The Effects of Resistance Exercise, Resistance Training, and a Multi-Ingredient High Caffeine Pre-Exercise Supplement on the p38 and ERK1/2 Cellular Signaling Proteins.

By

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Submitted to the graduate degree program in Department of Health Sport & Exercise Sciences and the Graduate Faculty of the University of Kansas in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

Mitogen Activated Protein Kinases (MAPKs) have been implicated in cellular signal transduction leading to cellular growth and differentiation in skeletal muscle following exercise. This dissertation provides a review of the published literature describing the role and actions of the MAPK pathways particularly the ERK1/2 and p38 pathways in response to exercise and to nutritional supplements. Two separate but related investigations were conducted. The first sought to elucidate the effect of various permutations of acute resistance exercise programming using a high concentric velocity (high power) back squat exercise on the mitogen activated protein kinases ERK1/2 and p38. The second investigation examined the effect of 8 weeks of resistance training with and without a multi-ingredient caffeinated pre-workout supplement on the same two MAPK pathways (p38 and ERK1/2), and other performance variables. Taken together these two studies show the response of ERK1/2 and p38 to resistance exercise programing variables and resistance exercise training status. This information taken in light of the previous research has significant implications for strength and conditioning coaches, trainers, and therapists who are seeking to increase muscular strength and hypertrophy for any number of reasons.

RESEARCH ABSTRACT FOR STUDY 1

PURPOSE: The purpose of this study was to determine the effect of three different high power back squat protocols and the associated muscular power production on the MAPKs, ERK1/2 and p38. Power fatigue across sets was also considered **METHODS:** Nine recreationally trained

males (21.4±1.67 years, 202.9±21.4 lbs.), completed each of three speed squat protocols in randomized order. The three testing protocols consisted of 5 sets of 10 repetitions at 30% of 1RM; 5 sets of 5 repetitions at 70% of 1RM; and 5 sets of 3 repetitions at 90% of 1RM. Average and peak power measurements were obtained by Tendo Weightlifting Analyzer for 7 of the subjects. Resting and post exercise blood lactate and muscle biopsies were taken. Western blot analysis was completed for ERK1/2 and p38 as well as their phosphorylaed counterparts. **RESULTS:** Blood lactate increased similarly between the three testing protocols. Analysis of power measures identified a significant main effect for load (F=10.23, p= 0.004), but not for set, p >0.05). The average power was lower for the 90% protocol than for the 30% protocol (F= 28.96, p<0.01). A significant interaction (F= 4.98, p< 0.001) was found differentiating the power production between the highest and lowest loads. This study did not show any increase in the ratio of ERK1/2 phosphorylation following exercise. And only a relative difference in 38 was found between the exercise protocols (F2,16) = 5.514, p=0.015). **CONCLUSIONS:** Resistance load appears to affect power fatigue across five sets of resistance exercise. The heaviest load had the highest power fatigue, while the light and moderate loads did not show any evidence of power fatigue. This study found no change in the ERK1/2 or p38 phosphorylation following any of the protocols. The exercise modality, training status of the subjects, and/or the timing may account for these findings.

RESEARCH ABSTRACT FOR STUDY 2

PURPOSE: The purpose of this study was to examine the acute and training effects with and without a multi-ingredient high caffeine pre-workout supplement on squat and bench press power, vertical jump, and the mitogen activated protein kinase (MAPK) pathways (ERK1/2 and p38). METHODS: Twenty four of thirty physically active collegiate (19.5±0.269 yr) males completed this double blind placebo controlled investigation. Subjects in the Experimental group (EXP) (*n*=14) consumed a multi-ingredient, high caffeine (450mg) pre-exercise supplement 10-15 minutes before each training session. Subjects in the Control group (CON) (*n*=10) drank a non-caloric placebo at the same times. Both groups completed an eight week resistance training program (3days/wk). Squat and bench press power and vertical jump were measured before and after the 8 weeks of resistance training. Muscle biopsies were also taken before and after the first and last workouts of the training program. Separate repeated measures ANOVAs were performed to test for differences between EXP and CON groups for power as well as total, phosphorylated and the relative ratio of phosphorylated to total ERK1/2 and p38. RESULTS: Squat power measurements showed a significant group by time interaction ($F_{1/22}$ =4.857, p=0.038). Vertical Jump and bench press power did increase with training, but did not differ between groups. Not only did ERK phosphorylation and the ratio of phophorylation increase after exercise ($F_{(1,22)}$ = 4.854, p = 0.38; $F_{(1,22)}$ = 5.159, p=0.033) but there was evidence that the ERK1/2 exercise response was is reduced after weeks of training ($F_{(1,22)}$ = 6.607, p=0.017). P38 was increased following training, but there was no effect of exercise bout on the phosphorylation on p38 in this study. DISCUSSION: Only one of the three power measurements, squat power, was benefited by the supplementation. EXP group than the CON group, the supplement did not affect any ERK1/2 or p38 measures. Eight weeks of pre-workout supplementation may provide some muscle

performance benefit when combined with resistance exercise training. This study supports previous findings that ERK1/2 phosphorylation following exercise is blunted by exercise training.

ACKNOWLEDGMENTS

I would like to acknowledge and thank the following people for their contribution and help during this dissertation process. I would first of all like to think my chair, Dr. Andrew Fry for his dedication and mentorship throughout this project. I would also like to thank Dr. Phil Gallagher, who has been invaluable resource both in terms of academic advising and regarding all practical laboratory learning. The advice, guidance and mentorship from these two individuals has shaped my professional career. I would also like to thank all of the members of my committee: Dr Philip Gallagher, Dr. Phillip Vardiman, Dr.Herda, and Dr Orr. I appreciate the assistance that you have given me especially during the writing phase of this project. I know that each of you has committed a great deal of time and effort to this process and deeply appreciate your efforts.

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My parents, Marge and Lee Kudrna, provided me the solid foundation for all of my academic studies, the courage to venture out and explore new things and places, and the confidence of knowing I can always come home. Also to Catherine Rustemeyer who always there to support and spoil me in so many ways.

Finally, I would like to thank those to provided me moral support, who have been there through every trial and difficulty, who pulled me through discouragement and frustration, and did not allow me to give up. Among this group I include My family, especially my parents, and my closest friends, especially Nicole. Lastly and Mostly, to God, the source of all good things.

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CHAPTER 1: INTRODUCTION

Muscle cells are capable of responding to diverse environmental stimuli. Chronic responses to such stimuli cause muscle cells to adapt over time. The metabolic, mechanical, environmental, and hormonal trigger coordinated cellular signaling pathways that ultimately alter gene expression within the cell. The differential gene activation dependent on these stimuli allow for the up or down regulation of the genes that will be most crucial for adaptation and resistance to the same cellular perturbation or condition if it were experienced again in the future. To state the same more concisely, the altered gene expression (termed adaptation) that is observed in response to a cellular stress or stimuli, must be linked by some cellular mechanism. Thus, cellular signaling pathways and interactions are critical mechanisms responsible for the adaptation response.

Mitogen activated protein kinase (MAPK) pathways have been implicated as one of many contributors to the cellular responses following resistance training. The MAPKs are a family of ubiquitous proteins, prevalent in mammalian cells. These MAPK signaling pathways transmit cellular signals via phosphorylation of threonine, and tyrosine residues (reviewed in Widegren, Ryder, & Zierath, 2001). Within the 'classical' MAPK pathways there are four primary parallel MAPK signaling pathways that are affected by different upstream kinase cascades (Widegren et al., 2001). As you can see in the Figure 1 below, these pathways appear to be entirely separate independent of other signaling cascades, however this is not necessarily the case as will be discussed more completely in chapter two .

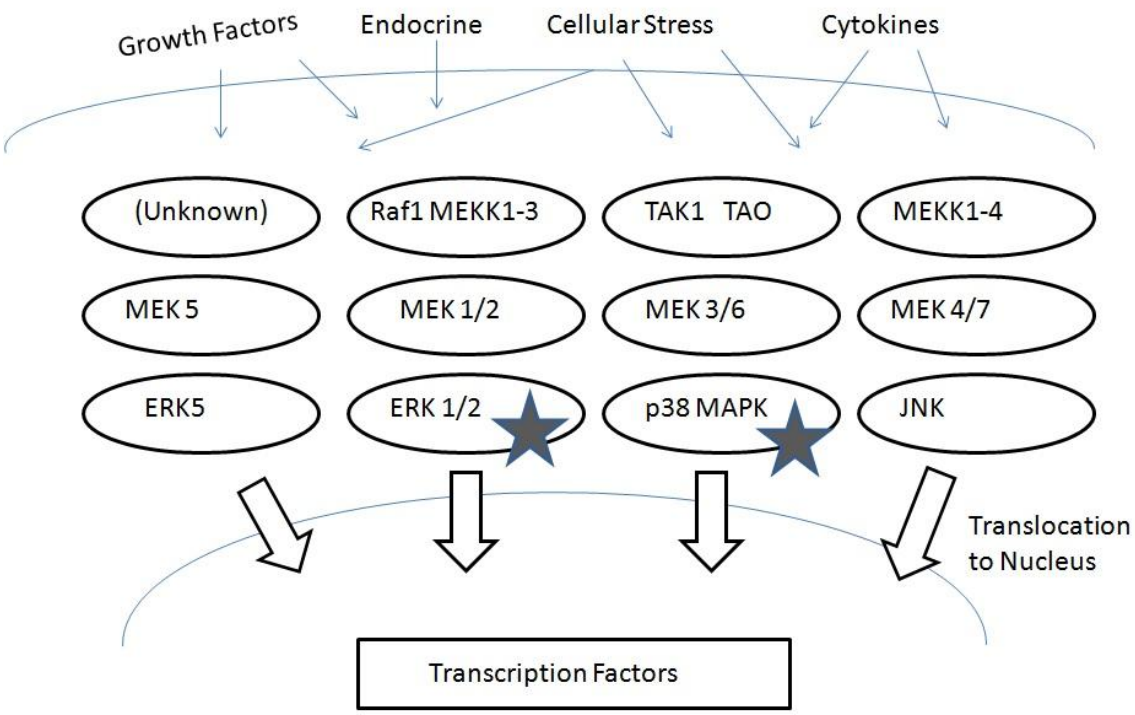

Figure 1. Simplified MAPK Cascade based upon the review by Widegren (2001). The Stars highlight the two pathways that are the focus in this paper.

Many extracellular and intracellular stimuli for MAPK activation have been identified. These include hormones, cytokines, mechanotransduction, and metabolic disturbances (Li et al., 2005; Martineau & Gardiner, 2001; Widegren et al., 1998; Wright, 2007; Yan, Li, & Akimoto, 2007). Once activated, the MAPK proteins will activate or modify one or many other downstream protein kinases, gene promoters for example, altering gene expression within the cell (M Gibala, 2009). The MAPK pathways, together, have important regulatory functions in almost every aspect of the myocyte activity. These include cellular proliferation, differentiation in stem cells as well as hypertrophy, mitochondrial biogenesis of differentiated myocytes. Each of these will be discussed in greater detail in chapter 2 of this work.

Because the MAPK pathways are crucially involved in the some of the most important cellular functions in skeletal muscle, they are also considered a therapeutic target particularly for the treatment muscle wasting diseases and the age related loss of strength and muscle mass (Williamson, Gallagher, Harber, Hollon, & Trappe, 2003), as well as cancer and inflammatory diseases (Malemud, 2007). MAPK pathways also affect muscle size and strength relating to athletic performance (Bassel-Duby & Olson, 2006). In addition to skeletal muscle hypertrophy, the MAPK pathways appear to be involved in the progression of insulin resistance and diabetes in the skeletal muscle (Cusi et al., 2000; Hawley, 2004; McGee & Hargreaves, 2004; Vichaiwong et al., 2009). Finally, the MAPK pathways represent a point of convergence and mechanism of control complementary to many other signaling pathways. The medical, performance, and basic research related implications of the MAPK pathways are not mutually exclusive and there are many potential benefits to understanding the MAPK pathways more fully.

STATEMENT OF THE PROBLEM

MAPKs are shown to respond to a wide variety of exercise and contraction types and it is likely that these stimuli affect the MAPK pathways by multiple mechanisms. What remains to be elucidated is the differential changes in the ERK1/2 pathway in human skeletal muscle following different types of resistance training. Also it is known that the MAPK pathways respond to a wide variety of stimuli related to nutrition and dietary supplementation. Lastly the response of these pathways to consistent resistance training has not been clearly elucidated.

RATIONALE FOR THE INVESTIGATIONS

While MAPKs especially ERK phosphorylation and activity increases following resistance exercise, there is very little research examining the exact relationships between this, the training status, and resistance exercise prescription variables. A more complete understanding of the relationship between resistance exercise and resistance exercise training would allow a more precise and efficient application of resistance exercise for sporting populations, the general population, and the elderly. It is likely that a more thorough understanding of the cellular response to exercise would be of the greatest benefit to those for whom muscle hypertrophy is most crucial, such as is the case in elderly individuals who are frequently limited in daily function and self-care because of muscle atrophy. The therapeutic application of resistance exercise for such individuals is necessary to preserve function, maintain independence, and retain quality of life.

STATEMENT OF THE PURPOSES

INVESTIGATION I:

The purpose of this study was to determine the MAPK (ERK1/2 and p38) responses to three different protocols of maximal power back squat and elucidate the role of muscular power in resistance training on the changes in these MAPKs.

INVESTIGATION II:

The purpose of this study was to examine the effects of resistance exercise and a high-caffeine, multi-ingredient pre-workout supplement on the phosphorylation of p38 and ERK proteins before and after 8 weeks of training. In order to clearly delineate the contribution of the exercise and the supplement plus exercise a double blind placebo controlled training design was utilized.

STATEMENT OF HYPOTHESES

INVESTIGATION I: THE EFFECT OF THREE DIFFERENT HIGH POWER SQUAT PROTOCOLS ON MAPK EXPRESSION AND PHOSPHORYLATION

1. Acute bouts of resistance exercise will increase the phosphorylation of ERK1/2.
2. A dose response relationship will exist between the muscular power of the resistance exercise and the relative changes in phosphorylation of the

MAPKs (ERK1/2, and p38). Muscular power is most likely to be highest in the low or moderate intensity loads

3. Within the context of this study's high power resistance exercise protocols MAPK p38 will be activated to a greater extend in the exercise protocol with the highest volume (total number of repetitions) but not intensity of work as measured by the load or relative load used. The p38 protein is unlikely to be related to power output.

INVESTIGATION II: THE EFFECT OF EIGHT WEEKS OF RESISTANCE TRAINING WITH AND WITHOUT PRE-WORKOUT SUPPLEMENTATION ON MAPK EXPRESSION AND PHOSPHORYLATION.

1. Acute bouts of resistance exercise will increase the phosphorylation of ERK 1/2.
2. Habituation to a total body resistance exercise bout via regular resistance training will assuage relative MAPK responses to exercise at the same relative intensity.
3. The pre-exercise supplement will produce a MAPK response to an acute bout of resistance exercise that is different than the placebo based upon the possible actions of one or more of the ingredients of the supplement.

RATIONALE FOR THE SCOPE OF THE INVESTIGATIONS

PHILOSOPHICAL RATIONALE FOR APPLIED RESEARCH

While basic scientists have at their ready a wide range of research tools and techniques to undertake the complete biochemical study of the MAPK or any other cellular

signaling pathway, the studies presented here take a decidedly applied approach to the problem at hand. Basic molecular and biochemical analysis of the cellular signaling process is vital to the advancement of our understanding in this area, but without a robust body of more applied studies the implications of such basic research remains theoretical. The assumption that whole muscle in a biologically complex human being, having an endocrine, immune, and neruoregulatory systems, would respond identically to analogous but not identical stimuli (bicycling vs. vacuum induced cyclical stretch) is abhorrently arrogant and demonstrably false.

The proposed studies were not undertaken to produce new evidence of the mechanistic actions of the MAPK pathways, this research was undertaken in an effort to corroborate the previously identified basic observations in live whole humans undertaking resistance exercise as they might their 'native habitat'. The exercise programs used in this study are substantially similar to those that athletes or avid exercisers might complete and performed in a setting more similar to the typical gym. Thus this research was designed to be more externally valid than previous resistance training investigations on the same topic.

CHAPTER 2: REVIEW OF LITERATURE

INTRODUCTION

The mitogen activated protein kinase (MAPKs) pathways have been implicated as one of cellular responses following resistance training and many other exercise and nutritional stimuli. The MAPKs are a family of ubiquitous proteins, prevalent in mammalian cells including skeletal muscle. These MAPK signaling pathways transmit cellular signals via phosphorylation (reviewed in Widegren et al., 2001). There are four parallel MAPK signaling pathways that are

affected by different upstream kinase cascades, (shown in figure 1 in chapter 1), (Widegren et al., 2001). While the pathways work in conjunction with one another and there is evidence of crosstalk between these pathways (Widegren et al., 2001), it is thought that each is particularly sensitive to different stimuli (Widegren et al., 1998) and that associated scaffolding proteins, proteins that physically link kinases within a cascade. Similarly the role of the MAPK proteins as regulators of muscle specific gene promoters, (Aziz, Liu, & Dilworth, 2010).

Many extracellular and intracellular prompts for MAPK activation have been identified. These include hormones, cytokines, reactive oxygen species, mechanotransduction, and metabolic disturbances, to list a few. (Li et al., 2005; Makanae, Kawada, Sasaki, Nakazato, & Ishii, 2013; Martineau & Gardiner, 2001; Widegren et al., 1998; Wright, 2007; Yan et al., 2007). Once activated, the MAPK proteins will differentially affect other downstream protein kinases and gene promoters, altering gene expression within the cell (M Gibala, 2009). The MAPK pathways, together, have important regulatory functions in cellular hypertrophy, differentiation, mitochondrial biogenesis and apoptosis.

Because the MAPK pathways are crucially involved in the some of the most important cellular functions, they are also considered a therapeutic target particularly for the treatment of muscle wasting diseases and the age related loss of strength and muscle mass (Williamson et al., 2003), as well as cancer and inflammatory diseases (Malemud, 2007). MAPK pathways also affect muscle size and strength relating to athletic performance (Bassel-Duby & Olson, 2006). In addition to skeletal muscle hypertrophy, the MAPK pathways appear to be involved in the progression of insulin resistance and diabetes in the skeletal muscle (Cusi et al., 2000; Hawley, 2004; McGee & Hargreaves, 2004; Vichaiwong et al., 2009). Finally, the MAPK pathways represent a point of convergence and mechanism of control complementary to many

other signaling pathways. The therapeutic, sport performance, and basic research related implications of the MAPK pathways are not mutually exclusive and there are many potential benefits to understanding the MAPK pathways more fully.

Among the many signaling stimuli that the MAPKs are associated with, the cyclic adenosine mono-phosphate (cAMP) and GTP-binding protein coupled receptors (GPCR) present a particularly interesting scenario. GPCRs and cAMP are upregulated by exercise and various nutraceuticals, which are herbal other chemical substances that affect various tissues beyond the expected role of foods. These cellular receptors are also common therapeutic drug targets. Thus a better understanding of this pathway is an important first step in developing more effective and targeted therapeutic interventions and drugs to combat muscle wasting diseases or any of the other conditions where the MAPKs might play a role.

The purpose of the following review is threefold. In the first section a general overview of the MAPK signaling cascades as they are understood to occur in skeletal muscle Special emphasis will be placed upon the p38 and ERK1/2 pathways. In the second section a more thorough review of the literature related to the role of these same two MAPK pathways in important skeletal muscle differentiation, hypertrophy, senescence regulation, and insulin resistance. The third portion of this review will examine the relationship of the MAPK signaling cascades to various forms of exercise, nutritional and orthomolecular compounds. Finally in the conclusion I will present the argument that there is sufficient basic science evidence to support further research into the nutritional and exercise interventions to affect the p38 and ERK1/2 pathways in humans.

SECTION I: THE MITOGEN ACTIVATED PROTEIN KINASES SIGNALLING CASCADES

The mitogen activated protein kinases are actually at least four parallel signaling cascades that have a common scheme of action. There are actually many total MAPKs in mammalian cells that can be grouped into four/five functional families. One family includes the extracellular related protein kinases (ERK1 and ERK2), collectively ERK1/2. A second extracellular related protein kinase of a much larger size, ERK 5, is prevalent in mammalian cells. In addition to the ERK1/2 MAPKs, there are two common isoforms of the c-Jun NH_2 Terminal kinase (JNK1 and JNK2), collectively JNK1/2. Lastly, there is the p38 protein so named for its 38kD size.

Each of these MAPKs is activated by upstream mitogen activated protein kinase kinases (MAPKKs or MKK). Each parallel branch of the MAPKs has one or more specific MKK associated with it. MKKs are themselves protein kinases that act to activate MAPKs via dual phosphorylation on both tyrosine and threonine residues (Garrington & Johnson, 1999). The MKKs are also activated by phosphorylation by upstream mitogen activated protein kinase kinase kinases (MAPKKK or MKKK). The MKKK are capable of phosphorylating MKKs on serine and threonine residues. Again each of the MAPK cascade families has a specific MKKK associated with it. Lastly upstream of the MKKKs are a variety of GTP binding proteins and other low molecular weight proteins that could be considered MKKKKs (Garrington & Johnson, 1999). GTP related proteins such as Ras and Cdc42 are the proteins responsible for linking receptor activity to the MAPK pathways.

The activity of these GTP related proteins may be aided by scaffold proteins that help to bring the selected G-protein subunits and kinases of a pathway together (Garrington &

Johnson, 1999; Mayor, Jurado-Pueyo, Campos, & Murga, 2007). Combined, the specificity of scaffold proteins, MKKKKs and MKKKs and MKKs and MAPKs create a very complex system of specific signaling (Raman, Chen, & Cobb, 2007). A problem in defining the precise MKKKK and GTP-binding and especially the scaffold protein interactions is that the majority of this research is performed in *saccharomyces cerevisiae* or isolated cells and these interactions may not clearly represent the complexities of a multicellular organism responding to more complex and varied stimuli simultaneously. Studies in human and animal models have demonstrated general activation patterns of the MAPK, especially within the three most studied MAPK signaling pathways in skeletal muscle, p38, JNK1/2 and ERK1/2.

MAPKs a Junction Checkpoint in Signal Cascades

Collectively, the MAPKs appear to be junctions for several major signaling pathways within the cell. Some of the cellular pathways associated with MAPK changes are, mechanical deformation or mechanotransduction (Boppart, Burkin, & Kaufman, 2006; Boppart, Hirshman, Sakamoto, Fielding, & Goodyear, 2001; Martineau & Gardiner, 2001), beta-adrenergic signaling (Crespo, Cachero, Xu, & Gutkind, 1995; Frost, Nystrom, & Lang, 2004; Zheng et al., 2000), calcineurin signaling (Rauch & Loughna, 2008), calcium-calmodulin signaling (Xu et al., 2002) cytokine signaling (de Alvaro, Teruel, Hernandez, & Lorenzo, 2004) and many others. These varied cellular signals all seem to activate one or more of the MAPK proteins.

Because the MAPK pathways are perfectly positioned to propagate signal transduction to a variety of stimuli, the MAPKs are also a good candidate mechanism of trans-pathway regulatory action. The MAPK family ERK1/2, for example, has been shown to be a major regulator of tuberous sclerosis complex (TSC) which in turn regulates mammalian target of rapamycin (mTOR) (Kwiatkowski & Manning, 2005). Therefore, ERK in particular can

influence or modify signals of nutrient and energy availability in mTOR, a known regulator of protein synthesis. Similarly p38 has been shown to regulate the important skeletal muscle gene 'master regulator' MyoD, as well as play a critical role in the PGC-1 signalling pathway that is associated with insulin sensitivity, energy availability, and mitochondrial biogenesis.

The upstream, and downstream targets of the different isoforms varies in response to its activating stimuli, the cell type or stage of the cell cycle, and possibly hundreds of other factors with the cell (Aziz et al., 2010; Guttridge, 2004; Liu et al., 2012). Thus contrary to the simplified classical model of the MAPK cascades that is depicted in Figure 1 (in chapter 1), the complexity of the signaling cascades of ERK1/2 and p38 cannot be understated.. Figure 2 below demonstrates that complexity for just one aspect of p38 function. Figure 2 shows a proposed mechanism by which MyoD which was previously thought to act in an MAPK independent mechanism is now believed to be reliant on p38 for its demethylation and activation of the skeletal muscle specific genes in differentiating skeletal muscle cells (Aziz et al., 2010). In the figure p38 not only phosphorylates MEF2d, but it is done as part of a complexed protein structure. Part B of figure 2 demonstrates the MAPKs are not true linear signaling cascades, but the amount of crosstalk is significant(Junttila, Li, & Westermarck, 2008). Finally part C of figure 2 shows that these cascades do respond to more than one upstream signal, and that there is significant crosstalk not only among MAPK pathways, but as shown in the case of ERK1/2, there is crosstalk with other signaling pathways (Berki, Boldizsar, Szabo, Talaber, & Varecza, 2001). This evidence Thus this single example of one of p38's known roles shows that the previously held simplified model of separate, true, and linear cascades for the MAPKs is incorrect.

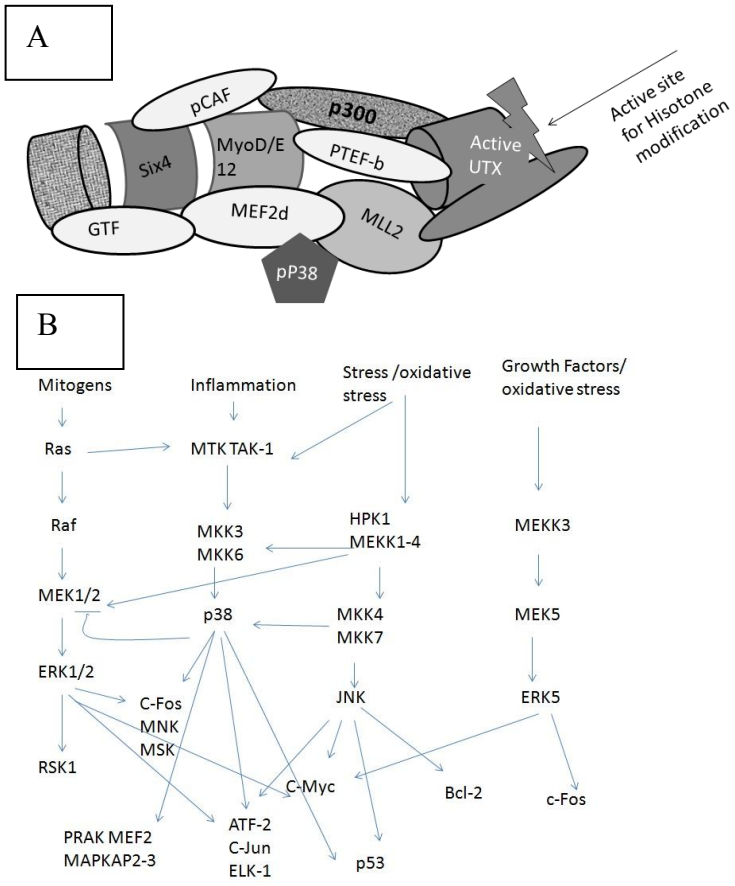

Figure 2a. Proposed model of p38 action in activation of repressed muscle specific genes during differentiations. The complexed protein shown in A above is proposed to activate a demethylation enzyme. Modeled after proposed interatctions presented in Aziz et al. (2010). Figure 2b.Demonstrates crosstalk within the MAPK cascades and the multiple and overlapping downstream targets proposed for these cascades. The figure is base upon the work of Junttila et al. (2008).

JNK PATHWAY

The first of the four pathways to be examined here is the JNK pathways. JNK MAPKs were originally identified as stress activated protein kinases. There are actually three JNK isoforms but only JNK1 and JNK2 are fund in muscle (Krishna & Narang, 2008). The MAPKKs responsible for JNK activation are the mitogen activated protein kinase kinase 4 and 7 (MKK4 and MKK7) and the MKKKs associated with JNK are MEKK1-4 (Krishna & Narang, 2008). Additionally the JNKs are activated by the upstream kinases RAC1/2, Cdc 2, and SEK1. JNK becomes maximally activated when phosphorylated on both threonine and tyrosine residues.

Like other MAPKs the activation of JNK can result in downstream changes in gene expression as well as modification of other kinases. More than 50 possible JNK substrates have been identified, though little research has been performed to clarify most of these interactions (Bogoyevitch, Ngoei, Zhao, Yeap, & Ng, 2010). JNK is capable of phosphorylating transcription factors like c-jun, jun D, jun B, and ATF-2 (reviewed in Krishna & Narang, 2008). One of the most often studied JNK associated events is the upregulation of the AP-1 promoted genes (Krishna & Narang, 2008). JNK has been shown to play a critical role in cytokine signaling, especially in response to TNF-α (Borer, 2003) and are involved in cytokine production (Frost et al., 2004). JNK has also been linked to carbohydrate metabolism and insulin (Hirosumi et al., 2002; Moxham, Tabrizchi, Davis, & Malbon, 1996), epinephrine (Napoli et al., 1998), and cell apoptosis (Krishna & Narang, 2008). Also, JNK is activated by several forms of exercise and muscle overload (Boppart et al., 1999; Carlson, Fan, Gordon, & Booth, 2001; Goodyear, Chang, Sherwood, Dufresne, & Moller, 1996). Over-expression of JNK in mice also increased the phosphorylation of several other proteins including ERK 1/2 and its downstream target p90, AKT

and its downstream target p70, and Glycogen synthase kinase 3, (Fujii et al., 2004). This is further evidence of th junctional role of MAPKs.

ERK 1/2

The ERK 1/2 pathway was both the first identified and most researched among all the MAPK pathways (Krishna & Narang, 2008). This pathway has gained a great deal of attention because it responds strongly to many types of exercise as well as growth factors (Krishna & Narang, 2008). The common activation pathways for ERK1/2 include MEK1 and MEK2 as well as MKK and Raf MKKKs. Upstream of this are small G-proteins like Ras. Of all the MAPKs, ERK has the greatest amount of evidence for transport to the nucleus upon activation (Krishna & Narang, 2008). ERK does have substrates at the cell membrane, in the cytoplasm, and within the nucleus, however. Some of the many substrates include phospholipase 2, MEF-2, c-myc, and CREB (Krishna & Narang, 2008).

The genes and protein substrates activated by ERK are involved in a wide variety of cellular activities including cell differentiation (Al-Khalili, Kramer, Wretenberg, & Krook, 2004), growth, survival (Aronson et al., 1998), and apoptosis (Krishna & Narang, 2008). More specifically ERK is activated by insulin (Napoli et al., 1998), various forms of exercise (Martineau & Gardiner, 2001; Osman, Hancock, Hunt, Ivy, & Mandarino, 2001; Yu, Blomstrand, Chibalin, Krook, & Zierath, 2001), as well as GPCRs (Krishna & Narang, 2008).

THE P38 PATHWAY

The p38 MAPK pathway shares commonalities with both the JNK and ERK pathways. There are four isoforms of p38 (α, β, γ, and δ) and these isoforms appear to be

selectively phosphorylated by different upstream MKKs (Krishna & Narang, 2008). MEK3 and MEK6 are the most traditionally associated with p38, but the MKKs in the JNK pathway (MKK4 and MKK7) also activate some forms of p38, thus accounting for the crosstalk between these two pathways. MAPK p38 has been shown to respond to MTK1, AKL, ASK1, and TAK1, all MKKKs. Lastly, the same G proteins in the ERK pathways (Ras, Raf) can activate p38 as well.

When activated, p38 may translocate to the nucleus, but like ERK there appear to be a variety of p38 substrates at the membrane, in the cytoplasm, and in the nucleus (Krishna & Narang, 2008). Many transcription factors are phosphorylated and activated by p38 including: ATF-1, ATF-2, Sap1, p53, MEF2A and NFAT. PGC-1α, a transcriptional co-activator associated with mitochondrial genes, also seems to be activated by p38 (Knutti, Kressler, & Kralli, 2001). Other evidence calls into question any direct interaction between p38 and PGC-1α, but instead demonstrates that p38 activity removes the PGC-1α repressor p160 myb (Fan et al., 2004; Knutti et al., 2001).

Physiological activation of p38 has been shown for growth factors , interleukins, and insulin like growth factor (Krishna & Narang, 2008). MAPK p38 has been implicated as a major mechanism in skeletal muscle atrophy signaling (Glass, 2005). Increased levels of p38 phosphorylation (Kim et al., 2009) or activity (Zhang, Chen, & Fan, 2007) have been shown to exert transcriptional control over atrogin 1, also called muscle atrophy F-box (MAFbx), and Muscle specific ring finger 1 (MuRF-1). The p38 induced increases in MAFbx and MuRF-1 have been observed with disuse atrophy (Zhang et al., 2007), cast immobilization (Kim et al., 2009), and TNF-induced atrophy, (Li et al., 2005). It is also been observed in serum starved cell cultures (Kim et al., 2009), and reactive oxygen species (Li et al., 2005). Because the FOX-O transcription factor has also been shown to be responsible for MuRF-1 and atrogen-1

transcription, one hypothesis is that p38 directly activates FOXO (Li et al., 2005).

This proves again the xomplexity as p38 is both associated with cellular differentiation in skeltal muscle as well as atraophy in other circumstances.

ERK 5

Little is known about the contribution of ERK 5 (also known as BigMAPK) in skeletal muscle (Kramer & Goodyear, 2007). Both ERK5 and MAPK/ERK kinase 5, its upstream kinase, have been found in significant quantities in the skeletal muscle (Zhou, Bao, & Dixon, 1995). Its contribution to the skeletal response to exercise has not been clearly established, but it is known that during the differentiation of myoblast to fused myotubes, the relative expression of ERK5 does not change (Al-Khalili et al., 2004) . Future research may elaborate its involvement or lack there-of in the signaling mechanisms within skeletal muscle.

SECTION II. PRIMARY IMPLICATIONS FOR P38 AND ERK1/2 SIGNALLING IN MUSCLE DIFFERENTITAION, GROWTH, AND HYPERTOROPHY.

EMBRYONIC STEM CELL AND SKELETAL MUSCLE SATELLITE CELL DIFFERENTIATION

Differentiation is a complex, vital, and often irreversible cellular activity and is controlled by combination of signaling cascades. To put the regulation of muscle into perspective consider that there are at least 5 separate signaling cascades that affect muscle size either by stimulating hypertrophy or atrophy, (Guttridge, 2004). For a simplified overview of the most completely understood of those pathways see the review by Guttridge (Guttridge, 2004). Arguably the MyoD signaling is of greatest interest here. MyoD is considered the ‘master regulator’ or skeletal muscle gene products (Aziz et al., 2010). MyoD expression and function

seems to be a defining characteristic of functional muscle cells so its expression during the cellular differentiation process is a crital component (Aziz et al., 2010; Liu et al., 2012).The MAPK proteins especially p38 are known to play a crucial role in the differentiation and development of myoblasts and myocites (Aziz et al., 2010; Knight et al., 2012; Liu et al., 2012). In this section, evidence of the mechanisms by which p38 and ERK1/2 are involved in the regulation of muscle hypertrophy will be considered.

Durring myocyte differentiation mesodermal stem cells are the precursors to myocytes. The mesodermal cells exit the cell cycle and differentiate into mononuclear monoblasts. This process is regulated by the muscle regulatory factors (MRFs) a family which includes MyoD, Myf5, myogenin and MRF4 (Liu et al., 2012). This differentiation is also controlled tightly by the p38 protein kinase signaling pathways. Fascinatingly, it appears that several of the isoforms of p38 play a unique and separate roles in this process. The p38γ isoforms has been shown to prevent the cell from exiting the cell cycle and becoming a differentiated cell (Gillespie et al., 2009). Newer research clearly shows that the asymmetric cell division in the proliferation phase is associated with the Par Complex activation of p38α/β occurs in one daughter cell while myoginin and MyoD initiate the differentiation in the other daughter cell (Troy et al., 2012). The p38α isoform, on the other hand, is required for differentiation to occur (Liu et al., 2012). Specifically p38α has been shown to regulate several muscle specific genes, chromatin remodeling enzymes, and also the expression of at least one of the MRF family proteins, as myogenenin in reduced in p38α knockdown cells. (Liu et al., 2012). Up-regulating myogenein leads to a down regulation of genes involved in progression of cell differentiation, therefore p38α and p38γ appear to regulate opposite outcomes for the cell. Stated more simply p38α either initiates or supports the exit from the cell cycle and differentiation into a myocyte

(Liu et al., 2012), while the p38γ isoform prevents the differentiation (Gillespie et al., 2009). It has also been observed that loss of both p38γ and α within a cell produce irreversible quinescence, (Jones et al., 2005).

In an effort to further understand the binding homology differences between p38α and γ isoforms and to understand more fully the opposing roles these proteins play in the differentiation, Knight et al. (2012), found that though the binding domains are 75% identical (H. Wang, Xu, Xiao, Jiang, & Wu, 2008), It is in fact there localization, and not their substrate specificity that determines the oppositional roles of these two isoforms. Specifically p38α appears to increase substantially in the cytoplasm during the differentiation process, and that p38γ cannot fulfill the cytosolic role of p38α (Knight et al., 2012). A second observation that helps to explain the contradictory roles of p38 in the differentiation process, is that both p38α and γ appeared to produces different outcomes based upon the stage of the cell cycle, cell phenotype, and/or another regulatory mechanism yet to be discovered (Jones et al., 2005; Knight et al., 2012). I It is likely that future research will clarify that many mechanisms are responsible for additional modification of the p38 protein kinase activity.

While differentiation of the stem cell into a myoblast through the aforementioned mechanisms, p38α has an additional role as well in the fusion of the myoblasts into the functional multi-nucleated myocyte as well (Liu et al., 2012). This second role is equally essential for normal muscle development. Liu et al. (2012) dramatically demonstrated that the p38α knockdown cells were able to differentiate when myogenein was over expressed to compensate, but that myoblast fusion into myocytes was not possible without adequate levels of p38α. Further investigation into this showed that p38α regulates several genes previously shown to be important for myoblast fusion. Liu et al. found that tetraspanin CD53 was likely the primary protein

involved in this fusion process as it is not only up-regulated during fusion but also localizes to the site of the fusion along the membrane (Liu et al., 2012). Furthermore, a tetraspanin CD 53 knockdown mouse model had significantly reduced muscle size (Liu et al., 2012).

Clearly p38 has a critical role in the differentiation process of skeletal muscle in mammalian cells as well as fusion of myoblasts. It is no great leap therefore to see that it must play a significant role in muscle hypertrophy as a regulator and essential part of the satellite cell differentiation and fusion to the existing myofibers. The role of ERK1/2 in the proliferation and differentiation is only just becoming more clear. There is far less known about the role of ERK1/2 on cellular differentiation than p38. What is known is that ERK1/2 and MEK1/2 phosphorylation increased as well as cellular differentiation and myogenesis when the antagonist protein kinase Cθ isoform was knocked down (Marino et al., 2013). Contradictory findings from Feng et al. (2013), on the other hand show that inhibition of ERK1/2 enhanced myoblast differentiation so long as the ERK1/2 was available for early myogenic processes. This study also demonstrated that a microRNA secquence miR-133 was tied into a negative feedback loop with ERK1/2 and its phosphorylation (Feng et al., 2013). miR-133 is known to regulate protein at the post transcription level. A possible interpretation of these findings and a way to resolve the apparent conflict with the Marino et al. research would suggest that ERK1/2 is necessary for proliferation of the undifferentiated cells, but that differentiation can only occur when ERK1/2 and its phosphorylation are decreased or alternately by the modifying actions of the ERK1/2 activity antagonist miR-133 (Feng et al., 2013; Marino et al., 2013).

A POSSIBLE ROLE FOR ERK1/2 AND P38 IN G PROTIEN RECEPTOR SIGNALLING

The class of membrane receptors that associate with GTP-binding proteins (G proteins) are often identified as G protein coupled receptors (GPCRs). There are many types of GPCRs that have been further classified into adenosine, adrenergic, angiotensin II, endothelin receptor types, and other families based on homology and function (Borer, 2003). GPCR super family all contain seven membrane spanning segments, where the extracellular portion is associated with agonist binding specificity and the intracellular of the protein is associated with G protein specificity (reviewed in Lynch & Ryall, 2008). Each of these categories has multiple receptor subtypes and isoforms.

GPCR are by definition associated with G-proteins and use some specific G-protein for signal transduction. In addition to the wide variety of GPCRs and their agonist binding specificities, there are also G protein specificities for each receptor type. G-proteins are heterotrimeric proteins with subunits identified as α, β and γ. Both the α and γ subunits are attached to the membrane by a covalently linked lipid tail in most cases. The α subunit also has a GTP/GDP binding and hydrolysis site as well.

In the inactive state, the trimeric G protein is associated with GDP. Cytosolic interaction with an activated GPCR causes the trimeric G protein to release GDP and associate with GTP instead. The α subunit will undergo a conformational change following GTP binding that can (but not always) cause the alpha subunit to dissociate from the βγ complex. Both the α and the βγ subunits can play a role in the signaling pathways.

G-proteins are varied in their structure and function. Based upon the target specificity, the G proteins are further classified as G_s, G_i, $G_{q/11,}$ and $G_{12/13}$, (Post & Brown, 1996). Each receptor

type is likely to recruit predominantly one type of G protein. Beta adrenergic signaling predominantly couples with $G\alpha_x$ and $G\alpha_i$ subunits to activate adenylyl cyclase and α_2 adrenergic receptors recruit primarily $G\alpha_{q/11}$ (reviewed in Lynch & Ryall, 2008). Further complexity arises in this system with at least 27α, 7β, and 12γ subtypes that can form in any combination (Lynch & Ryall, 2008). Thus, although the family of beta receptors may all appear to be similar, they will have a combination of agonist specificity and G protein specificity, resulting in different catecholamines activating different receptors and having different effects within different cell types.

Beta-agonist Stimulated Transduction

There is a great deal of research demonstrating that β-agonists induce hypertrophy and/or repair of cardiac, smooth, and skeletal muscle (Beitzel, Sillence, & Lynch, 2007; Izevbigie & Bergen, 2000; Lynch & Ryall, 2008; Ryall et al., 2008; Zheng et al., 2000). There are three subtypes of β-adrenergic receptors that are expressed in varying quantities in different tissues. Of these subtypes, the β_1 and β_2 appear to be most plentiful in skeletal muscle (Lynch & Ryall, 2008) and the β_2 receptors appear to be the primary stimulators of cAMP (Roberts & Summers, 1998). cAMP itself is not the only possible activator of downstream kinases however. Both the G_α and $G_{\beta\gamma}$ are capable of activating various signaling proteins upstream of the MAPKs. First, the role of cAMP in MAPK signaling will be explored, followed by possible direct G-protein signaling.

$G\alpha_s$ is the G protein subunit that is responsible for the activation of adenylyl cyclase, causing the conversion of ATP into cAMP. cAMP then interacts with various cell proteins to begin the kinase signaling cascades, including the MAPKs. Specifically cAMP can interact with an

exchange protein directly activated by cAMP (EPAC) or one of several other activators of Rap-1 (Goldsmith & Dhanasekaran, 2007). From there the Rap-1 will signal one of the Raf isoforms, and from there the cascade continues to MEK1/2 and ERK1/2 (Goldsmith & Dhanasekaran, 2007). This direct pathway leading to ERK activation may not hold true for all cell types, in other instances the ERK activation observed following GPCR activation is mediated by protein kinase A (PKA) . The specific activation mechanism of ERK in skeletal muscle has not yet been determined.

The activation of p38 by $G\alpha_s$ is likely to be caused by a PKA mediated pathway as well. Beta adrenergic induced phosphorylation of p38 is not direct, but is instead PKA dependent in cardiomyocytes (Zheng et al., 2000), but the exact proteins connecting PKA activation to MKK3 and MKK6 (upstream of p38), have not been identified (Goldsmith & Dhanasekaran, 2007). In PKA dependent ERK1/2 phosphorylation, the upstream protein Ras is likely involved. Thus, both ERK1/2 and p38 may be activated in a PKA dependent manner, while more direct cAMP signaling is possible in some cell types, though it has not been clearly established for skeletal muscle. JNK does not apparently respond to $G\alpha_s$ signaling (Goldsmith & Dhanasekaran, 2007).

The inhibitory G_i class of G-proteins are also able to stimulate ERK1/2 through the involvement of phospholipase C (Goldsmith & Dhanasekaran, 2007). There is however no evidence that p38 can be activated in this manner. Instead G_i inhibits adenylyl cyclase activity and would theoretically then inhibit PKC stimulation of p38 as well as ERK1/2.

β-adrenergic receptor activity may also play an important role in the muscle repair and hypertrophy following injury, (Beitzel et al., 2007; Ryall et al., 2008). Ryall et al. (Ryall et al., 2008) found that a single injection of formoterol resulted in significantly higher strength at seven

days without any detrimental cardiac hypertrophy. Moreover Beitzel and colleagues (Beitzel et al., 2007) found that there was a threefold increase in receptor density following injury, which increased the ability of regenerating muscle to respond to beta agonists. In that study, the receptor density in fenoterol (an agonist) treated animals did decrease, but the cAMP levels following isoproterenol were only reduced in the fast twitch extensor digitorum longus muscle of the rats (Beitzel et al., 2007) indicating a muscle type specificity. Elevated adenylyl cyclase activity and cAMP production may also be the mechanism by which the β-agonist is able to increase the rate of injury repair. Thus, the next piece of the puzzle is to understand the signaling that is occurring downstream of cAMP. There have been no specific mechanisms of action determined with regard to muscle injury and repair.

ESTROGEN, ESTROGEN –RELATED RECEPTOR, AND VITAMIN D RECEPTOR SIGNALING

Estradiol and its estrogen receptors (α and β subtypes) are important regulators of cellular activity including activation of Elk-1transcription factor and the cAMP response element binding protein (CREB) that are known to increase or decrease downstream transcription (Ronda et al., 2007). Similarly while the vitamin D receptor differs significantly in homology, it does share many similarities in the downstream signaling pathways with the estrogen signaling axis (Ronda et al., 2007). Estrogen signaling is dependent upon both the ERK1/2 and the p38 MAPKs, as well as protein kinase C and src (Ronda, Buitrago, & Boland, 2010). Similarly it has been shown that there are significant anti-apoptotic effects of estradiol are mediated by p38 (Ronda, Vasconsuelo, & Boland, 2010). While estradiol does interact with both of its receptor types, there may be additional mechanisms by which the steroid hormone alters cellular signaling that are yet

to be elucidated. It has been noted that activation of both the estrogen (Hatae, Takami, Lin, Honda, & Inoue, 2009) and the vitamin D receptor (Thota et al., 2014) up-regulates the synthesis and the translocation of estrogen receptors to the cell membrane in various cell types. It appears that this rapid increase in estrogen receptor number occurs by an ERK1/2 dependent mechanism (Hatae et al., 2009). The up-regulation of estrogen receptors may create a positive feedback mechanism by which cells become increasingly sensitive to estrogen signals. While there are a host of similarities between the estrogen receptor and the vitamin D receptors, the unique role of vitamin D signaling is discussed more completely in the 'nutrition: vitamins' section later in the paper.

The Estrogen-related receptors are named for their close structural similarities to the estrogen receptors, but do not bind estradiol, in fact its physiologically significant ligand has not been clearly established (Murray & Huss, 2011). Even so this receptor appears to regulate myocyte differentiation as well as a host of metabolism related genes via an ERK1/2 dependent mechanism (Barker et al., 2014).

Testosterone Stimulated Muscle Hypertrophy

While the exact mechanism remains unknown, administration of testosterone to rats resulted in an increase in p38 phosphorylation and was linked to the resultant muscle hypertrophy by concomitant administration with a p38 inhibitor (Brown, Hikim, Kovacheva, & Sinha-Hikim, 2009). This may be related to its role in the activation of differentiation of satellite cells as it was also associated with up-regulated Notch1 and Notch2 proteins, which are thought to be transmembrane receptors related to proliferation and differentiation (Brown et al., 2009).

OBESITY, INSULIN RESISTANCE AND TYPE II DIABETES

Because of the treatment potential, involved one of the most exciting areas of research is the role of p38 in insulin resistance. Koistinen (2003) showed that there was significantly higher basal p38 phosphorylation in insulin resistant skeletal muscle obtained from type II diabetics. This may indicate that p38 plays some role in the maladaptive physiologic changes. Similar changes to p38 phosphorylation state have also been observed in diabetic adipocytes as well (Carlson, Koterski, Sciotti, Poccard, & Rondinone, 2003). At this time it appears that the changes in p38 do not cause the muscle to take on the insulin resistant characteristics (Koistinen et al., 2003). The up-regulated p38 levels may instead be the result of poor energy availability within the cells such as the reduced availability of glucose within the cell (Koistinen et al., 2003). Hirosumi, (2002) clearly shows that many members of the MAPK family respond to cytokines that may be elevated in the diabetic or insulin resistant condition. Thus the unregulated p38 levels may also be more directly caused by the inflammatory markers associated with diabetes and insulin resistance.

In addition to aberrant basal phosphorylation levels, Koistinen (2003) also found that in vitro exposure of normal and insulin resistant muscle to insulin produced divergent changes. Whereas normal muscle showed an increase in p38 phosphorylation following insulin exposure, the insulin resistant muscle actually saw a significant drop in phophorylation from its elevated basal condition. More recent evidence takes this a step further showing that exercise training can not only lower basal phosphorylation levels in the insulin resistant muscle (Vichaiwong et al., 2009), but that increased phosphorylation of p38 was associated with increased GLUT 4 transporter protein mRNA post exercise (Hussey, McGee, Garnham, McConell, & Hargreaves, 2012). This may indicate that the elevated p-p38 in insulin resistant

skeletal muscle is not maladaptive but instead a reactive and positive adaptation given the reduced function of the insulin regulated glucose transport.

What is known definitively is that p38γ is tightly associated with the PGC-1α a major regulator of gene products associated with substrate utilization including mitochondrial biogenesis as more completely described in the review article by Lira, Benton, Yan, and Bonen, (2010). Because of this potentially therapeutic effects the relationship of p38 to insulin resistant skeletal muscle and exercise should be investigated further.

While p38 is the MAPK typically associated with insulin resistance there is also evidence that the ratio of phosphorylated to total ERK2 is lower in obese rats than their lean counterparts (Osman et al., 2001). Furthermore, seven weeks of exercise training for these rats restored some of the ERK2 activity both at rest and following insulin administration (Osman et al., 2001).

SECTION III: EXERCISE AND NUTRITIONAL MODULATORS OF P38 AND ERK1/2 SIGNALLING

EXERCISE, CONTRACTION, AND STRETCH

Traditional endurance training, long duration moderate intensity (Benziane et al., 2008), as well as short bouts of intense interval exercise have been shown to increase the activation of p38 and this has been associated circumstantially (M. Gibala et al., 2009) and causally (Little, Safdar, Cermak, Tarnopolsky, & Gibala, 2010) with up-regulated expression of Proliferator Activated Receptor-γ Coactivator-1α (PGC-1α). For a more complete review of the relationship between p38 and PGC-1α see Lira et al. (Lira et al., 2010). It should also be noted that there is evidence that exercise not only affects the ERK1/2 and p38 pathways of the

exercising muscle, but p38 of non-exercised limbs also seems to respond indicating that there may be both local and systemic pathways of activation (Widegren et al., 1998) .

Muscular contraction has been shown to activate both p38 and ERK1/2 in some (Galpin, Fry, Chiu, Thomason, & Schilling, 2012; Moore, Atherton, Rennie, Tarnopolsky, & Phillips, 2011; Ryder et al., 2000), but not all (Terzis et al., 2010) studies. Furthermore, the type of contraction or other modifications to the resistance training or contraction protocols may all be contributing to the skeletal muscle adaptations and the MAPK signaling response to exercise as it has in other signaling pathways (Terzis et al., 2010). Hulmi et al. (2012) found that a hypertrophy protocol, high volume and moderate intensity), produced significantly greater increases in ERK1/2 phophorylation over a maximal strength (low volume, high intensity) protocol while Terzis et al. (2010) did not see a significant change in ERK1/2 in any of their protocols. These two studies were in agreement that p38 was equally increased in all exercise protocols indicating that it is not differentially activated by modifications to the resistance training protocol (Hulmi et al., 2012; Terzis et al., 2010).

In addition to the volume and intensity, power, rate of force development and frequency may also have a role. Type II muscle fibers had had markedly higher phosphorylation of p38 following lengthening contractions (Tannerstedt, Apro, & Blomstrand, 2009; Wretman, Widegren, Lionikas, Westerblad, & Henriksson, 2000). Thus activities that produce a greater rate of force development, and those that require recruitment of a greater number of type II muscle fibers would produce greater whole muscle changes in p38 phosphorylation. Franchi et al. (2014) found that the morphological characteristics of the muscle, fascicle length and the pennation angle, were differently affected by either concentric or eccentric exercise training. These differences were circumstantially associated with MAPK activation in the eccentric group

but not in the concentric group (Franchi et al., 2014). Lastly, the frequency of training appears to affect the activation of several signaling pathways in rats (Coffey et al., 2007). Frequent training bouts with incomplete recovery between bouts increased the p38 activity likely in association with increased cytokine signaling or phosphorylation of AMPK and may suppress IGF-1 and Akt associated hypertrophic activity. ERK1/2 was not tested in this study (Coffey et al., 2007). Much further research is necessary to fully elucidate the roles each resistance training variable has on the cellular response to the exercise.

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Aging

Age appears to also influence the MAPK response to exercise and muscle contraction. In comparison to young adult skeletal muscle muscle from aged rats demonstrated a much blunted increase in ERK1/2 phosphorylation immediately following contraction (Parkington, LeBrasseur, Siebert, & Fielding, 2004). There were however no differences 6 hours after the contraction, nor where there any differences between the young and old muscle's expression or phosphorylation of ERK1/2 in the basal state (Parkington et al., 2004). This stunted signaling was found to be true in both the tibialis anterior and in the plantaris muscles (Parkington et al., 2004). This does however conflict with a previous study in humans that showed a significantly higher phosphorylation of both ERK1/2 and p38 in the basal state of older subjects (Williamson et al., 2003).

A second discrepancy is that Williamson et al. (2003), showed a decrease in ERK1/2 and p38 phosphorylation following resistance exercise. From these two studies it can be concluded that ERK1/2 phosphorylation does not increase as much or is in fact decreased

following exercise in aged muscle, but the contradictory findings regarding differences in p38 signalling are hard to resolve between these two studies (Parkington et al., 2004; Williamson et al., 2003). These studies explored the immediate and intermediate timed effects of exercise on ERK1/2 and p38. When measured 24 hours after the exercise bout ERK1/2 phosporylation was significantly increased compared to young subjects while p38 phosporylation was not different between the age groups (Kosek & Bamman, 2008). Kosek & Bamman followed up with 16 weeks of resistance training 3 times per week, and tested the response to exercise after the 16 weeks as well. Interestingly the older men had much elevated p38 phosphorylation after 16 weeks of training compared to the initial exercise bout (Kosek & Bamman, 2008). Kosek & Bamman (2008) like Parkington (2004) attributed this to "overstress" of the muscle of the aged individuals, but in this case they were referring to chronic overstress and not acute overstress though that notion was not well supported.

Training Status

It is known that exercise training produces diminishing returns over time. What is not well understood is the molecular signaling changes that account for this ceiling effect. The role of training status in MAPK activation is a fascinating area that has only just begun to be studied. An early study by Yu et al. (2003) found that there was greater MAPK activation in untrained vs trained male after intervals of cycling. This seems to indicate that the blunted response to exercise with training is accompanied by reduced activation of the associated MAPK and other signaling activities. Similar results were observed in other non-MAPK signaling cascades (Coffey et al., 2006). Contradictory evidence shows ERK1/2 activation was not different after 16 weeks of training in young men however (Kosek & Bamman, 2008).

Mechanotransduction

One of many possible mechanism by which exercise activates the MAPK proteins is mechanotransduction (Wozniak, Kong, Bock, Pilipowicz, & Anderson, 2005). Furthermore mechanotransduction as an upstream activator of ERK1/2 and or p38 would also explain why passive stretch can induce some of the same alterations in phosphorylation state of the MAPKs as exercise, resistance training, and muscle contractions of various types (Boppart et al., 2001; Hanke et al., 2010; Rauch & Loughna, 2005, 2008). Similarly, mechanotransduction would help to explain the dose response relationship between amount of tension and the degree of change in MAPK and other cellular signaling pathways (Martineau & Gardiner, 2001). Not all studies agree that the MAPK ERK1/2 and p38 are among the downstream targets of mechanotransduction (Dentel, Blanchard, Ankrapp, McCabe, & Wiseman, 2005), or that their role is essential in muscle hypertrophy (Sasai et al., 2010). In fact Dentel et al., (2005) adds credence to the idea that p38 responds to the metabolic (cAMP) and not the force producing related stimuli of exercise. Kosek & Bamman (2008) propose that the increased p38 phosphorylation state in older men compared to younger men post resistance training is evidence that the decreased hypertrophic response in older men is caused by the elevated p38 phosphorylation as it is an indicator of "over-stress" to the muscle. This would indicate the p38 is acting as an antagonist to the Distrophin associated protein complex signaling cascade (one of many mechanotransduction pathways). Mechanotransduction is not a single pathway, but instead involves multiple different parallel and series signaling components including a variety of autocrine signals (Adams & Bamman, 2012). A complete review of the intricacies of mechanotransduction is well beyond the scope of this paper. Two excellent reviews on the topic include Adams and Bamman (2012), and Wang& Thampatty (2006).

Timecourse of ERK1/2 and p38 changes in response to exercise and mechanical perturbations

Possibly the best direct study of the topic of the timecourse of the alterations of the MAPK proteins was performed by Carlson et al. (2001) who performed oblation of the gastrocnemius in rats and then sacraficed the animals after 1, 6, 12, and 24 hours of overload. Under these conditions the p38 remained elevated across all timepoints while the ERK 2 was increased for the first 12 hours and then returned to baseline (Carlson et al., 2001). This doesn not readily equate the human exercise model however in that the oblation model constitutes a continual resistance stimulus that does not end for the durration of the 24 hours. Studies in humans that have used resistance activity that is intermittenent and finite have demonstrated increased ERK1/2 ranging from minutes into the exercise bout (Galpin et al., 2012) and up to two hours post exercise (Taylor, Wilborn, Kreider, & Willoughby, 2012).

NUTRITIONAL INFLUENCES AND DIETARY SUPPLEMENTS

Some evidence suggests that there are nutrients and nutraceutical agents that also have the ability to alter MAPK phosphorylation (Ulrich-Merzenich, Zeitler, Vetter, & Kraft, 2009). It is unlikely that the nutritional components can directly affect activity of the MAPKs, but instead may alter the cellular status of the various stimuli that MAPKs are sensitive to. As examples, both forskolin and caffeine have been shown to increase cellular cAMP (T. E. Graham, Battram, Dela, El-Sohemy, & Thong, 2008; Seamon, Padgett, & Daly, 1981) . Both of these are available in supplement form and marketed to bodybuilders and the fitness minded, but the cellular mechanisms of their effectiveness have not been established. Because ERK1/2 and p38 are implicated in so many cellular signaling pathways these two kinases are also prime targets for activation of the various signaling mechanisms that neutraceuticals, phytonutrients, and vitamins

activate ultimately leading in many cases to altered protein expression. Examples of this interaction are included here, but an exhaustive list is beyond the scope of this paper. Furthermore, a review of this topic does not seem to be available.

Commercialized multi-ingredient supplements

Beyond these isolated compounds nutritional and ergogenic supplements have become increasingly common as competitive athletes and recreational exercisers alike seek to maximize performance and aesthetic outcomes from their efforts. A plethora of multi-ingredient pre and post workout supplements flood the market advertising dramatic enhancements to the exercise program. As Ormsbee, (2012) notes, the need for double blind placebo controlled trials on such supplements to help limit the marketers "misappropriation of the underlying science." After first having identified the concern over these poorly understood multi-ingredient concoctions, it is also important to note that multiple previous studies have demonstrated these market available supplements do provide significant but modest augments to exercise training alone, (Falk, Heelan, Thyfault, & Koch, 2003; Hoffman et al., 2008; Ormsbee et al., 2012).

Amino Acids & Creatine

Amino acid supplementation is widely known to affect the mTOR hypertrophyic pathway, but much less is known about the effect if that any amino acids have on the MAPK pathways. Karlsson et al.(2004) found BCAA supplementation before exercise had no effect on the ERK1/2 or p38 pathways. Sparse research on the role of creatine yields no effect over exercise alone (Deldicque et al., 2008) and a contradictory finding indicating p38 is involved in creatine's hypertrophic properties (Deldicque et al., 2007).

Caffeine potentially affects the MAPK cascades

There are many studies demonstrating the ergogenic effects of caffeine ingestion in skeletal muscle contraction (Reading, Murrant, & Barclay, 2003; Sipido, 2007), lipid mobilization (Donsmark, Langfort, Holm, Ploug, & Galbo, 2003), and possibly carbohydrate metabolism, but there is a general lack of research into the mechanisms of these actions (T. E. Graham et al., 2008) and the results of such studies are far from conclusive as others refute such findings, (T. E. Graham, Helge, MacLean, Kiens, & Richter, 2000). There are several possible mechanisms by which caffeine could affect muscle signaling and function both directly and indirectly. First caffeine can indirectly alter cellular contractile and metabolic functions via activity of the adenosine receptor rich CNS. Caffeine ingestion is also associated with increased levels of epinephrine and possibly also norepinephrine and thus can cause an increase in beta-adrenergic receptor signaling (reviewed in T. E. Graham et al., 2008). The generally accepted mechanism of caffeine signaling involves the inhibition of adenosine G_i associated GPCRs and thus the increase of cAMP (T. E. Graham et al., 2008). A meta-analysis of several smaller (low sample number) studies demonstrates that caffeine is most effective at raising cAMP levels when combined with exercise (T. E. Graham et al., 2008). Thus the efficacy of caffeine's cAMP increase may also be enhanced by allosteric modifications to either the receptor or some other related signaling component. The contribution of nor-epinephrine, epinephrine, and acetycholine have not been adequately studied adequately to determine if they are a cause of the exercise/caffeine synergism.

While the above mentioned association between caffeine and cAMP is well established, very little is known about the cellular signaling from that point. There are at least three cellular pathways that may be conveying the caffeine signals. These include the protein kinase A pathway (PKA),

the protein kinase C pathway (PKC), and calcium calmodulin signaling. Involvement of the calcium calmodulin cascade, supported by evidence in cardiomyocytes (Magne, Couchie, Pecker, & Pavoine, 2001; Sipido, 2007) and skeletal muscle (Reading et al., 2003), is beyond the scope of this paper. Hormone sensitive lipase activity within skeletal muscle appears to be up-regulated following caffeine exposure, but this occurs by a mechanism other than the ERK1/2 related changes that occur following exercise (Donsmark et al., 2003). This may also be related to the yet to be determined involvement of the MAPKs.

Caffeine is a widely utilized stimulant in multi-ingredient sports supplements and as a standalone ergogenic aid. This is particularly true for supplements that are marketed toward resistance training athletes of all levels. Caffeine is known to affect muscle in a couple different ways including changes in sarcoplasmic reticulum activity and endocrine activity (Reviewed inTarnopolsky, 2008, 2010), but interestingly there is also evidence that caffeine may alter cellular signaling in Saccharomyces cerevisiae (Truman, Kim, & Levin, 2009), and in mammals is known to affect enzymatic activity regulating glycogen, (Chesley, Howlett, Heigenhauser, Hultman, & Spriet, 1998) or not (T. E. Graham et al., 2000).

It is thought that the majority of caffeine's muscle specific regulation is due to its role as an adenosine receptor antagonist (T. E. Graham et al., 2008). At least for different adenosine receptor types have been identified including type 1 and 2 which has been shown to be expressed on the cell membrane of skeletal muscles (Lynge & Hellsten, 2000; Lynge, Schulte, Nordsborg, Fredholm, & Hellsten, 2003). Given the prevalence of adenosine receptors in skeletal muscle caffeine should raise cAMP levels and thus result in MAPK phosphorylation changes (Schulte & Fredholm, 2003a, 2003b) particularly for p38 though this is contested by (Lynge et al., 2003). Graham (2001), has in fact shown that cAMP is elevated following exercise with caffeine

compared to exercise alone. What has not been shown is whether this is also associated with changes in the MAPK phosphorylation status or downstream targets of the MAPKinases. By comparing Lynge et al. (2003) and Graham (2008) the co-contributions of exercise and caffeine in signaling has not been tested and appears to be different than caffeine alone.

Two significant confounding variables challenge the elucidation of this information. First caffeine in vivo likely increases epinephrine and or norepinephrine levels (T. E. Graham et al., 2000). Thus in vivo, it would be difficult to separate caffeine's direct action on the skeletal muscle with those changes that result by neurological and endocrine interaction. Based upon the work of Napoli (1998) it is reasonable to assume that such neurological and endocrine interactions might contribute to changes in JNK and/or p38 activity, but is unlikely to affect ERK1/2 phosphorylation. The second confounding factor is the role that calcium regulation and signaling plays in both exercise, caffeine, and endocrine responses within the cell. By multiple underlying causes Calcium-calmodulin signaling could also result in changes to MAPK phosphorylation and activity (T. E. Graham et al., 2008).

Based upon the complex and multifaceted signaling mechanism that occur when exercise is performed with caffeine, it is likely that p38 and other MAPK cascade signaling proteins are in fact activated. Future investigations into the role of long term caffeine supplemented exercise should consider the possibility that MAPK signaling cascades are contributing the cellular adaptation process.

Direct interaction between caffeine and MAPKs has been observed via a novel phosphorylation of MAPK analog in budding yeast, (Truman et al., 2009). No direct interactions between MAPK and caffeine have been identified in mammalian cells, however.

Vitamins alter MAPK signaling in skeletal muscle

Methylcobalamin, an active analog of vitamin B12 in animals, has been shown to increase C2C12 cell proliferation and migration in a ERK1/2 dependent manner (Okamoto et al., 2014). Furthermore, cells exposed to this form of B12 also demonstrated resistance to apoptosis also by a ERK1/2 dependent mechanism (Okamoto et al., 2014). Vitamin C on the otherhand reduced muscular hypertrophy due to overload via synergist oblation and was associated with decreased p70s6k phosphorylation as well as reduced ERK1/2 phosphorylation (Makanae et al., 2013). Though it remains unclear whether the decreased phosphorylation of ERK1/2 was a causal or merely a coincidental event in the decreased hypertrophy, the authors proposed that the anti-oxidant activity of vitamin C may have reduced reactive oxygen species thus reducing ERK1/2 phosphorylation via that mechanism (Makanae et al., 2013).

In addition to vitamin C and B12, there is strong evidence that vitamin D_3 affects cellular gene expression via the MAPK pathways though vitamin D_3 can be syntheysized by humans with exposure to UV radiation, it is also listed as an essential dietary nutrient. $1\alpha(OH)_2D_3$, the active steroid form of vitamin D_3 in mammals has been shown to increase phosphorylation of the ERK1/2, p38, and AKT kinases, by a combination of vitamin D receptor dependent(Choi, Park, Cho, & Lee, 2013; Ronda et al., 2007), and independent mechanisms(C. Buitrago, Pardo, & Boland, 2013). Some of the findings in this area include the phosphorylation ofP38 and ERK1/2 as well as the downstream targets of p38 CREB and Elk-1transcription factors following exposure to $1\alpha(OH)_2D_3$ (Ronda et al., 2007). $1\alpha(OH)_2D_3$ also increased Akt phosphorylation in a src dependent manner and perhaps via PI3K as well (C. G. Buitrago, Arango, & Boland, 2012). Further study demonstrated that knockdown of the vitamin D receptor did no completely eliminate the activation of these signaling cascades and leave open the

possibility that there are additional intermediaries, mechanisms, and signaling cascades involved including possibly calcium signaling, and protein kinase C (C. Buitrago et al., 2013). It was further noted that the $1\alpha(OH)_2D_3$ associated cellular changes were both similar to and synergistic with 17β-estradiol (Ronda et al., 2007).

In addition to the cellular signaling cascades activated in normal cells, $1\alpha(OH)_2D_3$ administered by injection to rats normalized markers of inflammation, muscle damage following a high intensity muscle damaging exercise protocol (Choi et al., 2013). This indicates that the additional vitamin D_3 was able to mitigate the muscle damage, possibly by decreasing the cytokine and immune cell response following the exercise damage as has been observed in humans previously (Barker et al., 2014). The supplementation group had lower creatine kinase levels, lower lactate ddgydrogenase levels in the plasma as well as reduced phosphorylation of AMPK, p38, ERK1/2, and Akt (Choi et al., 2013). Similarly NF-κB was also reduced in the supplement rats(Assy et al., 2011; Choi et al., 2013). While the phosphorylation states of these protein kinases was normalized, the effect on the subsequent adaptation was not studied. Further research needs to establish whether this blunted hypertrophic signaling response is also associated with reduced adaptive hypertrophy or myosin heavy chain expression compared to the non-D_3 supplement controls. On the other hand Choi et al. points out that this may be one mechanism to explain previous research findings that vitamin D_3 can reduce muscle soreness in healthy (Barker et al., 2013) and diseased populations (Assy et al., 2011).

Resveratrol

The phytonutrient resveratrol is found in high concentrations in grape skin, red wine, and Japanese knotweed extracts. While it's health benefits have been touted for many

years, the mechanisms behind its actions are not yet fully understood. Previously resverstrol has been studied for its effect on vascular endothelium, but there is also evidence that resveratrol leads to healthy adaptation in skeletal muscle as well. Deng et al. demonstrated that resveratrol which has a chemical structure similar to estradiol can also bind to the estrogen receptor and modulate muscular glucose uptake via improved insulin sensitivity, and by non-insulin dependent mechanisms (Deng, Hsieh, Huang, Lu, & Hung, 2008). It was further demonstrated that ERK1/2 and p38 were necessary for the early phase response (1 hour) to resveratrol in C2C12 cells, and p38 and PI3k were required for adaptations observed at 14hours (Deng et al., 2008). The immediate co-activation of ERK1/2 and p38 is highly indicative of that observed in estradiol activation of the estrogen receptor (Ronda et al., 2007) . The late phase p38 activation concurrent with PI3k is similarly characteristic of src mediated cross-talk (C. G. Buitrago et al., 2012).

Forskolin

Forskolin, a diterpene extract from the Coleus forskohlii plant native to India, has been used in traditional Ayruvedic medicine, traditional Indian healing practice, to treat a variety of diseases (Ammon & Muller, 1985). Forskolin is a potent stimulator of adenylyl cyclase resulting in dose dependent increased concentrations of cAMP (Ammon & Muller, 1985; Roberts & Summers, 1998; Seamon et al., 1981). This increase in cAMP is both its apparent mechanism of action and the reason that the effects of forskolin mimic β-agonists which also increase cAMP concentrations within the cell (Ammon & Muller, 1985).

Forskolin has been shown to produce increased lipolysis (Lynch & Ryall, 2008) , increased protein synthesis (Thompson et al., 1996), and increased muscle hypertrophy (Godard, Johnson, & Richmond, 2005); again these effects are similar to those observed following B_2AR agonist

administration. Of primary interest is the study by Goddard and colleagues which demonstrated forskolin stimulated increases in lean body mass while simultaneously reducing adiposity when supplementation occurred in conjunction with an exercise program (Godard et al., 2005). This increased muscle mass accretion and reduction in body fat due to forskolin is reminiscent of that observed with various B_2AR agonists (reviewed in Lynch & Ryall, 2008). Based upon the parallels between the B_2AR signaling and that observed in Forskolin signaling, it seems plausible that there may be some shared signaling components between them.

While it is known that forskolin increases cyclic AMP via activation of adenylyl cyclase (Litosch, Hudson, Mills, Li, & Fain, 1982), the signaling pathway that downstream of cAMP has not been as well established, especially in skeletal muscle. Many proteins and pathways have been explored as downstream responders to forskolin including the PI3K-AKT pathways (Richmond, 2007; Richmond, Touchberry, & Gallagher, 2009), protein kinase C (PKC) (Iijima et al., 2002) and the MAPK pathways (Cao, Medvedev, Daniel, & Collins, 2001; Crespo et al., 1995; Iijima et al., 2002) in various cell types. As mentioned above, the strongest evidence is for cAMP to affect MAPKs via the PKA pathway, except for possibly ERK1/2.

Specific evidence linking p38 MAPK activation following forskolin administration comes from a study performed with adipocytes. Cao and colleagues (Cao et al., 2001) have been demonstrated that p38 is phosphorylated in response to both forskolin, protein kinase A agonist (Isoproterenol), as well as a B_3AR agonist (CL316243). Similarly, Zheng and colleagues (Zheng et al., 2000), found that cardiomyocytes also increased p38 activity in a protein kinase A (PKA) dependent manor.

As mentioned, PKA may not be the only intermediary for cAMP signaling, however. p38 was also temporally associated with protein kinase C (PKC) phosphorylation in response to forskolin in cardiomyocytes (Iijima et al., 2002). In this same study however ERK, and not p38, was identified as the critical kinase leading to phosphorylation of p70/85S6K a downstream target associated with protein synthesis (Iijima et al., 2002). Again as mentioned above the MAPKs may respond to G_i proteins via PKC, but p38 has not been shown to work by this mechanism.

CONCLUSION

The purpose of this review has been to examine the stimulatory role of G protein coupled receptors, especially β-adrenergic receptors, in cAMP related MAPK signaling. This review began by defining the general schema of MAPK activation and examined how the parallel but related MAPKs are activated by a complex set of upstream MKKs MKKKs and MKKKKs, which allow this set of protein kinases to act as a signaling junction for many different cell stimuli. A brief overview of the three key MAPKs in skeletal muscle signaling clearly demonstrated the differential activation patterns of the MAPKs in response to key stimuli including steroid hormones like estradiol and vitamin D_3. The ERK1/2 and p38 changes that are observed in response to exercise and nutritional and supplementation were also briefly reviewed. This brief glimpse into these ubiquitous, variable, and inherently complicated cellular signaling cascades only more emphatically demonstrate the importance for a cell to tightly regulate these proteins and there kinase activity .

Though much more research needs to be completed to identify how these two proteins and their various isoforms interact with other signaling mechanisms to couple isolated

and integrated stimuli with a coherent set of protein modifications and gene transcription adaptations. Additionally, it is clear that the exact mechanisms of this complex signaling system remain undetermined. Furthermore, there is a need for applied human research demonstrating how complex systems of stimuli like resistance exercise training and nutritional supplementation might affect both the cellular signaling cascades as well as the adaptive response on a more global scale.

CHAPTER 3: METHODOLOGY

STUDY 1. SKELETAL MUSCLE MAPK ACTIVATION DURING HIGH POWER RESISTANCE EXERCISE

STUDY DESIGN

The purpose of this study was to determine the MAPK (ERK1/2 and p38) responses to three different resistance exercise bouts where the back squat exercise was performed with maximal concentric velocity resulting in the greatest power production possible given the bar's load. In order to elucidate these responses to different resistance exercise loads and different power outputs, this study used a repeated measures design in which each of the study volunteers completed each of three resistance exercise bouts in a randomized order. A muscle biopsy was taken before and within ten minutes after the exercise bout. These muscle samples were then used to determine changes in the MAPK phosphorylation occurring during each exercise bout. To ensure complete physical recovery from the previous testing sessions, subjects completed each treatment trail no less than one week apart. Also to prevent muscular fatigue and in order to minimize the effect of outside physical activity on the measures of MAPK phosphorylation, subjects were asked to refrain from exercise for 36 hours before each testing session. A timeline of the study is shown below (Figure 3.1) to elucidate the data collection points and the

organization of the study.

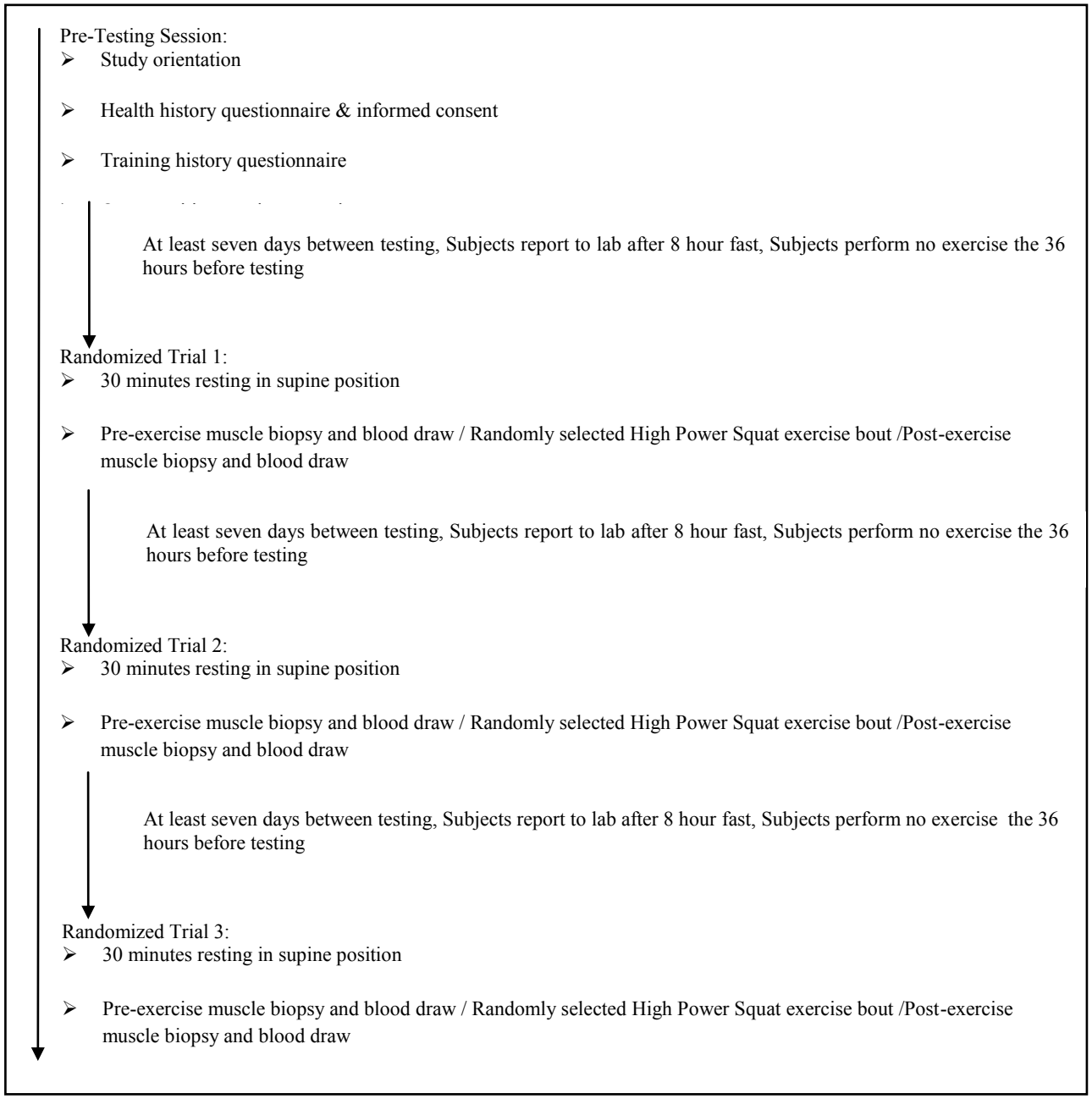

Figure 3. Study I Timeline

Nine healthy active collegiate males who had squatted regularly participated in this study. Prior to participation, each volunteer completed a pre study orientation in which informed consent was

obtained in accordance with the Human Subjects Committee of the University of Kansas, Lawrence, KS. (See Appendices A and B). A health history questionnaire to assess the health status of the volunteers (See Appendicies C) . In order to qualify for the study volunteers must have had good general health, been physically active, non-obese (BMI less than 28kg/$m^{2)}$, non-smoker, normotensive and free of metabolic and cardiovascular disease. A training history questionnaire (Appendix D) was used to determine their familiarity with the barbell squat exercise and their habitual patterns of lower body resistance exercise.

Each participant also performed unloaded squats for evaluation by the researcher to ensure the subjects all had safe and consistent squat technique prior to the maximal squat protocol. Subjects also had to demonstrate their ability and comfort in achieving the minimum parallel squat depth for the study. All subjects in the study had at least 2 years of resistance training experience, though they did not all have 2 consecutive years experience. All had been resistance training consistently for at least 3 months leading up to the study. Finally all subjects squatted at least once per week in the months leading up to the study.

METHODS

Anthropometric and Strength Measures.

The height and weight of each subject was measured by stadiometer and body composition was determined using the Dual Energy X-ray Absorptiometry (DEXA) method. One repetition maximum strength testing was tested. Lastly each subject completed one repetition maximum testing for the back squat exercise in accordance with the NSCA protocol published in the Essentials of Strength and Conditioning textbook (Baechle & Earle, 2008). The squat maxes were carefully monitored for safe and consistent technique. The required squat depth was parallel as

defined by the greater trochanter level with the top of the patella as visually determined by the research team.

DATA COLLECTION.

For each of the three exercise conditions the athletes completed a standard relative warm-up using a barbell before beginning the measured exercise sets. Figure 3.2 displays the warm-up and the exercise protocols used in this study. The set and repetition scheme of this study was intended to typify the recommendations for development of muscular strength and power (90% 1RM: High Load), muscular Hypertrophy (70% 1RM Load: Moderate Load) and muscular endurance (30%1RM : Low Load). For all indicated loads in the warm-up the actual load was rounded up to the nearest loadable 5lb. increment. For any indicated loads at or below 45lbs. an unloaded bar was used. For the work sets that were measured the bar was loaded to the nearest (up or down) loadable 5 lb increment. A rest interval of two minutes was given between all warm-up sets. For the moderate and low intensity protocols two minutes was enforced between all work sets. The high intensity load protocol utilized a 3 minute rest between work sets, however. All warm-up sets were performed using a standard Olympic style Barbell, while all work sets(Jennings, Viljoen, Durandt, & Lambert, 2005) were performed on a ProSpot Fitness® Rack (Pompano Beach, CA). Squat depth was monitored by the research team and a verbal cue was given if subjects failed to achieve full depth, but the repetition was not repeated.

Table 1. Set, Rep & Load for the Three Protocols

Exercise Treatment	Warm-ups Sets x Reps (% target load [TL])	Work sets Sets x Reps (% 1RM)
90% 1RM High Load	1 x 10 @ 45 lbs 1 x 5 (~50% TL) 1 x 3 (~70% TL) 1 x 2 (~90% TL)	5 x 3 (90%)
70% 1RM Moderate Load	1 x 10 @ 45 lbs 1 x 5 (~50% TL) 1 x 3 (~70% TL) 1 x 2 (~90% TL)	5 x 5 (70%)
30% 1RM Low Load	1 x 10 @ 45 lbs 1 x 5 (~50% TL) 1 x 3 (~70% TL) 1 x 2 (~90% TL)	5 x 10 (30%)

Maximal concentric velocity was asked of the subjects on each repetition of the experimental sets. Based upon previous information that type II muscle fibers would respond with greater changes in p38 than type I fibers, this method was used to increase the activation of type II fibers within the muscle. Power generated was estimated using a FitroDyne® unit (Slovak Republic), which has previously been established as a reliable method of measuring power in barbell lifting. Power estimates were based upon the system mass (bar weight+ body mass of the lifter). From these measures set average power and set average of peak power was calculated as the arithmetic mean of the repletion average power as mean of the repetition peak power respectivey.

BIOPSY AND BLOOD DRAW PROCEDURES.

In order to obtain muscle samples for analysis, a needle punch biopsy system was used before and after each exercise protocol. This system used a spring loaded, mechanical action needle to obtain a 15-20 mg of muscle tissue. To prepare the subjects for the baseline biopsy taken before each treatment protocol, subjects arrived at the lab after an 8 hour fast and instructed to relax in a reclined position for 30 minutes to return muscle activity to basal levels. The biopsy site was then shaved, sterilized with betadine solution, and anaesthetized with ~3ml of 1% lidocane (without epinephrine) by local injection. After allowing 5 to 10 minutes a sterile needle was used to break the skin in preparation for the actual biopsy needle. The biopsy was obtained by needle punch method using the Angiotech ProMag system and 14 guage disposable biopsy needles from the same company, (Angiotech Medical Device Technologies, Gainsville FL.). Samples were immediately removed from the needles and frozen in liquid nitrogen for further analysis.

Following the pre-exercise biopsy sterile and flexible pressure wraps were placed over the wound site. When the bandages where securely in place the volunteers began their warm-up and exercise protocol. Immediately following the exercise protocol the subjects returned to the supine resting position for the post-exercise biopsy. The post- exercise biopsy occurred in a similar manner to the pre-exercise biopsy, except that the same puncture site was used without additional anaesthetization. Additional lidocane was not always administered because the exercise protocol for this study was quite short (12-20 minutes in duration). Subjects who felt that they had not retained the anesthetic effects were offered a second injection of lidocane . The post exercise biopsy sample (though from the same site) was taken proximally to the pre-exercise biopsy as determined by the angle of the biopsy needle over the puncture site. Post-exercise biopsies were completed within 5-10 minutes of the cessation of exercise.

Blood was also drawn from the antecubital vein of the arm. Approximately 5-10cc of blood were collected and divided between untreated Vacutainer™ test tubes (for serum) and Heparin treated Vacutainer™ test tubes (for plasma). The blood samples were allowed to clot in untreated Vacutainer™ test tubes. The tubes were subsequently centrifuged for 20 minutes at 2000 RPM). After which the plasma and serum were pipette into eppendorf tubes and stored at -20° to -70°. Blood was drawn at the identical six time points as the muscle biopsies. At the same time as Vacutainer™ blood collection, blood Lactate measures were analyzed using the Acusport™ Blood lactate analyzer.

SAMPLE PREPARATION AND ANALYSIS

In order to extract the cellular proteins for analysis, each muscle sample was weighed and homogenized in 1:10 (weight: volume) T-per® Tissue Protein Extraction Reagent (Thermo Scientific, Rockford, IL) with 10 µl/ml Halt®™ phosphatase inhibitor and Halt™ protease inhibitor (100x, Thermo Scientific, Rockford, IL) and 3.4 µl/ml of PMSF. The samples where then chilled for 30 minutes with intermittent vortexing every ten minutes, then separated in a centrifuge at 3000g for 3 minutes at 4°C. The pellet was discarded and the supernatant was stored at -80°C until further analyses could be completed.

Protein concentration of the extracted muscle tissue was determined using the Micro BCA™ protein assay in triplicate (Thermo Scientific, Rockford, IL was performed. The absorbance readings for the samples and standards where read at a wavelength of 562nm using a Synergy HT plate reader (Bio-Tek®, Winooski, VT). Upon determination of the protein concentration of the samples, an equal volume:concentration of 5X Lane Marker Sample Buffer (Thermo Scientific, Rockford, IL) and 2:1 (volume: concentration) of HES buffer was added to the samples.

Western Blot analysis was performed by separating the samples in 12% SDS-Page gels, followed by a transfer to Amersham Hybond-P™ PVDF membrane (GE healthcare, Little Chalfont, Buckinghamshire UK). Membranes where then blocked in SuperBlock® blocking buffer in TBS (Pierce, Rockford, IL) for one hour with gentle rocking. Primary antibodies for total p38 and phospho-p38 (Thr 180/Try 182), (Santa Cruz Biotechnology Inc., Santa Cruz, CA) isolated from two different host animals were added to the blocking buffer at the manufacturer's recommended dilutions and incubated at room temperature for two hours with gentle shaking. The membranes where then washed 4x5 minutes in Phosphate buffered Saline (PBS) plus 0.1% Tween-20. Next, IR-Dye™ (700 and 800nm wavelengths) linked secondary antibodies specific to the two separate host animals of the primary antibody were diluted 1:10,000 in Superblock® with 0.1% Tween-20 and .02% SDS and incubated for one hour protected from light while gently rocking. Finally membranes where rinsed 4x5 minutes in PBS with Tween, followed by PBS alone and dried overnight in preparation for imaging. Infrared image detection of both 800 and 700 nm IR linked secondary antibodies was accomplished with use of the Odyssey® Scanner (LI-COR Biotechnology, Lincoln, NE).

STATISTICAL ANALYSIS

First, the total p38 infrared intensities (I.I.) were much lower than the intensities for the phosphorylated antibody. This is a common issue and is merely related to the affinities of the antibodies used. Thus the raw values were expressed as a ratio to the first timepoint. This method corrected for variations in blot intensity . The mean density and standard deviation ($M \pm SD$) was calculated including each of the four subjects. Three separate repeated measures ANOVAs were used to assess differences between the total, phosphorylated and the ratios of total to phosphorylated p38. The alpha level accepted for statistical significance in this study was $\alpha \leq$

0.05. Statistical Package for the social Sciences (PASW, Chicago, IL) software was used to perform these statistical analyses.

STUDY 2. THE EFFECTS OF RESISTANCE EXERICSE AND A PRE-WORKOUT DIETARY SUPPLEMENT ON ERK AND P38 PHORPHORYLATION

STUDY DESIGN.

The purpose of this study was to examine the acute and training effects with and without a multi-ingredient high caffeine pre-workout supplement on squat and bench press power, vertical jump, and the mitogen activated protein kinase pathways (ERK1/2 and p38). In orders to clearly delineate the contribution of the exercise and the supplement plus exercise a double blind placebo controlled training design was utilized. Subjects were randomly assigned to either the experimental (EXP) or the placebo control (CON) group.

SUBJECTS.

Thirty recreationally trained men between the ages of 18 and 35 with good health were recruited to participate in this study, which was reviewed and approved by the Human Subjects Committee of the University of Kansas (Lawrence, KS) (See Appendix E). The subjects were informed of the requirements of the study and their rights as volunteers using the approved informed consent documents (appendix F), Health History and caffeine tolerance questionnaire (appendix G), a exercise training history questionnaire (Appendix H). For safety of the volunteers, individuals who had health concerns or risk factors listed in the Human Subjects Committee Approved List of Exclusionary Criteria (appendix E) were excluded from participation in the study.

BASELINE TESTING

In order to assess changes in body weight and composition following the exercise training, each subject's height, weight and body composition was determined by stadiometer, scale, and Dual

Energy X-ray absorptiometry (DEXA) (Lunar Protogy; General Eectric, Waukesha, WI), respectively. One repetition Maximum (1RM) strength testing was also performed for the lifts that would make up the first day of exercises for the training program. These lifts were the squat, bench press, Leg extension, leg curl and lat pull-down exercises. The 1RM testing was done in accordance with the guidelines set forth by the National Strength & Conditioning Association (Baechle & Earle, 2008). Subjects were asked to keep a food log for the three days preceding the first day of exercise training as well.

TESTING AND BIOPSIES.

On the first day of the exercise training muscle biopsy and blood samples were taken before and after the actual exercise session. Subjects reported to the lab following an eight hour fast. Subjects were asked to rest comfortably for 30 minutes to return to resting state following arrival at the lab. A muscle sample and blood sample was collected from each subject before beginning the exercise or consuming the pre-exercise supplement .

All muscle biopsies were obtained from the vastus lateralis of by percutaneous needle biopsy by either Dr. Andrew Fry or Dr Philip Gallagher. To prepare the subject's leg was for the muscle biopsy, the thigh was shaved and cleaned with with Betadine solution.. Two to Three mL of 2% lidocane solution was injected into the skin and the surrounding tissues of the leg. Next, a 1cm incision was made through all lays of the skin and into the adipose and connective tissue below. A Bergstrom needle (Bignel Surgical Inc., Essex, United Kingdom) double-chop technique (Staron, 1991) and suction (Evans, Phinney, & Young, 1982) . The incision was closed with adhesive wound closures and covered with sterile and compressive dressing before they proceeded on to the resistance exercise bout.

The same methods were utilized to collect a second muscle and blood sample following the exercise bout. Muscle samples were sectioned, flash frozen and stored in liquid nitrogen for later analysis. Blood samples were allowed to clot, centrifuged for 20 minutes at 3000G at 4°C. The blood's serum was removed and frozen for later analysis.

Post Exercise Training muscle biopsies and blood samples were collected in the same manner as those collected on the first day of exercise training. The subjects reported to the lab following an 8 hour fast and samples were collected prior to and just after the last and exercise training bout that matched the first training bout in relative (but not absolute) intensity, volume, and allotted rest intervals between exercises.

EXERCISE TRAINING PROTOCOL

Following the initial testing day that included exercise, each subject reported to the laboratory three times per week for exercise training. The exercise training was standardized for all participants using relative loads based upon the pre-testing strength measures. Each participant worked with one of the research staff for the first part of the study to ensure exercises were performed properly. After the weeks of the study when the participants felt capable, they worked with each other and used each other as spotters but with the constant supervision of the researchers.

The exercise training was designed to be quite challenging such that no individual even those who were less well conditioned prior to this experiment would find adapt more quickly than the increases in volume and load. The training program consisted of three days per week of total body resistance training. Each day used a combination of core lifts (major multi-joint free weight lifts), accessory lifts (lifts to target smaller quantities of muscle mass), and exercises to target the trunk and abdomen. For the core lifts (back squat, bench press, leg

extensions leg curls, incline bench press, deadlift, and bicep curls) the participant completed strength testing and load was prescribed relative to their maximal strength in that exercise. While the bicep curl is not typically programmed or considered a core lift, it was in this case because of the preferential training of that muscle group/exercise by the subjects.

The accessory exercise for this study (standing press, barbell split squat, dips, pull-ups, and the abdominal exercises (weighted sit-up, weighted Russian twist, supine leg raise, weighted v-ups, weighted twisting crunches, and Supine wipers, where programmed without indicating an appropriate load. For all of these exercises the subject was to complete two sets of 12-15 to fatigue/failure recording the weight and repetitions. The subjects were encouraged to increase as they were able adding weight or increasing to the maximum allowable 15 repetitions per set, but these exercises where not otherwise programmed for progressive overload. For the pull-up and dip exercise subjects used an assisted machine (Stairmaster Gravitron assisted chin/dip; Vancouver, WA) if they were unable to complete adequate repetitions with body weight. Conversely, subjects added weight via belt or other method of their choosing as they were able to accommodate loads higher than body weight. Abdominal exercises were weighted with rubberized bumper plates, metal plates, medicine balls as was deemed appropriate and safe for the specific accessory exercises.

The eight week training program was progressive including fluctuations in both volume and load. Day two of the first week of training consisted of additional 1RM testing for lifts used in the training program, but not in the first day of the training program. After increasing linearly for the first four weeks, The end of the fourth week offered a lower volume higher intensity training session. Week five of the program began with 1RM re-testing to evaluate any progress made in the short phase of exercise training. The prescribed loads were adjusted to match the new strength measures in the following weeks. Week seven, like week 4 has a

reduction in volume and an increase in load. Week 8 has the lowest volume, but contained the post training 1RM testing. The final day of the training program like the first day of the training program were identical in volume and relative load (loading relative to the most recent 1RM testing), and where testing days in which muscle biopsies were collected. The exact training program is shown in Appendix J where the load indicates the percentage of maximum load.

SUPPLEMENTATION

Each subject in this study was randomly assigned to either the EXP or CON group referring to the pre-exercise supplementation the individual received prior to each exercise session. The EXP group received a commercially available multi-ingredient workout supplement called Supercharge ,(Labrada Nutrition, Houston, TX). The nutritional information panel for the experimental supplement is shown in a table in appendix K. The manufacturer proposed that the most significant active ingredients for the supplement included charbohydrate in the form of maltodextrin, corn syrup, and modified food starch, as well as, Taurine, arginine, caffeine, creatine monohydrate, betain, and the amino acids alanine, glutamine, and histidine. Several other ingredients were included and can be seen in appendix K. The CON goup received a low-glycemic color and flavor matched placeebo that contained polydextrin and/or manitol to match the flavor of the EXP drink and silica to match the texture of the drink. The drink also included typical food coloring and flavoring ingredients.

The preparation of the EXP and CON pre-exercise supplements was blinded to the researchers and the subjects. The dry powder supplements were measured and placed into opaque disposable containers labeled for the specific subjects. When the subjects arrived at the laboratory for their exercise training (or testing as in the case of the first and last days chilled tap water was added to the bottles (approximately 12oz.). The mixture was shaken to dissolve the

drink mix completely and then consumed by the subject. The grouping was not unblinded until the a subject completed the study.

Because of the high caffeine content of the EXP supplement, the dosage given to the subjects was reduced by half for the first week and a half of training sessions following the initial day of training with muscle biopsies. That is to say that the subjects received a serving size of '2 scoops' (32g of powdered supplement) for the initial day of testing, but the following sessions they received only '1 scoop' (16g) dissolved in the same amount of water. This was done to allow for some habituation to the caffeine, hoping to avoid serious caffeine related side effects. The end of this acclimation period and throughout the remainder of the study the subjects were given the full 32g dose of either the EXP supplement or the CON placebo. To further monitor for negative side effects of the supplement, each subject filled out a short questionnaire asking about any suspected side effects (appendix I) , and blood pressures were monitored to ensure subjects did not develop chronic hypertension. This monitoring was completed weekly for the duration of the study.

SAMPLE PREPARATION AND ANALYSIS

Protein Extraction

The p38 and ERK protein was extracted from , each muscle sample, weighed and homogenized in 1:10 (weight: volume) T-per® Tissue Protein Extraction Reagent (Thermo Scientific, Rockford, IL) with 10 µl/ml Halt®™ phosphatase inhibitor and Halt™ protease inhibitor (100x, Thermo Scientific, Rockford, IL) and 3.4 µl/ml of PMSF. The samples where then chilled on ice for 30 minutes and agitated by vortexing every ten minutes. The solution was centrifuge at 3000g for 3 minutes at 4°C. The pellet was discarded and the supernatant was stored at -80°C until further analyses could be completed.

In order to determine the concentration of protein in the extracted muscle tissue, a Micro BCA™ protein assay (Thermo Scientific, Rockford, IL) was performed for each sample in triplicate according to the manufacturer's instructions. The absorbance readings for the samples and standards where read at a wavelength of 562nm using a Synergy HT plate reader (Bio-Tek®, Winooski, VT). Upon determination of the protein concentration of the samples, an equal volume:concentration of 5X Lane Marker Sample Buffer (Thermo Scientific, Rockford, IL) and 2:1 (volume: concentration) of HES buffer was added to the samples and stored at -8o°C until further analysis could be performed.

Western Blot analysis began by separating the muscle samples in 12% SDS-Page gels, followed by a transfer to Amersham Hybond-P™ PVDF membrane (GE healthcare, Little Chalfont, Buckinghamshire UK). Membranes where then blocked in SuperBlock® blocking buffer in TBS (Pierce, Rockford, IL) for one hour with gentle rocking. Primary antibodies for the target proteins (total p38 and phos pho-p38 and total and Phospho ERK1/2) isolated from two different host animals were diluted in the same blocking buffer at the manufacturer's

recommended dilutions and incubated at room temperature for two hours with gentle shaking. The membranes where then washed 4x5 minutes in Phosphate buffered Saline (PBS) plus 0.1% Tween-20. Next, IR-Dye™ (700 and 800nm wavelengths) linked secondary antibodies specific to the two separate host animals of the primary antibody were diluted 1:10,000 in Superblock® with 0.1% Tween-20 and .02% SDS and incubated for one hour protected from light while gently rocking. Finally, membranes where rinsed 4x5 minutes in PBS with Tween, followed by PBS alone and dried overnight in preparation for imaging.

Infrared image detection of both 800 and 700 nm IR linked secondary antibodies was accomplished with use of the Odyssey® Scanner (LI-COR Biotechnology, Lincoln, NE) and recorded as infrared intensities (I.I.) which are analogous to optical densities. For all samples ratio of phosphorylated to total I.I. will be analyzed as an indication of the effect that the exercise bout or the exercise training may have on the phosphorylation state of p38 and ERK.

STATISTICAL ANALYSIS.

Statistical Descriptors of the subjects including average and standard deviation for height, weight, body composition, and training history variables were calculated. For the main comparison the I.I. for the total and phosphorylated p38 and ERK were compared between pre and post testing Seperate repeated measures ANOVAs were run comparing the ratio of phosphorylated to total p38 and ERK separately. For all hypothesis testing the alpha level accepted for statistical significance in this study was $\alpha \leq 0.05$. Statistical Package for the Social Sciences (SPSS/PASW, Chicago, IL) software was used to perform these statistical analyses.

CHAPTER 4: RESULTS

STUDY 1. SKELETAL MUSCLE MAPK ACTIVATION DURING HIGH POWER RESISTANCE EXERCISE

Recall that the purpose of this study was to determine if there was a differential response to three different volumes and intensities of maximal concentric velociey (high power) back squat protocols. The three protocols investigated were the High Intensity (5 sets of 3 repetition at 90% or 1RM load), Moderate Intensity (5 sets if 5 repetitions at 70% of 1RM load, and Low Intensity (5 sets of 10 repetitions at 30% of 1RM load. Nine healthy college aged (21.44±0.56 yr.) males participated in the study. Raw data for their physical characteristics including height (177.80± 5.056cm) and weight (78.68 ± 9.712kg), and their one-repetition maximum in the back squat (115.7±40.26 kg) are reported in appendix L along with the testing order for each subject.

To determine if the three exercise protocols resulted in significantly different blood Lactate response a repeated measures 2x3ANOVA (general linear model) was calculated using the Timepoint (pre- vs. Post-exercise) and the three protocols (High, Moderate, and Low intensity). There was a significant effect for timepoint $F_{(1,8)} = 93.29$, $p<0.0001$. There was no main effect for the protocol ($p >0.05$), nor was there a significant interaction, timepoint x Protocol, ($p >0.05$). Blood lactate values for each subject are included in appendix L along with the mean and standard deviation for each timepoint and protocol. The lactate values are shown in Figure 4.

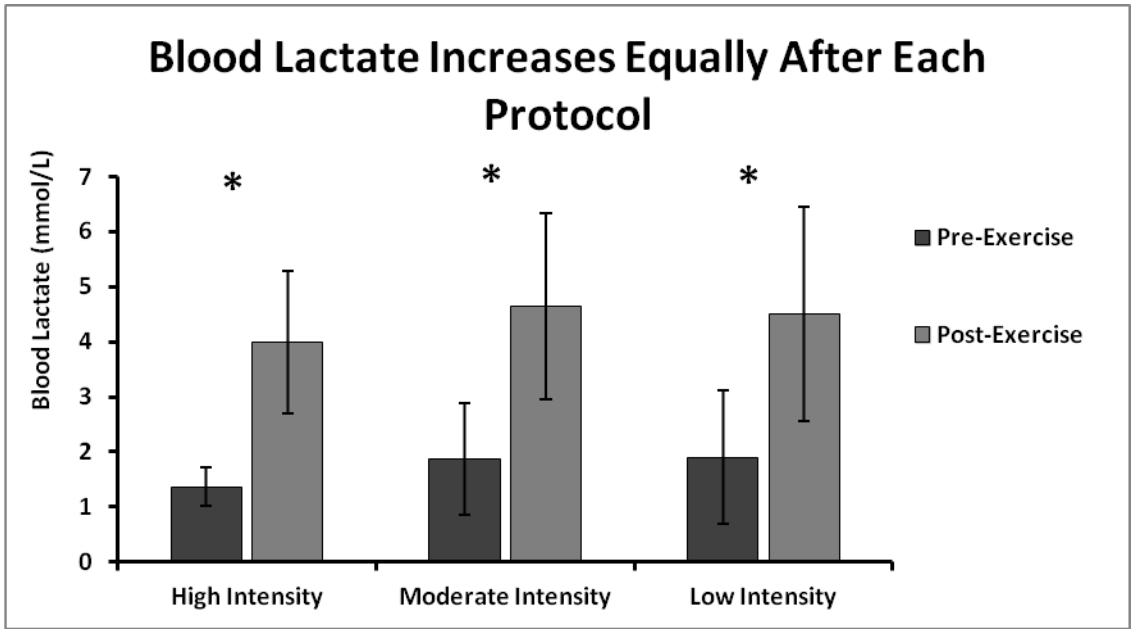

Figure 4. Blood Lactate was significantly and equally increased by each of the three protocols. * indicates $p < 0.05$ compared to associated pre-exercise value. There was no difference between protocols.

Of major interest in this study was the role that power production might play in the physiological response. As such the power produced while performing the concentric portion of the prescribed squats for each protocol was estimated using the Tendo Weightlifting Analyzer. An average and peak power was recorded from each repetition. A set average power was calculated by taking an average of the repetitions for each set. The set peak power is likewise an average of the peak power measures from each repetition within a given set. Lastly a grand mean of average power and grand mean of peak power was calculated by finding the mean of the set average and peak values respectively for each protocol. Appendix L includes set peak and set average power by by protocol for each subject. A mean and standard deviation for set and the grand mean and standard deviation for each protocol's peak and average power is also included there. Note that missing values are attributable to one of two procedural errors on the part of the

research team. Either the Tendo unit was not attached by the research assistants to the weightlifting bar thus no data was collected, or the data file was not saved properly before the next trial began.

Separate repeated measures ANOVAs was calculated to determine if the set average power and set peak power measures were significantly different between the (grand average of average power), $F_{2,16)} = 11.359$, $p = 0.003$. Planned contrasts were utilized to determine between which protocols the average power differed. The Low Intensity protocol (30%1RM) produced average power measures significantly higher than both the High Intensity ($F_{(1,8)} = 33.169$, $p < 0.001$) or Moderate Intensity ($F_{(1,8)} = 10.78$, $p = 0.011$) protocols. There was however no difference between the High Intensity and Moderate Intensity protocols ($p = 0.177$). The grand mean of peak power was not significantly different between the protocols ($F_{(2,10)} = 0.774$, $p = 0.487$).

To determine the impact of power fatigue across the three different protocols two separate repeated measures 3x5 ANOVAs (general linear model) were completed for the peak and average power measures respectively. In this statistic the three protocols (High, Moderate, and Low Intensity) are compared within and across the 5 sets. For the set average power measures there was a significant main effect for protocol ($F_{2,10)} = 10.23$, $p=0.004$), but not for set ($F_{4,20)} = 0.833$, $p = 0.52$). When the set average power was evaluated by pair wise comparisons, it was determined that set averages were significantly lower for the High Intensity protocol compared to the Low Intensity protocol ($p=0.009$), but no other differences between protocols (main effect) were observed. A significant interaction (protocol x set; $F(_{8.40)} = 4.984$, $p < 0.001$) was identified and a post hoc analysis showed that the High Intensity protocol alone resulted in significantly lower average power measures in the fifth set compared to the first and

second sets of that same protocol. Stated more directly, there was a significant decline in set average power by the fifth set, and this pattern was unique to the High Intensity protocol. Figure 3 below shows demonstrates the set average power decline of 16% that is observed in the High Intensity protocol only. The 3x5 repeated measures ANOVA for peak power across sets did not indicate any significant main effects or interactions ($p > 0.05$ in all instances). Figure 5 demonstrates the trend and differences between protocols.

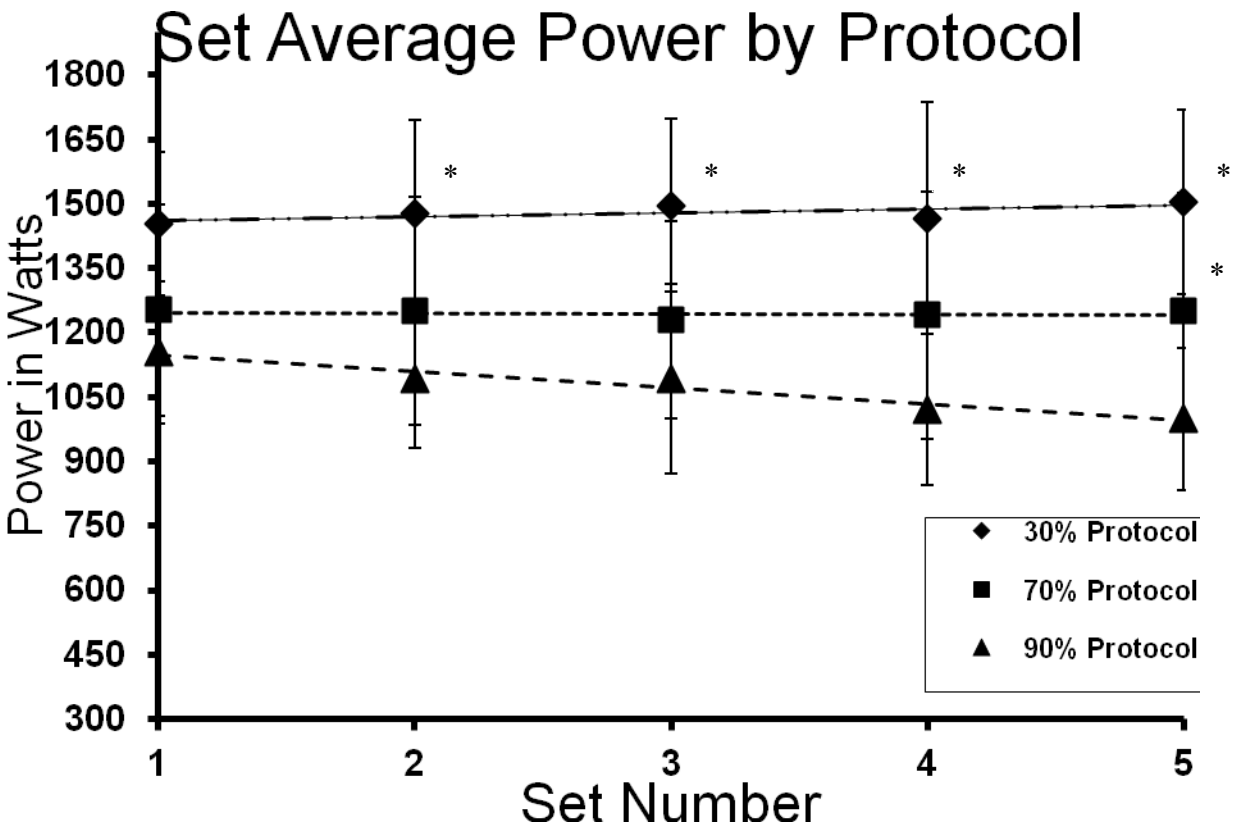

Figure 5. Set Average Power by Protocol. A) The set average power declines by 16% in the High Intensity (90% protocol) which is significantly different than the trends within the Low and Moderate Intensity protocols. * indicates the set average is significantly different from the 90% protocol for the same set.

Western Blot optical density information for the total and phosphorylated versions of ERK1/2 and P38 are given in appendix L. Statistical analysis of this data was performed by first performing separate 3x2 repeated measures ANOVAs for each protein, where the three treatment groups were compared across the pre- and post exercise bout. For total ERK1/2 there was no significant main effect for treatment, but there was a significant main effect for the pre-post-timepoint $F_{(1.8)} = 8.149$, $p < 0.021$. The interaction effect was not significant. The same pattern was true for the phosphorylated ERK1/2 protein with a significant main effect for timepoint ($F_{(1.8)} = 6.725$, $p < 0.032$). For the pre-Post comparison combined across all treatments both the ERK1/2 and the phosphorylated ERK1/2 were significantly lower after exercise compared to before. The results of these analyses are shown in Figure 6.

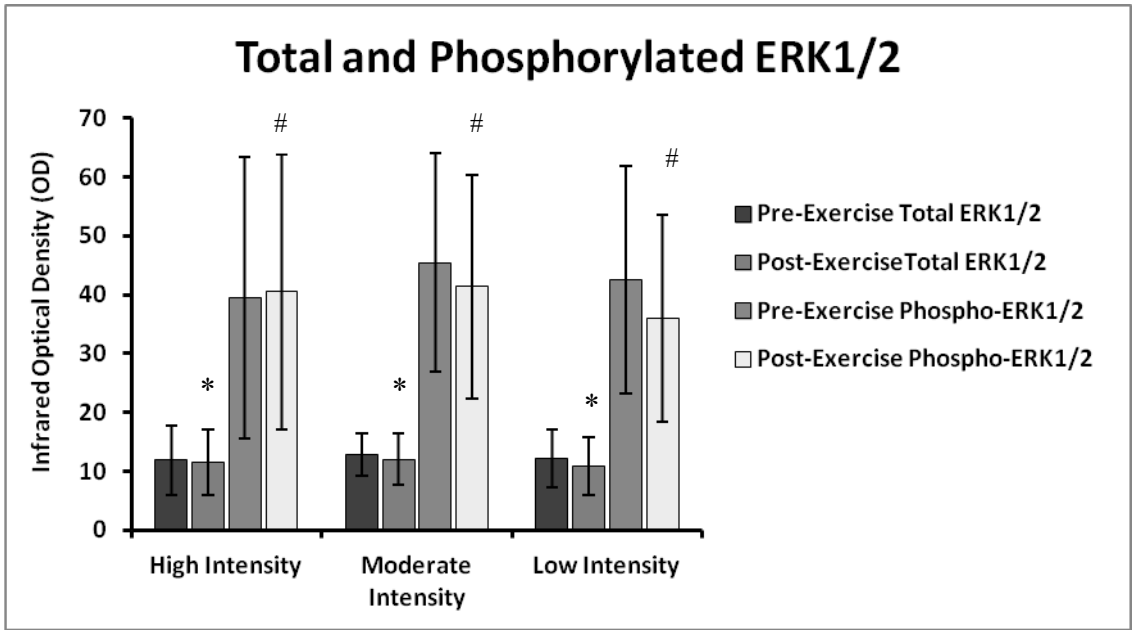

Figure 6. The total and phosphorylated ERK1/2 pre- and post exercise for all three exercise conditions. There was no significant difference between the protocols for ERK1/2 ($F_{(2,16)}$ = 0.585, p=0.528) or Phospho-ERK1/2 ($F_{(2,16)}$ = 0.831, p=0.454) . * highlights that ERK1/2 was significantly lower following exercise ($F_{(2,8)}$ = 8.149, p=0.021) This was a main effect thus is the combination of all three protocols. # Likewise indicates there was a main effect for pre- to post exercise Phospho-ERK1/2($F_{(1,8)}$ = 6.725, p=0.528) . There was no significant interaction (protocol x timepoint) ($F_{(2,16)}$ = 0.385, p=0.032).

To further clarify the findings a set of simple repeated measures ANOVAs were also completed using the relative change in total ERK1/2 and the relative change in phosphorylated ERK1/2 respectively. This analysis would thus more clearly compare effects of the exercise bouts while eliminating any confounding difference in the basal protein expression levels. There was no significant difference between treatments for the relative change in ERK1/2 ($F_{(2.16)}$ = 0.564, p = 0.58)1 or phosphorylated ERK1/2 ($F_{(2.16)}$ = 1.593, p = 0.234) see Figure 7.

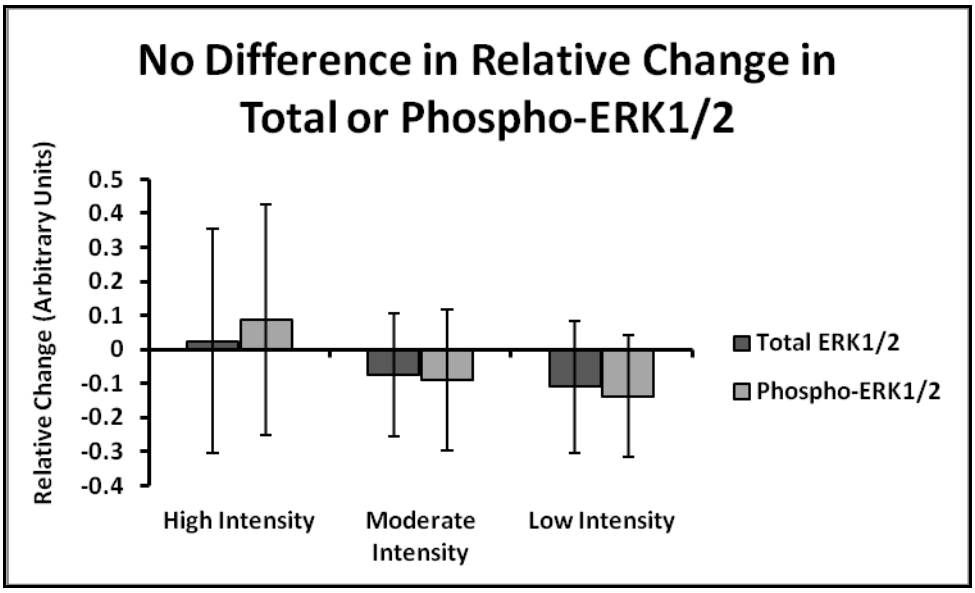

Figure 7. There is no significant difference in relative change in total or phosphorylated ERK 1/2 ($F_{(2.16)} = 0.564$, $p = 0.581$ & $F_{(2.16)} = 1.593$, $p = 0.234$ respectively)

Lastly the fold change in the ratio of phosphorylated to total ERK1/2 was analyzed by repeated measures ANOVA in the manner as mentioned previously. There was a significant difference in the relative fold change in the ratio of phosphorylated ERK1/2 to total ERK1/2 ($F_{(2,16)} = 5.514$, $p=0.015$). Planned contrasts identified that there the High Intensity protocol resulted in a significantly different change in phosphorylation ratio compared either the Moderate Intensity ($p =0.026$) or Low Intensity ($p = 0.017$) power squat protocols (see Figure 8).

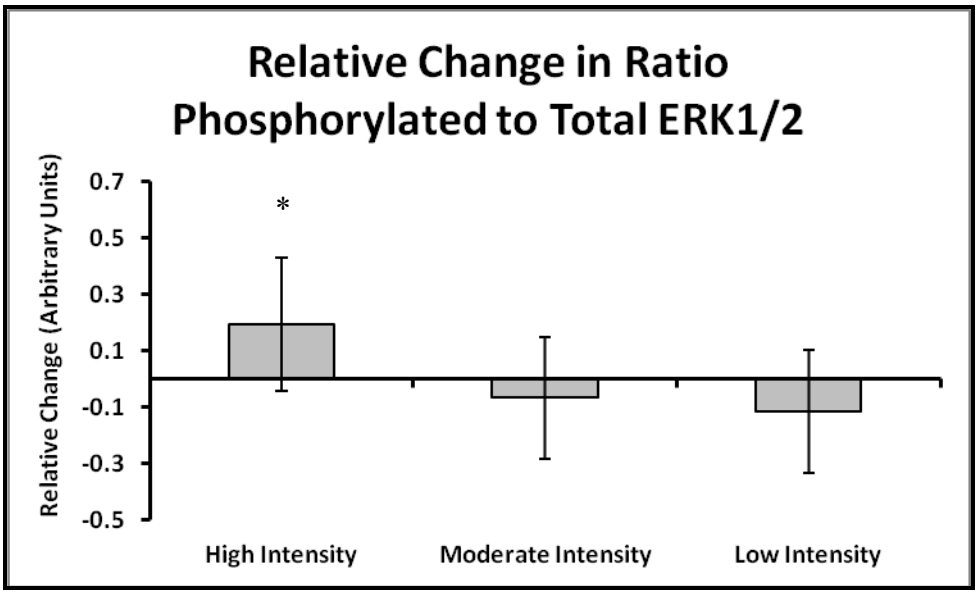

Figure 8. There is a significant difference in the relative change in the ratio of phosphorylated to total ERK1/2 between protocols. Planned contrasts identified that the High Intensity protocol resulted in a significantly different change in phosphorylation ratio compared either the Moderate Intensity ($p = 0.026$) or Low Intensity ($p = 0.017$) power squat protocols.

The 3x2 ANOVAs for total p38 found no differences between treatment days, the pre-post- exercise timepoint, or an interaction of these two variables ($p>0.05$). The phosphorylated p38 did have a significant main effect for the treatment ($F_{(2.16)} = 4.252$, $p < 0.033$). But there was not a significant main effect for timepoint (pre-/ post) nor was the interaction significant. Planned contrasts further discriminate the significant difference identified between the treatments to be a significantly lower values on the High Intensity treatment group than the other two treatment days (Figure 9).

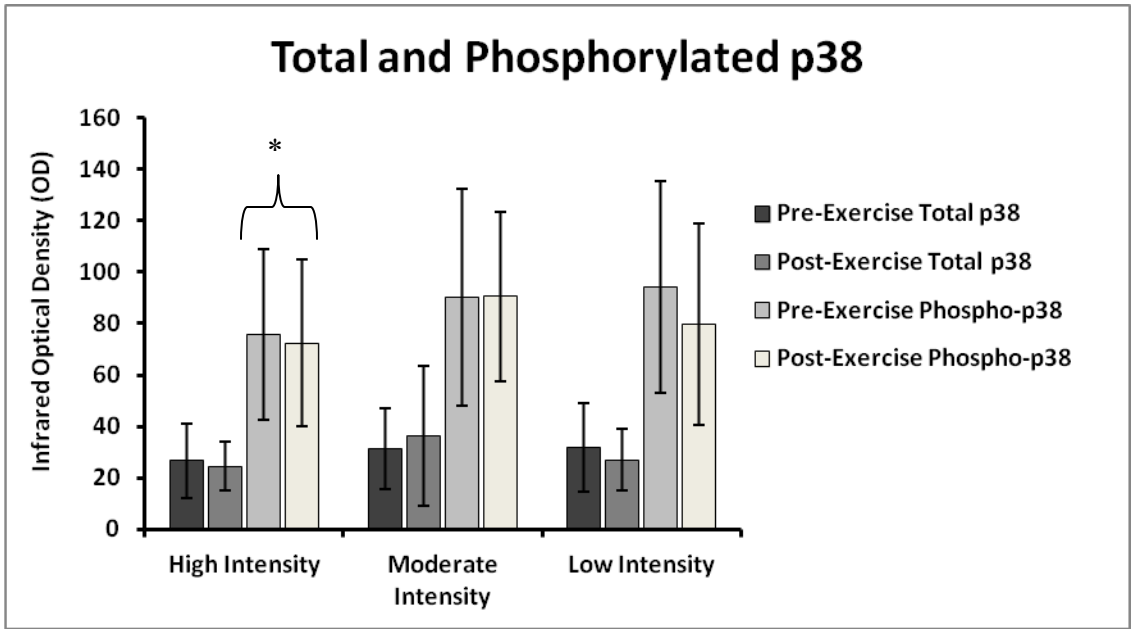

Figure 9. Total and phosphorylated p38 by protocol and timepoint. There were no significant differences in total p38 by day ($F_{(1.8)} = 3.127$, $p = 0.036$) , timepoint ($F_{(1.8)} = 252$, $p = 0.622$) or interaction (protocol x timepoint)($F_{(2.16)} = 1.537$, $p = 0.251$). * indicates a significantly lower phosphor-p38 on the high intensity day $F_{(2.16)} = 4.252$, $p = 0.036$. There was no significant main effect for timing (pre- post-exercise) ($F_{(1.8)} = 2.585$, $p = 0.147$) or for the interaction (protocol x timpoint, $F_{(2.16)} = 4.252$, $p = 0.436$.

As was performed for the ERK1/2 proteins, a second set of repeated measures ANOVAs was performed using the relative change in the expression of the p38 and phosphor-p38 proteins from pre to post exercise bouts in order to more clearly identify the potential differences that might be caused by the exercise. There was no difference in the relative change in total p38 protein expression between the exercise bouts ($F_{(2.16)} = 0.562$, $p = 0.581$). Nor was there a significant difference between treatments for the relative change in the phosphorylated p38 levels $F_{(2,16)} = 1.974$ $p = 0.171$; See Figure 10). As with the ERK1/2 protein the fold change in the ratio of phosphorylated to total ERK1/2 was analyzed by repeated measures 1x3 ANOVA. There was no

significant difference in the relative change in the p38 phosphorylated to total ratio ($F_{(2,16)}$ = 1.221, p= 0.32; see Figure 11).

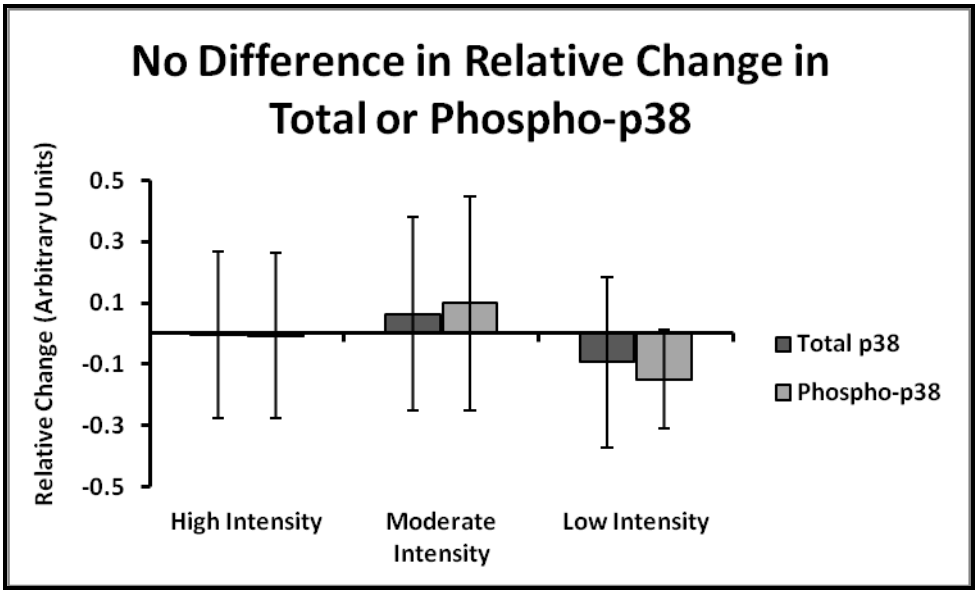

Figure 10. There was no difference in the relative change of total or phosphorylated p38 between exercise protocols (p >0.05).

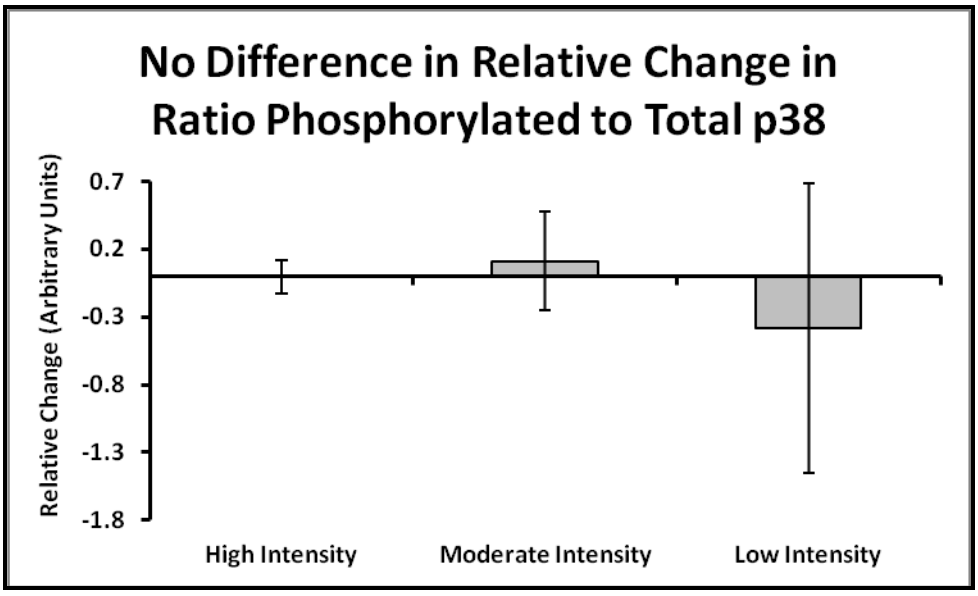

Figure 11. There is no significant difference between the relative change in the ratio (phosphorylated: total) p38 ($p>0.05$).

STUDY 2. THE EFFECTS OF RESISTANCE EXERICSE AND A PRE-WORKOUT DIETARY SUPPLEMENT ON ERK AND P38 PHORPHORYLATION

Twenty four subjects completed the study, (N=14Supplement group N=10 in the Placebo Group. Of the six subjects who left the study, five chose do so based due to the time commitment and lack of enjoyment, and one was removed from the study after exceeding the allowable number of missed training sessions. Descriptive characteristics for the subjects along with the raw data for this study are displayed in appendix M.

This study was part of a larger investigation and a previously published companion study (Moodie, 2008), and a jointly published research abstract (Kudrna et al., 2011) have also been published. Since a number of these findings are important for a complete understanding of the current research, a summary of the findings reported in the dissertation of Nicole Moodie is included in appendix N. Notably, the appendix includes the results for changes in body mass, lean body mass, and strength measures.

Jointly published (Kudrna et al., 2011), muscle performance data includes squat and bench press power measured by Tendo Weightlifting Analyzer at 70% of 1RM, and vertical jump performed at the beginning and end of the 8 week training study. Each of these three variables was analyzed by mixed model repeated measures ANOVA (group x time). Squat power was significantly increased after training ($F_{(1,23)} = 46.178$, $p < 0.001$), and the supplement group's response was significantly better than the placebo group ($F_{(1,23)} = 6.291$, $p = 0.02$). There was a significant increase in bench press power from beginning to end of the study ($F_{(1,23)} = 4.855$, $p = 0.38$) , but no difference was found between the supplement and placebo groups ($F_{(1,23)} = 0.903$, $p = 0.352$). Vertical Jump was significantly increased after training ($F_{(1,23)} = 55.164$, $p < 0.001$).

There was no significant in vertical jump improvement difference between groups $F_{(1,23)} = 0.014$, $p = 0.905$.

The remainder of the findings are entirely new and have not been previously reported. In order to analyze the results of the western blots the raw optical density values which varied widely from blot to blot were expressed as a relative or fold change from the initial pre-training pre-exercise sample. Repeated Measures 2x2 Mixed Models ANOVA was performed for the relative expression of ERK1/2 and phosphorylated ERK1/2 using the group (supplement or placebo as categorical factor; data displayed in Figure 12). For Total ERK1/2 there was no significant main effect or interaction effects among any of the variables. For the relative values of phosphorylated ERK1/2 there was a significant main effect for timing (before vs. after the exercise bout; $F_{(1,22)} = 4.854$, $p = 0.38$), but there was no main effect for training, and no interaction effects associated with the supplement. Combined analysis for pre-training and post-training showed that relative phosphorylation was significantly higher following exercise compared to the pre exercise bout levels as seen in (Figure 13).

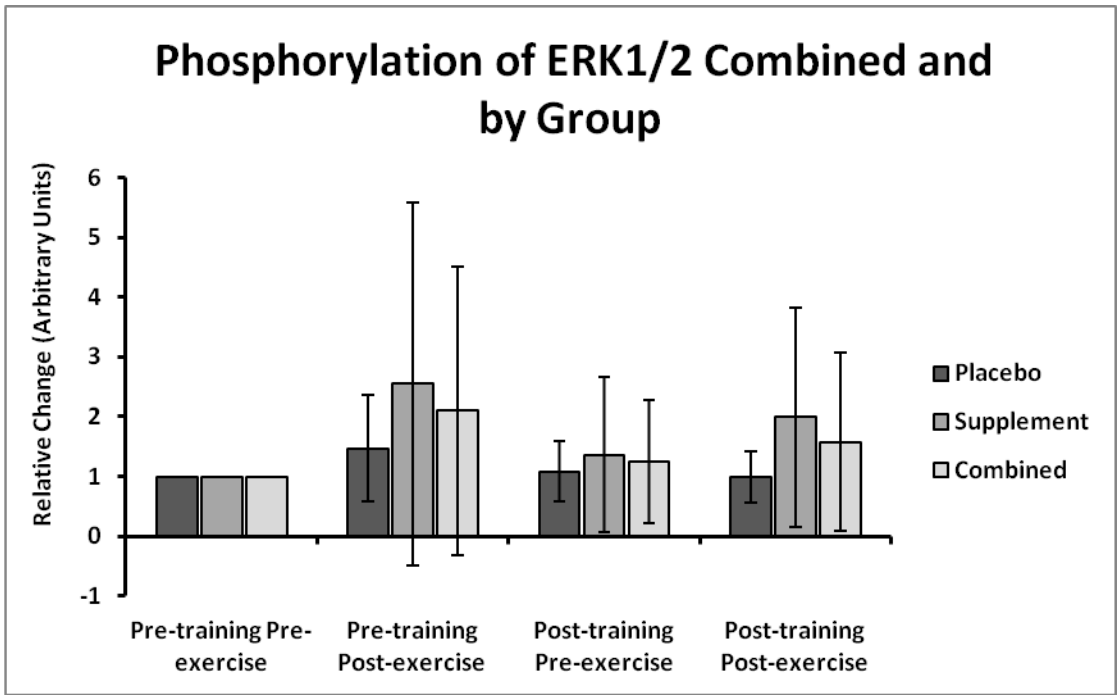

Figure 12. Phosphorylation of ERK1/2 Combined and by group. There is a significant main effect for pre-/post-exercise comparison which is more clearly demonstrated in Figure 13 below. For the relative values of phosphorylated ERK1/2 there was a significant main effect for timing (before vs. after the exercise bout; $F_{(1,22)} = 4.854$, $p = 0.38$), but there was no main effect for training, and no interaction effects associated with the supplement.

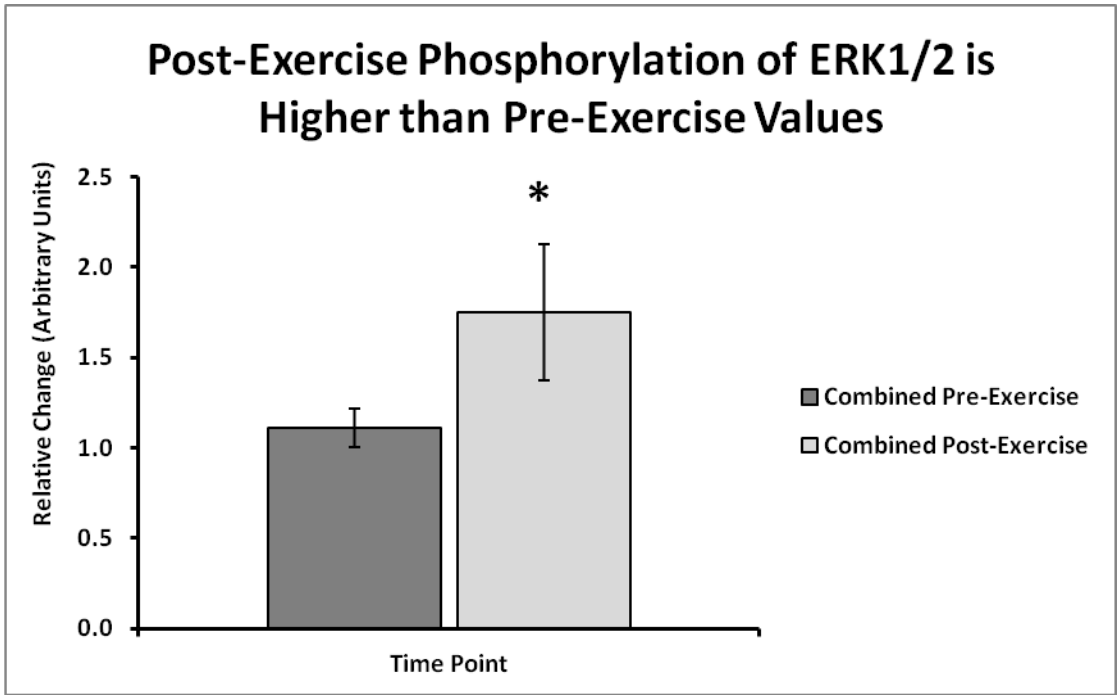

Figure 13. Post Exercie phosphorylation of ERK1/2. For the relative values of phosphorylated ERK1/2 there was a significant main effect for timing (before vs. after the exercise bout; $F_{(1,22)} = 4.854$, $p = 0.38$).

The ratio of phosphorylated to total ERK1/2 for each time point was calculated using the raw score values and also analyzed by a 2x2 mixed model ANOVA. Figure 14 demonstrates all of the phosphorylated to total ERK1/2 ratio data. For the ratio of phosphorylated to total ERK1/2 there was a significant main effect for the combined comparison of pre- to post-exercise measures ($F_{(1,22)} = 5.159$, $p=0.033$) as is shown in Figure 15. There was also a significant interaction effect for training status x exercise timing (pre-post training x pre-post exercise ; $F_{(1,22)} = 6.607$, $p=0.017$) as is highlighted in Figure 16.

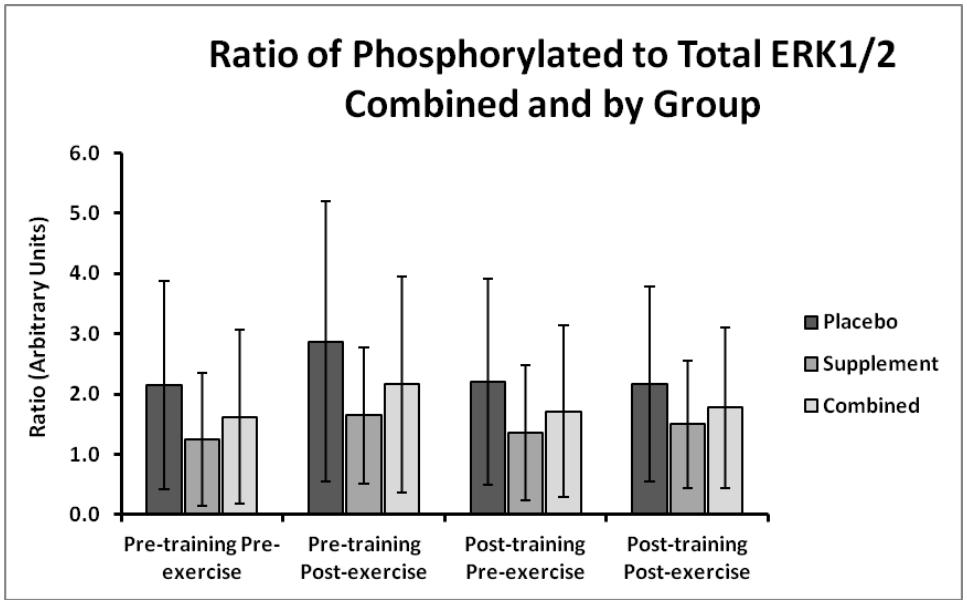

Figure 14. Phospho:Total Ratio of ERK1/2 By group and Combined. For the ratio of phosphorylated to total ERK1/2 there was a significant main effect for the combined comparison of pre- to post-exercise measures ($F_{(1,22)}$ = 5.159, p=0.033) as is shown in Figure 15. There was also a significant interaction effect for training status x exercise timing (pre-post training x pre-post exercise ; $F_{(1,22)}$ = 6.607, p=0.017) as is highlighted in Figure 16.

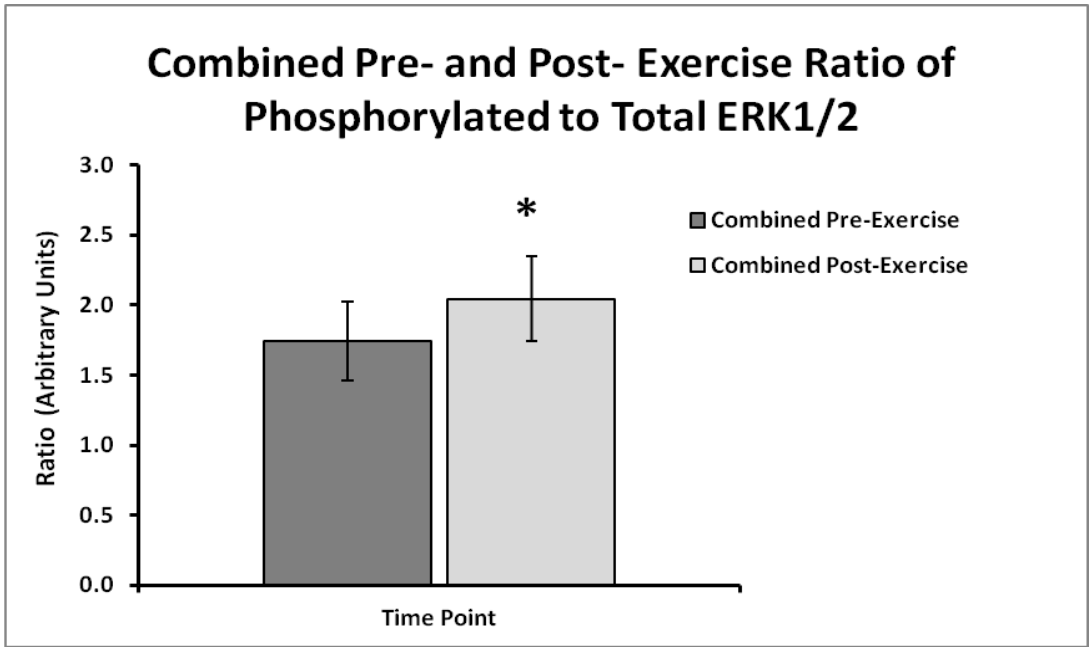

Figure 15. The Phosph:Total Ratio of ERK1/2 was significantly increased after exercise ($p < 0.05$).

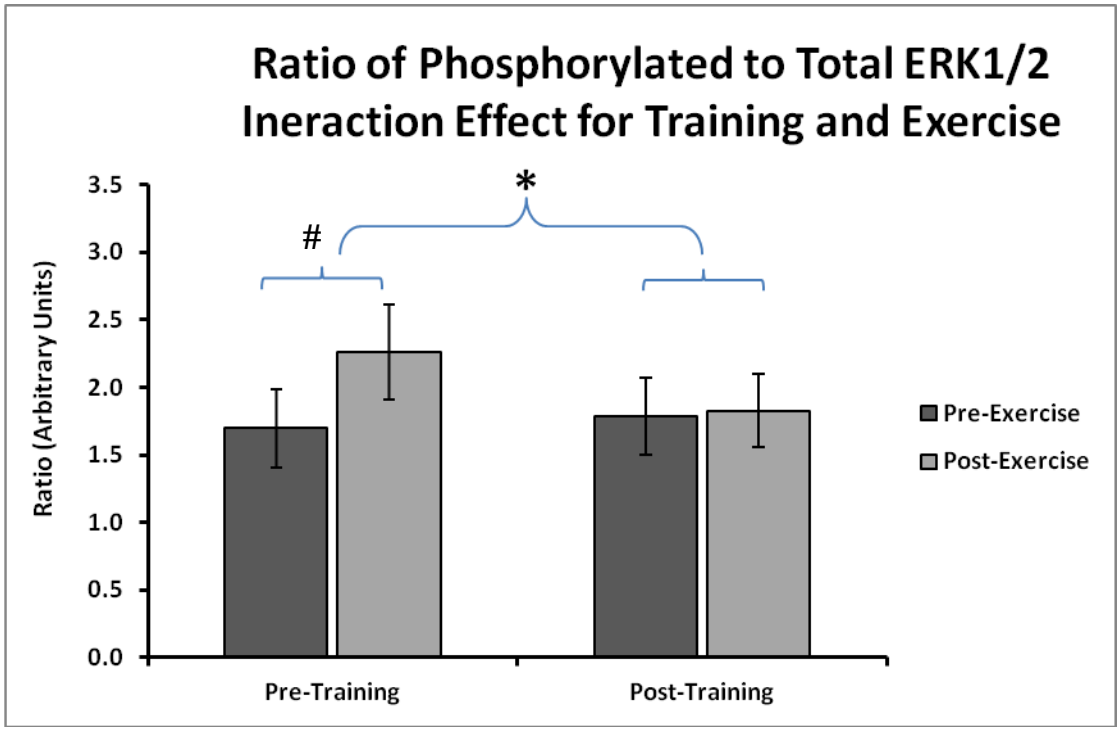

Figure 16. The changes in ERK1/2 were significantly reduced after training ($p < 0.05$). The * indicates a significant interaction effect demonstrating that the change associated with exercise is affected by exercise training. The # indicates a significant post hoc analysis showing that ERK1/2 phosphorylation expressed as a ratio was significantly altered in the pre-training measures, but not the post-training measures.

Repeated Measures 2x2 Mixed Models ANOVA was performed for the relative expression of p38 and phosphorylated p38 using the group (supplement or placebo as categorical factor) as was completed for the ERK1/2 protein. For total p38 there was a significant main effect for training status $F_{(1,22)} = 8.124$, $p=0.09$). This indicates that the combined pre-and post-exercise p38 values were higher after training as compared to before. There was no main effect for exercise bout nor was the interaction significant. The group (placebo vs. supplement) did not significantly affect the levels of p38. Figure 17 and 18 show the p38 values by group and combined and the Pre- to Post- training comparison. The relative phosphorylated p38 values

followed the same pattern as the total p38. There was a significant main effect for training (pre- to post-training; $F_{(1,22)}$ = 8.288, p=0.09). As with the total p38. These pre- to post training analyses should be interpreted with caution since the use of relative expression of the values results in a Standard deviation of zero for on set of samples. As such this leads to an increase in the likelihood of a II error. Figure 19 and 20 demonstrate the differences in phosphorylated p38 by group and combined and show the combined pre- to post training effect respectively.

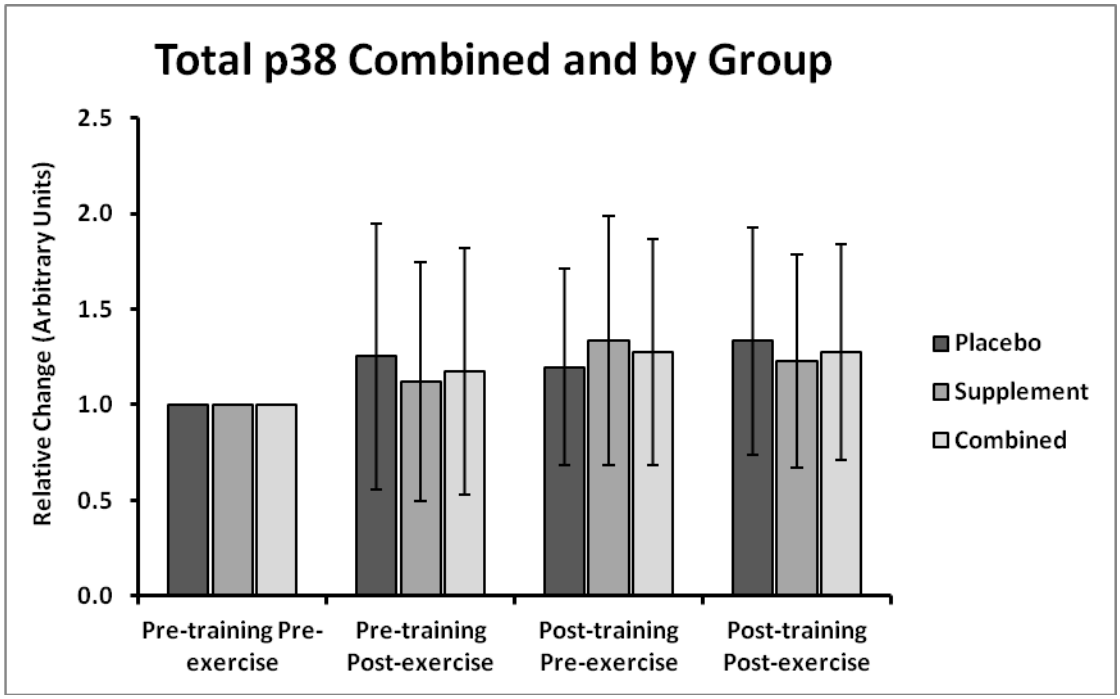

Figure 17. Total p38 combined and by group. For total p38 there was a significant main effecct for training status $F_{(1,22)}$ = 8.124, p=0.09). This indicates that the combined pre-and post-exercise p38 values were higher after training as compared to before . There was no main effect for exercise bout nor was the interaction significant. Figure 17 and 18 more clearly demonstrate these differences.

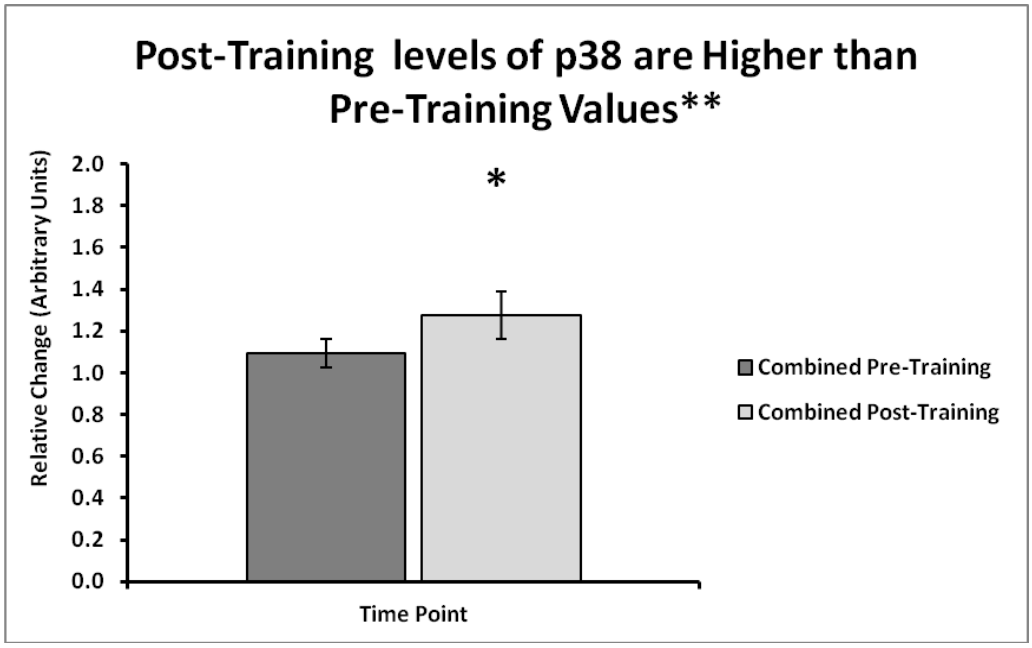

Figure 18. Post Training levels are higher than pre training levels, but should be interpreted with caution as the standard deviation is not accurately accounted for.

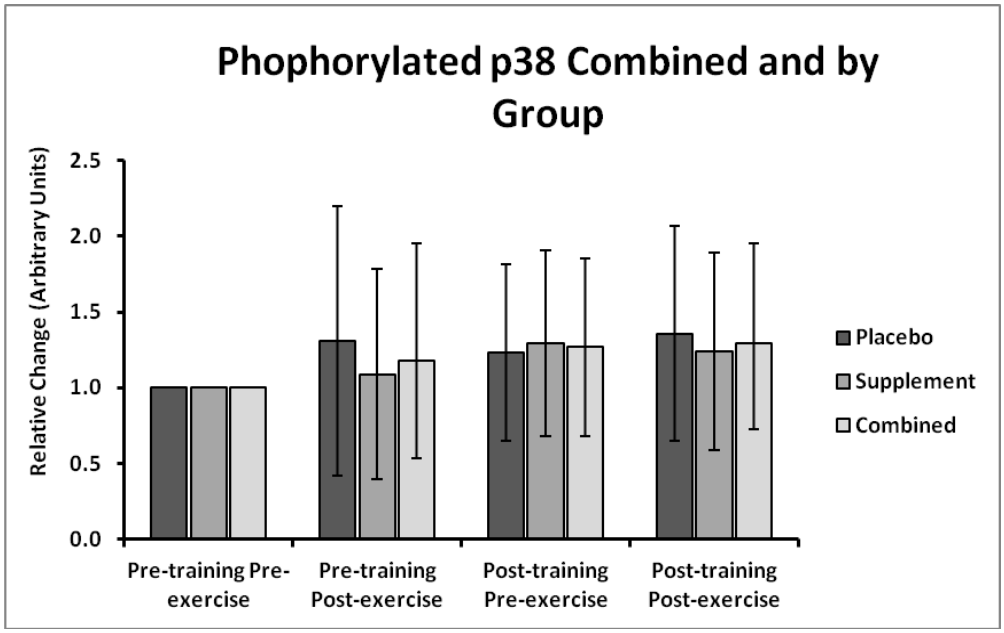

Figure 19. Phosphorylated p38 combined and by group. There was a significant training effect which is more clearly shown in figure 20.

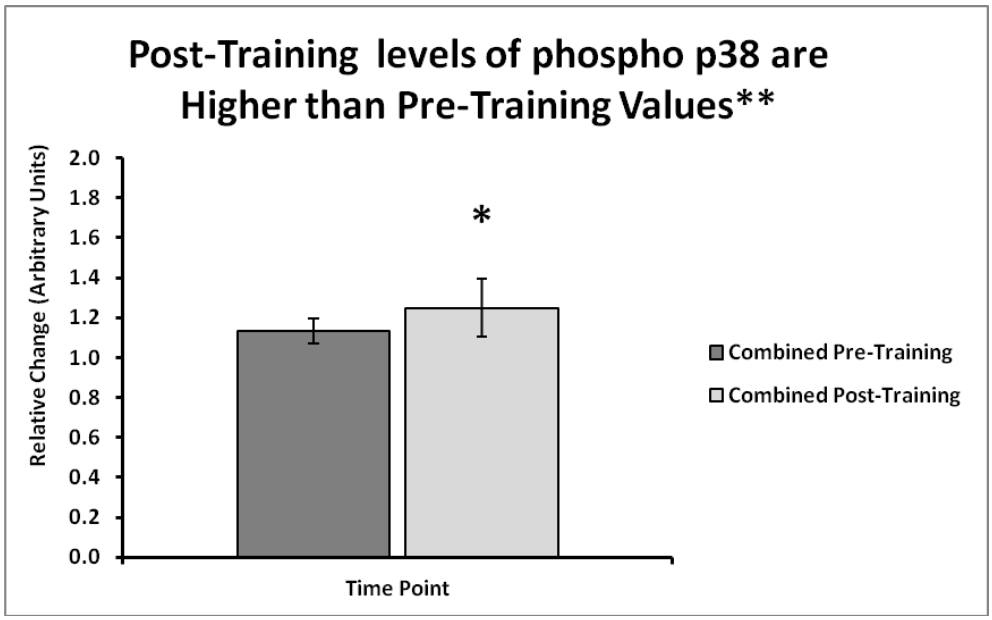

Figure 20. Post Training levels of Phosphorylated p38 are higher than pre training levels ($F_{(1,22)}$ = 8.288, p=0.09), but should be interpreted with caution as the standard deviation increases the chance of a type II error.

The ratio of phosphorylated to total p38 for each timepoint was caluculated using the raw score values and analyzed by 2x2 mixed model ANOVA. Figure 21 demonstrates all of the phosphorylated to total ERK1/2 ratio data. For the ratio of phosphorylated to total p38 there no significant main effects for training or Exercise (p>0.05). There were also no significant interactions between these variables (p>0.05). The supplement also had no significant impact on the ratio of p38 phosphorylation (p >.0.05).

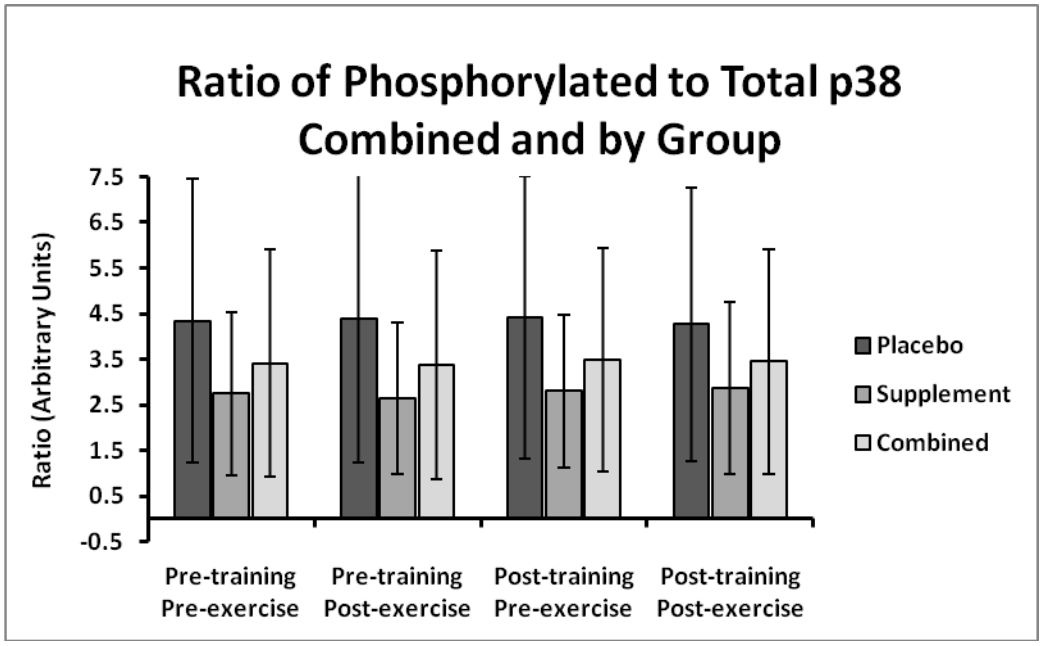

Figure 21. Phospho:Total Ratio of p38 combined and by group. No significant differences were identified.

CHAPTER 5: DISCUSSION

STUDY 1. SKELETAL MUSCLE MAPK ACTIVATION DURING HIGH POWER RESISTANCE EXERCISE

This study sought to determine if there was a difference in the physiological response to the three different high power squat exercise bouts. This study showed that lactates were increased following each exercise bout, but that there was no difference in the elevation of the lactates between groups. Since the protocols all consisted of intermittent exercise and rest the lactate response was apparently limited. In short there was no distinguishing difference between the lactate response based upon the exercise protocol.

The power measures taken during the study demonstrated that the low intensity protocol produced significantly higher average power in agreement with previous research (Zink, Perry, Robertson, Roach, & Signorile, 2006). There was however no difference between the protocols for peak power which was unexpected. The load appeared to affect power fatigue across five sets of resistance exercise. The heaviest load had the highest power fatigue (13% difference from set 1 to set 5). The moderate and light loads did not show any evidence of power fatigue. It should also be noted that five sets at 30% 1RM may be indication of a warm-up effect an not one of power fatigue. Thus this data indicates that at the intensities most frequently utilized to develop power, there is no evidence of decreased power over 5 sets of speed squats. Practitioners attempting to train for power at higher loads (i.e. 90%) should be aware that power fatigue may occur in as few as 5 sets. Also notable is the lack of difference in the average of peak power across the 5 sets at dramatically different loads.

This study showed that ERK1/2, Phosphorylated ERK1/2 were found to decrease following the exercise bout contradictory to what has been shown previously (Galpin et al., 2012; Moore et al., 2011; Ryder et al., 2000). When analyzed further it was determined that the High Intensity protocol tended to increase whereas the other two protocols showed a trend toward decrase resulting in a significant difference from each other. It may be that the decreased total ERK1/2 following exercise is due to it's complexing with larger proteins and lost during centrifugation. That however is only speculator. Against previous research the relative change in the ratio of phosphorylated to total ERK1/2 did not differ between the exercise protocols. This weak response to ERK1/2 might be attributable to an inadequate volume or intensity of exercise. The highest intensity protocol did produce the greatest trend toward a ERK1/2 phosphorylation.

The p38 and phosphorylated p38 also showed no difference from pre to post exercise contrary to what was expected base upon most(Coffey et al., 2006; Galpin et al., 2012; Karlsson et al., 2004; Yu, Blomstrand, Chibalin, Wallberg-Henriksson, et al., 2001) but not (Williamson et al., 2003) all previous research. As with the ERK1/2 the volume or intensity of the squat exercise may not have been adequate. It is also possible the timing was not the most advantageous to identify the phosphorylation changes, though it is not inconsistent with the literature. It was the intention of this study to examine the relationship between power and the ERK1/2 and p38 response but without significant changes in either erk1/2 or p38, it is impossible to determine the relationships between these items.

There are a number of possible reasons that the MAPK changes in response to these exercise bouts were less than hypothesized. Among them, the stimuli may have been insufficient to stimulate the muscle. Most previous research has utilized leg extension as at least part of the methodology. The back squat, while it does typically recruit the vastus lateralis, may

not activate that muscle group adequately in this study. The exercise protocol most close to this studies is the use of heavy clean pulls as described in Galpin (2012). In addition to the mode of exercise the volume or the ratio of work to rest may have been a factor.

Finally, the prior training of the subjects may have been the reason for the lack of MAPK response. Based upon previous research(Coffey et al., 2006; Yu, Blomstrand, Chibalin, Wallberg-Henriksson, et al., 2001) and the second study of this work, it is possible that the careful selection of subjects who were competent and practiced in the back squat exercise was part of the reason the subjects did not respond to the exercise bouts. Habitual exercise training appears to blunt or eliminate the MAPK response to similar exercise (Coffey et al., 2006), but this has not previously been observed in recreationally trained resistance exercisers. .

Study 2. The Effects of Resistance Exericse and a Pre-Workout Dietary Supplement on ERK and p38 Phorphorylation

Subject Dropout and Supplement Tolerance

The nutritional supplement utilized in this study seemed to be fairly well tolerated by the subjects. There were no major adverse reactions reported by the subjects. Prudent precautions were taken to eliminate caffeine sensitive and caffeine naive subjects from the study. Also precautions were taken to help the supplement group adapt to the high caffeine loads by increasing the caffeine to the full dose in week two (see methodology). Such precautions are essential to ensuring that a high caffeine supplement such as this one does not cause serious adverse reactions.

One adverse reaction that was observed in the supplement, but more so the placebo group was stomach upset and vomiting during the exercise training. Because this was

more prevalent in the placebo users it may have contributed to the disproportionate withdrawal of subjects selected to the placebo group. Upset stomach during the exercise training may be attributable to the consumption of the supplement or placebo immediately prior to the exercise training. Though the ingredients in the placebo were all generally recognized safe by the FDA, there may have been an unexamined factor such as osmolarity of the drinks or there may have been different gastric emptying rates due to one or more of the different ingredients within the supplement and placebo. Individuals considering the addition of any supplement to their training or competition nutritional plan should take into account the possible digestive disturbances. It is recommended that all persons considering this or any pre-exercise supplement consider important health, safety, efficacy, and other related nutritional issues as is recommended by the American College of Sports medicine and the American Dietetic Association (Rodriguez, Di Marco, & Langley, 2009).

POWER PERFORMANCE MEASURES

Both groups increased measures of power in the 70% of maximum back squat, the 70% of maximum bench press, and vertical jump. Increased power performance even at the same relative intensity is an expected outcome of resistance exercise training and agrees with the previously published literature, (Chtara et al., 2008; Cormie, McBride, & McCaulley, 2009; Pesta et al., 2014; Petrella, Kim, Tuggle, & Bamman, 2007). The supplement was associated with significantly greater improvements in the squat power than the placebo group, while there was no such difference in the other two power measures, bench press and vertical jump. It remains unclear by what mechanism the supplement might disproportionately affect the power improvements. One possible explanation is that the caffeine which has been shown to acutely increase muscle power and contraction velocity (Pallares et al., 2013) could have over the course

of the 8 weeks of training contributed to a chronic training effect. The dose of caffeine used in this study were lower than the effective doses identified in Pallares et al. (2013) given the loads prescribed. Nonetheless, The increase in leg power with a MIPS (supplement) does agree with previous research (Ormsbee et al., 2012), though the methods of power testing were different.

A possible explanation for the vertical jump being unaffected by the supplement compared to the 70% of squat power measurement is that the training program which highlighted muscle hypertrophy and strength gains was inadequate to cause changes in the vertical jump which is a much higher velocity movement (Cormie et al., 2009). It is important to note that the training program was not directly designed to increase power and the subjects were not instructed to perform the training movements with maximal velocity. Thus the adaptations to the 70% of 1RM squat power was much more specific to the training undertaken during the 8 week study. Finally, the fact that the bench press power test was not different between groups thought the force would have been similar to their training loads might be related to the fact that the subjects had a greater percent change in their squat maxes compared to their bench press maxes. Thus both the strength and power improvements were greater in the lower body than in the upper body.

ERK1/2 Observations

The results of this study indicate that the levels of phosphorylated ERK1/2 and the ratio of phosphorylated to total ERK1/2 increased following a resistance exercise bout has been observed in most previous studies (Galpin et al., 2012; Moore et al., 2011; Ryder et al., 2000). One interesting difference between this study and others is that the exercise bout was not simply isolated to the muscle specifically being assessed by biopsy (Moore et al., 2011) or to a

single exercise or modality (Galpin et al., 2012; M Gibala, 2009; M. Gibala et al., 2009) or a lower body only training program (Kosek & Bamman, 2008). This study may be the first to show that ERK1/2 phosphorylation (and the ratio of phosphorylation to total) is significantly increased following a whole body resistance exercise protocol that is typical of muscle hypertrophy training. While it may not have been unexpected it does confirm that whole body resistance exercise does not preclude the cellular signaling mechanisms that have been observed under more tightly controlled and muscle group specific laboratory protocols.

One of the most interesting finding of this study is the significant interaction between training status and the response to a single resistance exercise bout. As was demonstrated in Figure16, when expressed as a ratio of phosphorylaed to total ERK1/2 there was a significant diminution of the ERK1/2 response following training. This is study clearly indicates a training effect that may be part of the cellular signaling mechanism associated with the ceiling effect of exercise training. The habitual exposure to similar exercise resulting in decreased ERK1/2 phosphorylation has been noted previously (Coffey et al., 2006; Yu, Blomstrand, Chibalin, Wallberg-Henriksson, et al., 2001), but these studies used previously trained individuals and thus do not preclude the possibility that it is a characteristic of successful training that the subjects would respond in the manner that they did.

One study previously undertook to examine the training response in resistance exercise naïve subjects using a stronger pre-post testing design (Kosek & Bamman, 2008). Kosek & Bamman, did not find any training effect for ERK1/2 as has been shown in this study. A second discrepancy with findings with the Bamman study was that they had concluded that young but resistance exercise naïve individuals did not demonstrate an elevated phosphorylation of ERK1/2 after training, and had no training associated reduction in the ERK1/2 response. Whereas

the current study indicates that regular recreational resistance exercise is not enough to prevent a training associated elimination of the ERK1/2 response. The most likely reason for the discrepancy is that Kosek & Bamman took muscle biopsies a full 24 hours after the exercise bouts at the beginning and end of the study. The current study took biopsies more immediately as is more common in the literature. While the 24 hour time point was useful in identifying aberrant signaling in the older men of the Kosek & Bamman study, this study clearly shows acute immediate effects on the ERK1/2 pathway. Thus this study adds clear and important evidence of a training effect on ERK1/2 phosphorylation response to an exercise bout .

P38 OBSERVATIONS

Unlike as was observed for ERK1/2, p38 phsophorylation expressed relative to the initial pre-training pre-exercise value or expressed relative to the total p38 did not increase following exercise. Most (Coffey et al., 2006; Galpin et al., 2012; Karlsson et al., 2004; Yu, Blomstrand, Chibalin, Wallberg-Henriksson, et al., 2001) but not all previous research (Williamson et al., 2003) shows that p38 phosphorylaton is increased following unaccustomed resistance exercise in young men. Reasons this study may differ from the majority of studies might include the use of a non-targeted whole body resistance training program that included abdominal and not lower body exercise as its final exercises. Thus the timing of the post exercise biopsies may not have been ideal to catch the transient increase in p38 phosphorylation.

This study di find a significant increase in the amount of p38 and phosphorylated p38 and from pre-training to post training, but should be evaluated with a great deal of caution given that expressing the quantities as relative to the initial value while necessary for comparison of the Western blots, it does create a standard deviation of 0 for the initial sample set. Thus there

is an increased chance of a type II error in the analysis. Assuming this information is not erroneous, it is contradictory to Yu et al., (2001). A major difference between the studies is that Yu et al. compared trained runners to sedentary controls whereas the present study followed recreationally active individuals as they became resistance exercise trained to a greater extent over the course of 8 weeks. Tannerstedt et al. (2009) showed that type II fibers phosphorylated p38 at a much higher rate than type I muscle fibers following eccentric contractions. While there is no other evidence to support the notion, it is possible that there is a fiber type difference associated with the amount of p38 present in the muscle. A hypertrophy based resistance training program would selectively increase the volume of type II fibers in proportion to type I in the muscle. This may be an area for future research.

THE EFFECTS OF THE MIPS SUPPLEMENT

The multi-ingredient pre-workout supplement tested in this study did improve in some muscle performance variables to a greater extent than the placebo including the 1RM bench press, and the 70% of 1RM squat power, but the majority of strength and power measures were not improved by consumption of the supplement. There are several reasons this may have occurred. The subjects of this study consumed the supplement only three times per week just prior to their resistance training bouts. The manufacturer does recommend that the product be taken daily. Since one of the ingredients of the supplement is creatine and studies do show that daily lower doses of creatine are can be as effective as larger short term doses, the dosing and timing used in this study may not have been adequate to observe all of the possible benefits of the supplement (Volek & Rawson, 2004). And as always a longer intervention period may have been needed to distinguish a greater difference between the two groups performances. As for the effects of the supplement on the MAPK pathways it is possible that the effects were simply to

small to be detected by western blot and that a truly quantitative method would be necessary to distinguish any role that they play. Similarly perhaps the supplement must be consumed daily in order to produce observable differences.

APPENDIX A: HUMAN SUBJECTS COMMITTEE LAWRENCE APPLICATION FOR PROJECT APPROVAL: SKELETAL MUSCLE MAPK ACTIVATION DURING HIGH POWER RESISTANCE EXERCISE

2/2008 HSCL #_____
(to be assigned)
UNIVERSITY OF KANSAS
Human Subjects Committee Lawrence
Application for Project Approval

1. Name of Investigator(s) Dr. Andrew. Fry,
2. Department Affiliation Health Sport and Exercise Science
3. Campus or Home Mailing Address: 1301 Sunnyside Ave; Robinson Rm101D; Lawrence, KS 66045
a. Email address:__acfry@ku.edu_____
Phone Number(s): (a) Campus: ___785-864-0784_____
(b)_____;
5. Name of Faculty Member Responsible for Project: _ Dr. Andrew Fry_____
HSCL must receive faculty approval via hard copy signature or email notification before a student application may be processed.
a. Email address of Faculty Member:_____
6. Type of investigator and nature of activity. (Check appropriate categories)
☒ Faculty or staff of University of Kansas
☐ Project to be submitted for extramural funding; Agency:_____
KU/KUCR project number:_
(HSCL must compare all protocols in grant applications with the protocols in the corresponding HSCL application)
☒ Project to be submitted for intramural funding; Source: New Faculty General Research Program
☐ Project unfunded
☐ Other: _____
☐ Student at University of Kansas: ☐ Graduate ☐ Undergraduate ☐ Special
☐ Class project (number & title of class): _____
☐ Independent study (name of faculty supervisor): _____
☐ Other (please explain): _____
☐ Investigators not from the Lawrence campus but using subjects obtained through the University of Kansas
7.a. Title of investigation: Skeletal Muscle MAPK Activation During High Power Resistance Exercise
7.b. Title of sponsored project, if different from above: N/A

8. Individuals other than faculty, staff, or students at Kansas University.
Please identify investigators and research group:
None

9. Certifications: By submitting this application via email or hard copy I am certifying that I have read, understand, and will comply with the policies and procedures of the University of Kansas regarding human subjects in research. I subscribe to the standards and will adhere to the policies and procedures of the HSCL, and I am familiar with the published guidelines for the ethical treatment of subjects associated with my particular field of study.

Date: _____ Date: _____

Signature: _____ Signature: _____
First Investigator Faculty Supervisor

Signature:_____
Second Investigator

Principal Investigator: Dr. A. C. Fry HSCL #: _____

Project Title: Skeletal Muscle MAPK Activation During High Power Resistance Exercise

10. Please answer the following questions with regard to the research activity proposed:
(Please write "Yes" or "No.")

Does the research involve:

yes a. drugs or other controlled substances?

yes b. payment of subjects for participation?

no c. access to subjects through a cooperating institution?

yes d. substances taken internally by or applied externally to the subjects?

no e. mechanical or electrical devices (e.g., electrodes) applied to the subjects?

yes f. fluids (e.g., blood) or tissues removed from the subjects?

yes g. subjects experiencing stress (physiological or psychological)?

no h. deception of subjects concerning any aspect of purposes or procedures (misleading or withheld information)?

no i. subjects who could be judged to have limited freedom of consent (e.g., minors, developmentally delayed persons, or those institutionalized)?

yes j. any procedure or activities that might place the subjects at risk (psychological, physical, or social)?

yes k. use of ☐interviews, ☐focus groups, X questionnaires, ☐audio or ☐video recordings? (check all that apply)

no l. data collection over a period greater than one year?

yes m. a written consent form will be used? Note: HSCL makes the final determination on waiver of consent form.

yes n. will the research involve receiving, accessing, collecting, compiling and/or maintaining

information that relates to the past, present, or future physical or mental health or condition of an individual, the provision of health care to an individual, or the past, present, or future payment for the provision of health care to an individual?

11. Approximate number of subjects to be involved in the research: 12

Complete the following questions on this page. Please do not use continuation sheets.

This investigation will explore the effects of three different resistance exercise bouts on the Mitogen Activated Protein Kinase (MAPK) cellular signaling pathway for musclular hypertrophy. The three exercise bouts in this study represent the continuum that exists between resistance training load and the velocity with witch the load can be moved. Maximal power has been shown to occur at approximately 70% of the repetition maximum (CITE). The effect that power plays in regulating cellular signals of hypertrophy such as the MAPK pahway have not been investigated previously. The purpose of this study is to compare changes in the MAPK cellular hypertrophy pathway following three bouts of resistance training that generate different levels of muscular power.

12. Project Purpose(s):

Eight (8) young male volunteers (18-30y) will be recruited from KU and the local community for participation in our study. The inclusion criteria for all volunteers require: good general health, a history of consistent physically activity, non-obese (BMI less than 28kg/m2), non-smoking, normotensive, and free of metabolic or cardiovascular diseases. Due to gender differences in hormonal fluctuations and typical responses to resistance training only males will be used in this investigation.

13. Describe the proposed subjects (age, sex, race, or other special characteristics). If there is a physical or mental health condition that characterizes the subjects to be included in the study, please indicate this here as well.

14. Describe how the subjects are to be selected. Please indicate how you will gain access to, and recruit these subjects for participation in the project. That is, will you recruit participants through word-of-mouth, fliers or poster, newspaper ads, public or private membership or employee lists, etc. (If subjects are to be recruited from a cooperating institution, such as a clinic or other service organization be aware that subjects' names and other private information, such as medical diagnosis, may not be obtained without the subjects' written permission.)

Subjects will be recruited from a current list of potential volunteers that have indicated that they would be interested in participating in a research studies in the Applied Physiology Lab. Additional recruiting methods will be comprised of word of mouth and flyers posted around the KU campus and throughout the Lawrence area. All forms of recruitment will include investigator contact information (laboratory phone numbers and e-mail addresses). Potential subjects will be informed of the study design and inclusion criteria via phone, e-mail, and/or face to face communication.

The purpose of this study is to examine changes in the MAPK pathway following resistance training designed to utilize different levels of muscular power. The three experimental protocols for the study are 1) Five sets of 10 repetitions at 30% of RM; 2) Five sets of five repetitions with 70% of 1RM; 3) Five sets of three reptitions at 90% of 1RM. A needle biopsy sample will be taken from the vastus lateralis muscle before and after each of the three exercise protocols. Analysis of the MAPK protein concentration and phosphorylation ratios will be accoplished by western imunnoblot analysis.

Descriptive data for subject descriptives will be expressed as means and Standard Deviations. MAPK protien concentrations will be expressed as a ratio of phoshorylated to total protein and interblot comparisons will be made by using a standard control sample on each blot. Repeated measures ANOVA will test for statistical differences for percent change following each of the three exercise protocols.

10a. Lidocaine will be used as a local anesthetic during all muscle biopsies. In order to further reduce the risk of allergic reaction (very rare), a prebiopsy questionaire will be used to determine if potential subjects have had allergic reactions to the anesthetic in previous dental proceedures.
10b. Subjects will receive a $125 honorarium for their time.
10d. In addition to the three exercise protocols used in this study subjects will have to complete a one (1) repetition maximum (RM) protocol for the squat exercise used in this study. The three experimental protocols for the study are 1) Five sets of 10 repetitions at 30% of RM; 2) Five sets of five repetitions with 70% of 1RM; 3) Five sets of three reptitions at 90% of 1RM.
10f. A total of six (6) muscle biopsies will be performed.
A muscle biopsy of the vastus lateralis will be obtained from each subject at before exercise exercise and immediately post exercise for each of the three exercise protocols for quantification of intramuscular muscle proteins an cellular signalling activation. Muscles biopsies will be performed by Philip Gallagher, PhD. The sample of muscle to be collected will be about 75-150 mg (the size of a pea) . Testing sessions will be separated by seven days.
10g. Resistance exercise can be mildly painful lasting a few seconds and occationally muscle sorenesss can occur which can last for up to 72 hours. Rarely significant musculoskeletal injuries can occur. The use of physically active regular exercisers for our subjects will greatly reduce the risks of injury and severe soreness.
Muscle biopsy – muscle biopsy often causes a dull "cramp-like" pain and delayed soreness. There may also be tenderness at the incission site a few days.
10j. Muscle biopsy – there is a small risk of bleeding, infection, and scarring of the skin. Temporary numbness of the skin near the biopsy site occurs rarely.
10k. A health history questionnaire will be used to help in determining the eligibility of subjects. A physical activity questionaire establishes recent exercise habbits. A training history questionaire will assess long term exercise participation habbits. A pre-biopsy questionaire will be used to assure the subject has not previous alergic reactions to lidocane, a history of abnormal blood clotting, or current blood thining medications. Laboratory proceedures exist to protect the subjects health information from third parties
10m. Informed consent will be completed and obtained by each subject for all procedures in accordance with the Human Subject Committee – Lawrence of the University of Kansas.
10n. See item 10k.
Abstract of the proposed procedures in the project (must be complete on this page). (The abstract should be a succinct overview of the project without jargon, unexplained abbreviations, or technical terminology. Here is where you must **provide details about Yes answers to items under question 10.a through 10.n of the application: drugs, cooperating institutions, medical information requested, security measures and post-project plans for tapes, questionnaires, surveys, and other data,** and detailed debriefing

Submit one complete application and supporting documents with your application. Supporting documents may include consent forms, information statement, oral consent procedures, assent procedures, questionnaires/ surveys/research measures, advertisements recruiting participants e.g. flyers, classified ads, debriefing procedures. You may send all materials via email attachment to dhann@ku.edu; Campus Mail to HSCL Youngberg Hall; or U.S. Mail to HSCL, Youngberg Hall, 2385 Irving Hill Road, Lawrence, KS 66045-7563.

APPENDIX B: INFORMED CONSENT DOCUMENT: SKELETAL MUSCLE MAPK ACTIVATION DURING HIGH POWER RESISTANCE EXERCISE

TITLE
Skeletal Muscle MAPK Activation During High Power Resistance Exercise

Informed Consent

INTRODUCTION

You are invited to participate in a research study examining the different physiological responses to various resistance exercise protocols in human skeletal muscle. This study will be conducted at the University of Kansas, and 8 subjects are being sought to participate. The Department of Health, Sports, and Exercise Sciences at the University of Kansas supports the practice of protecting human subjects participating in research. The following information is provided to help you make an informed decision on whether or not to participate in the present study. Please feel free to ask any questions.

PURPOSE OF THE STUDY

The purpose of this study is to examine cellular signaling responses following three different resistance training bouts; 1)high load low power, 2) moderate load high power or 3) high load low power. Additionally, the hormonal responses to these lifting protocols will also be studied. This investigation will help us to understand how exercisers adapt to their various resistance exercise programs. It may also help us to understand and develop more effective exercise protocols so that exercisers can more easily obtain desired benefits from their exercise programs.

BASIS FOR SUBJECT SELECTION

You are eligible to participate in this study if you meet the following inclusion criteria: Volunteers must be male, between the ages 18-30 years old. Volunteers must also be in good general health, non-obese (body mass index < 28 kg/m^2), non-smokers, and have normal blood pressure. It is also important that subjects in this study be free of metabolic and cardiovascular diseases, a diagnosed bleeding disorder, or abnormal clotting or be taking a blood thinning medication or anti-inflammatory (aspirin, ibuprofen, naproxen). Persons with sensitivity to scarring, those that cannot exercise because of an orthopedic problem, or those that have been treated for a heat related injury will not be eligible for this study as well. Finally, if you are allergic to the local anesthetic or to betadine, or have had allergic reactions to other anesthetics (e.g., Novacaine), then you will be disqualified from the study.

PROCEDURES

A time-line of the testing procedures and an overview of the testing sequence for the pre-testing and test days are presented below. All procedures will be conducted in the Applied Physiology Laboratory at the University of Kansas and will be supervised by trained personnel.

Timeline of Testing Procedures for all subjects

Pre-Testing:

Health History Questionnaire
Pre-Biopsy Screening Questionnaire
Age, Height, and Weight Measurements
Familiarization with exercise protocols
Training History
1RM Squat Testing

Test-Day 1 (one week after pre testing):

Muscle Biopsy #1 & Blood Draw #1 (following an 8 hour fast)
Resistance Exercise Bout (randomly assigned)
Muscle Biopsy #2 Blood Draw #2 within five minutes of exercise completion

Test-Day 2 (One week after previous testing):

Muscle Biopsy #3 & Blood Draw #3 (following an 8 hour fast)
Resistance Exercise Bout (randomly assigned)
Muscle Biopsy #4 & Blood Draw #4 within five minutes of exercise completion

Test-Day 3 (One week after previous testing):

Muscle Biopsy #5 & Blood Draw #5 (following an 8 hour fast)
Resistance Exercise Bout (randomly assigned)
Muscle Biopsy #6 & Blood Draw #6 within five minutes of exercise completion

Post Biopsy Follow-Up (24 hrs after each testing day)

All subjects are to report to the Applied Physiology Laboratory 24 hours after each set of biopsies (following each testing day) to ensure the biopsy site is healing normally.

1) Pre-testing Protocol – Health history and pre-biopsy questionnaires, anthropometric data and determining appropriate weight for each group will be obtained no later than three (3) days prior to Test-Day 1. These protocols will take approximately 30-40 minutes.

2) Exercise Protocols - For this study the exercise bouts will consist of the two legged squat exercise. Each subject will first determine a one repetition maximum (1 RM; i.e., the most weight that can be lifted one time) for the squat exercise. Subsequently each subject will complete each one of three squat exercise protocols over the course of the next three weeks. The three experimental protocols for the study are 1) Five sets of 10 repetitions at 30% of RM; 2) Five sets of five repetitions with 70% of 1RM; 3) Five sets of three reptitions at 90% of 1RM. The rest intervals between exercise sets for the above protocols will be 2 minutes for the low load low power protocol (1), 2 minutes for the high power protocol (2), and three minutes for the high load low power protocol (3). Each protocol will take 20-25 minutes to complete.

3) Muscle Biopsies – You have been informed that the purpose of this study will be to examine the difference between a high intensity and a low intensity resistance protocols in regards to human skeletal muscle signal pathways and muscular growth. By obtaining a small sample of your muscle tissue (¼ the size of a pea), the different types of proteins in your muscle may be determined which will be helpful in the evaluation of health and exercise performance. All

muscle tissue samples (biopsies) will be taken from the outer portion of the front of the thigh using a needle biopsy technique. Andrew Fry, Ph.D. has performed over 400 biopsies over the past decade and Philip Gallagher, PhD., Assistant Professor of HSES, has performed over 200 muscle biopsies over the past year and has assisted on over 1000 biopsies over the past six years on various populations (athletes, sedentary people, elderly etc.) with no significant complications or nothing more than minimal adverse reactions have occurred. The procedure is being overseen by Jeff Burns, M.D. who is on the faculty in the Dept. of Neurology at the KU Medical Center in Kansas City, Kansas. Dr. Burns supervises the procedure, but will not be physically present for the biopsies. The total size of each muscle biopsy will be approximately ¼ the size of a pea (30 mg or less). You will be placed on an examination table lying down on your back (supine) so that the muscles of the leg are relaxed. The skin will be thoroughly cleaned with antiseptic solution (Betadine) using sterile cotton swabs after which a surgical cover will be placed around the sampling site. If you are allergic to Betadine, an alternative antiseptic solution will be used to clean the skin. A small amount (2ml or 2cc) of a local aesthetic (2% Lidocaine) will be injected into the tissue under the skin around the site to be sampled. During this injection you may feel a slight burning sensation. Following the injection of the numbing agent into your thigh, a minimum of five (5) minutes will be allowed to pass to ensure adequate time for the agent to take effect in the area where the biopsy will be taken. A 14 gauge biopsy needle (slightly larger than the needle used when donating blood) will be inserted into the middle of the muscle (muscle belly) at a depth of 3 cm (about 1 inch). During the time that the sample is being taken (about 5 seconds) you may feel some deep pressure and cramping that will be moderately painful. You have been informed that if you have been previously diagnosed as having a bleeding disorder, a blood clotting problem, take blood thinning medication or have sensitivity to scarring, you will tell the researcher and not participate in the biopsy procedure.
Following the biopsy procedure, firm and constant pressure will be placed on the wound to stop any bleeding. Since there is not an incision, the biopsy site will simply be covered with a large Band-Aid. The procedure will take about 20 minutes.
Sterile disposable instruments and sterile gloves will be used for the preparation of the site and the sterile biopsy needle will be used only once and then disposed. Approximately 30 mg (¼ the size of a pea) of skeletal muscle tissue will be removed. With this muscle biopsy technique there is little possibility of a blood related infection since the needles are sterile in accordance with the standards of the American medical Asociation, and are used only once. A total of six (6) biopsies will be performed over the course of this study (see timeline, above).

4) Blood Draws – We will measure the amount of specific hormone and cytokine levels found in your blood prior to, after and following the exercise bout. Six blood draws will be performed during this study (see time line above). In this procedure, a small amount of blood will be taken from the antecubital vein (inside of the elbow joint) for each blood draw by a trained phlebotomist using a needle and a syringe. Approximately 10 cc of blood (~ 2 tablespoons) will be drawn for each time-point. The blood draw will take about 5 minutes.

RISKS
Blood Draws – The blood sample has a small risk of infection and bruising of the area, but this is rare. The needle stick for the blood draw will be mildly painful and only lasts a couple of seconds.

Muscle biopsy – The use of local anesthetics will result in a slight burning sensation, lasting approximately 5 seconds. There is an extremely low risk of allergic reactions to the local injection (1 in 1 million). There is a small chance of bleeding from the biopsy site. The principle concern would be prolonged bleeding which would produce a bruise in the area. This would extend the muscle soreness, but is adequately treated with rest, ice, a compression bandage, and keeping the leg elevated as much as possible. Nausea, dizziness, and fainting can occur in remote cases (1 in 100) during the biopsy process. As a result, the subjects will be in a supine position during the biopsy procedure. There is a minimal risk of infection (1 in 1000) and irritation associated with the biopsy procedure. The use of aseptic techniques, careful cleaning of the skin and keeping the area dry will minimize the risk of infection. There is a minimal risk of bruising (1 in 100) from the biopsy procedure. This will be minimized by placing ice over the site following the procedure and by applying a compression bandage on the site for the 24 hours following the biopsy. In rare instances (1 in 200), some motor nerves may be damaged which may cause local muscle atrophy (decrease in the size of muscle fibers, with a small dimple on the skin). Since there is no incision, scarring is unlikely.

Resistance Exercise: During the strength testing and exercise sessions there is a risk of injury or problems even though all subjects are screened for health problems. The risk and discomforts that are associated with this type of test include muscle fatigue, lightheadedness, chest discomfort, knee and/or back injury, and very rarely death. The potential for death during or immediately following the test (or any vigorous exercise) is approximately 0.5 per 10,000 tests, according to the American College of Sports Medicine. You will experience muscle soreness due to the eccentric component of the resistance exercise. The determination of your one-repetition maximum (1 RM) will require maximal exercise. The three exercise bouts in this study will consist of 1) Five sets of 10 repetitions at 30% of RM; 2) Five sets of five repetitions with 70% of 1RM; 3) Five sets of three reptitions at 90% of 1RM. Each testing session will occur one week apart and the exercise bouts will occur in a randomized order. Laboratory personnel will stay in contact with you after a testing/training session to ensure that you are comfortable. You will be given a 24 hr contact number (Applied Physiology Laboratory personnel) to convey any type of unusual discomfort.

In all of these procedures, great care will be taken to employ "universal precautions" for the handling of blood and infectious materials to ensure your safety. All instruments and bandages that will come into contact with broken skin will be sterile.

FOLLOW UP CARE

Following the procedure you will be provided with a biopsy care sheet, extra bandages and contact information for Andrew Fry, Ph.D. and Philip Gallagher, Ph.D. After 24-hrs you must report back to the testing coordinator to check the wound. At this time the bandage will be removed and properly disposed of and a new sterile dressing placed over the wound. You will again be asked to report to the test coordinator after 3-days and be contacted via phone one week after the biopsy or whenever necessary to ensure normal recovery. The biopsy procedure of this type is not likely to result in any scarring at the site, and all post-treatment care will aid in

reducing the potential for scarring. All care will be taken to aid in the healing of the wound. The entire biopsy procedure will be performed under sterile conditions. All testing staff and associated personnel will be trained in first aid and will be familiar with emergency procedures.

There have been no other major complications reported in the scientific literature as a result of taking small tissue samples from the skeletal muscle using the percutaneous needle biopsy technique described above. This procedure has been performed on numerous subjects by qualified personnel in many institutions worldwide with only slight discomfort being reported. During the muscle biopsy it is common to feel a strong cramping sensation in the muscle while the biopsy is being performed. However, muscle function is not impaired. In fact, subjects have been reported to continue participation in sporting events immediately following a muscle biopsy. It is common for subjects to experience mild soreness, moderate pain and bruising near the biopsy site, similar to a "Charlie-horse" the day after the procedure. In order to allow the biopsy site to heal properly and minimize the risk of infection, you should not get the biopsy site wet for 24-hours and avoid prolonged exposure to water for 4-days. Daily showers are acceptable (after the first 24-hours), but baths, swimming, sauna's etc. should be avoided for 4-days following the biopsy procedure.

BENEFITS

You will gain an increased understanding of your skeletal muscle function. A copy of all personal data from the tests will be provided to you and your data will be completely explained to you by a member of the investigation team.

The results of this investigation will provide a greater knowledge of the acute cellular responses that exist with different resistance exercise protocols and will aid in understanding the underlying causes of muscle hypertrophy. These findings will not only have implications on those healthy individuals that are trying to gain muscle mass, but also patients that are subject to prolonged periods of bed rest or immobilization and the elderly population.

PAYMENT TO SUBJECTS

You will receive a $125 honorarium for your participation to compensate for your time and effort. Investigators will ask you to fill out paperwork including your social security number in order to comply with federal and state tax and accounting regulations.

IN CASE OF EMERGENCY CONTACT PROCEDURE

In the event of a research related injury or adverse reaction, please contact Andrew Fry, Ph.D. at 785-864-0784 (office), Phil Gallagher, Ph.D. 785-864-0784 (office) or 785-550-6300 (cell), or Becky Kudrna at 785-864-0773 (office), 573-694-8871 (cell).

EMERGENCY CARE AND COMPENSATION IN CASE OF INJURY

In the unlikely event that any injury or illness occurs as a result of this research, the University of Kansas, their officers, agents, and employees, do not automatically provide reimbursement for medical care or other compensation. In cases of emergency, consistent with the Kansas Tort Claims Act, you would be responsible for payment of expenses related to treatments or associated with such complications except in a case where neglect can eventually be proven.

The following information is provided in accordance with HEW regulations: "In the event of injury, the Kansas Tort Claims Act provides for compensation if it can be demonstrated that the injury was caused by the negligent or wrongful act or omission of a state employee acting within the scope of his/her employment." You have been informed that payment for treatment of any injury or illness must be provided by your or your third-party payer, such as a health insurer. If any injury or illness occurs in the course of research, or for more information, you will notify the investigator in charge.

INFORMATION TO BE COLLECTED

To perform this study, researchers will collect information about you. This information will be obtained from the health history and physical activity questionnaires, muscle biopsy, and muscle function evaluation. Your name will not be associated in any way with the information collected about you or with the research findings from this study. The researchers will use a study identification number or initials in place of your name.

Dr. Andrew Fry, and his research team will use the information collected about you. The researchers will not share information about you with anyone not specified above unless required by law or unless you give written permission.

Permission granted on this date to use and disclose your information remains in effect indefinitely. By signing this form you give permission for the use and disclosure of your information for the purposes of this study at any time in the future.

CONFIDENTIALITY

Information obtained from this research will be kept confidential, including questionnaires, medical history, laboratory findings, or performance data. Records will be open to FDA inspection if deemed necessary, in accordance with FDA regulations. Records of the research may also be subpoenaed by court order or inspected by federal regulatory authorities. Data from this study may be used in reports, presentations, and publications, but the results of each individual will be kept confidential.

REFUSAL TO SIGN CONSENT AND AUTHORIZATION

You are not required to sign this Consent and Authorization form and you may refuse to do so without affecting your right to any services you are receiving or may receive from the University of Kansas or to participate in any programs or events of the University of Kansas. However, if you refuse to sign, you cannot participate in this study.

CANCELLING THIS CONSENT AND AUTHORIZATION

You may withdraw your consent to participate in this study at any time. You also have the right to cancel your permission to use and disclose information collected about you, in writing, at any time, by sending your written request to: Andrew Fry, Ph.D., University of Kansas, 1301 Sunnyside Avenue, Robinson Center Room 101DJ, Lawrence, Kansas 66045. If you cancel permission to use your information, the researchers will stop collecting additional information about you. However, the research team may use and disclose information that was gathered before they received your cancellation, as described above.

PARTICIPANT CERTIFICATION

I have read this Consent and Authorization form. I have had the opportunity to ask, and I have received answers to, any questions I had regarding the study and the use and disclosure of information about me for the study. I understand that if I have any additional questions about this study you may call Dr. Andrew Fry (785-864-0784) or e-mail: acfry@ku.edu. I understand that if I have any additional questions about my rights as a research participant, I may call (785) 864-7429 or write the Human Subjects Committee Lawrence Campus (HSCL), University of Kansas, 2385 Irving Hill Road, Lawrence, Kansas 66045-7563, email dhann@ku.edu.

I agree to take part in this study titled 'Differential cellular response to various resistance exercise protocols' as a research participant. I further agree to the uses and disclosures of my information as described above. By my signature I affirm that I am at least 18 years old and that I have received a copy of this Consent and Authorization form.

_____ _____

Print Subject's Name Signature of subject

_____

Date

_____ _____

Print Name of Person Obtaining Consent Signature of Person Obtaining Consent

_____

Date

_____ _____

Print Name of Witness Signature of Witness

_____

Date

RESEARCHER CONTACT INFORMATION

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APPENDIX C: MEDICAL HISTORY QUESTIONNAIRE: SKELETAL MUSCLE MAPK ACTIVATION DURING HIGH POWER RESISTANCE EXERCISE

APPENDIX D: EXERCISE TRAINING HISTORY QUESTIONARE: SKELETAL MUSCLE MAPK ACTIVATION DURING HIGH POWER RESISTANCE EXERCISE

Exercise Training History Questionnaire

TRAINING HISTORY QUESTIONAIRE

1. At what age did you start resistance training?

2. Since then how regular, have you been with resistance training?

a. Have / do you take breaks from resistance training longer than 2 weeks? How frequently?

b. Have / do you take breaks from resistance training longer than 1 month? How frequently?

c. Have / do you take breaks from resistance training longer than 3 months? How frequently?

d. When, and for what reasons did you take such breaks?

3. When you first became serious about resistance training what was you main reason?

    a. Sport(s): List_____level of competition_____

    b. Fitness

    c. Competitive Lifting: (powerlifting, olympic lifting, strongman, other)

    d. Rehabilitation

    e. Other: Explain_____

4. What is your reason for resistance training now?

a. Sport(s): List_____level of competition_____

b. Fitness

c. Competitive Lifting: (powerlifting, olympic lifting, body building, strongman, other)

d. Rehabilitation

e. Other: Explain_____

5. In the last 6 months, How many times per week have you typically resistance trained? (Include multiple sessions per day separately).

a. Time per week upper body _____

b. Times per week lower body _____

c. Total times per week _____

6. In the last 6 months, How many weeks have you participated in resistance training one time or **LESS** per week? (include vacations, breaks, and schedule conflicts).

7. In the last 6 months, How many weeks have you participated in resistance training three times or **MORE** per week? (Include multiple sessions per day separately).

8. When you lift, do you usually work at a percentage of your known maximum or by what other method do you choose a weight for each lift?

9. Estimate your 1RM Max, or provide how much you think that you could lift for a given number of repetitions.

LIFT	I have done this exercise in	Predicted Maximum	Weight I usually	Number of Repetitions I

	the last 6 months	Weight	use	usually do
BENCH PRESS	Yes No			
INCLINE BENCH	Yes No			
PARRALLEL SQUAT	Yes No			
FULL SQUAT (below parallel)	Yes No			
DEADLIFT	Yes No			
STANDING PRESS	Yes No			
LUNGES (WITH BARBELL)	Yes No			
LEG EXTENSION	Yes No			
LEG CURLS	Yes No			
BICEP CURLS	Yes No			
PULL-UPS	Yes No			
DIPS	Yes No			

10. Create in the space below a representation of your TYPICAL exercise session in the last 6 months. If you perform lower and upper body movements on separate days list each day separately below.

EXERCISE **SETS AND REPS** **POUNDAGES**

How many times per week do you participate in stretching? _____

11. How many years/months have you been performing cardiovascular exercise?

12. Since you began have / do you take breaks from cardiovascular training longer than 2 weeks? How frequently?

13. Since you began, have / do you take breaks from cardiovascular training longer than 1 month? How frequently?

14. Have / do you take breaks from cardiovascular training longer than 3 months? How frequently?

15. When, and for what reasons did you take such breaks?

16. What is your Current reason for participating in cardiovascular training now?

    a. Sport(s): List_____level of competition_____

    b. Health / Fitness: Explain _____

    c. Rehabilitation

    d. Other: Explain_____

17. In the last 6 months, How many times per week have you <u>typically</u> participated in cardiovascular exercise? (Include multiple sessions per day separately).

18. In the last 6 months, How many weeks have you participated in cardiovascular exercise one time or **LESS** per week? (include vacations, breaks, and schedule conflicts).

19. In the last 6 months, How many weeks have you participated in cardiovascular exercise training three times or **MORE** per week? (Include multiple sessions per day separately).

20. When you run, row, swim, etc, do you usually work at a percentage of your Heart Rate Maximum, aim for a particular time goal, or by what other method do you choose your intensity?

21. Create a TYPICAL weeks exercise log of cardiovascular exercises below. Use only the last six months as a guide. Include type of exercise, the duration, and the intensity or pace at which you perform this task. If you monitor your HR please include that information as well. Provide as much detail as possible use additional paper if needed.

	TYPE OF EXERCISE	DURATION	PACE	INTENSITY
MONDAY				
TUESDAY				
WEDNESDAY				
THURSDAY				
FRIDAY				
SATURDAY				

SUN				

APPENDIX E: HUMAN SUBJECTS COMMITTEE LAWRENCE APPLICATION FOR PROJECT APPROVAL: EFFECTS OF RESISTANCE EXERCISE AND A PRE-WORKOUT DIETARY SUPPLEMENT ON PHYSIOLOGICAL ADAPTATIONS

HSCL #_____

(to be assigned)

UNIVERSITY OF KANSAS
Human Subjects Committee Lawrence
Application for Project Approval

1. Name of Investigator(s) Philip Gallagher, Andy Fry, Phill Vardiman, Becky Kudrna, Nicole Moodie
2. Department Affiliation Health Sport and Exercise Science (HSES)
3. Campus or Home Mailing Address: Robinson 101DJ
   a. Email address:philku@ku.edu
   Phone Number(s): (a) Campus: 785.864.0773 (b) Home: 785.550.6300
5. Name of Faculty Member Responsible for Project: Philip Gallagher, Andy Fry, Phill Vardiman
HSCL must receive faculty approval via hard copy signature or email notification before a student application may be processed.
   a. Email address of Faculty Member: philku@ku.edu
6. Type of investigator and nature of activity. (Check appropriate categories)
   ☒ Faculty or staff of University of Kansas
   ☐ Project to be submitted for extramural funding; Agency:_____
   KU/KUCR project number:_____
(HSCL must compare all protocols in grant applications with the protocols in the corresponding HSCL application)
   ☐ Project to be submitted for intramural funding; Source: _____
   ☐ Project unfunded
   ☒ Other: Funded by Labrada Nutrition Inc.
   ☐ Student at University of Kansas: ☐ Graduate ☐ Undergraduate ☐ Special
   ☐ Class project (number & title of class): _____
   ☐ Independent study (name of faculty supervisor): _____
   ☐ Other (please explain): _____
   ☐ Investigators not from the Lawrence campus but using subjects obtained through the University of Kansas
7.a. Effects of resistance exercise and a pre-workout dietary supplement on physiological **adaptations**

7.b. Title of sponsored project, if different from above: N/A

8. Individuals other than faculty, staff, or students at Kansas University.
   Please identify investigators and research group:
N/A

9. Certifications: By submitting this application via email or hard copy I am certifying that I have read, understand, and will comply with the policies and procedures of the University of Kansas regarding human subjects in research. I subscribe to the standards and will adhere to the policies and procedures of the HSCL, and I am familiar with the published guidelines for the ethical treatment of subjects associated with my particular field of study.

Date: _____ Date: _____

Signature: _____ Signature: _____

First Investigator Faculty Supervisor

Signature:_____ Signature: _____

Second Investigator Third Investigator

Principal Investigator: Philip Gallagher HSCL #: _____

Project Title: Effects of Resistance Exercise and a Pre-Workout Dietary Supplement on Physiological Adaptations

10. Please answer the following questions with regard to the research activity proposed:
(Please write "Yes" or "No.")

Does the research involve:

Yes a. drugs or other controlled substances?

Yes b. payment of subjects for participation?

No c. access to subjects through a cooperating institution?

Yes d. substances taken internally by or applied externally to the subjects?

No e. mechanical or electrical devices (e.g., electrodes) applied to the subjects?

Yes f. fluids (e.g., blood) or tissues removed from the subjects?

Yes g. subjects experiencing stress (physiological or psychological)?

No h. deception of subjects concerning any aspect of purposes or procedures (misleading or withheld information)?

No i. subjects who could be judged to have limited freedom of consent (e.g., minors, developmentally delayed persons, or those institutionalized)?

Yes j. any procedure or activities that might place the subjects at risk (psychological, physical, or social)?

Yes k. use of ☐interviews, ☐focus groups, ☒questionnaires, ☐audio or ☐video recordings? (check all that apply)

No l. data collection over a period greater than one year?

Yes m. a written consent form will be used? Note: HSCL makes the final determination on waiver of consent form.

Yes n. will the research involve receiving, accessing, collecting, compiling and/or maintaining information that relates to the past, present, or future physical or mental health or condition of an individual, the provision of health care to an individual, or the past, present, or future payment for the provision of health care to an individual?

11. Approximate number of subjects to be involved in the research: 30

Complete the following questions on this page. Please do not use continuation sheets.

12. Project Purpose(s):

The purpose of the present proposal is to examine the effectiveness of a pre-workout dietary supplement combined with a supervised resistance exercise program. Physiological variables of interest include lean body mass, body composition, muscle performance, and the accompanying endocrine and inflammatory profile. **Psychological variables of interest include body image, effort and enjoyment of training, and happiness from pre- to post-study.**

Aside from examining the effectiveness of the supplement, the tissue analysis following the resistance training component of this study could provide information concerning the role of resistance training in preventing and treating various disease states associated with inflammation and muscle wasting.

The following hypotheses are postulated;

H_1: The combination of dietary supplement and resistance exercise will result in greater improvements in body composition compared to resistance exercise and a placebo supplement.

H_2: The combination of dietary supplement and resistance exercise will result in greater improvements in muscular performance compared to resistance exercise and a placebo supplement.

13. Describe the proposed subjects (age, sex, race, or other special characteristics). If there is a physical or mental health condition that characterizes the subjects to be included in the study, please

The volunteers will be between the ages of 18 and 35, healthy, non-obese (BMI <28 kg/m^2), non-smoking, normotensive and free of metabolic diseases, cardiovascular diseases, kidney diseases, **and history of seizures**. Subjects will be screened using a health-history questionnaire for contraindications to exercise by American College of Sports Medicine (ACSM) guidelines. Subjects taking any medications or supplements that could interact with the ingredients in the supplement/placebo will be excluded from the study. If the primary investigator believes a medication may pose a risk the information will be forwarded to Dr. Burns for further review. Resistance trained subjects (3 or more days / week for at least 1 year) will be selected to mimic those who are generally more likely to use a supplement of this kind. In addition, subjects currently using drugs that reduce inflammation (aspirin, ibuprofin, NSAIDs, etc.) will be excluded from this investigation. Due to gender differences in hormonal fluctuations and responses to resistance training, only males will be recruited. Prior to giving their consent to participate in this study, the individuals will be fully informed of the risks and benefits associated with the using this nutritional supplement or placebo, completing the exercise program, as well as muscle biopsies and blood sampling. Written consent will be required in accordance with the Institutional Review Board at the University of Kansas.

indicate this here as well.

14. Describe how the subjects are to be selected. Please indicate how you will gain access to, and recruit these subjects for participation in the project. That is, will you recruit participants through word-of-mouth, fliers or poster, newspaper ads, public or private membership or employee lists, etc. (If subjects are to be recruited from a cooperating institution, such as a clinic or other service organization be aware that subjects' names and other private information, such as medical diagnosis, may not be obtained without the subjects' written permission.)

Subjects will be recruited from a current list of potential volunteers that have indicated that they would be interested in participating in research studies in the Applied Physiology Lab. Additional recruiting methods will include word of mouth and flyers posted around the KU campus and throughout the Lawrence area. All forms of recruitment will include investigator contact information (laboratory phone numbers and e-mail addresses). Potential subjects will be informed of the study design and inclusion criteria via phone and/or e-mail.

15. Abstract of the proposed procedures in the project (must be complete on this page). (The abstract should be a succinct overview of the project without jargon, unexplained abbreviations, or technical terminology. Here is where you must provide details about Yes answers to items under question 10.a through 10.n of the application: drugs, cooperating institutions, medical information requested, security measures and post-project plans for tapes, questionnaires, surveys, and other data, and detailed debriefing procedures for deception.

The purpose of this study is to examine the effect of a pre-workout energy drink mix used during an eight week strength training exercise program on muscular strength and hypertrophy. Thirty physically active, resistance trained, college age (18-35y) males will be recruited from the university and local community (Lawrence, Kansas) for this investigation. Subjects will be randomly assigned into 2 groups: 1) Experimental group (EXP) (n=15) and 2) control group (CON) (n=15). All subjects will report to the laboratory at least one week prior to the beginning of the training protocol in order to assess maximal strength and familiarize the subjects with the exercise protocol. Subjects in the EXP will be instructed to drink the supplement 15 minutes before each training session. Subjects in the CON will be instructed to drink a non-caloric placebo mixture at the same times. Both groups will complete an eight week exercise program consisting of three days of strength training per week. Each training session will be designed to increase total body muscular strength and hypertrophy. All subjects will complete the same training protocol. The weights lifted will be assigned relative to each individual following their initial maximal strength testing. Subjects will have their blood drawn for analysis of plasma markers of the endocrine response (testosterone, growth hormone, cortisol), hypertrophy (IGF-1) and systemic inflammation (cytokines) immediately before and after the first training bout, and immediately before and after the final training bout. Muscle biopsies will occur prior to and immediately following the first and last training bouts. The muscle biopsies will be analyzed for markers of skeletal muscle hypertrophy and cytokines. These findings may determine the effectiveness of the pre-workout energy drink mix on weight, lean body mass, muscular strength and hypertrophy following a resistance training program.

10a. Lidocaine will be used as a local anesthetic during muscle biopsy procedures.

10b. Subjects will receive a $200 honorarium for their time.

10d. **An individual associated with the Applied Physiology Laboratory, but not on the research team for this particular project will be assigned the task of randomizing the subjects into placebo and experimental supplement groups. This individual will also be responsible for preparing the supplement for each subject prior to their exercise sessions. The supplement and placebo mix provided by Labrada Nutrition, Inc. will be labeled 'A' or 'B' but will not provide any information about their contents so that this study can be conducted in a double blind fashion. Only this assigned individual will know the contents of the containers. This individual will mix the designated amount of supplement or placebo with a specific amount of water, and monitor the participant as they consume the drink 15 minutes prior to exercise.** The subjects will be aware that they are consuming either the EXP supplement or the CON placebo, but the content of what is ingested will not be revealed until the end of the study.

The placebo drink will consist of a low-glycemic flavor and color matched drink. The drink will be sweetened with polydextrin and/or mannitol. The drink will also include typical food coloring and flavoring ingredients. The experimental supplement is a blend of several known nutritional supplements and ergogenic aids. The "Nutritional Information Panel" as it is typically included on

their commercially available powder supplement has been provided by the company and is also attached as a separate documents (super_charge_orange.JPG). A "Supplement and Placebo Ingredient Information" form is also attached in order to provide further information on these drinks.

10f. *Blood Sample*s: Blood samples will be obtained by a trained phlebotomist from the antecubital vein, using a 22-gauge needle and a 10 cc syringe, when the subject is in a fasted state prior to any muscle function tests. Blood samples will be centrifuged immediately at 3000 g for 20 min at 4°C. The plasma and serum will be stored at -80°C until analyses for CK and inflammatory cytokines are performed.

*Muscle Biopsie*s: Percutaneous needle biopsies will be obtained from the dominate leg. The biopsy will be obtained with the subject in a fasted state prior to muscle function test. The muscle biopsy (~100 mg/sample) will be obtained using manual suction and will be taken from the *vastus lateralis* under local anesthesia (2 % lidocaine) at a depth of ~2 cm. The sample will be divided into two sections (40-50 mg each) and immediately frozen at -80°C for later analysis of stress kinases. All muscle biopsies will be performed in the Applied Physiology Lab at the University of Kansas either by Dr. Phil Gallagher or by Dr. Andy Fry. Dr. Gallagher and Dr. Fry have performed over 200 muscle biopsies in the last year and assisted on over 1000 over the past eight years with no major complications.

10g. *Testing* – The following tests will be performed at the beginning, middle, and end of the study for all subjects.

Body Weight/Anthropometric testing – Body weight will be measured with a calibrated electronic scale. Height will be measured by stadiometer. Limb circumferences will be measured using a measuring tape.

Body Composition – Fat mass, lean body mass, and % fat will be determined using a dual-energy X-ray absorptiometry (DEXA).

One RM Strength – Maximal strength for the upper and lower body will be determined using 1 RM tests for the barbell bench press and the barbell high bar parallel squat.

Upper and Lower Body Power and Velocity – Average and peak power and average and peak velocity at 70% 1 RM loads for the bench press and barbell squat will be determined using a Fitrodyne external dynamometer. This is a simple device attached to the bar or weight stack during resistance training.

Vertical Jump Height and Power – Lower body power using body weight for resistance will be determined from a standing vertical jump test. Vertical jump height will be determined, and jump power will be calculated using the Harman equation.

Rate of Force Development (RFD) – RFD for both the upper body (chest press) and lower body (knee extension) will be determined from specially modified resistance exercise machines. Force transducers will be placed in-series with the resistance cable to detect isometric force at 200 HZ sampling rate. These data will permit determination of maximum isometric force, RFD, and the explosive strength deficit as described by Zatsiorsky and Kraemer.

Dietary Recall – Subjects will be asked to record all food and drink consumption for three days prior to their first and last muscle biopsies.

Psychological & Other Survey Assessments – All subjects participating in the EXP and CON groups will complete the following survey assessments at each test time;

1 - Task & Ego Orientation in Sport Questionnaire
2 - Assessment of exercise enjoyment
3 - Assessment of body image
4 - Assessments of satiety and palatability
5 - Perceptions of training stresses

Exercise Training - EXP and CON groups will perform free weight exercises three days each week. The program will be periodized to provide an optimal stimulus for muscle hypertrophy and muscle performance adaptations. In general, large muscle mass, multi-joint exercises will be performed using loads at or near repetition maximum (RM) loads (e.g., 15 RM, 8-10 RM, 4-6 RM). One to two minutes inter-set rests will be used depending on the relative load for the exercise. Examples of exercises include the following: back squat, bench press, lat pull-down, barbell lunges, leg extension and curl, bicep curls, standing shoulder press, pull-ups, and dips

10j. *Muscle biopsy* – The use of local anesthetics will result in a slight burning sensation, lasting approximately 5 seconds. There is an extremely low risk of allergic reactions to the local injection (1 in 1 million). There is a small chance of bleeding from the biopsy site. The principle concern would be prolonged bleeding which would produce a bruise in the area. This would extend the muscle soreness, but is adequately treated with rest, ice, a compression bandage, and keeping the leg elevated as much as possible. Nausea, dizziness, and fainting can occur in remote cases (1 in 100) during the biopsy process. As a result, the subjects will be in a supine position during the biopsy procedure. There is a minimal risk of infection (1 in 1000) and irritation associated with the biopsy procedure. The use of aseptic techniques and careful cleaning of the skin and keeping the area dry will minimize the risk of infection. There is a minimal risk of bruising (1 in 100) from the biopsy procedure. This will be minimized by placing ice over the site following the procedure and by applying a compression bandage on the site for the 24 hours following the biopsy. In rare instances (1 in 200), some motor nerves may be damaged which may cause local muscle atrophy (decrease in the size of muscle fibers, with a small dimple on the skin). There is likely to be a small scar (½-inch in length) where the incision for the biopsy is performed. This scar usually dissipates over a period of 6-12 months at which time the scaring is very modest.

Blood Draws – The blood draw has a small risk of infection and bruising of the area, but this is rare. The needle stick for the blood draw will be mildly painful and only lasts a couple of seconds.

DEXA Scan- The DEXA scan uses an FDA-approved X-ray absortiometry machine to examine body composition (relative amounts of fat mass and lean body mass). During this test the subject will be asked to lie face up, on a padded table, motionless for 7-15 minutes while the scanner arm of the DEXA machine passes over the entire body. The DEXA scan information is being used solely for descriptive and comparison purposes and to assess the accuracy of various modes of body composition assessment. This information is not intended to be used as a clinical assessment of any medical conditions. The Applied Physiology Laboratory complies with the University of Kansas philosophy of safety that all exposures to radiation, other hazardous materials, and risks from physical hazards shall be kept "as low as reasonably achievable." The amount of radiation that the subject has received in the past year will be evaluated and they will be asked to let us know if they have had any other X-rays within the last year. If they have recently undergone CT (Computed Tomography), PET, fluoroscopic, or nuclear medicine studies within the past year, they will be excluded from the study. The amount of radiation that each subject will receive from a whole body scan is the equivalent to a uniform whole-body exposure of 0.1 mrem. The DEXA measurements will be made by trained personnel who have either received accreditation from the International Society for Clinical Densiometry for diagnostic procedures, or who have received formal training by the manufacturer following installation of the DEXA for femur/spine/total body scans.

Strength Testing and Training Exercises– The strength training exercises may cause delayed onset muscle soreness. This soreness is temporary and is a normal result of this type of contraction. The subjects will be informed to expect muscle soreness to peak between 24-48 hours and dissipate completely 96 hours following the exercise sessions.

Supplementation-

Placebo – This drink will consist of a low-glycemic flavor and color matched drink. The drink will be sweetened with polydextrin and/or mannitol. There are no known risks to consuming a beverage of this sort except in those who have rare food allergies to the artificial colors or flavoring ingredients. No information on the occurrences rate are estimated to be less than 1 per 100 people, and lower in adults than children. Persons known to be allergic to one of the ingredients will be excluded from the study

The Experimental Supplement- The risks associated with each ingredient in the experimental pre-exercise supplement are listed in the provided attachment. Safety information for caffeine in doses similar to this are also provided in that document. **Questionnaires will be provided to all subjects at each exercise session to record any adverse effects (jitters, upset stomach, etc.) they might have as a result of taking the placebo or supplement. If they experience any problems that they feel might be related to the placebo or supplement after leaving the training session they will be asked to report these to lab personnel immediately.. In**

addition, blood pressure will be monitored once each week prior to supplementation in order to ensure the subject is not having an adverse reaction to the caffeine in the supplement. These questionnaires and blood pressure measures will be reviewed by the safety monitoring board.

None of the investigators in this study have consulting agreements, ownership interests or receive personal benefit from Labrada Nutrition Inc. Labrada Nutrition Inc. has not placed any restrictions on the publication of the effects of this supplement.

10k. During the preliminary meeting subjects will be asked to complete medical history, physical activity, and biopsy screening questionnaires to determine if they are eligible for participation in this study. At the beginning and end of the study, all participants will complete body image, effort and enjoyment of training, and happiness surveys. All questionnaires are attached. All documents will be stored in a secured file in the Applied Physiology Laboratory. **All subject contact information will be saved following the conclusion of the study in the event Labrada Nutrition implements a product recall.**

10m. Informed consent will be completed and obtained by each subject for all procedures in accordance with the Human Subject Committee – Lawrence of the University of Kansas. The consent form is attached.

10n. As discussed in 10k, medical history, biopsy screening, and activity questionnaires will be used to gain information concerning the individuals past and present health and activity levels. In addition body image, effort and enjoyment of training, and happiness surveys will be completed by participants at the beginning and end of the study.

Effects of Resistance Exercise and a Pre-Workout Dietary Supplement on Physiological Adaptations

Exclusionary Criteria (in no particular order)

- Non-male
- Under the age of 18
- Over the age of 35
- Does not meet activity requirements (exercise 3 or more days per week for at least 1 year)
- Obese (BMI <28 kg/m^2)

- Smoker
- Has adverse effects to caffeine (including but not limited to dizziness, lightheadedness, nausea, 'jitters')
- Uses anti-inflammatory medications or over-the counter drugs and cannot discontinue for the course of the study
- Uses other nutritional supplements and will not discontinue for the course of the study
- Takes other medications that may interact with ingredients in the placebo and/or supplement (including but not limited to anti-asthmatic medications, anti-hypertensive medications, blood thinners, anti-seizure medications, anti-anxiety medications, monoamine oxidase inhibitors-MAOIs)
- History of any of the following: myocardial infarction, angiography, coronary surgery, chest discomfort, high blood pressure, low blood pressure, shortness of breath upon light exertion, pulmonary disease, dizziness upon light exertion, heart palpitation, heart murmur, diabetes (I or II), circulation problems, stomach ulcers, kidney problems, metal implants, seizures
- History of allergies to local anesthetics
- History of allergies to iodine
- History of allergies to Band-Aids or other adhesives
- History of food allergies (including but not limited to ingredients in the placebo such as polydextrin or mannitol, or ingredients in the supplement such as caffeine, tyrosine, citrulline, malate, arginine, taurine, creatine, betaine, picamilon, niacin, beta-alanine, glutamine, histidine, vitamin A or vitamin E)

Submit one complete application and supporting documents with your application. Supporting documents may include consent forms, information statement, oral consent procedures, assent procedures, questionnaires/ surveys/research measures, advertisements recruiting participants e.g. flyers, classified ads, debriefing procedures. You may send all materials via email attachment to dhann@ku.edu; Campus Mail to HSCL Youngberg Hall; or U.S. Mail to HSCL, Youngberg Hall, 2385 Irving Hill Road, Lawrence, KS 66045-7563.

HSRC REQUESTED ADDENDUM TO THE APPLICATION

Supplement and Placebo Ingredient Explanation

Placebo:

The placebo drink will consist of a low-glycemic flavor and color matched drink. The drink will be sweetened with polydextrin and/or mannitol. There are no known risks to consuming a beverage of this sort except in those who have rare food allergies to the artificial colors or flavoring ingredients. No information on the occurrences rate are estimated to be less than 1 per 100 people, and lower in adults than children. Persons known to be allergic to one of the ingredients will be excluded from the study. The health history questionnaire asks potential subjects to identify food allergies so that these individuals may be excluded from the study.

Supplement:

Caffeine. The experimental exercise supplement contains 450 mg of caffeine. The US Food and Drug Administration (FDA) has carefully reviewed and deemed caffeine safe and effective. In 1958, caffeine was placed on the Food and Drug Administration's generally recognized as safe (GRAS) list. The FDA does regulate the amount of caffeine in cola and 'pepper' sodas, but not in other foods or drinks.

Caffeine is a known stimulant and diuretic, though its actions as a diuretic have recently been questioned in scientific literature. Typical positive effects of caffeine ingestion include increased mental concentration, and increased physical work capacity. Ingestion of caffeine can also cause 'jitters', nervousness, and dizziness or light headedness, and palpitations. These symptoms increase with the amount of caffeine consumed. Other common risks of excessive caffeine consumption especially in caffeine sensitive individuals include elevated heart rate, anxiety/panic disorders, and heart arrhythmias. Caffeine tolerance is highly individualized (A. Graham, Schultz, Mayo-Smith, Ries, & Wilford, 2003). Some individuals experience negative side effects with even small amounts of caffeine ingested (40mg), while others tolerate doses over 500mg without negative side effects. Caffeine sensitivity is decreased in habitual caffeine consumers, such as persons who drink coffee or tea daily, but individuals may experience symptoms of caffeine if they consume more than their usual amount of caffeine. **Body size is also be associated with greater caffeine tolerance such that caffeine consumption is often expressed relative to body size.** Because the amount of caffeine that causes sensitivities is highly individualized, there is no calculatable risk level for these side effects. We will be using the Caffeine Questionnaire to attempt to determine individuals who would be overtly sensitive to the quantity of caffeine in the study and exclude them from the study.

One cup of brewed or drip coffee has between 80 and 175mg of caffeine per 8oz depending on the type of bean, roast, and method of processing,100mg per 8oz is considered the average amount. A typical restaurant (McDonalds) serving of coffee is 12 or 16oz. and the typical home coffee mug is 11oz, but travel mugs are typically 16oz. The 20oz VENTI® size coffee also sold at Starbucks® has 415mg of caffeine. Nearly the same as in this supplement. These are the values of the regular brewed coffees without 'espresso shots' or the other coffee drinks like Café Americano®. (http://www.starbucks.com/retail/nutrition_beverage_detail.asp Thus this supplement

has caffeine equivalent to 4.5-8oz servings of coffee, or 3.75 McDonalds small coffees, or 2.25 Large Coffees.

A review of caffeine in sports performance has also been sent to you as a separate document (L. M. Burke, 2008). Caffeine is no longer considered an illegal performance enhancer by the World Anti-Doping Association (monitor Olympic and International Sports) because they deemed caffeine not to be dangerous in quantities that would increase sports performance (L. M. Burke, 2008). Additionally, many studies on caffeine have been performed using the standard research doses of 2-6mg/kg bodyweight (a table of studies provided in the article(L. M. Burke, 2008)). Other evidence suggests doses of caffeine up to 13mg/kg will improve athletic performance, (Sokmen et al., 2008). In the above information, I addressed the fact that habitual caffeine users are less sensitive (A. Graham et al., 2003) . Two specific studies have addressed the ergogenic benefits in non-habituated athletes ((Jacobson, Weber, Claypool, & Hunt, 1992; Woolf, Bidwell, & Carlson, 2009). The first study, (Jacobson et al., 1992) examine caffeine doses of 7mg/kg in individuals consuming less than 100mg of caffeine per day and Woolf et al used a dose of 5mg/kg in individuals with less than 50mg caffeine per day in their diet. Jackobson reported "no side effects" while in Woolf et al. some subjects reported jitters, but no other ill effects. I would like to point out once again that these studies utilized dosages at more than 1.5 times greater than we are proposing in subjects that are similarly or less caffeine habituated than the subjects we will use in our study.

We understand that caffeine can have serious side effects particularly in sensitive individuals. In an attempt to prevent the negative caffeine related side effects in our subjects they will answer several questions concerning past and present caffeine consumption. Subjects will be included in this study if they have previously consumed moderate to large amounts of caffeine (300mg/day or more) without experiencing any excessively uncomfortable negative side effects. We also ask our subjects to refrain from excessive outside caffeine consumption during the study. Our informed consent document warns subjects of typical caffeine side effects and asks them to report any supplement side effects to us. A person reporting uncomfortable effects can thus be identified and removed from the study via the process described in the IRB application

L-Tyrosine. The supplement contains 500 mg of tyrosine. Tyrosine is a nonessential amino acid (meaning the body is capable of producing it from other nutrients) that is made from phenylalanine, an essential amino acid. Tyrosine is a precursor for dopamine and norepinephrine and has been associated with improved alertness, regulate appetite, reduced body fat and an antidepressant effect. Tyrosine is commonly found in meats and dairy products. A maximum safe dose has not be established for tyrosine, but it is suggested that a person should not consume more than 12,000 mg/day. Typical tyrosine supplements sold over the counter contain 500-1500mg/dose. Thus, this supplement contains less than a typical tyrosine supplement. Overconsumption of tyrosine (which is unforeseeable in this study) can cause gastric upset, diarrhea, and migraine headaches.

L-Citrulline-di-malate. The supplement contains 1000 mg of L-citrulline-di-malate. Citrulline is a nonessential amino acid formed from the essential amino acid lysine. Citrulline plays a role in the removal of ammonia from the blood via the urea cycle. No clear benefit of citrulline supplementation has been determined at this time. No dosage recommendations currently exist for this supplement. However, a published research study reported that 6g/day for 15 days of citrulline supplementation did not cause any adverse side effects in men. Because of the small amount in this product, we do not anticipate you will experience any side effects due to this ingredient.

Arginine Alpha-ketogluterate. This supplement contains 1742 mg of arginine alpha-ketogluterate. This is another naturally occurring amino acid derivative. Arginine is associated with improved growth hormone production leading to reduced body fat and increased muscle mass, improved creatine production, and ammonia detoxification. Previous studies have found that supplementation with 6,000-15,000 mg/day of arginine alpha-ketoluterate in healthy young men was well tolerated and did not produce negative side effects. Some have suggested a common dose for arginine is 2,000-3,000 mg taken by mouth three times per day. Because of the combined amount of ingredients that are variations of arginine in this product (arginine alpha-ketogluterate and di-arginine malate) is lower than doses used in previous studies, we do not anticipate any risks to due to arginine. Potential side effects associated with high doses of arginine include stomach discomfort, including nausea, stomach cramps, or an increased number of stools were common. Other potential side effects include low blood pressure and changes in numerous chemicals and electrolytes in the blood. Examples include high potassium, high chloride, low sodium, low phosphate, high blood urea nitrogen, and high creatinine levels.

Taurine. This supplement contains 1,000mg of taurine. Taurine is an amino acid derivative found naturally in protein rich foods. This nonessential amino acid is associated with the transport of electrolytes within the cell as well as with blood pressure regulation. The recommended amount of taurine supplementation (beyond food sources) should not exceed 3,000mg per day. Because of the small amount of taurine in this product, we do not anticipate any risks to you due to this ingredient.

Di-arginine malate. The supplement contains 2242 mg per serving of di-arginine malate. This is another form of the amino acid arginine. Arginine is associated with improved growth hormone production leading to reduced body fat and increased muscle mass, improved creatine production, and ammonia detoxification. Previous studies have found that supplementation with 6,000-15,000 mg/day of arginine alpha-ketoluterate in healthy young men was well tolerated and did not produce negative side effects. Some have suggested a common dose for arginine is 2,000-3,000 mg taken by mouth three times per day. Because of the combined amount of ingredients that are variations of arginine in this product (arginine alpha-ketogluterate and di-arginine malate) is lower than doses used in previous studies, we do not anticipate any risks to due to arginine. stomach discomfort, including nausea, stomach cramps, or an increased number of stools were common. Other potential side effects include low blood pressure and changes in numerous chemicals and electrolytes in the blood. Examples include high potassium, high chloride, low sodium, low phosphate, high blood urea nitrogen, and high creatinine levels.

Creatine monohydrate. The supplement contains 2750 mg of creatine monohydrate. Creatine is a substance that is produced naturally in the body and stored in the musculature of animals to contribute to the production of energy. Creatine monohydrate is the most common supplemental form of creatine. Research has shown that creatine monohydrate supplementation is associated with improved muscle strength and power, increases in lean body mass, faster recovery between high intensity exercise bouts, and reduced muscular fatigue. Doses of creatine used to improve training intensity and recovery range from 1000 to 24,000 mg per day. Some individuals have used as much as 80 to 160 mg per pound of lean body weight (10,000 to 24,000 mg total). Generally creatine is used regularly for time periods of two to three months. Large doses of creatine may cause dehydration and gastrointestinal distress. However, because the combined dose of creatine monohydrate and di-creatine malate is at the lower end of the typical creatine dose, few side effects are anticipated. No adverse long-term effects have been reported from long-term clinical trials using creatine monohydrate.

Betaine anhydrous. The supplement contains 1000 mg of betaine anhydrous. Betaine consumption has been associated with increased creatine consumption in animals. While studies have examined exercise performance changes following betaine supplementation, none have examined if changes occur in creatine concentration in humans. No recommended doses of betaine have been found, however a previous study using betain supplementation in humans involved consumption of 1,250 mg of betaine twice per day for fifteen days.

Di-creatine malate. This supplement contains 125 mg of di-creatine malate, a form of creatine that is bound to malic acid which is also involved in energy production. As previously mentioned large doses of creatine may cause dehydration and gastrointestinal distress. However, because the combined dose of creatine monohydrate and di-creatine malate is at the lower end of the typical creatine dose, few side effects are anticipated.

Picamilon. This supplement used in this study contains 50 mg of picamilon. Picamilon is a combination of niacin with γ-Aminobutyric acid (GABA), an inhibitory neurotransmitter in the central nervous system. Picamilon is naturally occuring found in particularly high quantities in makerel and wheat bran, but also in walnuts and spinach. During digestion niacin and GABA are separated and act independently on the body. Niacin (common vitamin) acts as a vasodilator, allowing for relaxation of the smooth muscle that surrounds the vasculature. Niacin doses of 1500-6,000 mg/day have been shown to induce side effects including skin flushing and itching, dry skin, as well as gastrointestinal complaints. Issues such as liver toxicity, hyperglycemia, orthostasis, and cardiac arrhythmias have also been reported in higher doses. The dose of picaminion in this supplement is unlikely to cause any side effects except possibly a niacin induced flushing. GABA could activate its receptors and allow for decreased CNS activity, increasing mental focus and increasing sleepiness. Occasionally other side effects such as anxiety and headache have been reported as well. There are no dietary recommendations for GABA, but it is found in large quantities in organ meats like liver, kidney and brain. Dietary supplements sold in the US contain 50-750mg of GABA. A tolerable limit set in some nutrition texts is 500mg/day. With 59mg of picomilon in this suplement there appears to be no risk.

Beta-Alanine. This supplement contains 750 mg of beta-alanine, a naturally occuring amino acid derivative of the amino acid alanine. Beta-alanine has been shown to decrease fatigue and increase work production in humans. No side effects have been reported for beta alanine doses of up to 800 mg per day.

N-Acetyl-L-Glutamine. This supplement contains 500 mg of N-Acetyl-L-Glutamine. L-glutamine has been shown to neutralize cortisol, a hormone that is released during high intensity exercise. This could allow for more efficient muscle growth as well as aid in the exercise recovery process. Glutamine is also essential for proper brain functioning as well as the immune system. Glutamine supplementation doses have ranged from 500 to over 20,000 mg per day. Due to the small amount of this ingredient in the supplement, no side effects are anticipated due to this ingredient.

L-Histidine. This supplement contains 125 mg of L-histidine. It has been suggested that this amino acid is essential to the growth and repair of tissue which is very important for those participating in high intensity activities. Additionally, histidine has been used as a treatment for arthritis due to its anti-inflammatory effects. Exact benefits of the long-term usage of this supplement by athletes are unclear. Typical supplemental doses of L-histidine range from 1,000 to 5,000 mg per day. Due to the

small amount of this ingredient in the supplement, no side effects are anticipated due to this ingredient. (E. R. Burke & Gastelu, 1999)

Vitamin A. The experimental supplement contains 4600 IU of Vitamin A. The recommended daily allowance of Vitamin A is 3,000 IU for males over the age of nineteen. The tolerable upper limit intake level for Vitamin A is 10,000 UL. Recent research has suggested acute toxicity related to this vitamin can occur at doses of 25,000 IU/kg of body weight. Chronic toxicity can also occur if high doses of this vitamin are taken on a regular basis. It has been shown that this will occur when taking 4,000 IU/kg of body weight daily for 6–15 months. While the amount of Vitamin A in this supplement is higher than the RDA it is also lower than the upper intake level as well as the levels shown to cause acute or chronic toxicity. However, individuals with renal problems will not be included in this study as a precaution as high doses of Vitamin A place a large stress on the kidneys.

Vitamin E. The experimental supplement contains 8 IU of Vitamin E. The recommended daily allowance (RDA) of this vitamin is 22.4 IU for males over the age of fourteen. This being considered we do not see Vitamin E toxicity as a risk for this study considering the relatively low dose included in the supplement.

References

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All other information (not directly cited above) was taken from Burke & Gastelu (1999), which is reference 6 above.

APPENDIX F: INFORMED CONSENT DOCUMENT: THE EFFECTS OF RESISTANCE EXERICSE AND A PRE-WORKOUT DIETARY SUPPLEMENT ON ERK AND P38 PHORPHORYLATION

Effects of Resistance Exercise and a Pre-Workout Dietary Supplement on Physiological Adaptations

Informed Consent

INTRODUCTION

You are invited to participate in a research study examining the effectiveness of a pre-workout dietary supplement combined with resistance exercise on various physiological variables. Thirty subjects are being sought to participate in this study at the University of Kansas. The Department of Health, Sports, and Exercise Sciences at the University of Kansas supports the practice of protection for human subjects participating in research. The following information is provided to help you make an informed decision on whether or not to participate in the present study. Please feel free to ask any questions. This study was requested and is funded by Labrada Nutrition Inc.

PURPOSE OF THE STUDY

The purpose of this study is to examine the effectiveness of a pre-workout dietary supplement combined with an 8-week resistance training program on physiological measures. This supplement contains large amounts of caffeine (450mg); the equivalent of 4.5 cups of coffee. The supplement is not currently commercially available and is not approved by the Food and Drug administration (FDA), as no supplements are approved by the FDA. Aside from examining the effectiveness of the supplement, the resistance training component of this study could provide information concerning the role of resistance training in preventing and treating various disease states associated with inflammation and muscle wasting. The physiological variables that will be measured include body weight, body composition, strength and power, as well as blood and muscle markers of skeletal muscle growth (hypertrophy) and the inflammatory response to training. Psychological variables of interest include body image, effort and enjoyment of training, and happiness from pre- to post-study. The findings of this study will determine if the use of this pre-workout supplement in addition to an 8-week training plan results in changes in weight, lean body mass, muscular strength and hypertrophy that are different from the changes in these variables that result from the 8-week training plan alone.

BASIS FOR SUBJECT SELECTION

In order to participate in this study you must be male, between the ages of 18 and 35, healthy, non-obese (BMI <28 kg/m^2), non-smoking, and free of metabolic cardiovascular, kidney diseases, as well as free of a history of seizures. You will be screened for participation using a health-history questionnaire for contraindications to exercise by American College of Sports Medicine (ACSM) guidelines. If you are currently taking any medications or supplements that may interact with ingredients in the supplement/placebo involved in this study you will be excluded from the study. In

order to participate in this study you must be recreationally trained (exercise 3 or more days / week for at least 1 year) in order to mimic those who are generally more likely to use a supplement of this kind. In addition, if you currently use drugs that reduce inflammation (aspirin, ibuprofin, NSAIDs, etc.) on a regular basis you will be excluded from this investigation. You will also be asked to refrain from using these drugs during the course of this study. Due to gender differences in hormonal fluctuations and responses to resistance training only males will be recruited.

PROCEDURES

A time-line of the testing procedures and an overview of the training program for the present study are presented below. All procedures will be conducted in the Applied Physiology Laboratory (APL) at the University of Kansas and will be supervised by trained personnel. It is important for you to note that this study will require your presence in the Applied Physiology Laboratory for three exercise sessions per week (approximately five hours per week) for eight weeks, as well as two testing sessions for one week prior to the start of the training program (approximately 2.5 hours).

Timeline of Procedures for Control and Experimental Groups:

Pre-Screening:

All subjects

Health History Questionnaire
Pre-Biopsy Screening Questionnaire
Physical Activity Questionnaire
Caffeine Intake and Tolerance Questionnaire
Body image, effort and enjoyment of training, and happiness surveys
Body Weight, Anthropometric Tests, Body Composition Testing using DEXA Scan

Testing Week 0, Day 1:

1-Repetition Maximum (RM) on all exercises in the training protocol

Testing Week 0, Day 2 (3 days after Testing Day 1):

Power Testing for Bench Press and Back Squat (70% 1-RM); Vertical Jump Test
Rate of Force Development Testing Using an Isometric Bench Press

Training Week 1, Day 1:

Report to the APL following an 8 hour fast
Turn in 3-day dietary recall
30 Minute Rest
Blood Draw #1, Biopsy #1
Drink Supplement/Placebo
Exercise Session
15 Minute Rest
Blood Draw #2, Biopsy #2

Training Week 5, Day 1:

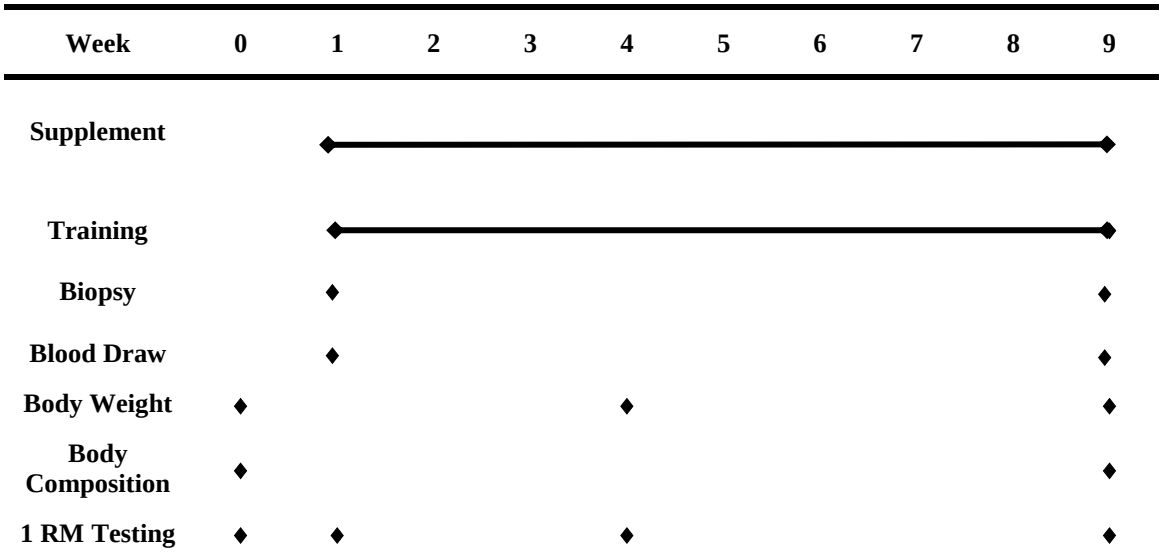
Body Weight Testing
Strength Testing (1-RM) on all exercises in the training protocol will be substituted for normal workout.

Training Week 5, Day 2:
Power testing for bench press and back squat (70% 1-RM); vertical jump test
Rate of force development testing using an isometric bench press
These tests will be incorporated with the bench press and squat sessions within the normal workout.

Training Week 8, Day 1:
Strength Testing (1-RM) on all exercises in the training protocol will be substituted for normal workout.

Training Week 8, Day 2:
Power Testing for bench press and back squat (70% 1-RM); vertical jump test
Rate of force development testing using an isometric bench press
These tests will be incorporated with the bench press and squat sessions within the normal workout.
Body image, effort and enjoyment of training, and happiness surveys

Training Week 8, Day 3:
Report to the APL following an 8 hour fast
Turn in 3-day dietary recall
30 Minute Rest
Blood Draw #3, Biopsy #3
Drink Supplement/Placebo
Exercise Session
15 Minute Rest
Blood Draw #4, Biopsy #4
DEXA Scan



Power Testing	♦	♦			♦					♦
Rate of Force Development	♦	♦			♦					♦
Psych Surveys	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦
Supplement Side Effect Survey	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦

1) Pre-testing Protocol – Health history, physical activity, **caffeine intake and tolerance**, body image, effort and enjoyment of training, and happiness, and pre-biopsy questionnaires, and anthropometric data will be obtained approximately 1 week prior to Testing Day 1. These procedures will take approximately 1 hour. The DEXA scan uses an FDA-approved X-ray absortiometry machine to examine body composition (relative amounts of fat mass and lean body mass). During this test you will be asked to lie face up and motionless on a padded table for 7-15 minutes while the scanner arm of the DEXA machine passes over your entire body. The scanner will not enclose or touch you, and you can wear your regular clothing (no metal or jewelry allowed). The DEXA scan information is being used solely for descriptive and comparison purposes. This information is not intended to be used as a clinical assessment of any medical conditions.

2) Exercise Testing - All strength testing will be done using free weights or weight machines and following standard repetition maximum strength testing guidelines. You will be asked to warm-up for five minutes using a stationary leg cycle. Following this you will perform warm-up sets followed by maximal effort lifts for each of the following exercises: back squat, bench press, lat pull-down, barbell lunges, leg extension and curl, bicep curls, standing shoulder press, pull-ups, and dips. Three days later you will return to the lab and perform power tests for the bench press and the back squat. This will involve a warm-up, followed by a set of 10 repetitions at 70% of your bench press and back squat maximum. Following this you will perform a rate of force development test for the bench press and leg extension exercises. Force transducers will be placed in-series with the resistance cable to detect isometric force for these exercises. For this test you will begin to perform the exercise as normal by generating force to move the weight, however the weight will be fixed and will not move. Following this test you will perform three maximal vertical jumps with approximately 3 minutes of rest between each. This will allow for the calculation of lower body power.

3) Dietary Recall – All subjects will be asked to record food and drink consumption (including portion sizes) for three consecutive days prior to their first (training week 1, day 1) and last muscle biopsies (training week 8, day 3). Subjects will be given an explanation of portion sizes prior to completion of the dietary recall.

4) Supplementation – All subjects will be asked to consume either a supplement or placebo drink 15 minutes prior to each exercise session. The supplement/placebo mix containers will be labeled as either A or B, however the experiment will be double blind in nature as neither the research staff or the subjects will know which drink is the placebo and which is the supplement. An individual associated with the Applied Physiology Lab, but not directly involved in this research project, will be assigned the duty of randomizing subjects into placebo and supplement groups. They will also be responsible for

mixing the supplement drinks, and monitoring the subjects as they consume the supplement. As this experiment is double blind, no other individuals associated with the study (research staff or subjects) will know which mix is the supplement and which is the placebo until after the study is completed. Questionnaires will be provided to you at each exercise session to record any adverse effects you might have as a result of taking the placebo or supplement.

5) Exercise Training – All subjects will report to the lab for training three times per week for a period of eight weeks. These training sessions will be supervised by Applied Physiology Laboratory personnel. All subjects will complete an exercise program consisting of the following exercises: back squat, bench press, lat pull-down, barbell lunges, leg extension and curl, bicep curls, standing shoulder press, pull-ups, and dips. The training program will be relative to each subject and exercise will be prescribed as a percentage of their 1-RM on each exercise. We will also ask you to fill out a pre/post exercise survey to asses your anticipation of training, recovery, strength, and muscle pain. These surveys will take less **than** 5 minutes to complete. Subjects are asked to refrain from exercise other than that which is prescribed by this study and takes place during the assigned lab training hours.

6) Muscle Biopsies – You have been informed that one of the purposes of this study is to measure the cellular markers of muscle growth and inflammation from the muscle samples that we collect. By obtaining a small sample of your muscle tissue (size of a pencil eraser or small finger nail), the different types of proteins in your muscle may be determined which will be helpful in the evaluation of health and exercise performance. All muscle tissue samples (biopsies) will be taken from the outer portion of the front of the thigh using a needle biopsy technique by either Philip Gallagher or Andy Fry. Philip Gallagher, PhD., Assistant Professor of HSES, and Andy Fry, PhD., Professor of HSES, have performed over 200 muscle biopsies over the past year and have assisted on over 1000 biopsies over the past six years on various populations (athletes, sedentary people, elderly etc.) with no significant complications and nothing more than minimal adverse reactions. The procedure is being overseen by Jeff Burns, M.D., who is a medical doctor in Neurology at the KU Medical Center in Kansas City, Kansas. Dr. Burns supervises the procedure, but will not be physically present for the biopsies. The total size of the muscle biopsy will be approximately the size of a pea. You will be placed on an examination table lying down on your back (supine) so that the muscles of the leg are relaxed. The skin will be thoroughly cleaned with antiseptic solution (Betadine) using sterile cotton swabs after which a surgical cover will be placed around the sampling site. If you are allergic to Betadine, an alternative antiseptic solution will be used to clean the skin. A small amount (2ml or 2cc) of a local aesthetic (2% Lidocaine) will be injected into the tissue under the skin around the site to be sampled. During this injection you may feel a slight burning sensation. If you are allergic to the local anesthetic, or have had allergic reactions to other anesthetics (i.e.: Novocain) then you will be disqualified from the study. Following the injection of the numbing agent into your thigh, a minimum of five (5) minutes will be allowed to pass to ensure adequate time for the agent to take effect in the

area where the incision will be made. A small incision (1 cm) will then be made in the skin overlying the muscle and the biopsy needle inserted into the middle of the muscle (muscle belly) at a depth of 3 cm (about 1 inch). During the time that the sample is being taken (about 5 seconds) you may feel some deep pressure and cramping that will be moderately painful. You have been informed that if you have been previously diagnosed as having a bleeding disorder, a blood clotting problem, take blood thinning medication or have sensitivity to scarring, you will tell the researcher and not participate in the biopsy procedure.

Following the biopsy procedure, firm and constant pressure will be placed on the wound to stop any bleeding. The incision site will be closed with a Steri-Strip and covered with a large Band-Aid and the site compressed using a 10 cm strip of sterile elastic surgical stocking for a period of 24-hrs. You have been informed that the procedure will take about 20 min.

Sterile disposable instruments and sterile gloves will be used for the preparation of the site and the reusable biopsy needle will be thoroughly sterilized (via steam and heat autoclave) after each biopsy. Approximately 100 mg (size of a pencil eraser or small finger nail) of skeletal muscle tissue will be removed. With the invasive skeletal muscle biopsy technique there is the possibility of a blood related infection (HIV, Hepatitis B), but the reusable needles will be cleaned and sterilized using an autoclave, a generally accepted method of cleaning surgical instruments.

A total of **four (4)** biopsies will be performed over the course of the study (see timeline, above).

7) Blood Draws – We will measure hormonal markers as well as levels of inflammation found in your blood prior to and following the first and last exercise bouts. Four blood draws will be performed during this study (see timeline, above). In this procedure, a small amount of blood will be taken from a vein for each blood draw by a trained phlebotomist using a needle and a syringe. Approximately 10 cc of blood (~ 2 teaspoons) will be drawn for each time-point. The blood draw will take about 5 minutes.

RISKS

1) DEXA scan – The examination of fat and lean body mass using the DEXA scan involves the use of X-rays. Any time an individual is exposed to radiation there is a potential risk. The University of Kansas has adopted the philosophy of safety that all exposures to radiation, other hazardous materials, and risks from physical hazards shall be kept "as low as reasonably achievable." There are certain limitations placed upon this procedure to achieve that aim. We also want to evaluate the amount of radiation that you have received in the past year, so if you have had other X-rays, let us know. If you have recently undergone CT (Computed Tomography), PET, fluoroscopic, or nuclear medicine studies within the past year, you cannot participate in the study. The parts of your body that will receive the

most radiation are the skin, although your whole body will be exposed to the radiation. The amount of radiation that you will receive from a whole body scan is the equivalent to a uniform whole-body exposure of 0.1 mrem. Although you will be receiving a small amount of exposure, the risk from radiation exposure of this magnitude is too small to be measured directly and is considered to be negligible when compared with other everyday risks. For example, you would receive radiation exposure of approximately 80 mrem on a transatlantic airline flight of 8 hours, or 30-40 mrem during a typical chest x-ray. The Radiation Safety Officer at the University of Kansas can provide you with more information about radiation exposure if you are interested. The DEXA measurements are being made by trained personnel who have either received accreditation from the International Society for Clinical Densiometry for diagnostic procedures, or who have received formal training by the manufacturer following installation of the DEXA for femur/spine/total body scans. The system is equipped with a Class II Laser device. A Class II rating indicates a low power visible laser that is not normally hazardous to eyesight but has the potential to be hazardous if viewed directly for an extended period of time. Because of the potential hazard DO NOT stare directly into the beam while the laser is in operation. The beam should not be pointed directly in the eye of the participant.

2) Muscle biopsies – The use of local anesthetics will result in a slight burning sensation, lasting approximately 5 seconds. There is a risk of allergic reactions to the local injection (1 in 1 million). There is a small chance of bleeding from the biopsy site. The principle concern would be prolonged bleeding which would produce a bruise in the area. This would extend the muscle soreness, but is adequately treated with rest, ice, a compression bandage, and keeping the leg elevated as much as possible. Nausea, dizziness, and fainting can occur (1 in 100) during the biopsy process. As a result, the subjects will be in a supine position during the biopsy procedure. There is a risk of infection (1 in 1000) and irritation associated with the biopsy procedure. The use of aseptic techniques, careful cleaning of the skin and keeping the area dry will minimize the risk of infection. There is a risk of bruising (1 in 100) from the biopsy procedure. This will be minimized by placing ice over the site following the procedure and by applying a compression bandage on the site for the 24 hours following the biopsy. In some instances (1 in 200), some motor nerves may be damaged which may cause local muscle atrophy (decrease in the size of muscle fibers, with a small dimple on the skin). There is likely to be a small scar where the biopsy is performed. This scar usually dissipates over a period of 6-12 months at which time the scaring is very modest.

3) Blood Draws – The blood sample has a small risk of infection and bruising of the area. The needle stick for the blood draw will be mildly painful and only lasts a couple of seconds.

4) Supplement/Placebo—You will be asked to ingest a supplement or a placebo drink fifteen minutes before each training bout. This variation of the supplement is not currently available for commercial sale and is not approved by the Food and Drug Administration (FDA), as no supplements are approved by the FDA. The ingredients found in this supplement and information concerning the safety of their ingestion is listed below. In addition, blood pressure will be monitored once each week prior to supplementation in order to ensure your body is not having an adverse reaction to the caffeine in the supplement. These questionnaires and blood pressure measures will be reviewed by the safety monitoring board. Due to the possibility that other supplements may contain similar ingredients that might interact with ingredients in the study placebo/supplement and place you at risk or confound the results of this study, you are asked to refrain from using all other nutritional supplements or ergogenic aids during the course of this study.

Placebo:

The placebo drink will consist of a low-glycemic flavor and color matched drink sweetened with polydextrin and/or mannitol. Persons known to be allergic to one of the ingredients will be excluded from the study

Supplement:

Caffeine. The experimental exercise supplement contains 450 mg of caffeine. This amount is the equivalent of 4.5 eight-ounce cups of coffee. Caffeine is a known stimulant and diuretic. Side effects of caffeine ingestion can include increased mental concentration, increased physical work capacity, 'jitters', nervousness, and dizziness or light headedness. These symptoms increase with the amount of caffeine consumed so we ask that you refrain from consuming more than one additional serving of caffeinated beverages per day, (coffee, tea, energy drinks, colas) during the study. You will be included in this study if you have previously consumed moderate amounts of caffeine (300mg/day or more) without experiencing any uncomfortable negative side effects.

L-Tyrosine. The supplement contains 500 mg of L-tyrosine. Tyrosine a non-essential amino acid (meaning the body is capable of producing it from other nutrients) commonly found in meats and dairy products. Doses of 100 to several thousand mg of L-tyrosine are common. Overconsumption of tyrosine (which is unforeseeable in this study) can cause gastric upset, diarrhea, and migraine headaches.

L-Citrulline-di-malate. The supplement used in this study contains 1000 mg of L-citrulline-di-malate, an amino acid derivative. Previous research using citrulline in greater doses than the present study did not cause any adverse side effects in men, therefore we do not anticipate any risks to you due to this ingredient.

Arginine Alpha-ketogluterate. The supplement contains 1742 mg of arginine alpha-ketogluterate, another naturally occurring amino acid derivative. Previous research using arginine alpha-ketogluterate in greater doses than the present study did not cause any adverse side effects in men, therefore we do not anticipate any risks to you due to this ingredient.

Taurine. The supplement contains 1,000mg of taurine, an amino acid derivative found naturally in protein rich foods. The recommended amount of taurine supplementation (beyond food sources) should not exceed 3,000mg per day. Because of the small amount of taurine in this product, we do not anticipate any risks to you due to this ingredient.

Di-arginine malate. The supplement contains 2242 mg of di-arginine malate, a form of the amino acid arginine. Potential side effects include low blood pressure and changes in various chemicals and electrolytes in the blood. Examples include high potassium, high chloride, low sodium, low phosphate, high blood urea nitrogen, and high creatinine levels.

Creatine monohydrate. The supplement contains 2750 mg of creatine monohydrate, a substance that is produced naturally in the body and stored in the musculature of animals to contribute to the production of energy. Doses of creatine used to improve training intensity and recovery range from 1000 to 24,000 mg per day. Large doses of creatine may cause dehydration and gastrointestinal distress. However, because the combined dose of creatine monohydrate and di-creatine malate is at the lower end of the typical creatine dose, few side effects are anticipated.

Betaine anhydrous. The supplement contains 1000 mg of betaine which is associated with increased creatine consumption in animals. Previous studies using higher doses of betaine did not report side effects.

Di-creatine malate. This supplement contains 125 mg of di-creatine malate, a form of creatine that is bound to malic acid which is also involved in energy production. As previously mentioned large doses of creatine may cause dehydration and gastrointestinal distress. However, because the combined dose of creatine monohydrate and di-creatine malate is at the lower end of the typical creatine dose, few side effects are anticipated.

Picamilon. The supplement contains 50 mg of picamilon, a combination of niacin with γ-Aminobutyric acid (GABA), an inhibitory neurotransmitter in the central nervous system. Niacin acts as a vasodilator, allowing for relaxation of the smooth muscle that surrounds the vasculature. Because of the small amount of picamilon in this product we do not anticipate any risks to you due to this ingredient .

Beta-Alanine. This supplement contains 750 mg of beta-alanine, a naturally occuring amino acid derivative. Beta-alanine has been shown to decrease fatigue and increase work production in humans. No side effects have been reported for beta alanine doses of 800 mg per day.

N-Acetyl-L-Glutamine. This supplement contains 500 mg of N-Acetyl-L-Glutamine. L-glutamine has been shown to neutralize cortisol, a hormone that is released during high intensity exercise. This could allow for more efficient muscle growth as well as aid in the exercise recovery process. Glutamine supplementation doses have ranged from 500 to over 20,000 mg per day. Due to the small amount of this ingredient in the supplement, no side effects are anticipated due to this ingredient.

L-Histidine. This supplement contains 125 mg of L-histidine. It has been suggested that this amino acid is essential to the growth and repair of tissue. Typical supplemental doses of L-histidine range from 1,000 to 5,000 mg per day. Due to the small amount of this ingredient in the supplement, no side effects are anticipated due to this ingredient.

Vitamin A. The experimental supplement contains 4600 IU of Vitamin A. While the amount of Vitamin A in this supplement is higher than the RDA (3,000 IU) it is also lower than the upper intake level (10,000 IU) as well as the levels shown to cause acute or chronic toxicity (25,000 IU/kg). However, individuals with renal problems will not be included in this study as a precaution as high doses of Vitamin A place a large stress on the kidneys.

Vitamin E. The experimental supplement contains 8 IU of Vitamin E. The recommended daily allowance (RDA) of this vitamin is 22.4 IU for males over the age of fourteen. Because of the small amount of Vitamin E in this product we do not anticipate any risks to you due to this ingredient.

5) Exercise Testing and Training - During the strength testing sessions test there is a risk potential even though no health problems exist. The risk and discomforts that are associated with this type of test include muscle fatigue, lightheadedness, chest discomfort, and very rarely death. The potential for death during or immediately following the test (or any vigorous exercise) is approximately 0.5 per 10,000 tests, according to the American College of Sports Medicine. Following these tests however you will experience muscle soreness due to the eccentric component of the resistance exercise. This

soreness generally referred to as delayed on-set muscle soreness as it is most likely to occur in the days following the exercise bout. All exercise training will be prescribed relative to each subject and the principles of proper exercise progression and overload will be applied so as to reduce the risk of injury to the subject. Laboratory personnel will stay in contact with you after a testing/training session to ensure that you are comfortable. You will be given a 24 hr contact number for the Applied Physiology Laboratory personnel to convey any type of unusual discomfort.

In all of these procedures, care will be taken to employ "universal precautions" for the handling of blood and infectious materials to ensure your safety.

FOLLOW UP CARE

Following the procedure you will be provided with a biopsy care sheet, extra bandages and contact information for Philip Gallagher, Ph.D. After 24-hrs you must report back to the testing coordinator to check the wound. At this time the bandage will be removed and properly disposed of and a new sterile dressing placed over the wound. You will again be asked to report to the test coordinator after 3-days and be contacted via phone one week after the biopsy or whenever necessary to ensure normal recovery. The biopsy procedure often results in a small fine scar at the site of the incision, however, all care post-treatment will aid in reducing the potential for scarring. All care will be taken to aid in the healing of the wound. The entire biopsy procedure will be performed under sterile conditions. All testing staff and associated personnel will be trained in first aid and will be familiar with emergency procedures.

There have been no other major complications reported in the scientific literature as a result of taking small tissue samples from the skeletal muscle using the percutaneous needle biopsy technique described above. This procedure has been performed on numerous subjects by qualified personnel in many institutions worldwide with only slight discomfort being reported. During the muscle biopsy it is common to feel a strong cramping sensation in the muscle while the biopsy is being performed. However, muscle function is not impaired. In fact, subjects have been reported to continue participation in sporting events immediately following a muscle biopsy. It is common for subjects to experience mild soreness, moderate pain and bruising near the biopsy site, similar to a "Charlie-horse" the day after the procedure. In order to allow the incisions to heal properly and minimize the risk of infection, you should not get the biopsy site wet for 24-hours and avoid prolonged exposure to water for 4-days. Daily showers are acceptable (after the first 24-hours), but baths, swimming, sauna's etc. should be avoided for 4-days following the biopsy procedure.

BENEFITS

Over the course of this study you will receive information concerning your current body composition and fitness level as well as a personal exercise prescription. You will also have trained staff monitoring your exercise sessions and tracking your progress through the program. You will also gain an increased understanding of resistance training and program design. A copy of all personal data from the tests will be provided to you and your data will be completely explained to you by a member of the investigation team.

The results of this investigation will provide a greater knowledge of the influence of exercise training and use of this supplement on body composition, muscular strength and power, as well as blood and muscle markers of skeletal muscle growth (hypertrophy) and the inflammatory profile. These findings will not only have implications on those healthy individuals that are trying to gain muscle mass and lose body fat but also those whose focus is to improve muscular strength and power.

PAYMENT TO SUBJECTS

You will receive a $200 honorarium for your participation in this nine week study. Although not anticipated, if you need to discontinue the study due to an adverse event associated with the study, you will receive compensation. In the event that you do not complete the entire nine week study, the following payment plan has been devised: completion of ¼ of the study will result in a payment of $25; completion of ½ of the study will result in payment of $50; completion of over ½ of the study will result in full payment. Investigators will ask for your social security number in order to comply with federal and state tax and accounting regulations.

COMPENSATION FOR INJURY

The following information is provided in accordance with HEW regulations: "In the event of injury, the Kansas Tort Claims Act provides for compensation if it can be demonstrated that the injury was caused by the negligent or wrongful act or omission of a state employee acting within the scope of his/her employment."

IN CASE OF EMERGENCY CONTACT PROCEDURE
In the event of a research related injury or adverse reaction, please contact Philip Gallagher, Ph.D. at 785-864-0772 (office) or 785-550-6300 (cell), or the Applied Physiology Laboratory at 785-864-0773.

EMERGENCY CARE AND COMPENSATION IN CASE OF INJURY
In the unlikely event that any injury or illness occurs as a result of this research, the University of Kansas, their officers, agents, and employees, do not automatically provide reimbursement for medical care or other compensation. In cases of emergency, consistent with the Kansas Tort Claims Act, you would be responsible for payment of expenses related to treatments or associated with such complications except in a case where neglect can eventually be proven. You have been informed that payment for treatment of any injury or illness must be provided by you or your third-party payer, such as a health insurer. If any injury or illness occurs in the course of research, or for more information, you will notify the investigator in charge.

INFORMATION TO BE COLLECTED

To perform this study, researchers will collect information about you. This information will be obtained from the health history and physical activity questionnaires, muscle biopsy, and muscle function evaluation. Your name will not be associated in any way with the information or tissue collected from you or with the research findings from this study. The researchers will use a study identification number or initials in place of your name. Any data stored electronically will be stored on a password protected laboratory computer (not a laptop). Any paperwork associated with you will be stored in a locked file cabinet secured in the Applied Physiology Laboratory. All tissue collected will be stored securely in the Applied Physiology Laboratory. All tissue collected will be used to analyze markers of inflammation and protein synthesis.

Some persons or groups that receive your information may not be required to comply with the Health Insurance Portability and Accountability Act's privacy regulations, and your information may lose this federal protection if those persons or groups disclose it. The researchers will not share information about you with anyone outside of the Applied Physiology Laboratory personnel unless required by law or unless you give written permission.

Permission granted on this date to use and disclose your information remains in effect indefinitely. By signing this form you give permission for the use and disclosure of your information for the purposes of this study at any time in the future.

REFUSAL TO SIGN CONSENT AND AUTHORIZATION

You are not required to sign this Consent and Authorization form and you may refuse to do so without affecting your right to any services you are receiving or may receive from the University of Kansas or to participate in any programs or events of the University of Kansas. However, if you refuse to sign, you cannot participate in this study.

CANCELLING THIS CONSENT AND AUTHORIZATION

You may withdraw your consent to participate in this study at any time. You also have the right to cancel your permission to use and disclose information collected about you, in writing, at any time, by sending your written request to: Philip Gallagher, Ph.D., University of Kansas, 1301 Sunnyside Avenue, Robinson Center Room 101DJ, Lawrence, Kansas 66045. If you cancel permission to use your information, the researchers will stop collecting additional information about you. However, the research team may use and disclose information that was gathered before they received your cancellation, as described above.

PARTICIPANT CERTIFICATION

I have read this Consent and Authorization form. I have had the opportunity to ask, and I have received answers to, any questions I had regarding the study and the use and disclosure of information about me for the study. I understand that if I have any additional questions about this study I may call Prof. Philip Gallagher (785-864-0772) or e-mail: philku@ku.edu, Becky Kudrna, kudrna@ku.edu or Nicole Moodie nicolejg@ku.edu. I understand that if I have any additional questions about my rights as a research participant, I may call 785-864-7429 or write the Human Subjects Committee Lawrence Campus (HSCL), University of Kansas, 2385 Irving Hill Road, Lawrence, Kansas 66045-7563, email dhann@ku.edu.

I agree to take part in this study titled 'Effects of Resistance Exercise and a Pre-Workout Dietary Supplement on Physiological Adaptations' as a research participant. I further agree to the uses and disclosures of my information as described above. By my signature I affirm that I am at least 18 years old and that I have received a copy of this Consent and Authorization form.

_____ _____

Print Subject's Name Signature of subject

_____

Date

_____ _____

Print Name of Person Obtaining Consent Signature of Person Obtaining Consent

_____

Date

_____ _____

Print Name of Witness Signature of Witness

_____

Date

RESEARCHER CONTACT INFORMATION

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APPENDIX G: MEDICAL HISTORY FORM WITH CAFFEINE TOLLERANCE QUESTIONAIRE

APPLIED PHYSIOLOGY LABORATORY
UNIVERSITY OF KANSAS

MEDICAL HISTORY FORM WITH CAFFEINE TOLERANCE QUESTIONS

NAME:_____ DATE:_____

AGE: _____ HEIGHT: _____ WEIGHT: ___

A. Have you ever experienced any of the following conditions or procedures?

1. Myocardial Infarction	YES	NO
2. Angiography	YES	NO
3. Coronary Surgery	YES	NO
4. Chest Discomfort	YES	NO
5. Hypertension (high blood pressure)	YES	NO\
6. Hypotension (low blood pressure)	YES	NO
Systolic ≤ 100mmHg or Diastolic ≤ 60mmHg		
7. Shortness of breath upon light exertion	YES	NO
8. Dizziness upon light exertion	YES	NO
9. Pulmonary disease	YES	NO
10. Heart palpitation	YES	NO
11. Heart murmur	YES	NO
12. Diabetes	YES	NO
If "YES", Type I or Type II		

13. Extremity discomfort YES NO

14. Claudication (circulation problems cause leg pain) YES NO

15. Peptic Ulcers (stomach ulcer) YES NO

16. Kidney problems YES NO

17. Metal implants (including pins) YES NO

18. Seizures YES NO

19. Have you been told by a doctor that is it not safe or appropriate to exercise? YES NO

20. Does anyone in your family have a history of cardiovascular disease? YES NO

If "YES", who? _____

21. Do you smoke? YES NO

22. Are you currently using any anti-asthmatic medications? YES NO

23. Are you currently using any anti-hypertensive medications? YES NO

24. Are you currently taking any anti-inflammatory medications? YES NO

25. Are you currently taking any blood thinners (i.e.: coumadin, aspirin)? YES NO

26. Are you currently taking any anti-seizure or anti-anxiety medication? YES NO

27. Are you currently taking an MAOI (monoamine oxidase inhibitors)? YES NO

Common MAOIs include Phenelzine (Nardil), Tranylcypromine (Parnate), Isocarboxazid (Marplan), Moclobemide (Aurorix, Manerix, Moclodura), Selegiline (Selegiline, Eldepryl, Emsam), Nialamide, Iproniazid (Marsilid, Iporzid, Ipronid, Rivivol, Propilniazida), Iproclozide, and Toloxatone

28. Are you currently taking any other kind of medication? NO YES

i. If "YES", please list below:

_____

_____

_____

29. Are you currently taking any vitamin, mineral, or performance enhancing supplements? Please list them, with the dosage.

_____

_____

_____

30. Are you allergic local anesthetics (such as Novocain or lidocaine)? YES NO

31. Are you allergic to iodine (Betadine, tincture of Iodine)? YES NO

32. Are you allergic to Band-Aids or any other adhesive? YES NO

33. Have you ever been treated for a heat related illness

(heat exhaustion, heat stroke)? YES NO

34**. Do you have any food allergies? YES NO**

35. Are you allergic to any of the following?

(If you are unsure what the ingredient is, please ask for more information and you can also refer to ingredient explanations in the consent form)

Natrual Food flavaorings (corn syrup solids, modified food starch, medium chain triglycerides, talin, or di-alpha tocopherol) YES NO

Silica YES NO

The Artificial Sweetener Sucralose YES NO

Citric Acid YES NO

Natural Food Colors (beta-carotein) YES NO

Polydextrin YES NO

Mannitol YES NO

Caffeine YES NO

L-Tyrosine YES NO

L-Citrulline-di-malate YES NO

Arginine Alpha-ketogluterate YES NO

Taurine YES NO

Di-arginine malate	**YES**	**NO**
Creatine monohydrate	**YES**	**NO**
Betaine anhydrous	**YES**	**NO**
Di-creatine malate	**YES**	**NO**
Picamilon	**YES**	**NO**
Beta-Alanine	**YES**	**NO**
N-Acetyl-L-Glutamine	**YES**	**NO**
L-Histidine	**YES**	**NO**
Vitamin A	**YES**	**NO**
Vitamin E	**YES**	**NO**
Any other Artificial Food Colors (such as yellow 5)	**YES**	**NO**
Any artificial food flavorings	**YES**	**NO**

36. What is your current Cholesterol level? (If known)_____

37.What is your current Blood-Pressure? (must be measured by APL staff)_____

Caffeine Questionnaire

1. How many cups of caffeinated coffee, tea, soda, and energy drinks (red bull, full throttle etc.) do you consume on a typical weekday?

2. How many cups of caffeinated coffee, tea, soda, and energy drinks (red bull, full throttle etc.) do you consume on a typical weekend day?

3. Do you consider yourself addicted to caffeine? YES NO

4. Do you ever get headaches or feel unwell because you did not get caffeine or coffee at your usual time? YES NO

5. Do you feel that you need a caffeinated beverage to wake up in the morning? YES NO

6. If you take part in this study will you be able to reduce or eliminate caffeine from your diet? YES NO

7. Have you ever had too much caffeine such that it caused negative side effects like rapid heart rate, anxiety, rapid breathing, or jitters? YES NO

    a. <u>IF yes</u>, approximately how much caffeine (or how much soda, coffee, tea) did you consume? (you will find caffeine amounts for common foods on the next page)

    _____

    _____

    b. If NO, What is the most caffeine you have ever consumed in a 3 hour period? (or how much soda, coffee, tea) did you consume? (you will find caffeine amounts for common foods on the next page)

    _____

    _____

Caffeine Content of Popular Drinks

12-ounce beverage	**milligrams**
Red Bull (8.2 oz)	80.0
Jolt	71.2
Pepsi One	55.5
Mountain Dew / Diet Mountain Dew / Code Red	55.0
Mellow Yellow / Surge	52.8
Tab / RC Cola / Diet RC	46.8
Diet Coke	45.6
Dr. Pepper/ Diet Dr. Pepper / Mr. Pibb	41.0
Pepsi-Cola / Diet Pepsi / Wild Cherry Pepsi	37.5
Coca-Cola Classic	34.0
Snapple Flavored Teas (Reg. or Diet)	31.5
Nestea Sweet Iced Tea	26.5
Nestea Unsweetened Iced Tea	26.0
Lipton Diet Green Tea with Citrus (16.9 oz)	23.0
Barq's Root Beer	23.0
Lipton Brisk, All Varieties	9
Diet Rite Cola	0
Sprite /7-Up / Slice / Sierra Mist / Fresca	0
Mug / Diet Barq's Root Beer /A&W Root Beer	0
Sundrop Orange / Minute Maid Orange	0
8 Ounce Beverage	**milligrams**
Coffee, Drip	115-175
Coffee, Brewed	80-135
Coffee, Espresso (2 ounces)	100
Tea, iced	47
Tea, brewed, imported brands (avg.)	50
Tea, instant	30
Tea, green	15
Hot cocoa	14
Coffee, Decaf,	3-4

Dark Chocolate 10-30 mg per ounce Milk Chocolate 5-10mg / oz

APPENDIX H: EXERCISE TRAINING HISTORY QUESTIONAIRE: EFFECTS OF RESISTANCE EXERCISE AND A PRE-WORKOUT DIETARY SUPPLEMENT ON ERK & P38 PHOSPHORYLATION

Exercise Training History Questionnaire

22. At what age did you start resistance training?

23. Since then how regular, have you been with resistance training?

a. Have / do you take breaks from resistance training longer than 2 weeks? How frequently?

b. Have / do you take breaks from resistance training longer than 1 month? How frequently?

c. Have / do you take breaks from resistance training longer than 3 months? How frequently?

d. When, and for what reasons did you take such breaks?

24. When you first became serious about resistance training what was you main reason?

   a. Sport(s): List_____level of competition_____
   b. Fitness
   c. Competitive Lifting: (powerlifting, olympic lifting, strongman, other)
   d. Rehabilitation
   e. Other: Explain_____

25. What is your reason for resistance training now?

   a. Sport(s): List_____level of competition_____
   b. Fitness
   c. Competitive Lifting: (powerlifting, olympic lifting, body building, strongman, other)
   d. Rehabilitation

e. Other: Explain_____

26. In the last 6 months, How many times per week have you typically resistance trained? (Include multiple sessions per day separately).

a. Time per week upper body _____

b. Times per week lower body _____

c. Total times per week _____

27. In the last 6 months, How many weeks have you participated in resistance training one time or **LESS** per week? (include vacations, breaks, and schedule conflicts).

28. In the last 6 months, How many weeks have you participated in resistance training three times or **MORE** per week? (Include multiple sessions per day separately).

29. When you lift, do you usually work at a percentage of your known maximum or by what other method do you choose a weight for each lift?

30. Estimate your 1RM Max, or provide how much you think that you could lift for a given number of repetitions.

LIFT	I have done this exercise in the last 6 months	Predicted Maximum Weight	Weight I usually use	Number of Repetitions I usually do
BENCH PRESS	Yes No			
INCLINE BENCH	Yes No			
PARRALLEL SQUAT	Yes No			
FULL SQUAT (below parallel)	Yes No			
DEADLIFT	Yes No			
STANDING PRESS	Yes No			
LUNGES (WITH BARBELL)	Yes No			
LEG EXTENSION	Yes No			

LEG CURLS	Yes No			
BICEP CURLS	Yes No			
PULL-UPS	Yes No			
DIPS	Yes No			

31. Create in the space below a representation of your TYPICAL exercise session in the last 6 months. If you perform lower and upper body movements on separate days list each day separately below.

EXERCISE	**SETS AND REPS**	**POUNDAGE**

32. How many times per week do you participate in stretching? _____

33. How many years/months have you been performing cardiovascular exercise?

34. Since you began have / do you take breaks from cardiovascular training longer than 2 weeks? How frequently?

35. Since you began, have / do you take breaks from cardiovascular training longer than 1 month? How frequently?

36. Have / do you take breaks from cardiovascular training longer than 3 months? How frequently?

37. When, and for what reasons did you take such breaks?

38. What is your Current reason for participating in cardiovascular training now?

    a. Sport(s): List_____level of competition_____

    b. Health / Fitness: Explain _____

    c. Rehabilitation

    d. Other: Explain_____

39. In the last 6 months, How many times per week have you typically participated in cardiovascular exercise? (Include multiple sessions per day separately).

40. In the last 6 months, How many weeks have you participated in cardiovascular exercise one time or **LESS** per week? (include vacations, breaks, and schedule conflicts).

41. In the last 6 months, How many weeks have you participated in cardiovascular exercise training three times or **MORE** per week? (Include multiple sessions per day separately).

42. When you run, row, swim, etc, do you usually work at a percentage of your Heart Rate Maximum, aim for a particular time goal, or by what other method do you choose your intensity?

43. Create a TYPICAL weeks exercise log of cardiovascular exercises below. Use only the last six months as a guide. Include type of exercise, the duration, and the intensity or pace at which you perform this task. If you monitor your HR please include that information as well. Provide as much detail as possible use additional paper if needed.

	TYPE OF EXERCISE	DURATION	PACE	INTENSITY
MONDAY				
TUESDAY				
WEDNESDAY				
THURSDAY				
FRIDAY				
SATURDAY				
SUN				

APPENDIX I: SUPPLEMENT SIDE EFFECTS QUESTIONAIRE

Supplement Side Effects Questionnaire

Have you experienced any of the following since your last training session?

Dizziness or light headedness
YES NO
Nervousness or 'jitters'
YES NO
Indigestion
YES NO
Stomach discomfort
YES NO
Other: (please explain)

If so, when did this occur?

If you circled "yes" for any of the above, answer the next question according to the following scale:

1 = some, but no influence on normal functioning
2 = moderate, but minor influence on normal functioning
3 = severe; inability to maintain normal functioning

How would you rate the supplement side effects that you have experienced since your last training session?

1
2 3

Have you experienced any of the following since you ingested the supplement/placebo drink today?

Dizziness or light headedness
YES NO
Nervousness or 'jitters'
YES NO
Indigestion
YES NO
Stomach discomfort
YES NO
Other: (please explain)

If so, when did this occur? Circle all that apply.
Immediately (prior to workout)

During my workout
Immediately following my workout

If you circled "yes" for any of the above, answer the next question according to the following scale:

1 = some, but no influence on normal functioning
2 = moderate, but minor influence on normal functioning
3 = severe, inability to maintain normal functioning

How would you rate the supplement side effects that you have experienced since your last training session?

1

2 3

Are you experiencing any joint or muscle pain that has stemmed from the exercise program?
YES NO

If so, please explain.

APPENDIX J: EIGHT WEEK EXERCISE TRAINING PROGRAM

Name: Joeseph A. Example

Week 1

DAY 1	Date:		staff initials:					
Squat	**wt**	bar	45	65	65	65		
	reps	10	10	10	10	10		
Bench Press	**wt**	bar	45	65	65	65		
	reps	10	10	10	10	10		
Leg Extensions	**wt**	70	70					
	reps	10	10					
Leg Curl	**wt**	70	70					
	reps	10	10					
Lat Pulldown	**wt**	70	70					
	reps	10	10					
Standing Press	**wt**							
12-15	**reps**							
Weighted Sit-up	**wt**			**Wted Russian Twist (BH)**			**wt**	
12-15	**reps**			12-15			**reps**	

DAY 2	Date:		staff initials:					
Incline Bench	**wt**	Max						
	reps							
Deadlift	**wt**	Max						
	reps							
Bicep Curls	**wt**	Max						
	reps							
BB Split Squat	**wt**							
12-15	**reps**							
Dips	**wt**							
12-15	**reps**							
Pull-ups	**wt**							
12-15	**reps**							
Weighted Supine Leg Raise	**wt**			**Wtd Supine Wipers**			**wt**	
12-15	**reps**			12-15			**reps**	

Day 3	Date:		staff initials:					
Squat	**wt**	bar	45	65	65	65		
	reps	10	10	10	10	10		
Bench Press	**wt**	bar	45	65	65	65		
	reps	10	10	10	10	10		
Leg Extensions	**wt**	70	70					
	reps	10	10					
Leg Curl	**wt**	70	70					
	reps	10	10					
Lat Pulldown	**wt**	70	70					
	reps	10	10					
Upright Row	**wt**							
12-15	**reps**							
Wtd Twist Cruch/Elev. Legs	**wt**			**Weighted V-ups**			**wt**	
12-15	**reps**			12-15			**reps**	

NOTES:

Name: Joeseph A. Example

Week 2

DAY 1	Date:			staff initials:						
Squat	**wt**	bar	45	65	65	65	65	65		
	reps	10	10	10	10	10	10			
Bench Press	**wt**	bar	45	65	65	65	65	65		
	reps	10	10	10	10	10	10			
Leg Extensions	**wt**	70	70	70						
	reps	10	10	10						
Leg Curl	**wt**	70	70							
	reps	10	10							
Lat Pulldown	**wt**	70	70							
	reps	10	10							
Standing Press	**wt**									
12-15	**reps**									
Weighted Sit-up	**wt**			**Wted Russian Twist (BH)**				**wt**		
12-15	**reps**						12-15	**reps**		

DAY 2	Date:			staff initials:						
Incline Bench	**wt**	bar	45	65	65	65	65	65		
	reps	10	10	10	10	10	10			
Deadlift	**wt**	95	45	65	65	65	65	65		
	reps	10	10	10	10	10	10			
Bicep Curls	**wt**	70	70	70						
	reps	10	10	10						
BB Split Squat	**wt**									
12-15	**reps**									
Dips	**wt**									
12-15	**reps**									
Pull-ups	**wt**									
12-15	**reps**									
Weighted Supine Leg Raise	**wt**			**Wtd Supine Wipers**				**wt**		
12-15	**reps**						12-15	**reps**		

Day 3	Date:			staff initials:						
Squat	**wt**	bar	45	65	65	65	65	65		
	reps	10	10	10	10	10	10			
Bench Press	**wt**	bar	45	65	65	65	65	65		
	reps	10	10	10	10	10	10			
Leg Extensions	**wt**	70	70							
	reps	10	10							
Leg Curl	**wt**	70	70							
	reps	10	10							
Lat Pulldown	**wt**	70	70							
	reps	10	10							
Upright Row	**wt**									
12-15	**reps**									
Wtd Twist Cruch/Elev. Legs	**wt**			**Weighted V-ups**				**wt**		
12-15	**reps**						12-15	**reps**		

NOTES:

Name: Joeseph A. Example

Week 3

DAY 1	Date:			staff initials:							
Squat	**wt**	bar	45	65	65	65	65	65	65		
	reps	10	10	10	10	10	10	10	10		
Bench Press	**wt**	bar	45	65	65	65	65	65	65		
	reps	10	10	10	10	10	10	10	10		
Leg Extensions	**wt**	70	70								
	reps	10	10								
Leg Curl	**wt**	70	70								
	reps	10	10								
Lat Pulldown	**wt**	70	70								
	reps	10	10								
Standing Press	**wt**										
12-15	**reps**										
Weighted Sit-up	**wt**			**Wted Russian Twist (BH)**				**wt**			
12-15	**reps**						12-15	**reps**			

DAY 2	Date:			staff initials:							
Incline Bench	**wt**	bar	45	65	65	65	65	65	65		
	reps	10	10	10	10	10	10	10	10		
Deadlift	**wt**	95	45	65	65	65	65	65	65		
	reps	10	10	10	10	10	10	10	10		
Bicep Curls	**wt**	70	70	70							
	reps	10	10	10							
BB Split Squat	**wt**										
12-15	**reps**										
Dips	**wt**										
12-15	**reps**										
Pull-ups	**wt**										
12-15	**reps**										
Weighted Supine Leg Raise	**wt**			**Wtd Supine Wipers**				**wt**			
12-15	**reps**						12-15	**reps**			

Day 3	Date:			staff initials:							
Squat	**wt**	bar	45	65	65	65	65	65	65		
	reps	10	10	10	10	10	10	10	10		
Bench Press	**wt**	bar	45	65	65	65	65	65	65		
	reps	10	10	10	10	10	10	10	10		
Leg Extensions	**wt**	70	70								
	reps	10	10								
Leg Curl	**wt**	70	70								
	reps	10	10								
Lat Pulldown	**wt**	70	70								
	reps	10	10								
Upright Row	**wt**										
12-15	**reps**										
Wtd Twist Cruch/Elev. Legs	**wt**			**Weighted V-ups**				**wt**			
12-15	**reps**						12-15	**reps**			

NOTES:

Name: Joeseph A. Example

Week 4

DAY 1	Date:			staff initials:						
Squat		**wt**	bar	45	65	70	70	70		
		reps	10	10	8	8	8	8		
Bench Press		**wt**	bar	45	65	70	70	70		
		reps	10	10	8	8	8	8		
Leg Extensions		**wt**	70	70						
		reps	10	10						
Leg Curl		**wt**	70	70						
		reps	10	10						
Lat Pulldown		**wt**	70	70						
		reps	10	10						
Standing Press		**wt**								
	12-15	**reps**								
Weighted Sit-up		**wt**			**Wted Russian Twist (BH)**				**wt**	
	12-15	**reps**					12-15		**reps**	

DAY 2	Date:			staff initials:						
Incline Bench		**wt**	bar	45	65	70	70	70		
		reps	10	10	8	8	8	8		
Deadlift		**wt**	95	45	65	70	70	70		
		reps	10	10	8	8	8	8		
Bicep Curls		**wt**	70	70	70					
		reps	10	10	10					
BB Split Squat		**wt**								
	12-15	**reps**								
Dips		**wt**								
	12-15	**reps**								
Pull-ups		**wt**								
	12-15	**reps**								
Weighted Supine Leg Raise		**wt**			**Wtd Supine Wipers**				**wt**	
	12-15	**reps**					12-15		**reps**	

Day 3	Date:			staff initials:						
Squat		**wt**	bar	45	65	75	75	75		
		reps	10	10	6	6	6	6		
Bench Press		**wt**	bar	45	65	75	75	75		
		reps	10	10	6	6	6	6		
Leg Extensions		**wt**	70	70						
		reps	10	10						
Leg Curl		**wt**	70	70						
		reps	10	10						
Lat Pulldown		**wt**	70	70						
		reps	10	10						
Upright Row		**wt**								
	12-15	**reps**								
Wtd Twist Cruch/Elev. Legs		**wt**			**Weighted V-ups**				**wt**	
	12-15	**reps**					12-15		**reps**	

NOTES:

Name: Joeseph A. Example

Week 5

DAY 1	Date:			staff initials:								
Squat		**wt**	max									
		reps										
Bench Press		**wt**	Max									
		reps										
Leg Extensions		**wt**	Max									
		reps										
Leg Curl		**wt**	Max									
		reps										
Lat Pulldown		**wt**	Max									
		reps										
Standing Press		**wt**										
12-15		**reps**										
Weighted Sit-up		**wt**				**Wted Russian Twist (BH)**		**wt**				
12-15		**reps**				12-15		**reps**				

DAY 2	Date:			staff initials:								
Incline Bench		**wt**	bar	50		75		75		75		
		reps	10	10		10		10		10		
Deadlift		**wt**	95	50		75		75		75		
		reps	10	10		10		10		10		
Bicep Curls		**wt**	80	80		80						
		reps	10	10		10						
BB Split Squat		**wt**										
12-15		**reps**										
Dips		**wt**										
12-15		**reps**										
Pull-ups		**wt**										
12-15		**reps**										
Weighted Supine Leg Raise		**wt**				**Wtd Supine Wipers**		**wt**				
12-15		**reps**				12-15		**reps**				

Day 3	Date:			staff initials:								
Squat		**wt**	bar	50		75		75		75		
		reps	10	10		10		10		10		
Bench Press		**wt**	bar	50		75		75		75		
		reps	10	10		10		10		10		
Leg Extensions		**wt**	80	80								
		reps	10	10								
Leg Curl		**wt**	80	80								
		reps	10	10								
Lat Pulldown		**wt**	80	80								
		reps	10	10								
Upright Row		**wt**										
12-15		**reps**										
Wtd Twist Cruch/Elev. Legs		**wt**				**Weighted V-ups**		**wt**				
12-15		**reps**				12-15		**reps**				

NOTES:

Name: Joeseph A. Example

Week 6

DAY 1	Date:		staff initials:									
Squat	**wt**	bar	50	75	75	75	75					
	reps	10	10	10	10	10	10					
Bench Press	**wt**	bar	50	75	75	75	75					
	reps	10	10	10	10	10	10					
Leg Extensions	**wt**	80	80									
	reps	10	10									
Leg Curl	**wt**	80	80									
	reps	10	10									
Lat Pulldown	**wt**	80	80									
	reps	10	10									
Standing Press	**wt**											
12-15	**reps**											
Weighted Sit-up	**wt**			**Wted Russian Twist (BH)**				**wt**				
12-15	**reps**						12-15	**reps**				

DAY 2	Date:		staff initials:									
Incline Bench	**wt**	bar	50	75	75	75						
	reps	10	10	10	10	10						
Deadlift	**wt**	95	50	75	75	75						
	reps	10	10	10	10	10						
Bicep Curls	**wt**	80	80	80								
	reps	10	10	10								
BB Split Squat	**wt**											
12-15	**reps**											
Dips	**wt**											
12-15	**reps**											
Pull-ups	**wt**											
12-15	**reps**											
Weighted Supine Leg Raise	**wt**			**Wtd Supine Wipers**				**wt**				
12-15	**reps**						12-15	**reps**				

Day 3	Date:		staff initials:									
Squat	**wt**	bar	50	75	75	75	75					
	reps	10	10	10	10	10	10					
Bench Press	**wt**	bar	50	75	75	75	75					
	reps	10	10	10	10	10	10					
Leg Extensions	**wt**	80	80									
	reps	10	10									
Leg Curl	**wt**	80	80									
	reps	10	10									
Lat Pulldown	**wt**	80	80									
	reps	10	10									
Upright Row	**wt**											
12-15	**reps**											
Wtd Twist Cruch/Elev. Legs	**wt**			**Weighted V-ups**				**wt**				
12-15	**reps**						12-15	**reps**				

NOTES:

Name: Joeseph A. Example

Week 7

DAY 1	Date:		staff initials:						
Squat	**wt**	bar	50	70	80	80	80		
	reps	10	10	8	8	8	8		
Bench Press	**wt**	bar	50	70	80	80	80		
	reps	10	10	8	8	8	8		
Leg Extensions	**wt**	80	80						
	reps	10	10						
Leg Curl	**wt**	80	80						
	reps	10	10						
Lat Pulldown	**wt**	80	80						
	reps	10	10						
Standing Press	**wt**								
12-15	**reps**								
Weighted Sit-up	**wt**			**Wted Russian Twist (BH)**			**wt**		
12-15	**reps**					12-15	**reps**		

DAY 2	Date:		staff initials:						
Incline Bench	**wt**	bar	50	70	80	80	80		
	reps	10	10	8	8	8	8		
Deadlift	**wt**	95	50	70	80	80	80		
	reps	10	10	8	8	8	8		
Bicep Curls	**wt**	80	80	80					
	reps	10	10	10					
BB Split Squat	**wt**								
12-15	**reps**								
Dips	**wt**								
12-15	**reps**								
Pull-ups	**wt**								
12-15	**reps**								
Weighted Supine Leg Raise	**wt**			**Wtd Supine Wipers**			**wt**		
12-15	**reps**					12-15	**reps**		

Day 3	Date:		staff initials:						
Squat	**wt**	bar	50	70	85	85	85		
	reps	10	10	6	6	6	6		
Bench Press	**wt**	bar	50	70	85	85	85		
	reps	10	10	6	6	6	6		
Leg Extensions	**wt**	80	80						
	reps	10	10						
Leg Curl	**wt**	80	80						
	reps	10	10						
Lat Pulldown	**wt**	80	80						
	reps	10	10						
Upright Row	**wt**								
12-15	**reps**								
Wtd Twist Cruch/Elev. Legs	**wt**			**Weighted V-ups**			**wt**		
12-15	**reps**					12-15	**reps**		

NOTES:

Name: Joeseph A. Example

Week 8

DAY 1	Date:			staff initials:								
Squat		**wt**	max									
		reps										
Bench Press		**wt**	max									
		reps										
Leg Extensions		**wt**	max									
		reps										
Leg Curl		**wt**	max									
		reps										
Lat Pulldown		**wt**	max									
		reps										
Standing Press		**wt**										
12-15		**reps**										
Weighted Sit-up		**wt**				**Wted Russian Twist (BH)**			**wt**			
12-15		**reps**				12-15			**reps**			

DAY 2	Date:			staff initials:								
Squat Power test		**wt**	Max									
		reps										
Vertical jump		**wt**	Max									
		reps										
Isometric ROFD tests		**wt**	Max									
		reps										
Bicep curls		**wt**										
12-15		**reps**										
Dips		**wt**										
12-15		**reps**										
Pull-ups		**wt**										
12-15		**reps**										
Weighted Supine Leg Raise		**wt**				**Wtd Supine Wipers**			**wt**			
12-15		**reps**				12-15			**reps**			

Day 3	Date:			staff initials:								
Squat		**wt**	bar	55		75		75		75		
		reps	10	10		10		10		10		
Bench Press		**wt**	bar	55		75		75		75		
		reps	10	10		10		10		10		
Le												
L												
Standing Press		**wt**										
12-15		**reps**										
Wtd Twist Cruch/Elev. Legs		**wt**				**Weighted V-ups**			**wt**			
12-15		**reps**				12-15			**reps**			

Duplicates the initial tested workout with same relative but different absolute loads to reflect strength improvements

NOTES:

APPENDIX K: EXERCISE SUPER CHARGE NUTRITIONAL INFORMATION

	Amount
Calories	*64*
Total Carbohydrate	*16*
Sugar	*2g*
Vitamin A	*4600IU (92%)*
Vitamin E	*8IU (26%)*
"Super Charge Proprietary Blend"	*2242mg*
Nitrous Mallage (di-arginine malate)	
Taurine	*2000mg*
Arginine Alpha-Ketogluterate	*1742mg*
L-Citruline-di-malate	*1000mg*
L-Tyrosine	*500mg*
Caffeine	*450mg*
Phase 2 Strength & Endurance Complex	
CreaPure™ (creatine monohydrate)	*2750mg*
BetaPure™ (Betaine anhydrous)	*1000mg*
2CM™ (di-creatine malate)	*250mg*
Picatropin™ (picamilion)	*50mg*
Phase 3 Post Workout Recovery Complex	
Beta-Alanine	*1500mg*
N-Acetyl-L-Glutamine	*1000mg*
L-Histadine	*250mg*
Other ingredients: Maltodextrin, Natural Flavor (Corn Syrup Solids, Modified Food Starch, Medium Chain Triglycerides, Talin, di-Alpha tocopherol, Silica, Beta-carotene (color), Sucralose, Citric Acid.	

Nutritional Information Label for Supper Charge Supplement, as provided by the manufacturer; (Labrada nutrition, Houston TX.).

APPENDIX L: STUDY 1 DATA

STUDY 1

SUBJECT DESCRIPTIVE DATA AND TESTING ORDER

Subject	Age	Height	Weight	%BF	Max Squat	Testing Order
1	19	73.3	205.5	19.1	275	H-L-M
2	21	70.0	206.2	24.8	315	L-H-M
3	21	70.2	173.7	9.9	315	H-L-M
4	22	74.0	173.4	8.6	255	M-H-L
5	22	73.2	210.9	21.0	295	M-L-H
6	20	72.5	200.3	21.0	295	M-L-H
7	22	70.3	204.5	20.2	405	L-H-M
8	25	75.2	245.8	23.8	330	M-H-L
9	21	70.0	206.3	12.4	545	H-M-L

BLOOD LACTATE CONCENTRATIONS (MMOL/L)

Subject	High Intensity PRE	High Intensity Post	Moderate Intensity Pre	Moderate Intensity Post	Low Intensity Pre	Low Intensity Post
1	1.5	4.5	2.4	7.2	4.9	8
2	1.8	2.9	1.3	2.1	1.5	6.6
3	1.3	3	2.4	2.7	1.1	2.9
4	0.9	3.5	1.2	4.3	1.4	2.2
5	1.8	4.1	1.6	6.5	2.6	3.1
6	1.3	2.3	0.9	5.6	1	6.1
7	1.1	6.7	1.5	4.1	1.4	3.7
8	1.7	4.1	4.2	5.5	1.7	3.8
9	0.9	4.8	1.3	3.9	1.5	4.1

AVERAGE AND STANDARD DEVIATION (N=9) FOR BLOOD LACTATE CONCENTRATIONS (MMOL/L)

	High Intensity PRE	High Intensity Post	Moderate Intensity Pre	Moderate Intensity Post	Low Intensity Pre	Low Intensity Post
Average	1.37	3.99	1.87	4.66	1.90	4.50
Standard Deviation	0.36	1.30	1.01	1.69	1.21	1.95

POWER DATA

AVERAGE POWER HIGH INTENSITY (90% 1RM) EXERCISE BOUT

Subject Number	Set 1	Set 2	Set3	Set 4	Set 5	Average of Sets
1	916.0	788.3	673.3	653.3	653.3	736.9
2	1334.3	1246.7	1356.3	1217.7	1174.3	1265.9
3	1047.3	1108.7	1006.7	1027.0	952.0	1028.3
4	1271.0	1260.7	1343.3	1157.3	1126.3	1231.7
5	1010.0	1010.3	1017.0	960.7	1024.0	1004.4
6	1235.0	1152.0	1159.0	1104.0	1021.3	1134.3
7	1189.7	1189.7	1198.0	1122.0	1096.3	1159.1
8	1386.0	1162.0	1201.7	1089.7	1113.7	1190.6
9	999.3	909.7	879.7	849.7	832.0	894.1

MEAN AND STANDARD DEVIATION FOR THE AVERAGE POWER MEASURES FOR HIGH INTENSITY (90% 1RM) BOUT

	Set 1	Set 2	Set3	Set 4	Set 5	Grand Mean
Average	1154.3	1092.0	1092.8	1020.1	999.3	1071.7
Standard Deviation	166.0	159.2	221.9	175.8	165.9	172.9

AVERAGE POWER MODERATE INTENSITY (70% 1RM) EXERCISE BOUT

Subject Number	Set 1	Set 2	Set3	Set 4	Set 5	Average of Sets
1	1207.4	1179.0	1172.1	1157.8	1161.4	1175.6
2	1131.9	1108.4	1123.1	1036.5	1107.5	1101.5
3	1362.0	1239.8	1190.8	1208.4	1194.0	1239.0
4	1130.2	1123.6	1177.0	1167.6	1183.4	1156.4
5	779.2	800.2	813.3	769.0	803.1	793.0
6	1361.2	1408.0	1321.4	1350.4	1317.8	1351.8
7	1537.6	1472.2	1472.2	1567.8	1520.0	1514.0
8	1131.9	1108.4	1123.1	1036.5	1107.5	1101.5

MEAN AND STANDARD DEVIATION FOR THE AVERAGE POWER MEASURES FOR MODERATE INTENSITY (70% 1RM) BOUT

	Set 1	Set 2	Set3	Set 4	Set 5	Grand Mean
Average	1238.8	1234.3	1217.0	1217.6	1233.9	1228.3
Standard Deviation	234.2	252.8	217.5	276.7	260.6	245.4

AVERAGE POWER LOW INTENSITY (30% 1RM) EXERCISE BOUT

Subject Number	Set 1	Set 2	Set3	Set 4	Set 5	Average of Sets
1	1234.0	1241.8	1286.2	1189.0	1234.0	1237.0
2	1559.1	1588.5	1556.3	1544.2	1511.9	1552.0
3	1382.3	1283.1	1270.0	1151.4	1285.4	1274.4
4	1591.6	1656.7	1656.7	1715.8	1639.6	1652.1
5		1182.1	1216.7	1244.9	1190.0	1208.4
6	1254.0		1469.4	1207.4	1593.7	1381.1
7	1377.8	1491.0	1544.0	1621.3	1635.7	1534.0
8	1533.5	1619.6	1673.9	1669.3	1664.6	1632.2
9	1697.8	1765.8	1793.9	1858.3	1790.6	1781.3

** Missing Values reflect data that was either not collected or nor not saved properly as a result of assistant error.

MEAN AND STANDARD DEVIATION FOR THE AVERAGE POWER MEASURES

FOR LOW INTENSITY (30% 1RM) BOUT

	Set 1	Set 2	Set3	Set 4	Set 5	Grand Mean
Average	1453.8	1478.6	1496.3	1466.8	1505.1	1472.5
Standard Deviation	166.9	216.6	201.9	269.1	215.4	204.9

PEAK POWER FOR THE HIGH INTENSITY (90% 1RM) EXERCISE BOUT

Subject Number	Set 1	Set 2	Set3	Set 4	Set 5	Average of Sets
1	1381.0	1219.3	1098.0	1017.3	1017.3	1146.6
2	2486.7	2384.7	2450.3	2333.7	2224.0	2375.9
3	2095.0	2272.0	1986.0	2081.3	2061.0	2099.1
4	2521.3	2325.0	2449.0	2004.7	2077.0	2275.4
5	1511.3	1483.3	1441.0	1398.7	1462.3	1459.3
6	2360.0	2483.7	2498.0	2449.0	2380.3	2434.2
7	2193.7	2075.3	2244.0	2227.3	2362.3	2220.5
8	2667.7	2403.3	2291.3	2475.7	2523.7	2472.3
9	1903.0	1819.0	1831.3	1885.0	1837.0	1855.1

MEAN AND STANDARD DEVIATION FOR THE PEAK POWER MEASURES FOR HIGH INTENSITY (90% 1RM) BOUT

	Set 1	Set 2	Set3	Set 4	Set 5	Grand Mean
Average	2124.4	2051.7	2032.1	1985.9	1993.9	2037.6
Standard Deviation	450.4	449.3	492.7	492.0	486.2	463.2

PEAK POWER FOR THE MODERATE INTENSITY (70% 1RM) EXERCISE BOUT

Subject Number	Set 1	Set 2	Set3	Set 4	Set 5	Average of Sets
1						
2	2585.2	2585.2	2564.0	2546.8	2623.2	2580.9
3	2105.8	1868.4	1780.8	1816.2	1756.8	1865.6
4	1688.8	1751.6	1883.4	1783.2	1773.4	1776.1
5	2397.2	2469.4	2353.8	2389.8	2401.0	2402.2
6	2317.4	2247.6	2247.6	2256.2	2243.2	2262.4
7	2585.2	2585.2	2564.0	2546.8	2623.2	2580.9
8	2585.2	2585.2	2564.0	2546.8	2623.2	2580.9
9	2184.0	2480.4	2470.0	2589.6	2704.0	2485.6

** Missing Values reflect data that was either not collected or nor not saved properly as a result of assistant error.

MEAN AND STANDARD DEVIATION FOR THE PEAK POWER MEASURES FOR MODERATE INTENSITY (70% 1RM) BOUT

	Set 1	Set 2	Set3	Set 4	Set 5	Grand Mean
Average	2306.1	2321.6	2303.5	2309.4	2343.5	2316.8
Standard Deviation	311.3	336.2	313.2	333.2	386.8	326.0

PEAK POWER FOR THE LOW INTENSITY (30% 1RM) EXERCISE BOUT

Subject Number	Set 1	Set 2	Set3	Set 4	Set 5	Average of Sets
1	2190.7	2273.8	2241.8	2189.3	2136.9	2206.5
2	1986.7	1848.9	1792.6	1627.7	1801.3	1811.4
3	2318.5	2355.8	2386.7	2416.3	2337.1	2362.9
4		1496.9	1529.0	1571.6	1458.5	1514.0
5						
6	1856.4	2338.8	2337.3	2502.1	2316.0	2270.1
7	2140.6	2314.4	2394.5	2438.3	2397.5	2337.1
8	2339.1	2483.5	2538.0	2581.4	2475.2	2483.4
9						

** Missing Values reflect data that was either not collected or nor not saved properly as a result of assistant error.

MEAN AND STANDARD DEVIATION FOR THE PEAK POWER MEASURES FOR LOW INTENSITY (30% 1RM) BOUT

	Set 1	Set 2	Set3	Set 4	Set 5	Grand Mean
Average	2138.7	2158.9	2174.3	2189.5	2131.8	2140.8
Standard Deviation	188.7	353.1	369.5	420.7	371.3	348.2

Study 1

WESTERN BLOT RAW OD VALUES FOR TOTAL ERK1/2 (ARBITRARY UNITS)

Subject Number	High Intensity Pre	High Intensity Post	Moderate Intensity Pre	Moderate Intensity Post	Low Intensity Pre	Low Intensity Post
1	9.25	9.73	11.07	13.00	11.17	9.49
2	8.73	4.06	7.07	7.07	4.87	5.62
3	17.72	16.80	16.48	15.71	16.56	17.37
4	21.25	20.35	14.34	15.03	18.91	12.67
5	12.12	8.61	10.19	8.81	10.40	7.78
6	18.73	17.75	19.53	19.55	19.43	19.55
7	5.29	7.68	13.12	12.42	9.06	5.52
8	8.26	12.33	12.92	10.20	10.06	8.43
9	5.74	6.89	11.57	6.25	9.86	10.79

WESTERN BLOT RAW OD VALUES FOR PHOSPHO-ERK1/2 (ARBITRARY UNITS)

Subject Number	High Intensity Pre	High Intensity Post	Moderate Intensity Pre	Moderate Intensity Post	Low Intensity Pre	Low Intensity Post
1	19.67	23.40	27.18	32.20	28.69	24.44
2	27.51	13.12	22.19	23.31	16.49	18.12
3	49.84	56.26	54.93	48.70	54.72	58.89
4	80.76	78.85	56.20	59.02	63.40	43.31
5	46.23	37.61	41.20	36.34	44.23	29.79
6	69.21	67.21	83.11	78.06	76.65	68.64
7	14.50	23.25	41.13	38.60	28.91	18.56
8	32.23	47.75	50.81	41.51	40.18	31.98
9	15.16	17.33	32.24	14.37	28.99	30.22

Study 1.

WESTERN BLOT RAW OD VALUES FOR TOTAL P38 (ARBITRARY UNITS)

Subject Number	High Intensity Pre	High Intensity Post	Moderate Intensity Pre	Moderate Intensity Post	Low Intensity Pre	Low Intensity Post
1.00	21.11	19.42	22.13	17.75	23.76	19.92
2.00	31.82	18.66	30.37	35.77	27.30	25.67
3.00	9.42	10.73	12.17	9.62	8.46	6.25
4.00	29.82	39.95	38.45	34.89	41.30	30.26
5.00	31.14	28.32	29.35	36.48	31.89	27.87
6.00	25.27	27.98	31.59	37.98	34.99	24.72
7.00	59.48	36.92	67.53	100.89	67.28	42.96
8.00	17.69	23.80	31.26	43.98	38.73	45.87
9.00	15.55	15.46	19.22	10.52	13.44	20.31

WESTERN BLOT RAW OD VALUES FOR PHOSPHO-ERK1/2 (ARBITRARY UNITS)

Subject Number	High Intensity Pre	High Intensity Post	Moderate Intensity Pre	Moderate Intensity Post	Low Intensity Pre	Low Intensity Post
1	134.29	119.36	145.71	112.32	161.10	132.32
2	67.11	39.01	74.06	74.06	61.14	54.89
3	41.90	49.32	39.97	38.41	37.95	28.91
4	67.64	95.18	93.09	84.72	96.02	76.29
5	81.00	73.73	79.32	101.99	96.89	71.15
6	80.15	84.34	91.59	109.71	101.90	70.83
7	77.41	50.35	99.04	147.02	98.12	70.90
8	22.51	28.40	31.26	52.96	49.40	57.39
9	110.72	111.07	158.49	93.42	145.25	154.98

APPENDIX M: STUDY 2. DATA

MUSCLE SAMPLE AND PROTEIN CONCENTRATION DATA FOR STUDY 2

PRE-TRAINING VALUES

Subject	Time Point*	Muscle Sample Size (mg)	Mean BSA(μg/ml)	CV
1	PRE 1	19.4	26.598	8.4438
1	PRE 2	16.21	20.070	3.8280
2	PRE 1	13.88	21.365	1.1586
2	PRE 2	14.05	20.066	3.1056
3	PRE 1	16.31	23.726	6.2521
3	PRE 2	17.52	18.200	2.3923
4	PRE 1	15.79	22.628	0.7813
4	PRE 2	17.73	23.103	1.6163
5	PRE 1	12.4	36.312	0.6929
5	PRE 2	14.5	24.549	1.5212
6	PRE 1	15.75	24.219	1.9795
6	PRE 2	17.03	19.536	1.5479
7	PRE 1	17.26	28.793	1.2492
7	PRE 2	16.13	23.487	1.8885
8	PRE 1	16.73	24.164	1.1434
8	PRE 2	18.9	24.567	4.1379
9	PRE 1	13.78	24.183	1.4892
9	PRE 2	15.44	26.488	0.9499
10	PRE 1	17.81	14.596	5.0075
10	PRE 2	20.15	23.982	3.5908
11	PRE 1	13.89	19.737	2.7816
11	PRE 2	20.06	27.585	1.8978
12	PRE 1	16.27	27.439	9.5734
12	PRE 2	20.33	31.153	4.3052
13	PRE 1	16.8	23.067	2.2869
13	PRE 2	16.84	26.836	4.3736
14	PRE 1	16.5	22.577	2.8487
14	PRE 2	16.4	28.052	1.6247
15	PRE 1	13.38	25.176	3.0667
15	PRE 2	17.04	25.176	3.0667
16	PRE 1	13.92	25.101	0.2211
16	PRE 2	17.49	25.120	1.2210
17	PRE 1	15.41	30.687	3.0421
17	PRE 2	19.52	39.743	7.0418
18	PRE 1	16.65	33.174	1.4238

18	PRE 2	16.53	25.899	5.4043
19	PRE 1	20.1	31.633	1.2742
19	PRE 2	15.21	27.440	1.7675
20	PRE 1	18.7	25.992	0.5664
20	PRE 2	14.62	25.640	11.9074
21	PRE 1	16.08	27.681	5.1137
21	PRE 2	19.14	31.763	0.8643
22	PRE 1	16.55	29.425	0.7652
22	PRE 2	19.98	22.206	1.9923
23	PRE 1	15.21	20.481	7.7676
23	PRE 2	13.99	24.842	3.5013
24	PRE 1	19.22	32.432	8.3077
24	PRE 2	14.77	30.669	5.4544

* Timepoint of muscle biopsy. Pre indicates the biopsy was taken at the beginning of the study before the 8 weeks of exercise training. Post would indicate biopsies taken after the 8 weeks of training. The number 1 indicates that the sample was taken before the exercise session for that testing day. The numeral 2 indicates it is the second biopsy taken on that given testing day, after the exercise session for the day.

Muscle Sample and Protein Concentration Data for Study 2

POST -TRAINING VALUES

Subject Number	Timepoint*	Musle Sample Size	Mean BSA(μg/ml)	CV
1	Post 1	17.05	33.062	5.420
1	Post 2	18.96	27.885	1.830
2	Post 1	16.35	27.941	6.561
2	Post 2	16.7	27.347	5.529
3	Post 1	20.87	27.565	1.609
3	Post 2	19.75	23.803	0.269
4	Post 1	17.89	32.985	0.897
4	Post 2	18.96	33.711	1.599
5	Post 1	16.03	32.724	1.422
5	Post 2	18.45	27.230	1.086
6	Post 1	12.79	27.267	1.706
6	Post 2	20.66	38.330	0.366
7	Post 1	19.06	27.453	0.538
7	Post 2	17.61	27.062	1.801
8	Post 1	17.17	22.332	1.665
8	Post 2	17.02	25.330	2.884
9	Post 1	19.32	31.886	0.789
9	Post 2	17.37	23.244	0.773
10	Post 1	15.11	20.655	4.202
10	Post 2	14.82	23.021	1.222
11	Post 1	17.55	29.334	1.586
11	Post 2	14.48	23.486	1.647
12	Post 1	17.83	25.516	4.140
12	Post 2	18.48	33.916	4.329
13	Post 1	15.32	20.003	3.645
13	Post 2	18.33	22.890	1.798
14	Post 1	15.66	24.231	1.920
14	Post 2	16.31	27.416	1.850
15	Post 1	20.65	33.357	5.041
15	Post 2	17.58	26.820	1.357
16	Post 1	16.63	26.045	2.428
16	Post 2	19.5	33.076	0.782
17	Post 1	17.57	30.756	0.308
17	Post 2	17.32	25.899	1.274

18	Post 1	15.57	21.826	2.421
18	Post 2	92	25.880	8.802
19	Post 1	15.71	27.305	0.875
19	Post 2	17.05	30.775	2.725
20	Post 1	16.41	30.556	0.746
20	Post 2	16.65	27.871	0.299
21	Post 1	19.88	37.624	0.445
21	Post 2	18.99	27.798	2.422
22	Post 1	14.5	23.141	6.169
22	Post 2	16.56	26.227	1.052
23	Post 1	18.35	32.017	2.532
23	Post 2	18.05	30.738	2.066
24	Post 1	15.37	25.625	0.326
24	Post 2	17.54	34.044	1.004

* Timepoint of muscle biopsy. Pre indicates the biopsy was taken at the beginning of the study before the 8 weeks of exercise training. Post would indicate biopsies taken after the 8 weeks of training. The number 1 indicates that the sample was taken before the exercise session for that testing day. The numeral 2 indicates it is the second biopsy taken on that given testing day, after the exercise session for the day.

WESTERN BLOT RAW OPTICAL DENSITY VALUES FOR TOTAL ERK1/2 (ARBITRARY UNITS)

Subject Number	Group*	Pre 1	Pre 2	Post 1	Post 2
1	1	0.96	0.98	0.50	0.98
2	1	0.97	0.73	0.73	0.59
3	1	1.56	1.57	1.17	1.27
4	1	1.43	0.94	0.77	1.48
5	2	1.15	2.04	1.05	1.63
6	2	1.58	1.89	1.58	1.92
7	2	1.65	2.07	1.85	1.16
8	2	2.10	2.60	1.75	2.49
9	2	0.77	0.81	1.15	1.18
10	2	0.92	1.09	0.83	1.13
11	2	2.85	0.89	1.40	0.72
12	2	1.17	0.65	1.05	0.69
13	2	1.86	1.16	0.93	1.56
14	2	2.72	2.91	2.12	2.54
15	2	1.77	2.97	1.92	3.28
16	1	2.91	2.62	3.17	1.81
17	2	3.74	5.47	4.18	4.60
18	1	4.35	1.75	3.92	2.27
19	1	1.89	2.27	2.14	2.04
20	2	0.20	1.02	0.92	81.00
21	1	0.53	0.16	1.32	0.72
22	2	0.20	0.76	0.50	1.04
23	1	1.67	1.99	1.88	1.80
24	1	2.49	1.14	2.22	1.22

*Group: 1= Placebo/control group; . 2= Supplement/experimental group

WESTERN BLOT RAW OPTICAL DENSITY VALUES FOR PHOSPHO- ERK1/2 (ARBITRARY UNITS)

Subject Number	Group*	Pre 1	Pre 2	Post 1	Post 2
1	1	2.97	6.57	1.23	2.53
2	1	0.50	1.53	2.53	0.92
3	1	1.31	1.50	1.13	1.11
4	1	2.00	3.43	1.16	3.73
5	2	1.18	3.65	1.91	2.70
6	2	1.61	5.66	1.63	4.10
7	2	19.11	3.91	1.89	1.73
8	2	1.20	1.70	1.28	1.82
9	2	0.74	1.72	0.98	1.30
10	2	0.80	2.01	0.87	1.88
11	2	1.20	0.56	9.21	0.51
12	2	10.63	0.79	73.00	0.56
13	2	2.43	0.73	0.81	2.19
14	2	0.41	0.47	0.39	0.50
15	2	0.40	0.65	0.38	0.62
16	1	2.87	4.61	4.61	2.47
17	2	0.72	1.12	0.87	0.87
18	1	6.53	3.47	3.22	3.77
19	1	1.80	5.31	2.36	2.39
20	2	0.30	3.54	1.44	1.20
21	1	0.69	4.92	1.37	1.17
22	2	0.14	2.22	0.51	1.69
23	1	1.03	1.35	0.95	0.99
24	1	2.01	1.10	1.55	1.38

*Group: 1= Placebo/control group; . 2= Supplement/experimental group

WESTERN BLOT RAW OPTICAL DENSITY VALUES FOR TOTAL P38 (ARBITRARY UNITS)

Subject Number	Group*	Pre 1	Pre 2	Post 1	Post 2
1	1	52.44	43.29	58.06	80.13
2	1	70.86	104.96	82.57	74.23
3	1	20.89	27.41	18.90	25.97
4	1	82.37	86.03	72.21	65.44
5	2	2.33	4.28	3.39	5.03
6	2	4.33	3.70	3.01	3.94
7	2	7.27	8.70	12.30	11.39
8	2	20.42	21.90	29.49	21.01
9	2	28.02	28.99	29.79	36.25
10	2	2.68	7.20	8.53	5.56
11	2	6.51	8.04	7.09	7.70
12	2	6.80	11.14	6.66	9.92
13	2	9.53	3.14	12.36	10.08
14	2	36.37	13.31	33.93	26.67
15	2	30.43	19.35	29.65	16.07
16	1	8.02	5.73	7.27	7.85
17	2	31.09	17.34	14.80	16.78
18	1	27.22	26.94	29.92	26.08
19	1	25.46	28.69	30.94	30.32
20	2	21.48	23.55	36.72	14.26
21	1	18.69	12.65	10.43	24.46
22	2	16.30	18.58	27.38	32.83
23	1	7.21	22.20	16.95	20.94
24	1	11.92	15.24	21.28	16.55

*Group: 1= Placebo/control group; . 2= Supplement/experimental group

WESTERN BLOT RAW OPTICAL DENSITY VALUES FOR TOTAL P38 (ARBITRARY UNITS)

Subject Number	Group*	Pre 1	Pre 2	Post 1	Post 2
1	1	104.45	74.40	120.59	179.60
2	1	161.08	256.17	203.45	189.41
3	1	57.27	74.60	49.75	63.30
4	1	205.11	201.61	166.09	143.21
5	2	4.72	9.63	7.30	11.47
6	2	11.20	8.07	7.62	6.24
7	2	4.12	2.62	3.55	3.55
8	2	17.86	19.91	23.81	18.48
9	2	22.10	21.50	22.90	27.79
10	2	7.96	21.95	25.01	20.41
11	2	19.77	31.25	29.53	26.22
12	2	26.17	41.72	25.47	39.66
13	2	41.35	12.01	50.78	42.21
14	2	67.47	21.65	58.74	45.61
15	2	52.90	29.64	51.07	23.92
16	1	22.89	16.48	21.47	21.78
17	2	53.79	34.61	48.20	59.12
18	1	268.82	268.74	287.42	277.68
19	1	242.89	275.38	298.26	237.80
20	2	117.96	113.95	197.38	89.51
21	1	121.59	84.84	74.29	162.02
22	2	107.56	113.40	153.58	198.53
23	1	17.60	65.22	46.40	56.50
24	1	32.53	39.93	56.71	44.18

*Group: 1= Placebo/control group; . 2= Supplement/experimental group

APPENDIX N: SUMMARY OF FINDINGS PREVIOUSLY PUBLISHED BY NICOLE MOODIE

STUDY 2. FINDINGS PREVIOUSLY PRESENTED AND PUBLISHED IN DISSERTATION FORMAT:

Moodie, Nicole Jayne, (2008). The effects of a pre-workout supplement and eight weeks of resistance training on markers of inflammation. University of Kansas

Anthropometric Findings

- Body Mass Significantly increased over the eight weeks of training ($F_{(1,22)}$=23.29, p<0.01) ,but there was no significant difference in body mass gains between the supplement and placebo groups (p>0.05).
- Lean Body Mass increased significantly following the 8 weeks of resistance training, ($F_{(1,22)}$=220.320, p<0.01). Again there was no significant difference between the supplement and placebo groups.

Strength Measures

- Significant improvements were observed in the 1-repetition maximum of the back squat, bench press, leg extension, leg curl, and lat pull-down exercises (all p>0.05).
- Non-significant improvements were observed in the dead lift, incline press, and bicep curl exercises.
- The supplement group was not different than the placebo for all strength measures except bench press $F_{(1/22\ )}$=4.843, p=0.039 (Kudrna et al., 2011).

Inflammation Markers

- Significant differences in the Skeletal muscle IL-6 was observed across the four time points (pre- & post-training and pre-& post exercise; $F_{(3,69)}$=14.641, p<0.01 was lower pre- and Post Exercise in the post-training samples compared to the initial pre-exercise pre-training time point.
- No significant differences were observed in serum cytokine levels for: IL-1-α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IFN-γ, or TNF-α.

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