The Effects of a Pre-Workout Supplement and Eight Weeks of Resistance Training on Markers of Inflammation

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ABSTRACT

The purpose of the present study was to examine the effects of eight weeks of resistance training and pre-workout supplementation on lean body mass, strength, and markers of inflammation. Twenty four (n=24) healthy, recreationally active, college aged males (19.5 ± 1.3 years) volunteered for this study. Individuals were randomly assigned to supplement (SUP) or placebo (PCBO) groups and drank either a multi-ingredient supplement or color and taste matched placebo 15 minutes prior to exercise three times per week for 8 weeks. Strength and body composition testing was performed before and after the training period. Muscle biopsies and blood draws were performed immediately before and after the first and last exercise bouts. Skeletal muscle and serum samples were analyzed for markers of inflammation. Repeated measures ANOVAs revealed significant differences in lean body mass across subjects following training [$F(1, 22) = 20.320$, $p < 0.01$]. Significant differences in squat, bench press, leg extension, leg curl and lat pulldown 1-repetition maximum (RM) strength following training were also found, ($p < 0.01$). No significant differences in lean body mass [$F(1, 22) = 0.994$, $p > 0.01$] or strength were seen between SUP and PCBO groups ($p > 0.01$). Pairwise comparisons revealed significant decreases in skeletal muscle IL-6 when comparing pre to post training time points [PRE 1-POST1, $t(23) = 4.661$, $p < 0.01$; PRE2-POST2, $t(23) = 4.875$, $p < 0.01$]. No significant changes IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IFN-γ, or TNF-α were observed ($p > 0.01$). No significant differences in cytokine levels were found between SUP and PCBO groups ($p > 0.01$). Further research examining the cytokine response to resistance exercise and the effects of supplementation on the inflammatory response is warranted.

KEY WORDS: IL-6, TNF-α, cytokines, resistance training

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LIST OF ABBREVIATIONS

1-RM One repetition maximum

4E-BP1 Eukaryotic translation initiation factor 4E binding protein 1

AKT Another acronym for protein kinase B

AMPK Adenosine monophosphate-activated protein kinase

ATF-2 Activating transcription factor-2

Ca-III Carbonic anhydrase-III

EDL Extensor digitorum longus

Elk-1 Ets-like gene 1

Ets External transcribed spacer

GLUT4 Glucose transporter isoform 4

GSK-3 Glycogen-synthase-3

H-FABP Heart fatty-acid binding protein

IGF-1 Insulin-like growth factor-1

IKK IκB kinase

INF-γ Interferon-gamma

IL Interleukin

IRAK-1 Interleukin-1 receptor associated kinase-1

ISRE Interferon response element

JAK-STAT Janus kinase-signal transducer and activator of transcription

JNK c-Jun amino-terminal kinase

Jun:ATF Jun N-terminal kinase: activating transcription factor

LBM Lean body mass

LPS	Lipopolysaccharide
TNF-α	Tumor necrosis factor-alpha
TNF-α	Tumor necrosis factor-alpha receptor-1
TRADD	Tumor necrosis factor-alpha receptor-associated death domain
MAPK	mitogen-activated protein kinase
MHC	Myosin heavy chain
mRNA	Messenger ribonucleic acid
NFAT	Nuclear factor of activated T-cell
NFκB	Nuclear factor of κB
NO	Nitric oxide
PCBO	Placebo
PI3K	Phosphatidylinositol-3-Kinase
P70s6k	p70 ribosomal S6 kinase
PKB	Protein kinase B
STAT1	Signal transducer and activator of transcription 1
SUP	Supplement
ROS	Reactive oxygen species
SOCS	Suppressor of cytokine signaling
TLR	Toll-like receptor
TRAF-6	Tumor necrosis factor-α associated factor-6
VO_{2max}	Maximal ventilatory oxygen uptake

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CHAPTER I

INTRODUCTION

Cytokines are proteins manufactured and secreted by many cells of the body that play crucial roles in the proliferation, growth, and maturation of cells (112). Skeletal muscle is crucial to the production of several cytokines including interleukin (IL)-6, IL-8, IL-15, and possibly IL-4 (112). The cytokines that are produced within skeletal muscle are now more commonly called myokines. The production of IL-6 has been of particular focus to date as changes in plasma values of this protein are associated with both body composition and physical activity levels (40-41). In general, individuals who are obese and inactive have higher levels of IL-6 (41). Over production of IL-6, combined with a high level of tumor necrosis factor-alpha (TNF-α) has been proposed to lead to type II diabetes mellitus (113). Recent evidence has revealed that high basal levels of IL-6 are generally due to the hyperproduction of this cytokine by the adipose tissue (87). However, contraction of the skeletal muscle is also associated with an increase in IL-6. This conflicting evidence has led many to question the role for the skeletal muscle production of IL-6.

The increase in IL-6 in the skeletal muscle with acute exercise is associated with an increase in IL-6 receptor, and is also responsible for the dramatic increase in plasma IL-6 with exercise (74). Plasma levels of IL-6 have been shown to increase up to ten-fold immediately following one hour of aerobic exercise and gradually decrease to baseline during one hour of recovery. Research has shown that IL-6 plays a central role in metabolism; regulating both glucose uptake as well as lipolysis (4, 50, 114). Due to this positive role, IL-6 has been deemed an anti-inflammatory cytokine (121). TNF-α however, due to its role in protein degradation is

considered a pro-inflammatory cytokine. These two cytokines generally act in opposition to one another with the exception of individuals with insulin resistance and type II diabetes mellitus as previously mentioned (70-71, 104).

Both IL-6 and TNF-α are associated with a downstream signaling cascade of mediators that play a role in the regulation of metabolism. Specifically, IL-6 release is associated with an increase in IL-10, whose role is to inhibit the pro-inflammatory marker IL-1β which is linked to TNF-α release (135). Other cytokines downstream of IL-6 include IL-8 and IL-4 which respectively promote angiogenesis and skeletal muscle satellite cell proliferation (45, 67). IL-6, IL-10, and interferon (INF)-γ have been implicated as anti-inflammatory proteins, however studies have shown conflicting results on the role of IFN- γ in skeletal muscle degradation (110, 126, 146). While these cytokines act in response to the release of IL-6, IL-15 is not directly associated. IL-15 has been shown to increase with skeletal muscle contraction; however, it has not been shown to play a role within the skeletal muscle itself (21). Increases in IL-15 have been associated with reduced white adipose tissue (98, 117). Few studies have examined changes in IL-15 and acute and chronic exercise using a human model (120).

Due to the influence of cytokines on metabolism, the impact of carbohydrate ingestion prior to and during prolonged exercise has been examined. In general, these studies have revealed glucose ingestion during exercise attenuates the IL-6 and downstream response to exercise (39, 100). The implication of carbohydrate ingestion on IL-15 is unknown. Many other ingredients are commonly included in pre-workout supplements. These include, but are not limited to caffeine, creatine, and amino acids. The literature examining the *in vivo* inflammatory response to these additives are limited; however, the response to caffeine, some amino acids, and Vitamin E have been explored in cell culture and rodent models (47, 61, 64-65, 68, 148).

Analysis of the role of caffeine on fitness measures are varied; some revealing this supplement will improve strength, while others find no influence on strength or muscular endurance (11-12, 51, 149-150). Despite this, studies have suggested caffeine is associated with a decrease in circulating TNF-α (47, 65). Similarly, intake of the amino acid glutamine has been linked to an increase in strength following training, as well as a decrease in TNF-α in culture and mouse models (148). Some have revealed increases in strength and power with L-arginine and α-ketoglutarate supplementation; however, none have examined inflammation with their intake (22). B-alanine supplementation is associated with reduced fatigue and improved muscular endurance, but again no research has determined its influence on inflammation (61-62). Creatine supplementation has been widely studied revealing increases in strength, muscular endurance, and lean body mass; however, it too has not been examined from an inflammatory perspective (13, 85, 90, 142).

While studies have examined the skeletal muscle production of myokines during acute and chronic aerobic exercise as well as acute anaerobic exercise, few studies have examined the influence of regular resistance training on the inflammatory response. With pre-workout supplementation becoming increasingly popular, further research on the metabolic role of these products and their influence on resistance training adaptations and the immune response are necessary.

I. Statement of the Problem

Skeletal muscle IL-6 mRNA and thus plasma IL-6 and downstream cytokines as well as IL-15 have been shown to increase in response to acute aerobic and anaerobic exercise, respectively (99, 134). TNF-α and IL1-β however generally do not change with exercise (70). Chronic exercise training has been associated with an increase in IL-6 receptor and thus lower

circulating levels of basal IL-6 (50). To date few studies have examined the inflammatory response to a total body resistance training exercise bout, or a long-term resistance training program. In addition, no studies have examined the influence of a pre-workout supplement containing caffeine, creatine, various amino acids, among other ingredients on the inflammatory response to an acute bout of exercise.

Therefore, the following hypotheses were tested in college aged recreationally trained, healthy males:

1. An acute bout of total-body resistance training will result in an increase in serum IL-6, IL-10, IL-4 as well as an increase in IL-15 within skeletal muscle. TNF-α and IL-1β levels will remain unchanged.

   The pre-workout supplementation will not significantly alter anti or pro-inflammatory cytokines produced during an acute bout of total body resistance training.

2. An 8-week total body resistance training program will result in a decrease in basal serum IL-6, TNF- α and IL-1β, and an increase in skeletal muscle IL-15.

   The pre-workout supplementation will not result in any significant differences in cytokine production.

3. An acute bout of total-body resistance training following an 8-week total body resistance training program will result in an increase in serum IL-6, IL-10, and IL-4. This increase will be significantly lower than before training. The same bout of acute

exercise will be associated with a significantly greater IL-15 production within skeletal muscle.

The pre-workout supplementation will not significantly alter anti or pro-inflammatory cytokines produced during an acute about of total body resistance training following an 8-week resistance training program.

4. The 8-week resistance training program will be associated with an increase in strength and lean body mass.

The pre-workout supplementation will not significantly affect changes in strength or lean body mass.

II. Rational for the Investigation

Recent research has established the role of anti and pro-inflammatory cytokines on metabolism (112). Most research however, has focused on the effect of acute aerobic exercise and chronic aerobic training on inflammatory adaptation. Evaluating the effect of resistance training on the skeletal muscle IL-15 protein and body composition changes will allow for a further understanding of the role of this cytokine. Understanding the skeletal muscle and serum IL-6 and TNF-α response to this exercise could provide insight to proper training programs for individuals suffering from metabolic disorders including type II diabetes mellitus. Finally, supplementation of caffeine and amino acids that have previously shown to decrease the pro-inflammatory cytokine TNF-α will provide insight to possible effects of this supplement on metabolism in recreational weight lifters and its possible pharmacological use in diseased populations.

CHAPTER II

REVIEW OF LITERATURE

The Role of Cytokines in Skeletal Muscle

Research has shown that physically active individuals have lower plasma basal IL-6 than sedentary individuals (40-41, 113). The increase in exercise induced IL-6 mRNA has been shown to decrease with aerobic training, while the IL-6 levels in plasma are generally similar before and after training (42). Examination of rat soleus and extensor digitorum longus (EDL) muscles following eight weeks of aerobic training revealed significant decreases in IL-1β, IL-6, TNF-α, and IL-10 in the EDL (84). Another study demonstrated IL-6 receptor mRNA increases during an acute bout of exercise as well as following ten weeks of training (72). It is the changes in IL-6 receptor that can be associated with any changes in IL-6 due to training (72).

While IL-6 rises in response to acute exercise, the subsequent rise in IL-6 receptor in skeletal muscle tissue during an acute exercise bout has been linked to glucose regulation (50). Research has shown the rise in IL-6 is also associated with increased glucose uptake due to increases in intrinsic glucose transporter, GLUT4 (4, 114). Exercise following training has shown reduced gene expression of IL-6 and IL-6 receptor as well as reduced IL-6 plasma concentration (3). In addition, it has been shown that glucose ingestion during exercise and exercise training reduces the change in IL-6 receptor by blunting the IL-6 response, thus reducing the overall increase in IL-6 receptor following aerobic training (3).

IL-6 release is also associated with an increase in free fatty acids within the blood and an increase in lipolysis (4, 140). Current research suggested skeletal muscle contraction induces an anti-inflammatory effect by the release of IL-6 and its downstream signaling, while at the

same time the adipose tissue has a pro-inflammatory response (122). These differences could be due to increased lipolysis necessary for energy production during exercise (122). Similarly, a previous study demonstrated rats with an IL-6 deficiency develop obesity at greater rates than wild-type rats (145). In addition, IL-6 infusion over 18 days resulted in decreased body weight in the IL-6 deficient rats (145). This further identifies the role of IL-6 in the increase in fatty acids during exercise. The link between IL-6 and substrate metabolism could be adenosine monophosphate-activated protein kinase (AMPK) (4). IL-6 has been shown to increase AMPK in skeletal muscle, which is associated with increases in fatty acid oxidation and glucose uptake (4).

Despite these positive changes elicited by IL-6, the inflammatory marker is also associated with the activation of the protein, suppressor of cytokine signaling (SOCS) (113). SOCS3 increases in the skeletal muscle and even more so in the liver (110). This protein is commonly associated with insulin resistance. These differences in the role of IL-6 have led to confusion concerning the benefit of this marker in the anti-inflammatory response. It has been suggested there is an upper limit to the positive effects of IL-6. Hyperproduction of IL-6 is related to not only insulin resistance and type 2 diabetes, but a myriad of problems related to metabolism including obesity and atherosclerosis. The overproduction of this cytokine occurs along with receptor dysfunction of the glucocorticoid receptor. IL-6 production from adipose tissue also accounts for this overproduction of IL-6, as adipose is the main source of increased IL-6 at rest (87, 97). Release of IL-6 from adipose tissue is not associated with the increase in IL-6 with exercise. Similarly, hyperproduction of IL-6 does not allow for the typical exercise effect on IL-6. In addition, it has been shown that insulin resistance in skeletal muscle may be directly related to fat cell size (125). The Akt/PKB pathway is directly related to glucose uptake into

skeletal muscle. Skurk et al. (85) revealed that secreted markers from isolated fat cells reduce insulin stimulated Akt/PKB phosphorylation contributing to obesity-linked insulin resistance. These changes were seen across several markers secreted from the tested adipocytes including but not limited to IL-6 and IL-8. The difference in the basal levels of IL-6, and the changes with IL-6 in response to exercise could be the keys to distinguishing between detrimental and normal changes in IL-6 production.

IL-6 has also been implicated in the regulation of tumor necrosis factor (TNF)-α. While many have suggested the combination of the two play a role in a variety of diseases, more recent evidence has shown that TNF-α is the pro-inflammatory marker (71). When TNF-α is present there is decreased GLUT4 translocation which is related to decreased skeletal muscle glucose storage (111). TNF-α and IL-1β have been implicated in the cause of muscle damage and wasting in many diseases (123). One of the most significant health-related benefits of increases in IL-6 during exercise and the subsequent increase in IL-6 receptor following training is its role on TNF-α. Research suggests IL-6 release during exercise suppresses TNF-α release (113). One previous study found an acute bout of exercise lowers TNF-α in individuals with high basal levels of TNF-α (71). In fact, IL-6 infusion has been shown to upregulate IL-1ra and TNF receptors. While the decrease in TNF- α is partly contributed to an increase in IL-6, it has been noted that this decrease still occurs in IL-6 deficient mice suggesting another mechanism yet to be determined plays a role in this change (128).

The differences in the influence of TNF-α and IL-6 have further supported the conclusion that anti-inflammatory and pro-inflammatory classes of cytokines exist. Many have studied the complex signaling pathways that contribute to the activation of these markers, but much about this process is still not understood. Further examination of the cytokine response to resistance

training exercise is necessary to more fully understand the role of cytokines within the skeletal muscle.

Cytokine Signaling within Skeletal Muscle

Research within the last decade has revealed the major role of the skeletal muscle in the production and release of cytokines (134). The origin of several cytokines were originally thought to be related to endotoxin stimulated monocytes and macrophages in association with the general inflammatory response to infection (35). It was suggested that the eventual activation of NFκB (nuclear factor of κB) was responsible for the transcription of genes related to cytokines IL-6, TNF-α and IL-1β (82). In this pathway lipopolysaccharide (LPS), a bacterial endotoxin found in the cell wall of Gram-negative bacteria, would bind to Toll-like receptor (TLR)-4 and cause MyD88 (myeloid differentiation primary-response protein 88) to move to its cytoplasmic domain (81). This then caused IRAK-1 (IL-1 receptor associated kinase-1) and TRAF-6 (TNF-α receptor associated factor-6) to activate IkappaB kinase (IKK)-NFκB pathway and this led to the production of IL-6 and other cytokines (82). However, it is now evident that many types of cells outside of the immune system can also produce IL-6. Skeletal muscle in particular has been shown to produce IL-6 in response to reactive oxygen species (ROS), inflammatory cytokines (TNF-α and IL-1β), and during contraction in human and animal models (86). Due to its production within the skeletal muscle, IL-6 is commonly called a myokine (112).

IL-6 production by the skeletal muscle was revealed in 2000 (134). It was suggested this increase is the source of the increase in plasma IL-6 during exercise (134). More recently it has been shown that the increase in IL-6 during exercise is purely due to the skeletal muscle (115). This was revealed when no change was seen in the number, percentage, and mean of IL-6

positive stained monocytes following prolonged cycling (129). A later study by the same laboratory revealed that the number of monocytes staining positive for IL-6 actually decreased following prolonged running (130).

In addition, the IL-6 mRNA in skeletal muscle has been shown to increase up to 100-fold at the end of a prolonged exercise bout (133). The nuclear transcription of IL-6 increases significantly at the start of exercise (73) allowing for an increase in IL-6 at approximately 30 minutes of exercise, and generally peaks when exercise is stopped (133). While increases in IL-6 produced by the skeletal muscle are significant, it is also important to note that limiting IL-6 release using 4 weeks of Vitamin C and E supplementation blocked the IL-6 release from muscle, but only decreased plasma IL-6 by 50% (42). This further proves skeletal muscle significantly contributes to circulating IL-6 but also draws attention to the fact that other cells are responsible for IL-6 production. Although adipose tissue has been associated with releasing IL-6 at rest, it has been shown that these tissues do not release IL-6 during exercise (87). Further research examining the IL-6 production of other cell types during exercise is necessary to fully understand the inflammatory signaling cascade

Several cytokine signaling theories currently exist. The cell signaling that occurs in response to LPS, ROS, and inflammatory cytokines has also been linked to the mitogen-activated protein kinases (MAPKs). During skeletal muscle contraction the role of this pathway could be of particular interest during states of muscular glycogen depletion. It has been previously shown that p38 MAPK is related to physiological stressors including skeletal muscle contraction. Contraction induced increases in stress kinases including c-jun amino-terminal kinase (JNK) 1 and 2 and p38 MAPK increase as the energy supply is low in a glycogen depleted state. It is

speculated that p38 activation may lead to activation of a particular transcription factor that binds to the regulatory region of the IL-6 gene during skeletal muscle contraction (27).

There are conflicting opinions regarding the influence of the NFκB pathway on IL-6 production during skeletal muscle contraction. Studies have revealed when nonsteroid anti-inflammatory drugs, substances known to inhibit the NFκB pathway, are administered during exercise, IL-6 is reduced suggesting this pathway plays an important role in the IL-6 production (78). Others have demonstrated that IκB which plays a role in the IKK-NFκB pathway does not increase the transcription of IL-6, suggesting this pathway does not play a role during contraction. Still others have revealed the NFκB pathway is associated with inhibition of differentiation related to muscle wasting and muscle damage conditions, suggesting this protein is more likely to have a pro-inflammatory effect (80).

Muscle glycogen levels also play a role in IL-6 production. Several studies have revealed IL-6 production in skeletal muscle is greater in tissues that have low glycogen levels (26, 101). When individuals ingest supplemental glucose during exercise the IL-6 release is inhibited, however IL-6 mRNA expression within the working muscle is not affected (39). Research has revealed that IL-6 within the plasma decreases as the IL-6 receptor production decreases, suggesting there is a mechanism of sensitivity of the muscle to the exercise induced effects of IL-6 when IL-6 levels are low (26). It is also suggested that the IL-6 receptor could be involved with energy restoration within the muscle (26). The role of epinephrine in IL-6 production has also been examined. As fuel in the form of carbohydrates is reduced during exercise, there is an increase in the sympathetic response causing an increase in epinephrine. Researchers questioned if this response could be related to the IL-6 response, however recent evidence does not support this theory (111). Still others have suggested that AMPK may be related to IL-6 production

within the skeletal muscle tissue (88). AMPK activity is also associated with low levels of muscle glycogen, and has been shown to be correlated with higher IL-6 production; however, no direct link between these two proteins has been proven.

Calcium activation of nuclear factor of activated T-cell (NFAT) has also been a suggested mechanism associated with IL-6 production during skeletal muscle contraction. It is commonly known that skeletal muscular contraction is associated with the sarcoplasmic reticulum release of calcium. NFAT is generally activated during sustained calcium release. However, studies have yet to demonstrate a significant increase in NFAT during exercise. It has been suggested however that the quick dephosphorylation by calcineurin and rephosphorylation by NFAT kinases and movement to the nucleus does not allow for significant changes in the abundance of this protein at a specific point during or immediately following exercise, making it difficult to examine its relationship with IL-6 production. However, research has shown the calcineurin pathway is upregulated through ionomycin treatment and resulted in an increase in IL-6 in a time and dose dependent manner (103). Cyclosporin A (30), an inhibitor of calcineurin pathway, is associated with an increase in TNF-α. These differences revealed that IL-6 and TNF-α are independently regulated (70), suggesting IL-6 release is directly related to the calcineurin pathway while TNF-α is related to the inactivity of this pathway.

Calcium has further been referred to as a potential regulator of IL-6 production through studies of the inflammatory response to exercise in different skeletal muscle fiber types. In general, these studies have revealed that type II fibers produce more IL-6 than type I fibers over the same time period (59, 122). Hiscock et al. (59) suggested this might be due to signaling of an unknown IL-6 transcription factor as a result of greater calcium influx in the type II contracting tissues. However, if duration is considered, prolonged exercise that relies heavily on

type I skeletal muscle will produce a low amplitude of Ca^{2+} influx which is known to activate calcineurin (9). Research has shown that inhibition of calcineurin using cyclosporin A and FK506 reduced IL-6 activity and thus proved the role of calcineurin on the production of IL-6 (9). These researchers also suggested calcineurin plays a significant role in the transcription of the IL-6 in types I and IIa fibers, as these levels increased as markers of calcineurin activity increased (10).

Still another possible mechanism of IL-6 production within the skeletal muscle exists. Recent studies have suggested that nitric oxide (NO) may also be associated with the regulation of gene transcription in skeletal muscle (132). Nitric oxide is significantly increased in contracting skeletal muscle. Research has shown that inhibiting NO during exercise reduces IL-6 mRNA production and increase of IL-6 protein release from skeletal muscle. Furthermore, the infusion of NO is associated with increased IL-6 in resting skeletal muscle (132).

It is also possible that a combination of these mechanisms is responsible for the increase in IL-6 with exercise. It has been shown that in B lymphocytes, the amplitude and duration of calcium release directly controls activation of NF-κB, c-Jun amino-terminal kinase (JNK), and NFAT (38). NF- κB and JNK are generally activated by large increases in calcium, while NFAT is regulated by longer, sustained calcium release. However, based on research examining IL-6 release in response to various exercise types, Febbraio and Pedersen (38) suggested the IL-6 release is more likely Ca^{2+}/NFAT regulated. In a review of IL-6 signalling pathways, Febbraio suggested the potential roles for each of the mechanisms discussed above (37). He suggested that an increase in Ca^{2+} during exercise activates IL-6 gene transcription. As exercise duration increases, the calcineurin-NFAT pathway is associated with increases in IL-6. In addition Febbraio suggests that increased exercise intensity, marked by a significant increase in Ca^{2+} flux,

activates NF-κB and JNK. Prolonged exercise that results in low glycogen availability is then associated with the activation of p38 MAPK and possibly the activation of NF-κB (37). No matter what the specific cause of the increase in IL-6 may be, research has shown that this cytokine is associated with the regulation of other inflammatory markers.

The Influence of IL-6 on Downstream Cytokine Signaling

It has been suggested that IL-6 is associated with the regulation of several cytokines including TNF-α, IL-1, and IL-10. TNF-α is associated with NF-κB stimulation (80). Specifically, the TNF-α receptor 1 'death domain' interacts with TNF receptor-associated death domain (TRADD), activating fas-associated death domain and kinase receptor interacting protein (110). These proteins are involved with the regulation of apoptosis through the TNF-α related activation of NF-κB. NF-κB is located in the cell cytoplasm bound to the inhibitory protein IκBα, however TNF-α upregulation results in the phosphorylation of IκBα. This allows for the release of IκBα, possibly due to the increase in mitochondrial reactive oxygen species related caused by TNF-α and eventual activation of the ubiquitin proteosome pathway (123).

Many studies have reported that TNF-α does not change during or immediately following prolonged exercise, with the exception of prolonged high intensity exercise such as marathon racing (70, 104, 127). In fact, several reports concerning the relationship between IL-6 and TNF-α now agree that IL-6 actually inhibits TNF-α and IL-1 release, while allowing for the release of IL-1ra and IL-10. However, others have shown that TNF-α has been suppressed in IL-6 knock-out mice, suggesting that while IL-6 may play a role in this process, there is another mechanism that limits TNF-α production at the start of exercise (71). Still others have focused on skeletal muscle fiber type and differences in cytokine expression. Rosa Neto et al. (122)

recently found TNF-α mRNA expression increased following exhaustive exercise in the extensor digitorum longus (EDL) but not soleus muscles in rats.

The absence of IL-6, TNF-α and IL-1β have been implicated in the repression of hormones related to skeletal muscle growth, specifically growth hormone and insulin-like growth factor-1 (75, 135). These changes are associated with a reduced protein expression throughout the body, including the reduced expression of differentiation factors specific to muscle. The activation of TNF-α receptor 1 (TNF-R1) and IL-1 receptor 1 (IL-1R1) are associated with the activation of the JNK pathway which causes resistance to IGF-1, among other hormones (135). Recently it has been shown that the presence of IL-1 in skeletal muscle myotubes is associated with an activation of p-38 MAPK and NF-κB signaling (80). In this specific study, the exposure to IL-1 over 48 hours resulted in a reduction in the width of the myotubes as well as a reduction in actin within the sarcomere. Kelley et al. (75) suggested this is likely due to IL-1β inhibition of typical IGF-1 signaling. Strle et al.(135), however; revealed IL-10 inhibits the function of IL-1β thus allowing for normal IGF-1 signaling. Since it has been previously shown IL-6 release from skeletal muscle during contraction is associated with an increase in serum IL-10 (131), it is possible than IL-6 actually contributes to the normal IGF-1 response.

Still other studies have suggested TNF-α may have a positive role on skeletal muscle growth. Plaisance et al. (116) demonstrated incubation of C2C12 myotubes with TNF-α for 24 hours increased protein synthesis. This increase was demonstrated by an increase in ERK1/2 and downstream targets as well as an increase in Akt and downstream targets GSK-3, p70s6k, and 4E-BP1. This upregulation was decreased when an inhibitor of TNF-α was administered. Similarly, when rapamycin was used, while mTOR was inhibited, there was no effect on the upregulation of P13K-Akt or MED-ERK activation due to the increased TNF-α. These findings

reveal more research is needed examining the influence of these cytokines on skeletal muscle function. This is contrary to previous work by de Alvaro et al.(34) which suggested the phosphorylation of p-38 resulted in the impaired activation of the PI3K-Akt signaling and thus glucose uptake, leading to insulin resistance.

Previous research has demonstrated that an increase in IL-10 via LPS is associated with an inhibition of IL-1α, IL-1β, IL-6, IL-8, and TNF-α gene transcription (35) suggesting IL-6 and IL-8 are targeted by IL-10 as well. Within the skeletal muscle however evidence has suggested this is not the case. Recently IL-6, IL-8, and IL-10 are considered anti-inflammatory cytokines, providing further evidence that the increase in cytokines following exercise is not the same as the cytokine response that is initiated by an infection (121). IL-10 has been associated with inhibiting the expression of proinflammatory cytokines as well as their receptors. Recent evidence has revealed IL-10 may specifically act to block the IL-1β phosphorylation of JNK, but not other pathways in MAPK signaling (135). This suggests IL-10 may play a role in preventing IGF-1 resistance and thus insulin resistance. However, again recent evidence suggests the response of this cytokine also might be a fiber type specific response, as IL-10 has been shown to increase in EDL rat muscle following extensive exercise without changes in soleus tissue (122).

Early studies of the effect of exercise on the inflammatory response demonstrated an increase in plasma IL-1 up to five days post eccentric aerobic exercise suggesting that this cytokine my play a role in the catabolic or anabolic process within the muscle (23). However, the release of IL-6 has been shown to be associated with inhibition of IL-1 (121). As previously discussed, IL-1β is a pro-inflammatory protein that often leads to protein degradation. However, IL-1ra blocks the binding of IL-1α and IL-1β to IL-1 receptors and effectively inhibits the effects of IL-1. IL-6 is considered the major inducer of IL-1ra. It is possible that earlier studies

exhibiting an increase in IL-1 for several days post exercise did not distinguish between IL-1ra and IL-1β (46).

IFN-γ is another cytokine associated with the adaptive immune response and commonly associated with the immune response to exercise. The role of IFN-γ, and whether this cytokine is involved in the inflammatory or anti-inflammatory process, is debated. Many have noted this cytokine is often increased with TNF-α in mouse and culture tissues (110, 146). The presence of TNF-α and INF-γ in myotubules have resulted in a decrease in myosin mRNA expression (1). Many assumed this suggested IFN-γ is an inflammatory cytokine (110, 126, 146). However, recent evidence suggests these two cytokines do not act in the same manner (28, 126). It has been reported that treatment with INF- γ did not affect reactive oxygen species activity, which has been demonstrated to be a common occurrence with an increase in TNF-α (126). In addition, 72 hours of IFN-γ did not result in any change in the MHC content. This evidence suggests IFN-γ and TNF-α may not play a similar role in the inflammatory response (126). In addition, this demonstrates that IFN-γ may not have a catabolic role.

An early study examining the inflammatory response to prolonged running revealed no change in IFN-γ post exercise (137). However, Cheng et al. (28) recently reported IFN-γ is expressed in both mRNA and protein levels in myoblasts within damaged muscle. This suggests the muscle itself may produce this cytokine following injury. This study also revealed blocking IFN-γ receptor in mice was associated with reduced myotubule proliferation as well as reduced fiber regeneration (28). In addition, following a more severe injury of the muscle such as a laceration, IFN-γ has been shown to improve the healing of the muscle while reducing fibrosis, or scar tissue formation (44). These findings suggest IFN-γ may be necessary for muscle

growth. It has also been suggested the immune role of this cytokine includes macrophage activation, antiviral and antitumor activity, as well as the production of free radicals (17).

The production of free radicals may reveal a role for IFN-γ following exercise or muscle damage. IFN-γ signalling is directly related to the JAK-STAT signaling from the IFN-γ receptor complex (109). More specifically, ligand binding of this complex activates Jak1 and Jak2 and the dissociation of STAT1 from this complex. STAT1 then translocates to the nucleus and binds to the gamma activating site (GAS) inducing transcription of IFN-γ promoters. One such promoter is IRF-1 which is responsible for identifying the interferon stimulation response element (ISRE) which also is associated with the expression of IFN-γ and subsequent free radical production (110).

As discussed, it has been demonstrated that IL-6 produced within the skeletal muscle in response to contraction produces a downstream signaling of cytokines related to the inflammatory state. The production and release of these markers are associated with the signaling crucial to tissue repair. The differences in the release of these IL-6 are often related to the duration, intensity, and type of the exercise stimulus.

Influence of Exercise Duration, Intensity, and Mode on Cytokine Signaling

The influence of exercise on the cytokine response has largely focused on the role of IL-6, as this myokine is the first activated and elicits the most pronounced increase during exercise. In addition, IL-6 is associated with the increase of several other cytokines as well as an inhibition of pro-inflammatory markers. Serum levels of IL-6 have been shown to significantly increase during prolonged aerobic exercise in active and inactive individuals (18-19, 49, 73-74, 87, 105-106, 108, 133-134, 139). These changes were shown to be greater in a nonactive population (49). An examination of five minutes of intense aerobic exercise showed a slight, but not

significant increase in IL-6, suggesting the duration of exercise might be key to changes in IL-6 (18). Studies of prolonged aerobic exercise have all shown an increase in IL-6 however the duration of that increase varies. Many studies have revealed IL-6 peaks at the end of exercise (105, 133). One study revealed IL-6 returned to baseline after one hour of recovery from 60 minutes of cycling at 70% of VO_{2max} (139). Still others have reported IL-6 has a more gradual decrease over time, with IL-6 declining over a four hour recovery period following two or more hours of exercise (106, 108, 127). Another study even reported a peak in IL-6 at three hours post exercise (18). In general, only exercise lasting more than one hour has been shown to result in 10-fold increases in IL-6 (112). Some have suggested that the intensity as opposed to the duration of the exercise might be the key in explaining these differences (108).

Changes in IL-6 during and following anaerobic exercise have been debated. IL-6 has been shown to increase immediately during anaerobic sprint intervals (95). Another study showed that circuit weight training (5 exercises performed at 3 sets of 10 repetitions) resulted in an increase in IL-6; however, this was not significant (19). A recent study found no significant changes in plasma IL-6 following resistance exercise bouts of 50%, 75%, 90%, and 110% of one-repetition maximum for the bench press exercise (138). Some have suggested the increase in IL-6 following exercise is independent of muscle damage (18, 127); although this is debated (19).

Others have examined changes that occur with cytokines and regular training. Main (91) examined significant changes in serum IL-10, TNF-α, and IL-12 over the course of a 12 week training period in elite rowers. These cytokines, as well as IL-6 gradually increased over time peaking just before a taper began. IL-1β levels, while not significant, were at their lowest at this point. Significant changes were also noted between cytokines as exercise duration and intensity

changed. Specifically, a significant interaction was observed between training distance and training duration for all measured cytokines except for IL-6 and IL-1β. Only one study to date has examined the influence of regular resistance training on the serum cytokine response. This study revealed an acute bout of exercise following resistance training results in an increase in IL-6, IL-1ra, and IL-10 as well as the pro-inflammatory IL-1β (69). These findings however are in contrast to previous analysis of aerobic training. Fischer et al. (42) found skeletal muscle mRNA for IL-6 is actually decreased following aerobic training, and plasma IL-6 is generally the same before and after training. Keller et al. (72) revealed an acute bout of exercise following training results in an increase of IL-6 receptor mRNA suggested any changes in IL-6 as a result of training are actually due to changes in the IL-6 receptor.

Some have suggested that the type of exercise performed effects the inflammatory response to exercise. For instance some studies have suggested that exercises that focus on areas of smaller muscle mass may result in a significant increase in IL-6 (58, 102, 138). It has been suggested that running causes the greatest inflammatory response (112). Other studies have suggested eccentric exercise which is commonly associated with muscle damage, does not cause significantly greater IL-6 response than concentric exercise but may affect the recovery process (89, 147). These studies revealed plasma IL-6 may peak up to six hours following eccentric exercise and may take up to 96 hours or more to return to baseline levels; both of which are longer time periods than are typical for other types of exercise. To date, no studies have examined the local inflammatory response to a resistance training program. However previous information suggests type, duration, and intensity of exercise all play a significant role in the IL-6 response.

Additional Myokines: IL-8, IL-15, IL-4

IL-8

While much of the literature has focused on IL-6, two other anti-inflammatory markers, IL-8 and IL-15, are also considered myokines as evidence has shown they are also produced within the skeletal muscle (112). As previously discussed, NO production is associated with IL-6 production. Evidence has also shown that NO is also associated with the transcription of IL-8 in skeletal muscle (132). Akerstrom et al. (2) revealed increases in IL-8 mRNA following three hours of cycling and the same response after three hours of knee extensor exercise at 60% of maximum. Similarly, Frydelund-Larsen et al. (45) showed IL-8 receptor protein was elevated levels at 3, 4.5, 6, 9, and 24 hours following three hours of cycling. Despite changes within the skeletal muscle, neither study found significant changes in plasma IL-8 suggesting this myokine has an immediate localized effect (2, 45). However, post marathon plasma IL-8 has been shown to peak 0.5 hours after exercise (107). Frydelund-Larsen et al. (45) also examined CD105, the TNF-β receptor which is expressed in endothelial cells in response to hypoxia (exercise), and IL-8 receptor protein. Their study revealed CD105 was coexpressed with IL-8 receptor protein levels. This finding suggests IL-8 is primarily located within the microvascular endothelial cells and may play a role in angiogenesis within the skeletal muscle.

IL-15

Previous evidence has indicated that IL-15 is the most prevalent cytokine in skeletal muscle, and has the highest IL-15 content of the body (120). In contrast to the role of IL-8, IL-15 is associated with decreased protein degradation within the skeletal muscle. Specifically, IL-15 has been shown to decrease the activity of the ubiquitin/proteasome pathway (21). Busquets et al. (21) further state IL-15 does not play a role in an increase in protein synthesis. In fact, IL-

15 may play a role in the inhibition of TNF-α releated apoptosis. However, increases in IL-15 mRNA have been expressed in skeletal muscle following an acute bout of resistance exercise in untrained individuals (97). This increase took place without increases in skeletal muscle or plasma IL-15 protein. Quinn et al. (119) had also previously demonstrated that IL-15 stimulated a five-fold increase in myosin heavy chain (MHC) expression in already differentiated myocytes. This increase in MHC occurred in the absence of IGF-1 suggesting that this change must occur through another mechanism. These findings reveal IL-15 could play a role in the skeletal muscle preservation during periods of disease when IGF-1 levels are low including cancer or sepsis (118). Another study revealed an increase in plasma IL-15 protein immediately following acute resistance training exercises, as well as following chronic training (120). This study examined the influence of 10 weeks of resistance training involving 3 sets of 6-10 repeptitions of 13 exercises. Skeletal muscle hypertrophy following training has been linked to changes within specific untranslated regions within the IL-15ra gene, suggesting the presence of this myokine effects phenotype (120).

More recently, several studies have suggested IL-15 may play a significant role in the regulation of body composition (5, 24, 100, 117). Carbo et al. (24) revealed that seven days of IL-15 administration to rats resulted in no change in muscle mass but a 33% reduction in white adipose tissue and decreased lipoprotein lipase. Alvarez et al. (5) revealed similar findings in a later study of leptin receptor negative and leptin deficient rats. Later analysis of transgenic mice that overexpressed IL-15 demonstrated no change within the skeletal muscle, but revealed significant decreases in body fat and increases in bone mineral density without changes in any other cytokines (117). Nielsen et al. (98) examined the relationship between IL-15 mRNA expression, plasma IL-15 and obesity levels in a cohort of 199 individuals to reveal a negative

relationship between fat mass and circulating IL-15. Within the same study, the overexpression of IL-15 in mice revealed a decreased body fat accumulation in the trunk with no change in subcutaneous fat. Further research is needed examining the changes in IL-15 and body composition following exercise programs in human models.

IL-4

Although IL-4 is not yet deemed a myokine, recent evidence suggests it may also be produced within the skeletal muscle. Few studies reporting the inflammatory response to exercise have examined IL-4. However, Horsley et al. (67) reported IL-4 played a role in myogenesis when using a transgenic mouse model. More recently, Lafreniere et al. (79) revealed in human cells IL-4 plays a role in the second step of myogenesis during which myoblasts fuse with myofibers, and thus suggesting IL-4 contributes to hypertrophy of the skeletal muscle. In addition, these researchers demonstrated IL-4 also attracts other cell types, as well as myogenic precursor cells to the site of muscle injury; thus, decreasing the myoblast migration rate. The upregulation of IL-4 in skeletal muscle is associated with the calcineurin/NFAT pathway (66, 152). Specifically, the activation of NFATc2 is associated with myotubular growth and results in the activation of IL-4 transcription (66).

The study of IL-8, IL-15, and IL-4 as myokines has been a relatively recent development when compared to the study of IL-6. Little is known about the influence of exercise mode, frequency, intensity, and duration on these proteins. Similarly, examination of pathways responsible for the increase in these proteins is warranted.

Pre-Workout Nutritional Supplementation and the Cytokine Response

Nutritional supplements are often consumed before or during both aerobic and anaerobic events to improve performance. These supplements can range from energy sources including various forms of carbohydrates and proteins, to vitamins, minerals, amino acids and many other varieties. Few studies have examined the use of these substances prior to exercise and their influence on the inflammatory response.

Carbohydrates

Carbohydrates are commonly ingested prior to as well as during long-term aerobic exercise as a means to prevent skeletal muscle glycogen depletion and thus reduce amino acid oxidation (54, 144, 151). Van Loon et al. (141) suggested that carbohydrate supplementation every thirty minutes during prolonged cycling actually resulted in an increase in glycogen synthesis. In addition, glucose supplementation has been shown to improve attention as well as mood and reduce overall fatigue during prolonged physical activity (83).

Ingestion of carbohydrates before or during a resistance training workout has been associated with an increase in post-exercise insulin and growth hormone levels which can be linked to an increase in protein synthesis (29). In addition, Haff et al. (52) concluded that carbohydrate ingestion (0.3g/kg body weight) prior to a resistance training bout improved performance of multiple squat sets performed four hours post training. Haff et al. (53) later revealed carbohydrate consumption prior to isokinetic leg exercise significantly improves performance as noted by increases in total and average work. Additionally, plasma glucose was significantly higher in the supplementation group while no changes in free fatty acids or lactic acid were revealed.

As previously mentioned, carbohydrate ingestion prior to exercise has been shown to significantly influence the inflammatory state. More specifically, consumption of a 6% carbohydrate drink before and every 15 minutes during 2.5 hours of exercise results in a significantly different inflammatory response than consumption of a placebo (101). This study revealed higher plasma glucose and insulin accompanied by lower levels of IL-6 and IL-1ra cytokines with carbohydrate supplementation. Similarly, Stanley Chan et al.(25) revealed individuals in a glycogen deprived state produced higher levels of IL-6 and IL-8 mRNA during and following 60 minutes of cycling. No significant changes were found in IL-1β, IL-15, and TNF-α. Febbraio et al. (38) had previously revealed similar findings and also showed IL-6 release was attenuated in subjects who ingested a 6.4% carbohydrate solution at 15 minute intervals throughout the exercise. Despite this, there was no increase in skeletal muscle IL-6 mRNA expression. Fewer studies have analyzed the cytokine response to carbohydrate supplementation and resistance training. Koch et al. (77) found carbohydrate intake resulted in increased post exercise plasma glucose without significant differences in the lymphocyte response to resistance training; however, intramuscular or plasma cytokines were not examined.

Caffeine

Caffeine is also a common ingredient in pre-workout supplements. This common ingredient is a form of methylxanthines which are known antagonists to adenosine receptors (32). While many supplements list caffeine as an ingredient, others list methylxanthines. Past research on the effects of caffeine consumption on performance has produced conflicting results. Early studies of caffeine supplementation revealed caffeine ingestion prior to aerobic exercise significantly improved performance (15, 31). Bergland et al. revealed caffeine consumption of 6mg/kg body weight resulted in an improved 21km cross country race times at both low and high

altitudes (15). Similarly, Costill et al. (31) noted that 330mg of caffeine consumption one hour prior to cycling resulted in a longer time to exhaustion. In addition, caffeine intake was associated with increased lipolysis and lower ratings of perceived exersion.

Later studies of the effects of caffeine have focused on its influence on strength and power. Woolf et al. (149) demonstrated an increase in peak power during Wingate ergometer testing as well as an increase in bench press 1-RM in well-trained athletes following ingestion of a shake containing 5mg/kg body weight of caffeine and 0.125g/kg body weight of carbohydrates. Green et al. (51) found ingestion of 6mg/kg body weight of caffeine 1 hour prior to exercise reduced fatigue in well-trained subjects. These subjects were asked to complete 3 sets of leg press to fatigue at a weight equal to their 10-RM. Those who had ingested the caffeine were able to complete more repetitions during their final set of the exercise than those who had consumed a placebo.

Despite these studies suggesting caffeine aids in performance enhancement, many studies have revealed conflicting data. For example, Woolf et al. (150) found no difference in 40 yard dash, 20 yard shuttle, or 1-RM bench press performance in collegiate football players following 5mg/kg body weight of caffeine. Similarly, Beck et al. (12) found no change in leg press 1-RM or endurance or bench press endurance following a supplement containing yerba mate, guarana, and black tea extract, all of which contain caffeine. However, this study did reveal and increase in bench press 1-RM. In other studies, caffeine did not significantly change 1-RM bench press, running time to exhaustion, VO_2peak or body composition (11 7, 92). While many factors may have played a role in these differing results, Fisher et al. (43) suggested the subjects' tolerance to caffeine may explain why the findings from these studies have varied. This study revealed that individuals who habitually had caffeine in their diet (788±75mg/day) acquired a tolerance to the

substance. These individuals did not see any significant changes in aerobic performance following the consumption of 5mg/kg body weight of caffeine.

While these previous studies examined the role of caffeine in aerobic and anaerobic measures, fewer studies have looked at the influence of caffeine on markers of inflammation. One study examined the influence of caffeine and paraxanthine, a byproduct of caffeine within the body, on cytokine and TNF-α response (65). A cell culture model demonstrated that when these substances are added in an amount typical of human consumption to blood mononuclear cells, TNF-α was significantly reduced. In addition, no changes in IL-10 or IL-1β were found. Dray et al. (36) revealed three different doses of caffeine, all typical of human consumption, resulted in a decrease in TNF-α gene expression in human adipocytes in culture. Similarly, Geraets et al. (47) found a decrease in TNF-α gene expression at 4 hours and 24 hours post treatment of 1,7 dimethylxanthine, a byproduct of paraxanthine. This study also showed a decrease in TNF-α in patients suffering from chronic obstructive pulmonary disorder. It was suggested these changes were associated with a disruption of the NFκB pathway. Finally, in healthy subjects, 1,7 dimethylxanthine consumption was associated with a decrease in IL-6. To date, no studies have examined the influence of caffeine and exercise on markers of inflammation.

Creatine

The use of creatine supplementation has been extensively studied (13, 56, 85, 90, 143). These studies have examined the role of creatine in skeletal muscle hypertrophy, strength and endurance. No studies to date have examined creatine supplementation and inflammation. Many creatine studies employ both a loading and a maintenance supplementation phase. In general, this consists of 20g of creatine monophosphate/day for a period of 5 days followed by 5-10g of

creatine/day for several weeks of training. These phases of supplementation have been associated with increases in strength and body mass (13, 143). Creatine supplementation has also been associated with increased muscular endurance as measured by bench press fatigue (56, 85). However, other studies have shown increases in strength with lower creatine doses (20, 56, 64, 85). In addition, Herda et al. (56) compared 30 days of 5g of creatine supplementation with low (1.25g/day) and high (2.50g/day) polyethylene glycosylated (PEG) creatine to find strength increased in all groups. Despite this, no change in body mass, power, or endurance were observed in those consuming PEG creatine.

Changes in strength associated with creatine supplementation are associated with increases the intracellular stores of total creatine which contributes to an increase in anaerobic capacity and muscle mass when supplementing a resistance training program (109). This increase has been linked to skeletal muscle hypertrophy due to an increase in growth hormone delivery, contractile protein synthesis, and reduced protein catabolism (109). Despite positive changes in overall body mass, lean body mass, muscular strength and endurance following creatine supplementation, these studies have not shown changes in body fat percentage. Others have suggested if the supplementation dose not adequately increase the skeletal muscle creatine stores, these changes will not occur. While many studies examining creatine supplementation have revealed positive performance adaptations, still others have revealed differing results, and it has been suggested inadequate creatine concentration within the muscle has been the differing factor. Other studies have also suggested the use of small samples of elite athletes and resistance training programs not designed to increase strength may also explain the differences found in these studies.

Amino Acids

Pre-workout supplements commonly include a combination of the above ingredients as well as other nutrients including amino acids. The amino acids of interest in the current study include arginine, glutamine, tyrosine, and alanine. L-Arginine has been suggested to be involved in protein synthesis including creatine synthesis, and the removal of ammonia. Supplementation of high doses of L-arginine (30g intravenous infusion) has been shown to be a physiological precursor of nitric oxide (NO), a mediator of vasodilation (16). Smaller doses of L-arginine (3g/day) have been linked to a decrease in exercise induced plasma lactate and an increase in L-citrulline (124). These authors suggested the inverse relationship between L-citrulline and lactate support the theory that L-arginine increases the nitric oxide pathway during exercise. Another study examined the influence of L-arginine and α-ketoglutarate on strength and power changes following an 8 week supplementation and resistance training program (22). This study revealed supplementation of 6g of L-arginine and 6g of α-ketoglutarate per day for 8 weeks resulted in an increase in strength as measured by 1-RM bench press as well as an increase in power as determined by Wingate testing. Despite these positive changes, no changes in body composition (total mass, fat mass, lean body mass, or body fat) or aerobic capacity were observed in this study. To date, no studies have examined the influence of L-arginine on inflammation.

Glutamine is commonly included in pre-workout supplements as depletion of this amino acid has been associated with an increase in muscle catabolism (6). Thus, supplementing glutamine is an attempt to reduce the catabolism during the exercise bout. Recent evidence has shown that glutamine supplementation of 0.9g/kg of LBM per day during a six week resistance training program result in significant increases in strength or LBM when compared to changes

within a placebo group. However, glutamine has been closely linked to leukocyte function and may play a central role in immune function. Specifically, several studies have examined the role of this amino acid on cytokines. In a cell culture sepsis model, glutamine was associated with decreased TNF-α in circulation and an increase in heat shock protein 72. A similar study revealed high levels of glutamine was associated with decreased TNF-α at both 4 and 24 hours following the addition of the amino acid to culture (148). Meador et al. (94) examined glutamine supplementation and force production using a mouse model. In this study, plantar flexor isometric force production was measured two hours after LPS injection. This resulted in a 33% decrease in maximal force production and an increase in TNF-α and IL-6. However, when glutamine was supplemented at 1g/kg BW, there was no significant change in pre to post force measures. TNF-α levels were the same while IL-6 levels were lower than the non-supplemented trial suggesting glutamine plays a role in maintaining force production during acute inflammation. In contrast, Hiscock et al. (60) suggested 3.5g glutamine ingestion at 60 minutes and 105 minutes into a cycling bout did not significantly change IL-6 production. It is possible that these differences in IL-6 release following supplementation could be due to the type of exercise employed, the model used, or the supplement dose. Further study is necessary to determine the role of glutamine on the inflammatory response to exercise.

As previously stated, alanine and taurine are other amino acids commonly included in pre-exercise supplements. Alanine has been associated with an increase in intramuscular carnosine, which is associated with pH buffering as well as the regulation of calcium sensitivity and electron coupling (55). This study revealed both lower doses (3.2g/d) and moderate doses (6.4g/day) of β-alanine for four weeks resulted in significant increases in carnosine. Similarly, Hill et al. (57) revealed an increase in carnosine following 10 weeks of β-alanine

supplementation in cyclists. Hoffman et al. (61) examined 4.8g/day of β-alanine supplementation on an acute bout of resistance exercise (6 sets of 12 repetitions of the squat at 70% 1-RM). Following supplementation subjects were able to perform approximately 22% more repetitions of the squat exercise, suggesting β-alanine aided in improvements in muscular endurance. Similarly, an study of three weeks of β-alanine supplementation in college football athletes resulted in an increase in bench press training volume and lower subjective feelings of fatigue among athletes (62). Despite these changes, no differences were seen in fatigue ratings following high intensity exercise. However, Kendrick et al. (76) examined 10 weeks of resistance training and β-alanine supplementation (6.4g/day) and found no significant differences in muscular endurance, strength or body composition. When comparing the effects of β-alanine and taurine supplementation and a downhill running muscle damage protocol in rats, Dawson et al. (33) revealed taurine was associated with an increase in circulating glutamate, a decrease in lactate dehydrogenase, and improved running performance. β-alanine was also associated with a decrease in lactate dehydrogenase but also a significant decrease in body weight following 24 hours of exercise. The authors concluded that this data suggested taurine has a cytoprotective effect to attenuate muscle injury. To date, no research has provided information concerning the role of these amino acids on inflammation.

Additional Ingredients

Other common pre-workout supplement ingredients of interest are betaine, citrulline, Vitamin E and tyrosine. Few studies have examined the role of betaine on performance, however Hoffman et al. (63) revealed 1.25g ingested twice a day for 15 days resulted in an improved lower body muscular endurance as noted during a fatiguing squat protocol. No differences were seen in bench press endurance or bench press throw, vertical jump, or Wingate

power tests. Similarly, Maresh et al. (93) did not find any significant differences in lower or upper body endurance, isometric bench or squat measures, vertical jump or bench press power following 15 days of betaine supplementation.

Citrulline malate has been associated with an increase in oxidative energy production in individuals with fatigue (14). This increase in aerobic function following 15 days of supplementation could have been associated with an increased malate supply and therefore increase in ATP production from the tricarboxylic acid cycle. Giannesini et al. (48) also found a decrease in pathological fatigue in rats supplementing 1g creatine malate/kg of body weight three times per day over a 48 hour time period following LPS injection. In a human model, Sureda et al. (136) found that supplemental L-citrulline prior to a cycling stage race increase plasma arginine availability and thus increased NO synthesis in peripheral blood mononuclear cells. This study suggests that citrulline may be a beneficial supplement for aerobic exercise, yet few studies have focused on its role in resistance training.

Few studies have examined the supplemental roles of Vitamin E and tyrosine. One study did reveal 100mg/kg of body weight of tyrosine ingestion decreased symptoms, improved mood, and reduced performance impairments in individuals placed under cold and hypoxia stress for 4.5 hours (8). While no studies have examined the roles of betaine, citrulline, or tyrosine and the inflammatory response, Vitamin E has been shown to have a beneficial role. Specifically, 48 days of ingestion of 800IU/day was associated with a reduction in IL-1β release following a downhill running bout (97). Despite this there were no differences in TNF-α release between supplement and placebo groups. Another study examined three days of Vitamin E supplementation in skeletal and cardiac tissue in culture and determined this exposure decreased IL-6 and IL-1β response to LPS and prevented NFκB activation in these tissues (68). Further

research is necessary to determine the role of many ingredients included in common supplements on inflammation.

Conclusion

In summary, the current understanding of the inflammatory response includes a role for skeletal muscle in the production and release of many cytokines including IL-6, IL-8, IL-15 and possibly IL-4. The release of IL-6 has been shown to result in downstream effects on other markers of inflammation including IL-10, TNF-α and IL-1β. These cytokines play important roles in metabolism regulation. Because myokines are produced within the skeletal muscle, exercise has been shown to influence the presence of these markers. While most research has examined the roles of duration, frequency, intensity and mode of exercise on the inflammatory state, few studies have examined the influence of a total body resistance bout, or chronic resistance training on plasma or tissue levels of the aforementioned cytokines. Furthermore, the influence of pre-workout supplements on the inflammatory state remains to be determined. Caffeine and glutamine supplementation have previously been associated with decreases in TNF-α. The influence of Vitamin E on TNF- α has also been examined, however no role has been determined. To date, no studies have examined the roles of other common supplement ingredients including creatine, tyrosine, or the amino acids arginine, alanine, or taurine. Similarly, the influence of a combination of these ingredients on adaptations to acute or chronic resistance training has not previously been examined.

CHAPTER III

METHODS and PROCEDURES

Experimental Design

Thirty college aged, recreationally trained subjects were randomly assigned to either supplement (SUP) or placebo (PCBO) groups. The study was double blind in nature as only the researcher preparing the supplement and placebo drinks was aware of the individuals who were assigned to either group. The nine week study consisted of a two pre-testing sessions during week one, followed by eight weeks of training and testing. A timeline of the testing and training procedures can be seen in Figure 1. All subjects completed pre-testing strength and body composition testing the week prior to the training program start. Subjects were asked to complete a log of their food and drink consumption for three days prior to the first training day. During the first day of the training program subjects reported to the Applied Physiology Laboratory (APL) following a four hour fast. Blood samples and muscle tissue samples were obtained before exercise. Subjects then consumed either the SUP or PCBO drink provided to them and waited fifteen minutes to begin their workout. Fifteen minutes following the workout an additional blood draw and muscle biopsy were performed.

Following the first training day subjects reported to the APL three days per week for their training sessions. Prior to each workout subjects consumed their assigned drink (SUP or PCBO) and rested fifteen minutes until beginning their workout. Workouts completed were primarily designed to increase skeletal muscle hypertrophy by using a combination of core and accessory lifts.

Testing of the 1-RM was again completed at the beginning of week five. This data allowed for the proper progression of the subjects' training sessions. Strength and body composition post testing were again completed during the final week of the study. Prior to the final day of training subjects again were asked to complete a three-day food and drink log and report to the APL following an overnight fast. Muscle biopsies and blood draws were again obtained before and fifteen minutes after the SUP/PCBO drink and workout session. The workout performed was the same workout that was performed during the first day of training however the loads were changed based on the subjects' new 1-RM values.

Subjects

Thirty, non-smoking, recreationally active college aged males (*n*=30) volunteered to participate in this investigation. All subjects gave written informed consent to the study protocol which was in accordance with the Declaration of Helsinki and approved by the Human Subjects Committee, Lawrence KS requirements (see Appendix A). Subjects health history, exposure to materials used in the biopsy protocol, physical activity level, and caffeine intake were screened via questionnaires prior to their admission to the study (see Appendices B, C, D, E). Individuals were selected as participants if they met the following inclusionary criteria: between 18 and 35 years of age; exercised 3 days per week for at least 1 year; non-obese; non-smoker; no history of cardiovascular or metabolic disease risk; no history of computed tomography (CT), PET, or nuclear medicine studies within the last year; no history of allergies to local anesthetics, adhesives, or ingredients in the placebo or supplement; no history of adverse reactions to caffeine; and no use of anti-inflammatory medications or medications that may adversely react with the placebo or supplement.

Testing Protocol

All subjects completed two pre-testing sessions prior to the beginning of the training program. The first day involved 1-RM testing of the squat, bench press, leg extension, leg curl, and lat pulldown exercises. Subjects performed warm-ups of 8-10 repetitions at 70% of their predicted 1-RM and 3-5 repetitions at 85% of their predicted 1-RM. Subjects were given 2 minute rest periods between warm-up sets and 3-4 minutes rest between maximal effort attempts. On the second day of pre-testing, body composition measurements were taken. Individuals' weight was measured using an electronic scale, height was measured using a stadiometer, and body composition was determined using a Lunar Prodigy Dual X-Ray Absorptiometry (DXA) Scan (General Electric, Waukesha, WI).

During the first week of the training program, subjects completed additional 1-RM testing. This testing took place on the second training day of the week and involved testing exercises that would only be performed during the second training day of each week for the remainder of the program. The exercises tested on this day included the dead lift, incline press, and bicep curl. The warm-up and 1-RM protocol followed the same guidelines previously described.

Mid-point testing was completed five weeks into the training program, 1-RM for the squat, bench press, leg extension, and leg curl were again measured. These measurements were taken during the first training day that week. Following their 1-RM testing, subjects then completed the remainder of their workout. Subjects' weight was again measured at this point.

Post-testing occurred during weeks seven and eight of training. During week seven of the program 1-RM testing was completed for the dead lift, incline bench, and bicep curl exercises on the second training day. Immediately following testing subjects completed the

remainder of their workout for the day. During week eight of the training program, subjects repeated the pre-testing procedures during days 1 and 2 of their training week. Again, during the first day 1-RM was determined for squat, bench press, leg extension, leg curl and lat pulldown. During the second day weight and height were again measured and post-training body composition was determined using the DXA scan.

Training Protocol.

The goal of the exercise training program was to increase muscular hypertrophy and strength. The exercises performed varied with each day, however each day included both core and accessory lifts. Day one and day three of each week were similar and included back squats, bench press, leg extension, leg curl, lat pulldowns, a back or shoulder exercise and weighted abdominal exercises. Day two of the training program included dead lift, incline bench press, split legged squats, bicep curls, pull-ups, dips, and abdominal exercises. The detailed list of the exercises included in the program is presented in Table 3-1.

The exercises that were considered core exercises, or those of the main focus of the program, were the squat, bench press, leg extension, leg curl, lat pulldown, deadlift, incline press, and bicep curls. The weight lifted for these exercises was based on the individuals' 1-RM. The weight for weeks 1-4 were based on the pre-testing 1-RM. Following the mid-point testing at week 5, the weight was adjusted according to changes in the new 1-RM. Finally, the last workout session was adjusted according to the post-testing 1-RM. The percentage of the 1-RM lifted for these exercises varied week to week to progressively overload the musculature and allow adaptation. A breakdown of the weekly training volume can be found in Table 3-2 and Table 3-3.

The accessory exercises included standing overhead press, pull-ups, dips, split legged squat, high pull, and abdominal exercises. However, following mid-point testing many subjects were having difficulty lifting a load associated with their new 1-RM for the lat pulldown exercises, so these were moved to the accessory category with the exception of the final workout. For their accessory lifts, subjects were instructed to lift a weight that would fatigue them in 12 to 15 repetitions. That is, the subject could not possibly lift the weight more than 15 times. For the pull-up and dip exercises subjects were allowed to use an assisted machine if needed, while others were instructed to add weight when their body weight became too easy. Subjects were instructed to take approximately 90 seconds to two minutes rest between all sets of both core and accessory exercises.

All exercise sessions were monitored by individuals working in the APL. Initially, subjects were trained on a one-on-one basis. However, after approximately a week and a half when the subjects were comfortable with the workouts subjects were allowed to work out in small groups yet still monitored by APL staff. Close attention was paid to the sets, repetitions, rest, and weight being lifted to ensure that subjects were completing the workouts properly.

Supplementation

Subjects were randomly assigned to SUP and PCBO groups. The supplement contained 64 calories, all from carbohydrates, as well as, but not limited to the following ingredients: nitrous malate, taurine, arginine alpha-ketoglutarate, L-citrulline, L-tyrosine, caffeine, creatine, betaine, picamilon, beta-alanine, N-acetyl-L-glutamine, L-histidine, vitamin A and Vitamin E. The nutrition label for this supplement can be seen in Appendix F. The placebo contained a low-glycemic flavor and color matched drink sweetened with polydextrin and/or mannitol.

Each drink was prepared by measuring two scoops (32 grams) of the assigned powder into a water bottle. Upon each subject's arrival to the lab for training approximately twelve ounces of water was added to the mixture, the bottle was shaken and given to the subject. The subject consumed the drink and waited fifteen minutes before beginning their training session. Following the initial training and biopsy session the dose of the placebo and supplement was reduced to half for one and a half weeks. This was done in an attempt to help the subject become accustomed to drinking it prior to their workout. After this, all subjects consumed the full dosage of the drink. During each visit to the lab all subjects completed a Supplement Side-Effect Questionnaire (See Appendix G) to examine if individuals were experiencing any problems such as nausea or light-headedness immediately following the use of the drink or any other problems later in the day or the days between sessions. Blood pressure was monitored once each week and recorded on this questionnaire. These Side-Effect Questionnaires were filed and reviewed by the study's Data Safety Monitoring Board.

Blood and Muscle Sample Collection

All muscle tissue samples were taken from the vastus lateralis using a needle biopsy technique by either Dr. Philip Gallagher or Dr. Andy Fry. Subjects were asked to lie on an examination table so that the muscles of the legs were relaxed. The skin was thoroughly cleaned with Betadine prior to the procedures. Approximately 2ml of 2% Lidocaine was injected into the tissue under the skin around the site sampled. Following the injection of the numbing agent, a minimum of five minutes passed to ensure adequate time for the agent to take effect. During this time approximately 10ml of blood was drawn from the antecubital vein in the arm. Once the numbing agent had taken effect on the leg a small incision (1 cm) was then made in the skin overlying the muscle, the biopsy needle inserted into the muscle belly at a depth of

approximately 3 cm, and a sample was obtained. Following the biopsy procedure, firm and constant pressure was placed on the wound to stop any bleeding. The incision site was closed with a Steri-Strip or butterfly Band-Aid, covered with a large Band-Aid, and the site was compressed using a pressure wrap. Muscle samples were sectioned, flash frozen and stored in liquid nitrogen until later analysis. Blood samples were centrifuged at 3000g's for 20 minutes and stored in the -80° freezer for later use.

Blood and Muscle Sample Analysis

All muscle samples were analyzed for IL-6 and IL-15 protein. Approximately 20mg of each muscle sample was homogenized in extraction buffer using a glass on glass tissue grinder. Homogenized samples were centrifuged at 4°C at 3,000g's for 4 minutes. Supernatant was separated from the pellet and the sample was diluted (1:1000), and total protein concentration determined using a bicinchoninic acid (BCA) protein assay (Pierce; Rockford, IL).

Muscle samples were then diluted with 5x buffer and heated for 3 minutes. Samples were loaded at a protein concentration of 80µl and ran on a 5% stacking and 10% separating gel at 0.03mA for 1 hour. Proteins were transferred to hydrophobic polyvinylidene difluoride (PVDF) membranes at 0.02mA for 2 hours. Membranes were blocked for 1 h in a Tris-buffered saline with 5% nonfat dry milk on a rocker at room temperature. Membranes were then incubated on a rocker overnight in a 1:1000 IL-6 and IL-15 antibody/TBST and 1% nonfat dry milk solution. Following the overnight incubation, membranes were rinsed 3 times for 5 minutes in TBST before one hour incubation in horseradish peroxidase conjugated secondary antibody, and again rinsed 3 times. Membranes were then incubated in chemiluminescence for 5 minutes. IL-6 protein bands were visualized and quantified using densitometry (Alpha Innotech; San

Leandro, CA). Multiple attempts at the detection of IL-15 in practice tissue as well as subject samples were unsuccessful. Thus, the analysis of IL-15 was not completed for this study.

Blood serum samples from each time point were analyzed using a Q-Plex Human Cytokine Inflammation (9-PLEX) array (Quansys Biosciences; Logan, UT). Cytokines analyzed included IL-1α, IL-1β, TNF-α, IFN-γ, IL-2, IL-4, IL-6, IL-8 and IL-10. Antigen standards were loaded at 1:9, 1:27, 1:81, 1:243, and 1:729 ratios of standard to sample diluent. Serum samples were loaded at 1:2 ratio of sample to sample diluent. 30µl of each standard and sample were loaded in duplicate onto a 96 well microtiter plate. This plate was placed on a room temperature shaker for one and a half hours at 300rpm. After this incubation the plate was washed three times using a plate washer. 30µl of detection solution was added to each well. The plate was again incubated at room temperature and 300rpm for one and a half hours. The plate was washed three times using the previous plate washer program. 30µl of streptovidin was then added to each well. This solution will incubate in the plate for 15 minutes at room temperature and 300rpm. After this incubation the plate was washed six times. 40µl of a substrate mixture was added to each well. The plate was immediately imaged for 4 minutes using the Alpha Innotech system (Alpha Innotech; San Leandro, CA). The resulting images were visualized and quantified using densitometry (Q-View, Quansys Biosciences; Logan, UT).

Statistical Analysis.

Descriptive statistics were determined for subject age, anthropometric measures, and body composition by group. Separate repeated measures analysis of variance (ANOVA) tests were conducted to examine main effects for strength, body composition, and markers of inflammation, as well as group interactions. The independent variable, the subject group, consisted of two levels: the group placebo group and the supplement group. The dependent

variables were 1-RM values for eight strength tests, weight, lean body mass and body fat, 9 serum cytokine measures and 1 myokine measure. The specific cytokines of interest were serum IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IFN-γ, and TNF-α, as well as IL-6 within skeletal muscle. Four paired-samples *t*-tests were used to examine a main effect for IL-6 protein within the skeletal muscle. Significance was determined based on an alpha level of 0.01. This conservative approach was taken due to the high number of dependent variables.

Table 3-1: Training Program Exercises by Day.

Day 1	Day 2	Day 3
Core Exercises:		
Squat	Dead lift	Squat
Bench Press	Incline Press	Bench press
Leg Extension	Bicep Curls	Leg Extension
Leg Curl		Leg Curl
Lat Pulldown*		Lat Pulldown*
Accessory Exercises:		
Standing Overhead Press	Split Legged Squat	High Pull
Weighted Sit-up	Pull-ups	Weighted Twist
Russian Twist	Dips	Weighted V-ups
	Weighted Supine Leg Raises	
	Weighted Wipers	

* Following week 5, Lat Pulldown was moved to the Accessory Exercises category

Table 3-2: Training Volume Progression by Week and Exercise for Lower Body Core Lifts.

	Squat	Dead Lift	Leg Extension/Leg Curl
Week 1			
Sets/Reps	3/10	N/A*	2/10
Load (%1-RM)	70%		80%
Week 2			
Sets/Reps	4/10	4/10	2/10
Load (%1-RM)	70%	70%	70%
Week 3			
Sets/Reps	5/10	5/10	2/10
Load (%1-RM)	70%	70%	75%
Week 4			
Sets/Reps	3/8; 3/6†	3/8	2/8; 2/6†
Load (%1-RM)	75%; 80%†	80%	80%; 85%†
Week 5			
Sets/Reps	1-RM; 3/10**	3/10	1-RM; 2/10**
Load (%1-RM)	70%	75%	80%
Week 6			
Sets/Reps	4/10	3/10	2/10
Load (%1-RM)	70%	75%	80%
Week 7			
Sets/Reps	3/6; 3/5†	N/A*	2/6
Load (%1-RM)	80%; 85%†		80%; 85%†
Week 8			
Sets/Reps	1-RM; 3/10**	N/A	1-RM; 2/10**
Load (%1-RM)	70%		80%

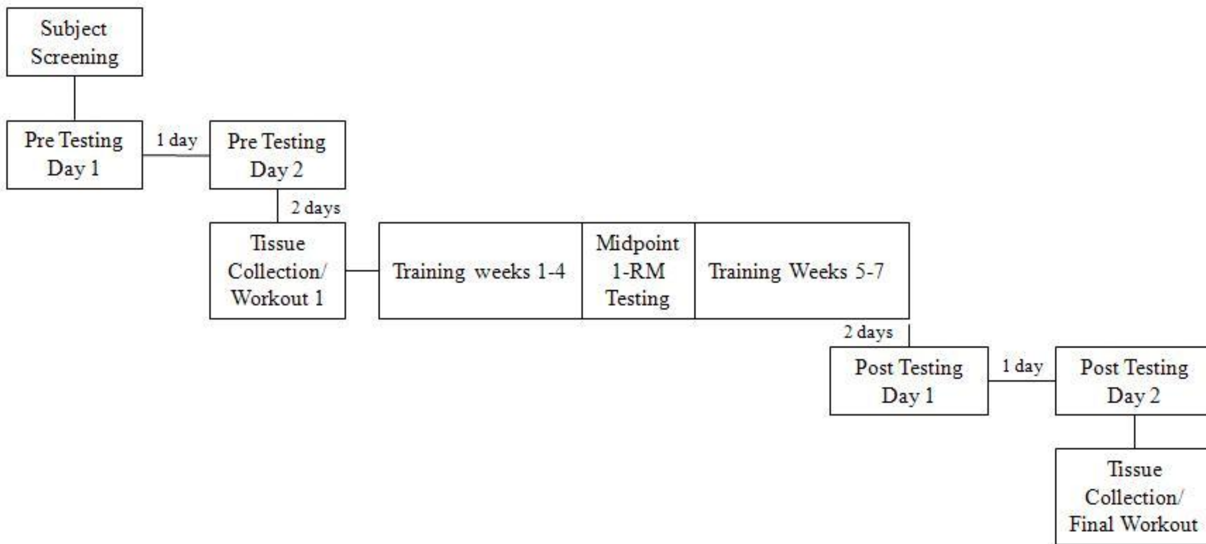
* 1-RM testing took place on this day for this exercise
† The first protocol is for Day 1; the second is for Day 3
** 1-RM testing took place on D1 this week

Table 3-3: Training Volume Progression by Week and Exercise for Upper Body Core Lifts.

	Bench	Incline	Lat Pulldown	Curls
Week 1				
Sets/Reps	3/10	N/A*	2/10	N/A*
Load (%1-RM)	70%		80%	
Week 2				
Sets/Reps	4/10	4/10	2/10	3/10
Load (%1-RM)	70%	70%	75%	80%
Week 3				
Sets/Reps	5/10	5/10	2/10	3/10
Load (%1-RM)	70%	70%	75%	80%
Week 4				
Sets/Reps	3/8; 3/6†	3/8	2/8; 2/6†	3/10
Load (%1-RM)	75%; 80%†	80%	80%; 85%†	80%
Week 5				
Sets/Reps	1-RM; 3/10**	3/10		3/10
Load (%1-RM)	70%	75%		85%
Week 6				
Sets/Reps	4/10	3/10		3/10
Load (%1-RM)	70%	75%		85%
Week 7				
Sets/Reps	3/6; 3/5†	N/A*		N/A*
Load (%1-RM)	80%; 85%†			
Week 8				
Sets/Reps	1-RM; 3/10**	N/A	1-RM; 2/10**	N/A
Load (%1-RM)	70%		80%	

* 1-RM testing took place on this day for this exercise
† The first protocol is for Day 1; the second is for Day 3
** 1-RM testing took place on D1 this week

Figure 3-1: Study Timeline.



CHAPTER IV

RESULTS

The purpose of this study was to examine the effects of eight weeks of resistance training and supplementation on anthropometric, strength, and inflammatory changes in healthy, recreationally active college-aged males. Strength and lean body mass measures were determined before and after the training protocol. Muscle and blood samples were obtained before (PRE1) and after an acute bout of resistance training (PRE2) prior to (POST1) and following (POST2) the eight week training program. Serum samples were analyzed for the presence of IL1-α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IFN-γ, and TNF-α. Muscle tissue was analyzed for IL-6 protein content.

Descriptive Data

Twenty four of the thirty recruited males completed the study (n=14 SUP, n=10 PCBO). Five subjects discontinued the study due to the time commitment required, while one subject was disqualified for missing training sessions. Pre-training descriptive statistics for each group, including subjects' age, height (HT), weight (WT), body fat percentage (BF), and lean body mass (LBM) are represented in Table 4-1.

Anthropometric Changes

Pre and post training mean and standard deviation values for weight, lean body mass, and body fat are presented in Table 4-2. A repeated measures ANOVA revealed a significant main effect for weight within subjects; $F\ (1,22) = 23.293$, $p < 0.01$. There was no significant group interaction for weight; $F\ (1,22) = 0.320$, $p > 0.01$. Similarly, a repeated measures ANOVA found a significant main effect for LBM measures within subjects; $F\ (1,22) = 20.320$, $p < 0.01$.

No significant interaction between SUP and PCBO was observed; $F(1,22) = 0.142$, $p > 0.01$. No significant differences in body fat percentage were found within subjects; $F(1,22) = 1.174$, $p > 0.01$; or between groups $F(1,22) = 0.284$, $p > 0.01$. Changes in anthropometric measures can be examined in Figure 4-1. Estimated marginal means for anthropometric changes within subjects is presented in Table 4-4.

Strength Changes

Mean pre and post-training strength scores are presented in Table 4-2. Separate repeated measures ANOVAs revealed significant main effects for time when analyzing the 1-RM measures of the squat, bench press, leg extension, leg curls, and lat pulldown exercises within subjects. This reveals all subjects improved their strength in those exercises. No significant changes were revealed for the dead lift, incline press or bicep curl exercises. Estimated marginal means within subjects for strength measures are presented in Table 4-4. Examination of the changes in strength by group revealed no significant interactions for time and group in squat, bench press, leg extension, leg curl, lat pulldown, dead lift, incline press, or bicep curl 1-RM measures between SUP and PCBO groups. The results of these statistical tests are reported in Table 4-3. Graphical representation of these changes can be observed in Figure 4-2.

Inflammatory Changes

A repeated measures ANOVA was used to examine differences in IL-6 within skeletal muscle across four time points: before the first exercise bout (PRE1); after the first exercise bout (PRE2); before the last exercise bout (POST1); and after the final exercise bout (POST2). A significant main effect for time within subjects was found $F(3,69) = 14.641$, $p < 0.01$. Paired samples *t*-tests revealed significant differences in skeletal muscle IL-6 content within subjects for PRE1 to POST1, $t(23) = 4.661$, $p < 0.01$; PRE1 to POST2, $t(23) = 4.875$, $p < 0.01$; PRE2 to

POST1, $t(23) = 5.211$, $p < 0.01$; and PRE2-POST2, $t(23) = 6.171$, $p < 0.01$. Changes in skeletal muscle IL-6 across time points are seen in Figure 4-3. The estimated marginal means for these time points are as follows: PRE1 18004611.48±2568563.34; PRE2 16211177.074±1617595; POST1 7645092.47±825379.42; POST2 6870461.19±563966.46. There was no significant interaction for IL-6 protein presence and SUP/PCBO groups at any time points; $F(3, 20) = 0.586$, $p > 0.01$.

Mean and standard error for serum cytokine levels across the four time points are presented in Table 4-6. Statistical analyses of these levels were performed using densitometry values. Mean and standard error for these values are presented in Table 4-5. Separate repeated measures ANOVAs found no significant main effects for time when analyzing IL1-α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IFN-γ, or TNF-α levels within subjects; revealing no changes in serum cytokines in the subjects. No significant interactions for time and groups were observed, suggesting the serum cytokine levels did not differ between SUP and PCBO groups. The results from these analyses are presented in Table 4-7.

Table 4-1: Pre and Post-Training Age and Anthropometric Measures. Age is reported in years. WT and LBM are reported in kilograms (kg). BF is reported as a percentage of WT. *Denotes a significant main effect for time ($p < 0.01$).

Exercise	SUP				PCBO			
	Pre		Post		Pre		Post	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age	19.29	1.55	-	-	19.80	1.49	-	-
Height	180.34	4.06	-	-	180.44	8.81	-	-
Weight	77.90	7.21	79.57	7.54*	77.89	7.52	79.69	8.90*
Lean Body Mass	61.70	4.13	63.25	4.03*	61.75	4.07	63.06	4.58*
Body Fat	16.84	6.03	16.26	6.40	16.31	8.53	16.08	6.83

Table 4-2: Pre and Post-Training 1-RM Scores.

Exercise	SUP				PCBO			
	Pre		Post		Pre		Post	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Squat	112.50	21.64	145.94	23.30	107.50	22.16	135.22	20.10
Bench Press	79.54	10.95	93.18	14.06	80.45	15.75	89.54	16.94
Leg Extension	89.12	17.40	18.18	12.60	87.72	9.83	114.54	11.90
Leg Curl	77.27	12.97	89.28	8.79	74.09	10.28	86.36	7.26
Lat Pulldown	85.87	14.04	97.24	12.38	88.40	10.84	97.04	9.46
Dead Lift	124.35	30.45	49.02	17.59	126.36	18.56	145.45	25.96
Incline Press	75.16	13.50	82.30	12.89	72.27	11.32	80.39	15.83
Bicep Curls	32.46	7.09	39.44	6.02	34.54	6.49	43.18	6.54

Table 4-3: Changes in 1-RM Strength Determined by Separate Repeated Measures ANOVAs. *Denotes a significant main effect for time ($p > 0.01$). No significant time x group interactions were observed.

1-RM measure	Time		Time x Group	
	F	p	F	p
Squat	145.604	0.000*	1.271	0.272
Bench Press	121.069	0.000*	4.843	0.039
Leg Extension	88.963	0.000*	0.143	0.709
Leg Curl	47.079	0.000*	0.005	0.942
Lat Pulldown	69.207	0.000*	1.287	0.269
Dead Lift	4.438	0.047	2.106	0.161
Incline Press	0.022	0.884	2.438	0.133
Bicep Curls	0.961	0.338	3.214	0.087

Table 4-4: Pre and Post-Training Whole Muscle Estimated Marginal Means Within Subjects.

Measure	Pre		Post	
	Mean	SE	Mean	SE
1-RM				
Squat	110.41	4.39	141.47	4.53
Bench Press	79.92	2.62	91.66	3.07
Leg Extension	88.54	2.95	116.66	2.49
Leg Curl	75.94	2.40	88.06	1.66
Lat Pulldown	86.93	2.50	97.15	2.20
Dead Lift	125.18	5.20	141.47	7.40
Incline Press	73.95	2.50	75.66	5.30
Bicep Curls	33.30	1.38	36.55	1.38
Anthropometric				
Weight	77.90	1.46	79.62	1.60
LBM	61.72	0.82	63.17	0.85
BF	16.50	1.20	16.10	1.30

Table 4-5: Pre and Post-Training Serum Cytokine Densitometry Values.

Cytokine		Pre 1		Pre 2		Post 1		Post 2	
	Group	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
IL-1α									
	SUP	6617.25	6948.04	4541.59	1563.63	7335.45	9895.73	7075.07	9570.05
	PCBO	14009.98	19738.27	10495.81	10239.08	12856.38	18982.20	9025.32	9943.96
IL-1β									
	SUP	6512.11	8463.32	5027.79	2226.44	8343.43	16206.12	9080.32	16038.84
	PCBO	9778.40	12499.99	8907.17	2226.44	4686.25	4964.30	11134.06	12093.84
IL-2									
	SUP	4691.16	7765.34	4966.88	5609.76	4140.05	9222.95	5360.58	11802.77
	PCBO	9340.48	12375.50	7873.89	8494.28	4212.15	7913.53	3508.71	5108.77
IL-4									
	SUP	9731.62	9551.58	5764.49	1524.45	8869.24	15262.18	8959.44	14703.73
	PCBO	9848.36	9551.58	9926.99	9059.22	9165.31	8325.87	9006.95	8843.95
IL-6									
	SUP	4061.75	3304.51	3935.99	1423.00	7375.49	12256.46	8514.21	14693.37
	PCBO	8840.14	9327.52	9329.54	9437.93	8674.52	14045.15	7930.89	7562.96
IL-8									
	SUP	20706.92	9047.53	17277.20	6619.07	17897.04	11917.11	19902.60	13041.13
	PCBO	22532.55	15783.00	20948.90	11170.10	24988.88	9001.02	24886.79	8430.56
IL-10									
	SUP	14500.41	16659.75	13199.16	10787.46	17745.26	20694.89	19931.67	20964.14
	PCBO	9593.77	6350.87	12820.30	11046.57	10006.73	3625.69	15324.66	9030.96
IFN-γ									
	SUP	7436.42	6931.06	5131.08	1467.05	7960.22	9563.96	8958.59	12323.93
	PCBO	9756.77	10423.77	8830.50	9236.71	9058.32	5570.35	9045.73	5183.85
TNF-α									
	SUP	11440.37	6981.66	8395.79	2913.18	12463.63	15262.19	12824.64	15200.77
	PCBO	14553.07	16218.53	12192.75	11404.57	11329.22	6136.46	12801.24	8566.89

Table 4-6: Pre and Post-Training Serum Cytokine Levels. Data is presented as mean ± standard error. Values presented are in pg/ml.

Cytokine		Pre 1		Pre 2		Post 1		Post 2	
	Group	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*
IL-1α									
	SUP	0.00	9.70	0.00	0.20	0.04	13.95	0.00	13.49
	PCBO	46.15	27.83	21.87	14.43	38.18	26.76	11.72	14.02
IL-1β									
	SUP	32.21	12.95	21.02	3.14	45.94	24.80	51.47	24.54
	PCBO	56.69	19.13	50.16	16.62	18.52	7.59	66.86	18.50
IL-2									
	SUP	0.00	4.72	0.00	3.41	0.00	5.61	0.00	7.18
	PCBO	2.35	7.52	0.00	5.17	0.00	4.81	0.00	3.11
IL-4									
	SUP	28.23	9.87	8.14	1.57	23.87	15.78	24.32	15.20
	PCBO	28.83	9.47	29.22	9.36	25.37	8.60	24.56	9.14
IL-6									
	SUP	10.32	3.80	9.60	1.63	29.00	14.09	35.41	16.90
	PCBO	37.25	10.72	40.01	10.85	36.32	16.15	32.13	8.69
IL-8									
	SUP	10.21	1.11	8.14	0.82	8.51	1.47	9.73	1.61
	PCBO	11.32	1.95	10.36	1.38	12.81	1.11	12.74	1.04
IL-10									
	SUP	42.15	12.74	37.27	8.25	54.31	15.83	62.50	16.03
	PCBO	23.76	4.85	35.85	8.45	25.31	2.77	45.24	6.90
IFN-γ									
	SUP	2.57	1.21	0.60	0.25	3.02	1.67	3.88	2.15
	PCBO	4.56	1.82	3.77	1.61	3.96	0.97	3.95	0.90
TNF-α									
	SUP	2.52	0.32	1.82	0.13	2.74	0.70	2.83	0.70
	PCBO	3.22	0.74	2.68	0.52	2.49	0.28	2.82	0.39

Table 4-7: Changes in Serum Cytokines Determined by Separate Repeated Measures ANOVAs. No significant main effects (time) or interactions (time x group) were observed ($p > 0.01$).

Cytokine	Time		Time x Group	
	F	*p*	*F*	*p*
IL-1α	0.624	0.608	0.667	0.582
IL-1β	2.129	0.129	1.194	0.337
IL-2	0.322	0.810	0.447	0.722
IL-4	0.376	0.771	0.622	0.609
IL-6	0.147	0.931	0.556	0.650
IL-8	1.020	0.405	0.258	0.855
IL-10	2.567	0.083	0.626	0.606
IFN-γ	0.639	0.598	0.341	0.796
TNF-α	1.737	0.192	0.216	0.884

Figure 4-1: Pre to post-testing percent change results for anthropometric measures. Data presented represent mean and standard error values. *Denotes a significant main effect for pre and post-training measures ($p < 0.01$). No significant time x group interactions were observed.

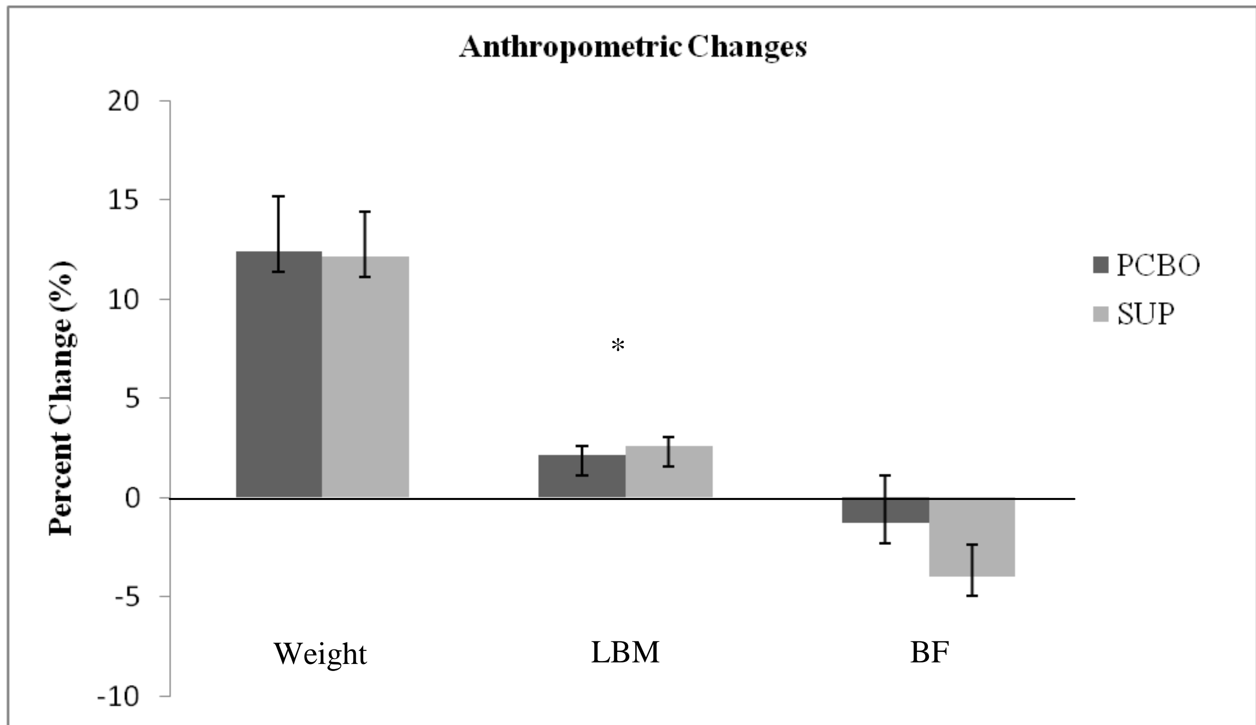


Figure 4-2. Pre to post-testing percent change results for 1-RM strength measures. *Denotes a significant main effect for pre and post-training measures ($p < 0.01$). No significant time x group interactions were observed.

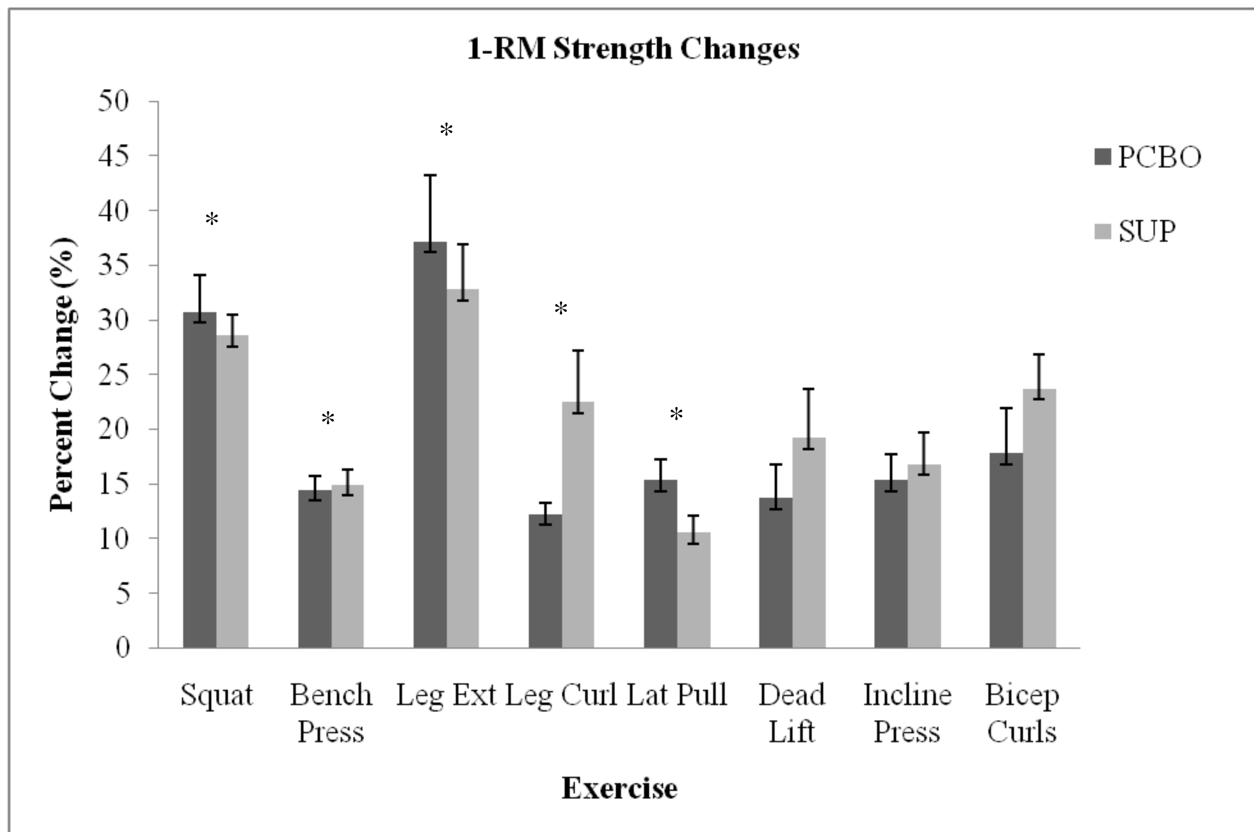
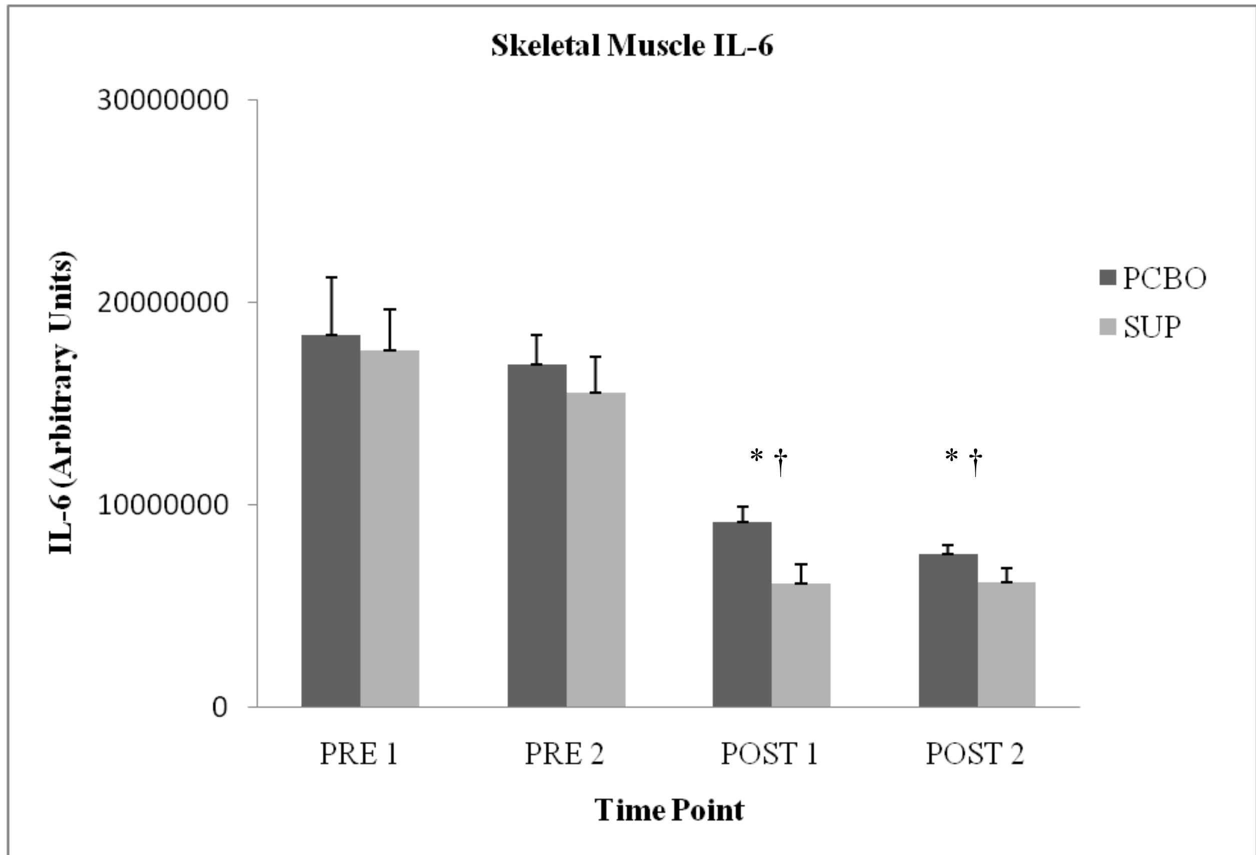


Figure 4-3: Changes in skeletal muscle IL-6 protein content across four time points. *Denotes a significant within subject difference in PRE 1 and POST 1 IL-6 content ($p < 0.01$). †Denotes a significant within subject difference in PRE 1 and POST 2, and PRE 2 and POST2 IL-6 content. ($p < 0.01$). No significant time x group interactions were observed.



CHAPTER V

DISCUSSION

Skeletal muscle contraction has been implicated in the production of cytokines, specifically IL-6 (134). This cytokine, deemed a myokine due to its production within the muscle, initiates a downstream cascade of changes in anti and pro-inflammatory markers. IL-6 is associated with an increase in the anti-inflammatory IL-10 (35). IL-6 and IL-10 are known to inhibit the pro-inflammatory markers TNF-α and IL-1β, respectively (35, 135). Together, these responses significantly influence metabolism as IL-6 and TNF-α play a role in regulating glucose metabolism. Prolonged endurance exercise results in an increase in IL-6 production within the skeletal muscle which is coupled with an increase in GLUT4 and IL-6 receptor (4, 72, 114). Thus, changes in IL-6 production and release in skeletal muscle have been linked to exercise training for those who are active and non-obese and have lower levels of resting IL-6 (40-41, 113). To date, the IL-6 response to an acute bout of anaerobic exercise is debated. Few studies have examined the influence of resistance training on the inflammatory state including changes in IL-6, IL-10, TNF-α, IL-1β as well or other cytokines including IL-8, and IFN-γ.

Resistance training studies have examined the effectiveness of supplementation of creatine and amino acids among other substances on strength and lean body mass changes. No studies have examined the influence of these types of supplements on the inflammatory response to exercise. The purpose of the present study was to determine the effects of supplementation and eight weeks of resistance training on strength, lean body mass, and markers of inflammation in recreationally active college-aged males. Following baseline anthropometric and strength testing, subjects were randomized into supplement and placebo groups, and completed eight

weeks of total body resistance training. Skeletal muscle biopsies and blood draws were obtained immediately before and after exercise day one as well as before and after the final training day in order to examine the tissue response to IL-6 and serum markers of IL-4, IL-6, IL-8, IL-10, TNF-α, and IFN-γ. Western blot analyses of skeletal muscle tissue for changes in the presence of IL-6 protein were analyzed. Multiplex-ELISAs were used to examine changes in serum cytokine levels.

Anthropometric Changes

Few of the ingredients in the supplementation used in the present study have been related to changes in body composition; however, the combination of creatine supplementation and resistance training have been related to increases in lean body mass (13, 109). Generally, past training studies that have elicited these positive results are associated with a creatine loading phase of up to 20g/day for 5 days followed by 5-10g/day for several weeks of training (13, 109). Training and creatine supplementation are generally not associated with changes in overall body fat percentage (13, 56, 85, 90, 143). The present study elicited increases in lean body mass in all subjects, suggesting these changes were a result of the resistance training, and the supplementation protocol did not play a role in this increase. It is likely that the lack of a creatine loading phase, or the smaller dose of creatine within the supplement was not enough to elicit greater changes in the SUP group. Similar to previous studies, no significant differences in body fat were seen in either subject group. The anthropometric findings of this study support the proposed hypothesis that eight weeks of resistance training would elicit improvements in lean body mass in all subjects without significant differences between SUP and PCBO groups.

Strength Changes

Previous studies have examined the use of common supplement ingredients including caffeine, creatine, and amino acids. Most caffeine supplementation studies have focused on the acute exercise response and have shown improvements in aerobic performance with conflicting results on anaerobic performance. Caffeine supplementation of 5-6mg/kg body weight has been associated with increases in peak power and reduced fatigue (149). Other studies have shown no changes in 40-yd dash, 20-yd shuttle, 1-RM leg or bench press, or endurance bench press tests following consumption of similar doses of caffeine (7, 11, 92, 150). No studies to date have reported the effect of caffeine supplementation on a resistance training program. Unlike caffeine supplementation, creatine supplementation of 1.25g/day to 10g/day has shown improvements in strength, power, and reduced fatigue (13, 56, 85, 90, 143). Many amino acids including arginine, glutamine, arginine, and taurine, are also commonly supplemented. A previous study revealed an intake of 6g of L-arginine and 6g of alpha keto-glutarate per day for 8 weeks was associated with increases in 1-RM bench press and power determined by Wingate testing (22). Other studies suggested a smaller 3g/day dose decreased plasma lactate and increased L-citrulline with in the blood (124). Despite these positive changes in past studies, the supplement studied in the present project contained only 1.742g of a combined arginine-alphaketoglutarate. Similarly, the supplement contained 1g of N-Acetyl L-Glutamine, while past studies have suggested much higher doses of 0.9g/kg of LBM combined with six weeks of resistance training result in significant increases in strength and LBM (6). Previous research has also demonstrated doses of 3.2g/day to 6.4g/day of alanine are associated with increases in intramuscular carnosine, which effective in pH buffering and the regulation of calcium sensitivity (55). The supplement used in the present study contained 1.5g of beta-alanine.

The small dose of each of these ingredients within the supplement may provide an explanation for the results of the present study. While significant increases in squat, bench press, leg extension, leg curl, and lat pulldown, were found across all subjects, there were no significant differences in strength between SUPP and PCBO groups for any strength measure, supporting the original hypothesis. While these ergogenic aids have elicited positive effects on exercise performance in the past, the low doses of each within the pre-workout supplement may not have been enough to stimulate effects on performance greater than that of resistance training alone. No significant differences in dead lift, incline press, or bicep curl 1-RM strength were found across subjects. It is likely that this was because these exercises were performed once a week, while the squat, bench press, leg extension, leg curl, and lat pulldown exercises were performed twice per week.

Inflammatory Changes

Previous research on the influence of resistance training on markers of inflammation is scarce. To date, no studies have examined the influence of a pre-exercise supplement and resistance training program on the cytokine response within skeletal muscle tissue or the systemic response within serum. The present study revealed significant changes in skeletal muscle IL-6 protein content were observed following eight weeks of training. Basal levels of IL-6 following the training program were significantly lower than both basal and post-exercise pre-training values. These findings are in contrast to the original hypothesis which suggested IL-6 protein concentration would be greater following eight weeks of resistance training. In addition, no previous studies have examined changes in IL-6 protein content within skeletal muscle following a resistance training program. Despite this, the information obtained from the present analysis is consistent with previous studies of aerobic training that found reduced gene

expression of IL-6 following training (42). This previous analysis revealed an increase in IL-6 receptor following training is likely the cause in reduced protein expression. It is possible that regular resistance training might also increase IL-6 receptor mRNA, thus reducing IL-6 protein presence within the skeletal muscle following training.

Despite this training effect, no significant differences in IL-6 protein were observed following an acute bout of exercise. This too is in contrast to the original hypothesis as previous studies of prolonged aerobic exercise that have revealed acute exercise increases IL-6 and IL-6 receptor mRNA expression (72). Studies examining changes in skeletal muscle IL-6 have debated whether changes in this protein are associated with exercise intensity, duration, type of exercise or musculature associated with the exercise (108). Some have suggested only exercise lasting more than one hour has been shown to elicit 10-fold increases in IL-6 protein (112). This research suggested running has elicited the greatest IL-6 response; however, as mentioned few studies have examined the IL-6 response to resistance training. While it is possible that the exercise bout was not long enough to elicit a significant increase in IL-6 protein within the musculature, it is more likely that the exercise specifically focusing on the vastus lateralis muscle, the muscle that was sampled, was not long enough in duration to elicit a response. In addition, since these exercises (squat, leg extension) were performed within the first fifteen minutes of the exercise bout, and little is known about the time frame associated with increases of IL-6 protein within the muscle in response to resistance training, it is possible the production of this protein may have peaked prior to the muscle sampling. While conflicting studies exist, most of those examining the skeletal muscle production of IL-6 protein following aerobic exercise have determined IL-6 peaks immediately following exercise of the vastus lateralis muscle (running, cycling) (105, 133, 139).

The present study also revealed that an acute bout of exercise did not significantly alter the systemic inflammatory state in recreationally trained individuals, or the same individuals following an eight week resistance training program. These findings support the original hypothesis as there were no significant differences in serum IL-1α, IL-β, IL-2, IL-4, IL-6, IL-8, IL-10, IFN-γ, or TNF-α at any of the four time points. Previous literature has indicated that the increase in plasma IL-6 that accompanies exercise is due to significant increases in the skeletal muscle production of IL-6 (134). The fact that in the present study there was not a significant increase in skeletal muscle production of IL-6 can explain the changes, or lack thereof, within the serum inflammatory markers. Since changes in many of the other cytokines including IL-10, TNF-α, and IL-1β are related to the presence of IL-6, the absence of changes in IL-6 could indicate why no significant differences in these measures were observed. It is possible that the recreationally trained state of the subjects may have limited the changes in cytokines seen following an acute bout of exercise. Studies of aerobic exercise have revealed serum levels of IL-6 increase significantly more in untrained subjects (50).

Izquierdo et al. (69) also did not find significant differences in serum IL-1α, IL-β, or IL-10 following an acute bout of lower body resistance exercise in previously trained individuals. However, these authors reported significant increases in IL-6 at 45 minutes following an acute exercise bout as well as increases in post-exercise markers of -1α, IL-β, IL-6 and IL-10 following seven weeks of resistance training. Differences between this data and the present study can be explained by differences in the testing protocol. In the present study, blood samples were obtained immediately before and after a whole-body resistance training program lasting approximately one hour. Izquiredo et al. (69) collected blood samples at various time points before and after five sets of 10 repetitions of the leg press exercise. Previous studies have

reported exercises that focus on a smaller area of muscle mass may result in a significant increase in serum IL-6 (58, 101, 137); thus, the lower body only testing protocol may be responsible for the differences in serum IL-6 and IL-10 content between the two studies. Also, while IL-10 was significantly higher than baseline during, immediately after, and at 15 and 45 minutes post exercise in the previous study, IL-6 was only significantly higher at 45 minutes post exercise. It is possible that during the present study this peak in IL-6 was not seen due to the lack of increases in muscular IL-6 protein production as previously discussed, or possibly due to the immediate post exercise blood draw. This time point was chosen as few studies had examined the cytokine response to resistance training. Previous studies had revealed an increase in IL-6 immediately following anaerobic sprint intervals (95), and most aerobic training studies suggested IL-6 peaked immediately following exercise and gradually declined during exercise recovery (105, 132). Still others have found results similar to those of the present study, reporting no significant changes in serum IL-6 following circuit weight training (18) or following short bouts of maximal resistance exercise (138).

Several *in vitro* studies have examined the influence of caffeine on the cytokine response. These studies have suggested caffeine reduces TNF-α in human blood mononuclear cells, adipose cells, and IL-6 and TNF-α in mouse models (36, 66). Similarly, glutamine supplementation has been associated with decreases in TNF-α *in vitro* (149). This amino acid has also been shown to assist in maintenance of force production during acute IL-6 and TNF-α inflammation (96). Vitamin E, which is an ingredient in the supplement in the current study, might also have a positive role in inflammation as it has been shown that 800IU/day is associated with decreased IL-1β production following a downhill running bout (94). Other in vitro studies

have suggested this vitamin decreases IL-6 and IL-1β (68). In the present study no significant differences between SUPP and PCBO groups were observed for serum inflammatory markers. While previous studies have suggested caffeine (36, 65) glutamine (97) and Vitamin E (96, 68) might positively affect these markers by reducing pro-inflammatory cytokines, this is the first study to examine the effect of pre-workout supplementation on these markers. It is possible, as suggested previously, that the doses of glutamine and Vitamin E within the supplement investigated in this study were not high enough to illicit an effect on the production of cytokines. Despite this, it is difficult to suggest the same is true for caffeine as the dose of this ingredient was approximately 5.8g/kg of body weight for the average subject weight. It is unknown how this dose compares to previous *in vitro* studies. Further studies are necessary to examine the *in vivo* effect of caffeine consumption on resistance training programs as well as the cytokine response in humans.

Summary

The complex cytokine signaling that occurs as a result of skeletal muscle contraction has been extensively studied. Many of these studies have suggested the duration, intensity, and type of exercise performed will influence the extent of inflammatory changes following exercise, however still little is known about what causes these changes. To date, most literature has focused on the impact of prolonged aerobic exercise on cytokine signaling. These studies suggest IL-6 release from the skeletal muscle increases following an acute bout of exercise and are directly supported by an increase in plasma IL-6, IL-10, IL-15, decreases in IL-1β and TNF-α, and increases in IL-8 within the skeletal muscle. Studies of aerobic training have suggested that regular training reduces skeletal muscle IL-6 mRNA expression, possibly due to an increase in IL-6 receptor within the skeletal muscle. Other than the present study, no studies have

examined the skeletal muscle inflammatory response to resistance training. This study suggested an acute bout of total body resistance training does not elicit significant increases in skeletal muscle or serum IL-6, or serum IL-1α, IL-1β, IL-2, IL-4, IL-8, IL-10, IFN-γ, or TNF-α. These findings do not support the hypothesized response to an acute bout of exercise, or to the training program.

As discussed, it is possible these findings differ from similar studies which have examined the inflammatory response of serum markers because of the type of exercises employed, the time points at which samples were collected, or the training state of the subjects. The subject pool, specifically their training history and their caffeine consumption prior to the study are also considered limitations to the present study. These variables were examined using a self-report prior to subject selection. It is possible if the subjects were not honest with their answers to these surveys that the fitness level and/or tolerance to caffeine may have been different than intended and thus affected the outcome of the study.

Little is known about the skeletal muscle IL-6 production during resistance training. Because of this, it is possible that the time at which muscle samples were collected could also be a limitation of this study. In addition, even less is known about the effect of pre-workout supplementation on the inflammatory response. While many of the ingredients within the supplement tested might have a role in changing inflammation, these ingredients should be tested individually and not as a mixed supplement in order to fully understand their role on the inflammatory response.

It is also possible that the muscle biopsy itself may have altered the inflammatory response within either the skeletal muscle itself or within the systemic response. However, since the percutaneous biopsy technique is the best method for obtaining human skeletal muscle

samples and the same procedures were used for each biopsy, it was assumed this inflammatory response was similar in each case. In order to ensure any changes seen within the skeletal muscle itself were due to the exercise bout and not a local inflammatory response, biopsies performed on the same day were performed on opposite legs.

Following the biopsy process as well as during protein analyses all samples were handled in a manner aimed at limiting any degradation or contamination of proteins. All gels and solutions used in the Western Immunoblotting and ELISA processes were made fresh to eliminate contamination as well as ensure the same protocols for the analysis of each sample. The Western Immunoblotting process is commonly used for the analysis of proteins within skeletal muscle and had previously been used for the analysis of IL-6 within this lab. The use of the Multiplex ELISA for analysis of markers of inflammation was relatively new to this lab; however, several practice samples were tested using this methodology to ensure proper detection of proteins.

Practical Implications

The present study has demonstrated the cytokine response to acute and chronic resistance training is likely different than the inflammatory response to aerobic exercise. Despite this, changes in basal skeletal muscle IL-6 reveal the potential for a training effect following eight weeks of resistance training. In addition, the use of a multi-ingredient supplement did not significantly change markers of inflammation this college-aged, recreationally active population. Further analysis of the inflammatory changes that occur following acute resistance exercise and chronic resistance training are warranted. These studies could provide useful exercise training insight for individuals suffering from diseases related to altered inflammatory profiles.

This study also examined the use of a multi-ingredient supplement prior to resistance training exercise on changes in strength and lean body mass. While many previous studies have found ingredients within the supplement effective in improving LBM and 1-RM strength measures, this study has revealed the combination of these ingredients may not result in the same positive performance outcomes. It is possible the dose of each ingredient within the supplement was lower than those previously reported to promote such changes.

Implications for Future Research

The cytokine signaling network is very complex and thus allows for many directions of research. It is important to continue to examine the cytokine response to exercise as changes in inflammation following regular training programs might provide effective means of controlling diseases marked by an altered cytokine state such as type II diabetes. Because much research has focused on aerobic exercise, further studies should examine the cytokine response to resistance exercise in both active and inactive individuals. Further examination of the inflammatory response to supplementation *in vivo* using separate studies for each ingredient within the supplement, and higher doses of each ingredient used could also provide insight to possible pharmacological interventions for diseases associated with cytokine disturbances.

Conclusions

The eight weeks of resistance training implemented in the present study resulted in expected changes in strength and lean body mass. Analysis of the tissue collected suggested an acute bout of total-body resistance exercise does not significantly increase skeletal muscle IL-6, however decreases in IL-6 with regular resistance training were reported and are in accordance with previous findings following aerobic training. Contrary to other studies acute or regular resistance exercise training did not result in any significant alterations of the inflammatory

markers IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IFN-γ, or TNF-α. In addition a pre-workout supplement containing caffeine, creatine, and amino acids among other ingredients did not significantly affect strength, lean body changes, or markers of inflammation associated with regular resistance training.

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APPENDIX A
Informed Consent

Approved by the Human Subjects Committee University of Kansas, Lawrence Campus (HSCL). Approval expires one year from 9/24/2009. HSCL#18136

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. The inclusion criteria is listed below:

Inclusionary Criteria

- Male
- Over the age of 18
- Under the age of 35
- Meets activity requirements (exercise 3 or more days per week for at least 1 year)
- **Non-Obese** (BMI <28 kg/m^2)
- Non-Smoker
- Has not experienced adverse effects to caffeine despite regular ingestion (including but not limited to dizziness, lightheadedness, nausea, 'jitters')
- Does not use anti-inflammatory medications or over-the counter drugs or can discontinue for the course of the study
- Does not uses other nutritional supplements or will discontinue for the course of the study
- Does not take 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)
- No 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
- No history of allergies to local anesthetics
- No history of allergies to iodine
- No history of allergies to Band-Aids or other adhesives
- No 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, food coloring)
- **No history of computed tomography (CT), PET, fluoroscopic, or nuclear medicine studies within the past year.**

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

Week	0	1	2	3	4	5	6	7	8	9
Supplement		◆	─	─	─	─	─	─	─	◆
Training		◆	─	─	─	─	─	─	─	◆
Biopsy		◆								◆
Blood Draw		◆								◆
Body Weight	◆				◆					◆
Body Composition	◆									◆
1 RM Testing	◆	◆			◆					◆
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 nor 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. <u>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.</u>

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 part of your body that will receive the most radiation is 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. An extended version of this information is attached as an appendix and we encourage you to read that as well. 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 mdenning@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 B
Medical History Questionnaire

APPLIED PHYSIOLOGY LABORATORY
UNIVERSITY OF KANSAS

MEDICAL HISTORY FORM

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) Systolic ≤ 100mmHg or Diastolic ≤ 60mmHg	YES	NO
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 If “YES”, Type I or Type II	YES	NO
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? YES NO

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)

Natural 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)

APPENDIX C

Biopsy Screening Questionnaire

Department of Health, Sport and Exercise Sciences

Applied Physiology Laboratory

Muscle Biopsy Screening Form

To help us ensure your safety and wellbeing, please answer the following questions honestly.

1. Have you ever had an adverse or allergic reaction to a local numbing agent (e.g. during dental procedures)?
   Yes No

2. Are you allergic to iodine based skin cleansers (i.e. Betadine, tincture of Iodine, etc)?
   Yes No

3. Are you allergic to Band-Aids or any similar adhesive dressings?
   Yes No

4. Do you have a tendency towards easy bleeding or bruising (e.g. with minor cuts or shaving)?
   Yes No

5. Please list any medications that you are taking including. Aspirin, Coumadin, Anti-inflammatories, Plavix, etc.

6. Have you ever had excessive scarring from a cut or a scrape?
   Yes No

7. Have you ever fainted or do you have a tendency to faint when undergoing or watching medical procedures?
   Yes No

8. Will you seek medical attention if you have any concerns about the biopsy site including: excessive redness, swelling, infection, pain or stiffness of the leg?
   Yes No

9. Are you willing to visit the Applied Physiology Lab to comply with the follow-up care and assessment of the biopsy site?
   Yes No

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 your or your third-party payer, such as my health insurer. If any injury or illness occurs in the course of research, or for more information, you will notify the investigator in charge.

Subject Name (print):____

Subject Signature:____

Date: __________________

Witness Signature:____

APPENDIX D
Physical Activity Questionnaire

Exercise Training History Questionnaire

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 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			

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	POUNDAGE'S

11. How many times per week do you participate in stretching? ______________

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

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

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

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

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

17. 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____

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

19. 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).

20. 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).

21. 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?

22. 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				
SUNDAY				

APPENDIX E
Caffeine Intake Questionnaire

Caffeine Intake and Tolerance 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. IF yes, 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 F
Supplement Label

Super Charge Orange

Supplement Facts

	Intense Workout		Super Intense	
Serving Size:	1 scoop (16g)		2 scoops (32g)	
Serving Per Conatiner		50		25
Amount Per Serving		**DV%**		**DV%**
Calories	32	†	64	†
Total Carbohydrate	8 g	3%	16 g	6%
Sugars	0 1 g	†	0 2 g	†
Vitamin A (beta-carotene)	2300 IU	46%	4600 IU	92%
Vitamin E (dl-Alpha Tocopheryl)	4 IU	13%	8 IU	26%
Super Charge® Proprietary Blend				
Phase 1- Energy/Hemo-Dilation Complex				
Nitrous Malate™ (di-arginine malate)	1121 mg	†	2242 mg	†
Taurine	1000 mg	†	2000 mg	†
Arginine Alpha-ketoglutarate	871 mg	†	1742 mg	†
L-Citrulline-dl-malate	500 mg	†	1000 mg	†
L-Tyrosine	250 mg	†	500 mg	†
Caffeine(Methylxanthines)	225 mg	†	450 mg	†
Phase 2- Strength & Endurance Complex				
CreaPure™ (creatine monohydrate)	1375 mg	†	2750 mg	†
BetaPure™ (betaine anhydrous)	500 mg	†	1000 mg	†
2CM™ (di-creatine malate)	125 mg	†	250 mg	†
Pikatropin™ (picamilon)	25 mg	†	50 mg	†
Phase 3- Post-Workout Recovery Complex				
Beta-Alanine	750 mg	†	1500 mg	†
N-Acetyl-L-Glutamine	500 mg	†	1000 mg	†
L-Histidine	125 mg	†	250 mg	†

*Percent Daily Values are based on a 2,000 Calorie diet.

† Daily Value not established.

Other ingredients: Maltodextrin, Natural Flavor (Corn Syrup,Solids, Modified Food Starch, Medium Chain Triglycerides, Talin, dl-Alphatocopherol), Silica, Beta-carotene (color), Sucralose, Citric Acid.

APPENDIX G
Supplement Side Effects Questionnaire

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

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.

Wednesday Blood Pressure Reading: ____________

APPENDIX H
Raw Data

Whole Muscle Data

Subject	Group	Height	Weight	Weight(kg)	Body Fat	LBM (kg)
1	2	69.3	180.5	82.04	20.8	62.04
2	2	70.3	169.6	77.10	18.0	59.87
3	2	68.2	145.4	66.09	14.5	54.04
4	2	76.0	175.9	79.97	12.0	67.31
5	1	72.6	171.5	77.95	16.1	65.25
6	1	68.3	174.5	79.34	21.1	59.54
7	1	75.0	163.9	74.50	10.6	63.35
8	1	70.4	187.8	85.36	20.6	64.51
9	1	70.3	164.8	74.93	13.4	62.20
10	1	71.7	161.1	73.22	8.4	63.82
11	1	71.4	177.7	80.79	23.0	59.24
12	1	69.8	204.1	92.77	30.7	61.35
13	1	71.6	173.0	78.63	11.2	66.55
14	1	70.0	163.8	74.45	11.7	62.65
15	1	70.6	143.1	65.06	12.9	53.87
16	2	70.7	155.7	70.77	8.9	61.39
17	1	69.7	150.2	68.27	18.7	52.85
18	2	69.7	165.1	75.05	7.9	66.23
19	2	77.0	192.2	87.36	20.0	65.69
20	1	72.1	190.6	86.63	20.3	66.07
21	2	68.7	163.1	74.15	18.8	57.42
22	1	70.6	173.1	78.70	16.4	62.54
23	2	66.5	200.7	91.22	29.8	61.12
24	2	74.0	165.3	75.15	11.9	62.40

Whole Muscle Data

Subject	Group (#)	Pre Squat Max	(kg)	Pre Bench Max	(kg)
1	2	245	111.36	225	102.27
2	2	235	106.81	160	72.72
3	2	165	75.00	135	61.36
4	2	225	102.27	155	70.45
5	1	225	102.27	165	75.00
6	1	260	118.18	195	88.63
7	1	205	93.18	160	72.72
8	1	235	106.81	165	75.00
9	1	235	106.81	180	81.81
10	1	240	109.09	175	79.54
11	1	215	97.72	165	75.00
12	1	365	165.90	185	84.09
13	1	280	127.27	175	79.54
14	1	295	134.09	215	97.72
15	1	185	84.09	135	61.36
16	2	270	122.72	230	104.54
17	1	205	93.18	135	61.36
18	2	335	152.27	205	93.18
19	2	195	88.63	145	65.90
20	1	295	134.09	215	97.72
21	2	245	111.36	195	88.63
22	1	225	102.27	185	84.09
23	2	265	120.45	175	79.54
24	2	185	84.09	145	65.90

Whole Muscle Data

Subject	Group (#)	Pre Leg Ex Max	(kg)	Pre Leg Curl Max	(kg)
1	2	230	104.54	190	86.36
2	2	170	77.27	180	81.81
3	2	180	81.81	140	63.63
4	2	190	86.36	170	77.27
5	1	120	54.54	210	95.45
6	1	200	90.90	170	77.27
7	1	200	90.90	170	77.27
8	1	220	100.00	180	81.81
9	1	220	100.00	160	72.72
10	1	200	90.90	170	77.27
11	1	190	86.36	180	81.81
12	1	175	79.54	170	77.27
13	1	220	100.00	200	90.90
14	1	245	111.36	160	72.72
15	1	130	59.09	90	40.90
16	2	190	86.36	170	77.27
17	1	160	72.72	150	68.18
18	2	180	81.81	150	68.18
19	2	230	104.54	190	86.36
20	1	245	111.36	200	90.90
21	2	200	90.90	150	68.18
22	1	220	100.00	170	77.27
23	2	170	77.27	120	54.54
24	2	190	86.36	170	77.27

Whole Muscle Data

Subject	Group (#)	Pre Lat Pulldown Max	(kg)	Pre Deadlift Max	(kg)
1	2	230	104.54	355	161.36
2	2	190	86.36	275	125.00
3	2	170	77.27	215	97.72
4	2	160	72.72	245	111.36
5	1	190	86.36	305	138.63
6	1	200	90.90	295	134.09
7	1	190	86.36	205	93.18
8	1	140	63.63	245	111.36
9	1	230	104.54	255	115.90
10	1	190	86.36	275	125.00
11	1	200	90.90	155	70.45
12	1	200	90.90	355	161.36
13	1	200	90.90	345	156.81
14	1	220	100.00	365	165.90
15	1	115	52.27	225	102.27
16	2	220	100.00	315	143.18
17	1	160	72.72	205	93.18
18	2	225	102.27	305	138.63
19	2	190	86.36	285	129.54
20	1	210	95.45	365	165.90
21	2	180	81.81	275	125.00
22	1	200	90.90	235	106.81
23	2	200	90.90	275	125.00
24	2	180	81.81	235	106.81

Whole Muscle Data

Subject	Group (#)	Pre Incline Max	(kg)	Pre Curls Max	(kg)
1	2	185	84.09	75	34.09
2	2	130	59.09	80	36.36
3	2	125	56.81	45	20.45
4	2	145	65.90	60	27.27
5	1	155	70.45	75	34.09
6	1	185	84.09	80	36.36
7	1	145	65.90	65	29.54
8	1	145	65.90	50	22.72
9	1	165	75.00	80	36.36
10	1	145	65.90	55	25.00
11	1	225	102.27	65	29.54
12	1	180	81.81	80	36.36
13	1	165	75.00	85	38.63
14	1	190	86.36	105	47.72
15	1	115	52.27	55	25.00
16	2	175	79.54	90	40.90
17	1	130	59.09	50	22.72
18	2	195	88.63	95	43.18
19	2	135	61.36	80	36.36
20	1	205	93.18	80	36.36
21	2	175	79.54	80	36.36
22	1	165	75.00	75	34.09
23	2	175	79.54	80	36.36
24	2	150	68.18	75	34.09

Whole Muscle Data

Subject	Group (#)	Post Wt (kg)	Post % Fat	Post Lean Mass	Post LBM (kg)
1	2	84.00	19.2	142.73	64.88
2	2	76.59	16.7	133.39	60.63
3	2	68.36	16.7	119.62	54.37
4	2	81.77	10.7	149.57	67.98
5	1	79.97	14.6	142.86	64.94
6	1	82.95	23.4	133.32	60.60
7	1	76.63	10.3	144.98	65.90
8	1	87.22	22.6	140.91	64.05
9	1	75.70	13.3	138.25	62.84
10	1	77.29	7.8	150.17	68.26
11	1	81.88	23	131.91	59.96
12	1	95.45	29.7	141.08	64.12
13	1	77.47	9.9	146.07	66.39
14	1	75.72	12.2	139.35	63.34
15	1	64.81	10.7	121.18	55.08
16	2	71.34	8.9	136.08	61.85
17	1	70.47	16.8	123.42	56.10
18	2	75.31	8.3	145.08	65.94
19	2	91.25	19.3	151.39	68.81
20	1	87.47	18.9	149.43	67.92
21	2	77.56	22.3	126.37	57.44
22	1	80.90	14.5	145.25	66.02
23	2	96.88	29.3	143.90	65.40
24	2	73.84	9.4	139.35	63.34

Whole Muscle Data

Subject	Group (#)	Post Squat Max	(kg)	Post Bench Max	(kg)
1	2	295	134.09	255	115.90
2	2	280	127.27	165	75.00
3	2	235	106.81	150	68.18
4	2	275	125.00	175	79.54
5	1	300	136.36	190	86.36
6	1	335	152.27	225	102.27
7	1	250	113.63	185	84.09
8	1	330	150.00	190	86.36
9	1	315	143.18	215	97.72
10	1	390	177.27	205	93.18
11	1	275	125.00	205	93.18
12	1	420	190.90	220	100.00
13	1	375	170.45	190	86.36
14	1	335	152.27	250	113.63
15	1	250	113.63	145	65.90
16	2	300	136.36	240	109.09
17	1	265	120.45	165	75.00
18	2	370	168.18	220	100.00
19	2	275	125.00	160	72.72
20	1	340	154.54	260	118.18
21	2	305	138.63	210	95.45
22	1	315	143.18	225	102.27
23	2	375	170.45	225	102.27
24	2	265	120.45	170	77.27

Whole Muscle Data

Subject	Group (#)	Post Leg Ex Max	(kg)	Post Leg Curl Max	(kg)
1	2	245	111.36	210	95.45
2	2	215	97.72	180	81.81
3	2	220	100.00	170	77.27
4	2	250	113.63	185	84.09
5	1	260	118.18	220	100.00
6	1	245	111.36	180	81.81
7	1	265	120.45	205	93.18
8	1	290	131.81	200	90.90
9	1	260	118.18	170	77.27
10	1	235	106.81	200	90.90
11	1	250	113.63	210	95.45
12	1	315	143.18	210	95.45
13	1	260	118.18	210	95.45
14	1	280	127.27	180	81.81
15	1	195	88.63	170	77.27
16	2	245	111.36	185	84.09
17	1	245	111.36	165	75.00
18	2	235	106.81	180	81.81
19	2	300	136.36	220	100.00
20	1	265	120.45	220	100.00
21	2	275	125.00	205	93.18
22	1	275	125.00	210	95.45
23	2	260	118.18	180	81.81
24	2	275	125.00	185	84.09

Whole Muscle Data

Subject	Group (#)	Post Lat Pulldown Max	(kg)	Post Deadlift Max	(kg)
1	2	230	104.54	365	165.90
2	2	200	90.90	N/A	N/A
3	2	180	81.81	265	120.45
4	2	185	84.09	255	115.90
5	1	220	100.00	365	165.90
6	1	210	95.45	325	147.72
7	1	200	90.90	300	136.36
8	1	190	86.36	325	147.72
9	1	245	111.36	310	140.90
10	1	240	109.09	335	152.27
11	1	215	97.72	285	129.54
12	1	235	106.81	365	165.90
13	1	210	95.45	350	159.09
14	1	235	106.81	365	165.90
15	1	145	65.90	290	131.81
16	2	235	106.81	335	152.27
17	1	185	84.09	265	120.45
18	2	235	106.81	365	165.90
19	2	220	100.00	325	147.72
20	1	235	106.81	405	184.09
21	2	210	95.45	330	150.00
22	1	230	104.54	305	138.63
23	2	235	106.81	405	184.09
24	2	205	93.18	235	106.81

Whole Muscle Data

Subject	Group (#)	Post Incline Max	(kg)	Post Curls Max	(kg)
1	2	205	93.18	90	40.90
2	2	N/A	N/A	N/A	N/A
3	2	140	63.63	75	34.09
4	2	165	75.00	75	34.09
5	1	170	77.27	80	36.36
6	1	205	93.18	90	40.90
7	1	170	77.27	80	36.36
8	1	160	72.72	80	36.36
9	1	185	84.09	85	38.63
10	1	185	84.09	75	34.09
11	1	165	75.00	80	36.36
12	1	200	90.90	100	45.45
13	1	175	79.54	100	45.45
14	1	220	100.00	105	47.72
15	1	120	54.54	70	31.81
16	2	190	86.36	100	45.45
17	1	155	70.45	65	29.54
18	2	210	95.45	100	45.45
19	2	N/A	N/A	N/A	N/A
20	1	230	104.54	105	47.72
21	2	195	88.63	90	40.90
22	1	195	88.63	100	45.45
23	2	205	93.18	105	47.72
24	2	150	68.18	80	36.36

Protein Extraction/Concentration Data

PRE 1

Subject	Group	Sample Size (mg)	Mean BSA
1	2	19.4	26.6
2	2	13.88	21.37
3	2	16.31	23.73
4	2	15.79	22.63
5	1	12.4	36.42
6	1	15.75	24.22
7	1	17.26	28.79
8	1	16.73	24.16
9	1	13.78	24.18
10	1	17.81	14.6
11	1	13.87	19.74
12	1	16.27	27.44
13	1	16.8	23.07
14	1	16.5	22.58
15	1	13.38	25.18
16	2	13.92	25.1
17	1	15.41	30.69
18	2	14.65	33.17
19	2	20.1	31.63
20	1	18.7	25.99
21	2	16.08	27.68
22	1	16.55	29.43
23	2	15.21	20.48
24	2	19.22	32.43

Protein Extraction/Concentration Data

PRE 2

Subject	Group	Sample Size (mg)	Mean BSA
1	2	16.21	20.84
2	2	14.05	20.07
3	2	17.52	18.20
4	2	17.73	23.10
5	1	14.50	24.55
6	1	17.03	19.54
7	1	16.13	23.49
8	1	18.90	24.57
9	1	15.44	26.49
10	1	20.15	23.98
11	1	20.06	27.59
12	1	20.33	31.15
13	1	16.84	26.84
14	1	16.40	28.05
15	1	17.04	25.18
16	2	17.49	25.12
17	1	19.52	39.74
18	2	16.53	25.90
19	2	15.21	27.44
20	1	14.62	25.64
21	2	19.14	31.76
22	1	19.98	22.21
23	2	13.99	24.84
24	2	14.77	30.67

Protein Extraction/Concentration Data

POST 1

Subject	Group	Sample Size (mg)	Mean BSA
1	2	17.05	33.06
2	2	16.35	27.94
3	2	20.87	27.57
4	2	17.89	32.98
5	1	16.03	32.72
6	1	12.79	27.27
7	1	19.06	27.45
8	1	17.17	22.33
9	1	19.32	31.89
10	1	15.11	20.66
11	1	17.55	29.33
12	1	17.83	25.52
13	1	15.32	20
14	1	15.66	24.23
15	1	20.65	33.36
16	2	16.63	30.76
17	1	17.57	26.05
18	2	15.57	21.83
19	2	15.71	27.31
20	1	16.41	30.56
21	2	19.88	37.62
22	1	14.5	23.14
23	2	18.35	32.02
24	2	15.37	25.63

Protein Extraction/Concentration Data

POST 2

Subject	Group	Sample Size (mg)	Mean BSA
1	2	18.96	27.89
2	2	16.7	27.35
3	2	19.75	23.8
4	2	18.96	33.71
5	1	18.45	27.23
6	1	20.66	38.33
7	1	17.61	27.06
8	1	17.02	25.33
9	1	17.37	23.24
10	1	14.82	23.02
11	1	14.48	23.49
12	1	18.48	33.92
13	1	18.33	22.89
14	1	16.31	27.42
15	1	17.58	26.82
16	2	19.5	25.9
17	1	17.32	33.08
18	2	17.92	25.88
19	2	17.05	30.77
20	1	16.65	27.87
21	2	18.99	27.8
22	1	16.56	26.23
23	2	18.05	30.74
24	2	17.54	34.04

Western Immunoblotting Densitomety Values (arbitrary units)

Subject	Group	Average PRE1	Average PRE2
1	2	18697360	30813810
2	2	32405688	16797921
3	2	7620946.667	13378720.67
4	2	23989664	33144001
5	1	11173092.33	34387213.33
6	1	7060534	9032679.333
7	1	9272912	9621170
8	1	10921680	9802786.333
9	1	15362594.67	12941473.67
10	1	17504065	14802852
11	1	17646318	20637173.33
12	1	18605730	19563226.67
13	1	27352052	22455450
14	1	26148096	9698803.333
15	1	7100025	11617289.33
16	2	17043891.33	15955480.67
17	1	60660550	19058466.67
18	2	33823884	18897016
19	2	13826782	13434446.67
20	1	9956805	12056146.33
21	2	9711920	9751560
22	1	8327172.667	11355557
23	2	20460578.67	10793979.33
24	2	6017496	6234972.333

Western Immunoblotting Densitomety Values (arbitrary units)

Subject	Group	Average POST1	Average POST2
1	2	10093835	8361418.667
2	2	8299383.333	5285510.667
3	2	16283800	9700831.333
4	2	8077039	10628220
5	1	7567199.333	6016708.667
6	1	4770268	5454923.667
7	1	3596622	4946281.333
8	1	4918086.667	4775516
9	1	7405092	9110728
10	1	4705536	8182230
11	1	3891372.667	4428390
12	1	3699333.333	3138294.333
13	1	6446258	3683189
14	1	4696800	6285740.333
15	1	5396841	5953321.333
16	2	4602645	10738884
17	1	17772506.67	12372425
18	2	13562985.33	11761656.67
19	2	7082670	5338410
20	1	4267121.333	5263954
21	2	6366822	4973696
22	1	6589823.333	7252674
23	2	2523709.667	2407310
24	2	14778345.67	6167304

ELISA Data

Subject	Group	IL1α PRE1	IL1α PRE2	IL1β PRE1	IL1β PRE2	IL2 PRE1	IL2 PRE2
1	2	63123.77	35754.8	6929.235	5585.552	2038.24	2038.98
2	2	34984.79	5783.87	33647.05	3446.618	30153.4	1941.63
3	2	1855.35	21810.04	3603.113	32385.27	1907.42	23923
4	2	3231.417	5024.976	33022.84	26010.34	2147.62	10390
5	1	5814.66	5887.885	2442.382	3476.842	1745.09	1813.3
6	1	3720.5	3483.958	15876.07	8807.283	2754.69	2360.33
7	1	5245.962	3678.905	3184.286	2760.052	2309.25	13911.2
8	1	3028.975	4624.296	8218.431	6317.618	1623.82	8715.85
9	1	1257.688	1779.542	1779.542	5087.517	1257.69	1779.54
10	1	4045.906	3675.427	2829.771	2896.575	1602.58	1465.05
11	1	6828.188	5865.294	2877.174	2823.893	1216.11	1549.98
12	1	5431.558	4813.864	3742.398	3932.094	1332.52	9646.4
13	1	5287.639	5682.099	2691.183	3102.923	1673.44	1392.18
14	1	5964.772	6949.579	5475.72	5614.073	1798.68	1957.24
15	1	2532.407	1715.167	1715.167	7782.5	2532.41	1715.17
16	2	5686.976	4972.769	5149.45	6152.643	17428.3	18355.9
17	1	8424.781	6018.533	5089.406	5290.656	2637.16	1895.07
18	2	5232.413	7521.589	2752.511	3173.693	1540.15	1420.92
19	2	4140.963	4258.536	2887.314	3382.031	31761.8	15244.4
20	1	5145.663	5240.125	2319.896	3450.761	14053.7	18897.7
21	2	4625.969	5618.992	2184.794	2373.684	1707.88	1725.33
22	1	29912.93	4167.698	32928.14	9046.318	29139.1	2437.29
23	2	8248.238	7538.587	2652.889	3046.234	2047.53	1853.83
24	2	8969.9	6673.975	4954.859	3515.675	2672.38	1844.91

ELISA Data

Subject	Group	IL4 PRE1	IL4 PRE2	IL6 PRE1	IL6 PRE2	IL-8 PRE1	IL8 PRE2
1	2	7569.71875	6632.7071	31192.143	21655.306	32103.844	29219.4
2	2	32843.92188	6101.3009	17376.164	3375.9792	42164.367	18910.4
3	2	5993.571023	32900	2148.8586	30052.3	16161.143	41541.6
4	2	18872.44231	17782.528	13480.656	12572.575	55161.365	35594.6
5	1	4854.457895	4312.3611	2559.6143	3007.2935	21097.835	18594.6
6	1	5252.016667	3733.4811	3555.375	3753.2024	15134.007	16340.4
7	1	12363.2619	7595.7955	3224.1008	3296.0625	19315.643	8966.89
8	1	7731.1875	7542.5625	5018.5915	7746.7104	28022.644	18975.8
9	1	30042.33333	5019.4869	2361.9722	3640.8421	19039.722	13759.3
10	1	4918.075569	4734.8153	2252.7917	2662.1734	13581.636	14417
11	1	5135.169913	4600.9895	2421.5143	3587.2292	30552.25	34246.2
12	1	4982.315789	8204.1667	2797.2569	5893.125	16766.438	12488.2
13	1	4575.75	4930.9318	2312.1082	2627.5263	19377.805	20166.6
14	1	3936.904762	5645.125	3428.3158	3965.5381	20421.225	19535.6
15	1	6078.027778	5323.7944	2621.8	2691.0432	10135.977	10575.5
16	2	7719.008373	10626.435	5492.625	5783.2566	8392.1125	8988.65
17	1	6176.214976	4544.3333	3355.4783	3789.9391	23850.304	23185.7
18	2	3491.258333	4634.9386	3258.0812	2995.4348	17373.072	19057.1
19	2	6578.535897	6838.4471	4608.0625	4384.2847	7010.4405	7054.45
20	1	6855.166667	8177.1	6061.4063	5040.6335	8617.0303	9720.84
21	2	3726.538636	4507.8505	4722.536	2109.26	16913.791	14799.6
22	1	33341.80707	6338.0341	14894.245	3360.5852	43984.385	20908.6
23	2	5398.885417	4598.0909	2731.1563	2871.7895	10120.313	18843.1
24	2	6289.741228	4647.6404	3391.1402	7495.2974	19925.11	15480.3

ELISA Data

Subject	Group	IL10 PRE1	IL10 PRE2	IFNγ PRE1	IFNγ PRE2
1	2	13010.633	11985.038	10526.22321	8407.582707
2	2	7988.486	9477.4688	33075.78125	7155.991667
3	2	6339.6244	34604.58	7181.84375	32682.26087
4	2	21643.571	13852.015	23804.98011	15452.69173
5	1	6598.5675	8625.2639	5049.120773	5734.409091
6	1	59478.222	32756.087	6003.391667	4527.857143
7	1	7693.4708	4604.6458	5737.5625	2847.393939
8	1	43995.75	28351.115	7030.113636	4655.402174
9	1	7518.3048	9984.5179	5304.633152	6447.5625
10	1	6170.4042	7544.7403	5493.666667	4983.619048
11	1	7767.5	8279.3571	5626.998551	5174.78125
12	1	5790.5378	6912.5852	5494.761696	3760.740489
13	1	4770.1833	4422.5208	5004.451128	6040.1875
14	1	9993.1591	12200.585	6076.915179	7622.704545
15	1	21700.5	13320.658	4856.75	4742.068182
16	2	2155.256	2250.6857	1450.461905	1712
17	1	8995.8889	9036.4271	8903.487154	6790.47488
18	2	8510.9688	8420.1193	4410.47619	6143.269737
19	2	1511.0125	1861.1354	1675	1834.354167
20	1	2124.88	3122.5518	2485.178571	2424.119048
21	2	9468.8563	5937.939	3907.181704	4384.8
22	1	10408.362	35627.287	31042.87054	6083.921053
23	2	7537.3684	9617.2667	5214.742063	5483.121212
24	2	17772.01	30196.781	6321.10462	5048.954545

ELISA Data

Subject	Group	TNFα PRE1	TNFα PRE2	IL1α POST1	IL1α POST2
1	2	16289.938	12194.4	62687.8387	32731.89167
2	2	38050.15	9579.798295	5626.45	6662.704861
3	2	6043.9892	35186.77083	1679.90476	20751.24943
4	2	49727.921	30582.0767	2875.16667	3873.277193
5	1	9778.3708	9072.17193	4670.81667	4402.70303
6	1	18452.767	12742.37778	4636.36508	3522.3125
7	1	8551.4762	4327.882997	5744.57292	5138.133224
8	1	15337.267	9146.794444	2810.95833	3210.882576
9	1	8419.1875	7927.147727	5394.4	3631.333333
10	1	7148.6807	8225.045139	5052.07644	8485.111111
11	1	8526.419	7788.061265	5265.24554	4575.1
12	1	9683.5278	6266.7	3952.50833	4429.416667
13	1	7021.1389	7401.02381	5824.67143	4836.139394
14	1	10362.694	10566.05	5202.57986	8964.46875
15	1	11254.625	7741.315789	40778.0479	39448.23864
16	2	1787.5455	2378.412879	5063.06667	6033.217949
17	1	11645.717	9065.828571	10817.7783	5804.226316
18	2	7889.4778	9019.246528	6059.20556	5804.226316
19	2	1912.8148	1687.021739	10817.7783	6059.205556
20	1	2215.4333	3066.653846	1456.4697	1605.625
21	2	7347.9104	6086.53125	26716.153	1307.270833
22	1	31767.903	14204.09375	1089.90805	997.394958
23	2	6859.6667	7861.604978	1879.17017	1290.492424
24	2	9621.3153	7351.638095	5159.13889	5739.750877

ELISA Data

Subject	Group	IL1β POST1	IL1β POST2	IL2 POST1	IL2 POST2
1	2	7209.15035	9061.453557	2125.12319	1759.1417
2	2	2436.447222	3429.40625	1257.03704	1263.8523
3	2	2942.858553	32838.55466	1568.0625	17750.875
4	2	17912.82955	11804.68254	1689.25	2562.5414
5	1	1548.019608	1848.940476	1576.95833	1418.9167
6	1	13845.80114	16998.38393	2914.125	4056.3958
7	1	1820.454545	1879.568452	1710.91111	1797.9722
8	1	6677.75	9788.455592	1796	2635.3346
9	1	2184.840351	1895.198684	1603.93333	1098.9667
10	1	2315.4375	2759.194444	1370.30667	6496.875
11	1	2776.672222	2665.718421	1369.66667	1699.8288
12	1	2018.3	1953.961538	1965.8625	1929.8542
13	1	11882.01877	10296.6865	2186.81731	1838.3021
14	1	2867.327083	5747.883152	1038.97727	1780.8956
15	1	62935.42308	62602.00507	36142.25	46063.225
16	2	4711.377778	8608.308333	2096.42535	4565.0468
17	1	3333.04386	3799.383838	1738.63968	1628.6626
18	2	3543.846726	3799.383838	1894.29412	1628.6626
19	2	3333.04386	3543.846726	1738.63968	1894.2941
20	1	1605.625	2771.483333	1456.4697	1605.625
21	2	1307.270833	33654.48148	26716.153	1307.2708
22	1	997.394958	2117.654762	1089.90805	997.39496
23	2	1290.492424	2553.953947	1879.17017	1290.4924
24	2	2175.209821	2046.551487	1157.4087	1065.0128

ELISA Data

Subject	Group	IL4 POST1	IL4 POST2	IL6 POST1	IL6 POST2
1	2	8292.4688	7862.2375	47107.21875	18865.61
2	2	4604.6439	4503.3561	2229.026316	3071.559
3	2	4559.3947	33466.132	2409.84375	24679.71
4	2	16858.531	11091.918	6134.682065	5430.857
5	1	3409.1576	3574.7754	1624.274123	1947.342
6	1	5939.7083	6706.6474	3280.776786	3921.551
7	1	5575.7091	5968.7614	1861.035197	3679.04
8	1	5430.5	7305.8146	4438.875	7470.86
9	1	4088.4931	2711.7484	2159.346316	2250.374
10	1	4972.649	4650.8059	7309.113636	6671.304
11	1	3640.8278	3635.2159	2226.539474	2873.059
12	1	3202.6667	3553.8215	1571.05848	3360.693
13	1	7539.0632	6074.5132	11017.04545	6855.068
14	1	3012.4915	6065.4511	2912.596491	5986.727
15	1	61666.027	59799.133	48831.3	59207.47
16	2	3946.6053	5780.2829	2564.313158	6329.356
17	1	7169.1592	6736.6201	2745.842424	4774.744
18	2	6443.0313	6736.6201	2932.332609	4774.744
19	2	7169.1592	6443.0313	2745.842424	2932.333
20	1	4839.7763	4774.05	7711.801136	5692.177
21	2	30230.077	6068.0271	14745.76645	6985.551
22	1	3683.1842	3874.9026	5567.27381	4508.612
23	2	5484.9737	3997.9956	4153.742424	3374.373
24	2	4064.2727	4119.9437	1722.509058	2864.858

ELISA Data

Subject	Group	IL8 POST1	IL8 POST2	IL10 POST1	IL10 POST2
1	2	32011.643	17923.615	17837.3095	15091.7424
2	2	14005.524	18176.232	7327.08571	7181.71053
3	2	19125.286	35693.79	6825.79365	35333.2235
4	2	37487.565	32427.758	10912.2336	12526.1571
5	1	22323.303	19797.926	6239.225	6793.88651
6	1	14679.452	17118.471	57529.2141	57915.6153
7	1	14749.063	11123.893	6333.27083	8802.81548
8	1	17095.109	18446.433	43515.5	50507.3023
9	1	14493.977	11443.781	4558.88636	4460.59596
10	1	10435.906	7843.9524	9748.08807	10497.2905
11	1	19428.768	30138.751	9455.43333	9310.90625
12	1	10196.625	16312.291	4655.0625	6360.18651
13	1	19288.053	13443.397	12455.3153	16360.5089
14	1	13450.385	22281.235	6488.73684	11566.5969
15	1	57283.693	58939.545	63914.1417	64286.5222
16	2	22513.852	34914.669	13901.7242	25501.5333
17	1	15076.45	28707.681	7980.77964	19279.2352
18	2	30405.347	28707.681	8933.50417	19279.2352
19	2	15076.45	30405.347	7980.77964	8933.50417
20	1	11902.431	11480.708	7844.89638	6568.83239
21	2	38674.878	14938.835	9245.99048	12001.2083
22	1	10155.357	11558.366	7715.206	6333.20707
23	2	18970.098	14057.958	11162.3125	9244.99048
24	2	21618.219	21622.093	5940.64145	8153.375

ELISA Data

Subject	Group	IFNγ POST1	IFNγ POST2	TNFα POST1	TNFα POST2
1	2	11544.48864	10534.60885	14109.50595	12055.80159
2	2	6778.71875	7087.766304	6297.410714	8410.766667
3	2	5111.080702	22238.4366	6234.780952	35409.71198
4	2	16896.17763	12028.58333	21794.97222	15942.27083
5	1	3517.969697	3456.75744	6601.422807	5579.527778
6	1	5796.9	6608.738636	18363.90625	20416.89123
7	1	3754.548718	3546.760766	5541.292614	5596.59375
8	1	5142.312121	6150.327122	12040.125	14475.32589
9	1	4192.033333	3334.09375	4477.235119	4029.695489
10	1	6744.906015	6893.1875	6713.405844	6471.809211
11	1	4602.212121	4462.532164	7632.67619	7636.78125
12	1	3585.058081	4188.737374	5021.029762	6139.152778
13	1	13001.17506	10709.88587	15591.09673	11353.7803
14	1	4745.430556	8749.056818	7135.428571	13480.95417
15	1	40116.47727	51111.6622	63557.29602	63187.59447
16	2	5244.702381	6632.202381	8291.674242	15172.5
17	1	6929.90625	7074.139394	9079.698864	9716.526316
18	2	5837.007576	7074.139394	9198.680556	9716.526316
19	2	6929.90625	5837.007576	9079.698864	9198.680556
20	1	5052.052676	5061.15625	7000.496528	5903.796491
21	2	20697.84596	9022.136364	22603.70982	8352.9375
22	1	4262.108333	4073.277778	5735.791228	5556.540179
23	2	7244.016667	5257.956522	7623.1875	5988.129934
24	2	4299.277273	4744.555556	8058.633333	7765.153533

ELISA DATA

Standard Curve Densitometry Values

IL-1α-Plate 1		IL-1β-Plate 1		IL-2-Plate 1	
63408.21622	1 to 16	64454.027	1 to 16	64091.875	1 to 16
63627.4375	1 to 32	64567.1081	1 to 32	64093.5185	1 to 32
62059.84	1 to 64	63578.1515	1 to 64	63136.4545	1 to 64
58641.95238	1 to 128	61357.5333	1 to 128	54543.7778	1 to 128
12814.0625	Blank	4437.16667	Blank	12792.1333	Blank
IL-1α-Plate 2		IL-1β-Plate 2		IL-2-Plate 2	
62984.57895	1 to 16	63732.4667	1 to 16	63950	1 to 16
63486.26667	1 to 32	64600.2973	1 to 32	63773.4	1 to 32
60717.95652	1 to 64	62911.5625	1 to 64	57712.4762	1 to 64
62351.8125	1 to 128	62511.7097	1 to 128	54050.4	1 to 128
12003.7619	Blank	3779.23077	Blank	11036.7619	Blank
IL-1α-Plate 3		IL-1β-Plate 3		IL-2-Plate 3	
64375.7619	1 to 9	65535	1 to 9	63675.9737	1 to 9
63492.28125	1 to 27	63585.973	1 to 27	63477.1071	1 to 27
37837.36364	1 to 81	59213.5185	1 to 81	37801.9333	1 to 81
20089.16667	1 to 243	29027.619	1 to 243	21222.4667	1 to 243
11773.95455	1 to 729	12259.4737	1 to 729	6679.88889	1 to 729

ELISA DATA

Standard Curve Densitometry Values

IL-4-Plate 1		IL-6-Plate 1		IL-8-Plate 1	
62106.58824	1 to 16	62778.28571	1 to 16	63677.8	1 to 16
62595.48485	1 to 32	57426.5	1 to 32	63663.94118	1 to 32
62575.65385	1 to 64	47105	1 to 64	64067.5	1 to 64
56414.45455	1 to 128	20656.57895	1 to 128	59848.125	1 to 128
12032.83333	Blank	4242.259259	Blank	7669.157895	Blank
IL-4-Plate 2		IL-6-Plate 2		IL-8-Plate 2	
62958.33333	1 to 16	63501.40625	1 to 16	63409.95455	1 to 16
62212.51429	1 to 32	57899.38462	1 to 32	64820.38235	1 to 32
60080.20833	1 to 64	32156.78261	1 to 64	62001.59259	1 to 64
50245	1 to 128	27123.40909	1 to 128	59503.66667	1 to 128
11460.2	Blank	3485.5	Blank	6948.277778	Blank
IL-4-Plate 3		IL-6-Plate 3		IL-8-Plate 3	
64134.2093	1 to 9	63829.82143	1 to 9	63781.55556	1 to 9
63298.10526	1 to 27	51210.26923	1 to 27	62454.44737	1 to 27
31768.96154	1 to 81	22068.17391	1 to 81	46509.95455	1 to 81
15977.72222	1 to 243	10106.63636	1 to 243	20597.5	1 to 243
13380.57895	1 to 729	5409.111111	1 to 729	12183.52381	1 to 729

ELISA DATA

Standard Curve Densitometry Values

IL-10-Plate 1		TNF-α-Plate 1		IFN-γ-Plate 1	
15784.3913	1 to 16	60885.71429	1 to 16	64048.5	1 to 16
13308.57143	1 to 32	61538.56522	1 to 32	62663.23333	1 to 32
10232.68182	1 to 64	56314.69231	1 to 64	58122.57692	1 to 64
7849.681818	1 to 128	39742.81818	1 to 128	51201.5625	1 to 128
5444.636364	Blank	6620.695652	Blank	6336.26087	Blank
IL-10-Plate 2		TNF-α-Plate 2		IFN-γ-Plate 2	
38908.56522	1 to 16	64287.9375	1 to 16	63284.97059	1 to 16
22095.5	1 to 32	62547.69231	1 to 32	61719.60714	1 to 32
11670.61111	1 to 64	58187.9375	1 to 64	58269.5	1 to 64
9557.625	1 to 128	47865.125	1 to 128	44207.125	1 to 128
5171.368421	Blank	6242.16	Blank	6301.25	Blank
IL-10-Plate 3		TNF-α-Plate 3		IFN-γ-Plate 3	
61324.36364	1 to 9	63100.02439	1 to 9	64946.06818	1 to 9
17518.38095	1 to 27	56836.38462	1 to 27	62766.29032	1 to 27
6468.733333	1 to 81	27588.14286	1 to 81	30360.58333	1 to 81
4945.130435	1 to 243	14535.05556	1 to 243	12834.55556	1 to 243
4734.933333	1 to 729	8764.5	1 to 729	7836.772727	1 to 729