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The Effect of Betaine Supplementation on Performance and Muscle Mechanisms

Jenna M. Apicella

University of Connecticut - Storrs, jenna.apicella@gmail.com

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The Effect of Betaine Supplementation on Performance and
Muscle Mechanisms

Jenna Mae Apicella

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Master of Arts Thesis

The Effect of Betaine Supplementation on Performance and
Muscle Mechanisms

Presented by

Jenna Mae Apicella, B.S.

Major Advisor

Carl M. Maresh, Ph.D.

Associate Advisor

Jeffrey M. Anderson, M.D.

Associate Advisor

William J. Kraemer, Ph.D.

Associate Advisor

Jeff S. Volek, Ph.D.

University of Connecticut

2011

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Abstract

Background: Recent research has shown that betaine supplementation can increase strength and power performance. To further investigate the ergogenic effects of betaine supplementation a balanced, randomized, double-blind, repeated measures crossover study was designed to examine select hormonal and muscle signaling responses during supplementation.

Methods: Twelve recreationally active men (age, 19.7 ± 1.23 years; height, 172.6 ± 5.61 cm; weight, 84.27 ± 15.3 kg; body fat, $18.7 \pm 7.0\%$; BMI, 28.2 ± 4.0) with a minimum of 3 months of resistance training including back squat and bench press participated in the study. A within crossover design was utilized and subjects were randomly assigned to either betaine or placebo group. Subjects performed an acute exercise test (AET) consisting of maximal vertical jumps, isometric bench press, isometric squat, and a box lift test before and after 14 days of supplementation (twice a day) with either betaine (1.25 g dissolved in Gatorade) or placebo (same volume of Gatorade alone). During each AET, blood samples were obtained at pre, mid, post, +5, and +15 and were analyzed for GH, IGF-1 and cortisol. Also during each AET, muscle biopsies were obtained from the vastus lateralis at pre and +10 and were analyzed for select muscle signaling proteins (Total Akt, Akt S473, p70 S6k1, p70 S6k1 T389, and AMPK T172). Following a 14 day washout period, subjects crossed over to the other supplementation protocol and performed the same pre and post AETs.

Results: Following betaine supplementation, there was a significant increase in isometric squat force production and number of boxes lifted during the box lift test. However, there were no significant differences in isometric bench press and vertical jumps between

supplementation groups. There was a significant increase in area under the curve (AUC) for GH and IGF-1 and a significant decrease (AUC) for cortisol following 14 days of betaine supplementation. Following betaine supplementation, there was an increased in muscle Total Akt. Phosphorylation of Akt at S473 and phosphorylation of p70 S6k at T389 were maintained following betaine supplementation while these values were decreased during placebo supplementation. Phosphorylation of AMPK at T172 was decreased following betaine and placebo supplementation.

Conclusion: Betaine supplementation increased force production and muscular endurance. These performance measures correspond to increases in anabolic hormones (GH and IGF-1) and maintenance of anabolic muscle signaling (Total Akt, Akt and p70 S6k phosphorylation) responses, as well as the decrease in catabolic hormone (cortisol) and inhibitory muscle signaling (AMPK phosphorylation) following 14 days of betaine supplementation

Chapter 1: Introduction

Skeletal muscle plasticity allows the body to adapt to a wide range of stimuli. On the two ends of the exercise spectrum, endurance training and resistance training provoke different adaptations in muscle. While endurance training focuses on improving work capacity and decreasing fatigue mainly via increased mitochondrial biogenesis, resistance training focuses on improving strength and power via increased protein accretion and neural recruitment. For resistance training, the acute program variables (exercise choice, exercise order, load, volume, and rest intervals) dictate the specific stimuli resulting in the specific muscle adaptations³⁰.

Resistance exercise creates an anabolic environment by mediating both hormonal and molecular signaling cascades. From a hormonal perspective, an acute bout of resistance exercise increases testosterone, growth hormones (GHs), and Insulin-Like Growth Factor 1 (IGF-1). Testosterone is a key signal to protein synthesis and works through the androgen receptor to regulate gene transcription. GH and IGF-1 bind to their respective receptors and signal through phosphatidylinositol 3-kinase (PI-3K)⁴⁵. PI-3K potentiates the anabolic signal to the mammalian target of rapamycin (mTOR), which is considered a key regulator of muscle signaling and protein synthesis. mTOR further signals downstream to the 70 kDa ribosomal protein S6 kinase (p70 S6k) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) to initiate the translation of mRNA and protein synthesis²⁰. Although testosterone is considered a major promoter of muscle protein accretion⁵⁰, for the scope of this study, we will focus entirely on mTOR signaling.

On the contrary, endurance training stimulates a different pathway to exert its effect. Specifically, endurance training stimulates adenosine monophosphate-activated kinase (AMPK) / peroxisome-proliferator-activated receptor γ coactivator 1 α (PGC-1 α) pathway which stimulates mitochondrial biogenesis and other endurance training adaptations²⁸. Interestingly, the AMPK / PGC-1 α pathway inhibits the mTOR pathway and decreases protein synthesis¹⁰. Due to the specificity of training adaptations and the commonality of concurrent training (both resistance training and endurance training), the interaction of the mTOR and AMPK pathway has been of great interest.

As interest on the muscle signaling pathways has grown, more research has focused on the impact of nutrition and supplementation on these pathways. Indeed, nutritional interventions (most predominantly branch chain amino acids³⁴) have been shown to increase signaling and protein synthesis. Similarly, other supplements may exert their ergogenic effects through signaling pathways.

Recent studies have shown improvements in performance with betaine supplementation^{21,31}, but have not shown the mechanism for this improvement. Some suggested that betaine supplementation may aid athletic performance due to its role as an osmolyte and a methyl donor to produce creatine^{11,32}, but further research is necessary to show its true mechanism of action.

Therefore, the purpose of this study was to examine the effect of betaine supplementation on performance, and hormonal and muscle signaling responses in order to more effectively identify a mechanism of ergogenic action. We hypothesized that betaine supplementation would increase strength and power performance via increased

anabolic signaling hormonally, as well as potentiating the anabolic signal through the mTOR pathway.

Chapter 2: Review of Literature

For the purpose of this literature review, the muscle signaling pathway and betaine supplementation will be reviewed. To begin, muscle signaling will be reviewed in a broad scope. Then the AMPK / PGC-1 α or endurance signal cascade will be discussed. Subsequently, the Akt / mTOR Pathway, which is of greatest interest, will be reviewed in detail, as resistance exercise is the primary focus of this study. Next the review of literature will examine betaine supplementation, specifically beginning with an overview of the biological roles of betaine. Then the review of literature will focus on recent studies examining the ergogenic effect of betaine supplementation on human performance. Finally, the possible interaction of muscle signaling and betaine supplementation will be examined.

Muscle Signaling

Muscle Signaling Overview

Skeletal muscle adaptations to exercise are specific to the modality of the training³⁷. The mechanical signal of exercise activates a specific signaling cascade that result in a transient change in gene transcription, which over frequent bouts of exercise will result in increased protein synthesis⁸. The increase in protein synthesis is specific to the exercises performed and to the motor units recruited. On the two ends of the spectrum, resistance training and endurance training, result in different mechanical signals in the muscle, and resultant different molecular activation and overall protein synthesis⁹. Resistance exercise, characterized by short duration high intensity contraction, promotes protein accretion, muscle hypertrophy and strength gains, while endurance training, characterized

by longer duration low intensity contractions, promotes increased mitochondrial density and resistance to fatigue⁹. Thus, the form of exercise stimulates a certain signal cascade which results in the overall genotypic and phenotypic changes associated with the given training.

The key regulatory and rate-limiting step in protein synthesis is the initiation of messenger RNA (mRNA) translation⁴. The initiation of protein translation begins with the assembly of the preinitiation complex at the AUG start site on the 5-end of the mRNA. The formation of the preinitiation complex and thus the beginning of protein translation depends on the binding of initiator methionyl-tRNA to the 40S ribosomal subunit to form the 43S preinitiation complex, which then binds to the mRNA to form the 48S preinitiation complex. These translation initiation steps are regulated by specific signal cascades⁴. Thus, translation initiation and, therefore, protein synthesis is regulated by the signal cascade produced by the given activity performed.

As previously stated, endurance training represents one end of the physical activity spectrum, where altered gene expression results in the muscle phenotype with improved resistance to fatigue⁸. Resistance to fatigue and oxidative capacity is mainly determined by the mitochondria. Indeed, endurance training results in increased mitochondrial density and enzyme activity, together termed 'mitochondrial biogenesis' due to a signal cascade that results in the increase in mitochondrial transcription factors, specifically through the AMPK / PGC-1 α signaling pathway⁸. On the contrary, resistance training adaptations include increased muscle cross-sectional area and neural recruitment. The increase in muscle hypertrophy is regulated by the increase in transcription factors for protein synthesis, specifically induced by the phosphorylation of

the protein kinase B (Akt) / mammalian target of rapamycin (mTOR) signaling pathway^{8, 56}. Interestingly, these two pathways are inversely related, where an increase in the AMPK / PGC-1 α activity causes a decrease in the Akt / mTOR pathway activity and vice versa⁹. Thus, diverse contractile activity (i.e. mixed or concurrent training) may not be optimal for endurance or resistance specific training adaptations (Figure 2.1, Hawley et al.²⁰).

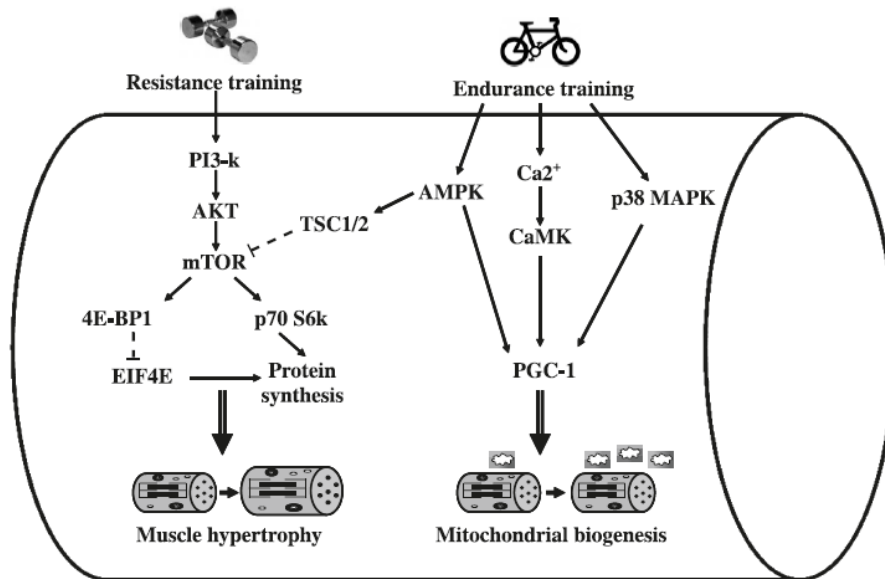


Figure 2.1: Intracellular signaling networks mediating exercise-induced skeletal muscle responses to resistance- and endurance-based exercise training programs. Resistance-based exercise induces an increase in the activity of the PI-3K/Akt/mTOR signaling cascade to regulate protein synthesis and degradation, which over a prolonged period results in muscle hypertrophy. Endurance-based exercise activates signaling pathways involved in metabolic homeostasis, comprising of AMPK / p38 mitogen-activated protein kinase (MAPK) / PGC-1 α axis. Activation of AMPK by endurance exercise inhibits mTOR signaling via tuberous sclerosis complex (TSC) and suppresses resistance-exercise-induced muscle-protein synthesis. 4EBP1 – eukaryotic initiation factor 4E-binding protein 1; CaMK – calmodulin-dependent protein kinase; EIF4E – eukaryotic translation initiation factor-binding protein; p70 S6k – 70 kDa ribosomal protein S6 kinase; TSC1/2 – tuberous sclerosis complex 1 and 2.²⁰

AMPK / PGC-1 α Pathway

It is well known that muscle contraction from exercise results in an energetic challenge to the muscle. As adenosine triphosphate (ATP) is used for energy, adenosine monophosphate (AMP) increases. AMPK is considered an energy sensor of the cell. It consists of a catalytic subunit (AMPK α) and two regulatory subunits (AMPK β and AMPK γ)²⁸. AMPK γ binds two AMP molecules cooperatively and has a high sensitivity to small changes in AMP concentration. Furthermore, ATP binds to AMPK α to allosterically regulate and inactivate the enzyme²⁸. Therefore, AMPK not only responds to AMP, but also the ATP to AMP ratio. The binding of AMP to AMPK alters the conformation to allow for upstream protein kinases, specifically the tumor suppressor LKB1²⁸, to phosphorylate the α subunit on threonine 172 in order to activate it²⁴.

When activated, AMPK has regulatory effects on glucose uptake, fatty acid oxidation, glycogen metabolism, and protein synthesis. Overall, AMPK inhibits energy consuming pathways (i.e. protein synthesis) and activates pathways to release energy (i.e. carbohydrate and fatty acid catabolism)²⁰. AMPK has been shown to increase the glucose transporter GLUT4 on the muscle plasma membrane to increase glucose uptake²⁴, and promote fatty acid oxidation by inhibition of acetyl-CoA carboxylase and activation of malonyl-CoA to increase glucose availability²⁰. Furthermore some studies, though still tentative, have shown that AMPK activates hormone sensitive lipase (HSL), which is thought of as the rate limiting enzyme in intramuscular triglyceride (IMTG) breakdown, to further liberate energy²⁴. As an energy conserving kinase, AMPK also acts to reduce protein synthesis. AMPK does this by down regulation of the mTOR pathway. It is thought that AMPK phosphorylates tuberous sclerosis complex 2 (TSC2) at

threonine 1227 and serine 1345, which improves its ability to inhibit mTOR activity, as well as by directly phosphorylating mTOR at threonine 2446, which prevents Akt phosphorylation of mTOR at serine 2446 and further signaling³⁴. As expected, many studies^{24, 28, 34} show that treatment with aminoimidazole carboxamide ribonucleotide (AICAR), an AMPK activator, results in decreased protein synthesis, as well as down regulation of Akt, mTOR, and downstream targets, p70 S6k1 and 4E-BP1.

Beyond immediate cellular homeostasis responses, AMPK directly phosphorylates PGC-1 α , which controls transcriptional factors for mitochondrial biogenesis^{33, 54}. By maintaining cellular homeostasis via liberating energy and inhibiting energy consuming processes, as well as increasing mitochondrial biogenesis the AMPK / PGC-1 α pathway results in the endurance training phenotype one would expect.

Akt / mTOR Pathway

The Akt / mTOR pathway is the primary focus of this study, as we are specifically looking at strength and power gains. To appreciate the entire picture of Akt / mTOR anabolic signaling (Figure 2.2, adapted from Spiering et al.⁴⁵) one must consider the many interactions during resistance exercise.

As previously stated, resistance exercise promotes muscle hypertrophy and strength gains in part through the Akt / mTOR signaling cascade that culminates with the increased mRNA translation initiation and overall increase in protein synthesis. To begin, mechanical deformation of skeletal muscle (i.e. resistance exercise) activates calcineurin, mitogen activated protein kinase (MAPK) and insulin-like growth factor signal cascades specific to the mechanical stimuli (i.e. frequency and intensity)⁸. Also, neural activation

and the release of calcium from the sarcoplasmic reticulum is specific to the contractile activity⁸. In fact, the muscle contraction itself stimulates mTOR directly through phosphatidic acid production⁴⁵. Thus, the muscle actions during resistance exercise directly (through mechanical deformation) and indirectly (through the hormonal responses) stimulate signal cascades and muscle growth⁴⁶.

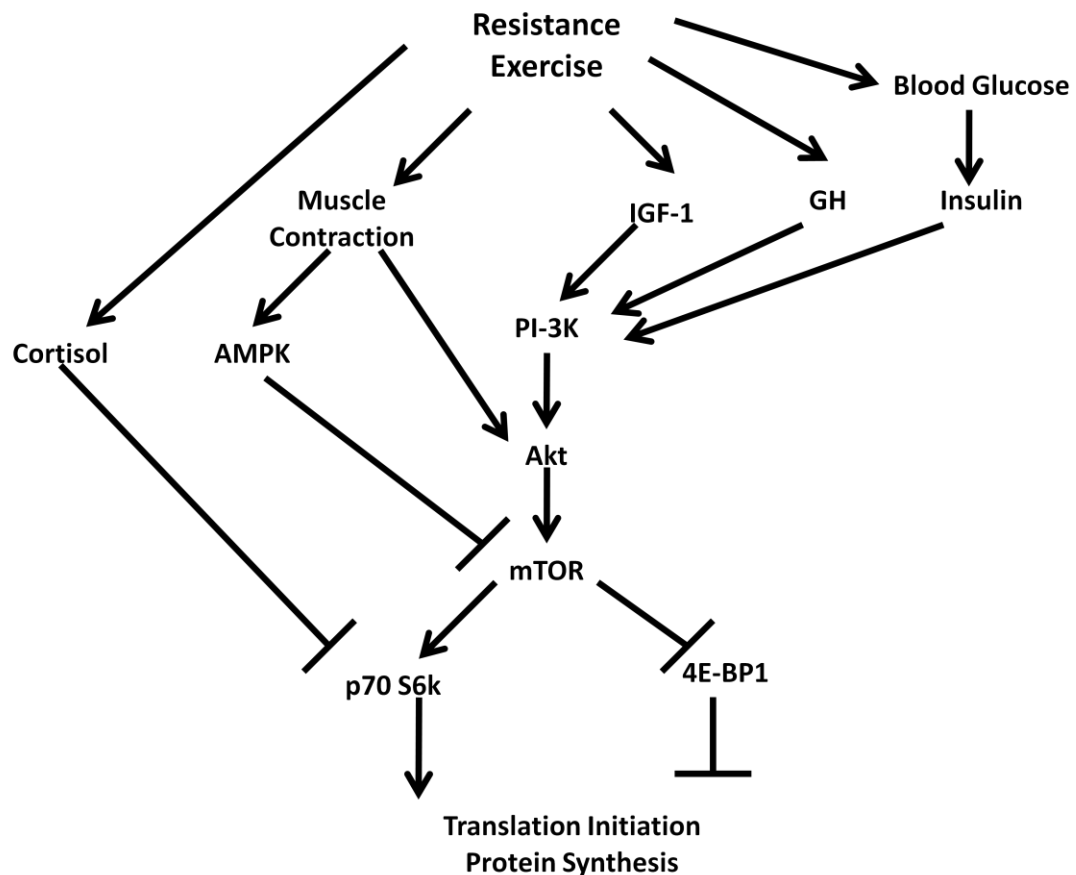


Figure 2.2: The many effects of resistance exercise on Akt / mTOR signaling pathway. The contractions from resistance exercise not only have their own actions (i.e. stimulating AMPK and Akt), but also incite hormonal responses, specifically, the increase in cortisol, insulin-like growth factor -1 (IGF-1), growth hormone (GH). Furthermore, the slight increase blood glucose as the need for energy increases causes an increase in insulin. The overall balance of this signaling cascade results in the adaptations made. (Adapted from Spiering et al.⁴⁵)

Muscle fiber actions, as well as hormonal responses through PI-3K phosphorylation, phosphorylate Akt⁵⁶. Akt signaling is important as it is a central factor and contributes to downstream phosphorylation of targets. When Akt is phosphorylated (either by PI-3K or mechanical stimuli) at serine 473 (Akt S473), it is then able to phosphorylate mTOR. Specifically, Akt S473 phosphorylates and activates mTOR on the serine 2448 residue (mTOR S2448), which increases mTOR activity and favors mTOR to subsequently phosphorylate p70 S6k and hyperphosphorylate 4E-BP1. mTOR S2448 phosphorylates p70 S6k on the threonine 389 residue (p70 S6k T389) and activates it. p70 S6k T389 then can phosphorylate the S6 subunit of the 40S ribosomal protein, which hyperphosphorylates the ribosomal protein S6 and then increases translation of mRNAs encoding translational factors. 4E-BP1 normally inhibits eukaryotic translation initiation factor 4E (eIF4E) by binding to it, but when mTOR S2448 hyperphosphorylates 4E-BP1 it releases from eIF4E. By releasing eIF4E from 4E-BP1 it is able to form the translation initiation complex eukaryotic translation initiation factor 4F (eIF4F) and bind to the cap structure of the mRNA and promote translation initiation.^{4, 45}

Hormonal responses to resistance exercise are well documented^{29, 45, 46}. Although they depend partly on the acute program variables (exercise choice, exercise order, load, volume, and rest intervals) of the exercise protocol, the responses are generally similar⁴⁶. Human GH (22 kD) acutely increases during and following resistance exercise of sufficient volume and intensity. The magnitude of this increase seems to be related to the total work performed where moderate to high intensity exercise, high in volume with relatively shorter rest intervals, results in the highest GH responses²⁹. When GH binds to

its membrane receptor, it activates janus kinase 2 (JAK2) signaling which then signals to PI-3K to continue to promote the anabolic signal ⁴⁶. Similarly, acute resistance exercise causes an increase in IGF-1 post exercise, as well as alters IGF binding proteins and existing IGF-1 biological activity ⁴⁶. Furthermore, IGF-1 binds to the receptor and induces the phosphorylation of insulin receptor substrate (IRS). IRS then activates PI-3K which phosphorylates Akt and continues the signal as described above ³⁴.

Insulin is a potent anabolic signal and has significant positive effects on protein synthesis when amino acids are available. Hence, ingestion of carbohydrate and/or amino acids pre, peri, or post workout is recommended to utilize this potent anabolic signal ²⁹. Analogous to IGF-1, insulin binds to the IRS receptor and activates PI-3K and Akt signaling ⁸.

On the contrary to the anabolic signals of GH, IGF-1 and insulin, cortisol is a catabolic glucocorticoid and has an inhibitory effect on protein synthesis. Acute resistance exercise can impose a major stress to the body and, accordingly, stimulates cortisol release. Similar to GH, cortisol release seems to be highest during high volume, moderate to high intensity workouts with short rest periods ²⁹. Also, it has now been showed that glucocorticoids have a direct inhibitory effect on the Akt signaling pathway, specifically on p70 S6k and 4E-BP1 phosphorylation ^{43, 44}. Ultimately, cortisol may reduce the efficiency of p70 S6k T389 phosphorylation via conformation autoinhibition ⁴². Also, 4E-BP1 is dephosphorylated by glucocorticoids causing it to remain bound to the eIF4E⁴³. The inhibitory stimulus is more powerful in p70 S6k and cannot be recovered with IGF-1, where 4E-BP1 inhibition is reversed ⁴⁴.

Overall, Figure 2.2 summarizes the interaction of the Akt / mTOR signaling pathway and resistance training mechanical and hormonal responses. It is important to note the strong inhibitory effect of cortisol and AMPK on protein synthesis, as both are normal responses to resistance training. Thusly, this results in a balancing between the stimulatory effects and inhibitory effects.

Akt / mTOR signaling is a constantly evolving field of inquiry, where interactions and effects from supplementations are yet to be evaluated. Recent research has focused on nutritional interactions, as well as concurrent workout effects. Since the Akt / mTOR pathway plays an integral role in protein synthesis and overall training adaptations, further research regarding these interactions is warranted.

Betaine Supplementation

Betaine Overview

Originally isolated in the 1860's by German chemist Carl Scheibler from sugar beets (*Beta vulgaris*)³², Betaine has been known by other names, including trimethylglycine, glycine betaine, l-cysteine, and oxyneurine¹¹. It is a neutral zwitterionic compound³² and a methyl derivative of glycine¹¹. Along with sugar beets, many other foods, such as wheat bran, wheat germ, spinach, pretzels, and shrimp, have high betaine content, although their levels vary highly with different sources (i.e. environmental conditions during growth) and cooking methods¹¹. Another way humans obtain betaine is from dietary choline, as the liver and kidney can oxidize choline to betaine in a two-step enzyme-dependent reaction^{11,49}. Though study results vary slightly, it is estimated

that the average daily intake of betaine is between 100 – 300 mg^{32,49}, but some studies have shown that dietary intakes of 9 – 15 g per day is still safe¹¹.

Once ingested, betaine is rapidly absorbed in the duodenum of the small intestine. Peak serum concentrations of betaine are seen approximately 1 – 2 hours postprandial¹¹. Betaine is known to have two major roles in the body: a methyl group donor and an organic osmolyte. When betaine is catabolized, which primarily occurs in the mitochondria of the liver and kidney, a series of transmethylation reactions occur; most significantly a methyl group is transferred from betaine to homocysteine¹¹. Homocysteine is methylated and converted to methionine, while betaine becomes dimethylglycine¹¹. This reaction is catalyzed by betaine-homocysteine methyltransferase (BHMT)^{11,32}. By remethylating homocysteine to methionine, betaine conserves methionine, detoxifies homocysteine, and produces S-Adenosylmethionine (SAM)¹¹. SAM is used in many other biological reactions as a direct methyl donor, specifically it participates in the pathways of protein, creatine, phospholipid, and carnitine synthesis, as well as DNA methylation¹¹.

When betaine is not catabolized, it is an organic osmolyte that can regulate cell volume^{11,49}. Interestingly, BHMT is osmoregulated to divide the two separate roles of betaine⁴⁹. Along with cell volume regulation, betaine is a “compensatory” solute that stabilizes proteins and is especially effective in countering the denaturing effect of urea⁴⁹. In particular, betaine protects muscle myosin ATPases and prevents urea build up in muscle¹¹ that may lead to inhibition of protein synthesis. Furthermore, Brigotti et al. showed the addition of betaine actually increased translation of globin mRNA by rabbit reticulocyte lysates⁵, supporting the hypothesis that betaine is essential to maintain

osmolarity and facilitate a hospitable environment for protein synthesis and perhaps other necessary physiological functions.

Betaine has also been linked to a variety of other positive actions. It is a lipotrope that has been shown to prevent and reduce fat accumulation in the liver in both animal and human studies ¹¹. Betaine even shows antiatherosclerotic actions ¹¹, reduces aortic inflammatory responses ⁴⁹, and decreases fatty acid synthesis and tissue lipids in animal studies ³². Furthermore, low betaine plasma levels and high urinary betaine loss is correlated with elevated plasma lipids and other components of the metabolic syndrome, while markers of obesity (BMI, percent body fat and waist circumference) have a negative association with betaine plasma concentrations ³².

Betaine is commonly included in animal feed, as it has shown to increase muscle mass, and decrease fat mass in many studies ³². Eklund et al. ¹⁵ speculated the carcass composition change could be due to a methionine sparing effect allowing more efficient use of dietary protein, though explanations vary among studies. Betaine has been shown to increase muscle mass, decrease fat mass or both in poultry ⁵⁷ and pigs ³⁶ in many different studies ¹⁵. Furthermore, betaine supplementation in untrained horses caused decreased levels of lactate accumulation post exercise ⁵². These beneficial effects seen in animals combined with the many essential roles of betaine demonstrated in humans, specifically as an osmolyte and a methyl donor for creatine synthesis in skeletal muscle, support the contention that betaine supplementation might enhance performance in humans.

Betaine and Human Performance

Although the studies on betaine supplementation and human performance are limited, they show the need for more research, as results seem promising. As suggested by Craig ¹¹ and Lever and Slow ³², betaine supplementation may reduce fatigue and improve athletic performance via its role as an osmolyte and a methyl donor to produce creatine *de novo*. Furthermore, Craig et al. ¹² showed that the mean betaine content of sweat in adolescent girls was $232 \pm 84 \mu\text{mol/L}$, which is seven times greater than betaine content of typical female plasma. Though this study is the only study that shows the betaine content of sweat, it further supports that during strenuous exercise when sweating is elevated, betaine supplementation may be beneficial to replace the betaine loss in sweat.

Armstrong et al. investigated the influence of betaine supplementation on strenuous running and sprinting ¹. This study focused on betaine as an organic osmolyte investigating its effect on cardiovascular function and thermal homeostasis during rehydration, specifically looking at its potential ergogenic effects with endurance performance. Well-trained competitive runners first dehydrated to -2.7% body mass, then consumed 1 L of rehydration fluid (with or without betaine (5g/L) in water or a sports drink containing carbohydrates and electrolytes) before they began the running performance trial. The running trial performance consisted of 75 min at 65% VO_2 max followed by a timed sprint to volitional exhaustion at 84% VO_2 max. Results showed a nonsignificant trend of increased sprint duration with betaine supplementation (16% and 21% increase with betaine and water or sports drink, respectively). These trends corresponded with thermal sensation ratings of the subjects throughout the protocol.

Interestingly, plasma lactate during the betaine and sports drink trial was greater than during the sports drink alone immediately and 15 minutes after completion of the workout, but due to the complexities of lactate turnover, little could be said whether this effect was due to enhanced clearance of lactate from the muscle, enhanced muscle glycolysis, or another unknown mechanism. Betaine supplementation increased energy production via aerobic metabolism, as seen by increased oxygen consumption during the betaine and sports drink trial compared to the sports drink alone. In addition, during recovery the loss of water from plasma was greater during the betaine and water rehydration fluid than with water alone, but no difference was seen in the sports drink trials. This phenomenon was explained by increased transport of betaine across the muscle membrane creating an osmotic gradient, and the sports drink osmolality was great enough to create entirely different gradients. ¹

The Armstrong et al. study showed some promise in betaine supplementation, but the study also pointed out some flaws that made it harder to interpret the results. First, the standard deviations of the sprint time were large making it hard to find a significant difference among trials. They hypothesized that a more homogenous sample may have made the findings significant. Second, betaine supplementation was only ingested once 45 minutes before the exercise trial, but not entirely before exercise (i.e. after the dehydration protocol). Studies have shown that betaine peaks approximately 1 -2 hours in the serum following supplementation ¹¹. Also, since dehydration already took place before supplementation, the protective effect of betaine as an osmolyte may have been harder to observe. Furthermore, a single acute supplementation may not be enough to induce ergogenic effects, similar to creatine supplementation, as betaine is also an

intracellular component ¹. Overall, Armstrong et al. was able to show that betaine supplementation altered metabolism, but its definitive benefits and mechanisms, and to what activities it might be best suited for, were not determined.

As choline is a precursor to betaine, it is also of interest to look at the effect of choline supplementation on endurance performance. Despite some studies showing decreases in plasma choline during strenuous prolonged exercise ²⁶, choline supplementation did not increase cognitive or physical performance in most cases ³⁹, and furthermore overall did not seem to increase endurance performance ^{26, 39, 51}. It is important to note that choline is not solely oxidized to betaine in the body, it is also acetylated in cholinergic neurons to acetylcholine (a neurotransmitter) and is utilized in other physiological pathways ⁴⁹. Because of these alternate pathways, choline supplementation is not hypothesized to be directly the same as betaine supplementation, but since more research has been done on choline supplementation and endurance training, it is still of interest to mention.

Betaine supplementation is hypothesized to increase creatine synthesis. This suggests that betaine supplementation may have more of an applicability to power and resistance exercise. The two studies that investigated the effect of betaine supplementation on power and strength had similar results with some minor differences.

Hoffman et al. ²¹ utilized a double-blinded, between group design where 24 recreationally active men were matched and randomly assigned to either a betaine or placebo group. The subjects were tested for two consecutive days at baseline (prior to any supplementation), then began consuming the betaine supplement (1.25 g of betaine twice daily) or placebo, and then were tested again on day 7 and day 14 of supplementation for

muscular endurance, strength, and power. Specifically subjects performed vertical jumps, bench throws, and as many repetitions as possible at 75% 1 RM for bench press and back squats on day 1 of testing and Wingate anaerobic tests on the following day. Hoffman et al.²¹ showed no difference in the bench throws, bench press, vertical jumps, or anaerobic power tests. Contrarily, subjects with betaine supplementation performed significantly more squat repetitions until exhaustion on day 7 and nonsignificantly, but trending towards ($p = 0.06$) more squat repetitions on day 14. Furthermore, subjects with betaine supplementation performed more squat repetitions at 90% or more peak power on day 7 and day 14 and more squat repetitions at 90% or more of mean power on day 14. This may support that betaine supplementation would allow athletes to improve the quality of their workouts causing increased performance. Hoffman et al.²¹ suggested that betaine supplementation increased creatine and therefore, increased local muscular endurance and power. They also attributed the differences seen in the squat and bench press exercise due to the widespread use of bench press in recreationally active men workouts and the less common utilization of the squat exercise, as well as the possibility of larger muscle mass being affected to greater extent. Furthermore, it has been shown that power exercises must be performed regularly to see improvement, and since the power exercises in the protocol was not a common part of the subjects' workouts, improvements may not have been seen.²¹

Lee et al.³¹ performed a similar study, but utilized a randomized double-blinded within treatment design with 12 recreationally active men that had been resistance trained for at least 3 months including the squat exercise. After baseline testing, subjects underwent supplementation of betaine (1.25 g of betaine in 300 ml of sports drink) or

placebo (300 ml of sports drink alone) twice daily. Subjects were tested on vertical jump, isometric squat, squat jumps, back squat at 85% 1 RM, isometric bench press, bench throw, and bench press at 85% 1 RM. Subjects were tested on day 0, 1, 14, and 15 (the same protocol was repeated the following day to measure the effects of betaine on recovery). Lee et al.³¹ showed that betaine supplementation significantly increased the following measures on both day 14 and day 15: power output for 2 of the 4 sets of vertical jump, increased force production during isometric squat, increased force production during isometric bench press, and increased bench throw power. No significance was seen in squat jump power or repetitions until exhaustion of back squat or bench press at 85% 1 RM. Overall, Lee et al. supported the hypothesis that betaine supplementation would enhance force production and power performances and be a useful ergogenic aid for some athletes.³¹

Although there are some differences seen between the Hoffman et al.²¹ and Lee et al.³¹ studies, most of these differences can be attributed to the slight changes in subject population and protocol. Hoffman et al. did not specify that the subjects have back squats in their regular exercise routine, nor did they have the subjects repeat the power exercises in their workouts in between testing. These may have caused the lack of significant results in the power and squat exercises observed. In contrast, subjects in the Lee et al. study were accustomed to the squat exercise and had a standardized exercise protocol in between workouts very similar to the protocol to ensure subjects were use to the power exercises. Despite these differences, both studies showed improvement in performance with betaine supplementation over time, supporting its use as an ergogenic aid.

It is important to note that although both studies showed improved performance, neither confirmed a definitive mechanism of enhancement. This warrants the need for more research regarding betaine as an ergogenic aid for strength and power athletes, specifically to determine a mechanism of action.

Muscle Signaling and Betaine Supplementation

Although there is no direct research examining the effect of betaine supplementation on muscle signaling pathways, current research shows that there could potentially be a significant interaction. As previously stated, animal research shows an increase in lean muscle deposition when feed is supplemented with betaine ¹⁵. Building on the animal feed studies, some have examined hormones and growth factors as the responsible mediators causing the change in carcass characteristics. This is of interest because these factors contribute to the mTOR signaling pathway as stated previously.

Huang et al. ⁴⁰ supplemented finishing pigs feed with 0.125% betaine for 42 days. Similar to other studies, pigs supplemented with betaine showed improved carcass traits (increased lean percentage, increased longissimus muscle area, decrease fat percentage, decrease backfat thickness). Along with these changes, pigs supplemented with betaine had a 45.61% increase in serum GH, 55.5% increase in serum IGF-1, and 42.34% increase in serum insulin compared to the control pigs. Also Huang et al. ⁴⁰ observed a significant decrease in fatty acid synthase activity and an increase in hormone sensitive lipase activity within adipose tissue and an increase in serum free fatty acid in betaine supplemented pigs. Moreover, the increase in GH, IGF-1, and insulin would

hypothetically be able to stimulate PI-3K and carry the signal further down the Akt / mTOR pathway to promote protein synthesis.

As GH is pulsatile, Huang et al.²² further looked at GH in the same finishing pigs to show that basal GH, mean GH, and GH pulse amplitude was increased with betaine supplementation. Also, betaine supplemented pigs had a decrease in serum urea nitrogen concentration and an increase in serum total protein level, further supporting the hypothesis of increased protein synthetic rates with betaine supplementation. Overall, Huang et al.²² showed that betaine supplementation increased GH, IGF-1, and insulin to potentiate the anabolic signal in these animals.

Similarly, Choe et al.⁷ supplemented laying hens feed with either 0, 300, 600, or 1200 ppm of betaine for four weeks. Hens supplemented with 600 or 1200 ppm betaine had significantly greater serum IGF-1. This correlated with IGF-1 gene expression in the liver. Choe et al.⁷ based the betaine enhancement on original findings of Kasai et al.²⁷, who showed that glycine, a betaine metabolite, induces an increase in blood GH via a dose dependent manner (higher glycine dose (up to 12 g) caused a higher release of GH) indicating glycine has a pivotal role in hypothalamic pituitary function. Additionally, Choe et al.⁷ showed that betaine supplementation of 600 ppm increased blood IGF-binding protein -3 (IGFBP-3) and therefore was able to extend the half-life of IGF-1 and enhance its action.

Furthering this research question, Yan⁵⁵ explored the effects of betaine supplementation on the hypothalamus and pituitary of finishing pigs. Yan showed finishing pigs supplemented with betaine had increased deposition of betaine on the hypothalamus, but not on the pituitary. This resulted in the increase in GHRH gene

transcription and secretion from the hypothalamus. Therefore, one could postulate betaine supplementation causes an increase in GHRH, which leads to an increase in GH release from the anterior pituitary⁵⁵. The increase in GH release would cause the increase in muscle growth and fat degradation, and the decrease in fat synthesis, which would result in the leaner, more muscular finishing pig seen with betaine supplementation.

On the catabolic side of hormonal responses, Matthews et al.³⁵ examined the cortisol response in pigs receiving betaine supplementation. It is important to note that this study was looking at multiple conditions and the supplementation period was not a set time, but rather until the pigs reached a certain weight. No significant difference in cortisol was observed with 0.25% betaine supplementation. Furthermore, there was a significant interaction effect with betaine supplementation and pen space where there was a greater gain in weight when pigs were given greater space and supplemented with betaine. This may indicate that the pigs need space to move and exercise in order for betaine supplementation to increase protein synthesis and overall weight gain.

Parker et al.³⁸ also examined betaine supplementation and cortisol, but was primarily interested in dehydration of steers during transportation rather than quality of carcass. Steers were examined after 24 and 48 hours of transportation. Again, there was no significant difference seen in cortisol with betaine supplementation.

Although neither of these studies showed differences in cortisol response, the methodology was not optimized to look at betaine supplementation alone. Matthews et al.³⁵ did not have a set period of time of supplementation and Parker et al.³⁸ only supplemented at a single time point. Additionally, the raw data presented in both studies did show a nonsignificant decrease in cortisol with betaine supplementation. It could be

speculated that prolonged betaine supplementation could provide a more pronounced difference.

Overall, animal studies have shown that betaine supplementation can increase GHRH, GH, IGF-1, and insulin in finishing pigs and laying hens. The studies proposed that these hormonal changes result in the phenotypic change in these animals (increase in lean muscle tissue and decrease in adipose tissue). Mechanistically, the increase in GH, IGF-1 and insulin not only can exert their actions directly, but also through muscle signaling. Although no research has been done examining betaine supplementation and these hormonal changes or potential muscle signaling effects in humans, the animal research, as well as human performance results, support this thought.

Therefore, the purpose of this study was to examine at the effect of betaine supplementation on human performance, hormonal responses, and muscle signaling responses in order to more effectively determine a mechanism of ergogenic action. We hypothesized that betaine supplementation would increase strength and power performance via increased anabolic signaling hormonally, as well as potentiating the anabolic signal through the Akt / mTOR pathway.

Chapter 3: Methods

Experimental Approach

In order to assess the effects of betaine supplementation we designed a randomized, double-blinded crossover study. Subjects performed an acute exercise test (AET) protocol before and after two weeks of supplementation with either betaine dissolved in a sports drink (Gatorade, fruit punch flavor, Chicago, Illinois) or a placebo (Gatorade, fruit punch flavor). Each AET began with 10 maximal vertical jumps without pause, followed by a maximal 10 second isometric bench press, then a maximal 10 second isometric squat, concluding with 10 minutes of box lifting. Subjects were given 2 – 5 minutes of rest between exercises, depending on the specific exercise. Vertical jumps, isometric squat, and isometric bench press were performed on a force plate (Accupower, Athletic Republic, Fargo, ND) to determine force and power output. The number of boxes lifted during the box lift test was used to evaluate muscular endurance and work capacity. During each AET, blood samples were obtained at pre, mid, post, +5, and +15 and were analyzed for GH, IGF-1 and cortisol. Also during each AET, muscle biopsies were obtained from the vastus lateralis at pre and +10 and were analyzed for select muscle signaling proteins (Total Akt, Akt S473, p70 S6k1, p70 S6k1 T389, and AMPK T172). Following a 14 day washout period, subjects crossed over to the other supplementation protocol and performed the same pre and post AETs.

Experimental Timeline

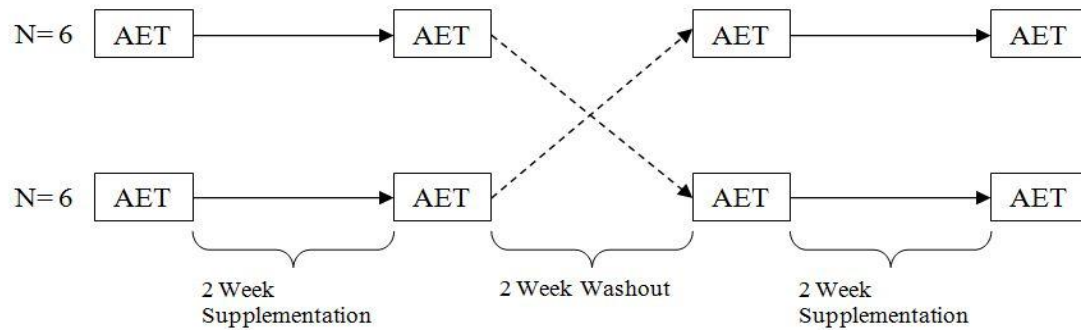


Figure 3.1: Overall experimental timeline of randomized crossover study design.

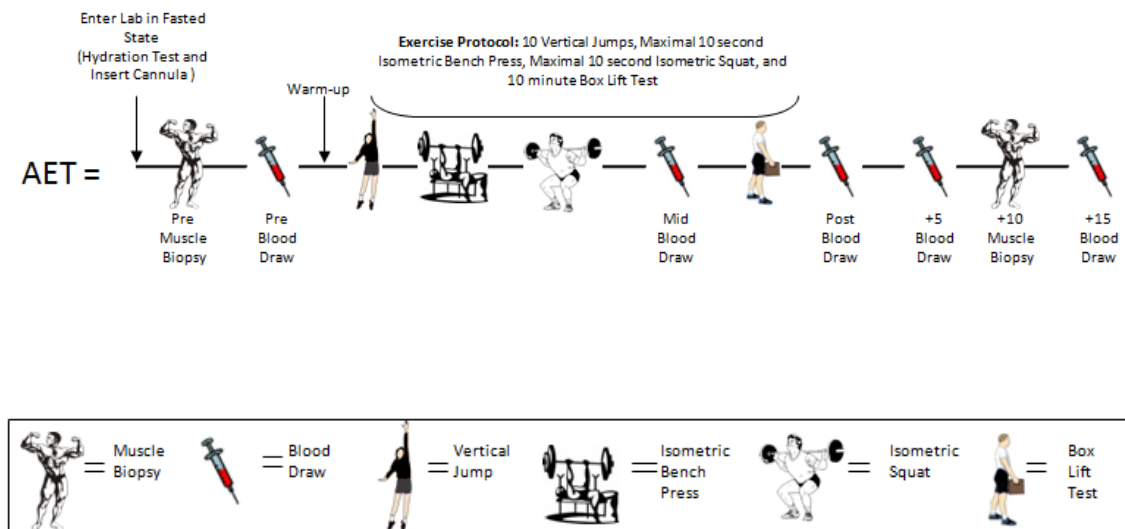


Figure 3.2: Acute exercise test (AET) protocol. Pre – Baseline; prior to any exercise; Mid – Middle of exercise protocol; Post – post exercise protocol; +5, +10, +15 – time point (in minutes) based on end of exercise protocol.

Subjects

Twelve recreationally active college men volunteered to participate in this study (Table 1). Subjects had been resistance training with the inclusion of the bench press and back squat exercises at least 2-3 times per week for the preceding three months, though the subjects averaged over four years of general resistance training. The subjects were given verbal and written explanation of the study requirements and associated risks, and then signed an informed written consent to participate which was approved by the University of Connecticut Institutional Review Board.

Table 3.1: Descriptive characteristics of subjects.

| Variable | Mean \pm Standard Deviation |
|-----------------|-------------------------------------------------|
| Age (yr) | 19.67 \pm 1.23 |
| Height (cm) | 172.59 \pm 5.61 |
| Weight (kg) | 84.27 \pm 15.30 |
| Body Fat (%) | 18.71 \pm 7.01 |
| BMI | 28.19 \pm 4.02 |

Procedures

Experimental Controls

Healthy college-aged male subjects were recruited as subjects. Subjects were excluded if they had any previous medical history that may have complicated the study or results. Also, subjects were excluded if they reported taking any drugs (recreational drugs or prescription drugs that may have an interaction), protein supplements, carbohydrate supplements, creatine, or other dietary supplements that may have caused confounding results. Subjects that fit within our inclusion criteria were reviewed by the medical monitor to ensure their safety and the validity of our results.

For three days prior to the first AET, subjects recorded their daily food and beverage intake and replicated this diet before each additional AET. Also during the three days preceding an AET, subjects refrained from alcohol, caffeine, and any exhaustive exercise. Subjects maintained a physical activity log throughout the study and replicated their physical activity and exercises from the first two week supplementation period during the second two week supplementation period. Additionally, subjects performed a standardized whole-body resistance workout weekly during the study to ensure that their level of conditioning was maintained. Subjects fasted for 12 hours prior to their arrival for the scheduled AET with the exception of water. Subjects were instructed to drink an extra 0.5 L of water prior to going to bed and when they awoke to ensure that they were in a euhydrated state when they arrived in the laboratory. Hydration was measured by urine refractometry (Model A300CL, Spartan, Japan). Subjects were considered euhydrated if their urine specific gravity (USG) was 1.020 g/ml or below. If subjects were not euhydrated, they drank water ad libitum and were evaluated for hydration status

in 30 minute increments until they had a USG of 1.020 g/ml or below. Subjects did not begin any specimen collection or exercise protocol until they were euhydrated to control for any variations due to hydration status. Also, to control diurnal hormonal changes and performance variations subjects arrival times were consistent with each subject among AETs.

Prior to the beginning of the AETs, subjects came in for a familiarization visit to allow them to become accustomed to the exercise protocol. At this time the subjects' height was measured via a tape measure to the nearest 0.1 cm and total body weight was recorded to the nearest 0.1 kg on a digital scale (OHAUS Corp., Fordham Park, NJ). In addition, body composition of the subjects was determined via Dual Energy X-ray Absorptiometry (DEXA) (Lunar, Prodigy, GE). Following anthropometric assessments, the subjects were taught a standardized light warm-up consisting of 5-minutes on a stationary bicycle followed by dynamic stretches, including heel kicks, lunges, high knees, high kicks, and bodyweight squats. This warm-up was repeated prior to the exercise protocol on each AET day for control purposes. Furthermore, during this visit subjects were familiarized with the specific exercises comprising the AET. The vertical jumps, isometric squat, and isometric bench were performed on the force plate (Accupower) as would be done during the AETs. Subjects were familiarized with proper isometric bench press position. Specifically, the bar was placed approximately 6 inches above the chest, about 1/3 of the way up with elbows at approximately a 120° angle. The bar was locked to the nearest rack position on the Smith Machine (LifeFitness, Schiller Park, IL). This position was noted and used for all the following AETs to ensure consistency. For the isometric squat exercise, the subject assumed a quarter squat position

and the bar was locked to the nearest rack position on the Smith Machine. Again, this rack position was noted and used for all the following AETs. For both exercises, subjects were given a 3-2-1 count to gradually increase pressure and push to maximal exertion over 10 seconds. Subjects were introduced to the box lift test and practiced lifting the box correctly and placing it on the platform.

Protocol

To begin AET days, as previously stated, subjects arrived at the laboratory at their designated time. Their hydration level was measured via urine refractometry (Model A300CL, Spartan, Japan). After euhydration was ensured, a trained phlebotomist inserted an indwelling Teflon cannula into the antecubital vein and the Pre blood sample was obtained. Next, the Pre muscle biopsy was obtained.

Subjects then performed the standardized warm-up protocol. Next they executed ten maximal vertical jumps without pause. Subjects were given time to recover before they began the isometric bench press. The rack was locked to the assigned position and the subject assumed the isometric bench press position with standard grip width. Subjects were told to gradually increase pressure with constant back, gluteal, and foot contact as previously instructed during the familiarization visit. Subjects pushed to maximal exertion for 10 seconds. Following another rest period, the squat rack was locked to the assigned position and subjects assumed the isometric squat position. Subjects pushed to maximal exertion for 10 seconds. All the preceding exercises were performed on a force platform (Accupower) to measure power (W) and force (N). Immediately following the isometric squat, the Mid blood sample was drawn.

Following the rest period, subjects began the box lifting test. The box lifting test consisted of lifting a 18.14 kg metal box with handles on to a 1.32 m high platform from 0.914 meters away. Two identical boxes were used and an assistant immediately returned each box to the start position using a slide system, which allowed a box to always be available for the subject to lift. During this 10 minute period the subjects lifted the box to the platform as many times as possible. Immediately after completion of the box lift test, the Post blood draw was taken. Five minutes after completion of the protocol the +5 blood was drawn. Subjects were transported to the biopsy room and at +10 the post-exercise muscle biopsy was obtained. Finally, the +15 blood was drawn. Also, verbal encouragement was given to achieve maximal output for each exercise.

Blood Draws

A trained phlebotomist inserted an indwelling Teflon cannula into an antecubital vein of the subject. The cannula was kept patent with 10% heparin/saline solution lock. Prior to each blood draw, 3 ml of blood was extracted and discarded to avoid inadvertent saline dilution of the blood. During each blood draw, 15 ml of blood was collected into appropriate tubes for obtaining plasma and serum. Blood tubes were centrifuged at 3000 rpm for 15 minutes at 4°C and resultant serum and plasma was aliquoted and stored at -80°C for later analyses.

Muscle Biopsies

A trained physician performed the percutaneous needle biopsy procedure on the subjects' vastus lateralis muscle using the technique described by Bergstrom³ modified

with suction to obtain a small (~100g) muscle sample. First, the skin was cleaned with an alcohol wipe and disinfected using betadine. Then local anesthetic (8-10 cc of 1% Lidocaine) was injected in a cone-like fashion 45 degrees proximal and 45 degrees distal to the biopsy site to ensure that the muscle fascia and subcutaneous tissue is anesthetized, but the muscle sample to be extracted is not harmed. After sufficient time for the anesthetic to exert its effect, a small incision (~1 cm) was made through the skin and the muscle fascia using a #11 surgical scalpel. A sterile biopsy needle was introduced to the muscle through the incision. Suction was used and the inner cannula was advanced to cut the muscle tissue. The needle was removed and the incision was covered with sterile gauze and compression was applied to prevent bleeding. The incision was then closed with sutures. The muscle sample obtained was cleared of excess blood and connective tissue, flash frozen in liquid nitrogen, and immediately stored at -80°C for later analyses.

During each AET, Pre and Post muscle biopsies were taken from the same leg, approximately 3 cm apart. Two separate sites were used to avoid immune/inflammatory responses confounding with the muscle signaling proteins of interest. For the following AET, the opposite leg was used to allow the maximum amount of time for recovery.

Supplement

Subjects ingested 1.25 g of betaine (Danisco Inc. Ardsley, NY) dissolved in 300 ml of a sports drink (Gatorade, fruit punch flavor) or a placebo consisting of 300 ml of the same sports (Gatorade, fruit punch flavor) twice a day for two weeks. The sports drink (Gatorade, fruit punch flavor) nutritional value is as follows: 69.6 kcal, 0 g fat, 0 g protein, and 17.4 g carbohydrate. Following the second AET subjects did not take any

supplementation for two weeks as a washout period. Following the washout period, subjects crossed over to the other supplement condition (betaine or placebo) and completed another two week supplementation period. The study was double blinded, so the subjects and primary researchers were unaware of the supplement the subjects received. Furthermore, subjects could not identify whether or not they were receiving the supplement or placebo, as taste and texture was identical.

Biochemical Analyses

Blood biochemical analyses (unpublished observations from the study) for GH, IGF-1, and cortisol were performed via enzyme-linked immunosorbent assays (ELISA).

Muscle samples were homogenized under cold conditions to inhibit any enzymatic processes in lysis buffer [Tissue Protein Extraction Reagent (TPER), 10 µl/ ml Protease and Phosphatase Inhibitor, and 10 µl/ml EDTA Solution (Thermo Scientific, Rockford, IL), more specifically consisting of Tris (pH 7.4) supplemented with aprotinin, bestatin, E-64, leupeptin, sodium fluoride, sodium orthovanadate, sodium pyrophosphate, b-glycerophosphate, and 0.5 M EDTA] and centrifuged at 10,000xg for 10 minutes at 4°C. The supernatants were extracted and a small portion (5 µl) was used to determine total protein concentration via the Bradford assay method (utilizing Coomassie Plus Reagent (Thermo Scientific) and the absorbance at 595 nm) to give the total protein concentration of the samples.

Once the total protein concentrations were known, the samples were diluted to a standard of 3 mg/ml with lysis buffer (as described above) and 5X Laemmli buffer (3% w/v Tris Base, 10% (w/v) Sodium Dodecyl Sulfate (SDS), 20% Glycerol, 0.2% (w/v)

Bromophenol Blue, 5% (w/v) Beta-Mercaptoethanol). Samples were then loaded (30 µg total protein per well) in duplicate on a 4 – 15% Tris-HCl gradient gel (Bio-Rad Laboratories, Hercules, CA) with Precision Plus Kaleidoscope Protein Standards (Bio-Rad Laboratories) in each end well as a molecular weight marker. The gel was then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in electrophoresis buffer (14.4% (w/v) Glycine, 3.02% (w/v) Tris Base, 1% (w/v) SDS, 1 L dH₂O) and ran at 200V for approximately one hour to allow total protein separation on the gel. Proteins were then transferred electrophoretically [50V for 1.5 hours in transfer buffer (14.4% (w/v) Glycine, 3.02% (w/v) Tris Base, 800 ml dH₂O, 200 ml Methanol) at approximately 4°C] to a polyvinylidene fluoride (PVDF) membrane (Bio-Rad Laboratories).

Following the transfer, the PVDF membrane was incubated for one hour at room temperature in a blocking solution. The blocking solution was optimized for the protein of interest. When the protein of interest was Akt Total, Akt phosphorylated at Serine 473 (Akt S473), or p70 S6K Total the membranes were blocked in a solution of Tris Buffered Saline (TBS) with 0.5% Tween-20 (Sigma-Aldrich, St. Louis, MO) (TBS-T) and 5% non-fat dry milk (Bio-Rad Laboratories). When the protein of interest was p70 S6K phosphorylated at Threonine 389 (p70 S6K T389) or AMPK phosphorylated at Thr 172 (AMPK T172), the membrane was blocked in a solution of TBS-T and 5% bovine serum albumin (BSA) (Sigma-Aldrich).

After blocking non-specific sites, the membrane was rinsed in TBS-T with agitation 3 times for 5 minutes. The membrane was then incubated overnight at 4°C with agitation in primary antibodies against Akt Total, Akt S473, p70 S6k Total, p70 S6k

T389, or AMPK T172 (Cell Signaling Technology, Inc., Danvers MA). All the primary antibodies were rabbit-sourced, polyclonal, and were diluted 1:1000 in 5% BSA TBS-T solution.

Following the overnight incubation, the membrane was rinsed in TBS-T 5 times with agitation for 6 minutes. Membranes were then incubated for one hour at room temperature with agitation in the species-specific horseradish peroxidase-conjugated IgG secondary antibody (anti-rabbit IgG HRP linked antibody, Bio-Rad Laboratories) diluted 1:2000 in a 5% BSA TBS-T solution. After another subsequent five rinses in TBS-T with agitation, the membrane was briefly incubated in SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific) with slight rocking. The membrane was then developed in a dark room with X-ray film (Thermo Scientific) and developing chemicals (Kodak, Rochester, NY). The visualized bands were captured and quantified for densitometric values using a Kodak Imager (Image Station 4000MM Pro, Kodak).

Statistical Analysis

Performance results (unpublished observations from the study) were analyzed using a condition x time repeated measures analysis of variance (ANOVA). Hormonal results (unpublished observations from the study) were computed for area under the curve and then analyzed using a condition x time repeated measures ANOVA. Muscle signaling results were analyzed for homogeneity. Since densitometric values are measured in arbitrary units, values were log transformed to decrease variation. Data was then analyzed using a condition x time repeated measures ANOVA with LSD fisher post-hoc comparisons used to determine specific pairwise differences. Significance was set at $p \leq 0.05$ for all results.

Chapter 4: Results

Performance and hormonal results (unpublished observations from the study) are presented in order to see the full picture and comprehensively look at the effect of betaine supplementation, as examined in this project.

Performance Results

No significant differences were seen with betaine supplementation in the vertical jumps. There were also no significant differences in the isometric bench press. In contrast, there was a significant increase for the change (post supplementation – pre supplementation) in force production (Newtons) in the squat exercise following 2 weeks of betaine supplementation compared to the placebo (Figure 4.1). Also, there was a significantly greater number of boxes lifted following 2 weeks of betaine supplementation compared to the placebo condition (Figure 4.2).

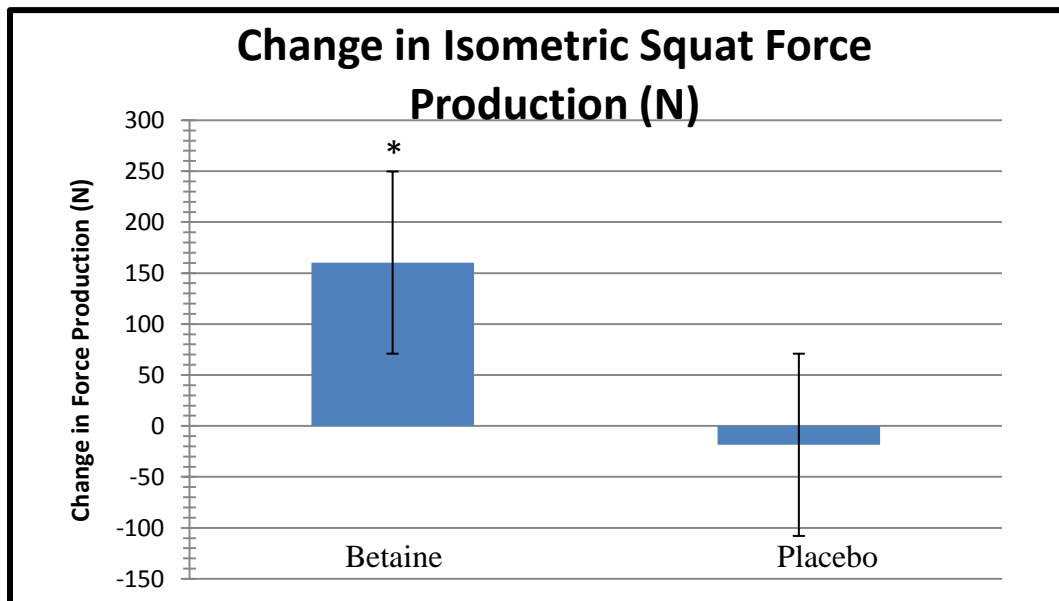


Figure 4.1: Change (post – pre supplementation) in force production (N) in squat exercise over two weeks of supplementation with betaine or placebo.

* Denotes significantly different from Placebo.

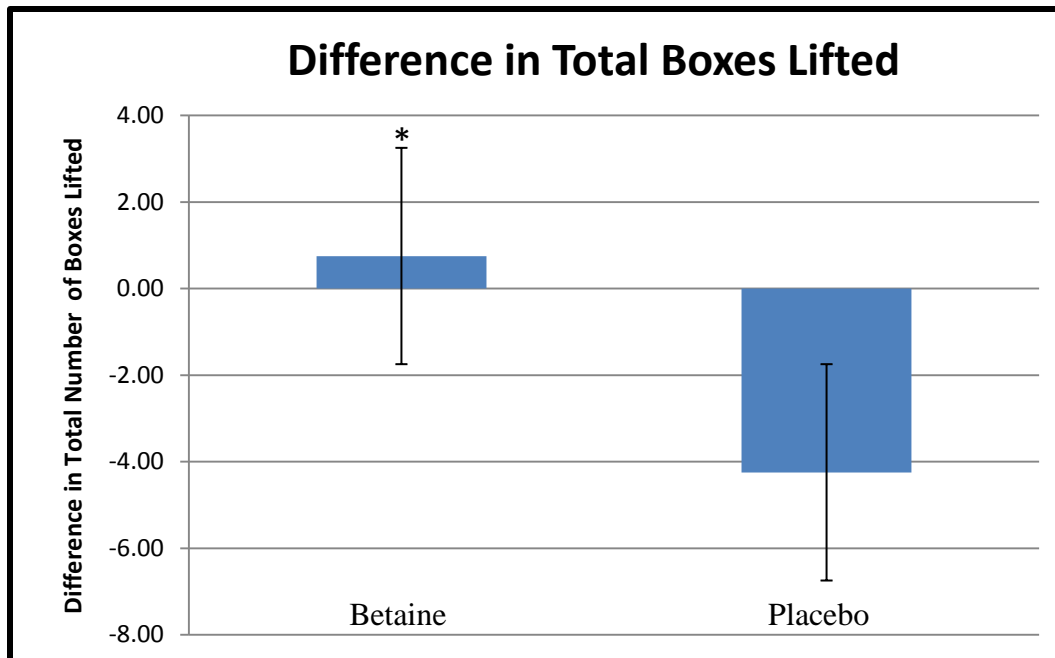


Figure 4.2: Change (post – pre supplementation) in total boxes lifted during the ten minute box lift test over two weeks of supplementation with either betaine or placebo.

* Denotes significantly different from Placebo.

Hormonal Results

There was a significantly greater area under the curve (for time points during the AET) in GH response (Figure 4.3) and IGF-1 response (Figure 4.4) following two weeks of betaine supplementation compared to placebo. There was also a significantly reduced area under the curve in cortisol (Figure 4.5) following two weeks of betaine supplementation compared to placebo. There was a significant time effect for glucose and lactate due to exercise, but these were not significantly different supplement.

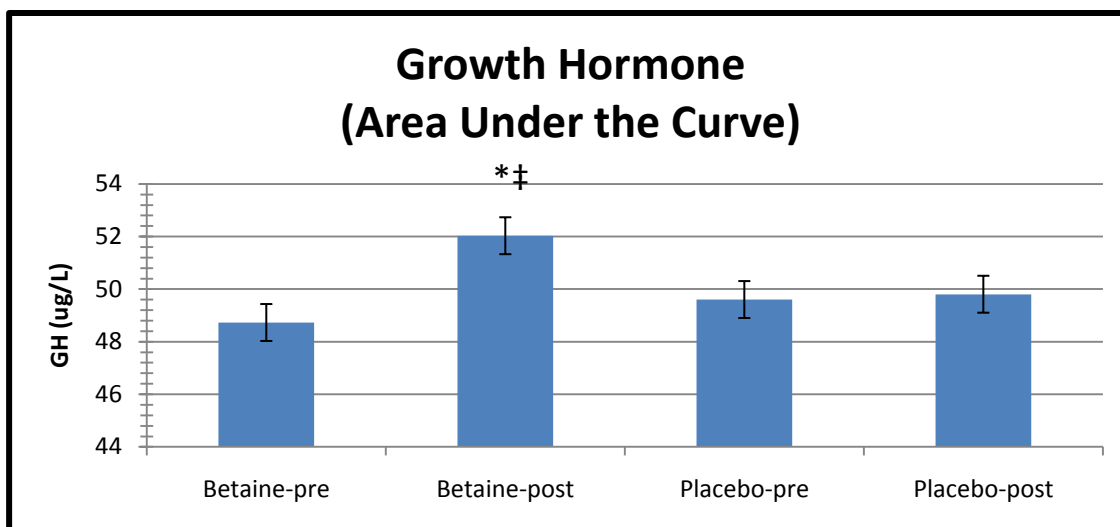


Figure 4.3: Significant difference in AUC for GH following 2-weeks of betaine supplementation.
 * Denotes significant differences between pre- and post-test for Betaine or Placebo.
 ‡ Denotes significant difference between Betaine Post-Test and Placebo Post-Test.

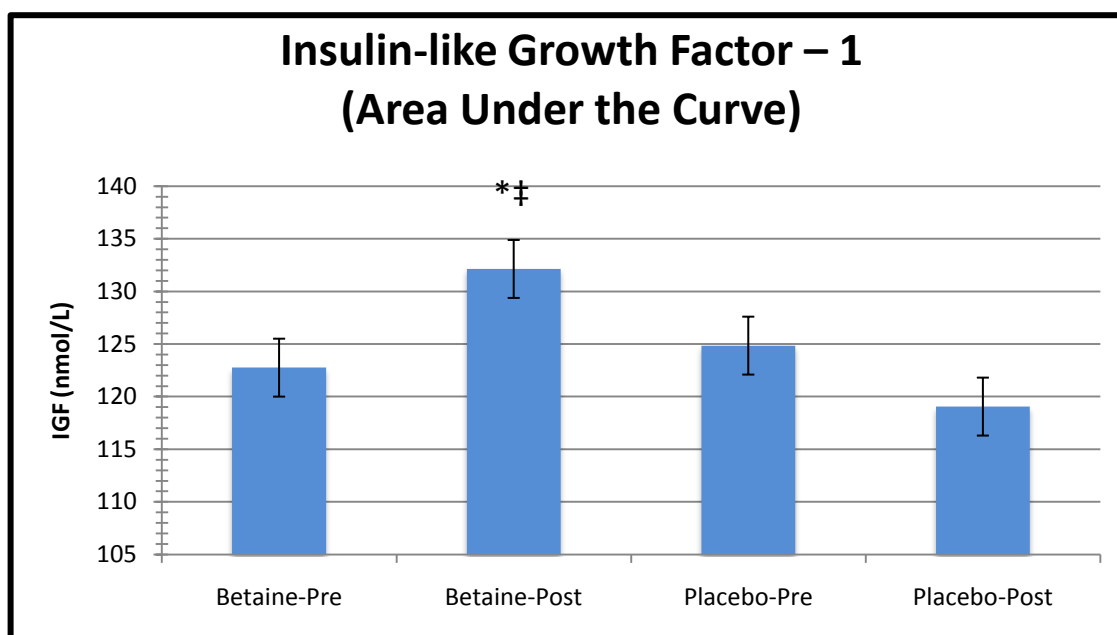


Figure 4.4: Significant difference in AUC for IGF-1 following 2-weeks of betaine supplementation.
 * Denotes significant differences between pre- and post-test for Betaine or Placebo.
 ‡ Denotes significant difference between Betaine Post-Test and Placebo Post-Test.

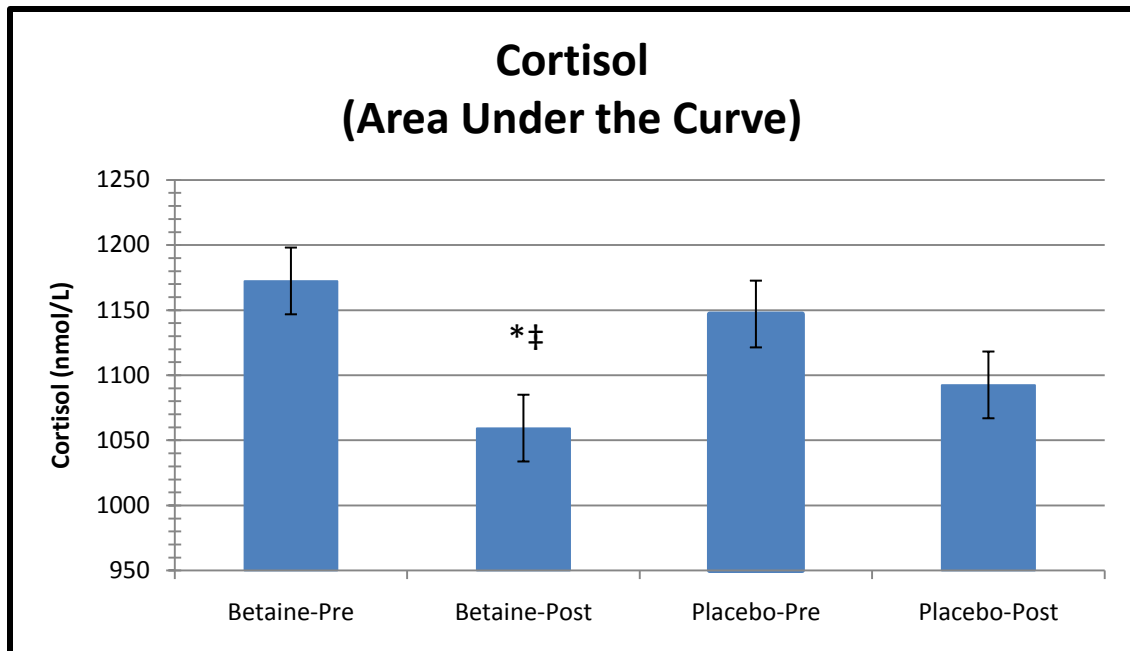


Figure 4.5: Significant difference in AUC for cortisol following 2-weeks of betaine supplementation.

* Denotes significant differences between pre- and post-test for Betaine or Placebo.

‡ Denotes significant difference between Betaine Post-Test and Placebo Post-Test.

Muscle Signaling Results

Akt Total, Akt S473, p70 S6k Total, p70 S6k T389, and AMPK T172

densitometric values (arbitrary units) values were log transformed and are presented as means with standard deviation bars. For all of these proteins there was no significant difference in pre-exercise and post-exercise per AET.

Akt Total pre-exercise was significantly increased ($p = 0.003$) after two weeks of betaine supplementation, and significantly greater than the Pre Placebo pre-exercise ($p = 0.00$) and Post Placebo pre-exercise ($p = 0.04$) (Figure 4.6 and 4.7). Akt Total post-exercise was significantly increased following betaine supplementation ($p = 0.017$), this also seen during placebo supplementation, but to a lesser degree ($p = 0.029$) (Figure 4.8).

Akt S473 was significantly lower post-exercise following placebo supplementation ($p = 0.016$), but was maintained during betaine supplementation (Figure 4.9).

p70 S6k Total was not significantly different during any time point of any trials (Figure 4.10).

p70 S6k T389 was significantly decreased pre-exercise after placebo supplementation ($p = 0.004$) while betaine supplementation maintained p70 S6k T389 pre-exercise (Figure 4.11). p70 S6k T389 was significantly decreased post-exercise after placebo supplementation ($p = 0.005$) while betaine supplementation maintained p70 S6k T389 post-exercise as well (Figure 4.12).

AMPK T172 was significantly decreased pre-exercise following betaine supplementation ($p = 0.001$). This was also seen during the placebo supplementation phase to a lesser degree ($p = 0.031$) (Figure 4.13). After two weeks of placebo and betaine supplementation both had a significant decrease in post-exercise AMPK T172 (both $p = 0.001$) (Figure 4.14).

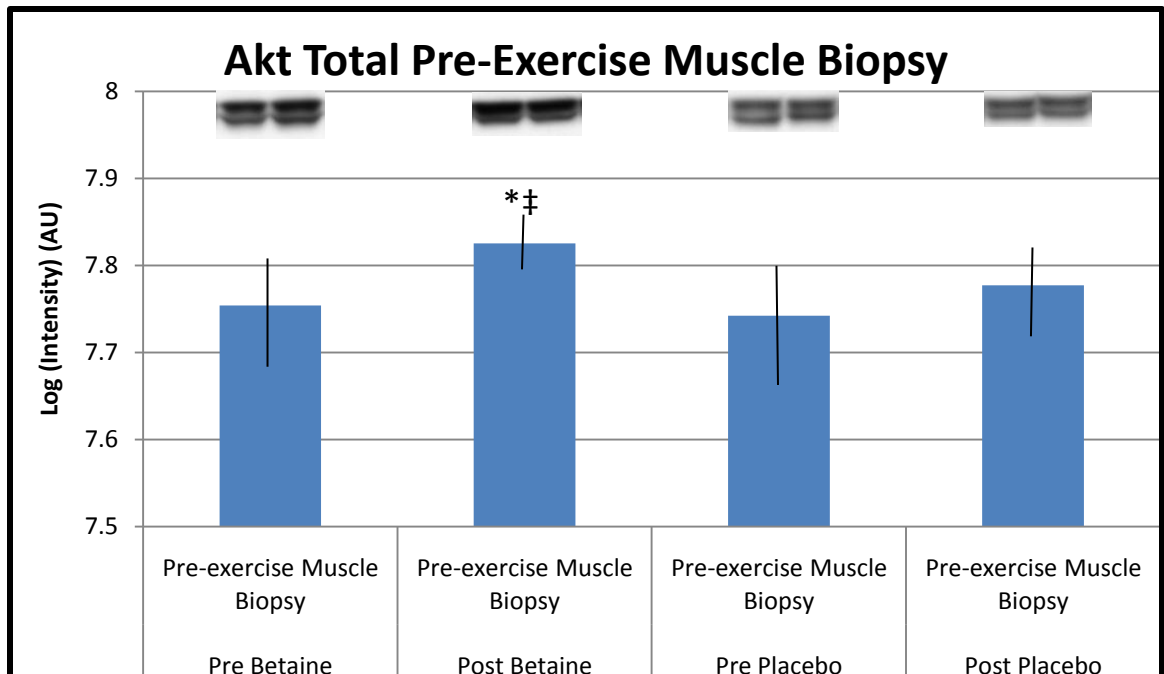


Figure 4.6: Pre-exercise muscle biopsy values of Akt Total.

* Denotes a significant difference between corresponding pre supplementation value (betaine or placebo).

‡ Denotes significant difference between both Pre Placebo and Post Placebo iterations.

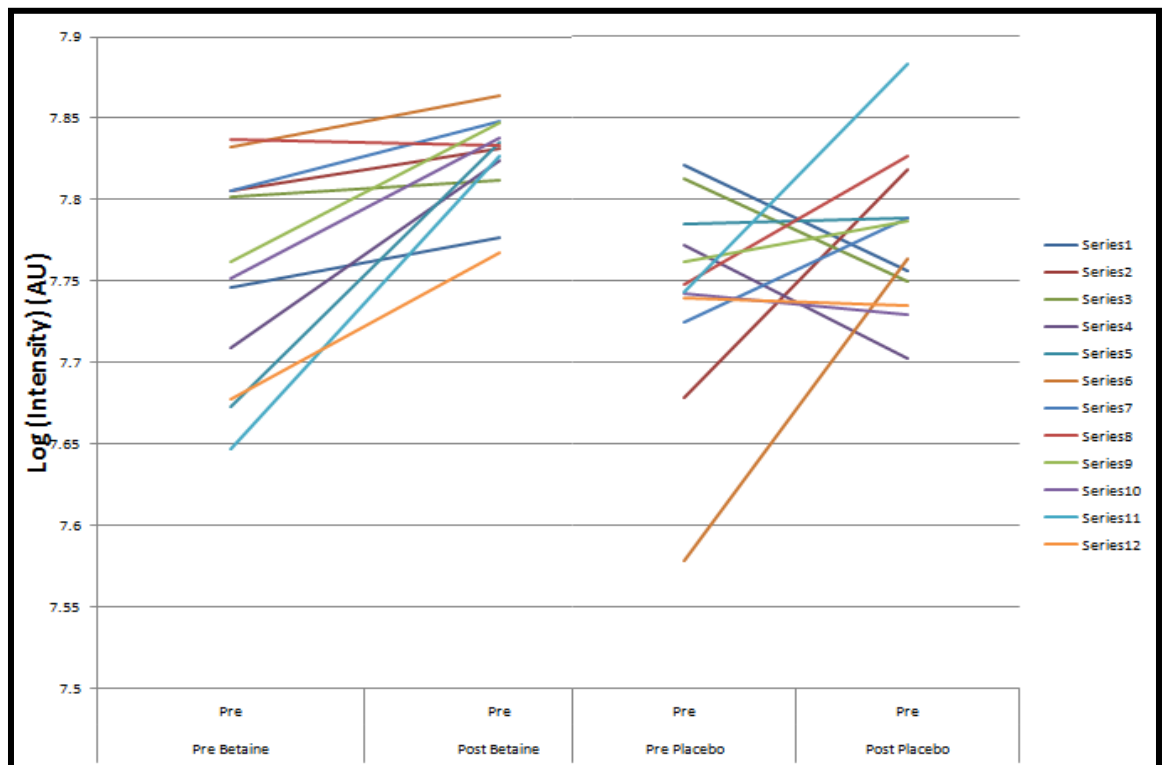


Figure 4.7: Pre-exercise muscle biopsy values of Akt Total for all subjects.

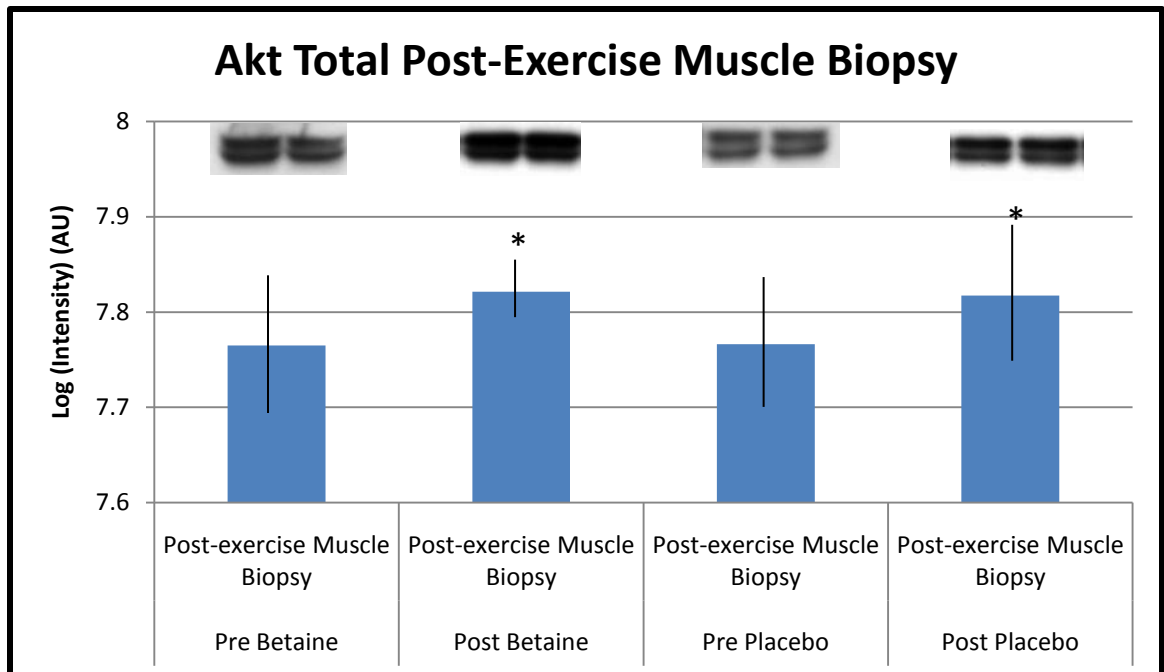


Figure 4.8: Post-exercise muscle biopsy values of Akt Total.

* Denotes a significant difference between corresponding pre supplementation value (betaine or placebo).

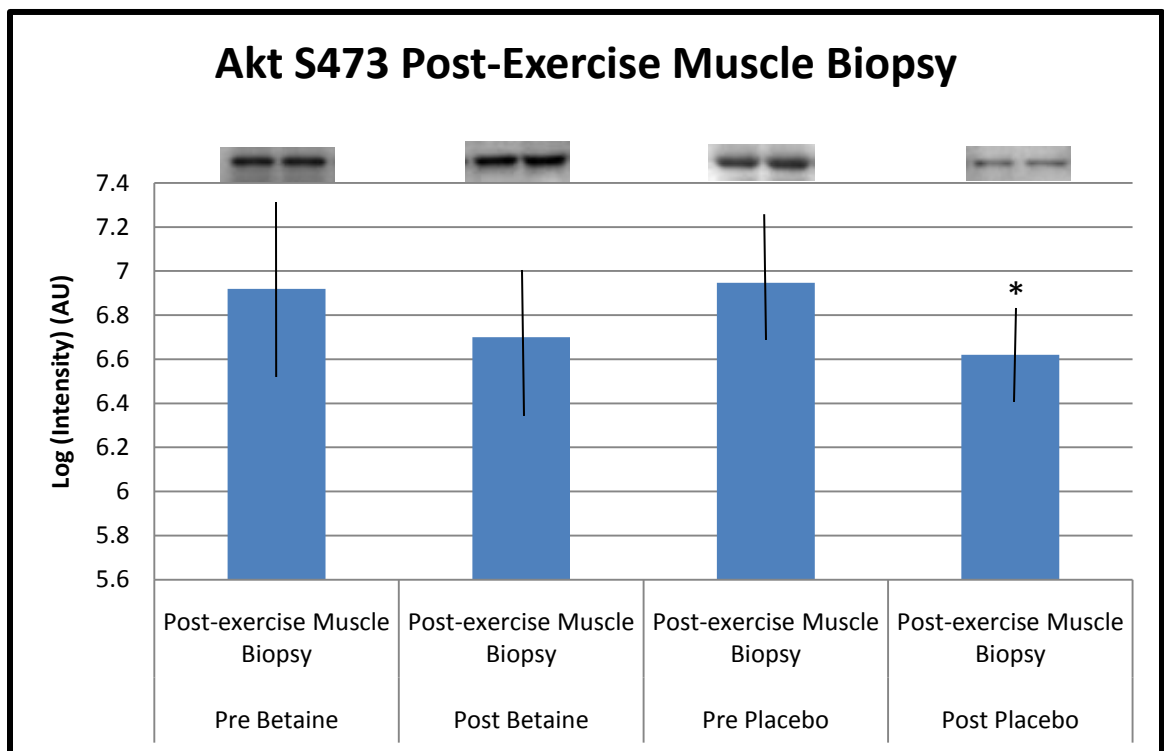


Figure 4.9: Post-exercise muscle biopsy values of Akt S473.

* Denotes a significant difference between corresponding pre supplementation value (betaine or placebo).

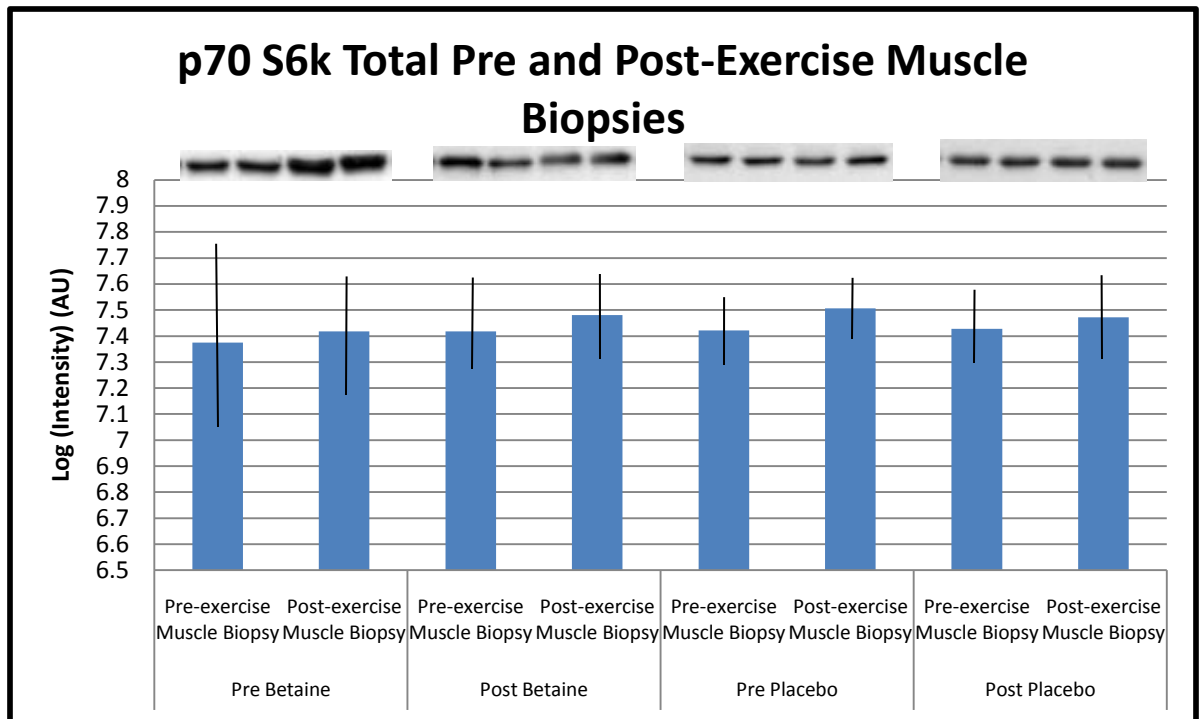


Figure 4.10 Pre-exercise and Post-exercise muscle biopsy values of p70 S6k Total.

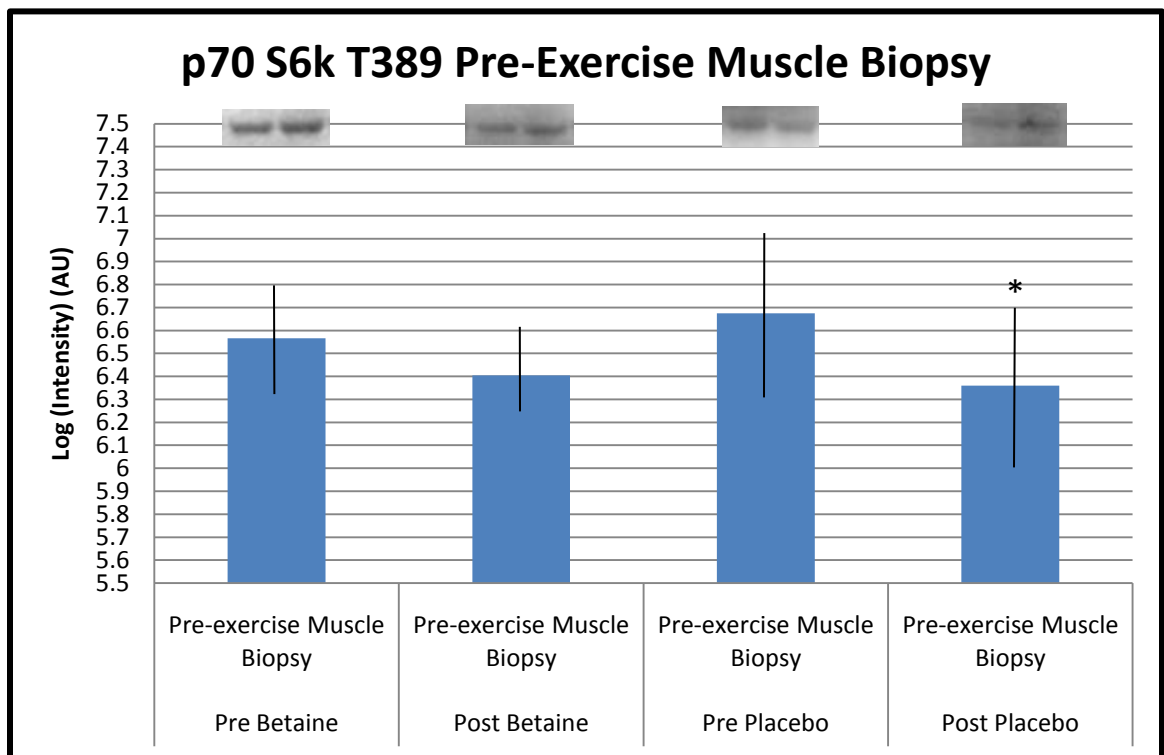


Figure 4.11 Pre-exercise values of p70 S6k T389.

* Denotes a significant difference between corresponding pre supplementation value (betaine or placebo).

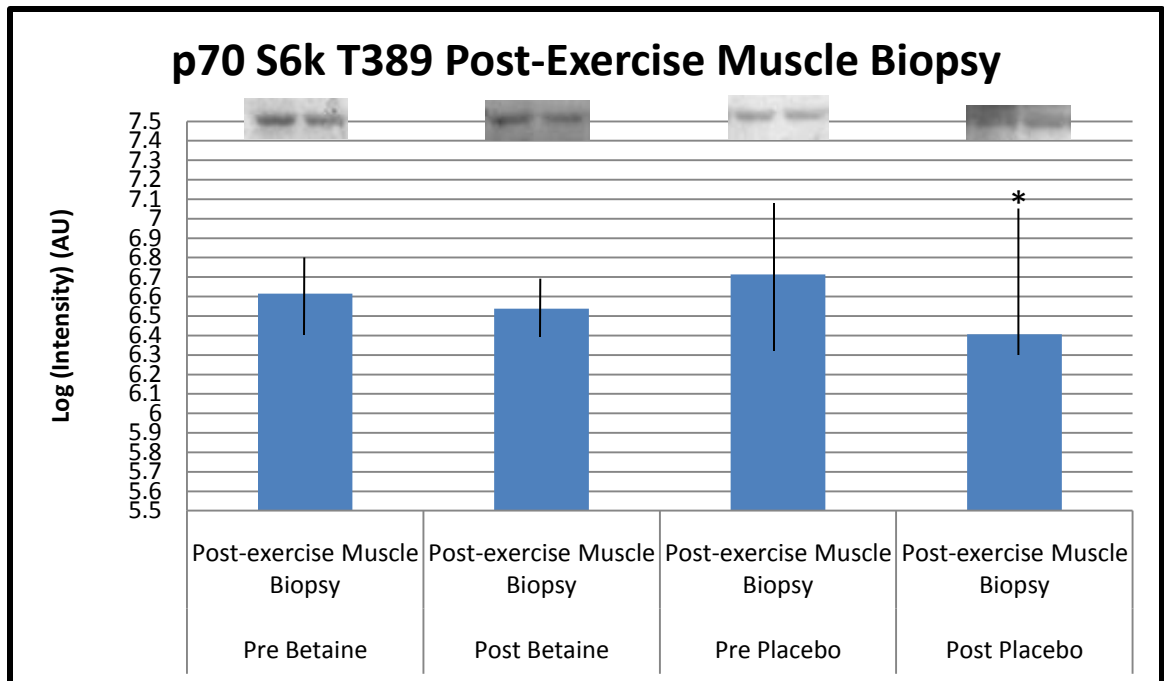


Figure 4.12: Post-exercise values of p70 S6k T389.

* Denotes a significant difference between corresponding pre supplementation value (betaine or placebo).

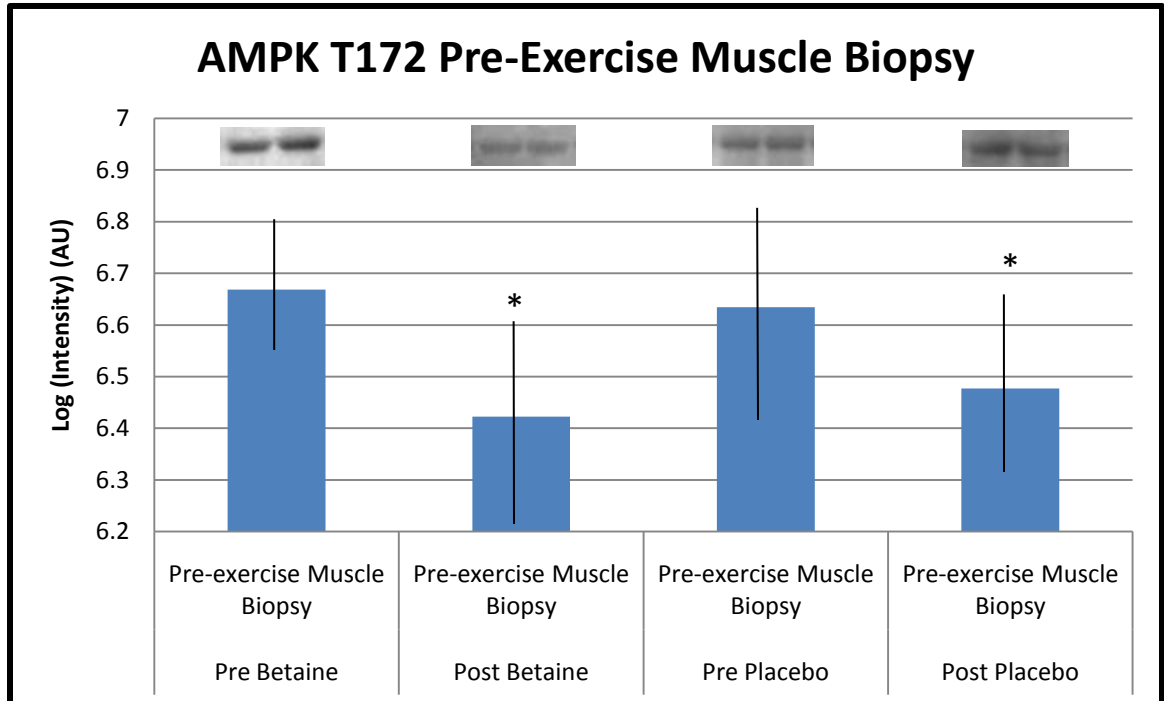


Figure 4.13 Pre-exercise values of AMPK T172.

* Denotes a significant difference between corresponding pre supplementation value (betaine (p = 0.001) or placebo (p = 0.031)).

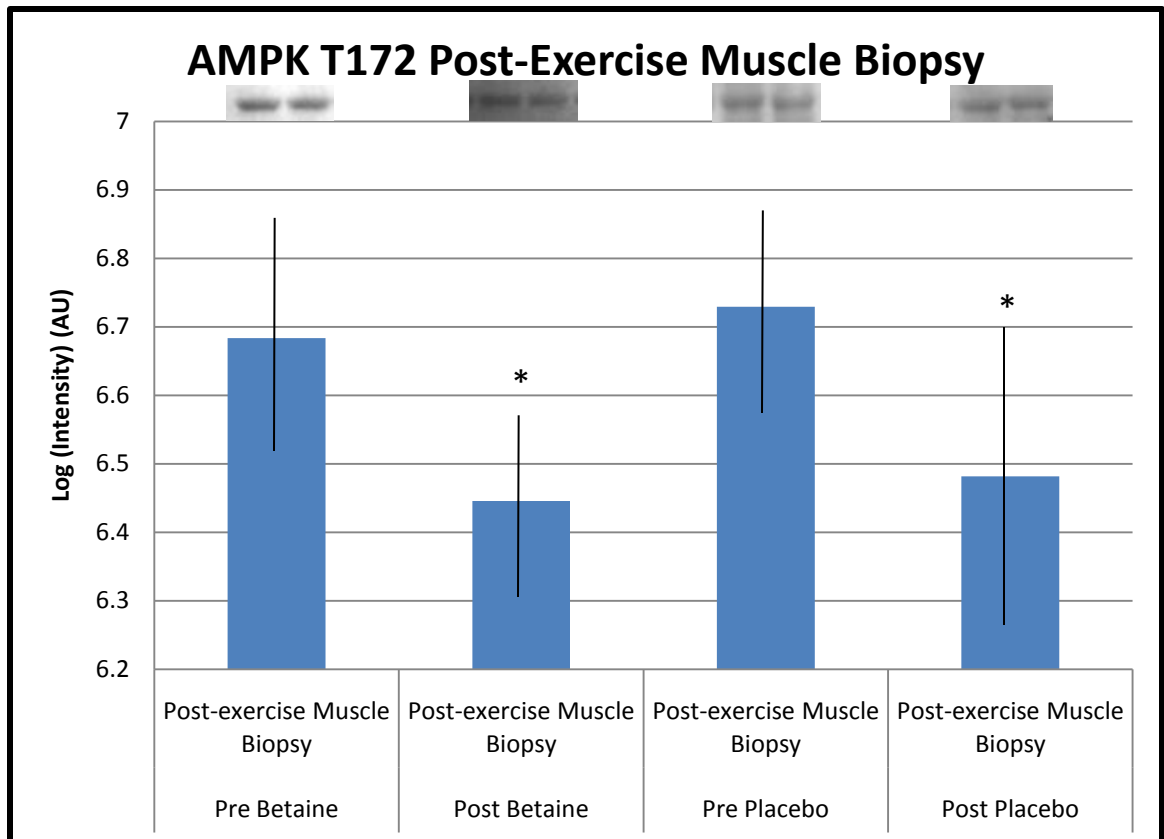


Figure 4.14: Post-exercise values of AMPK T172.

* Denotes a significant difference between corresponding pre supplementation value (betaine or placebo).

Chapter 5: Discussion

Previous research shows the importance of betaine within the human body. Most animal research ¹⁵ shows that betaine supplementation results in a leaner and more muscular animal. Although the results vary slightly, recent human research by Hoffman et al. ²¹ and Lee et al. ³¹ showed increases in muscular endurance, force production, and power. Agreeing with the previous research, our results showed significant increases in force production (increased isometric squat force) and muscular endurance (increased number of boxes lifted during box lift test) following betaine supplementation.

Mechanistically, animal studies ^{7, 22, 40} have recently shown that betaine supplementation can increase GH, IGF-1, and insulin. Although we did not report insulin measures, subjects had increased GH and IGF-1 following betaine supplementation. Contrary to other betaine supplementation studies that examined cortisol ^{35, 38}, we showed a significantly reduced area under the curve for cortisol following 14 days of betaine supplementation. These differences in results could be due to the longer supplementation period in our study and an exercise-focused methodology. Also, it is important to note that our study indicates the exercise-induced increase in circulating cortisol was attenuated following betaine supplementation rather than a decrease in resting levels all together. Thus, it could be speculated that betaine reduces the production and release of cortisol during stressful exercise. Since Yan ⁵⁵ observed an increase in betaine deposition in the hypothalamus, one could speculate that the betaine supplementation may have caused a decrease in the release of corticotrophin releasing hormone (CRH) from the hypothalamus, leading to decreased adrenocorticotrophic hormone (ACTH) from the anterior pituitary and finally, decreased cortisol from the adrenals.

Examining the hormonal data collectively provides a thought provoking picture since cortisol has been shown to inhibit GH release, specifically by blunting GH release in response to GHRH^{13, 41, 48}. Furthermore, CRH has also been shown to inhibit GH release stimulated by GHRH². Additionally, cortisol may increase somatostatin as another point of inhibition to GH². To our knowledge, no other study has reported both cortisol and GH responses to betaine supplementation. It could be suggested that the decrease in cortisol during the post betaine supplementation trial would decrease the inhibitory effects to allow the increase in GH release that was observed in our study. Also, though we did not measure GHRH, one would assume the increase in GH secretion originated with the increase in GHRH gene transcription and secretion from increased betaine deposition on the hypothalamus as Yan observed⁵⁵. It could also be speculated that the decrease in cortisol decreased somatostatin to allow the overall stimulus to the anterior pituitary to be more positive and allow increased GH secretion.

The changes observed in muscle signaling proteins correspond to the pattern of changes in hormonal responses. It cannot be determined whether any of the changes in muscle signaling were independent of hormonal changes. Interestingly, in our study there were no differences in any proteins within each AET. This finding is not very surprising as the study was primarily designed to look at performance measures, specifically force production. Because we wanted to study force production, the protocol utilized isometric exercises rather than concentric and eccentric action. Evidence shows that maximal eccentric actions cause the greatest increase in post-exercise p70 S6k phosphorylation rather than concentric or submaximal eccentric action¹⁶. Though some animal studies show similar RNA responses with concentric, eccentric, and isometric actions when force

production is equal ¹⁷, skeletal muscle can generate greater tension during eccentric action resulting in the greater signaling responses ¹⁶. Furthermore, isometric exercise was not able to increase the anabolic signal to atrophied rat muscle ¹⁹. Overall, isometric exercise may not be a sufficient stimulus to evoke immediate signaling responses post-exercise. Also, due to the box lift portion, the AET was not solely resistance exercise as 10 minutes of box lifting would rightly be considered partially oxidative. Therefore, the AET could be considered a concurrent workout and immediate post-exercise signaling responses may be mixed and harder to observe only 10 minutes into the recovery period.

Muscle signaling mechanisms are activated at the onset of contraction and continue through the recovery period hours after exercise ⁶. Studies show that Akt and p70 S6k phosphorylation peaks between the 30 – 60 minutes post exercise, but seems to remain elevated for 2 – 6 hours post exercise ⁶. Since the post muscle biopsy was only 10 minutes after exercise was completed and not longer into the recovery period, we may not have been able to capture the peak phosphorylation of the proteins. Similarly, AMPK phosphorylation is also seen within 30 – 60 minutes. Recent research has shown that pre-exercise feeding (specifically glucose ingestion ¹⁸) could attenuate the phosphorylation of AMPK T172 post-exercise. Despite the fact that betaine levels peak in the serum approximately 90 minutes post ingestion ¹¹, which has previously been utilized in betaine supplementation study protocols ^{21, 31}, our subjects were tested in a fasted state, so the results would not be confounded by acute pre-exercise feeding. Therefore, the study examined long-term (14 days) supplementation effects and avoided the confounding influence of acute consumption.

Although there were no significant differences observed within each AET, there were significant differences after supplementation. There was significantly more pre-exercise total Akt following betaine supplementation. Although this indicates more potential for translation initiation, due to the multiple isoforms and phosphorylation sites of Akt, it is not possible to conclude an increase of anabolic signal. Post-exercise Akt Total was increased following betaine supplementation. This post-exercise increase in Akt Total was also seen in the placebo group to a lesser degree. This may partially be explained by the placebo creating an overall positive energy balance due to increase caloric intake over the supplementation period. Overall, the changes in total Akt following betaine supplementation indicate a slightly higher potential to continue the anabolic signal downstream, but are not conclusive.

Despite the perhaps non-optimal timing of the post-exercise biopsy to detect Akt phosphorylation, there was a significant difference following supplementation between groups. While the placebo group had decreased post-exercise Akt S473, the betaine group was able to maintain the level of protein phosphorylation following the supplementation period. Therefore, it appears that the betaine supplementation allowed a greater stimulus to phosphorylate and consequently activate mTOR compared to the placebo group. Depending on the exact protocol, some studies have shown that resistance training itself decreases Akt S473 phosphorylation²³, so it was interesting to find that betaine supplementation diminished this decrease over the resistance training period.

Similarly, phosphorylation of p70 S6k T389 was significantly lower after placebo supplementation pre-exercise and post-exercise. These levels were maintained following betaine supplementation. p70 S6k T389 levels vary greatly due to training volume⁴⁷, fed

state⁵³, type of muscle action¹⁶, and cortisol inhibition⁴⁴. The decreased cortisol levels during the post betaine supplementation AET likely decreased the inhibitory effect to allow p70 S6k T389 phosphorylation and subsequently translation initiation. During the other AETs, the higher levels of cortisol likely inhibited the phosphorylation of p70 S6k at T389 to a greater extent. This indicates betaine supplementation was able to maintain protein synthesis while the placebo supplementation did not.

As stated previously, the exercise protocol was partially oxidative, so an AMPK response would be expected. Since AMPK is a systemic energy sensor²⁵ it is regulated by cellular energy state, as well as overall energy balance. Although the placebo (Gatorade) did not contain a large amount of calories, it is still possible that the 17.4 g of carbohydrates twice daily for two weeks could have created an overall positive energy balance and decreased AMPK activation in the post-supplementation AETs. Pre-exercise AMPK phosphorylation was decreased with greater significance in the betaine group, indicating betaine was able to decrease pre-exercise AMPK phosphorylation greater than placebo alone. Post-exercise AMPK phosphorylation was decreased after both supplementation periods indicating that the effect of betaine may not have been powerful enough after the stressful exercise stimulus to show a difference between betaine and placebo effects, but rather the speculated positive energy balance over the two weeks was able to cause a significant difference. Studies have shown that the decrease in muscle protein synthesis during resistance exercise is associated with an increase in AMPK activity¹⁴, so one could infer that the decrease in AMPK during the post supplementation trials would contribute to enhanced protein synthesis during these trials.

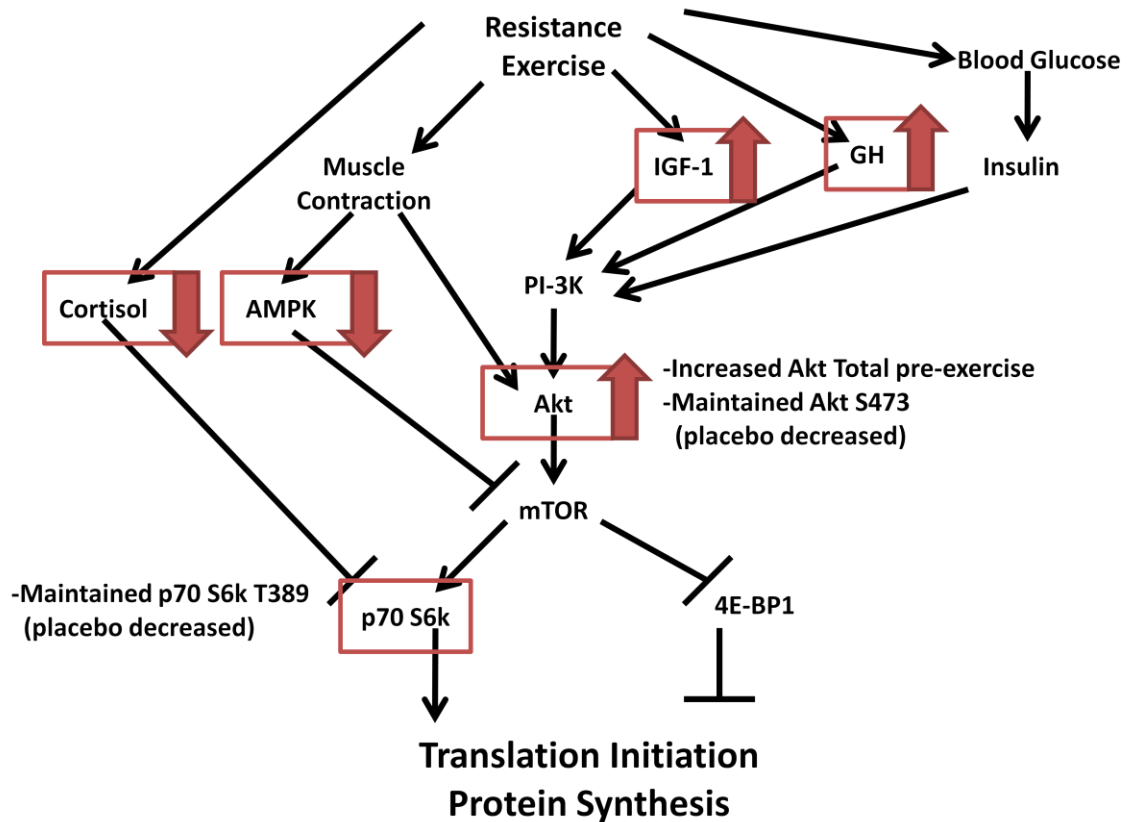


Figure 5.1: Overall effects of betaine supplementation on hormonal responses and the muscle signaling pathway.

Overall, betaine supplementation increased performance, as well as anabolic hormones and signaling to an extent. The increase in Akt Total pre and post-exercise, as well as the maintenance in Akt S473 post-exercise following betaine supplementation indicates a greater ability to signal further downstream. These results correspond to the increase in GH and IGF-1 AUC during the betaine supplementation. Although there was no change in p70 S6k Total, p70 S6k T389 phosphorylation was maintained during the betaine supplementation pre and post-exercise. The decrease in p70 S6k T389 during the post placebo supplementation trial is explained partially by the increased cortisol levels during this time, where the maintenance during betaine supplementation is explained by

the decrease in cortisol levels and therefore decreased inhibition to the muscle signaling pathway. Furthermore, the decrease in AMPK phosphorylation observed would decrease the inhibitory effect on the mTOR pathway, allowing a more positive anabolic signal. In total, these observations (Figure 5.1) suggest maintenance of protein synthesis during a concurrent workout, and provide a cellular basis to the ergogenic effect of betaine supplementation.

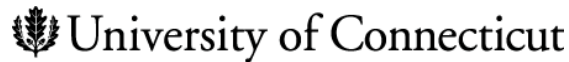
To conclude, our hypothesis was supported to an extent in each area. First, betaine supplementation enhanced strength performance as evident in increased force production and increased box lift performance. These performance changes correspond to increased anabolic hormone levels (GH and IGF-1) and decreased catabolic hormone levels (cortisol) following betaine supplementation. Lastly, the performance and hormonal improvements are consistent with the muscle signaling responses, including increased pre-exercise Akt Total, maintained Akt S473 and p70 S6k T389 phosphorylation, and decreased inhibition from AMPK T172.

Future research should focus on a purely resistance exercise protocol to look more closely at the anabolic signaling effects, as well as obtain a later muscle biopsy sample to more effectively examine signaling responses during recovery. Also, it would be interesting to study the insulin and 4E-BP1 responses to complete the paradigm presented. Additionally, to evaluate the hypothalamic point of action, it would be of interest to measure GHRH, CRH and other hormones secreted from the hypothalamus that may have an influence on performance. Also, since testosterone is a key regulator of protein synthesis and integral for examining the entire anabolic picture, it would be

interesting to examine the effect betaine supplementation may have. Finally, no study to our knowledge has examined the effect of betaine supplementation in women.

Appendix A

Consent Form for Participation in a Research Project



Principal Investigator: Carl M. Maresh, Ph.D.

Study Title: Betaine II: Study of Mechanisms in Skeletal Muscle.

1. **Invitation to Participate**

You are invited to participate in this study which will investigate the effects of a betaine supplement, mixed in Gatorade, on strength and power performance, circulating hormone concentrations, and muscle signaling proteins in response to an acute exercise protocol.

2. **Purpose**

This study is being conducted to see how betaine affects hormone concentrations and the proteins involved in muscle growth in response to exercise.

3. **Description of Procedures**

Study overview:

Betaine is a commonly found amino acid derivative usually extracted commercially from sugar beet molasses. It is found in a variety of foods. You would need to eat about 7 ounces of broccoli to consume the amount of betaine you will be supplementing each day during this study.

You will perform an acute exercise test (AET) protocol before and after 2 weeks of supplementation with either betaine dissolved in Gatorade or a placebo (Gatorade alone). After a 2 week washout (period of time when you do not take the betaine supplement or placebo), you will take the other supplement for another 2 week period. Before and after the second 2 week supplementation period you will perform an AET. By the end of the study, you will have taken both betaine and the placebo, but you will not know which you are receiving at those times. The AET protocol will consist of vertical jumps, isometric bench press, isometric squat, and box lifting. Muscle biopsies will be obtained before and 10 minutes after the AET. Blood samples will be collected before, once during, and 5 and 15 minutes after the AET. Before, during, and after the AET, heart rate and rating of perceived exertion will be measured; blood pressure will also be measured before, immediately after and 15 minutes after the AET.

Before you can be approved for participation you must complete a medical history questionnaire and a physical activity questionnaire to ensure that you meet all the inclusion and none of the exclusion criteria.

Inclusion:

To be considered eligible for this study you must be between the ages of 18-35 years. In addition, you must have been resistance training (at least 2-3 times per week) during the 3 months preceding the study.

Exclusion:

Your medical history form will be reviewed by our medical monitor (Jeffrey Anderson, M.D.). You will not be allowed to participate in this study if you have a pre-existing medical condition which may put you at risk while performing the strength-testing exercises or that might influence the outcomes of this study. Such conditions include, but may not be limited to, heart conditions or anomalies, respiratory conditions, blood pressure problems, kidney disease, musculoskeletal problems or previous orthopedic injuries that would limit the range of motion about the shoulder, elbow, hip, knee or ankle joint. Special attention will be given to exclude potential volunteers with lower back problems including herniated inter-vertebral discs. You cannot participate in this study if you have recently taken, currently take, or plan to take any hormonal substances such as testosterone, anabolic steroids or growth hormone. You will be screened by a registered dietician to ensure that you are not taking any nutritional supplements or adhere to atypical diets that may confound the results of this study. In addition, we reserve the right to dismiss you from this study at any time, if we believe you do not follow the instructions provided for this study.

Procedures:

This study will require that you come to the University of Connecticut Human Performance Laboratory (HPL) located in the basement of the Gampel Pavilion for five experimental visits (Visit 1,2,3,4,5) and one short follow-up visit the day after Visit 2,3,4,5. The follow up visit is for Dr. Maresh or Dr. Kraemer to inspect the biopsy sites to ensure proper healing.

Visit 1 (~1.0 hour)

On Visit 1, we will measure your height and weight. Then, a technician will measure your body composition using dual energy X-ray absorptiometry.

To familiarize you with the exercise protocol, you will first perform a light warm-up that includes 5-minutes of riding a stationary bicycle followed by moving stretches (heel kicks, lunges, high knees, high kicks, and unweighted squats). Then we will instruct and familiarize you with how to perform the following exercises: isometric bench press, isometric squat, vertical jumps and box lifting. We will give you plenty of rest throughout to ensure that you are not fatigued.

The box lifting exercise consists of lifting and moving a 40 lb metal box. The starting position for the box is on the floor, 3 feet from a 1.32 m high platform.

The objective of this exercise is to lift the box onto the platform as many times as possible in 10 minutes. Two identical boxes will be used in this experiment and an assistant will immediately return each box to the starting position using a slide system, thus a box will always be available for you to lift.

Visit 2-5 (Acute Exercise Test Visits) (~1 hour each)

You will arrive at the HPL after a 12-hour over-night fast (no eating or drinking anything except for water). Your hydration state will be tested by means of a urine sample prior to the AET. You will then lie down on a comfortable hospital bed so that we can insert a small flexible tube (i.e. catheter) into an arm vein for blood draws. Then you will be prepared for the first biopsy: We will use a local anesthetic to numb an area of your skin and thigh muscle before obtaining a very small amount of muscle (about the size of an unpopped popcorn kernel) via a muscle needle biopsy. This is the first of two muscle biopsies that will be taken during these visits. Since there are 4 AET visits, a total of 8 muscle biopsies will be performed.

The muscle biopsy involves taking a small piece of muscle tissue from a single incision site in your thigh muscle. Prior to the incision, the skin is cleaned and made sterile. Then the skin and tissue below are injected with local anesthetic to eliminate most of the associated pain. A small incision about the size of this dash " ____ " will be made through which a needle about the size of this letter "O" is advanced into the muscle. A piece of the thigh muscle is then removed with the needle. The incision site will be closed with a suture and a light dressing will be applied.

After the blood draw and biopsy, you will perform a light warm-up that includes 5-minutes of riding a stationary cycle followed by moving stretches (heel kicks, lunges, high knees, high kicks, and unweighted squats). After the warm-up you will complete 10 maximal vertical jumps without a pause between the jumps, a ten second maximal isometric bench press, a ten second maximal isometric squat and 10 minutes of box lifting. Force output will be measured during the jump exercise and the isometric bench and squat, and power will be measured during the jump exercise using a force platform. Before exercise, immediately after each exercise, and after 15 min of recovery your heart rate and rating of perceived exertion will be measured. To measure heart rate a heart rate monitor will be strapped around your chest just below the pectoral muscles. You will be asked to rate your perceived exertion (how hard you feel you are/were working) on a 0-10 point scale. The scale includes descriptors such as “weak” and “very strong”.

Immediately after you finish the exercise protocol, we will transfer you back to the bed so that we can obtain another blood draw and muscle biopsy from a different incision site as previously described. Once we have finished the last biopsy, the skin is closed with a suture and a light dressing is applied as before. During the experimental trial, 2 separate incisions on the same leg will be used to obtain the 2 biopsies. These incisions will be made 3 cm (approximately 1 inch) apart.

We will provide you with an informational take-home sheet that addresses care of the biopsy incision sites and will provide you with extra band-aids and topical antibiotic.

Supplementation:

After the first AET protocol you will ingest the appropriate supplement (1.25 g of betaine or placebo dissolved in 300 ml Gatorade) twice a day for 2 weeks after which you will perform a second AET protocol. After a 2 week washout period, you will crossover to the other supplement (betaine or placebo) and complete another 2 week supplementation period surrounded by AET protocols. You will not know which supplement you are taking during either supplementation period.

You will be asked to complete a diet record for the 3 days prior to each AET. These records will include everything that you eat and drink during this time period. You will be asked to replicate the diet you consume for the 3 days prior to the first AET for each subsequent AET.

Additionally, you must refrain from resistance training and / or exhaustive exercise, alcohol and caffeine during the 3 day period prior to each AET.

4. Risks and Inconveniences

Resistance Exercise

The performance of strenuous exercise may place you at risk for injury from over-exertion and/or accident. This study was designed to minimize the risk for injury. Additionally, we will use screening, selecting, and monitoring procedures that are designed to anticipate and exclude the rare individual for whom exercise might be harmful. However, for certain individuals, various underlying diseases may escape clinical detection. Therefore, strenuous exercise may be hazardous or may precipitate disability. Some disorders, such as disease/injury of the brain or lung circulation are rare and not readily diagnosed in the absence of symptoms; for these a history of tolerance to prior strenuous exercise must suffice. For other conditions, which might be more common, such as heart disease, the striking age-related incidence and the association of several identifiable risk factors with latent disease provides a rationale for a directed screening of certain subject candidates.

Performing lower-body resistance exercises such as the isometric squat and the box lifting exercise may cause back pain and, in rare instances, back injury. Additional risks associated with the exercises in this study involve the possibility of muscle strains or pulls of the involved musculature, delayed muscle soreness 24 to 48 hours after exercise, muscle spasm, and in extremely rare instances, muscle tears. Such risks are very low. Every effort will be made to make this investigation safe for your participation through subject familiarization, experienced testing personnel, warm-up and cool-down (i.e., stretching and low intensity activity-specific exercise), technique instruction and practice,

supervision, screening, monitoring and individualized exercise testing. All of these factors, including those previously outlined, should dramatically contribute to a reduction, if not an elimination, of any potential risks associated with this study. All of the laboratory research assistants are trained in CPR, and we have documented emergency procedures.

Blood Draws

We will take blood samples from a small, flexible tube (catheter) which will be inserted in a vein in your arm. There will be discomfort when the catheter initially punctures your skin. There is a small chance that you might develop a bruise at the site of the puncture and/or become dizzy / faint. Additionally, it is possible that an infection might occur; however, this is extremely rare. Insertion of the catheter will be done by a skilled technician using sterile techniques.

During Visits 2-5, we will draw about 1.2 table spoons (18 ml) of blood at each blood draw for a total of 6 table spoons (90 ml) per visit and thus about 1½ cups (360 ml) of blood for the entire study. The total is about 2/3 of the amount of blood typically taken for a Red Cross blood donation. As a safety precaution, you should avoid donating blood 2 weeks before the start of this study through 2 weeks after the end of this study.

Muscle Biopsies

In order to examine the effects of resistance exercise on muscle production of hormones, it will be necessary to perform a muscle biopsy on the thigh muscle of your leg. This area of your leg contains no major neural or vascular structures. The muscle biopsy technique has been employed thousands of times with human volunteers. First, a small portion of your leg will be cleaned using an antiseptic (betadine). Then, the biopsy procedure is accomplished by numbing a small portion of skin (size of a nickel) and underlying muscle, making a small incision (1/4 inch), and inserting a large needle into the muscle. The sample taken from the muscle amounts to the size of ½ to 1 unpopped popcorn kernel. This procedure is likely to cause pain, and a brief period of pressure sensation is usually experienced. It is possible that you might experience an accumulation of blood in the muscle and/or under the skin. In some cases, stiffness in the muscle may occur following the procedure but should dissipate within 48 hours. Such an accumulation of blood usually disappears in 1-2 weeks. In addition, there is a slight risk of infection resulting from the incision, but precautions will be taken to minimize such a possibility. Rarely nerve damage and temporary or permanent loss of sensation may occur. You will check in on the day following the biopsies to report any discomfort and insure proper healing of the incision. Ordinarily, small scars remain after the incision site has healed. In 5-10% of dark-skinned persons, however, the wound may heal with a fairly thick and conspicuous scar. If for some reason you have any concern regarding the incision site, you will have a contact number to immediately deal with the situation.

All biopsies will be done by a trained investigator (Jeff Anderson, M.D.) Following the biopsies, the skin will be closed via suture by Dr. Anderson. You must report to the HPL on the day after the biopsy procedures so it can be confirmed that the incisions are healing properly. Additionally, if you have any concerns about the incisions on the days following the biopsy, you should contact the study investigator so that a follow-up appointment with Dr. Anderson can be scheduled.

Body composition analysis

Body composition analysis for DEXA may involve some health risk, since any radiation exposure, no matter how low, may cause abnormal changes in cells. The body continuously repairs these changes, but it is conceivable such exposure could cause a cancer. The total exposure for a whole body scan (0.6 mrem) is approximately 1/10 of that during a standard chest x-ray (~6 mrem). Thus, the radiation levels are extremely low and the health risk is minimal.

Supplementation

The betaine supplement has no known adverse effects at the dose used in this study. The placebo contains non-active substances and carbohydrates to give it the same taste and appearance as the supplement compound.

Maintenance Training

During each of the 14-day periods, you will also complete two standardized resistance training workouts, each separated by one week, in order to maintain your training status throughout the study.

Various Inconveniences:

The time requirement of this study is about 5 hours (about 1 hours for Visit 1 and 1 hour each Visits 2-5). Additionally, you will be asked to keep a detailed dietary record for 3 days, which may add a small amount to the time requirements of the study. You will also be asked to fast over-night (12-hours) on one occasion.

5. Benefits:

By participating in this study you will learn how to perform isometric bench and squat exercises, vertical jumps and box lifts with correct technique and learn your body composition. We will explain all of your individual data collected from this study to you and interpret them along with answering any questions you might have in relation to these so that you can gain a maximum amount of educational understanding and use of your collected data. In general, this study will provide you a better understanding of how a betaine supplement impacts your hormone levels in response to a bout of resistance exercise.

6. Economic Considerations:

You will receive \$1000 if you complete all procedures involved in this study: If subject fail to finish the study, payments will be prorated as follows:

\$10 for completing visit 1

\$100 for completing visit 1 and 2
\$200 for completing visit 1, 2 and 3
\$400 for completing visit 1, 2, 3 and 4
\$1000 for completing visits 1, 2, 3, 4 and 5.

7. Confidentiality

All of the data collected will remain confidential, and you will never be identified by name in any reporting of the results. Furthermore, the results will not be shared with any person outside of the investigation without your consent. The results for this study will be kept in locked cabinets under the supervision of Dr. Carl Maresh.

You should also know that the UConn Institutional Review Board (IRB) and the Office of Research Compliance may inspect study records as part of its auditing program, but these reviews will only focus on the researchers and not on your responses or involvement. The IRB is a group of people that reviews research studies to protect the rights and welfare of participants

8. In Case of Illness or Injury

In the event you become sick or injured during the course of the research study, immediately notify the primary investigator, Dr. Carl M. Maresh (office 860-486-5322). If you require medical care for such sickness or injury, your care will be billed to you or to your insurance company in the same manner as your other medical needs are addressed.

If, however, you believe that your illness or injury directly resulted from the research procedures of this study, you may be eligible to file a claim with the State of Connecticut Office of Claims Commissioner. For a description of this process, contact the Office of Research Compliance at the University of Connecticut at 860-486-8802.

9. Voluntary Participation

You do not have to be in this study if you do not want to participate. If you agree to be in the study, but later change your mind, you may drop out at any time. There are no penalties or consequences of any kind if you decide that you do not want to participate.

10. Do You Have Any Questions?

Take as long as you like before you make a decision. We will be happy to answer any question you have about this study. If you have further questions about this project or if you have a research-related problem, you may contact the principle investigator (Dr. Carl M. Maresh, Ph.D., 860-486-5322). If you have any questions concerning your rights as a research subject, you may contact the University of Connecticut Institutional Review Board (IRB) at 860-486-8802.

Authorization:

I have read this form and decided that _____ will
(name of subject)

participate in the project described above. Its general purposes, the particulars of involvement and possible hazards and inconveniences have been explained to my satisfaction. My signature also indicates that I have received a copy of this consent form.

Signature:_____ Date:_____

Signature of Primary Investigator Date

or

Signature of Person Obtaining Consent Date

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