Conjugated Linoleic Acid Combined With Creatine Monohydrate and Whey Protein Supplementation during Strength Training

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By

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ABSTRACT

The purpose of this thesis was to determine the combined effects of protein, creatine, and conjugated linoleic acid (CLA) supplementation during resistance training. These nutritional supplements are popular during resistance training and we wanted to determine if they would have additive effects for improving body composition and strength. Forty-four participants (32 males, 12 females, mean age 20y) were randomized into three groups to receive: 1) 36 g/d protein (PRO), 2) protein and 9 g/d creatine (PRO/CR), or 3) protein, creatine and 6 g/d CLA (PRO/CR/CLA) for 5 weeks while resistance training on a four-day cycle (three days of resistance training, followed by one day of rest). Measurements at pre- and post-testing included body composition, muscle thickness of the elbow and knee flexors and extensors, and bench and leg press strength. There were time main effects ($p<0.01$) for strength, and muscle thickness. The PRO/CR/CLA group had significant increases in knee extensor muscle thickness over time compared to the other groups ($p<0.05$). There were no other differences between groups over time. The combinations of creatine and protein, or creatine, protein and CLA had no effects on body composition. It is concluded that combining protein, creatine, and CLA has minimal effects on muscular strength, muscle thickness, and body composition.

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LIST OF ABREVIATIONS

1-RM: One repetition maximum

AP: Air-displacement plethysmography

ADP: Adenosine diphosphate

ATP: Adenosine triphosphate

CLA: Conjugated linoleic acid

DEXA: Dual-energy x-ray absorptiometry

MRI: Magnetic resonance imaging

CHAPTER 1

REVIEW OF LITERATURE

1.1 Introduction

Protein, creatine, and conjugated linoleic acid (CLA) are naturally occurring nutritional components individually shown to improve strength and body composition when exercising. Taken in combination, protein and creatine have an additive effect (Burke et al., 2001; Cribb et al., 2007), while creatine and CLA in combination have also shown potential (Tarnopolsky et al., 2007).

Protein consumption in combination with exercise is important for muscle growth. Exercise increases the breakdown and rebuilding of muscle protein (Biolo et al., 1995a; Phillips et al., 1997; Phillips et al., 1999; Wolfe et al., 2000). Exercise also increases protein uptake into the muscle, thus it is beneficial to consume protein close to an exercise session to effectively rebuild muscle. When protein is consumed in close proximity to exercise while training, improvements over a placebo have been found to muscle protein synthesis rates, strength, and body composition (Anderson et al., 2005; Burke et al., 2001; Esmarck et al., 2001; Tipton et al., 2001).

Creatine is a natural nitrogenous amine found in red meats and fish, and produced by the liver in humans. In the muscle, creatine combines with phosphate to form phosphocreatine and is involved in re-synthesis of ATP. With creatine supplementation, muscle levels of creatine and phosphocreatine increase

(Greenhaff et al., 1994; Harris et al., 1992; Hultman et al., 1996; Willoughby et al., 2001). As a result, resynthesis of ATP during muscle contraction is improved, allowing a greater volume of work performed (Chrusch et al., 2001). Consuming creatine while exercising improves body composition and strength (Becque et al., 2000; Chilibeck et al., 2004; Cribb and Hayes, 2006; Kreider et al., 1998; Stone et al., 1999; Vandenberghe et al., 1997; Volek et al., 1999; Willoughby et al., 2001).

The biologically active isomers of CLA are found naturally in dairy products and meat of ruminants (Chin et al., 1994). Research on CLA in humans has increased after it was shown to significantly improve body composition (i.e. increased lean tissue and decreased fat mass) in animals (Akahoshi et al., 2002; Azain et al., 2000; Bhattacharya et al, 2005; Bhattacharya et al, 2006; Koba et al., 2002; Park et al., 1997; Park et al., 1999b; Poulos et al., 2001; Simon et al, 2005; Stangl et al., 2000; Takahashi et al., 2003, Terpstra et al., 2002; West et al; 2000). CLA's influence on body composition and strength in human subjects has showed varied results. CLA has improved body composition and strength when taken while exercising (Pinkoski et al., 2006; Thom et al., 2001), and body composition without exercise (Blankson et al., 2000; Gaullier et al., 2007; Mougios et al., 2001; Smedman et al., 2001; Watras et al., 2006). Other research has also shown CLA to have no influence on body composition or strength (Kreider et al., 2002; Nazare et al., 2007; Whigham et al., 2004; Zambell et al., 2000).

When consumed together, protein and creatine have increased strength and lean tissue mass to a greater extent than protein alone (Burke et al., 2001).

Creatine and CLA, taken together while exercising, has also improved lean tissue mass and isokinetic strength more so than a placebo (Tarnopolsky et al., 2007), however it is unknown if the results were due to one or both supplements. The purpose of the present study was to determine if the addition of CLA to protein and creatine would further improve strength and body composition in participants involved in a five week prescribed resistance training program. These nutritional supplements are popular during resistance training and we wanted to determine if they would have additive effects for improving body composition and strength.

1.2 Protein Supplementation and Exercise

The difference between muscle protein breakdown and synthesis is referred to as protein balance. Factors determining the quantity of breakdown and synthesis resulting from exercise are important to consider for maximal gains in lean tissue mass. A negative balance results in a loss of muscle mass, while greater synthesis than breakdown increases muscle mass. Important factors affecting protein balance include nutrient intake, timing of the nutrient consumption, and exercise. These factors will now be looked at in more depth.

Exercise and Muscle Protein Balance: Exercise affects muscle protein balance by increasing both synthesis and breakdown. After exercise, Biolo et al. (1995a) found a 51% increase in protein breakdown, while synthesis increased by 108%, leaving a positive net balance. After exercise, participants had an increase in

amino acid uptake into the muscle, a possible reason for the relatively larger synthesis. Another study looked at the effects of resistance training on protein balance at 3, 24 and 48 hours post-exercise. Protein synthesis increased by 112%, 65%, and 34% at these times, while breakdown increased 31% initially, dropped to 18%, and returned to normal levels by 48 hours (Phillips et al., 1997). These studies provide evidence of increased muscle protein turnover following exercise for up to 48 hours, partially caused by an increased uptake of amino acids into the muscle.

Another study measured the amount of protein synthesis and breakdown occurring after twelve participants performed resistance training exercise with one leg (Phillips et al., 1999). Within four hours of pleiometric exercise, there was significantly greater protein synthesis in the exercised legs of all individuals when compared to their unexercised leg. Additionally, participants with previous resistance training had no increase in muscle protein breakdown, while those with no resistance training experience had a significant increase in muscle protein breakdown. In summary, an isolated bout of exercise stimulated protein synthesis, while prior exercise training decreased the amount of protein breakdown occurring in the muscle. The current thesis study will therefore recruit individuals with previous resistance training experience.

Nutrition and Protein Balance: It is important for the body to have available protein in the muscle to promote synthesis. Protein is made available from the

muscle breakdown occurring following exercise; however, an external source is required as a portion of this protein is oxidized leaving the body in a negative balance (Biolo et al., 1995b). Additionally, as previously reported, individuals already performing a resistance training program had lower levels of muscle protein breakdown (Phillips et al., 1999), increasing the need for an external source of protein for synthesis. Exercise alone was shown to cause a negative protein balance in the muscles of individuals in a previously balanced state for two weeks (Gontzea et al., 1975).

Protein consumption increases amino acid concentrations in the muscle, providing an available source for synthesis. After consumption, the inflow of amino acids into the bloodstream is increased (arterial concentration × blood flow), stimulating inward transport of amino acids into the muscle (Biolo et al., 1995a). The type of amino acid consumed may also be important. Smith et al. (1998) flooded the bloodstream with amino acids (via injection) and found circulating essential amino acids, and not non-essential amino acids, were incorporated into the muscle protein at a significantly higher rate than normal ($P<0.01$). The current thesis study will use a whey protein supplement. Whey contains a high quantity of essential amino acids (Cribb et al., 2007), and has been shown to stimulate protein synthesis to a greater extent than other protein sources (Lands et al., 1999).

Exercise and Protein Supplementation: Both exercise and protein intake cause increases in protein synthesis (Biolo et al., 1995a; Phillips et al., 1997; Phillips et al., 1999; Wolfe et al., 2000). Protein consumption also increases amino acid uptake into the muscle (Biolo et al., 1995a). As a result, protein requirements are greater following exercise, and properly timing protein consumption after exercise has the potential to cumulatively increase protein synthesis.

One of the first studies to explore protein requirements when exercising found the amount of protein intake necessary for net protein balance was greater than the recommended daily allowance of 0.8g/kg/day (Meredith et al., 1989). Foundations of protein intake and nitrogen balance were challenged and established by Chittenden (1904) in his historical research, finding the required amounts of protein intake were approximately half of the, at the time, recommended amount of 118 g/day. By analyzing the muscle protein balance of participants taking various quantities of protein supplements following exercise, researchers found the required amount for a net balance to be 0.94g/kg/day. Biolo et al. (1997) investigated the effects of amino acid mixture infusion at rest, or following a single bout of resistance training. The effect was analyzed in the muscle biopsies and blood samples of six participants. Intravenous infusions caused similar increases in arterial amino acid concentrations in both groups, however, blood flow following exercise increased by 64%, increasing amino acid transport 30-100%. Additionally, muscle protein synthesis increased in both groups over time, yet the exercise group saw significantly larger increases (291 +/-

42% vs. 141 +/- 45%). These results provide evidence to support previous findings of increased protein synthesis following protein consumption.

Also looking at muscle protein synthesis following exercise, six participants completed a three-stage crossover design study (Tipton et al., 1999). Each stage had participants consume a different, randomly assigned formula after completing an exercise routine. Formulas included a mixed amino acid solution, an essential amino acid solution, and a placebo. Both amino acid supplements caused positive net protein balances, while the placebo resulted in a negative balance. The researchers noted adding non-essential amino acids is not necessary, as no additive effects were found. Similar results were found in participants consuming a protein/carbohydrate supplement following exercise (Rasmussen et al., 2000). Using a cross-over design, researchers looked at the effect of protein or placebo consumption one and three hours post-exercise on muscle protein synthesis. Measures were taken in six participants through tracers in blood samples and muscle biopsies. The protein/carbohydrate drink, taken at either time, caused significantly greater increases ($P<0.05$) in muscle protein synthesis over time, when compared to the placebo. In each of these studies muscle protein synthesis increased and resulted in a positive net protein balance when amino acids or protein were given shortly after exercise.

A longer duration study combining protein consumption with exercise, and involving participants with previous resistance training experience was done by Burke et al. (2001). Three groups were compared, consuming a protein

supplement, a protein and creatine supplement, or a placebo. The study was six weeks in duration, and involved 42 males, tested pre- and post-exercise intervention on strength and body composition measures. When compared to the placebo, protein alone caused significant increases ($p < 0.05$) in squat strength, knee flexion peak torque, and lean tissue mass. Protein and creatine combined caused additional increases ($p < 0.05$) in lean tissue mass and bench press compared to the protein group. These findings will be discussed in greater depth when creatine is reviewed. The increases in lean tissue mass and strength after six weeks validates the shorter duration protocol used in the present research.

Timing Protein Consumption with Exercise: Timing of protein intake is an important factor to consider when exercising. As previously reported, muscle protein synthesis increases immediately following exercise (Phillips et al., 1997). Synthesis remains elevated for up to 48 hours post exercise. Amino acid consumption increases protein synthesis independent of exercise, with the combination providing the greatest increases (Biolo et al., 1997). Research has looked into various factors concerning timing protein consumption relative to exercise.

In a 12-week long study with elderly men (average age of 74 +/- 1 year), effects of protein supplementation was compared when taken immediately, or two hours following exercise (Esmarck et al., 2001). As measured by muscle biopsies and MRI, cross sectional area and fiber area of the quadriceps increased

significantly if protein was taken immediately after, but not two hours post-exercise. Dynamic and isokinetic strength improved significantly ($p < 0.05$) by 46% and 15% when protein was taken immediately after exercise. Dynamic strength also increased when protein was taken two hours post-exercise (36%). It was concluded that immediate protein supplementation following exercise was best for hypertrophy and for increasing strength.

Tipton et al. (2001) compared pre- and post-exercise amino acid consumption. Following a crossover design, six participants took part in two trials in random order. The trials involved taking either a pre-exercise carbohydrate/essential amino acid supplement and a post-exercise placebo, or a pre-exercise placebo and a post-exercise carbohydrate/essential amino acid supplement. The exercise routine was described as an "intense leg resistance exercise bout". Prior to exercise, and again after completion, muscle biopsies and blood were taken from each participant. Amino acid delivery to the muscle was significantly higher in the group taking protein prior to exercise ($p < 0.05$), and subsequently muscle protein synthesis was greater. In a more recent study, Tipton et al. (2007) did a similar study using whey protein alone. There were no differences between groups taking the supplement before and after exercise. They reported being unsure how to explain the different responses between whey protein and the carbohydrate/essential amino acid supplements. Over a twelve-week study, Candow et al. (2006) found muscle thickness of the knee extensors to increase to a greater extent in men consuming protein before exercise than consuming protein

after exercise or placebo; however, there were no differences in lean tissue mass, other measures of muscle thickness and strength between participants across the different groups.

Anderson et al. (2005) also studied timing of protein consumption when exercising for fourteen weeks. Participants consumed protein or a carbohydrate placebo before and after exercise, and in the morning of a non-training day. Compared to baseline, only the protein group saw hypertrophy of both type I (18 +/- 5%, $p < 0.01$) and type II (26 +/- 5%, $p < 0.01$) muscle fibers; they also had significant improvements in squat jump height (9 +/- 2%, $p < 0.01$). The authors concluded protein consumption, before and after exercise, would be advantageous to those training for muscle hypertrophy, however only minor improvements would be expected to mechanical muscle function. Cribb and Hayes (2006) showed consuming protein and creatine before and after exercise led to significant improvements in body composition and strength over 10 weeks. They also had significantly greater increases in lean tissue mass and 1-repetition maximal squat and bench press tests compared to participants who performed the same exercise program, but instead consumed protein and creatine in the morning and evening. This provides further evidence of the importance of timing consumption relative to exercise.

The preceding research provides evidence that protein consumption and exercise both trigger protein synthesis. Protein consumption increases muscle protein stores, and when taken at specific times relative to exercise, can further

enhance delivery and synthesis. Lean tissue mass and strength gains are greatest when protein is consumed both before and immediately following exercise. In the current thesis study, participants will be required to consume supplements before and after exercise training to maximize the effectiveness of the supplements.

1.3 Creatine Supplementation and Exercise

Creatine monohydrate is involved in the synthesis of phosphocreatine, an important compound involved in the resynthesis of adenosine triphosphate (ATP) in the anaerobic alactic energy system (Persky et al., 2001). Supplementing a diet with creatine can theoretically allow muscle to perform a larger volume of work as a result of increasing phosphocreatine levels. As ATP is used, phosphocreatine replenishes stores by donating a phosphate ion to ADP. Increased stores allow greater recovery, thus a greater volume of resistance training can be achieved (Chrusch et al., 2001).

When increasing exercise volume, muscle protein breakdown and synthesis also increase. Adequate protein intake is then important to ensure its availability in the muscle when needed for synthesis (Biolo et al., 1995a). Evidence for the effectiveness of combining creatine and protein was shown by Burke et al., (2001), as the combination produced larger gains in lean tissue mass and one-repetition maximum bench press than protein alone. Likewise, combining creatine with protein is more effective for increasing lean tissue mass and strength than consuming creatine alone (Candow et al. 2008). Research shows evidence for

greater increases in lean tissue mass and strength measures when supplementing with creatine, however some is equivocal. Evidence supporting the inclusion of creatine in the current research will now be discussed.

Creatine Availability: Creatine is a natural nitrogenous amine found in red meats and fish, and produced by the liver in humans. Creatine is held in the human body in two forms, as free creatine, or as phosphocreatine (creatine attached to a phosphate ion). Important for high intensity exercise, phosphocreatine donates its phosphate ion to ADP to rapidly re-synthesize ATP. Skeletal muscle, which holds close to 95% of total body creatine, supplies enough ATP to endure approximately ten seconds of high intensity exercise at one time (Balsom et al., 1992). Consuming a creatine supplement increases free creatine and phosphocreatine levels in the muscle (Greenhaff et al., 1994; Harris et al., 1992; Hultman et al., 1996; Willoughby et al., 2001), potentially allowing for an increase in both ATP re-synthesis and volume of work performed.

Harris and associates (1992) studied the effects of supplementing with various doses of creatine monohydrate on blood plasma and skeletal muscle creatine concentrations. One gram of creatine had little effect on increasing muscle concentrations. When five grams of creatine were consumed, significant increases in creatine concentrations in the plasma and muscle (quadriceps femoris) were found; in one case an increase of 50% was found. Researchers found at least 20%

of absorbed creatine was converted to phosphocreatine in the muscle, allowing increased potential for ATP re-synthesis.

Hultman et al. (1996) explored two approaches of creatine supplementation for raising total muscle creatine levels. One group consumed a loading phase dose of 20g/day for six days, then maintained elevated muscle creatine concentrations by supplementing with 2g/day for the remaining 24 days. Another group consumed 3g/day for all 30 days of testing. Both approaches raised muscle creatine levels by approximately 20% after 30 days, though maximal levels were achieved after six days by consuming the loading dose, and after 28 days in those consuming 3g/day. The creatine levels returned to baseline thirty days after supplementing was stopped. Another study found average increases of 59% in serum creatine levels after 12 weeks of supplementing 6g/day (Willoughby et al., 2001). Significant differences between creatine and placebo groups were observed after two weeks, and continued to be present until completion. Because lower doses of creatine are effective over the long-term, the current thesis study will use a lower dose of creatine supplementation.

Creatine supplementation has the potential to increase muscle creatine levels, and phosphocreatine resynthesis after exercise (Greenhaff et al., 1994). Prior to consuming the supplement, the vastus lateralis muscles of eight participants were stimulated into isometric contractions. Muscle biopsies were taken immediately following, then again after 20, 60 and 120 seconds. The same tests were again performed after 10 days; the participants consumed 20 g/day of creatine for the

five days prior to testing. The rate of phosphocreatine re-synthesis was improved after the creatine supplementation. Increases in muscle creatine and phosphocreatine levels may contribute to positive training adaptations found with creatine supplementation. These positive training adaptations have been well documented and will be presented in the following section.

Creatine Supplementation and Exercise: This review will discuss creatine supplementation and the effect on body composition and strength measures. The majority of research chosen focuses on human participants performing short duration exercise, with preference given to strength training exercise. Creatine's effectiveness has been shown in a variety of populations, including males, females, and high level athletes (Becque et al., 2000; Kreider et al., 1998; Stone et al., 1999; Vandenberghe et al., 1997). Creatine caused greater increases of lean tissue mass and strength measures in males after six weeks (Becque et al., 2000), and females after ten weeks (Vandenberghe et al., 1997). Two studies explored effects of creatine taken during off-season training in Division 1 NCAA football players. After 28 days, participants supplementing with creatine significantly increased lean tissue mass, bench press, and repeated sprint performances (Kreider et al., 1998). Similar results were found by Stone et al. (1999), whose participants increased body mass, lean body mass, 1-RM bench press, and static vertical jump with creatine consumption for five weeks. Various quantities of creatine were consumed in these studies, ranging from a loading

phase followed by 2 grams/day to 20 g/day throughout the study. In addition, improvements in body composition and strength were seen in as little as four weeks, supporting the use of a shorter duration of training in the present research.

Another study, by Burke et al., 2000, showed participants consuming 7.7 g/day of creatine for 21 days were able to perform more total work, showed larger peak force and power improvements, and were able to maintain mean peak power for a longer duration than the placebo group. These results provided a basis of evidence from which it was decided to assess active male and female participants using a relatively low dose of creatine in this thesis study.

The increases in lean tissue mass with creatine supplementation may be due to an increase in muscle fiber size. After 12 weeks of creatine supplementation participants had significantly greater increases in area of type I, IIa, and IIb muscle fibers than placebo while following a periodized exercise program (Volek et al., 1999). Creatine also increased the volume of bench press performed during weeks five to eight; therefore it was concluded that the increases in fiber areas were likely a result of higher quality training sessions. Willoughby et al. (2001) found a group taking creatine (6g/day), while following a weight training program for twelve weeks, had significantly greater increases in body mass, lean tissue mass, thigh volume and muscle strength than the placebo. They also looked at participant muscle biopsies to gather information on type I, IIa and IIx myosin heavy chain mRNA and protein expression. After twelve weeks, Type I and IIx protein expression was greatest in the creatine group, while Type IIa was equal

between creatine and protein groups. Myosin heavy chain mRNA expression was greatest in the creatine group for all measures. In 2003, Willoughby and associates used the muscle biopsies collected in 2001 to further look at mRNA and protein expression of various myogenic regulatory factors. The combination of creatine and exercise significantly increased the muscle creatine kinase mRNA and protein expression of myogenin and myogenic regulatory factor-4. This was reported as a possible factor involved in increasing heavy chain expression.

As with protein supplementation, the timing of creatine supplementation may be important. Chilibeck et al., (2004) compared the effects of creatine supplementation to a placebo after single limb training. Participants were randomly divided into two groups, consuming creatine or placebo for six weeks. Participants trained four days per week, devoting two days to each side of the body. In the creatine group, creatine supplements were consumed after training one side of the body, and a placebo after the other; the placebo group consumed the placebo after training either side. After six weeks, elbow flexor muscle thickness was significantly increased on the side of the body following exercise with creatine. Creatine also caused significantly greater lean tissue mass gains in males than females. These findings substantiate previous findings of creatine's benefit while exercising, and show the importance of timing consumption relative to exercise. Further evidence of timing of creatine consumption was provided by Cribb and Hayes (2006). These researchers found timing protein and creatine consumption immediately before and after exercise more effectively increased

lean tissue mass and 1-RM squat and bench press, than consuming the same supplement in the morning and evening. This evidence justifies having participants in our study consume supplements in close proximity to performing their training program.

1.4 Conjugated Linoleic Acid Supplementation and Exercise

Conjugated linoleic acids (CLA) are a group of linoleic acid isomers containing conjugated double bonds. Linoleic acid is an 18 carbon chain fatty acid containing two double bonds, found at the ninth and twelfth carbons in the chain (cis-9 and cis-12). It is also defined as an n-6 (omega-6) fatty acid, as the first double bond from the omega end of the chemical structure occurs at the sixth carbon position. Conjugated double bonds are notable in structure as "double bonds separated in a molecule by only one single bond" (Beare-Rogers et al., 2001). Noted for their biologically activity, the cis-9,trans-11 (c9,t11) and trans-10,cis-12 (t10,c12) isomers of CLA appear frequently in research (Pariza et al., 2000).

The biologically active isomers are found naturally in dairy products and meat of ruminants, created as an intermediate during the biohydrogenation of linoleic acid by the rumen bacteria (Chin et al., 1994). The c9,t11 isomer is the primary CLA isomer in milk fat (Sehat et al., 1998), and has been named rumenic acid. CLA isomers can be created by other methods, and in other animals, as seen in the sera from a horse, which contained both active forms of CLA (Park and

Pariza, 1998). The two active isomers have different roles in their influence on body composition. When compared, the t10,c12 isomer is effective for reducing body fat in mice, possibly by reducing the uptake of lipids (Park et al., 1999a); whereas, Pariza et al. (2001) reported the c9,t11 isomer influenced growth and feed efficiency.

Interest in CLA began after it was shown to reduce cancer incidence (Ha et al., 1987). Researchers reported that mice treated with CLA, when compared to a control group, developed half as many tumors after sixteen weeks of exposure to a tumor promoting compound, 12-0-tetradecanoylphorbol-13-acetate. Both active isomers are reported to be involved in the inhibition of carcinogenesis (Pariza et al., 2001). Many studies have since explored the health benefits of CLA, including the potential to influence changes to body composition. Research exploring CLA's influence on body composition began with animal studies, while studies involving human participants have increased in recent years.

Conjugated Linoleic Acid and Body Composition in Animals. Many studies using mice and rats have found CLA to significantly decrease body fat mass, while also increasing lean tissue mass (Akahoshi et al., 2002; Azain et al., 2000; Bhattacharya et al, 2005; Bhattacharya et al, 2006; Koba et al., 2002; Park et al., 1997; Park et al., 1999b; Poulos et al., 2001; Simon et al, 2005; Stangl et al., 2000; Takahashi et al., 2003, Terpstra et al., 2002; West et al; 2000).

The first details of CLA's potential influence on body composition were reported by Chin et al. (1994). Researchers performed experiments to explore CLA's influence on growth and development in "pup" rats. The newborn rats were divided into three groups, consuming a control diet, or one containing 0.25% or 0.5% CLA. The 0.5% CLA diet produced the greatest increases in body mass after eight weeks. Significant differences in body mass between groups began after 10 days and remained until completion. There were no differences in caloric intake between groups throughout the study, thus gains in feed efficiency, i.e. weight gained per calorie consumed, were also reported. The suggested reasoning for the improved feed efficiency was a decrease in skeletal muscle catabolism due to CLA's influence on immune stimulation. The authors stated immune stimulation is associated with muscle catabolism, which is decreased by CLA, as found in past research (Cook et al. 1993, Miller et al., 1994). Furthermore, they reported muscle catabolism uses energy that could otherwise be used for growth. Thus by decreasing catabolism, energy can instead be used for growth, improving feed efficiency. Park et al. (1997) were the first to report the effect of CLA on body composition. Mice were fed a mixture of c9,t11 (50%) and t10,c12 (50%) CLA, which resulted in decreased fat mass and an increased lean tissue mass. The mice in the CLA group also ate a significantly smaller quantity of feed compared to the control group.

The same researchers later found increases in whole body protein prior to the reduction in body fat mass (Park et al., 1999b). Mice were fed a control diet,

or one supplemented with 0.5% CLA mixture containing a majority of c9,t11 or t10,c12 isomers. The increase in whole body protein was present within the first week, while the decrease in body fat mass did not occur until after the third week. The increase to whole body protein was identified as a potential factor in the decrease of body fat mass, because the majority of fat utilization occurred within the skeletal muscle. In a second experiment, performed later within the same study, the mice continued to have a consistently higher whole body protein and lower body fat up to four weeks after cessation of CLA supplementation (Park et al., 1999b). They concluded the lasting effects were due to CLA's influence on metabolism.

CLA impacts metabolism in mice, which increase metabolic rate in conjunction with reduction in fat stores with CLA treatment (Terpstra et al. 2002; West et al. 2000). A possible mechanism to explain CLA's role in increasing metabolism is its role in stimulating uncoupling protein expression in adipose tissue, which, in effect, would increase the metabolic rate (Choi et al., 2004; Roche et al., 2002; Warren et al., 2003).

Lean tissue mass gains from CLA supplementation have been attributed to a decrease in protein catabolism (Bhattacharya et al., 2005; Miller et al., 1994). Changes to lean tissue mass result from protein turnover, a term used to describe the breakdown and rebuilding of muscle protein. For gains to lean tissue mass, rebuilding of muscle protein (protein synthesis) must be greater than the muscle breakdown (protein catabolism). In theory, if protein catabolism is decreased

while protein synthesis remains unchanged, lean tissue mass would increase. Miller et al. (1994) found CLA to prevent loss of mass in mice, rats, and chicks following endotoxin injection, meant to inhibit growth. Groups taking a basal diet or fish oil supplement lost twice as much body weight as the group taking CLA. In a study involving CLA supplementation and exercise with mice, neither exercise nor CLA independently caused significant changes in lean tissue mass (Bhattacharya et al., 2005). However, when CLA was consumed in addition to an exercise program, the mice experienced a significant increase in lean tissue ($p<0.05$). The finding of greater lean tissue mass gains when CLA was combined with exercise supports the inclusion of a strength training program in the current study.

Conjugated Linoleic Acid and Body Composition in Humans. CLA became a popular supplement to study in humans after the success in animal models. To date, there is limited research in humans pertaining to body composition. Results of the currently available research has been somewhat varied.

One of the first studies to explore CLA's effect on body composition in humans was done by Blankson et al. (2000). Forty-seven overweight or obese participants were studied for 12 weeks to investigate the effect of supplementing with different doses of CLA against a placebo (olive oil). The CLA supplement was noted to be made up of the active isomers c9,t11 and t10,c12, equally divided. CLA was ingested in doses of 1.7g, 3.4g, 5.1g, and 6.8g, while 9g of the placebo

was consumed by the control group. The participants in each of the CLA groups had a significant decrease in body fat mass compared to the placebo group, however no additional benefits were found when supplementing in doses greater than 3.4g/day. No significant changes to lean tissue mass were found. Also comparing effects of doses of CLA, Mougios et al. (2001) provided twenty-two participants, divided into two study groups, with CLA or a placebo for eight weeks. CLA, equally composed of c9,t11 and t10,c12, was consumed in quantities of 0.7g/day for the first four weeks, and then 1.4g/day for the final four weeks. The study design provided participants with a dietary plan to follow; a dietary analysis noted no differences in energy consumption between groups. Measurements were taken prior to beginning supplementation, after four weeks, and again after completion. Participants taking 0.7g/day of CLA for four weeks, followed by 1.4g/day for four weeks, experienced a significantly greater reduction in body fat mass, sum of 10 skinfolds, and body fat percentage (calculated based on skinfolds). Consuming 0.7g/day of CLA for four weeks did not cause any significant effects to body composition; however CLA was significantly incorporated into serum lipids.

Smedman et al. (2001) divided 27 males and 26 females into two groups, which consumed either 4.2g/day of CLA or the equivalent amount of olive oil for 12 weeks. The CLA supplement, 85 % composed of equal amounts c9,t11 and t10,c12, was found to significantly decrease the proportion of body fat, most likely due to the reported increase in fatty acid metabolism. Another study, reporting a

limited sample size of 24 obese males, provided fourteen of the participants with 4.2g/day of CLA, which was 75% composed of the active isomers, equally divided. Following four weeks of supplementation, participants taking the CLA supplement had a significant decrease in their sagittal abdominal diameter (Riserus et al., 2001), which is the "distance between the anterior wall of the abdomen and back" (Anjana et al., 2004).

Watras et al. (2006) looked at CLA's potential to reduce weight gain during the winter holiday season. The study design saw forty overweight participants consume a CLA supplement, or placebo, for the six month period from August until the end of January. The CLA supplement was composed of 78% active isomers, divided equally, and was consumed in 3.2g/day quantities throughout the study; a placebo of safflower oil was used. Body weight was measured prior to supplementation, at the end of October, at the end of December (named the holiday months), and again in March. The majority of body composition testing was done at baseline and after six months. Participants consuming the placebo experienced significantly higher weight gain during the holiday months in comparison to the first three months studied. When compared to the CLA group, over the holiday months, the placebo group again gained a significantly larger amount of weight. The CLA group significantly decreased fat mass and body fat percentage over the six month period, and noted it was able to "prevent weight gain during the holiday season." Researchers also reported CLA was responsible

for causing significantly greater fat mass loss, and a decreased body mass index, in comparison to the placebo.

Gaullier and associates (2004, 2005) completed two continuous studies, each lasting 12 months, looking at the long term effects of CLA on body composition. In addition, they performed chemical analyses of blood serum and looked at adverse effect reports to evaluate the safety of the supplement. In the first study, researchers collected data from 180 participants, who were randomly assigned to one of three groups, providing participants with 4.5g/day of one of three supplement mixtures. One group received CLA (3.6g of active isomers, evenly divided) combined with a free fatty acid mixture, another received CLA (3.4g of active isomers, evenly divided) combined with a triacylglycerol mixture, and the third a placebo of olive oil. Both groups taking the CLA supplement experienced significantly greater decreases in body fat mass than the placebo group in tests performed at 6, 9 and 12 months (total losses of 1.7 +/- 3.0 kg and 2.4 +/- 3.0 kg, versus 0.2 +/- 3.3 kg). The CLA groups also had significant within-group increases in lean body mass after 12 months (0.6 +/- 2.0 kg and 0.7 +/- 1.8 kg versus 0.0 +/- 1.5 kg). In the follow-up study, 134 participants from the original study were given CLA for 12 additional months. Some of the significant findings included a decrease in cholesterol levels (plasma total and LDL), and a reduction in reported adverse effects. Participants who took the placebo in the previous study experienced a significant loss of body weight and body fat mass, while those who had previously taken CLA showed no further changes to body

composition. CLA was concluded to be a well tolerated supplement, able to reduce, and furthermore maintain, the loss of fat mass and body weight in overweight adults. Another study providing overweight and obese participants with a CLA supplement or placebo also reported a significant loss of fat mass when CLA was ingested for six months (-1.0 +/- 3.4 kg) (Gaullier et al., 2007). In this six-month study, participants supplementing with 4.5g/day of CLA oil, containing 75% active isomers equally divided, were studied for region-specific changes to body composition. CLA was found to cause weight loss in the leg region, and to a greater extent in females.

Despite the number of favorable findings, many have shown CLA to have no influence on body composition (Kreider et al., 2002; Nazare et al., 2007; Whigham et al., 2004; Zambell et al., 2000). These findings may have resulted from limitations in methodology, including small sample size, short treatment duration, or lack of CLA active isomer concentration. One such study looked at 17 participants, divided between two groups supplementing with CLA or a placebo for 64 days (Zambell et al., 2000). The CLA supplements used for this study were low in concentration of active isomers, containing 22.6% t10,c12 and 17.6% c9,t11. Another study, involving 50 participants, half taking a CLA supplement consisting concentrations of 37% of each of the active isomers, found no effect on body composition after 28 weeks of supplementation (Whigham et al., 2004). In this study, participants took CLA, or a placebo, in addition to following a low calorie diet. No differences were found between groups; however, any

changes to body composition resulting from CLA may have been masked by the low calorie diet, as participants lost between 10 and 20 percent of their initial body weight after twelve weeks. Nazare et al. (2007) used a sample population of forty-four healthy individuals. Active CLA isomers, each consisting of 35%, or a placebo were incorporated into yoghurt, and were consumed for 14 weeks, at 3.76g/day. No significant body composition changes were found, although the participants consuming CLA had significant increase in resting metabolic rate. The authors concluded the lack of body composition changes, despite having an increased resting metabolic rate, was likely due to the relatively short duration used in comparison to another study finding significant changes after one year (Gaullier et al., 2004).

The majority of research showing changes to body composition with CLA supplementation used a supplement composed of high concentrations of active CLA isomers, typically divided equally between the two. Another study, which used small quantities of active isomers of CLA, had participants engage in resistance training while supplementing (Kreider et al., 2002). Twenty-three male participants with previous weight training experience consumed 6g/day of CLA or 9 g/day of placebo for four weeks. The CLA, composed of 22.6% t10,c12 and 17.6% c9,t11, had no effect on body composition. This study had limitations due to the isomeric composition of the CLA supplement, a short duration, and a non-unified exercise program. Other studies of CLA in combination with exercise have shown greater benefits.

One of the first studies to look at the effects of CLA supplementation in combination with exercise was done by Thom et al. (2001). Twenty healthy participants (10 males and 10 females), took 1.8g/day of CLA or a placebo in combination with an exercise regimen. The participants were randomized into two groups, taking either 6 pills per day of CLA or a hydrogel placebo (two at breakfast, lunch and dinner). Body composition analysis was done through means of infrared light placed at the midpoint of the biceps. Researchers found a significantly lower body fat percentage in the CLA group at all testing times (4, 8 and 12 weeks), however the BMI and total body weight between groups did not differ; this could potentially be explained by a greater increase in lean body mass in the CLA group. Despite a lack of information on the exercise done by the participants, it can be concluded that CLA taken in low doses (1.8g/day) can reduce percent body fat, and is possibly aided by exercise. These improvements in body composition after 4 weeks supports the treatment duration used in the present research.

Pinkoski et al. (2006) investigated resistance training in combination with CLA supplementation. Seventy-six participants were randomized into two groups, receiving either CLA or a placebo, and followed a prescribed exercise routine. The supplement, composed of 67% active CLA isomers, evenly divided, was taken in 5g quantities per day. The study involved two phases, each lasting seven weeks, with the second following a crossover design. Following the first phase,

the participants consuming CLA had significant changes to their body composition in comparison to those taking the placebo. Changes included increased lean tissue mass, decreased fat mass, and a smaller amount of 3-methylhistidine in the urine (a marker of myofibrillar degradation). Seventeen of the participants proceeded to take part in a crossover study, consuming the opposite supplement as prior. This phase found participants, who previously took CLA and were now taking the placebo, to have an increase in body mass, fat mass, body fat percentage, and 3-methylhistidine. There were no changes in lean body mass in either group. The researchers concluded the CLA supplement had a small effect on body composition, and additionally caused a decrease in the muscle protein catabolism associated with resistance training, based on the findings of 3-methylhistidine.

A recent study, involving female participants, tested the combination of aerobic exercise with 3.6g/day of CLA for six weeks (Colakoglu et al., 2006). Fourty-four participants were divided into three groups, one of which combined CLA and exercise, another did not exercise but took CLA, while one exercised and consumed a placebo. CLA supplementation, in both groups, produced gains of lean tissue mass. A reduction of body weight was found in all groups, however changes were greatest in the exercise and CLA group, followed by the CLA-only group, and finally the exercise and placebo group. The researchers concluded that exercise and CLA could individually improve body composition, and had an additive effect when used in combination. These studies provide evidence for the advantages of supplementing a diet with CLA while exercising. Possible benefits

include greater gains in muscle protein turnover when resistance training, resulting from decreased protein catabolism, and increasing fat mass loss, possibly due to an increased resting metabolic rate. In additional, increases in lean tissue mass and strength after six weeks validates the shorter duration protocol used in the present research.

1.5 Combinations of supplements during resistance training

Creatine and Protein Supplementation with Exercise: The potential ergogenic effect of supplementing with both protein and creatine while exercising has been explored (Burke et al., 2001; Cribb et al., 2007).

Burke et al. (2001) was first to study the combination of protein and creatine, consumed while participants followed a prescribed resistance training program. Thirty-six males were divided into three groups, each given a different supplement combination. Groups received either protein and creatine, protein and a creatine placebo, or a placebo of both. After six weeks of resistance training, participants consuming both creatine and protein had significantly greater gains in lean tissue mass and bench press when compared to other groups. Knee extension peak torque was significantly higher in both groups consuming protein; no other differences were found between these groups.

Cribbs et al. (2007) had participants consume protein and creatine while following an eleven week resistance training program. Researchers used muscle

biopsies to examine muscle properties, including fiber type and cross-sectional area, contractile protein content, and creatine concentration. Strength and body composition measures were also taken. Thirty-three male recreational bodybuilders were divided into four groups, receiving either carbohydrates, whey protein, carbohydrates and creatine, or protein and creatine. Participants in groups consuming creatine and/or whey protein had significantly greater increases in muscle hypertrophy than those consuming carbohydrates. Increases were also found in squat, bench press, and pull down one-repetition maximal tests. A trend for creatine to increase myofibrillar protein content was found. More recently, Candow et al. (2008) found creatine and protein consumption, taken while exercising three days per week for ten weeks, could improve strength and body composition in elderly male participants. The low-dose of 0.1 g/kg body weight of creatine increased lean tissue mass, muscle thickness, and relative bench press strength, while also reducing protein breakdown compared to placebo.

Creatine and Conjugated Linoleic Acid Supplementation with Exercise: The effects of creatine combined with conjugated linoleic acid on strength and body composition were studied by Tarnolopolsky et al. (2007). Thirty-nine older adults (>65) performed resistance training exercises for six months while taking a dietary supplement combination of creatine and conjugated linoleic acid, or a placebo. In body composition tests, creatine and conjugated linoleic acid caused significant increases to lean body mass, while reducing fat mass. Lean tissue mass increased

significantly more in men than women. The supplement also increased isokinetic strength and muscular endurance significantly more than the placebo. Results cannot be attributed to one supplement, or the combination, due to the study design. The proposed research will further investigate the individual and combined effects of creatine and conjugated linoleic acid on body composition and strength.

1.6 Hypotheses

It is hypothesized that consuming protein, creatine, and CLA while exercising for five weeks will have the greatest influence on increasing lean tissue mass, muscle thickness, and strength, while also significantly decreasing fat mass, compared to consuming protein alone, or consuming creatine combined with protein. The effect of adding creatine to protein is hypothesized to influence these factors more so than protein alone.

CHAPTER 2

METHODS

2.1 Research Design

Male and female participants were randomly assigned to one of three supplement combination groups using a double blind study design: 1) whey protein and a placebo; 2) whey protein, creatine and a placebo; or 3) Whey protein, creatine and CLA. Participants in all groups performed a specified resistance-training program on their own time for a five week period while concurrently consuming the supplement as instructed. Before and after the resistance-training program, measurements were made of body mass, bench press strength, leg press strength, muscle thickness, fat mass, lean tissue mass, and caloric intake.

2.2 Participant Characteristics

Forty-four participants (12 females and 32 males), ranging in age from 18 to 32 volunteered for the study. Participants had previous strength training experience, an important inclusion criterion as the training program was completed on their own time. In addition, volunteers were not allowed to have taken creatine or CLA supplements in the month prior to participation, and were asked to not change their diet over the course of the study. Participants signed a consent form (Appendix B), and completed the Physical Activity Readiness Questionnaire (Appendix C), and were deemed to be healthy and have no pre-existing diseases.

2.3 Randomization and Supplementation

Participants were stratified by gender and then randomized to groups in a double blind fashion. A third party was responsible for the randomization of participants and the labeling of supplements to ensure everyone involved was blinded.

Quantities of protein and creatine provided to participants were based on those used in a previous study successfully finding increases in lean tissue mass and bench press strength (Burke et al., 2001; Esmarck et al., 2001). Participants received 36g/day of whey protein, and 9g/day creatine; those requiring a creatine placebo (protein-only group) were given a total of 45g/day of protein to equal the amount of nitrogen in the protein/creatine combination. Whey protein was used as it contains a high quantity of essential amino acids (Cribb et al., 2007), and has been shown to stimulate protein synthesis to a greater extent than other protein sources (Lands et al., 1999). Participants consumed 6g/day of CLA, or placebo (sunflower oil), based on a study successfully increasing lean tissue mass and decreasing fat mass in participants supplementing with metabolically active isoforms of CLA (cis-9, trans-11, and trans-10, cis-12) in combination with resistance training (Pinkoski et al., 2006). Weekly quantities of supplements, in powder form, were given to participants. They were instructed to mix the correct quantity of powder with water and consume at three specified times throughout the day (before workout, after workout and before bed). Timing protein and creatine

consumption immediately before and after exercise is an established strategy for maximizing supplement effectiveness (Cribb and Hayes, 2006). In all cases, a vanilla flavored supplement was used; the appearance and flavor of the three batches of supplements were identical.

2.4 Training Protocol

The resistance training program was similar to a previously studied protocol found to cause significant gains in muscular hypertrophy (Burke et al., 2001; Candow et al., 2001). Participants followed a repeating four-day training cycle over 5 weeks. The study was originally planned to extend for eight weeks, however due to complications with the supplement provider a five week program was adopted. Changes to body composition and strength have been found in research using durations of five weeks or less (Burke et al., 2000; Kreider et al., 1998; Stone et al., 1999; Thom et al., 2001). One cycle involved three days of training followed by one day of rest. The lifting intensities were set at 60-90% of the participant's pre-testing 1-RM, with participants performing a range of five to ten repetitions of an exercise in sets of three or four. For each set, participants selected an appropriate weight causing them to reach fatigue within the pre-determined range of repetitions, which are stated below.

Day one of the program involved exercises for the chest and triceps muscles. The specific exercises included bench press, incline bench press, flat bench dumb-bell flys, incline dumb-bell flys, cable triceps extensions, rope reverse triceps

extensions, and French curls (Candow, et al., 2001). Day two focused on the back and biceps, with exercises including chin-ups, low row, lat pull-downs, alternate dumb-bell row, standing EZ-curls, preacher curls, and alternate dumb-bell curls (Candow, et al., 2001). The third day focused on the leg, abdominal and shoulder muscles. The exercises on this day included squats, leg extensions, hamstring curls, standing calve raises, military dumb-bell press, upright row, shrugs, deltoid flys, and abdominal crunches (Candow, et al., 2001). On the fourth day, participants were instructed to rest from exercise; it was also the final day of the cycle.

The training program was divided into three blocks, consisting of three cycles each. During the first block, participants were instructed to perform exercises with repetitions of 10-12, in sets of four, with one minute of rest between sets. The second block progressed to four sets of 6-8 repetitions, with one and a half minutes of rest between sets. The third block again increased the strength component by moving to sets of three, with exercises repeated 4-5 times, and two minutes of rest between sets. The participants tracked their progress in a personal log book to ensure compliance to the program.

2.5 Measurements

2.5.1 Strength:

For the assessment of upper body strength, a one repetition maximal lift was performed using bench press Lever equipment (Pulse Fitness Systems; Winnipeg, Manitoba, Canada). Participants were instructed to place their backs against the bench while keeping their feet on the floor. Hands were placed on handles, approximately at shoulder width distance. The bar was then pushed away from the body to a maximal extension. A warm-up prior to the 1-RM attempt was used in which the participants performed 10 lifts at a comfortable weight. Two to four repetitions were then done at a higher weight, allowing participants and tester to gauge an appropriate starting point for the 1-RM attempt. After a short rest, participants selected a weight to do only once. If the attempt was successful, participants were given a three-minute rest and again attempted the 1-RM at a higher weight. This method was repeated until failure, with the last successful attempt recorded as their 1-RM.

The maximal strength of the lower body was tested on a bilateral leg press machine (Hammer Strength, Franklin Park, IL). This machine was adjusted for each participant's body; settings were recorded to ensure similar environments for pre- and post-testing. In this exercise, participants sat in the leg press machine and were instructed to keep their backs against the seat. They then placed their feet on the footpad, with knees bent at 90 degrees. Participants then pushed the weight

away from the body into knee extension without allowing the knees to lock. The warm-up and 1-RM testing were done in a similar fashion to the bench press protocol. The reliability of bench press and leg press have been measured (Chrusch et al., 2001), with intraclass correlation coefficients of 0.99 each on day to day measures.

2.5.2 Body Composition:

Body composition was assessed before and after the completion of the training program using whole-body dual-energy x-ray absorptiometry (DEXA). The DEXA is whole body scan that measures bone mineral free lean tissue mass, fat mass, and body fat percentage. Due to problems experienced with the DEXA during the course of pre-testing, air-displacement plethysmography (AP), using the BOD POD, was also employed to measure fat mass and lean tissue mass. Twenty participants were tested with the DEXA, while twenty-four with the BOD POD. Each participant was tested using the same method in pre- and post-testing.

The reliability of the DEXA was measured on 10 male participants (Chrusch et al., 2001) and was found to have a correlation coefficient (r) of 0.99 for both lean and fat tissue mass. The coefficients of variation were 0.54% for lean tissue mass and 2.95% for the body fat mass. Prior to having measurements taken, participants were instructed to avoid wearing any metal and to refrain from any intense exercise. A lab technician was hired to do all of the DEXA testing to ensure accuracy.

Air-displacement plethysmography (AP) was tested using the BOD POD, a two chamber method of measuring body composition. The BOD POD determines a subject's body volume, from which body fat and lean tissue percentage are calculated (McCrory, Mole, Gomez, Dewey, and Bernauer, 1998). The coefficient of variation (CV) of the BOD POD was tested to be 0.87% by Candow et al. (2008). Before the AP testing was done, participants were instructed to refrain from high intensity exercise, remove all jewelry, and wear specified clothing. Females were asked to wear a bathing suit and a swimming cap, while males wore spandex shorts and a swimming cap. Height and weight measurements were then taken and entered into the associated BOD POD computer software, which uses them in calculations to determine body fat percentage. The BOD POD was programmed to do two tests on each participant and produce an average reading of the results. If the software determined the two tests were not to be acceptably close, a third test was done, and an average was taken of the two closest results.

2.5.3 Muscle Thickness

Muscle thickness measurements were done on the elbow and knee flexors and extensors. The assessment was done using B-Mode ultrasound (Aloka SSD-500, Tokyo, Japan). Coefficients of variation were determined at each site by Candow et al. (2008) as follows: Elbow flexors (2.6%), elbow extensors (2.1%), knee flexors (2.3%), knee extensors (2.1%). To avoid any potential effects of muscle swelling following exercise, both the initial and final measurements were

done prior to any strength testing. In addition, final measures were done two days, at minimum, after completion of the resistance-training program for similar reasons. All measurements were done on the right side of the body.

Elbow flexors and extensors: Ultrasound testing of muscle thickness was done at the midpoint of the arm for the biceps and triceps. The midpoint was found by measuring the length from the acromion process to the olecranon process. A mark was made at the midpoint, and parallel marks were then placed on anterior and posterior points of the arm. A transparent sheet, precut in the middle to resemble the shape of the ultrasound probe, was placed over the referenced midpoints. The middle of the precut area was placed over the marked reference point, and an outline of the precut area was traced onto the participant's arm with a non-permanent marker. A permanent marker was then used to trace any obvious body landmarks (scars, moles, birthmarks) onto the transparent sheet. The tracings were stored and used during post-testing to ensure the participant's muscle thickness was taken at the exact same place on the arm.

For the elbow flexor measurement, participants were seated while having their right arm resting on a table in a supinated position. To measure elbow extensor muscle thickness, participants would stand with their arm hanging naturally and relaxed.

Prior to taking muscle thickness measurements, a gel was applied to the ultrasound probe. The probe was then placed in the traced area of the subject's

arm and gently pressed onto the skin until a distinct image could be seen on the ultrasound screen. This image was frozen on screen by the researcher for further evaluation. The thickness (cm) was measured at three points on the image (proximal, middle, and distal), and was recorded. This process was repeated two more times, for a total of three values at each of the three points (proximal, middle, and distal). The two closest measurements at each point were averaged for a representative value; in the case where the three values were equally separated, the middle value was used. Once there was a representative value for each of the three points (proximal, middle, and distal), they were averaged to give an overall value for the site.

Knee extensors and flexors: Ultrasound testing of muscle thickness was done at the midpoint of the leg for the knee extensors and flexors. The midpoint was found by measuring the length from the greater trochanter of the femur to the lateral condyle of the femur. A mark was made at the midpoint, and then parallel marks were placed on anterior and posterior points of the leg.

To measure knee extensors thickness, participants removed any clothing covering the testing site, then sat with the back of their knee joint at the edge of the table. They were instructed to relax their leg and allow it to hang naturally. For knee flexors thickness measurements, participants were instructed to lie on their stomach with their entire body on top of a table. Procedures used for lower body muscle thickness testing were the same as upper body, in terms of recording

landmarks, use of the ultrasound probe, performing multiple trials, and data recording

2.5.4 Dietary Assessment

To test if diet was a contributing factor to our results, we assessed caloric intake over a three day period for the participants, both before the study and near its completion. Each participant was given a booklet (Appendix D), and was asked to record all food and drink consumed over a period of two weekdays and one weekend day. The United States Department of Agriculture's (USDA) food intake assessment tool, entitled MyPyramid Tracker (www.mypyramidtracker.gov), was used to assess caloric intake, protein intake, carbohydrate intake, and fat intake for each participant. The three day food record has been used previously for assessing dietary intake (Burke et al., 2001; Candow et al., 2008; Chrusch et al., 2001).

2.5.5 Statistical Analyses

Data was analyzed using a 3 x 2 (group x time) factorial analysis of variance, followed by Tukey's post hoc test when significant interactions were found. The dependent variables tested were: lean tissue mass, fat mass, total body mass, 1-RM chest and leg press strength, and muscle thickness of elbow and knee flexors and extensors. Statistical significance was set at an alpha level of 0.05.

CHAPTER 3

RESULTS

3.1 Results

3.1.1 Participant Characteristics

Sixty-six individuals participated in the study pre-testing, including 47 males and 19 females. Forty-four participants (12 females and 32 males), ranging in age from 18 to 32 completed the study. All participants had previous strength training experience. The protein group included 11 males and 4 females, protein/creatine group included 11 males and 5 females, and protein/creatine/CLA group included 10 males and 3 females. Our initial analysis included gender as a factor. After finding that gender did not affect the responsiveness of the supplement, it was dropped as factor to increase the power of our analysis.

No significant differences were found between the three groups for any of the baseline characteristics (Table 3.1).

Table 3.1 Physical characteristics of participants at baseline (mean +/- standard deviation).

Group	**Age (y)**	**Height (cm)**	**Weight (kg)**
Protein	23.9 ± 2.4	175.3 ± 9.2	78.3 ± 15.4
Pro/Cr	22.6 ± 3.3	174.4 ± 11.5	75.1 ± 15.4
Pro/Cr/CLA	23.5 ± 2.3	176.9 ± 10.2	78.3 ± 13,8

No significant differences between groups.

3.2 Muscular Strength

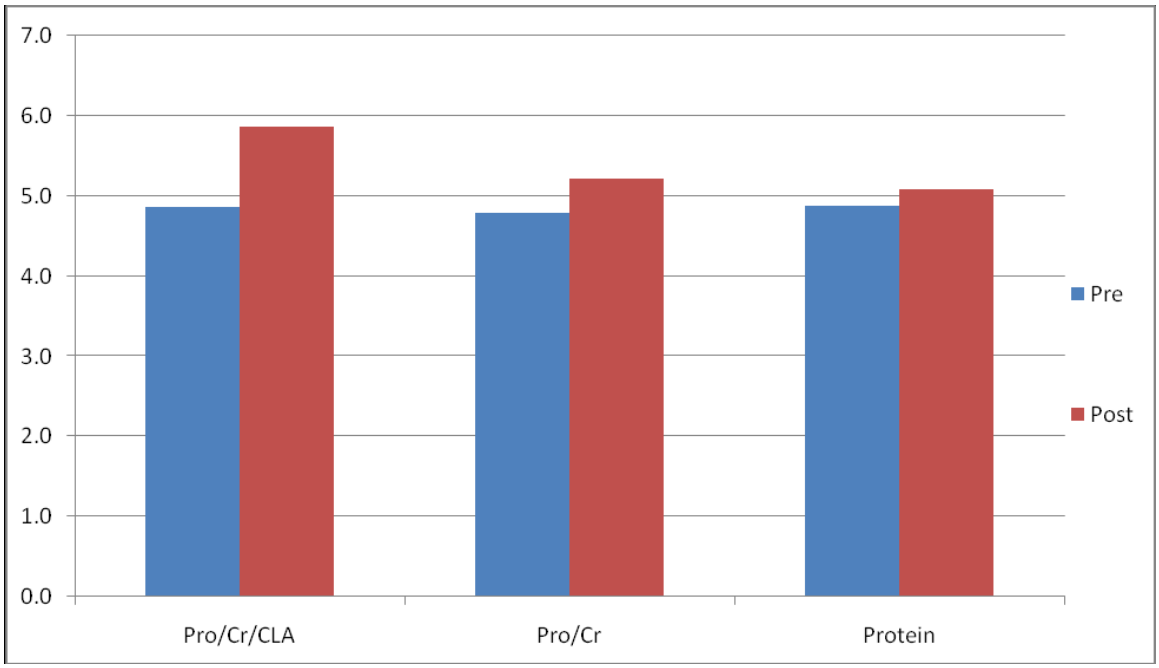
Bench Press 1-RM: For the bench press and leg press 1-RM there were significant time main effects, $F(1,37) = 35.9$ ($p<0.01$), and $F(1,38) = 22.5$ ($p<0.01$). No differences between groups were observed over time.

3.3 Muscle Thickness

Muscle Thickness: For overall muscle thickness there was a significant time main effect, $F(1,38) = 124.7$ ($p<0.01$). No differences between groups were observed over time.

There was a significant group x time interaction for knee extensor muscle thickness ($p=0.026$), with Tukey's post hoc test revealing significant changes over time in only the participants consuming protein, creatine, and CLA ($p<0.05$), as seen in Graph 3.1. Pre- and post-testing results were: protein (4.88 +/- 0.58 cm at baseline vs 5.08 +/- 0.56 cm after five weeks), protein/creatine (pre: 4.79 +/- 0.64 cm at baseline vs 5.22 +/- 0.82 cm after five weeks), protein/creatine/CLA (pre: 4.87 +/- 0.56 cm at baseline vs post: 5.86 +/- 1.36 cm after five weeks).

Graph 3.1. Muscle thickness (cm) pre- and post-testing for knee extensor muscle thickness.



Values are mean ± standard deviation. A significant group x time interaction was found ($p=0.026$), with Tukey's post hoc test revealing significant changes over time in only the participants consuming protein, creatine, and CLA ($p<0.05$)

3.4 Body Composition

Lean Tissue Mass: For lean tissue mass there was no significant time main effect, $F(1,37) = 0.2$ ($p=0.64$). No differences between groups were observed over time.

Fat Mass: For fat mass there was no significant time main effect, $F(1,37) = 2.0$ ($p=0.17$). No differences between groups were observed over time.

3. Diet

Analysis of 3-day food diaries, taken at the beginning and end of the study, showed no significant differences between groups. Caloric intake and macronutrient consumption pre- and post-training is shown in Table 3.2.

Table 3.2. Total calories (kcal/day) and macronutrient (g/day) consumption of protein, protein/creatine, and protein/creatine/CLA groups at the beginning and completion of training.

	Protein		Protein/Creatine		Protein/Creatine/CLA	
	Week 1	Week 5	Week 1	Week 5	Week 1	Week 5
Calories	2161 ± 333	2216 ± 595	2221 ± 419	2159 ± 540	2782 ± 1158	2341 ± 640
Protein	91 ± 11	99 ± 37	97 ± 25	99 ± 28	107 ± 50	96 ± 35
Carbs	261 ± 58	265 ± 80	282 ± 64	271 ± 74	356 ± 151	291 ± 94
Fat	75 ± 22	83 ± 25	76 ± 18	72 ± 21	108 ± 63	84 ± 29

Values are mean ± standard deviation. No significant differences were found between groups for pre-testing or post-testing means.

Table 3.3 Pre- and post-testing results (mean ± standard deviation) for bench press, leg press, lean tissue mass, fat mass, and muscle thickness.

Bench Press

	Pre	Post	% Change	p value
Protein (n=15)	265.3 ± 67.9	281.0 ± 61.1	6.7 ± 8.1	
Pro/Cr (n=15)	266.3 ± 60.8	299.0 ± 52.7	13.9 ± 9.2	0.06
Pro/Cr/CLA (n=13)	263.8 ± 64.1	293.8 ± 62.3	13.6 ± 13.5	0.54

Leg Press

	Pre	Post	% Change	p value
Protein (n=15)	463.2 ± 109.9	490.9 ± 109.3	6.2 ± 8.6	
Pro/Cr (n=15)	440.9 ± 123.7	475.4 ± 134.0	8.5 ± 7.2	0.33
Pro/Cr/CLA (n=13)	426.5 ± 76.0	470.8 ± 85.2	11.1 ± 11.1	0.52

Lean Tissue Mass

	Pre	Post	% Change	p value
Protein (n=15)	61.1 ± 7.3	60.8 ± 6.5	-0.7 ± 3.9	
Pro/Cr (n=15)	56.4 ± 8.2	56.5 ± 7.2	0.3 ± 3.1	0.15
Pro/Cr/CLA (n=13)	59.2 ± 4.7	59.8 ± 4.2	0.9 ± 1.6	0.15

Fat Mass

	Pre	Post	% Change	p value
Protein (n=15)	15.0 ± 6.7	15.5 ± 5.9	8.3 ± 17.7	
Pro/Cr (n=15)	13.5 ± 3.6	14.5 ± 4.1	7.5 ± 12.0	0.66
Pro/Cr/CLA (n=13)	14.8 ± 7.6	15.0 ± 7.0	5.6 ± 14.2	0.23

Muscle Thickness

	Pre	Post	% Change	p value
Protein (n=15)	19.0 ± 1.6	20.4 ± 1.7	7.6 ± 3.1	
Pro/Cr (n=15)	19.1 ± 1.5	20.9 ± 1.4	9.8 ± 5.4	0.11
Pro/Cr/CLA (n=13)	19.0 ± 1.5	21.5 ± 2.1	13.3 ± 6.5	0.14

No significant differences were found between groups in all categories.
P value represents the comparison of participants consuming a specific supplement to those not (ie. Pro/Cr p value compares Pro/Cr to Protein).

CHAPTER 4

DISCUSSION AND CONCLUSION

4.1 Discussion

This study explored the potential ergogenic effects of three supplements, consumed while following a resistance training program for five weeks. The supplement combination of protein, creatine and conjugated linoleic acid, consumed before and after exercise, and again in the evening, was hypothesized to improve strength and body composition to a greater extent than protein and creatine, or protein alone. The results did not support this hypothesis. A group by time interaction was found for knee extensor muscle thickness ($p < 0.05$), with post hoc analysis revealing significant changes over time in only those participants consuming protein, creatine and CLA. No significant differences were found between any groups in other variables measured.

Strength increases to bench press and leg press were found in all groups over time, however there were no changes to lean tissue mass ($P=0.64$) or fat mass ($P=0.16$). Increases in strength are a result of both "quantity and quality of the involved muscle mass" and the "extent to which the muscle mass has been activated" (Sale, 1988). Neuromuscular adaptations have been shown to precede increases in lean tissue mass. Chilibeck et al. (1998) found increases in leg press and bench press strength after ten weeks of resistance training, yet corresponding lean tissue mass increases were not found until 20 weeks. Researchers found

multi-joint exercises to have delayed hypertrophy in comparison to single-joint exercises, possibly requiring more complex neural adaptations. This information furthered results of research by Rutherford and Jones (1986), who reported strength increases in complex exercises were mainly a result of improved ability to coordinate muscle groups. In contrast, lean tissue mass gains, in addition to strength increases, were found after six weeks of resistance training while consuming protein and/or protein and creatine supplements (Burke et al., 2001). Increases to lean tissue mass were also found following a resistance training and creatine supplement program four weeks in duration (Volek et al., 2004). The majority of exercises performed in the present study involved multiple joint movements. As shown, the required neuromuscular adaptations which occur prior to muscle mass increases may explain the absence of lean tissue mass increases after five weeks.

Another potential explanation for increases in strength without corresponding increases in lean tissue mass, over a five week timeframe, is the design of the resistance training program. Participants progressed from performing four sets of 10-12 repetitions (2 minutes rest between sets), to four sets of 6-8 repetitions (1.5 minutes rest between sets), to finally three sets of 4-5 repetitions (1 minute rest between sets). In each set, participants selected an appropriate weight causing them to reach fatigue within the pre-determined range of repetitions. Participants were also instructed to choose a weight within the

range of 60-90% of pre-testing 1RM for appropriate exercise. Increases in muscle strength rely on multiple factors, including neural adaptations, as previously discussed, and increased muscle cross sectional area (ACSM, 2009). Muscular strength and hypertrophy are developed using training protocols incorporating various similarities, yet are optimized in a different ways. Maximizing strength gains in participants with previous resistance training experience, as was the case in the present study, requires training with, at minimum, 80% of their maximal 1RM (Hakkinen et al., 1985). High loads are also beneficial to muscle hypertrophy as they better recruit high threshold motor units and stimulate protein synthesis (McDonagh et al., 1984). High loads require exercises to be performed at slower velocities, increasing contraction time and time under tension, important factors in hypertrophy (Keogh et al., 1999).

Maximizing muscle hypertrophy differs from maximizing strength in that high loads are not conducive to maximize total work performed. Cronin and Crewther (2004) compared performances to exhaustion of 30, 60, and 90% 1RM. They found the highest load best influenced performance of single repetition force, however the lightest load allowed the greatest repetitions and greatest total work to be performed. A balance between work and load is important to exploit the benefits from each. Recent recommendations for muscle hypertrophy by the American College of Sports Medicine (2009) suggests three to six sets per exercise, the majority of which should use 6-12 repetitions. Alternatively,

increases in strength have been shown to be greatest in training incorporating less than six repetitions per set (Campos et al., 2002). Significant increases in muscle cross sectional area have been found when an additional set of 25-35 repetitions is added to a typical strength training program (3-5 repetitions), while the strength training program alone did not provide increases in cross sectional area (Goto et al., 2004). In this same study, the additional set caused increases to circulating growth hormone and testosterone. Increases in these hormones are important for muscle growth, and are related to total work done when exercising (Craig et al., 1994; Kraemer et al., 1990; Kraemer et al., 1991). Other factors associated with hypertrophy and total work done include increased contraction time, and increasing time under tension relative to rest (Keogh et al., 1999). The training protocol used in the present research progressed to increase load, decrease repetitions and sets, while also decreasing rest breaks. According to the majority of the exercise guidelines, this favors an environment for maximizing strength, possibly explaining the increase in both bench press and leg press 1RM in all groups over time, with no increases in lean tissue mass. In contrast, strength and lean tissue mass increases were found in previous research employing the same resistance training protocol for six weeks (Candow et al., 2001).

All participants in the present study were provided with protein, a dietary component well established for improving strength and lean tissue mass when consumed at proper times relative to exercise. Protein has been shown to cause significant increases ($p < 0.05$) in squat strength, knee flexion peak torque, and

lean tissue mass when compared to placebo after six weeks of resistance training (Burke et al., 2001). Other studies have also shown greater gains in strength, body composition, and/or muscle cross-sectional area when protein is consumed at proper times relative to exercise. Esmarck et al. (2001) found protein consumption and exercise increased muscle cross sectional area, in addition to dynamic and isokinetic strength, in elderly males after twelve weeks. Another study, by Anderson et al. (2005), found increases to cross sectional area of both type I and II muscle fiber types, and improvements in squat jump height when consuming protein while exercising for fourteen weeks. The difference in treatment duration possibly explains inconsistent results relative to the present study, while also providing further evidence supporting occurrences of neuromuscular adaptation. In the present study, participants consumed 36 – 45 grams of protein supplements per day for five weeks, however lean tissue mass was not affected.

Daily protein consumption was analyzed pre- and post-testing using a three-day food diary. Results of this analysis did not include the protein supplement. In all groups, average protein consumption ranged from 90-110 g/day without the supplement. For most participants this quantity would be greater than the recommended daily allowance of 0.8g/kg/day. The additional protein intake from the supplements made the total protein intake range from 125-155 g/day. Timing protein intake relative to exercise has been well established as an important factor in increasing protein synthesis in the muscle. Controlling

timing through supplement consumption was an important consideration in this study. However, properly timing intake of foods containing protein may be a viable alternative in future research as quantity of protein consumed was in excess of the recommended daily allowance.

Protein consumption was not compared against a placebo in this study due to previously established benefits, thus it is difficult to know if strength increased to a greater extent as a result. All groups experienced significant improvements in strength over time ($P<0.05$). Research in elderly participants shows protein does not always improve body composition and strength more than a placebo. After twelve weeks, Candow et al. (2006) found all groups experienced significant increases in strength, lean tissue mass, and muscle thickness, however found only muscle thickness of the knee extensors differed between those consuming protein and a placebo. Godard et al. (2002), also studying elderly male participants for twelve weeks, found no differences in strength or muscle mass with consumption of protein compared to placebo while exercising. It is difficult to speculate on the different findings, but perhaps older people do not have the capacity to respond to protein supplementation in the same way as younger people.

The addition of creatine did not cause any significant additional benefits than those found in the protein group. The addition of creatine was not found to influence changes over time more so than protein in all measurements: bench press ($P=0.06$), leg press ($P=0.33$), bench press and leg press combined ($P=0.10$), lean tissue mass ($P=0.15$), fat mass ($P=0.66$), or muscle thickness ($P=0.11$). In

opposition to these findings, creatine has shown potential as an ergogenic aid in past research.

In addition to increases beyond the placebo with protein consumption, Burke et al. (2001) found even greater gains when creatine was added to protein. Both bench press strength and lean tissue mass increased to a greater extent when protein and creatine were consumed, relative to protein and placebo groups. Differences in methodology may explain the contrast in results between the present study and Burke et al. (2001). A longer duration (6 vs. 5 weeks), and differences in the amount of sets and repetitions performed may have contributed to the significant changes found. Another important distinction was the inclusion of a true placebo group by Burke et al. (2001), allowing the participants consuming creatine to be compared against a larger sample of those who were not. In our research, 28 participants consuming creatine were compared to 15 not, while for Burke et al. (2001) 13 participants consumed creatine and 23 did not.

Creatine alone improved both body composition and strength in athletes over short durations of four weeks (Kreider et al., 1998) or five weeks (Stone et al., 1999). It has also shown similar improvements in both male and female participants (Becque et al., 2000; Vandenberghe et al., 1997). Burke et al. (2000) found participants consuming creatine for three weeks were able to perform more total work, and had greater improvements in peak force and power. The ability to reach greater training volume has also been shown (Volek et al., 1999). As previously discussed, performance of total work contributes to muscle

hypertrophy, possibly explaining consistent findings of increased lean tissue mass and muscle cross sectional area with creatine consumption when exercising.

The combination of CLA, creatine, and protein caused strength increases over time ($P<0.05$), however participants consuming CLA did not have significantly greater strength increases, in terms of bench press ($P=0.54$) or leg press ($P=0.52$). A possible explanation for limited findings is lack of power. With a setting of 80% power, and alpha=0.05, the required group size for significant increases to be present would have been 25 for bench press, and 61 for leg press, compared to our CLA group size of 13. Another study that was similar to our study in duration, found CLA did not cause greater increases in strength measures over a four-week training period compared to a placebo; however, there was a trend for overall strength to increase on CLA (Kreider et al., 2002). Supplements contained a low percentage of CLA isomers shown to be biologically active (c9,t11 and t10,c12) (Pariza et al., 2000). Studies using low amounts of active isomers have found limited results (Nazare et al., 2007; Whigham et al., 2004; Zambell et al., 2000). Statistically, small group sizes may have contributed to the limited findings in our study as well as other studies.

Bench press strength increased to a significantly greater degree in exercising males consuming CLA, compared to a placebo (Pinkoski et al., 2006). In the same study, no strength differences were found between groups of females, or in leg press strength in all participants. Differences in methodology possibly contributed to varied results including treatment duration (our five weeks

compared to seven), and group size, which varied in our study between 13-16 participants, compared to 38 participants per group used by Pinkoski et al. (2006). Improvements in chest press, knee extension, and body composition are dependent on treatment duration (Abe et al., 2000). Tested every two weeks during a 12-week resistance training program, males were found to significantly improve performance of the chest press after six weeks and knee extension after two. Women improved both factors after four weeks. After 12 weeks of resistance training, no changes were found to lean tissue mass. These results show how two additional weeks of training in the study by Pinkoski et al. (2006) might have affected strength improvement compared to the current study. This is true especially for the chest press measure, which according to Abe et al. (2000) is not improved until the 6-week time point.

Body composition measurements of lean tissue mass and fat mass did not show additional changes when CLA was consumed (P=0.15; P=0.23). Group size required for significant findings of lean tissue mass gains from CLA would be 69, based on calculations of 80% power, at alpha=0.05. These findings are similar to previous research showing CLA to have no influence on body composition in humans (Kreider et al., 2002; Nazare et al., 2007; Whigham et al., 2004; Zambell et al., 2000). Possible explanations for findings in these studies included limited duration, sample size, and active CLA isomers. Improvements to lean tissue mass and fat mass have been reported, however. CLA consumption in humans improved lean tissue mass after 12 months, while decreases in fat mass were seen

after six months (Gaullier et al., 2004). When taken while exercising for seven weeks, Pinkoski et al. (2006) found CLA to increase lean tissue mass and decrease fat mass. Tarnopolsky et al. (2007) also found greater lean tissue mass increases and fat mass decreases with creatine and CLA consumption while participants exercised for six months, compared to a placebo. The duration of the present study is considerably less than two of these studies (Gaullier et al., 2004; Tarnopolsky et al., 2007), possibly explaining different findings.

Knee extensor muscle thickness was found to increase only in participants consuming protein, creatine and CLA. Muscle thickness, measured by B-Mode ultrasound, was also tested in knee flexors, elbow flexors and elbow extensors. Combined muscle thickness increased over time ($P<0.05$), however no differences were seen between groups ($P=0.20$). Participants did not have greater overall increases with additional consumption of creatine ($P=0.11$) or CLA ($P=0.14$). Pinkoski et al. (2006) found similar muscle thickness increases of knee extensor and elbow flexors over seven weeks ($P<0.01$), yet found no additional benefit from CLA. Creatine was previously shown to increase muscle thickness of the elbow flexors in males after six weeks; however all other muscle thickness measures did not differ between groups (Chilibeck et al., 2004).

In summary, the combination of CLA, creatine, and protein significantly increased knee extensor muscle thickness over time, which was not found in other groups. Strength and muscle thickness increases were found in all groups over time ($P<0.05$). Additional benefits of creatine or CLA to strength or body

composition measures were not found. The relatively short five weeks of supplement consumption may have contributed to these findings.

4.2 Conclusion

The purpose of the present study was to determine if the addition of CLA to protein and creatine would further improve strength and body composition in participants involved in a five week resistance training program. Forty-four active male and female participants completed the study, ranging in age from 18-32.

Participants were randomly divided into three groups. Each was provided with a different nutritional supplement combination: protein, protein/creatine, or protein/creatine/CLA. During the five-week duration of the study, all participants followed the same strength-training exercise program, meanwhile consuming their supplement three times per day.

Pre- and post testing revealed significant changes in strength over time in all groups, however no changes in lean tissue or fat mass. Additionally, protein/creatine/CLA group had significantly greater changes in knee extensor muscle thickness than the other groups. These results provide information on which combinations of nutritional supplements might be effective during resistance training programs. This information can be used to further uncover the physiology involved in exercise, and in strategies for nutrient consumption while performing physical activity.

4.3 Recommendations

In future research exploring effects of CLA, creatine, and protein, it is important to explore effects of long term consumption. Performing this research for a length over 12 weeks may provide greater details on the impact of CLA on muscle growth, as this timeframe is associated with strength increases due to neurological adaptations.

The current study explored additive effects of CLA to creatine and protein. As creatine is a well-establish ergogenic aid, it is necessary to separate the effects of each in addition to protein. Research comparing strength and body composition changes over time between participants consuming creatine versus CLA, both individually and in combination, would be required to determine if CLA benefits are additional to creatine. Also worth exploring is the individual role of each of CLA's active isomers in relation to exercise. Comparing the c9,t11 and t10, c12 isomers may lead to a further understanding of their impact on body composition.

The participants involved in the present study were required to have previous resistance training experience, and be the range of 18-30 years of age. As the results of the current study are limited to this population, research involving participants of other demographics is important. As CLA is thought to decrease the breakdown of muscle following exercise, its influence on an elderly population regarding muscle wasting is warranted. Research into creatine's impact on reversing muscle mass deficits has been explored in elderly populations (Candow

et al., 2006), and would provide a model from which to study CLA's impact on the same.

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APPENDICES

APPENDIX A

Certificate of Ethics Approval

UNIVERSITY OF SASKATCHEWAN

Biomedical Research Ethics Board (Bio-REB) 13-Oct-2003

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	Bio #
Philip D. Chilibeck	Kinesiology	05-135

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT
College of Kinesiology
105 Gymnasium Place
Saskatoon SK S7N 5C2

SUB-INVESTIGATOR(S)
Gordon A. Zello

STUDENT RESEARCHER(S)
Nathan Jantz

SPONSORING AGENCIES
IOVATE HEALTH SCIENCES RESEARCH INC.

TITLE
The Additive Effects of Conjugated Linoleic Acid, Creatine Monohydrate, and Protein Supplementation for Increasing Muscle Mass and Muscular Strength

ORIGINAL APPROVAL DATE	CURRENT EXPIRY DATE
02-Oct-2005	30-Sep-2007

CERTIFICATION UPDATE
Amended Protocol (12-Oct-2006)
Amended Research Participant Information and Consent Form (12-Oct-2006)

APPROVED ON
13-Oct-2006

CERTIFICATION
The University of Saskatchewan Biomedical Research Ethics Board has reviewed the above-named research project at a full-board meeting (any research classified as minimal risk is reviewed through the expedited review process). The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research project, and for ensuring that the authorized research is carried out according to governing law. This Approval is valid for the above time period provided there is no change in experimental protocol or in the consent process.

ONGOING REVIEW REQUIREMENTS/REB ATTESTATION
In order to receive annual renewal, a status report must be submitted to the Chair for Committee consideration within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for further instructions: http://www.usask.ca/research/ethics.shtml. In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. This approval and the views of this REB have been documented in writing.

Michel Desautels, Ph.D., Chair
University of Saskatchewan
Biomedical Research Ethics Board

Please send all correspondence to:

Ethics Office
University of Saskatchewan
Room 305 Kirk Hall, 117 Science Place
Saskatoon SK S7N 5C8
Telephone: (306) 966-4053 Fax: (306) 966-2069

APPENDIX B

Participant Consent Form

CONSENT FORM

Research Participant Information and Consent Form

Title: The Additive Effects of Conjugated Linoleic Acid, Creatine Monohydrate, and Protein Supplementation for Increasing Muscle Mass and Muscular Strength

Sponsor: Iovate Health Sciences Research Inc.

Names of Researchers: Principal Investigator: Philip D. Chilibeck, Ph.D., College of Kinesiology, University of Saskatchewan, phone: 966-1072 or 343-6577, Co-investigators: Nathan Jantz, B.Sc. (student researcher, supervised by Phil Chilibeck), College of Kinesiology, University of Saskatchewan, phone: 966-1123, Gordon Zello, Ph.D., College of Pharmacy and Nutrition, University of Saskatchewan, phone: 966-5825

You are being invited to participate in a research study because we want to determine the effect of combining a number of nutritional supplements (protein, creatine, and conjugated linoleic acid) on muscle mass and strength during a resistance training program.

Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts.

If you wish to participate, you will be asked to sign this form. Your participation is entirely voluntary, so it is up to you to decide whether or not to take part in this study. If you do decide to take part in this study, you are free to withdraw at any time without giving any reasons for your decision and your refusal to participate will not affect your relationship with University instructors and your academic evaluations. Please take time to read the following information carefully and to discuss it with your family, friends, and doctor before you decide.

Purpose of the study: The purpose of the study is to compare the effects of protein alone, protein combined with creatine, and protein combined with creatine, and conjugated linoleic acid on muscle mass and strength, during seven weeks of strength training. Creatine monohydrate is a substance found in meat and fish products which, when given in higher amounts than usually consumed in the diet, has been shown to increase lean tissue mass. Conjugated linoleic acid is a fat compound found in meat and dairy products that when given in higher amounts than usually found in the diet, has been shown to increase lean tissue mass and reduce fat mass.

Possible benefits of the study: You might increase your muscle mass and strength by participating in this study. These benefits are not guaranteed.

Procedures:

If you agree to be in this study the following will happen:

You will initially be given a questionnaire (the physical activity readiness questionnaire) which assesses whether you are at a health risk from participating in exercise training. If there are possible health risks, with your permission we will send an additional form to your family physician for approval to allow you to participate in the study.

You will be randomized (by chance by a computer) into one of three groups: Group 1 will receive 36 grams of protein, 9 grams of creatine, and 6 grams of conjugated linoleic acid per day. Group 2 will receive 36 grams of protein, 9 grams of creatine, and 6 grams of placebo per day (a placebo is an inactive substance, that it looks identical to the test intervention but it contains no active ingredients). Group 3 will receive 45 grams of protein and 6 grams of placebo per day. All groups will receive their supplements in powder form and this will be mixed with water prior to consumption. Neither you nor the researchers will know which group you are in until the end of the study. The supplements will be divided into 3 doses to be taken before you exercise, after you exercise, and before you go to bed each day. You will receive the supplements in addition to your regular diet.

All groups will participate in six weeks of exercise (strength) training (6 days per week). The training program is designed to involve training of all major muscle groups over three consecutive days, followed by one day of rest. This cycle will be repeated over the seven weeks. Each training session will take one to one and a half hours to complete. You will be required to keep a detailed log of your training sessions.

Your muscular strength will be measured for three different exercises at the start of the study, and after the end of the training (6 weeks). These exercises include one upper body exercise (bench press) and two lower body exercises (leg press and knee extension/flexion).

Body composition (i.e. lean tissue, fat, and bone mass) will be assessed at the start of the study and after 6 weeks by dual energy X-ray absorptiometry. This involves lying still on a table for approximately 10 minutes while your body composition is assessed. Dual energy X-ray absorptiometry uses X-ray beams that are absorbed differently by fat, muscle, and bone. From this we can determine your fat, muscle, and bone mass.

Your muscle thickness will be measured twice: at the start of the study and after 6 weeks. Muscle thickness will be measured using ultrasound by placing a gel over your skin and applying a probe to your skin surface. Muscle thickness will be measured at the front and back of your upper arms, and thigh. This procedure will take 30 minutes.

You will be required to collect urine for 24 hours at two time points: At the start of the study and after 6 weeks. Prior to this urine collection, you will have to consume a meat-free diet for 3 days. The purpose of the urine collection is to measure a marker of muscle protein breakdown. Meat consumption affects the level of this marker; therefore, three days without meat is required.

You will be required to record all the food you eat in a food diary, for three days, at the start and end of the 6 week study.

Foreseeable risks, side effects or discomfort:

The exercise training and strength testing may result in muscle pulls or strains. You will be given a proper warm-up prior to exercising and this will minimize this risk.

There is radiation exposure from the dual energy X-ray absorptiometry but this is considered minimal. This is about the same amount of radiation as you would be exposed to during a return airplane flight from Saskatoon to Toronto, and is less than the amount of radiation you would be exposed to during a regular X-ray.

Creatine supplementation has been shown to be associated with minimal side effects, especially with the low dose given in this study. There have been anecdotal reports of increased muscle cramping or muscle pulls during creatine supplementation, but when this is compared to subjects receiving placebo, there is no differences in rates of occurrence of muscle cramping or pulls. Creatine supplementation has been shown, on two occasions, to worsen kidney function in individuals who already had kidney disease. If you have any problems with kidney function you should not participate in this study.

We previously conducted a study with conjugated linoleic acid supplementation (same dose over 7 weeks) in 80 subjects. Eight out of 40 subjects who received conjugated linoleic acid experienced upset stomach, nausea, or indigestion; whereas 7 out of 40 subjects who received placebo had the same side-effects.

We have previously conducted studies using a similar amount of protein supplement in young and older individuals with no reported side-effects. A high

protein diet may worsen kidney function in individuals with kidney disease. If you have any problems with kidney function you should not participate in this study. High protein intake may increase water loss and risk of dehydration. You should therefore make certain to consume an adequate amount of water while in the study.

There may be unforeseen and unknown risks during the study, or after the study has been completed.

Alternatives to this study:
You do not have to participate in this study to increase your muscle mass and strength. You can perform alternative exercises (i.e. free-body exercises such as push-ups or chin-ups instead of the exercise program in this study). You could also increase your protein, creatine, and conjugated linoleic acid consumption from your diet by consuming more high-protein sources, such as meats, milk, and eggs instead of receiving the protein and creatine supplementation in this study.

Research-Related Injury: There will be no cost to you for participation in this study. You will not be charged for any research procedures. In the event you become ill or injured as a result of participating in this study, necessary medical treatment will be made available at no additional cost to you. By signing this document you do not waive any of your legal rights.

Confidentiality: While absolute confidentiality cannot be guaranteed, every effort will be made to ensure that the information you provide for this study is kept entirely confidential. Your name will not be attached to any information, nor mentioned in any study report, nor be made available to anyone except the research team. It is the intention of the research team to publish results of this research in scientific journals and to present the findings at related conferences and workshops, but your identity will not be revealed.

Voluntary Withdrawal: Your participation in this research is entirely voluntary. You may withdraw from this study at any time. If you decide to enter the study and to withdraw at any time in the future, there will be no penalty or loss of benefits to which you are otherwise entitled.

If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrolment in the study will be retained for analysis.

If you have questions concerning the study you can contact Dr. Philip Chilibeck at 966-1072, 343-6577, or 230-3849 (24 hour cell) or Nathan Jantz (student researcher) at 966-1123.

If you have questions about your rights as a research subject, you can contact the Office of Research Services at the University of Saskatchewan at 966-4053.

By signing below, I confirm the following:

- I have read or have had this read to me and understood the research subject information and consent form.
- I have had sufficient time to consider the information provided and to ask for advice if necessary.
- I have had the opportunity to ask questions and have had satisfactory responses to my questions.
- I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.
- I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.
- I understand that I am not waiving any of my legal rights as a result of signing this consent form.
- I understand that there is no guarantee that this study will provide any benefits to me (if applicable).
- I have read this form and I freely consent to participate in this study.
- I have been told that I will receive a dated and signed copy of this form.

- I agree that my family physician can be contacted about my participation in this study:

_____Yes _____No

Participant's Signature:_____ Date: _____

Individual conduction the consent process:_____

Date: _____

APPENDIX C

Physical Activity Readiness Questionaire

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
☐	☐	**1. Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?**
☐	☐	**2. Do you feel pain in your chest when you do physical activity?**
☐	☐	**3. In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	**4. Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	**5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	**6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	**7. Do you know of <u>any other reason</u> why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to <u>all</u> PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

<u>Informed Use of the PAR-Q:</u> The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

Supported by: Health Canada Santé Canada

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APPENDIX D

3-Day Food Diary Booklet

3 Day Food Diary

INTRODUCTION

This booklet is used to record you detailed daily food intake. It is meant to give researchers some idea of your *usual* dietary intake. Therefore, it is very important that you do not alter your eating habits while taking part in this study. In other words, do not let the fact that you are writing down what you eat influence your choice of foods. The names of the participants in this study will be kept confidential.

The usefulness of the results of this study depends on the accuracy with which you record your daily food intake. Please write down full details on all the food and drink that you consume each day.

INSTRUCTIONS

1. The purpose of this diary is to record *all* the food (including drinks) which you eat for a three day period.

2. Two pages are provided for each day of the three day period.

3. After each meal or snack that you eat, please write down in detail each separate food item you consumed – including the type of food (e.g. processed cheese) and the amount of food in household measures (e.g. 1 cup of cooked spaghetti). A meal will have to be listed by its separate parts (e.g. fried steak – 8 oz., french fries – 1 cup, coleslaw – 3 tbsp.).

4. The best way to record the information is by carrying this diary around with you wherever you go. Before going to sleep, you should look over the diary and check that you have not missed anything. Remember to include snacks!

5. If you eat fast food, you can just list the type of food you ate (e.g. 1 Big Mac, 1 large fries, 1 chocolate milkshake).

6. The following pages explain the use of household measures, and the description of foods. A sample day's diet sheet is given. Please take the time to read these pages as it will help you to make your diet record more accurate.

Here is a sample: Date: Monday March 1, 1999

Time	Food Description	Amount	Code
9:30 a.m.	Waffles – white flour	3, 8" x 4"	
	Syrup – Aunt Jemima	½ cup	
	Yogurt - peach	125 mL	
	Coffee, tsp sugar	1 cup	
	Milk 2%	¼ cup	
10:30 a.m.	Chocolate chip cookies	3	
	Coffee, 1tsp sugar	1 cup	
	Milk (half and half – 10%)	¼ cup	
12:30 p.m.	Sandwich		
	- 2 slices whole wheat bread	2 slices	
	- mozzarella cheese (3"x1/4"x2")	2 slices	
	- salami	4 slices	
	- lettuce	1 leaf	
	- butter	1 tsp	
	- mayonnaise	1 tsp	
5:30 p.m.	Spaghetti	1 cup	
	Meat sauce	½ cup	
	Garlic toast	2 slices	
	Milk 1%	2 cups	
8:30 p.m.	Sandwich		
	- 2 slices 60% whole wheat bread	2 slices	
	- peanut butter	1 tbsp	
	- honey	1 tbsp	
	Milk 2%	2 cups	
	*** continue on next page if you need**		
	*** leave CODE column blank**		