THESIS

COMBINING CURCUMIN AND ALPHA LIPOIC ACID TO TREAT CARDIOMETABOLIC SYNDROME

Submitted by

Scott Edward Binns

Department of Health and Exercise Science

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Master's Committee:

Advisor: Christopher Bell

Elizabeth Ryan
Matthew Hickey

ABSTRACT

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INTRODUCTION: Obesity is associated with increased risk of developing cardiometabolic disease characterized by decreased insulin sensitivity, positive energy balance, and cardiovascular disease. Separately, the dietary compounds curcumin (CUR) and alpha lipoic acid (ALA) increase energy expenditure and insulin sensitivity. However the efficacy of combined CUR and ALA supplementation to improve characteristics of cardiometabolic health in overweight humans has never been evaluated.

METHODS: 11 overweight and sedentary adults were randomly assigned to a placebo (*n*=5; age: 44 ± 8 years; body mass index: 31.9 ± 2.1 kg/m^2; VO_{2peak}: 26.8 ± 4.1 ml/kg/min; (mean ± SE)) or CUR+ALA (*n*=6; age: 38 ± 7 years; body mass index: 32.2 ± 1.2 kg/m^2; VO_{2peak}: 24.9 ± 3.7 ml/kg/min) 12-week intervention. Measurements of resting metabolic rate (RMR) and thermic effect of feeding (TEF) via indirect calorimetry, and insulin sensitivity via the hyperinsulinemic euglycemic clamp technique were assessed in a pre/post treatment manner. Additionally, resting blood pressure, and body composition via dual energy x-ray absorptiometry (DEXA) were measured.

RESULTS: 12-weeks of CUR+ALA supplementation did not change RMR (1467 ± 89.9 to 1539 ± 94.4 kcal/day; *P*=0.239) or TEF AUC (*P*=0.51). Additionally, no change in glucose infusion rate occurred as a result of supplementation (12.8 ± 2.1 to 12.4 ± 1.2 mg/FFM kg/min; *P*=0.690). Resting systolic blood pressure was unaffected in the CUR+ALA group (116 ± 3.2 to

123.8 ± 25.4 mmHg; *P*=0.31). Body fat percentage did not change with CUR+ALA supplementation (43.8 ± 2.2 to 41.6 ± 1.9 body fat percent; *P*=0.162), however, body fat percentage decreased in all subjects regardless of treatment (*P*=0.002).

DISCUSSION: These preliminary data do not support the use of CUR+ALA to improve metabolic and cardiovascular health in overweight adults.

TABLE OF CONTENTS

CHAPTER I

LITERATURE REVIEW

Obesity is associated with increased risk of developing cardiometabolic disease characterized by decreased insulin sensitivity, positive energy balance, and cardiovascular disease. The combination of curcumin (CUR) and alpha-lipoic acid (ALA) may have additive beneficial effects on cardiometabolic disease. Both compounds have been shown to evoke increases in insulin sensitivity and energy expenditure and improve cardiovascular health in animals and humans. Characteristics of these supplements are described in this literature review.

Curcumin

Turmeric is a gold-colored spice and is derived from the highly aromatic and antiseptic rhizome of the plant *Curcuma longa.* Turmeric has been used by people of the Indian subcontinent for centuries in food, textiles, and for medicinal purposes including uses as an anti-inflammatory, antioxidant, antimalarial, insect-repellant and analgesic (1). Turmeric is consumed in some Southeast Asian communities in quantities as great as 1.5g per person per day (2). Dose escalation studies have confirmed a single administration as high as 12g in humans being well tolerated (2–4). Additionally, three months of repeated administration of 8g/day has no detrimental health effects in humans (3). Turmeric is composed of a wide variety of phytochemicals, one of which being CUR (2-5%). CUR is responsible for turmeric's gold color and is now recognized as the therapeutic component of turmeric.

CUR is a highly pleiotropic molecule (5), and has been used in treating complications associated with type 2 diabetes such as liver disorders, neuropathy, nephropathy, and vascular disease, as well as a chemopreventative agent (3). Some of the most well-supported mechanisms of action of CUR involve reduction in inflammation through inhibition of cytokines such as tumor necrosis factor alpha (TNF-α) and interleukins (6). Chronic exposure to inflammatory cytokines has been shown to contribute to the development of insulin resistance, failure of the hormone insulin to inhibit hepatic glucose output and promote peripheral glucose disposal (7). Inflammatory cytokine inhibition by CUR is thought to occur through regulation of nuclear factor-kappa B (NF-κB). NF-κB is activated by TNF-α and plays a central role in regulation of cytokines, adhesion molecules, and other mediators of the inflammatory response. CUR suppresses NF-κB in 15-week old *db/db* mice, a common model of obesity and diabetes (8,9). CUR has also been shown to improve insulin sensitivity through decreasing NF-κB activity and simultaneous suppression of inflammatory molecules in type 2 diabetic mice (10). Body weight and fat mass were also significantly reduced in the same mice administered CUR.

In addition to regulation of inflammatory pathways, CUR is believed to increase the activity of adenosine monophosphate-activated protein kinase (AMPK) (11–13). AMPK activation in skeletal muscle results in inhibition of acetyl-CoA carboxylase (ACC) activity thereby decreasing malonyl-CoA concentrations. Malonyl-CoA acts as an inhibitor of carnitine palmitoyltransferase-1 (CPT-1), the enzyme responsible for transfer of long chain fatty acyl-CoA into the mitochondria for β-oxidation thereby preventing entry of fatty acyl-CoA entry into the mitochondria (14). Treatment with CUR in type 2 diabetic rats and L6 myotubes causes reductions in ACC activity in addition to increased insulin-mediated glucose uptake and GLUT-4 expression on membranes in skeletal muscle of the rats and the L6 myotubes (15).

Recently, CUR has been shown to have an anti-diabetic effect via improvements in fatty acid oxidation in skeletal muscle (15). Individuals with type 2 diabetes invariably develop dysregulation in lipid oxidation, a characteristic reflected through elevated levels of plasma free fatty acids (FFA) and triglycerides (TG) (7). This characteristic favors accumulation of fatty acid intermediates, like diacylglycerol (DAG) and ceramide, both of which have been shown to inhibit insulin signaling through serine-phosphorylation of the insulin receptor by protein kinase-C-theta (PKC-θ) (16). The prevention of accumulation of fatty acid intermediates through increased fatty acid oxidation mediated through CUR could prevent inhibition of the insulin receptor and improve insulin sensitivity.

Similarly, intraperitoneal administration of CUR for 28 days in STZ-induced diabetic mice improved insulin sensitivity (17). Improvements in glucose tolerance were also demonstrated in high-fat diet induced obese and insulin resistant mice orally administered CUR (0.5g/kg/bw) for 15 days (18). In addition, CUR prevents diet induced hyperlipidemia in high-fat fed hamsters (19). Attenuation of hyperlipidemia could reduce accumulation of fatty acids in skeletal muscle and hepatocytes and the subsequent development of insulin resistance.

Despite the numerous beneficial effects CUR has been shown to elicit, many studies have also shown no effect in human and animal models. Pharmacokinetics, bioavailability, and the supra-physiological concentrations used in cell culture studies make translation and reproducibility difficult in humans. When CUR is administered orally to humans, bioavailability is low due to efficient first pass metabolism, limited gastrointestinal absorption, rapid elimination, and poor aqueous solubility (20). Limited research has been performed on specific transporters of CUR in the gastrointestinal tract, although, Na-dependent transporters are suspect (21). The pH of the lumen and foods consumed with CUR have also been shown to effect

gastrointestinal absorption (22). Foods high in fat content assist in gastrointestinal absorption of CUR (23). Serum concentrations of CUR after ingestion peak at 1-2 hours and gradually decline over the following 12 hours (3). Further, the half-life of CUR has been reported to be as short as 1.45 hours in rats to 6 hours in humans (23,24). Hepatic tissue has been shown to retain trace (10^{-8} M) amounts of CUR after oral ingestion of 3.6g/day for one week in humans (25). Such a small amount most likely did not have lasting physiological effects. Intravenous administration of CUR is possible and provides higher concentrations compared to orally administered CUR. However the logistics of intravenously administering CUR to insulin resistant and obese populations eliminates the convenience of administration via capsule.

Many derivatives of CUR, known as curcuminoids, are able to elicit similar or greater beneficial health effects compared to naturally occurring CUR. One example, CNB-001, is used as an alternative to CUR in many animal models because of its high bioavailability. CNB-001 was shown to attenuate body weight gain, serum triglycerides, and interleukin-6 in a high-fat diet fed mouse model (26). Interestingly, CNB-001 treatment lowered protein-tyrosine phosphate 1B (PTP1B) activity, a negative regulator of the insulin signaling pathway (26). The authors concluded that CNB-001 could be producing insulin-sensitizing effects by docking and inhibiting activity of PTP1B. Although CNB-001 is a promising alternative to naturally occurring CUR, further testing needs to be performed on its safety and efficacy in humans.

Compelling evidence also exists for the use of alternative curcuminoids in humans. The standardized preparation of CUR, demethoxycurcumin and bisdemethoxycurcumin, NCB-02, improved endothelial function and reduced markers of inflammation in type 2 diabetic patients to comparable levels of atorvastatin, a cholesterol lowering medication (27). The favorable effect of NCB-02 in this population is convincing due to the wide use across the United States and the

increased risk of developing type 2 diabetes associated with chronic use of statins (28). However, NCB-02 contains a greater amount of demthoxycurcumin and bisdemethoxycurcumin compared to commercially available CUR extracts. This characteristic limits its use and accessibility until compounds such as it itself are available to the general public.

Alpha-lipoic acid

Alpha-lipoic acid (ALA) is a naturally occurring dithiol compound and an essential cofactor to several enzymes involved in mitochondrial energy metabolism (29). ALA exists in both R- and S-enantiomeric forms, however, only R-ALA can be utilized in physiological settings (29,30). ALA is absorbed intact from dietary sources including muscle meats, heart, kidney, liver, and some fruits and vegetables (29,31). Gastrointestinal uptake of ALA is multifaceted and dependent on many different carrier proteins including the Na -dependent multivitamin transporter (29). Approximately 20-40% of orally ingested ALA appears in the plasma (32). The half life of ALA has been reported to be as short as 30 minutes in humans administered single-doses of .6g of ALA (33). Further, ALA uptake is decreased when administrated with food and absorption is followed by an equal rate of clearance by the renal system (34). In fact, 98% of radiolabeled ALA is excreted in the urine within 24 hours (35). Consequently, concentrations of free ALA are most likely negligible *in vivo* due to its transient nature and low cellular accumulation following oral intake. ALA has also been shown to cross the blood brain barrier and appear in the cerebral cortex within 60 minutes of administration in rats given doses of 0.025 g/kg bw (36).

ALA is proposed to have a range of antioxidant properties including free radical scavenging and regeneration of endogenous antioxidants (37). Such properties could prove

beneficial in states of chronic oxidative stress like type 2 diabetes and obesity. Free radicals such as, hydrogen peroxide, inhibit the insulin receptor and tyrosine phosphorylation of IRS-1 (38). ALA protects against oxidative stress-induced insulin resistance in 3T3-L1 adipocytes and L6 muscle cells treated with hydrogen peroxide (39). Research performed in type 2 diabetic humans demonstrated similar results with intravenous infusion of ALA significantly improving insulin sensitivity (39).

ALA is an essential cofactor for mitochondrial bioenergetic enzymes like pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase (αKD) (40). Both PDH and αKD are crucial to mitochondrial-specific pathways that generate energy from glucose. In addition, ALA has been shown to directly inhibit fatty acid synthesis and concomitantly increase fatty acid oxidation, thought to occur through reduction in the rate limiting enzymes of fatty acid synthesis: ACC, and fatty acid synthase (FAS) (41,42). However, more research in this field of study needs to occur due to the multifaceted nature of ALA as a biological agent.

Many studies have focused on the thermogenic effects of ALA in both animals and humans. Attenuation of weight gain in high-fat fed rats when supplemented with ALA was shown to occur through increases in AMPK activity and expression of peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC1-α) resulting in enhanced skeletal muscle energy metabolism (43). ALA has also been shown to increase energy expenditure through upregulation of uncoupling protein-1 expression in brown adipose tissue, a highly thermogenic tissue in rodents and potential target for weight maintenance in humans.

In addition to influencing energy expenditure, ALA can modify energy intake. Intraperitoneal and intracerebroventricular administration of ALA in obese type 2 diabetic mice has been shown to have an anorexic effect. This effect was attributed to decreased activity of

hypothalamic AMPK, a key regulator of food intake (14). Thus ALA administration not only affects energy expenditure but also intake.

Furthermore, ALA supplementation in obese rats has demonstrated beneficial effects on plasma lipid profile and stimulation of weight loss. In Spraque-Dawley rats fed a high-fat diet supplemented with ALA, total blood lipids, triglycerides, cholesterol, and FFAs were reduced compared to a control group fed a high-fat diet without ALA (44). Similar results have been found in studies evaluating the potential for a dose response to ALA on plasma lipid profile, however, no dose-response relationship was found (42). ALA has also been shown to modulate production and storage of hepatic intracellular lipid, resulting in reductions in steatosis in obese rats (45).

Conversely, many studies have reported no effect of ALA on metabolic parameters in humans. For example, 12-weeks of 6g/day had no affect on total cholesterol or oxidized LDL in end-stage renal disease patients (46). This study and others that fail to show benefits of ALA often attribute a lack of response to low bioavailability through limited absorption or high excretion and metabolism.

The combination of curcumin and alpha-lipoic acid

Separately, CUR and ALA have demonstrated similar effects on insulin sensitivity, glucose uptake, and weight loss in animal models. ALA has been touted as a potent antioxidant, a trait shown to rescue insulin sensitivity in insulin resistant individuals through the prevention of oxidative damage to the insulin receptor (Figure 1). Reductions in damage to the insulin receptor may prevent the progression of insulin resistance.

The thermogenic progression of ALA could also reduce adipose tissue mass and size. Hypertrophied adipocytes are responsible for the proinflammatory state associated with obesity and insulin resistance (47). Reductions in adipose mass could improve metabolic health through decreases in adipose-associated inflammation and oxidative stress.

The metabolic benefits of CUR are thought to occur through inhibition of pro-inflammatory pathways such as those affected by Nf-κB. Inflammation is associated with obesity and contributes to insulin resistance (48). CUR has been shown to increase fatty acid oxidation through activation of AMPK (Figure 1). Similar to ALA, this effect would act to prevent dysregulation of the insulin-signaling cascade.

Oral supplementation of CUR+ALA may also have an effect on cardiovascular health through reductions in inflammation and oxidative stress. Hypertension is linked to chronic inflammation and oxidative stress, both shown to decrease endothelial cell function (49). In theory, cardiovascular health should improve if CUR+ALA is efficacious in reducing damage to endothelial cells associated with inflammation and oxidative stress.

A limited number of studies have utilized the combination of CUR and ALA in treating metabolic health ailments. One study demonstrated the "anti-nociceptive effect" CUR+ALA and B-group vitamin supplementation has in patients with carpal tunnel syndrome (50). CUR+ALA supplementation has also been shown to protect against thioacetamide-induced liver cirrhosis in rats (51). Pertaining to weight loss and type 2 diabetes, only one study to date has investigated the effect CUR+ALA supplementation has on improving cardiometabolic health. Although not published, reductions in weight as well as improvements in insulin sensitivity were shown to occur in high-fat diet fed (60%kcal from fat) mice supplemented with CUR+ALA for 11 weeks (0.64 and 1.74g/kg/d, respectively). Such a study, however, has not been reproduced in humans.

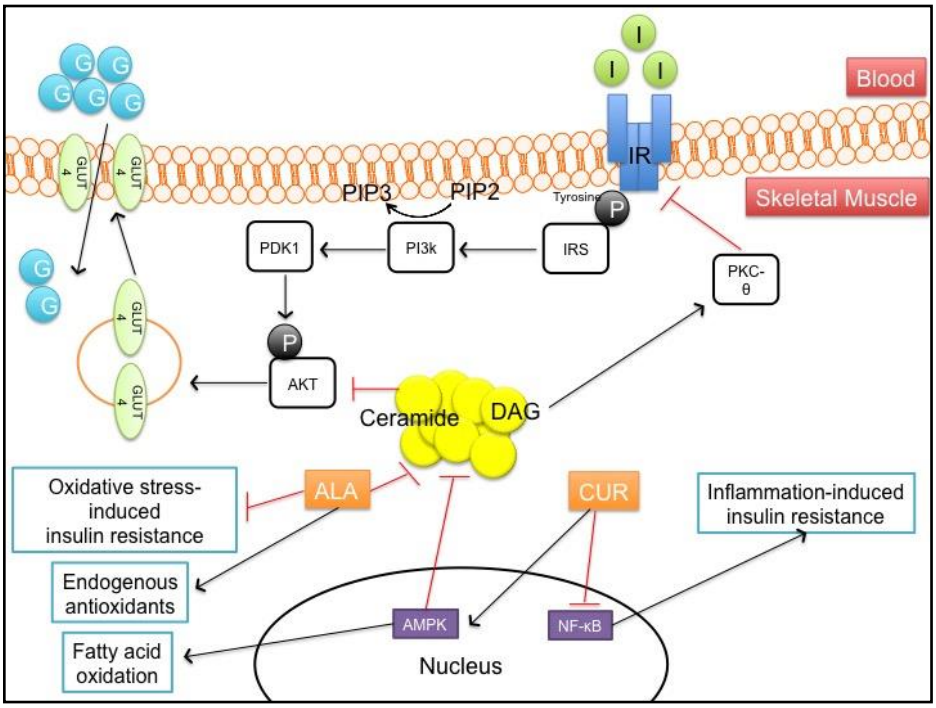


Figure 1. Once insulin (I) binds to its receptor, the insulin receptor (IR) phosphorylates phosphatidylinositol 3-kinase (PI3K) regulatory subunit, which causes the subsequent generation of phosphatidylinositol-3, 4, 5-triphosphate (PIP_3), responsible for activation of 3-phosphoinositide-dependent protein kinase (PDK1). The PDK1 enzyme activates protein kinase B (Akt/PKB) by phosphorylation, which acts through several proteins to translocate GLUT-4 vesicles to the plasma membrane and cause the subsequent entry of glucose (G) into the cell. Chronic oxidative stress and inflammation can cause insulin resistance through inhibition of the insulin receptor and downstream pathways. Curcumin (CUR) inhibits inflammatory pathways independently and through inhibition of nuclear factor kappa B (NF-κB). Activation of adenosine monophosphate-activated protein kinase (AMPK) by CUR also occurs, resulting in augmented fatty acid oxidation and storage. PKC-θ: protein kinase theta. IRS: insulin receptor substrate. ALA: alpha-lipoic acid.

Chronic inflammation and oxidative stress have a detrimental impact on insulin sensitivity and cardiovascular health, and are commonly exacerbated by obesity. An intervention that bolsters anti-inflammatory and antioxidant capacity would prove beneficial in treating and preventing the progression of cardiometabolic syndrome. Utilizing the combined beneficial effects of CUR and ALA could prove advantageous and have a direct impact on chronic inflammation and oxidative stress.

Statement of the problem

Obesity is associated with increased risk of developing cardiometabolic disease characterized by decreased insulin sensitivity, positive energy balance, and cardiovascular disease. The two supplements CUR and ALA both in combination and separately, demonstrated beneficial effects in isolated cells and animal models on insulin resistance, vascular disease, and prevention of weight gain. However, the combination of CUR+ALA has never been studied in humans and the potential for their combined effect on treating cardiometabolic disease could prove efficacious. Therefore the purpose of this study is to evaluate the efficacy of the combination of CUR+ALA in improving cardiometabolic health. We will measure a broad spectrum of cardiometabolic variables, as focusing on specific variables would exclude many factors associated with cardiometabolic disease.

Together, RMR and TEF are relatively easy to measure and responsible for roughly 80% of total daily energy expenditure (TDEE) in sedentary adults. Therefore, comparisons of each will be made pre and post supplementation of CUR+ALA to assess energy expenditure changes. We will also measure the respiratory exchange ratio and oxygen consumption during exercise. These measurements will provide us with insight into the third component of TDEE, thermic effect of activity.

We will measure insulin sensitivity via the hyperinsulinemic euglycemic clamp, the gold standard of measuring insulin sensitivity (52). Utilizing this method rather than others will provide us with glucose infusion rates used to maintain blood glucose levels at a specific plasma concentration, a direct measure of insulin sensitivity (52,53). Other methods lack this characteristic and merely reflect the whole-body response to a glucose load and not directly insulin sensitivity (54).

In order to fully assess the therapeutic effects CUR+ALA supplementation has on cardiometabolic disease we will also measure resting heart rate and blood pressure. Both components of cardiovascular health are tightly linked with obesity and diabetes. Due to the weight reducing effects CUR and ALA are shown to have in humans and animals, body composition will also be measured, specifically focusing on adipose tissue and fat free mass as both have an impact on insulin sensitivity and energy expenditure (55).

Hypothesis

CUR+ALA supplementation in obese/overweight individuals will cause weight loss and increase RMR and TEF and insulin sensitivity.

Resting heart rate and blood pressure will decrease as a result of 12-weeks of CUR+ALA supplementation.

Specific aims

1) To determine if 12-weeks of dietary supplementation of CUR+ALA compared with a placebo control increases insulin sensitivity in obese/overweight individuals.

2) To determine if 12-weeks of dietary supplementation of CUR+ALA compared with a placebo control increases energy expenditure through major components of TDEE.

3) To establish if 12-weeks of CUR+ALA supplementation compared with a placebo control decreases resting heart rate and blood pressure.

4) To quantify the changes on body composition and mass 12-weeks of CUR+ALA supplementation has compared with a placebo control.

CHAPTER II

COMBINING CURCUMIN AND ALPHA-LIPOIC ACID TO TREAT CARDIOMETABOLIC SYNDROME

INTRODUCTION

At approximately 111 million, the rising number of obese individuals in the United States is roughly equivalent to the entire population of Mexico (56). Consequences are reflected by the 2010 annual medical costs associated with obesity equivalent to $200 billion, more than the gross domestic product of New Zealand (57). These health costs largely arise from co-morbidities associated with obesity such as hypertension, which affects three-quarters of US adults, and individuals with type 2 diabetes, two thirds of whom are overweight or obese (58).

In response, augmenting energy balance through increased physical activity, and/or pharmaceutical interventions has been shown to aid in weight loss (59,60). Supplements are commonly used by Americans in their attempt to achieve the same health benefits as exercise and diet (61). However, many are ineffectual (or harmful) (62,63), and the need for scientific evaluation of supplements should occur before they can be ethically administered.

Two promising supplements are curcumin (CUR) and alpha-lipoic acid (ALA). Both have repeatedly demonstrated health benefits in mice and humans including protection from development of insulin resistance, cardiovascular disease, and cancer (1,5,64). Recent preliminary evidence has shown that dietary combination of CUR+ALA in obese mice resulted in increased energy expenditure and insulin sensitivity. However, this combination has yet to be

studied in overweight/obese humans. In light of animal studies, the potential efficacy of using this intervention to reduce the negative health complications associated with obesity in humans—specifically in regards to improving insulin sensitivity and increasing energy expenditure—is very high, and should therefore be investigated. We hypothesized that 12 weeks of CUR+ALA supplementation in overweight/obese humans would improve cardiometabolic health. Improvements would be manifested through increased insulin sensitivity and increased energy expenditure. Such improvements would also concomitantly lead to decreased resting blood pressure, resting heart rate, and reductions in body mass, specifically through reductions in body fat percentage.

METHODS

Subjects

Inclusion criteria consisted of age range between 18 and 70 years, body mass index greater than 25 kg/m^2, and weight stable for the previous 6-12 months. Exclusion criteria consisted of prior or current use of tobacco, current use of metabolic or cardiovascular medications, pregnant or breast-feeding, or contraindication(s) to exercise as identified via abnormal blood pressure response or abnormal cardiac rhythm (via 12-lead electrocardiogram (ECG)) at rest or during graded exercise. Subjects were encouraged to maintain their current physical activity level and diet. All procedures took place in the Human Performance Clinical Research Laboratory at Colorado State University. The experimental protocol was reviewed and approved by the Institutional Review Board at Colorado State University, Fort Collins, CO.

Subject screening

Each subject was required to partake in a screening visit prior to participation in the study. The screening visit consisted of measurements of blood pressure, heart rate, and analysis of resting cardiac rhythm via 12-lead ECG in supine, sitting and standing positions as well as during incremental stationary cycle erogmetry (Velotron Dynafit Pro, RacerMate, Inc., Seattle, WA, USA), until exhaustion. Participants were outfitted with a two-way non-rebreathing mouthpiece, valve, and headgear device (Hans-Rudolph, St. Louis, MO). The composition of expired gases, as well as ventilation, was measured continuously with a metabolic cart (Parvo TrueOne 2400 Metabolic Measurement System, Parvo Medics, Sandy, UT). Peak oxygen uptake (VO_{2peak}) and respiratory exchange ratio (RER) was calculated by using the four highest consecutive 15-second average VO_2 and corresponding RER values. Dual energy X-ray absorptiometry (Hologic, Discovery W, QDR Series, Bedford, MA, USA) was used to determine body composition.

Experimental protocol

A randomized, double-blind, placebo-controlled design was used for this study. Visits were organized as a pre/post 12-week intervention. Subjects underwent measures of the components of total daily energy expenditure (RMR, TEF, and metabolic response to standardized exercise), and insulin sensitivity via the hyperinsulinemic euglycemic clamp technique. In addition, body mass and composition via DEXA, and resting heart rate and blood pressure were determined.

Each subject was randomly assigned to either placebo or the CUR (2g/day) combined with ALA (.9g/day) treatment after the screening visit. To assess compliance, each participant

was given an excessive amount of capsules for their assigned treatment group. Each subject returned to the laboratory with their unused capsules at weeks 4 and 8. At this time, subjects were provided with capsules for the following 4 weeks. The number of capsules remaining upon return for refills was counted and used to check compliance to the intervention. Subjects who failed to consume 85% of the prescribed capsules were excluded from the remainder of the study.

Resting metabolic rate and thermic effect of feeding

Subjects arrived at the laboratory in the morning after fasting and avoiding vigorous exercise 24 hours prior to testing. Metabolic rate was measured using either a ventilated hood and indirect calorimetry system (Nighthawk Design, Boulder, CO) comprised of a gas analyzer (Perkin Elmer MGA 1100, MA Tech Services, Inc., St. Louis, MO) and ultrasonic flow sensor (ndd Medizintechnik AG, Zürich, Switzerland) or a metabolic cart (Parvo). We remained consistent in use of gas analyzer or Parvo with each subject and distribution of each was evenly distributed between all subjects in both CUR+ALA and placebo groups. The metabolic rate was measured before and after ingestion of a liquid mixed-meal (Ensure Plus, Ross Laboratories; 53% carbohydrate, 32% fat, 15% protein). Oxygen consumption and carbon dioxide production were averaged each minute during collection. The first 15 minutes of the collected gas exchange data were considered a habituation period and excluded from final analysis. The remaining 30 minutes were used to calculate RMR. Values were excluded from final analyses if they were greater than two standard deviations from the mean and/or if respiratory exchange ratio (RER) values were outside of physiological range (0.70-1.00).

After determination of RMR, subjects were fed the liquid mixed-meal, calorically equivalent to 40% of the measured RMR. Subjects were allowed 15 minutes to consume the meal. The postprandial rise in energy expenditure above fasting values represented the TEF, and was measured over the next 4 hours in 20 continuous minute intervals within a 30-minute period. Heart rate and blood pressure were recorded at the end of the initial RMR and at the end of each 20-minute interval.

Hyperinsulinemic-euglycemic clamp

The hyperinsulinemic-euglycemic clamp is considered the "gold standard" measure of insulin sensitivity (52). Briefly, an antecubital vein was catheterized and used for infusion of insulin and glucose, while the contralateral dorsal hand vein catheter was warmed via a heated blanket (Kaz, Inc., Hudson, NY, USA) for arterialized-venous blood sampling. Initially, a descending dose (127.6-40.0 mU/(m body surface area)^2/min) of insulin (Humulin, Eli Lilly and Co., Indianapolis, IN, USA) was administered in the first 10 minutes followed by a continuous dose (40 mU/(m body surface area)^2/min) from 10 to 180 minutes. Administration of 35% dextrose solution (2mg/(kg body mass)/min) began at 4 minutes with adjustments being made to maintain blood glucose concentrations of 90mg/dL. Arterialized venous blood samples (1ml) were obtained every 5 minutes and analyzed for blood glucose concentrations using an automated device (2300 STAT Plus Glucose Lactate Analyzer, YSI Inc., Yellow Springs, OH, USA). The mean rates of glucose infusion during the last 30 minutes of the clamp were used to describe insulin sensitivity.

Venous blood samples were collected immediately before the start of the hyperinsulinemic-euglycemic clamp. Approximately 10ml of venous blood was preserved with

K3 ethylenediaminetetraacetic acid (Vacuette EDTA tubes, Grenier Bio-One North America, Inc., Monroe, NC, USA) and 10ml transferred into a tube transferred into a serum separator tube (Vacutainer SST Tube with Silica Clot Activator, Polymer Gel, Silicone-Coated Interior, BD, Franklin Lakes, NJ, USA). Blood samples collected in chilled EDTA tubes were immediately placed in ice after collection and centrifuged (4°C and 3600rpm) within 60 minutes of collection to isolate plasma. Samples collected in SST tubes were allowed to chill at room temperature for approximately 30 minutes after sample collection. Samples were then centrifuged (4°C and 3600rpm) within 60 minutes of collection to isolate serum. All samples were stored at -80°C until analysis. Plasma insulin concentrations were measured in duplicate using an Insulin Human kit Enzyme-linked immunosorbent assay (ELISA) kit (Abcam, Cambridge, United Kingdom).

Metabolic response to standardized exercise

Measurements of energy expenditure were made utilizing indirect calorimetry (Parvo) during submaximal exercise on a cycle ergometer (Velotron) at three absolute workloads (50, 75, and 125 Watts) in 10-minute durations. During the last five minutes of each 10-minute bout, heart rate, blood pressure, RER, and VO_2 were measured.

Statistical analysis

A two-way analysis of variance (ANOVA; placebo vs. CUR+ALA) with repeated measures (Week 0 vs. Week 12) and Newman-Keuls post hoc analysis was used to examine changes from baseline to post intervention within groups. The level of significance was set at $P < 0.05$. Data are presented as mean ± standard error.

RESULTS

Subject characteristics

A total of 11 subjects were randomly assigned to either the placebo (*n*=5) or CUR+ALA group (*n*=6). Baseline subject characteristics are shown in **Table 1**. All individuals enrolled in the study were between the ages of 18-70 years old, exhibited poor-fair cardiorespiratory fitness based on ACSM guidelines (65), and were categorized as overweight or obese with a BMI greater than 25 kg/m^2. No differences in baseline characteristics were found between groups.

Body mass and composition

Treatment with CUR+ALA did not cause a change in body mass (**Figure 2A)**. Waist circumference, a predictor of disease risk, was unchanged from baseline to post-treatment in both treatments (*P*=0.677) **(Figure 2B).** Body fat percentage decreased in all subjects regardless of treatment (*P*=0.002) (**Figure 2C.**). However, no treatment-time interaction with CUR+ALA or placebo was found (*P*=0.162).

Resting metabolic rate and thermic effect of feeding

Pre- and post-treatment RMR are presented in **Figure 3A**. No differences were observed in the group average RMR post treatment in both groups (*P*=0.239). The TEF for each group is presented as area under the curve (AUC) for five hours following the standardized meal (liquid meal, RMR*0.4) (**Figure 3B)**. No difference in TEF AUC was observed following treatment in either condition (*P*=0.51).

Respiratory exchange ratio and oxygen consumption during varying intensities of exercise

Oxygen consumption (VO_2) was unchanged at varying intensities of cycle erogmetry exercise (50W, P=0.826; 75W, P=0.053; 125W, P=0.381) (**Figure 4A**). Group average RER was unchanged after treatment in both groups (50W, P=0.897; 75W, P=0.648; 125W, P=0.467) (**Figure 4B)**.

Resting heart rate and blood pressure

Resting heart rate and blood pressure were measured during the RMR and are represented as individual values in **Figure 5**. There was no interaction between the treatment and time and resting heart rate (P=0.080) or systolic (P=0.310) and diastolic blood pressure (P=0.382).

Hyperinsulinemic euglycemic clamp

Fasting glucose and insulin were measured prior to start of the hyperinsulinemic euglycemic clamp and are presented in **Figure 6**. No difference was observed after treatment for group averages of fasting glucose (P=0.60) or fasting insulin (P=0.304).

The glucose infusion rates during the last 30 minutes of the hyperinsulinemic euglycemic clamp represents insulin sensitivity (**Figure 7**). There was no interaction between treatment and time in glucose infusion rate (P=0.690)

Table 1. Baseline subject characteristics

Variable	Placebo	CUR+ALA	P Values
	n=5 (1 male, 4 female)	*n*=6 (1 male, 5 female)	NA
Age	44 ± 8	38 ± 7	0.64
Height (cm)	161.9 ± 15.7	165.2 ± 20.8	0.25
Body mass (kg)	83.1 ± 4.0	87.9 ± 3.8	0.42
Body mass index (kg/m^2)	31.9 ± 2.1	32.2 ± 1.2	0.89
Body fat (%)	40.1 ± 2.1	43.8 ± 2.2	0.28
Fat mass (kg)	33.2 ± 3.2	37.9 ± 2.2	0.24
Lean mass (kg)	46.6 ± 1.7	46.6 ± 3.3	0.99
Bone mineral content (kg)	2.2 ± 0.1	2.3 ± 0.1	0.67
VO_{2peak} (L/min)	2.2 ± 0.2	2.1 ± 0.3	0.90
VO_{2peak} (ml/kg/min)	26.8 ± 4.1	24.9 ± 3.7	0.74

Data are presented as mean±SE. NA: not applicable. VO_{2peak}: peak oxygen consumption. Differences between the treatment groups were examined using Student's *t*-test (P<0.05).

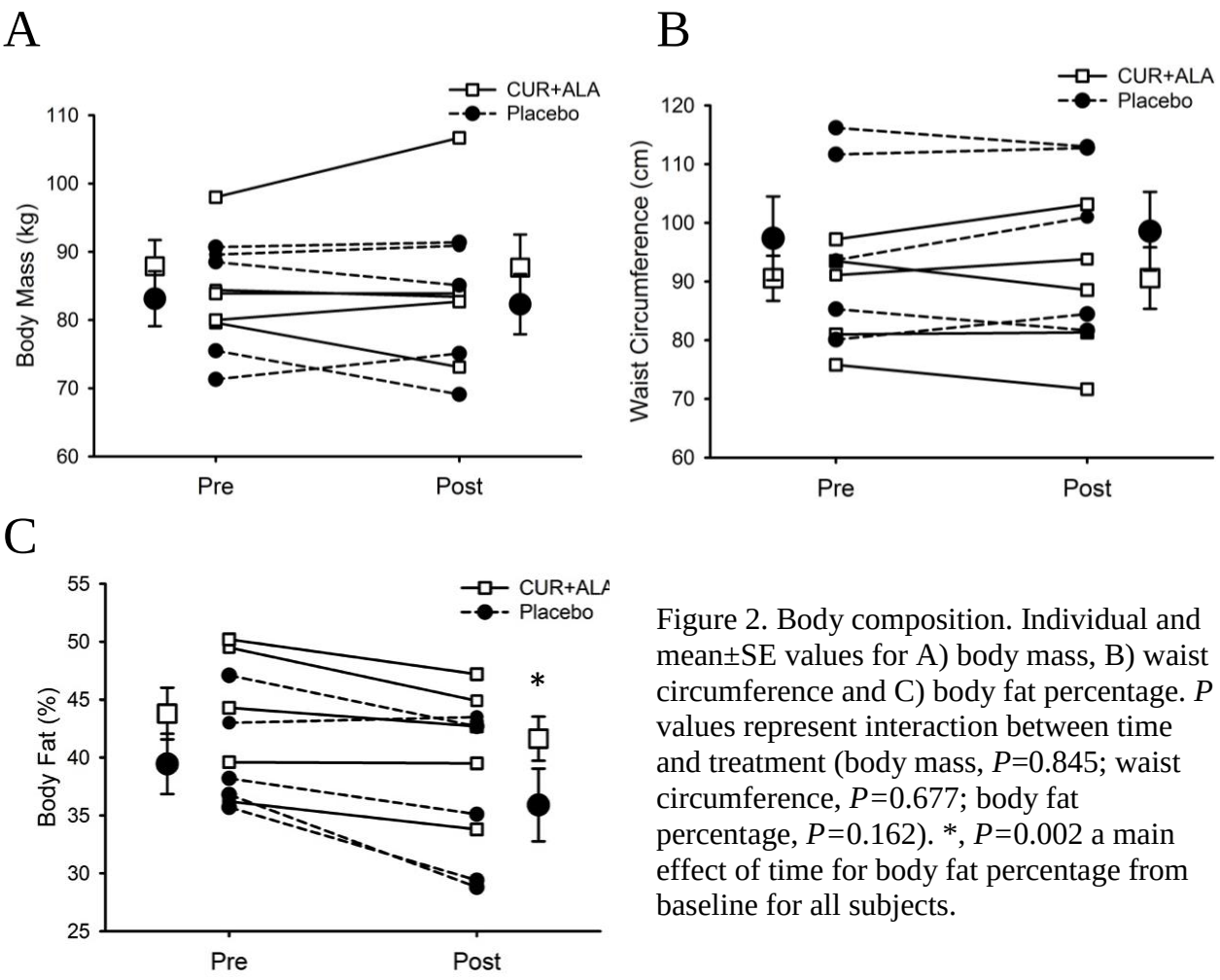
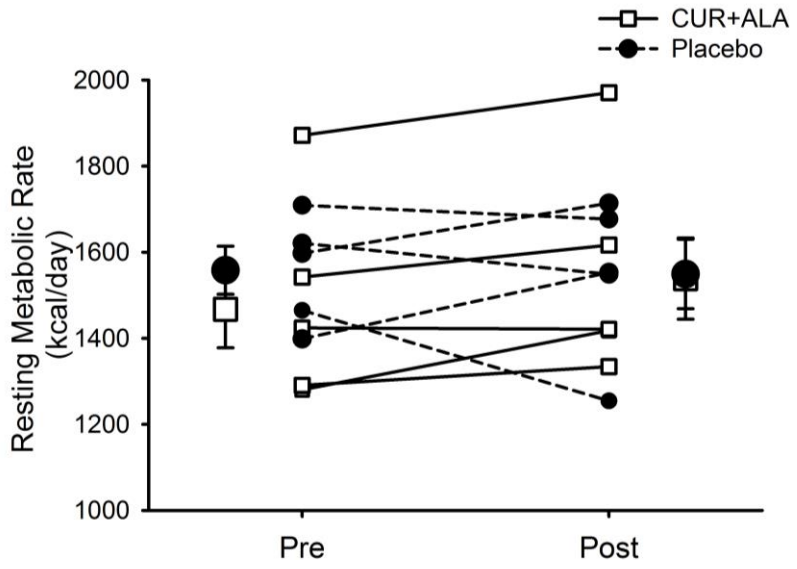


Figure 2. Body composition. Individual and mean±SE values for A) body mass, B) waist circumference and C) body fat percentage. *P* values represent interaction between time and treatment (body mass, P=0.845; waist circumference, P=0.677; body fat percentage, P=0.162). *, P=0.002 a main effect of time for body fat percentage from baseline for all subjects.

A



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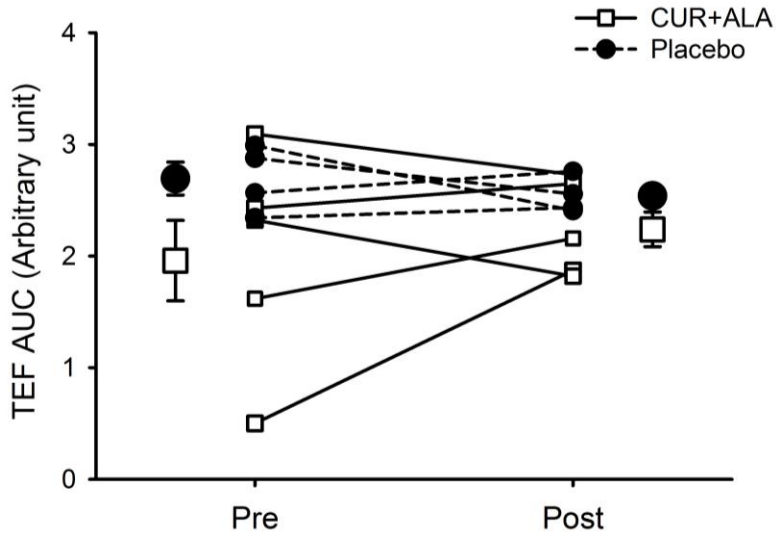
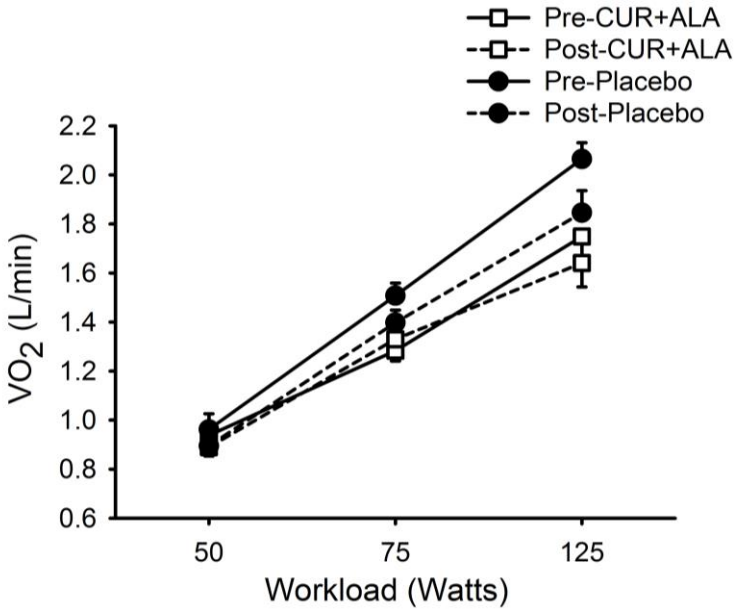


Figure 3. Resting metabolic rate and thermic effect of feeding. Individual and group mean±SE data are presented. A) Resting metabolic rate, P=0.239 for the effect of time and treatment, and B) thermic effect of feeding, area under the curve (AUC), P=0.051 for effect of time and treatment.

A



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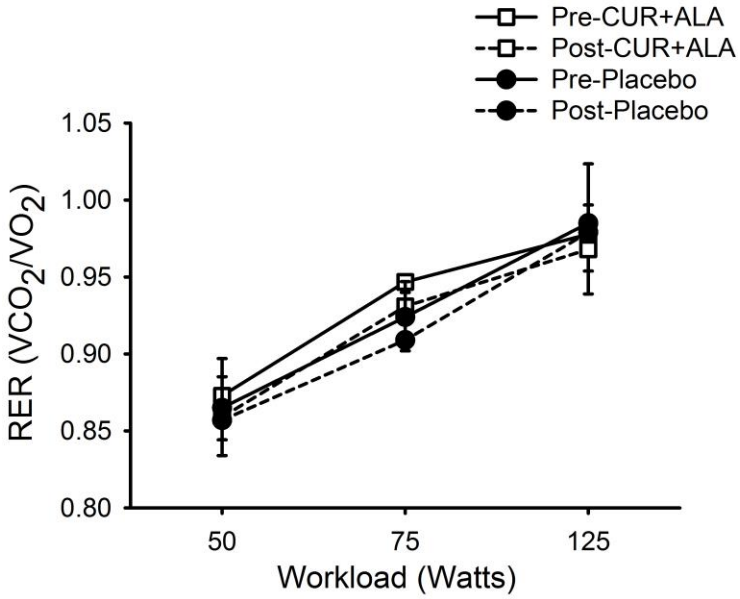
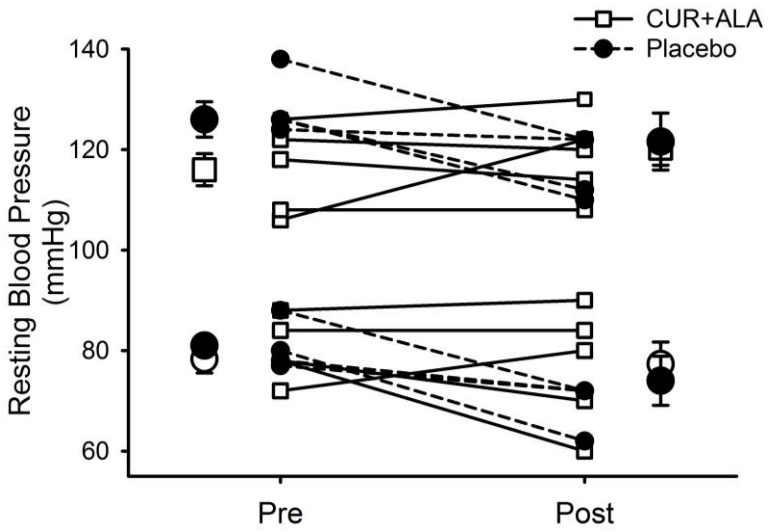


Figure 4. Oxygen consumption (VO_2) and respiratory exchange ratio during exercise at varying levels of intensity. *P* values presented for effect of time and treatment, A) VO_2 (50W, P=0.826; 75W, P=0.053; 125W, P=0.381) and B) RER, (50W, P=0.897; 75W, P=0.648; 125W, P=0.467). Data is presented as group mean±SE.

A



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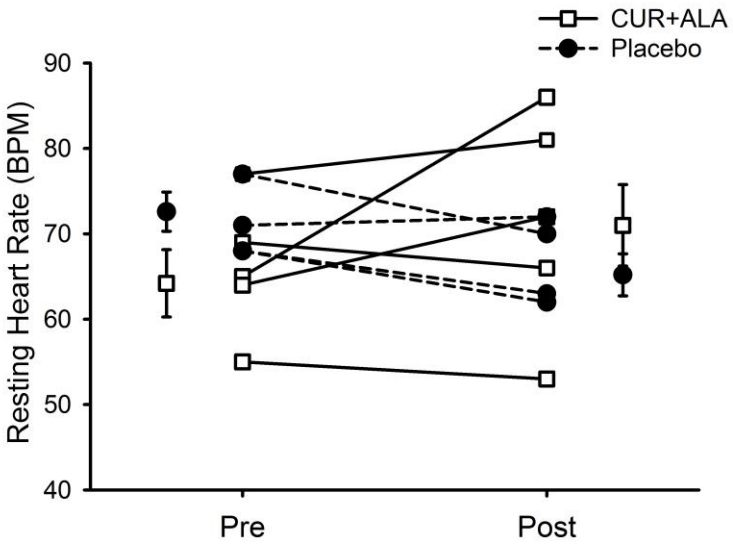
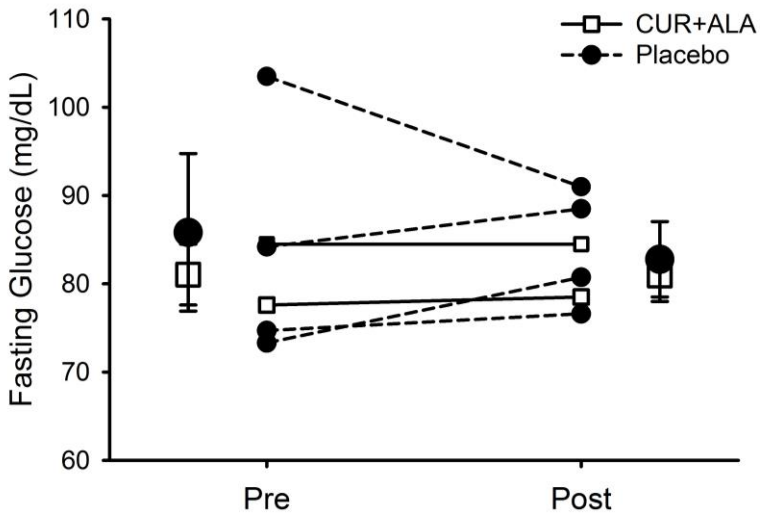


Figure 5. Resting heart rate and blood pressure. Individual A) systolic and diastolic blood pressure did not change due to treatment (P=0.31, P=0.382, respectively). No change occurred as an effect of treatment for B) resting heart rate, P=0.08.

A



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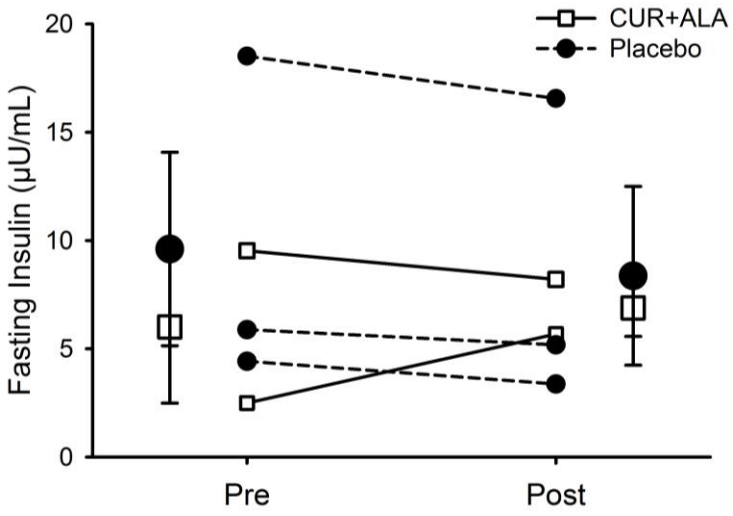

Figure 6. Individual fasting glucose and fasting insulin concentrations. A) Fasting glucose, and B) fasting insulin were measured prior to initiation of the hyperinsulinemic euglycemic clamp before and after treatment. No effect of time and treatment was found for glucose (P=0.60) or for insulin (P=0.304).

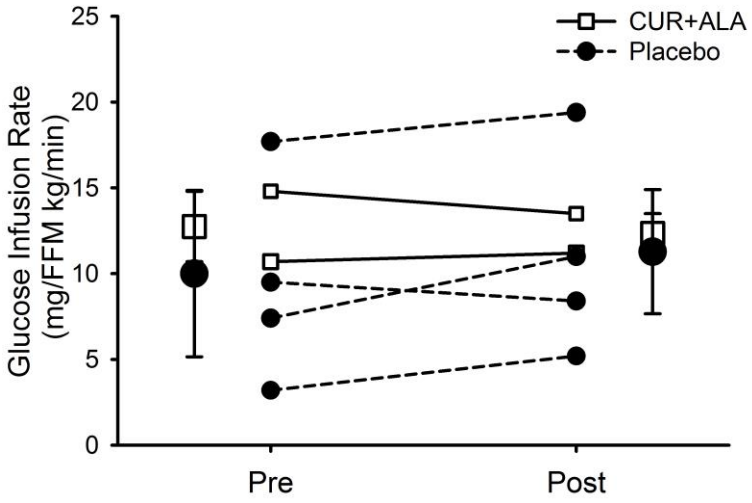

Figure 7. Glucose infusion rate during the last 30 minutes of the hyperinsulinemic euglycemic clamp before and after treatment. Individual glucose infusion rates are reported. No difference was found for time and treatment, *P*=0.690. FFM kg: total fat free mass in kilograms.

DISCUSSION

Separately, CUR and ALA have beneficial effects on cardiovascular and metabolic health (39,66–70). However, CUR and ALA in combination have never been evaluated in humans. In this pilot study, we determined that 12-weeks of oral CUR+ALA supplementation in overweight humans did not improve cardiometabolic health: no increase in RMR, TEF, or insulin sensitivity, no decrease in body mass, and no change in body composition.

Components of energy expenditure

Based on previous work and preliminary data showing the thermogenic properties of CUR+ALA in obese and type 2 diabetic mice, we proposed CUR+ALA would increase energy expenditure and result in weight loss in overweight humans. However, no change was detected

relative to a placebo control in RMR and TEF, two major components of TDEE. Consistent with this observation, body mass and composition were also unaffected.

CUR and ALA separately increase energy expenditure through activation of AMPK in skeletal muscle and hepatocytes (12,43). Heightened AMPK activity stimulates glucose uptake through increased GLUT-4 protein content and translocation (71). AMPK also causes phosphorylation of ACC, reducing malonyl-CoA production, an inhibitor of fatty acid entry into the mitochondria through CPT-1. This effect ultimately increases fatty acid oxidation, reducing accumulation of TG in hepatocytes and skeletal muscle (72). Insulin sensitivity is also positively affected through the same mechanism (73). However, AMPK activation was not measured in this study and we therefore cannot conclude CUR+ALA had an effect on this kinase and downstream mechanisms.

Insulin sensitivity

Previous studies have shown CUR and ALA separately increase glucose uptake in skeletal muscle through AMPK stimulated translocation of GLUT4 proteins (15). Additionally, AMPK activation by CUR and ALA is thought to suppress hepatic gluconeogenic pathways and enhance fatty acid oxidation (12). Increased fatty acid oxidation can prevent accumulation of fatty acid intermediates which activate PKC-θ, an inhibitor of the insulin receptor (16). We hypothesized that CUR+ALA would increase insulin sensitivity; however, no differences in glucose infusion rates were found in the treatment group indicating CUR+ALA did not increase whole-body insulin sensitivity. The lack of response to CUR+ALA supplementation on insulin sensitivity could be attributed to insufficient AMPK activation. This would result in no change in fatty acid oxidation rates, GLUT-4 translocation and content, or decreases in hepatic glucose

output. Additionally, PKC-θ activation by fatty acid intermediates may still occur and further insulin resistance.

Bioavailability of orally supplemented curcumin and alpha-lipoic acid

The lack of effect on cardiometabolic health from CUR+ALA supplementation may be a result of inadequate bioavailability. Although plasma concentrations of CUR and ALA were not measured, we hypothesize limited absorption in the gut and rapid elimination could have prevented CUR and ALA from having their therapeutic effect. In fact, low bioavailability of both CUR and ALA separately have previously been reported when administered orally in capsule form (68,74). Studies that have reported low bioavailability have accordingly shown limited improvements in metabolic and cardiovascular health.

Dosage is an important factor to mention when evaluating bioavailability. Based on previous studies, our dosage of 2.0g/day of CUR and 0.9g/day of ALA should have been sufficient to cause an increase in plasma concentration without adverse side effects. Previous studies have determined doses as low as 0.5g/day of CUR and 0.5g/day of ALA are able to appear in plasma after 2 hours post-ingestion in humans (75,76). Furthermore, doses as high as 12g/day of CUR and 1.2g/day of ALA have been shown to be well tolerated in humans (2,76). Unfortunately, increasing the dose may not result in higher plasma concentrations or beneficial effects. This relationship has previously been demonstrated in dose escalation studies of CUR and ALA where no significant difference in beneficial effects were detected in doses ranging from 1.2-8.0g/day for CUR and 0.6-2.4g/day for ALA (29,77). Consideration should also be made for the practicality of administering a larger amount and/or increasing the frequency of supplement administration. In our study subjects were required to consume a total of 6 capsules

accounting for 2.9g/total of combined CUR and ALA. Administering more than this amount may prevent many individuals from using CUR+ALA supplementation as a therapeutic agent due to inconvenience. The number of individual capsules, amount of supplement relative to diet, and the frequency in which one would need to consume a greater quantity of supplement could limit its use if the administered amount was increased. The appropriate dose used when translating animal to human studies is also an important factor. Based on previously developed equations which account for body surface area rather than weight, the dose provided in our study was equivalent to 0.274g/kg of CUR and 0.123 g/kg of ALA in mice (78). Such doses in animal studies have been shown to have beneficial effects, thus we can assume the doses used in this study were appropriately translated. Still, the absence of beneficial effects on cardiometabolic health in this study requires further evaluation of bioavailability.

Alternatively, more easily absorbed derivatives of CUR exist which possess greater therapeutic effects compared to the naturally occurring compound (2,79–81). These CUR derivatives exhibit higher efficacy in reducing inflammatory cytokines compared to natural CUR (81). CUR derivatives like CNB-001 have been shown to target and prevent activation of PTP1B, an inhibitor of insulin signaling in mice (26). However, no studies have been performed in humans utilizing such derivatives of CUR.

Intravenous administration of CUR+ALA is also a possible alternative to oral administration. Multiple studies have shown intravenous administration of CUR and ALA have greater bioavailability determined through plasma concentration compared to oral supplementation (76,82). However, this method is inconvenient and not easily accessible to the general public compared to oral supplementation.

Another method that may promote greater CUR+ALA bioavailability is delivery via liposomes. Liposomes are spherical vesicles made of a phospholipid-bilayer which can be used as a vehicle for lipid- and water-soluble drug administration (83). Liposomes have been shown to increase therapeutic index, reduce degradation, and prolong biological-half life of their contents (84). Utilization of liposomes have heightened drug absorption and activity for numerous drug classes including antitumor agents, antivirals, and antibiotics (85). The use of liposomes as a vehicle for delivery of CUR+ALA could provide greater bioavailability through prevention of degradation in the gut and increased absorption into blood and tissue.

Strengths and limitations

In this study we utilized a randomized, double-blind, placebo-controlled design. Baseline subject characteristics were not significantly different. A crossover design would have strengthened this study. However, the logistics of a washout period and the length of treatment make a crossover design burdensome to subjects. Radio labeled CUR has been shown to not be detected in serum of humans after 72 hours of ingestion (24). This evidence and other studies evaluating CUR in humans utilizing a washout period suggest a washout period of 7-14 days to be appropriate (23,86). However, such studies have only measured CUR in serum and plasma of humans and not in tissue, an area where CUR could accumulate and have a longer half-life.

The length of intervention was selected to investigate an effect of chronic administration of CUR+ALA rather than an acute dose. An acute dose would most likely not improve cardiometabolic health due to the chronic-nature and pathology of metabolic and cardiovascular disease. Indeed, an acute dose of an antioxidant like vitamin C is able to reverse endothelial dysfunction in people with hypertension (87), however weight loss with an acute dose is most

likely undetectable. A small increase in energy expenditure maintained over time will lead to a negative energy balance if energy intake is maintained and ultimately result in weight loss (88). This effect is difficult to capture over a short period of time, and is often reason why weight loss studies typically range from 4 weeks to 6 months in duration (89). Nevertheless, the short half-lives of CUR and ALA could more accurately represent an acute effect of each in the human body, and future studies should consider increasing frequency of doses throughout the day if to truly determine chronic effects of each compound.

Our sample size of 11 subjects is a limitation. Nevertheless, this study was designed to provide preliminary data and the lack of significant change in primary outcome variables is most likely an indication that CUR+ALA supplementation was not efficacious in treating cardiometabolic disease. We also had more females than males enrolled in the study and results may represent the effects of CUR+ALA supplementation in overweight women rather than men. Interestingly, CUR is absorbed more efficiently in women compared to men, a characteristic attributed to increased expression of hepatic drug efflux transporters in men (90). A smaller volume of distribution due to differences in body fat and blood volume may also be reason why women are reported to absorb more CUR than men. Also of importance to mention is the fact that many of the women in our study were post-menopausal. This characteristic can eliminate potential effects on absorption resulting from fluctuating hormones of menstrual cycle (91). Regardless, we were unable to observe any difference in absorption our study.

Another limitation of this study is the decision to not have separate CUR and ALA groups. Because of this, we were unable to tease out separate effects of each supplement. Regardless, our study hypothesis was focused on the combined effects of CUR and ALA. The thermogenic, anti-inflammatory and antioxidant properties of each compounds have been well

established in previous studies (39,64,66,67). The design of our groups in this study also meant we were able to have bigger subject numbers per treatment. Thus, the novelty of this study arrives from evaluating the combined effect of CUR and ALA.

Our study did not control for diet and physical activity during the 12-week supplementation period. Accordingly it is possible that modifications to diet or physical activity level could have occurred during the course of the study. Increases in energy expenditure of 100-200kcal/day from supplementation of CUR+ALA may not have been detected in this study and increases of that magnitude in kcal/day would easily be masked by alterations in physical activity and diet. Although an increase of 100-200kcal may seem insignificant, it could assist in weight maintenance (92). Such a role has been proposed for brown-adipose tissue thermogenesis in humans (93). In fact, ALA has been shown to cause brown-like remodeling of white adipose tissue in humans (94). In future studies dietary logs and physical activity monitors could be utilized to track and account for changes in such variables.

Summary

The goal of this pilot study was to determine if 12-weeks of oral supplementation of CUR+ALA in overweight humans would improve cardiometabolic health, assessed through changes in energy expenditure, insulin sensitivity, and cardiovascular health. However, no beneficial changes in these variables occurred as a result of CUR+ALA supplementations. Despite promising evidence from animal studies, these preliminary data do not suggest the use of CUR+ALA to improve cardiometabolic health in overweight humans.

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APPENDIX

Consent Form

Consent to Participate in a Research Study Colorado State University

TITLE OF STUDY:
Pilot Clinical Study Evaluating the Efficacy of Curcumin and Alpha-Lipoic Acid to Increase Insulin Sensitivity and Augment Energy Expenditure in Overweight/Obese Adult Humans

PRINCIPAL INVESTIGATOR:
Christopher Bell, Ph.D.
Department of Health and Exercise Science
Colorado State University
Fort Collins
CO 80523-1582

Telephone: 970-491-3495
Fax: 970-491-0445
Email: physiology@cahs.colostate.edu

WHY AM I BEING INVITED TO TAKE PART IN THIS RESEARCH?
You are an adult aged between 18 and 70 years. Your body mass index (weight in kg divided by height squared in m) is greater than 25 kg/m^2, and/or your fasting blood glucose concentration is greater than 100 mg/dL.

WHO IS DOING THE STUDY?
Christopher Bell, Ph.D., an associate professor in the Department of Health and Exercise Science at Colorado State University. Appropriately qualified staff and trained graduate and undergraduate students will assist.

WHAT IS THE PURPOSE OF THIS STUDY?
The purpose of this study is to determine the effect of short-term (12 weeks) dietary supplementation on energy expenditure (calories burnt) and control of blood sugar in adults. Specifically, the purpose of this study is to determine whether the dietary supplement will increase total daily energy expenditure, lead to weight loss, and/or improve control of blood sugar.

WHERE IS THE STUDY GOING TO TAKE PLACE AND HOW LONG WILL IT LAST?
The study will take place in the Human Performance Clinical/Research laboratory (HPCRL) in the Department of Health & Exercise Science (Moby Complex) at Colorado State University.

This whole research project will take place over a period of approximately two years. You will be asked to be involved for approximately 12-16 weeks. The total time of your participation will consist of 9 visits (total time ~ 26 hours).

WHAT WILL I BE ASKED TO DO?
You will be asked to visit our laboratories on nine separate occasions. Visit 1 will comprise a screening visit. Visits 2 and 7 will involve measurement of the rate in which you burn calories at rest and after eating. Visits 3 and 8 will involve exercise on an exercise bike. Visits 4 and 9 will involve measurement of blood sugar control. Visits 5 and 6 involve collecting the dietary supplement from the lab and dropping off any unused supplement. Visits 7, 8 and 9 will take place approximately 12 weeks after visits 2, 3 and 4; during this time you will eat four capsules per day, every day (2 capsules in the morning and 2 capsules in the evening). The capsules will either contain a placebo (something that has no effect) or a combination of natural ingredients.

Capsules
You will not know the contents of the capsules to which you have been assigned until the end of the study. The capsules will either contain a placebo (something that has no effect) or the following ingredients:

Curcumin – 0.5 g
Alpha-Lipoic Acid – 0.225 g

Curcumin is a natural ingredient in a spice usually found in Indian foods (e.g. curry). The spice is turmeric.

Alpha-Lipoic Acid is a very common ingredient in multivitamin formulas. It is also found in some types of meat (e.g. heart, kidney, and liver) and in small amounts in some fruits and vegetables.

We believe this combination of ingredients will improve your ability to control how many calories you burn and control your blood sugar.

You will be asked to swallow four capsules every day for 12 weeks: two capsules every morning, and two capsules every evening. At the beginning of every 4-weeks of the 12-week intervention (weeks 0, 4, and 8), you will be given capsules and asked to return any unused capsules at the end of the 4-weeks.

Visit 1 - Screening Visit ~ 2 Hours
The first visit to the HPCRL will be a screening visit. During this visit we will make sure that participation in this study is right for you.

This screening visit will include the following procedures:

Medical Questionnaire
You will be asked to answer several pages of questions related to your health, any illness you may have or have had, and medications you use or have used in the past.

Blood Pressure
We will measure your blood pressure using a standard blood pressure cuff (the same as in a doctor's office). Blood pressure will be measured during all of the tests performed in the lab with the exception of body composition. There are no known risks associated with this procedure. (Duration: 5 minutes)

Body Composition
We will measure how much fat you have in your body using a test called dual energy x-ray absorptiometry (DEXA). The DEXA test requires you to lie quietly on a padded table while a small probe gives off low-level x-rays and sends them over your entire body. This test gives very accurate measurements of your body fat and bone mineral density. We will also measure the circumference of your waist and hip using a tape measure. (Duration: ~ 15 minutes)

Exercise Stress Test
You will be asked to perform a vigorous exercise test. This test will tell us if your heart is healthy. You will be asked to walk on a motorized treadmill or ride an exercise cycle (cycle ergometer) for approximately 10-15 minutes. The exercise will become more difficult every 2 minutes. While you are walking/riding we will measure your heart rate with an electrocardiogram (ECG) and your blood pressure with a cuff placed around your upper arm. We will also ask you to wear a nose clip (something that stops you breathing through your nose) and ask you to breathe through a mouthpiece. This will let us measure the gases you breathe. Depending on your age, a physician may supervise the test. If we do not think your heart is healthy you will be referred to your primary care physician for further testing. There is a chance that you may not be allowed to take part in our study. (Duration: ~ 60 minutes)

Visit 2 and Visit 7 – Calories Burnt at Rest and During and After Feeding
We will measure the amount of calories you burn at rest. This is called your resting metabolic rate. This test will take place while you are lying down on a bed. A plastic transparent bubble will be placed over your head and shoulders and room air will be pumped in through a pipe. We will measure the air that you breathe in and out. There are no known risks associated with this procedure. You will perform this test twice; each test lasts approximately 1-hour.

You will then be asked to eat/drink a liquid meal. The meal will be consist of an energy drink called Ensure; this drink is available for purchase by anyone from most large grocery stores. The total volume you will be asked to consume will be based on your resting metabolic rate (i.e. the amount of calories you burn at rest). We will again

measure the amount of calories you burn after consuming the liquid meal. Like the one-hour test, you will be lying down with a bubble over your head while we measure the air that you breathe in and out. This time the test will last for 5 hours.

During these visits we will be taking blood from you. We will be taking approximately 120 ml (~8 table spoons) during each visit; this is a lot less than the amount that is typically taken when a person donates blood. Your blood will be tested for various things that are involved with your nerves, the amount of calories you burn at rest, and insulin and glucose (blood sugar). Your blood will be taken from veins in your arms or hands using needles and hollow plastic tubes called catheters.

In total, this visit will last approximately 6 hours.

Visit 3 and Visit 8 – Cycle Exercise

You will be asked to ride a stationary exercise bike for approximately 30 minutes. For the first 10 minutes the intensity will be very, very light. During the second 10 minutes the intensity will be increased but will still be moderate. During the final 10 minutes the intensity will be increased again such that it is quite difficult, but still achievable. Throughout the entire ride we will measure your heart rate with an electrocardiogram (ECG) and your blood pressure with a cuff placed around your upper arm. We will also ask you to wear a nose clip (something that stops you breathing through your nose) and ask you to breathe through a mouthpiece. This will let us measure the gases you breathe. You will only wear the mouthpiece during the final 5 minutes of each 10-minute period.

During these visits we will be taking blood from you. We will be taking approximately 80 ml (~6 table spoons) during each visit; this is a lot less than the amount that is typically taken when a person donates blood. Your blood will be tested for various things that are involved with your nerves, the amount of calories you burn at rest, and insulin and glucose (blood sugar). Your blood will be taken from veins in your arms or hands using needles and hollow plastic tubes called catheters.

During visit 8 we will also repeat the measurement of body composition.

Visit 3 will last ~45 minutes, visit 8 will last ~ 1 hour.

Visit 4 and Visit 9 – Measurement of Blood Sugar Control and Cutting Little Pieces of Muscle from Your Legs

This procedure is formally known as the hyperinsulinemic euglycemic clamp and often shortened to “The Clamp”. This procedure measures the ability of your body to control sugar. This test will take place early in the morning after a 12-hour fast, and abstention from alcohol, caffeine and exercise. We will inject sugar (glucose) and insulin (a naturally occurring substance produced by your body) into one of your veins. From another vein we will sample a very small amount of blood (~ 1 ml or 0.2 teaspoons) every 5-minutes for three hours (total amount of blood sampled ~ 36 ml or 7.3 teaspoons). We will measure the amount of sugar in each of these blood samples. We

will continue to inject insulin and sugar into your veins to try to keep the concentration of sugar in your blood the same. In order to prepare for the blood sugar tests, you will be asked to refrain from exercising for 48 hours, and avoid alcohol and caffeine for 12 hours. The night before the blood sugar test you will be asked to eat a standardized meal (a high-energy drink (e.g. Ensure) and a high-energy snack (e.g. Powerbar)).

Cutting Little Pieces of Muscle from Your Legs
This test is commonly called a muscle biopsy. During the muscle biopsy a drug (an anesthetic) will be injected into an area of your thigh to make it feel numb. A small incision (roughly 1/4 inch) will be made using a sharp sterile blade. A sterile probe will be inserted into your leg and a little piece of muscle (roughly the size of a sweet corn kernel) will be removed. This procedure will be performed at the beginning and the end of visit 4, and the beginning and the end visit 9.

Visits 4 and 9 will last approximately 5 hours.

SUMMARY OF VISITS
Visit 1 Screening
Visit 2 Calories Burnt at Rest and During and After Feeding
Visit 3 Cycle Exercise
Visit 4 Measurement of Blood Sugar Control and Cutting Little Pieces of Muscle from Your Legs
Collect 4-weeks of capsules (weeks 0-4)
Visit 5 Collect 4-weeks of capsules (weeks 4-8)
Visit 6 Collect 4-weeks of capsules (weeks 8-12)
Visit 7 Calories Burnt at Rest and During and After Feeding
Visit 8 Cycle Exercise and Body composition
Visit 9 Measurement of Blood Sugar Control and Cutting Little Pieces of Muscle from Your Legs

ARE THERE REASONS WHY I SHOULD NOT TAKE PART IN THIS STUDY? You will not be allowed to participate in these studies for any of the following reasons:

1) You are not aged between 18 and 70 years.
2) You are pregnant.
3) You are a nursing mother.
4) You smoke or have smoked during the previous two years.
5) Following: a) review of your medical history, b) physical examination, and, c) ECG and blood pressure at rest and during incremental exercise the research team have identified a physiological characteristic/condition that may increase the likelihood of an unfavorable event during the study.
6) Your participation has not been approved by a physician or by a senior member of the research team.
7) You are taking medications that would confound interpretation of the results of the studies.
8) You are unwilling and/or unable to perform cycle ergometer exercise.

9) Your body mass index is less than 25 kg/m^2 (we will calculate this for you) and your fasting blood glucose is less than 100 mg/dL.
10) You are participating in another study that may affect your body weight or blood sugar.

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?
It is not possible to identify all potential risks in research procedures, but the researcher(s) have taken reasonable safeguards to minimize any known and potential, but unknown, risks. The Human Performance Clinical Research Laboratory has emergency supplies including a medicine trolley equipped with heart machines and supplemental oxygen. The investigators have a great deal of experience with all of the procedures. Some of the procedures for which you are being asked to volunteer have a number of associated risks:

Body Composition
The risks associated with the DEXA are very low. The maximum radiation dose you will receive in this study is less than 1/3000th of the federal and state occupational whole body dose limit allowed to radiation workers. Put another way, you will receive less than 1.3 mrem from this scan and you already receive approximately 450 mrem /year from normal background radiation dose in Colorado. The more radiation you receive over the course of your life, the more the risk increases of developing a fatal cancer or inducing changes in genes. The radiation dose you receive from this scan is not expected to significantly increase these risks, but the exact increase in such risks is not known. There are no discomforts associated with this procedure.

Exercise Tests
There is a very small chance of an irregular heartbeat during exercise (less than 1% of all subjects). Other rare risks of a stress test are heart attack (less than 5 in 10,000) and death (less than 2 in 10,000). Wearing a mouthpiece and nose-clip can sometimes cause dryness in the mouth and mild discomfort. After any exercise your muscles might ache.

Capsules
If you feel unwell while taking the capsules you should stop, and contact the research team immediately. The capsules may give you an upset stomach or make you feel sick.

Blood Collection
When the needle goes into a vein, it may hurt for a short period of time (a few seconds). Also there may be minor discomfort of having the needle/plastic tube taped to your arm. In about 1 in 10 cases, a small amount of bleeding will occur under the skin that will cause a bruise. The risk of forming a blood clot in the vein is about 1 in 100, and the risk of significant blood loss is 1 in 1,000. Additionally, there is a risk that you may faint while having blood collected or having the catheter inserted in your vein.

Measurement of Calories Burnt at Rest and During and After Feeding
You may feel claustrophobic when you are under the bubble. You will be able to remove the bubble at any time during the tests should you feel claustrophobic.

Blood Sugar Test
The procedure involves placement of a catheter (hollow plastic needle) inside a vein thus the usual risks of blood collection apply (minor discomfort, bruising, fainting and blood clot (rare)). In addition there is a risk of hypoglycemia (low blood sugar); symptoms include hunger, nervousness and shakiness, perspiration, dizziness or light-headedness, sleepiness, confusion, difficulty speaking, and feeling anxious or weak. Although hypoglycemia can happen suddenly it can usually be treated very quickly by stopping the insulin infusion and continuing the glucose infusion, returning blood sugar concentration back to normal. To reduce the risk of hypoglycemia, blood glucose concentration is measured every 5 minutes. If blood glucose concentration falls below 65 mg/dL insulin administration will be stopped and glucose infusion continued until normal concentration (greater than 70 mg/dL) is resumed; this usually occurs very quickly (~ 5 minutes).

Muscle Biopsy
During the procedure you may feel discomfort associated with the injection of the numbing drug (the anesthetic) but during the actual muscle removal the discomfort should be minimal. There is a risk that you may faint during the procedure. There is also a risk of muscle cramp, bleeding, of loss of feeling in your leg, and of damage to a skin (cutaneous) nerve. The risk of infection and bruising is extremely small if you follow the instructions for caring for the incision. A very small and minor scar will remain as a result of the incision, but may not be noticeable. These procedures will be performed under surgically clean conditions. Emergency medical equipment will be available. You will be screened prior to the procedure for history of allergic reactions to Novocain (Lidocaine).

FUTURE USE OF BLOOD OR MUSCLE SAMPLES
It is possible that we may want to use any leftover blood or muscle tissue for future research not described in this consent form. We will keep private all research records that identify you (to the extent allowed by law) for both current *and* future use (please refer to the section, “WHO WILL SEE THE INFORMATION THAT I GIVE” for more information regarding privacy). Future research will pertain to physiological function. Choose only one of the following:

_____ I give permission for the use of my blood or muscle tissue collected as part of the current study only.

_____ **(your initials)**

_____ I give permission for the use of my blood or muscle tissue for the current study as well as for future studies.

_____ **(your initials)**

ARE THERE ANY BENEFITS FROM TAKING PART IN THIS STUDY?
There are no direct benefits in participating, however you will receive a copy of your results and information pertinent to your body composition (i.e. height and weight). You may lose weight. Your fasting blood sugar (glucose) may decrease. You will be provided with a copy of your DEXA scan; you may wish to have this interpreted by a medically qualified professional. Finally, this study has the potential to identify strategies that will improve health and physical performance.

DO I HAVE TO TAKE PART IN THE STUDY?
Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to which you are otherwise entitled.

WHAT WILL IT COST ME TO PARTICIPATE?
Other than transport to and from the lab, your participation should incur no costs.

WHO WILL SEE THE INFORMATION THAT I GIVE?
We will keep private all research records that identify you, to the extent allowed by law.

For this study, we will assign a code to your data (e.g. 123ABC) so that the only place your name will appear in our records is on the consent and in our data spread sheet that links you to your code. Only the research team will have access to the link between you, your code, and your data. The only exceptions to this are if we are asked to share the research files for audit purposes with the CSU Institutional Review Board ethics committee, if necessary. In addition, for funded studies, the CSU financial management team may also request an audit of research expenditures. For financial audits, only the fact that you participated would be shared, not any research data.

Your identity/record of receiving compensation (NOT your data) may be made available to CSU officials for financial audits.

Some of your blood and muscle will be sent to the University of Wyoming for analysis. The blood and muscle samples will be labeled with a code making it impossible to identify you from the samples.

CAN MY TAKING PART IN THE STUDY END EARLY?
Your participation in the study could end if you miss any of the scheduled appointments.

WILL I RECEIVE ANY COMPENSATION FOR TAKING PART IN THIS STUDY? You will receive $400 for taking part in this study ($150 for completing visits 2, 3 and 4, and $250 for completing visits 7, 8 and 9). Should your participation in the study end early, you will still receive feedback pertaining to your health and fitness.

WHAT HAPPENS IF I AM INJURED BECAUSE OF THE RESEARCH?
The Colorado Governmental Immunity Act determines and may limit Colorado State University's legal responsibility if an injury happens because of this study. Claims

against the University must be filed within 180 days of the injury. Participants should check with their insurance as to their coverage in the event of a research injury.

WHAT IF I HAVE QUESTIONS?
Before you decide whether to accept this invitation to take part in the study, please ask any questions that might come to mind now. Later, if you have questions about the study, you can contact the investigator, Christopher Bell, or members of the research team via email (physiology@cahs.colostate.edu) or telephone 970-491-3495. If you have any questions about your rights as a volunteer in this research, contact Janell Barker, Human Research Administrator at 970-491-1655. We will give you a copy of this consent form to take with you.

This consent form was approved by the CSU Institutional Review Board for the protection of human subjects in research on **08/15/2013**.

WHAT ELSE DO I NEED TO KNOW?
Your signature acknowledges that you have read the information stated and willingly sign this consent form. Your signature also acknowledges that you have received, on the date signed, a copy of this document containing 9 pages.

_____ _____
Signature of person agreeing to take part in the study Date

_____ _____
Printed name of person agreeing to take part in the study Time of Day

_____ _____
Name of person providing information to participant Date

_____
Signature of Research Staff