Effects of creatine and caffeine co-supplementation on body composition and muscle performance in trained adults

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4. Methods

4.1Participants

An *a priori* power analysis (G*Power v. 3.1.5.1) indicated that 48 participants are required. This calculation is based on a small effect size (Cohen's $f = 0.25$), an alpha level of .05, and a ß-value of 0.8 for a repeated measures, within-between analysis of variance (ANOVA) design. Males (18-30 years of age) who have been performing structured resistance training (> 3x/week for ≥ 6 months) prior to the start of the study will be recruited. Resistance trained participants are being recruited to help reduce the influence that neuromuscular learning has on changes in muscle mass and muscle performance over time. Prior to the start of supplementation and training, participants will fill out a questionnaire which assesses their readiness for participation in resistance training. Participants will also fill out a leisure time exercise questionnaire, which indicates the average number of times that strenuous (i.e. heart beats rapidly), moderate (i.e. not exhausting), and mild exercise (i.e. minimal effort) is performed per week. Participants will be excluded from the study if they have taken medications that affect muscle biology or creatine monohydrate ≤ 12 weeks prior to the start of resistance training and supplementation; if they have diseases that are known to affect muscle biology (i.e. Crohn's Disease), if they have pre-existing kidney or liver abnormalities, or if they plan to travel ≥ 1 week during the study where they have no access to a fitness facility. Habitual caffeine consumers are being included because regular caffeine consumption does not negate the beneficial effects of caffeine supplementation. Furthermore, habitual caffeine consumers who withdraw from caffeine may experience adverse symptoms which could negatively affect exercise performance. Participants will be instructed not to change their habitual diet or engage in additional physical activity that is not part of their normal daily routine or consume non-steroidal anti-inflammatory drugs during resistance training and

supplementation, as these interventions can affect muscle protein synthesis. The study will be approved by the Research Ethics Board at the University of Regina. Participants will be informed of the risks and purposes of the study before written consent is obtained.

4.2 Research Design

The study will be a double-blind, repeated measures design. In order to minimize group differences, participants will be matched according to age and weight and be randomized on a 1:1:1:1 basis to one of four groups: Creatine + Caffeine (CR-CAF; 0.1 g/kg of creatine monohydrate powder + 3 mg/kg of caffeine [micronized powder]); Creatine (CR; 0.1 g/kg of creatine monohydrate powder + 3 mg/kg of caffeine placebo [micronized cellulose powder]), Caffeine (CAF; 3 mg/kg of caffeine + 0.1 g/kg of creatine monohydrate placebo [maltodextrin]) or placebo (PLA; 0.1g/kg creatine monohydrate placebo + 3 mg/kg of caffeine placebo). An individual, blinded to supplement and group allocation, will be responsible for the preparation of study kits. Each study kit will contain the participants supplement for the duration of the study, detailed supplementation instructions, measuring spoons, supplementation compliance log, daily caffeine consumption log and a resistance training log. Supplement powders will be similar in energy content, color, taste, texture, and appearance. The creatine dosage of 0.1 g/kg has previously been shown to be effective for increasing muscle mass and muscle performance. The caffeine dosage of 3 mg/kg has been shown to increase muscle performance. Participants will be instructed to refrain from additional caffeine sources ≥ 3 hour prior to consuming their supplement so that a valid estimate regarding the effects of caffeine supplementation on muscle can be made. On training days, participants will mix their supplement powder in water and consume the solution 60 minutes prior to exercise. Sixty minutes was chosen because this is the approximate time it takes for peak plasma caffeine concentrations to occur after caffeine ingestion and pre-exercise

creatine supplementation has a beneficial effect on muscle performance. On non-training days, participants will refrain from consuming their supplement as the purpose of the study is to investigate the effects of pre-exercise creatine and caffeine co-supplementation. Adherence with the supplementation protocol will be assessed using compliance logs. Habitual daily caffeine consumption will be recorded using caffeine logs. Upon completion of the study, participants will be asked whether they thought they were administered creatine and caffeine, creatine, placebo, or unsure about what supplement they consumed. The primary dependent variables that will be measured at baseline and after the intervention include: (1) muscle thickness (elbow and knee flexors and extensors; ultrasonography), (2) muscle strength (1-RM leg press, chest press) and (3) muscle endurance (leg press and chest press; maximum number of repetitions performed for 1 set using 50% baseline 1-RM). In addition, participants will fill out a 3-day food diary during the first and final week of supplementation and training to determine whether total energy (kcal) and macronutrient intake changed over time.

4.3 Resistance Training Program

Participants will follow the same periodized, resistance training program for 6 weeks. The program will consist of three sets of 6, 8, 10 repetitions to muscle fatigue in order. Resistance training will start on the first day of supplementation and will consist of a split routine involving whole body musculature. Day 1 will involve chest and biceps musculature and include the following exercises in order: machine-based chest press, free weight incline bench press or dumbbell press, free-weight flat dumbbell press, machine-based pec-dec, free-weight standing barbell curl, free-weight alternate arm dumbbell curl and machine-based preacher curl. Day 2 will involve leg and core musculature and include the following exercises in order: free-weight squat, machine-based leg press, machine-based leg extension, machine-based leg curl, machine-based calf raise, and

machine-based weighted abdominal crunches. Day 3 will serve as a rest day from training. Day 4 will involve back and triceps musculature and include the following exercises in order: body weight or weight-assisted chin-ups, machine-based seated row, machine-based lat pull-down, free-weight alternate dumb-bell row, free-weight close-grip bench press, machine-based cable triceps bar extension, and machine-based cable triceps rope extension. Day 5 will involve shoulder and core musculature and include the following exercises in order: free-weight dumbbell press, free-weight upright row, free-weight shrugs, free-weight or machine based lateral deltoid flys, free weight or machine-based rear deltoid flys and machine-based weighted abdominal crunches. Day 6 will serve as a rest day from training. This cycle will be repeated for 6 weeks. Participants will maintain training logs to ensure adherence and compliance to the study and to determine total training volume (load x repetitions x sets).

4.4 Primary Dependent Variables

4.41 Muscle thickness

Muscle thickness (right side) of the elbow and knee flexors and extensors will be measured using B-mode ultrasound (LOGIQ e, GE Medical Systems) in the Exercise Physiology Laboratory, University of Regina. To measure elbow flexor and extensor muscle thickness, a small mark will be drawn on the lateral side of the arm to indicate 65% of the distance down from the acromion process to the olecranon process. A tape measure will be wrapped around the arm at the 65% mark and used as a reference, while another mark will be placed on the bulk of the biceps and triceps where the center of the ultrasound probe will be placed. To measure elbow flexor muscle thickness, each participant will place their right arm flat on a table with the belly of the biceps facing upwards and the forearm supinated. To measure elbow extensor muscle thickness, participants will stand with their back facing the researcher and elbows relaxed and extended.

To measure knee flexor and extensor muscle thickness, a small mark will be drawn on the lateral side of the leg to indicate 70% of the distance down from the greater trochanter to the lateral epicondyle of the tibia. A tape measure will be wrapped around the leg at the 70% mark and will be used to mark another reference point on the bulk of the vastus lateralis (knee extensor) and biceps femoris (knee flexor) where the center of the ultrasound will be placed. To measure knee extensor muscle thickness, each participant will be placed on a table in a seated position with the leg extended and relaxed. To measure knee flexor muscle thickness, each participant will be prone on the table with both legs extended and relaxed.

Precise markings using a permanent marker will be made on a transparency film sheet to ensure that the exact identical site is measured before and after the resistance training program by the same researcher. Water-soluble transmission gel (EcoGel 200, Eco-Med Pharmaceutical Inc., Mississauga, Ontario, Canada) will be placed on the measurement site to provide acoustic contact with the surface of the muscle. An 8-MHz scanning transducer head will be placed perpendicular to the muscle area. When the image is produced on the screen, the image on the monitor will be frozen. With the image frozen, a cursor will be enabled to quantify muscle thickness (cm) at three sites: proximal, mid and distal, as determined by divisions (1cm) on the monitor. Muscle thickness measurements will be determined from the monitor screen by measuring the distance from the bottom of the subcutaneous adipose layer to the surface of the humerus for the elbow flexors and extensors and to the surface of the femur for the knee flexors and extensors. Three muscle thickness measurements will be taken at each of the four muscle group sites. The values at each of three sites will be averaged to quantify one muscle thickness value that will be used for analysis.

The same researcher will perform all measurements. The reproducibility (coefficient of variation; intraclass correlation coefficient) of muscle thickness measurements will be determined

prior to supplementation and training by assessing 10 participants, > 24 hours apart. Baseline and post-testing muscle thickness measurements will be performed prior to assessing 1-RM strength and endurance. Post-testing muscle thickness measurements will be performed ≥ 48 hours after the last resistance training session.

4.42 Muscular Strength and Endurance

Machine-based leg press and machine-based chest press 1-RM will be performed in the Exercise Physiology Laboratory, University of Regina. To determine the 1-RM, participants will perform a 5-minute warm-up on a stationary cycle ergometer at a self- selected intensity and light passive stretching. Participants will then perform two warm-up sets of each exercise in the following order: 1 set of 10 repetitions using a load, which will be determined by each participant to be comfortable, and 1 set of 5 repetitions using a heavier weight. Two-minutes after the warm-up sets, the load will be progressively increased for each subsequent 1-RM attempt. Participants will rest (passively) for 2 minutes between 1-RM attempts. Participants will reach their 1-RM in ≤ 6 sets. To determine muscle endurance, participants will perform repetitions to volitional fatigue (1 set) using 50% baseline 1-RM for the squat and chest press.

To measure leg press 1-RM and endurance, participants will be positioned in a vertical leg press machine with both feet pushing up against the machine. Following a demonstration, participants will be instructed to lower the weight until an internal angle at the knees of 90° is achieved before returning to the upright position. The reproducibility (coefficient of variation; intraclass correlation coefficient) of leg press 1-RM will be determined prior to supplementation and training by assessing 10 participants, > 24 hours apart.

For chest press 1-RM and endurance, participants will be positioned in the bilateral chest press machine with both feet placed on the floor. Following a demonstration, participants will be

instructed not to lift their hips off the bench or arch their back during the lift. Participants will be instructed to grasp the bars (overhand grip) approximately shoulder width apart and push the load away from the body until full extension without locking the elbows and then lower the load back to the starting position. The reproducibility (coefficient of variation; intraclass correlation coefficient) of chest press 1-RM will be determined prior to supplementation and training by assessing 10 participants, > 24 hours apart.

Muscle strength and endurance will be assessed in order: (a) leg press strength, (b) chest-press strength, (c) leg press endurance, and (d) chest-press endurance. Five minutes of rest will separate each test. Muscle strength and endurance determination will be separated by 10 minutes of rest. Post-testing strength and endurance measurements will be performed ≥ 48 hours after the last resistance training session.

4.43 Diet

Dietary intake will be recorded during the first and final week of supplementation and resistance training to assess differences in total energy and macronutrient composition between groups. Participants will use a 3-day food booklet to record food intake for two weekdays and one weekend day. Participants will be instructed to record all food items, including portion sizes consumed for the three designated days. My FitnessPal will be used to analyze 3-day food records.

4.44 Adverse Event Assessment

In the case of an adverse event, participants will be asked to complete an adverse event form in order to provide details on the type of adverse event, the severity (i.e. mild, moderate, severe, or life threatening), the frequency, and the relationship to the intervention (i.e. not related, unlikely, possible, probable, or definite).

4.5 Statistical Analysis

A 4 (group: CR-CAF vs. CR vs. CAF vs. PLA) x 2 (time: pre- vs. post- training) repeated measures analysis of variance (ANOVA) will be used to determine differences between groups over time for the dependent variables of muscle thickness, muscle strength, muscle endurance and diet. If significant interactions are detected, a one-factor ANOVA on absolute change scores (Δ = post mean – pre mean) will be performed to confirm the interaction. A one-factor ANOVA will be used to assess baseline data and training volume. Significance is set *a priori* at an alpha level of $p < 0.05$. Cohen's *d* effect size (ES) for significant main effects and interactions will be calculated as post-training mean - pre-training mean / pooled pre-training standard deviation mean (Cohen, 1992). An ES of 0.00-0.19 is considered trivial, 0.20-0.49 is considered small, 0.50-0.79 is considered moderate, and ≥ 0.80 is considered large (Cohen, 1992). Statistical analyses will be performed using IBM® SPSS® Statistics, v. 24.