

**NCT03868631**

Muscle growth and development following a 12-week resistance training program in men and women consuming soy and whey protein supplements.

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## Methods

### Participants

Two hundred eighty-two people responded to the screening survey online from August 2016 through January 2017. Most survey respondents did not meet inclusion requirements or failed to reply to an email invitation to come in for a baseline lab visit. Sixty-one participants were randomized for study participation. Participants were healthy, nonsmoking males and females with a body mass index (BMI) between 18.5 and 29.9, ages 18-35 years who were recreationally active but had not participated in structured weight training for at least the previous 12 months. Exclusion criteria included adherence to a vegetarian or vegan diet; having any chronic disease; use of tobacco products; current pregnancy or lactation, being within six months post-partum, or intention to become pregnant in the next 12 weeks; allergy to whey or soy; change in body weight more than ten pounds in the previous three months; and any musculoskeletal injury or condition that would preclude full participation in a new exercise program. All participants provided written informed consent before enrollment in the study. The study was approved by the Institutional Review Board of Arizona State University and is in compliance with the Declaration of Helsinki as revised in 1983.

### Study design

A prospective, 2-group parallel-arm, randomized, double-blind study was conducted to compare the impact of soy or whey protein isolate supplements, matched for leucine content, on strength and lean body mass changes in response to resistance training. Enrolled participants were randomly assigned to receive either 19 grams of whey protein isolate or 26 grams of soy protein isolate daily. The varying amounts of protein supplement were chosen to ensure each contained two total grams of leucine. This amount of leucine was targeted for consumption as 10 grams of EAA (including ~1.8 g leucine) has been shown to be sufficient to maximally stimulate muscle protein synthesis (MPS) in young men and women, and that additional leucine (3.5 g) does not further augment MPS. Participants were stratified based on the baseline visit measurements for BMI, leg extension peak torque, and lean body mass, and randomly assigned to the treatment groups. Participants and all investigators except one were blinded to the group assignment; the investigators collecting and analyzing data were all blinded to group assignment. The investigator who knew group assignment remained uninvolved with data collection and assessment until the study was completed. Participants in both groups engaged in an identical supervised resistance training program, consisting of training on three nonconsecutive days per week for 12 weeks. Outcome measures (see below) were assessed prior to training, and after six and 12 weeks of training.

### Dietary protocol

Participants were clearly instructed to maintain their usual diet and physical activity level throughout the duration of the study, which was encouraged during each training session. Three times during the study (baseline, midway through the study, and during the 12th week of the intervention) participants completed three-day food logs (two weekdays and one weekend day). Participants were instructed by a Registered Dietitian Nutritionist (RDN) on how to complete the food logs completely and of the importance in being detailed and accurate in their records, including brand names when possible and precise amounts of each food, beverage, and condiment. All diet records were entered into Food Processor SQL Nutrition and Fitness Software by ESHA Research, Inc. (version 10.11.0, Salem, OR, USA) by the same person to ensure standardization. Total kilocalories, grams of protein, and percent

contribution of macronutrients were compared within participants and between group means to evaluate if participants maintained the composition of their diets during the intervention.

#### Supplement protocol

Protein powder was measured to the nearest gram using a MyWeight KD-8000 digital food scale. Daily protein was measured into individual plastic bags, and all portions required for the 12-week study were provided at the outset along with a shaker bottle to all participants. Protein supplements were either whey protein powder isolate (Bongards, Chanhassen, MN) or soy protein powder isolate (DuPont, Wilmington, DE). Leucine content of the products was determined from the USDA Nutrient database for soy protein isolate and from an analysis by the manufacturer for whey protein isolate since a product-specific analysis was available. The protein supplement was provided to participants in powdered form. Participants were instructed to consume the protein supplement daily by mixing the protein supplement with water. Participants were permitted to mix a non-nutritive sweetener with their protein powder if they wished, but not with milk or any other food substances or calorie-containing beverages. On workout days, the trainer observed the participant consume the protein powder immediately after the exercise session for added accountability. On non-workout days, participants were instructed to consume the protein between meals rather than concurrently with other food. This was to ensure timing of consumption would be ideal for maximizing the 24-hour muscle protein synthesis rate.

#### Resistance training protocol

The resistance training program consisted of whole body progressive resistance exercise training. Participants completed three workouts per week, each separated by at least 48 hours. Each workout began with an easy, self-selected five-minute warmup on the treadmill, elliptical, or stationary bike. Each resistance training session consisted of barbell bench press, incline chest press using a barbell, leg press, seated leg curl, leg extension, lat pull down, upright row, and abdominal exercises. Participants rested for 1-2 minutes between each set. The first workout of the week was not intended to take participants to complete muscular failure. However, the other two workouts per week were intended to elicit complete voluntary muscular failure. One-repetition maximum weight lifted was recalculated during the first workout of weeks 1, 4, 7, and 10 for the bench press, leg press, and knee extensions. Weeks 1-6 entailed lifting three sets of 10 repetitions at 60% of participants' one-repetition maximum (1-RM) for all exercises on the first workout of the week. The second and third workouts entailed lifting three sets of about 10 repetitions at about 70% 1-RM for each exercise. The precise weight lifted was increased as needed in order for participants to be completely fatigued by about repetition 10. Weeks 7-12 consisted of the same exercises but at a higher intensity. The first workout of each week consisted of four sets of eight repetitions at 70% 1-RM. The second two workouts of the week consisted of four sets of eight repetitions at 80% 1-RM. Similar to weeks 1-6, the actual weight lifted was increased above 80% 1-RM if needed in order for the participants to be completely fatigued after about repetition eight. In order to be included in analyses, participants could not have missed an a prior cut off of more than three training sessions in 12-weeks (completed at least 92% of sessions).

#### Study outcomes.

The following testing protocol was conducted prior to training and after six and 12 weeks of training. The only exception was that height was only measured during the pre-training period. Participants arrived to the lab having been asked to not perform exercise for a minima of 24 hours prior to the visit.

Participants' height and weight were measured using a SECA directprint 284 digital measuring station. Body composition was assessed using dual x-ray absorptiometry (DXA) (Lunar iDXA, General Electric Company, East Cleveland, OH, USA) which was obtained after voiding the bladder and participants laying down for 15 minutes to normalize fluid shifts. All scans were completed by the same certified radiology technician. Muscle thickness of the vastus lateralis (VL) and vastus intermedius (VI) of the self-determined dominate leg was assessed after 15 minutes of rest using ultrasound (uSmart 3300, Terason, Burlington, MA) and with a 15-4 Mhz linear transducer. Images were captured at 56% of the length from the greater trochanter of the femur to the lateral epicondyle. Circumference of the leg at this location was also measured. Image analysis was carried out using publicly available software (ImageJ, National Institutes of Health, USA). For the analysis of muscle thickness, ImageJ was first calibrated using the visible depth scale on each image. Muscle thickness (MT) of the VL and VI were measured. Briefly, MT of the VL was measured as the perpendicular distance between the border of subcutaneous fat and muscle to the deep aponeurosis. MT of the VI was measured as the perpendicular distance between the superficial aponeurosis and the superficial border of the femur. At each site, combined MT of the visible muscles (VL+VI) was also examined, measured as the perpendicular distance between the subcutaneous fat and muscle border to the superficial border of the femur. To account for changes in MT across the field of view (FOV) in each image, MT measurements were made at three locations across the FOV, corresponding to 10%, 50%, and 90% from left to right (based on pixel width of FOV). Measurements at the three locations across the FOV were averaged into one composite MT measurement for a given muscle. Following assessment of body composition and muscle thickness, isokinetic muscle strength was determined for the leg extensors and leg flexors on an isokinetic dynamometer (Computer Sports Medicine Inc. (CSMi), Stoughton, MA, USA). Briefly, participants were secured to the dynamometer to avoid any unnecessary movement. Participant performed two sets of three repetitions (30s rest in-between sets) on their self-reported dominant leg at 60 degrees per second (d/s). Participants were encouraged to produce maximal effort during each repetition. Peak torque was taken as the highest peak torque for flexion and extension from any of the sets.