Carbohydrates Alone or Mixing With Beef or Whey Protein Promote Similar Training Outcomes in Resistance Training Males: A Double Blind, Randomized Controlled Clinical Trial

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Running head: Protein-Carbohydrate supplementation

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Abstract

Beef powder is a new high-quality protein source scarcely researched relative to exercise performance. The present study examined the impact of ingesting hydrolyzed beef protein, whey protein, and carbohydrate on strength performance (1RM), body composition (via plethysmography), limb circumferences and muscular thickness (via ultrasonography), following an 8-week resistance-training program. After being randomly assigned to one of the following groups: Beef, Whey, or Carbohydrate, twenty four recreationally physically active males (n=8 per treatment) ingested 20 g of supplement, mixed with orange juice, once a day (immediately after workout or before breakfast). Post intervention changes were examined as percent change and 95% CIs. Beef (2.0%, CI, 0.2-2.38%) and Whey (1.4%, CI, 0.2-2.6%) but not Carbohydrate (0.0%, CI, -1.2-1.2%) increased fat-free mass. All groups increased vastus medialis thickness: Beef (11.1%, CI, 6.3-15.9%), Whey (12.1%, CI, 4.0, -20.2%), Carbohydrate (6.3%, CI, 1.9-10.6%). Beef (11.2%, CI, 5.9-16.5%) and Carbohydrate (4.5%, CI, 1.6-7.4%), but not Whey (1.1%, CI, -1.7-4.0%), increased biceps brachialis thickness, while only Beef increased arm (4.8%, CI, 2.3-7.3%) and thigh (11.2%, 95%CI 0.4-5.9%) circumferences. Although the three groups significantly improved 1RM Squat (Beef 21.6%, CI 5.5-37.7%; Whey 14.6%, CI, 5.9-23.3%; Carbohydrate 19.6%, CI, 2.2-37.1%), for the 1RM bench press the improvements were significant for Beef (15.8% CI 7.0-24.7%) and Whey (5.8%, CI, 1.7-9.8%) but not for carbohydrate (11.4%, -0.9-23.6%). Protein-carbohydrate supplementation supports fat-free mass accretion and lower body hypertrophy. Hydrolyzed beef promotes upper body hypertrophy along with similar performance outcomes as observed when supplementing with whey isolate or maltodextrin.

Keywords: Supplementation, Nutrition, Fat-Free Mass, Maximal Strength, Hypertrophy, Multi-ingredient.

Introduction

Whey protein based supplements have been promoted as the optimal protein source at maximizing resistance-training outcomes (Miller et al., 2014). Compared to other proteins, whey has greater bioavailability and solubility along with a higher concentration of branched-chain amino acid (BCAA), specifically leucine (Tang et al., 2007). These characteristics make whey an ideal amino acid source for maximizing muscle protein synthesis and the overall recovery process after resistance exercises in athletes (Kreider et al., 2010; Stark et al., 2012). Like whey, beef is a nutrient-rich, high-quality protein containing all the essential amino acids (EAA) in similar proportions to those found in human skeletal muscle (Chernoff, 2004). Few studies have analyzed the effectiveness of ingesting beef protein on resistance-training outcomes. Symons et al. (2011) reported 2-fold greater increases in muscle protein synthesis during a 5 h period following the ingestion of 340 g of lean beef combined with resistance exercise, compared to the ingestion of beef in resting conditions. Robinson et al. (2013) reported that 170 g of lean beef, providing 36 g of protein, ingested after performing 3 sets of an of unilateral leg resistance exercise resulted in greater rates of muscle protein synthesis compared to the ingestion of both 113 g and 57 g of beef containing 24 g and 12 g of protein, respectively. More recently, Negro et al. (2014) observed a significant increase in fat-free mass gains after an 8-week resistance-training program in males and females who consumed 135 g of tinned lean beef, providing 20 g of protein, compared to a non-supplemented group. Canned meat is more digestible than other meat sources (e.g., steak) as it does not generally cause any gastrointestinal distress, and its consumption is also practical (Negro et al., 2014). Beef protein is now available in powder-hydrolyzed form, which potentially enhances absorption when combined and ingested in liquid form

immediately after workout. The aim of the current investigation was to compare the effectiveness of combining an 8-week resistance training program with a commercially-available hydrolyzed beef protein powder (100% All Beef, Crown® Sport Nutrition, Spain), or whey isolate (Isolac, Carbery)) or a non-protein, maltodextrin supplement on body composition, muscle thickness, limb circumferences and strength performance in recreationally physically-active college males. The primary outcome of this study is muscular strength defined as one repetition maximum (1RM) for the bench press (BP) and parallel back squat (SQ). Secondary outcomes include indices of body anthropometry and hypertrophy.

Methods

Participants

Thirty regularly physically active participants met the inclusion criteria: (a) Males 18-40 year of age; (b) regular recreationally resistance training for at least 2 years performing bench press and squat using free weights as habitual exercises in their training routines (c) free from musculoskeletal limitations or injuries (d) agree not to ingest any other nutritional supplements during the study and (e) fluent in English. Exclusion criteria were: (a) a history of various metabolic conditions and/or diseases; (b) use of a variety of medications, including but not limited to those with androgenic and/or anabolic effects and/or nutritional supplements known to affect training outcomes such as creatine, proteins, etc. within 12 weeks prior to the beginning of the study, (c) current use of tobacco products.

All participants provided written informed consent in accordance with the Declaration of Helsinki. Procedures were approved by the University ethics committee and registered at ClinicalTrials.gov (NCT02425020) on 22nd April 2015.

Twenty-four of the 30 recruited participants completed all aspects of the study (Figure. 1).

Figure 1

This study utilized a randomized, double blind, parallel group, and controlled trial design. Participants were equally and randomly assigned to three treatment groups: Beef (n=10), Whey (n=10) and CHO (n=10). Participants were tested before and after an 8-week intervention period for measures of strength, body composition, limb circumferences and muscular thickness.

Prior to baseline assessments, participants performed six familiarization sessions aimed at minimizing any potential learning effects with the assessment and training procedures. Following the initial assessment, participants were matched by maximal strength in the SQ and BP. Assignment of participants to treatments was performed by block randomization, using a block size of three, and in a double blind fashion. Initial groups characteristics were equivalent at baseline: Beef: age 26±8 years, height 1.77±0.1 m, body mass 77.2±17.5 kg; Whey: age 26±4 years, height 1.80±0.1 m, body mass 74.9±9.5 kg; CHO: age 29±5 years, height 176±0.4 m, body mass 77.2±15.5 kg.

Training

All participants followed the same resistance training routine, three times per week, alternated with their normal recreationally physical activity for a total of 8 weeks. Workout sessions were carried out late in the afternoon or early evening. After a warm-up the participants performed a total of 3 circuits involving 1 set of the following exercises: 1) countermovement vertical jump 2) bench press; 3) parallel back squat; 4) upright row; 5) dumbbell alternate lunges; 6) shoulder press; 7) lateral hurdle jumps; 8) abdominal crunch. Every set involved 12 repetitions using the

heaviest possible load (except for the lateral hurdle jumps and the abdominal crunch that involved 20 repetitions per sets with no external overload). Experienced strength and conditioning coaches monitored all training sessions to ensure participants compliance to the training protocol. When participants were able to perform more than 12 repetitions per set, the load was slightly increased (between 2.5 to 5 kg). If less than 12 repetitions were completed, a minimum rest period of 15 sec was introduced until the participants were able to complete 12 repetitions per set. A ~30 sec rest period was permitted between exercises. Recovery between circuits was 2-3 minutes. All participants completed all lifts for each exercise. The average time to complete the workouts was 30 min.

Dietary Supplementation

The three products were presented as 20 g sachets of vanilla-flavored powder to be diluted in 250 mL of orange juice. The diluted drinks were similar in appearance, texture and taste, were isoenergetic, and dispensed in identical 500-mL bottles. The nutritional composition of each product is presented in Table 1. On training days, supplement was ingested just after training, whereas on non-training days product was administered in the morning, before having breakfast.

Table 1

Dietary Monitoring

Each participant's baseline diet (3 days, 2 weekdays, and 1 weekend day) was analyzed using Dietplan 6 software (Microsoft Forestfield Software Ltd. 14). Participants were instructed to maintain their normal diet throughout the intervention. In order to evaluate differences caused by the supplementation protocol, diet was analyzed again during the last week of the intervention.

Measurements and control of the intervention compliance

Measurements were determined over two sessions. Day 1 included (i) muscle thickness using ultrasonography, (ii) limbs circumferences and (iii) body composition via plethysmography. Day 2 included 1RM in BP and SQ. Prior to any testing session, participants were instructed to refrain from any vigorous activity and avoid caffeine ingestion for at least 48 h. All tests were performed at the same time of the day for the same participant.

After completing the initial evaluation, each participant received a batch of products, according to randomization, and began the intervention. The same testing procedures were repeated, at the end of the intervention. Tolerance, collected from adverse events and compliance with product intake (determined by an individual follow up of the participants) was evaluated continuously. Each participant was given 56 supplement packets and an opaque shaker plastic bottle to consume the supplement. Researchers regularly controlled consumption compliance using instant phone text message and asking participants on regularly weekly interviews. Acceptable supplementation compliance was set at ≥90% of dose consumption (51 doses). Average supplementation compliance was 98.6% (range: 95.1–100%) across all groups.

Body Composition

Body mass and height were assessed according the methods described by Ross and Marflel-Jones (1991). Whole body densitometry using air displacement via the Bod Pod® (Life Measurements, Concord, CA) was using in accordance with the manufacturer's instructions as detailed elsewhere (Dempster and Aitkens, 1995).

Limb circumferences

The circumferences of the right arm and thigh were measured using a constant

tension tape measure during maximal elbow extension or standing position respectively. Three measurements were made for both arm and thigh circumference. Averaging was performed to obtain mean values for both circumferences. Mid arm circumference was measured midway between the tip of the acromion and the olecranon process (Heymsfield et al., 1982) and the thigh circumference was determined at a point situated two thirds between the edge of the iliac crest and the proximal border of the patella (upper knee) (Bielemann et al., 2016) .

Muscle thickness

Right-side biceps brachialis and vastus medialis muscle thicknesses were measured in real time using an Diasus diagnostic ultrasound imaging unit (Dynamic Imaging, Livingston, Scotland UK) coupled to a 50 mm probe at a frequency of 7.5 MHZ while participants were lying supine at semi-recumbent position (45°) and with arms and legs completely relaxed.

The right upper limb was positioned supine with a 35° angle with respect to the trunk. The probe was placed perpendicular to the skin surface and bone tissues at two-thirds of the distance between the acromion process of the scapula and the lateral epicondyle of the humerus (Bradley and O'Donnell, 2002).

The right lower limb was positioned with the knee extended. The probe was placed perpendicular to the skin surface and bone tissues at 80% of the distance between the lateral condyle of the femur and greater trochanter (Bradley and O'Donnell, 2002). The probe, coated with a water-soluble transmission gel (Aquasonic 100 Ultrasound Transmission gel) to provide acoustic contact without depressing the dermal surface, was placed in the transversal plane and perpendicular to the skin surface and bone tissues at each of the marked sites. The placement site was carefully noted and the location was recorded on acetate paper, using moles and

small angiomas as reference points (identifiable markings viewed in the muscle) to ensure the same probe location during pre and post intervention. Thickness was calculated as the distance between superficial and deep aponeuroses measured at the ends and middle region of each 3.8 cm-wide sonograph. The intra- and inter-rater reliability of muscle thickness measurements performed by the expert investigator (MS) on the same scans in a preparatory study was excellent (>0.99). Therefore, the thickness measurements on vastus medialis and biceps brachialis at pre and post intervention can be compared confidently.

Three images of each muscle were obtained for each point and the average of the results was calculated. To favor reproducibility, probe placement was carefully noted for reproduction during the other test sessions. Furthermore, to ensure the intra-observer reliability of the muscle thickness all the participants (48 knees) were evaluated by the same author. In order to avoid any swelling in the muscles that could disturb the results, images were obtained at least 48 hours before and after the program intervention.

Strength tests

The 1RM value for both the BP and SQ using free weights was determined according to the methodology described by McGuigan (2016). To avoid any specific muscle group interaction, the order of testing for BP and SQ was randomized.

Intraclass Correlation Coefficient (ICCs) for the day-to-day reproducibility of the dependent performance measures were recorded at ICCs ≥0.90 and the coefficients of variation ranged from 1.0 to 2.5%.

Sample size determination

Based on the meta-analysis published by Naclerio and Larumbe-Zabala (2016) we expected to find moderate (f ≥0.25) significant within-between interaction effect

after a repeated measures analysis of variance (ANOVA). We performed a power analysis to determine the required sample size using G*Power 3.1. Assuming a significance level of 0.05, and a correlation among measures r=0.75, as determined by previous pilot studies, a 3×2 mixed ANOVA model required 24 participants (8 per group) to achieve a power ≥0.80. Preventing for a possible 15% attrition, we enrolled 10 participants per group.

Statistical Analysis

A descriptive analysis was performed and subsequently the Kolmogorov-Smirnov and Shapiro-Wilk test were applied to assess normality. Sample characteristics at baseline were compared between conditions using one-way Analysis of Variance (ANOVA). Changes pre to post treatment were assessed using a 2 (times) × 3 (treatments) repeated measures ANOVA. Delta scores (Δ) were calculated by subtracting test 1 values from test 2 values, dividing by test 1 and multiplying by 100; the scores were thus interpreted as percentages and used for determining relative changes from pre to post intervention and between conditions. One-sample *t*-tests of the Δ scores in each outcome variable were performed for each treatment condition [Alternative verbiage. Confidence intervals not crossing zero were considered statistically significant.]. Additionally, differences in Δ between treatment conditions were assessed throughout a one-way ANOVA. Bonferroni-adjusted post hoc analysis was performed for pairwise comparisons in all ANOVA models. Generalized eta squared (η_G^2) and Cohen´s *d* values were reported to provide an estimate of standardized effect size (small d=0.2, η_G^2=0.01; moderate d=0.5, η_G^2=0.06; and large d=0.8, η_G^2=0.14). Significance level was set to $p<0.05$. Results are reported as mean (standard deviation) unless stated otherwise. Data analyses were performed with Stata 13.1 (StataCorp, College Station, TX).

Results

Six participants (2 per each treatment group) dropped from the study due to personal reasons, not related with the intervention protocol. Correlation between pre and post measures was found larger than expected from the pilot study, ranging r=0.85 for vastus medialis to r=0.97 for fat-free mass (%). The post-hoc power analysis determined better sensitivity of the sample (f=0.187) assuming the same parameters as in our a priori power analysis. The final composition of the three groups was equivalent at baseline. Pre and post values, main time and group effects, as well as interactions between treatments and time, are provided in Table 2.

Table 2

Table 3 shows the dietary monitoring results determined before and after intervention. At baseline, no between-groups differences were observed. However, as a result of the nutritional intervention, all the three groups increased the amount intake of carbohydrates ($g \cdot kg \cdot d^{-1}$) and the protein groups (Beef and Whey) significantly increased the protein intake ($g \cdot kg \cdot d^{-1}$). Furthermore, only the Beef group showed a significant rise in fats meanwhile the three groups increased the energy intake, with no difference between them. Furthermore, the meal-by-meal analysis reveals that the during the intervention, the amount of proteins ($g \cdot kg \cdot d^{-1}$) ingested per meal was as follows: (1) Breakfast: Beef 0.32±0.11; Whey 0.30±0.09; CHO 0.30±0.05, (2) snack: Beef 0.25±0.08; Whey 0.24±0.08; CHO 0.22±0.04, (3) lunch: Beef 0.25±0.08; Whey 0.25±0.08; CHO 0.22±0.04, (4) snack: Beef 0.22±0.07; Whey 0.22±0.07; CHO 0.22±0.04, (5) post workout: Beef 0.22±0.05; Whey 0.23±0.03; CHO 0.00±0.00, 6) dinner: Beef: 0.43±0.12; Whey 0.50±0.19; CHO 0.48±0.08.

Table 3

Table 4 summarizes the results obtained from the delta comparison between time (pre and post intervention) and treatments (Beef vs. Whey vs. CHO).

Table 4

Compared to baseline, Beef showed significant relative improvements in fat-free mass, arm and thigh circumference, biceps brachialis and vastus medialis thickness, 1RM BP and 1RM SQ. The Whey group produced significant higher delta scores, in fat-free mass, vastus medialis thickness and 1RM BP and 1RM SQ. Meanwhile, the CHO group showed significant higher delta scores for biceps brachialis and vastus medialis thickness and 1RM SQ along with a strong trend to enhance 1RM BP. Figures 2 and 3 depict the relative changes observed for both strength and muscle thickness.

Figures 2 and 3

Comparison between treatments revealed significant between conditions effects only for the biceps brachialis thickness [$F(2,23)=9.08$, $p=0.001$, $\eta^2=0.48$] and a large effect size for the arm circumference [$F(2,23)=5.771$, $p=0.010$, $\eta^2=0.35$]. Pairwise comparisons revealed that Beef produced significant increases in biceps brachialis thickness compared to both Whey ($p=0.001$, $d=1.54$) and CHO ($p=0.026$, $d=1.02$) conditions (Figure 2). Additionally, Beef produced a larger increase in the arm circumference that was significantly different from Whey ($p=0.012$, $d=1.14$) and showed a strong trend ($p=0.057$, $d=1.02$) to be different from CHO. No other significant effects were determined.

Discussion

The main finding of the current investigation demonstrated that ingesting 20 g of beef protein mixed with 250 ml of orange juice immediately after workouts or before breakfast on non-training days, yielded comparable results to ingesting whey isolate

or carbohydrate following 8-weeks of resistance training. Although the three treatment groups showed positive effects in increasing strength and muscular thickness, the beef group was the only condition to achieve significant increases in tight and arm circumferences. Furthermore, beef produced the largest relative change in strength, fat-free mass, biceps brachialis thickness with a very similar increase of the vastus medialis thickness as observed for the whey protein group. Moreover, only the both protein conditions significantly increased fat-free mass (Tables 2 and 4).

To the best of the authors' knowledge, this is the first study to look at the effect of a hydrolyzed beef protein powder extract and comparing its effects with those elicited by whey protein and a non-protein isoenergetic nutrient at supporting resistance-training outcomes in young athletes. The ingestion of a post-workout protein-carbohydrate supplement induces a rapid glycaemia and hyperaminoacidemia, supported by an increased insulin sensitivity (Norton and Wilson, 2009). These events maximize amino acid uptake and muscle protein synthesis by prolonging mammalian target of rapamycin signaling (mTOR) during the post-training period (Farnfield et al., 2012).

The analysis of relative change reveals that the three treatment conditions appear to produce similar relative effects at supporting muscle hypertrophy and strength gains (Table 4 and Figures 2 and 3). The total energy provided under the three treatment conditions was almost similar. Herein, we propose two possible reasons explaining our results. First, it is conceivable that the amount of protein provided by Beef or Whey was insufficient in quantity to elicit significant differences vs. the CHO group, or second, the amount of protein consumed by the CHO condition relative to the participants normal diet was sufficient to support training adaptations. Specifically, with the exception of the protein ingested via supplementation, no

difference was noted for regular dietary protein ingestion (i.e., >0.20 to ~0.40 $g \cdot kg^{-1}$ per meal) with no between group differences observed at lunch or dinner, where the three groups consumed more than 0.40 $g \cdot kg^{-1}$. Despite not being ingesting protein immediately after training, the total daily protein ingested by the CHO condition was still within the recommended range for supporting resistance-training adaptations (Thomas et al., 2016). In fact, the recommended daily protein intake necessary to support training adaptations in physically active individuals ranges from 1.2 to 2.0 $g \cdot kg^{-1} \cdot d^{-1}$ (Thomas et al., 2016). According to the diet records, only 2 participants (1 Whey, 1 in CHO) were ingesting less than 1.2 $g \cdot kg^{-1}$ of protein meanwhile the rest of the participants were consuming between 1.2 and 2.6 $g \cdot kg^{-1} \cdot d^{-1}$.

The present results seems to support the premise that the main limiting factor for training adaptation would be the daily caloric intake (McLellan et al., 2014), being the total daily protein (Reidy and Rasmussen, 2016) or the timing of ingestion (Forbes et al., 2014) rather than the amino acid composition, more relevant factors affecting fat-free mass accretion during resistance training. Nonetheless, it is important to highlight that for the present investigation, diet was not controlled but only recorded over 3 days. Although this approach has been extensively used, providing a prepared and pre-packed diet to participants during the intervention or the days before a performance trial would offer an ideal scenario to standardize and control their diet (Jeacocke and Burke, 2010).

Both protein supplements were particularly rich in EAA including Leucine; which acts as a key amino acid to stimulate the muscle protein synthesis (Dideriksen et al., 2013). It has been estimated that between 20 g or $0.25g \cdot kg^{-1}$ (Witard et al., 2014) to 40 g or ~0.40 $g \cdot kg^{-1}$ (Macnaughton et al., 2016) of high-quality protein providing ~8 to ~20 g of EAA (~90 to ~230 $mg \cdot kg^{-1}$) and about 2 to 3 g of leucine (20

to 30 mg·kg^{-1}) consumed after exercise may maximize rates of muscle protein synthesis in young individuals. In the present study, participants allocated to Beef and Whey treatment conditions were supplemented with 0.22±0.05 and 0.23±0.03 g·kg^{-1} of protein respectively. The administered amount of protein was within the 90% confidence interval (0.18 to 0.30 g·kg^{-1}) to promote muscle protein synthesis after exercise and beyond which there was no further increase in young men under resting conditions (Morton et al., 2015). Whey isolate provided higher amount of EAA and leucine compared to the beef supplement (EAA 8.91 [139±22 mg·kg^{-1}] vs. 6.82 [94±22 mg·kg^{-1}], and leucine 1.93 [30±5 mg·kg^{-1}] vs. 1.32 [18±4 mg·kg^{-1}], respectively). Despite not reaching the recommended minimum absolute value, when expressed per kg of body mass, the beef powder reached the minimum requested amount of EAA and was very close to provide sufficient quantities of leucine. This rationale supports the notion that when the amounts of EAA and leucine reach a threshold, the effects on muscle protein synthesis and training adaptations seem to be similar regardless of the source (Reidy and Rasmussen, 2016). Maybe in addition to the amino acid profile, the nutrient density of the protein sources (e.g. iron, zinc, vitamin B12 or essential fatty acid included in beef) would also represent a relevant nutritional factor for supporting training outcomes (Phillips, 2012). The training protocol of the present study uses four squatting exercises but only one (upright row) determined a meaningful activation of the biceps brachialis. Thus, differences in the specific training volume performed per muscle groups could be the cause of the dissimilar results observed between the vastus medialis and the biceps brachialis thickness. Perhaps when performing very low training volumes per muscle group (e.g. 3 sets of 12 repetitions per workout) the ingestion of carbohydrate-protein supplements with a high micronutrient density such as a beef would be more

beneficial at supporting training outcomes compared to other isoenergetic mixtures containing whey or only carbohydrates.

Limitations of the current study are that our results may only be applicable for the assessed muscles, biceps brachialis and vastus medialis, in young men resistance trained individuals. Muscle thickness determination includes the deep fascia and intramuscular fat. Consequently, the amount of muscle could be over-estimated. Measurements were taken at one site per muscle, so they might not represent the whole biceps or thigh changes. Similar intervention protocols, including other exercise routines, should be assessed in different populations (e.g. women) measuring other muscles (anterior deltoids, triceps brachialis or vastus lateralis) and using other methods to estimate muscular hypertrophy (e.g. muscle biopsy or magnetic resonance imaging).

In Summary, the ingestion of a post-workout beverage mixing orange juice with proteins powders from beef or whey support fat-free mass accretion and lower body hypertrophy in young resistance trained athletes. In addition, hydrolyzed beef promotes higher hypertrophy response on the upper body along with similar outcomes in strength performance compared to the ingestion of whey isolate or only CHO.

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Authors declare are that they have no conflicts of interest relevant to the content of this manuscript.

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Figure captions

Figure 1. Flow diagram of participants throughout the course of the study.

Figure 2. Delta Score and 95% confidence interval determined per each treatment condition in Biceps Brachialis (A) and Vastus Medialis (B) muscular thickness.

* Significant respect to baseline; ᵠ Significant respect to both whey and CHO.

Figure 3. Delta Score and 95% confidence interval determined per each treatment condition in 1RM Bench press (A) and 1RM Squat (B).

* Significant respect to baseline.

Tables

Table 1. Nutritional composition of drinks per intake (20g of powder plus 250 ml of orange juice)

Nutrient	**Beef**	**Whey**	**CHO**
Energy value (kcal)	184	179	184
Carbohydrates (g)	25	25	45
Lipids (g)	1.54	0.30	-
Proteins (g)	16.40	18.00	-
Alanine	1.04	1.06	-
Arginine	1.06	0.38	-
Aspartic acid	1.50	2.29	-
Cysteine	0.16	0.48	-
Glutamic acid	2.58	3.34	-
Glycine	1.07	0.34	-
Histidine	0.55	0.31	-
Isoleucine	0.75	1.00	-
Leucine	1.32	1.93	-
Lysine	1.44	1.81	-
Methionine	0.39	0.44	-
Phenylalanine	0.65	0.61	-
Proline	0.81	1.17	-
Serine	0.65	1.05	-
Threonine	0.73	1.44	-
Tryptophan	0.187	0.39	-
Tyrosine	0.52	5.57	-
Valine	0.80	0.98	-
Total EAA	6.82	8.91	-

Notes: EAA, essential amino acids; CHO, Carbohydrates

Table 2 Treatment groups' description at baseline.

Variable	Beef (n=8)		Whey (n=8)		CHO (n=8)		Repeated measure ANOVA (2 times x 3 groups)
	Pre	**post**	**pre**	**post**	**pre**	**Post**	
Age (years)	25 (8)	--	26 (5)	--	29 (9)	--	Group: F(2,21)= 0.559, p=0.580
Height (m)	1.77 (0.1)	--	1.80 (0.1)	--	1.76 (0.0)	--	Group: F(2,21)=0.726, p=0.496
Body mass (kg)	76.9 (19.0)	77.66 (18.0)	78.0 (8.5)	78.4 (9.0)	78.1 (13.2)	78.4 (13.9)	Time: F(2,21)= 2.74, p=0.113 Group: F(2.21)=0.01, p=0.988 Time x group: F(2,21)=0.19, p=0.831
Fat (%)	17.83 (9.32)	14.84 (10.8)	15 (4.58)	14.2 (4.82)	17.16 (5.54)	17.45 (5.36)	Time: F(2,21)=1.34, p=0.261 Group: F(2,21)=0.48, p=0.624 Time x group: F(2.21)=1.07, p=0.361
Fat-free mass (%)	82.18 (9.32)	82.79 (8.76)	85.80 (4.58)	85.80 (4.81)	82.84 (5.54)	82.56 (5.35)	Time: F(2,21)=1.37, p=0.255 Group: F(2.21)=0.48, p=0.625 Time x group: F(2.21)=1.05, p=0.368
Fat (kg)	14.85 (10.82)	14.47 (10.84)	11.94 (4.08)	11.41 (4.81)	13.91 (7.48)	14.18 (7.32)	Time: F(2,21)=0.51, p=0.482 Group: F(2.21)=0.32, p=0.732 Time x group: F(2.21)=0.71, p=0.502
Fat-free mass (kg)	62.05 (10.28)	63.15** (9.47)	66.1 (5.75)	66.98** (6.12)	64.15 (7.28)	64.23 (7.27)	Time: F(2,21)=11.53, p=0.003 Group: F(2.21)=0.51, p=0.608 Time x group: F(2.21)=2.36, p=0.119
Arm Circumference (cm)	30.75 (4.49)	32.24** (4.92)	33.44 (2.56)	33.21 (2.29)	33.84 (5.26)	33.91 (4.47)	Time: F(2,21)=4.26, p=0.052 Group: F(2.21)=0.73, p=0.494 Time x group: F(2.21)=5.96, p=0.099
Thigh circumference (cm)	57.44 (6.52)	59.18* (6.28)	58.19 (3.86)	58.68 (4.01)	58.64 (6.99)	59.67 (4.76)	Time: F(2,21)=7.38, p=0.013 Group: F(2.21)=0.06, p=0.946 Time x group: F(2.21)=0.81, p=0.460
Biceps brachialis thickness (mm)	32.38 (3.83)	35.96** (4.35)	38.38 (6.83)	38.68 (6.25)	44.06 (18.65)	46.47** (21.68)	Time: F(2,21)=20.41, p=0.001 Group: F(2.21)=1.61, p=0.223 Time x group: F(2.21)=4.26, p=0.028
Vastus medialis thickness (mm)	31.26 (3.01)	34.68** (3.33)	33.95 (1.79)	38.06** (3.60)	35.88 (6.22)	37.96* (5.55)	Time: F(2,21)=46.5, p=0.001 Group: F(2.21)=2.09, p=0.148 Time x group: F(2.21)=1.59 p=0.228
1RM Bench Press (kg)	66.63 (21.33)	75.31** (18.96)	82.81 (15.03)	87.18 (13.90)	89.06 (31.34)	95.93* (24.05)	Time: F(2,21)= 6.07, p=0.001 Group: F(2.21)=2.11, p=0.147 Time x group: F(2.21)=0.57, p=0.575
1RM Squat (kg)	105.31 (30.19)	124.37** (26.10)	108.13 (14.38)	124.00** (20.16)	112.63 (32.50)	130.56** (26.40)	Time: F(2,21)=40.37, p=0.001 Group: F(2.21)=0.17, p=0.846 Time x group: F(2.21)=0.11, p=0.894

Note: All values are expressed as mean (standard deviation).

*p<0.05; **p<0.01 respect to pre intervention values.

Table 3. Descriptive analysis of the participant's diet composition

Treatment	Beef		Whey		CHO	
	Pre	post	Pre	post	pre	Post
Proteins $g \cdot d^{-1}$ $g \cdot kg^{-1} \cdot d^{-1}$ % of total energy	109.85 (23.90) 1.49 (0.46) 21 (3)	126.27 (23.91) * 1.69 (0.47)* 22 (3)	115.53 (22.06) 1.52 (0.45) 22 (3)	131.44 (26.08) 1.72 (0.52)* 23 (4)	111.01 (13.76) 1.45 (0.24) 21 (3)	110.98 (13.80) 1.44 (0.24) 20 (3)
Carbohydrate $g \cdot kg^{-1} \cdot d^{-1}$ % of total energy	3.11 (0.78) 45 (6)	3.41 (0.82)* 45 (7)	3.08 (1.38) 44 (5)	3.38 (1.39)* 45 (4)	3.05 (0.24) 45 (2)	3.62 (0.32)* 50 (3)*
Fats $g \cdot kg^{-1} \cdot d^{-1}$ % of total energy	1.01 (0.32) 33 (5)	1.19 (0.59)* 33 (8)	1.02 (0.19) 34 (4)	1.03 (0.20) 32 (4)	1.00 (0.23) 33 (5)	0.98 (0.24) 30 (4)
Energy $Kcal \cdot kg^{-1} \cdot d^{-1}$ Total daily energy	28.31 (7.05) 2077 (201)	31.94 (9.73)* 2346 (336)*	28.30 (8.84) 2150 (378)	30.46 (9.10)* 2323 (382)*	27.71 (3.11) 2132 (178)	29.86 (3.65)* 2304 (160)*

Notes: Values are means (SD); the post diet analysis includes the ingestion of the supplement for each of the treatment condition.

*$p<0.01$ significant difference from pre-intervention to post (last week of intervention)

Table 4. Average relative change (delta scores) and 95% confidence interval determined per each treatment condition.

Variable	Beef (n=8)		Whey (n=8)		CHO (n=8)	
	mean [95% CI]	P	mean [95% CI]	p	mean [95% CI]	P
Body mass (kg)	1.2% [-0.7, 3.1]	0.172	0.5% [-1.0, 2.0]	0.470	0.6% [-0.5, 1.7]	0.217
Fat (%)	0.1% [-11%, 11.35]	0.976	-4.4% [-15.6, 6.9]	0.389	1.9% [-1.6, 5.3]	0.339
Fat-free mass (%)	0.8% [-1.2, 2.9]	0.373	1.1% [-0.8, 3.0]	0.208	-0.3% [-1.0, 0.5]	0.451
Fat (kg)	1.5% [-11.3, 14.3]	0.788	-3.9% [-16.4, 8.7]	0.489	2.5% [-1.4, 6.4]	0.173
Fat-free mass (kg)	2.0% [0.2, 3.8]*	0.034	1.4 [0.2, 2.6]*	0.028	0.0% [-1.2, 1.2]	1.000
Arm Circumference (cm)	4.8% [2.3, 7.3]*$^{\theta}$	0.003	-0.5 [-2.9, 1.9]	0.644	0.6% [-2.6, 3.8]	0.657
Thigh Circumference (cm)	3.2% [0.4, 5.9]*	0.029	0.9 [-13.0, 3.0]	0.371	2.4% [-1.4, 6.2]	0.185
Biceps brachialis thickness (mm)	11.2% [5.9, 16.5]*$^{\pi}$	0.002	1.1 [-1.7, 4.0]	0.380	4.5% [1.6, 7.4]*	0.008
Vastus Medialis thickness (mm)	11.1% [6.3, 15.9]*	0.001	12.1 [4.0, 20.2]*	0.009	6.3% [1.9, 10.6]*	0.012
1RM Bench Press (kg)	15.8% [7.0, 24.7]*	0.004	5.8 [1.7, 9.8]*	0.012	11.4% [-0.9, 23.6]	0.064
1RM Squat (kg)	21.6% [5.5, 37.7]*	0.016	14.6 [5.9, 23.3]*	0.005	19.6% [2.2, 37.1]*	0.033

Data are presented as relative change (%) from baseline to follow-up and P-values are calculated via confidence intervals or Bonferroni adjusted Student t-tests for between group comparisons.

* Significant respect to baseline; $^{\theta}$ Significant respect to whey condition; $^{\pi}$ Significant respect to both whey and CHO conditions.