Intake of Animal Protein Blend Plus Carbohydrate Improves Body Composition with no Impact on Performance in Endurance Athletes

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ABSTRACT

The impact of animal blend protein supplements in endurance athletes is scarcely researched. We investigated the effect of ingesting an admixture providing orange juice and protein from beef and whey versus carbohydrate alone on body composition and performance over a 10-week training period in male endurance athletes. Participants were randomly assigned to a protein (CHO+PRO, n=15) or a non-protein isoenergetic carbohydrate (CHO, n=15) group. Twenty grams of supplement mixed with orange juice was ingested post-workout or before breakfast on non-training days. Measurements were performed pre- and post-intervention on body composition (by dual-energy X-ray absorptiometry), peak oxygen consumption ($\dot{V}O_{2peak}$), and maximal aerobic speed (MAS). Twenty-five participants (CHO+PRO, n=12; CHO, n=13) completed the study. Only the CHO+PRO group significantly ($p<0.05$) reduced whole body fat (mean±SD) (-1.02 ± 0.6 kg), total trunk fat (-0.81 ± 0.9 kg) and increased total lower body lean mass (+0.52 ± 0.7 kg), showing close to statistically significant increases of whole-body lean mass (+0.57 ± 0.8 kg, $p=0.055$). Both groups reduced ($p<0.05$) visceral fat (CHO+PRO, -0.03 ± 0.1 kg; CHO, -0.03 ± 0.5 kg) and improved the speed at MAS (CHO+PRO, +0.56 ± 0.5 $km{\cdot}h^{-1}$; CHO, +0.35 ±0.5 $km{\cdot}h^{-1}$). Although consuming animal blend protein mixed with orange juice over 10 weeks helped to reduce fat mass and to increase lean mass, no additional performance benefits in endurance runners were observed.

Keywords: Whey; beef; lean mass; trunk fat; visceral adipose tissue; aerobic, runners.

Introduction

The current daily protein recommendation for regular endurance exercisers is between 1.2 to 1.6 (Thomas et al., 2016) or up to 1.8 g·kg·body mass for trained endurance athletes (Jager et al., 2017). Accordingly, Kato et al. (2016), using the amino acid oxidation method, suggested an average daily consumption of 1.65 or up to 1.83 g·kg·body mass to satisfy protein requirements in endurance trained males. Such an amount of daily protein intake should be administered evenly spaced throughout the day. Moreover, the consumption of protein during the post-workout time has been proposed as a pragmatic and sensible strategy (Kerksick et al., 2017) for supporting recovery and the adaptational processes (Doering et al., 2016). While no ergogenic outcomes may be evident, research has reported that the post-workout ingestion of protein and carbohydrate admixtures are effective to attenuate markers of muscle damage, decrease muscular soreness (Kerksick et al., 2017), and maintain or increase muscle mass in endurance athletes compared to the ingestion of only carbohydrate (D'Lugos et al., 2016). Consequently, the post-workout ingestion of protein-carbohydrate admixtures may attenuate muscle disruption and optimize changes in body composition but this practice may not have a meaningful effect on performance compared to the ingestion of carbohydrate alone (McLellan et al., 2014).

Both whey and beef are high-quality protein sources with a very similar amino acid composition to that found in skeletal muscle (Cruzat et al., 2014). Although whey contains higher concentrations of leucine, which seems to be an important essential amino acid for starting muscle protein synthesis (Naclerio and Larumbe-Zabala, 2016), beef is a source of heme-iron, zinc, vitamin B12, and essential fatty acids that are relevant nutrients in supporting muscle remodeling (Phillips, 2012). Indeed, the ingestion of a post-workout hydrolyzed beef protein was effective to protect muscle mass in male endurance athletes (Naclerio et al., 2017). On the other hand, whey is composed of several bioactive fractions (glycomacropeptide, β-lactoglobulin, α-lactalbumin and lactoferrin), with multiple health (Zapata et al., 2017) and weight control benefits (Miller et al., 2014). Although the positive effects of protein supplementation to support lean mass in endurance athletes is well documented (Doering et al., 2016), its effects to reduce total and abdominal fat have been mainly observed in overweight and obese adults (Arciero et al., Ormsbee, 2014). The aim of the current study, therefore, was to compare the effects of combining a 10-week endurance training program with one of the following commercially available products: (i) Beef and Whey protein blend (Crown® Sport Nutrition, Spain) providing hydrolyzed 100% All Beef and whey isolate (Optipep, Carbery) mixed with orange juice; and (ii) non-protein, carbohydrate-only (maltodextrin and orange juice), on body composition and performance in well-trained male endurance runners. The primary outcomes measures were whole body fat mass, whole body lean mass, total trunk fat mass, trunk lean mass, visceral fat mass, total (right and left) upper and lower body limb lean and fat mass. Secondary outcomes measures included peak oxygen consumption, and maximal aerobic speed. Based on the available literature, we hypothesized that compared to an isoenergetic-only carbohydrate supplement, the post-workout ingestion of a carbohydrate-protein admixture would protect muscle mass, and promote fat reduction with no additional performance benefit in well-trained endurance athletes.

Methods

Participants

After a pre-screening of the individuals characteristics and training background, thirty endurance athletes met the inclusion criteria: (a) >18–45 years of age; (b) only those who consistently trained between 6 to 10 hours per week (four to seven workout per week) for the last five years were considered for the study; (c) free from musculoskeletal limitations. Exclusion criteria were: (a) history of metabolic conditions and/or diseases; (b) consuming any medication including those with androgenic and/or anabolic effects, nutritional supplements affecting performance and body composition (e.g. creatine, essential amino acids, proteins, dehydroepiandrosterone, etc.) during the previous 8 weeks prior to the start of the study; (c) current use of tobacco products; (d) the presence of any soft tissue or orthopedic limitations.

Compliance was confirmed verbally and prior to providing written consent. The study was approved by the Institution Ethics Committee for Clinical Research (ID: 2016 RM/05). All experimental procedures were conducted in accordance with the Declaration of Helsinki and registered as Clinical Trial at ClinicalTrials.gov, U.S. National Institutes of Health (Identifier: NCT02954367).

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

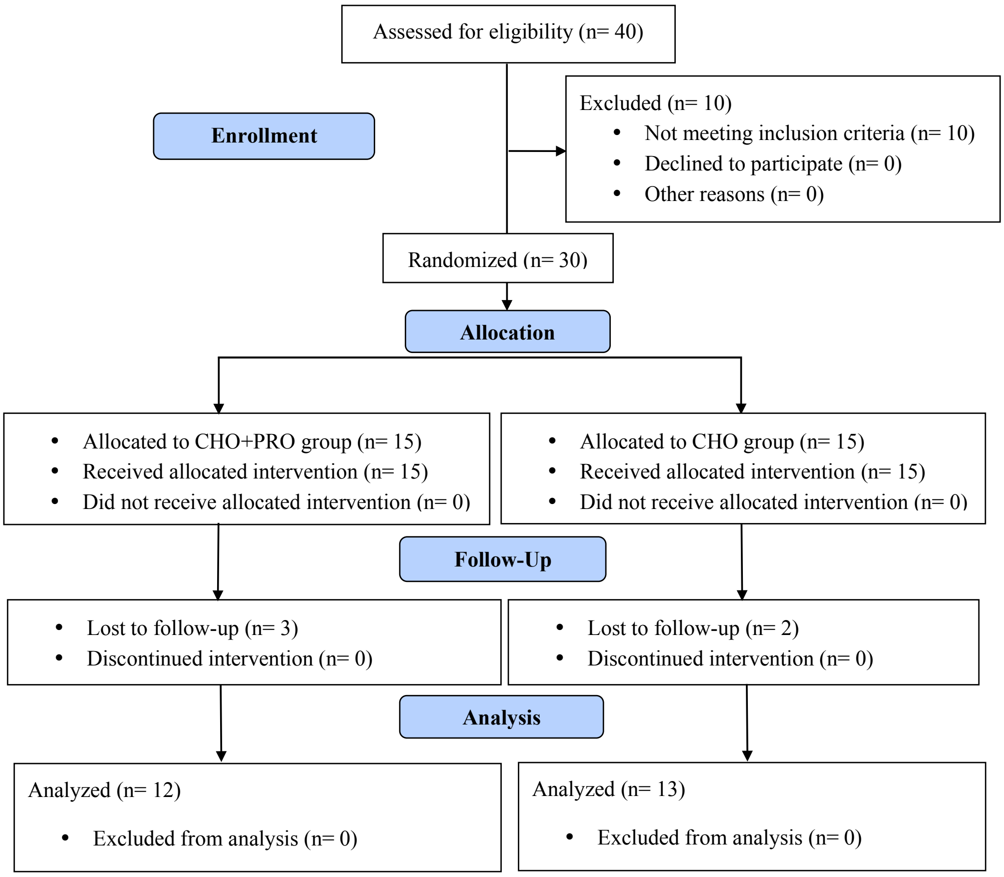

Figure 1: Flow diagram of participants throughout the course of the study.

The study was designed as a double-blind, two parallel group, randomized control trial for between-participant comparisons. After assessing for eligibility, the participants were randomly allocated into two equal-size treatment groups: protein (CHO+PRO), n=15; or carbohydrate only (CHO), n=15. Following a pre-assessment of body composition and performance, the participants were matched by their fat, fat-free and $\dot{V}O_{2peak}$ values. In a double-blind fashion, the assignment of participants to two treatments was performed by block randomization using a block size of two. Initial groups characteristics (mean ± SD) were not significantly different at baseline: CHO+PRO: age 30.3 ± 8.8 years, 1.74 ± 0.59 m height, 68.9 ± 4.4 kg body mass, 60.5 ± 7.3 ml/kg/min^{-1}$\dot{V}O_{2peak}$; CHO: 34.1 ± 7.8 years, 1.76 ± 0.51 m height, 66.2 ± 4.0 kg body mass, 61.49 ± 6.8 ml/kg/min^{-1}, $\dot{V}O_{2peak}$.

Sample size estimations were calculated assuming a two group by two repeated measures model, where the α-error probability was set at 0.05 and the statistical power was established at 0.80 $(1-\beta)$. Based upon an effect size of $\eta^2=0.035$ for the primary outcome variable, fat mass (kg), and an interaction effect between groups conducted upon an interim analysis of the first 12 participants, a sample size estimation of n=24 was determined as appropriate. Nonetheless, assuming an anticipated attrition rate of 20%, we enrolled 15 participants per group.

Assessments

Before and after a 10-week intervention period, measurements of body composition followed by an endurance test were determined. Prior to the assessments, 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.

Body mass, whole body fat mass, whole body lean mass, total trunk fat mass, estimated visceral fat mass, and fat and lean mass for upper and lower limbs (right and left) were measured using dual-energy X ray absorptiometry (General Electric Healthcare, Madison, WI). These measurements were performed in standardized conditions, in the morning and in a fasted state.

A progressive to volitional exhaustion running test was used to determine peak oxygen consumption ($\dot{V}O_{2peak}$) and maximal aerobic speed (MAS). After a general warm-up, starting at 10 $km \cdot h^{-1}$, running speed was increased by 0.3 $km \cdot h^{-1}$ every 30s until volitional exhaustion. Gas exchange data were collected continuously using an automated breath-by-breath system (Ultima^{TM} Series, MGC Diagnostic Corporation, St. Paul, Minnesota, USA Vmax 29C); which was calibrated according to the manufacturer's instructions. The volume calibration was performed at different flow rates with a 3-L calibration syringe allowing an error <3%. The calibration of gas analyzers was performed automatically using reference values of environmental gases and cylinders (16% O_2, 4% CO_2). $\dot{V}O_{2peak}$ was recorded as the highest $V^{\cdot}O_2$ value obtained for any continuous 30s period. The maximal aerobic speed (MAS) was associated with the last completed 30s stage before exhaustion (Esteve-Lanao, Foster, Seiler, & Lucia, 2007).

Control of training

All participants were trained by the same coach. All of them committed to follow a 10-week training program using a polarized intensity distribution (Esteve-Lanao et al., 2007). Participants trained 5 to 6 sessions per week controlling the duration, distance and quantified intensity by continuous heart rate registration. All the participants trained during the afternoon (12 to 6:00 pm).

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. To evaluate differences caused by treatments, diet was analyzed again during the last week of the intervention.

Supplementation and Control of the Intervention Compliance

The two supplements were presented as 24 g sachets of vanilla-flavored powder diluted in ~250 mL of orange juice. The mixed drinks were similar in appearance, texture and taste, and were isoenergetic. The nutritional composition of each product is presented in Table 1. On training days, supplements were ingested within 20 min after training, whereas on non-training days supplement was administered before breakfast. To avoid missing doses, on non-training days, automatic text messages were sent to all the participants. Additionally, participants were allowed to drink water at libitum but not to consume any food during the training sessions.

After completing the first assessment, each participant was given a batch of one of the two products, assigned according to randomization.

Tolerance collected from any adverse events and compliance with supplement intake (determined by an individual follow-up) was evaluated continuously. Only participants who completed the 70 days of treatment with a minimum of 4 sessions per week (40 workouts in total) were analyzed. The diary training report was used to determine participant compliance.

Table 1. Nutritional composition of drinks per intake (24 g of powder plus 250 ml of orange juice)

Nutrient	CHO+PRO	CHO
Energy value (kcal)	204	204
Carbohydrates (g)	27.70	50.10
Lipids (g)	1.05	0
Proteins (g)	19.84	0.40
Alanine (g)	1.14	-
Arginine (g)	0.82	-
Aspartic acid (g)	1.94	-
Cysteine (g)	0.33	-
Glutamic acid (g)	3.33	-
Glycine (g)	0.79	-
Histidine (g)	0.48	-
Isoleucine (g)	1.16	-
Leucine (g)	1.76	-
Lysine (g)	1.82	-
Methionine (g)	0.45	-
L-Ornitine	0.02	
Phenylalanine (g)	0.67	-
Proline (g)	1.08	-
Serine (g)	0.88	-
L-Taurine	0.02	
Threonine (g)	1.13	-
Tryptophan (g)	0.28	-
Tyrosine (g)	0.58	-
Valine (g)	1.13	-
Total EAA (g)	**10.64**	**-**
Heme Iron (mg)	1.93	**-**
Zinc (mg)	2.26	**-**
Potassium (mg)	2012.16	**-**
Magnesium (mg)	15.90	**-**
Selenium (µg)	2.88	**-**
Calcium (mg)	59.25	**-**
Folic Acid (µg)	10.04	**-**
Niacin (mg)	13.04	-
Vitamin B 6 (mg)	0.04	**-**
Vitamin B 12 (µg)	0.39	**-**

Notes: EAA: essential amino acids; CHO+PRO: supplement admixture including orange juice mixing with a beef and whey protein blend, CHO: supplement admixture including orange juice mixing with maltodextrin.

Statistical Analysis

A descriptive analysis was performed and subsequently the Kolmogorov-Smirnov and Shapiro-Francia test were applied to assess normality. Sample characteristics at baseline were compared between conditions (CHO+PRO vs. CHO) using two-tailed independent samples t test. Changes from pre to post treatment in body composition, and performance were assessed using a 2 (treatments) × 2 (times) repeated measures ANOVA. As suggested by Castañeda et al. (1993), changes over time were analyzed using a priori Bonferroni-adjusted pairwise comparisons. 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 0.05 but p values between >0.05 and 0.1 were considered indicative of a trend. Results are reported as mean ± SD unless stated otherwise. Data analyses were performed with Stata 13.1 (StataCorp, College Station, TX).

Results

Due to non-intervention related reasons, five participants (3 from CHO+PRO and 2 from CHO) dropped out of the study. At baseline, all the analyzed variables were not significantly different between groups. Table 2 shows the dietary monitoring results, determined before and after the intervention.

At baseline, no between-group differences were observed. However, as a result of the intervention, CHO+PRO group significantly increased both the protein and carbohydrate intakes while CHO group increased the consumption of carbohydrates. Despite no changes observed in the overall caloric intake, both groups increased the energy contribution from carbohydrates and decreased the proportion from fat. However, only CHO+PRO increased the proportion of energy from proteins. Despite the observed changes, no between-treatment differences were observed at post-intervention. No complaints about any negative symptoms (i.e. hypoglycemic reaction) or gastric discomfort due to the ingestion of supplement were reported. Table 3 summarizes the pre and post values of the analyzed variables.

Table 2. Descriptive analysis of the participants diet composition

Treatment	CHO+PRO **(n=12)**		**CHO (n=13)**	
	Pre	**Post**	**Pre**	**Post**
Protein				
$g \cdot d^{-1}$	122.5 ± 23.4	143.2 ± 29.5*	125.1 ± 28.6	125.4 ± 26.3
$g \cdot kg^{-1} \cdot d^{-1}$	1.7 ± 0.3	2.1 ± 0.4*	1.9 ± 0.4	1.9 ± 0.4
% of total energy	21 ± 0.4	23 ± 0.3*	22 ± 0.4	21 ± 0.3
Carbohydrate				
$g \cdot d^{-1}$	255.6 ± 102.9	304.5 ± 108.0*	238.82 ± 73.9	281.9 ± 59.3*
$g \cdot kg^{-1} \cdot d^{-1}$	3.6 ± 1.4	4.3 ± 1.5*	3.6 ± 1.1	4.2 ± 0.9*
% of total energy	41 ± 0.6	47 ± 0.5*	41 ± 0.6	48 ± 0.5*
Fat				
$g \cdot d^{-1}$	97.6 ± 27.8	103.98 ± 31.01	96.07 ± 29.6	93. 5 ± 21.1
$g \cdot kg^{-1} \cdot d^{-1}$	1.4 ± 0.4	1.48 ± 0.40	1.42 ± 0.4	1.4 ± 0.3
% of total energy	38 ± 0.5	30 ± 0.3*	38 ± 0.5	31 ± 0.4*
Energy				
Total daily energy	2433.5 ± 726.7	2561.0 ± 797.7	2339.8 ± 600.9	2373.9 ± 471.5
$Kcal \cdot kg^{-1} \cdot d^{-1}$	34.8 ± 10.5	36.4 ± 10.5	34.7 ± 8.3	35.2 ± 6.4

Notes: Pre and post intervention values are presented as mean ± standard deviation
*$P<0.05$; **$P<0.001$ and ^{T} $p<0.10$ from pre to post-intervention (last week of intervention).
CHO+PRO = participants ingesting orange juice mixed with beef and whey protein, CHO participants ingesting orange juice mixing with maltodextrin.

Main time effects were observed for body mass [F(1,23)=7.86, p=0.010, η_G^2=0.26], whole body fat [F(1,23)=15.83, p=0.001, η_G^2=0.41], whole body lean mass [F(1,23)=4.75, p=0.040, η_G^2=0.17], total trunk fat mass [F(1,23)=12.04, p=0.002, η_G^2=0.34], visceral fat mass [F(1,23)=14.83, p=0.001, η_G^2= 0.39], total lower body limb fat mass [F(1,23)=6.07, p=0.022, η_G^2=0.21] and total lower body limb lean mass [F(1,23)=5.06, p=0.034, η_G^2=0.18]. No interaction or between-groups effects were identified. Pairwise comparisons revealed that only CHO+PRO significantly reduced body mass (p=0.039). Both groups reduced whole body fat mass (CHO+PRO, p=0.004; CHO, p=0.024), but neither group increased trunk or upper body lean mass. No change in arm fat was observed. Furthermore, only CHO+PRO produced a significant increase in the total lower body limb lean mass (p=0.016) along with a very close to significant increase (p=0.055) in the whole-body lean mass. Additionally, both groups showed close to significant decreases in total lower body limb fat mass (CHO+PRO p=0.098; CHO p=0.075).

Table 3. Descriptive analysis of the body composition and performance variables

Variables	CHO+PRO **(n=12)**				**CHO (n=13)**			
	Pre	**Post**	**Change**	**ES**	**Pre**	**Post**	**Change**	**ES**
Body mass (kg)	69.6 ± 4	68.8 ± 4*	-0.87 ± 0.9	0.63	67.2 ± 3.6	66.5 ± 4.3^{t}	-0.67 ± 1.6	0.49
Whole body fat mass (kg)	14.5 ± 3.4	13.4 ± 2.8**	-1.02 ± 0.6	0.92	14.1 ± 2.8	13.4 ± 2.3*	-0.74 ± 1.3	0.67
Whole body lean mass (kg)	53.1 ± 3.3	53.6 ± 3.4^{t}	+0.57 ± 0.8	0.58	51.6 ± 3.8	51.9 ± 3.7	+0.28 ± 1.0	0.29
Total trunk fat mass (kg)	6.8 ± 2.1	6.0 ± 1.5**	-0.81 ± 0.9	0.94	6.3 ± 1.5	5.9 ± 1.4	-0.39 ± 0.8	0.45
Trunk lean mass (kg)	24.2 ± 1.8	24.0 ± 1.7	-0.19 ± 0.9	0.20	23.3 ± 1.6	23.4 ± 1.4	+0.13 ± 0.8	0.15
Visceral fat mass (kg)	0.34 ± 0.1	0.31 ± 0.1**	-0.03 ± 0.1	0.82	0.32 ± 0.1	0.28 ± 0.1*	-0.03 ± 0.5	0.72
Total lower body limb fat mass (kg)	4.9 ± 1.0	4.7 ± 1.0^{t}	-0.22 ± 0.2	0.47	5.1 ± 1.0	4.9 ± 1.0^{t}	-0.24 ± 0.58	0.54
Total lower body limb lean mass (kg)	18.9 ± 1.4	19.4 ± 1.6*	+0.52 ± 0.7	0.75	18.4 ± 1.6	18.5 ± 1.7	+0.10 ±0.6	0.16
Total upper body limb fat mass (kg)	1.7 ± 0.5	1.7 ± 0.6	+0.01 ± 0.1	0.01	1.7 ± 0.5	1.5 ± 0.4	-0.11	0.30
Total upper body limb lean mass (kg)	6.4 ± 0.6	6.6 ± 0.7	+0.22 ± 0.6	0.38	6.1 ±0.8	6.2 ± 0.8	+0.02 ± 0.5	0.04
$\dot{V}O_{2peak}$ (ml·kgm·min^{-1})	61.0 ± 5.6	61.2 ± 4.0	+0.24 ± 2.8	0.07	60.1 ± 6.9	60.8 ± 5.0	0.15 ± 3.7	0.04
Maximal aerobic speed (km·h^{-1})	17.8 ± 1.3	18.4 ± 1.0**	+0.56 ± 0.5	1.01	17.7 ± 1.0	18.1 ± 0.9*	+0.35 ±0.5	0.64

Note: Values determined at pre, post and the corresponding calculated change (post – pre) are presented as mean ± standard deviation. Pairwise comparison *$p<0.05$; **$p<0.01$ respect to pre-intervention values. ^{t}p >0.05 and <0.1. ES= Cohen’s d, effects size for two dependent means. CHO+PRO = participants ingesting orange juice mixing with beef and whey protein, CHO participants ingesting orange juice mixing with maltodextrin.

Only CHO+PRO significantly decreased total trunk fat (p=0.004, Figure 2A). However, both treatments decreased visceral fat (CHO+PRO, p=0.009; CHO, p=0.016, Figure 2B). No statistically significant differences between groups were observed after intervention in any of the body composition variables.

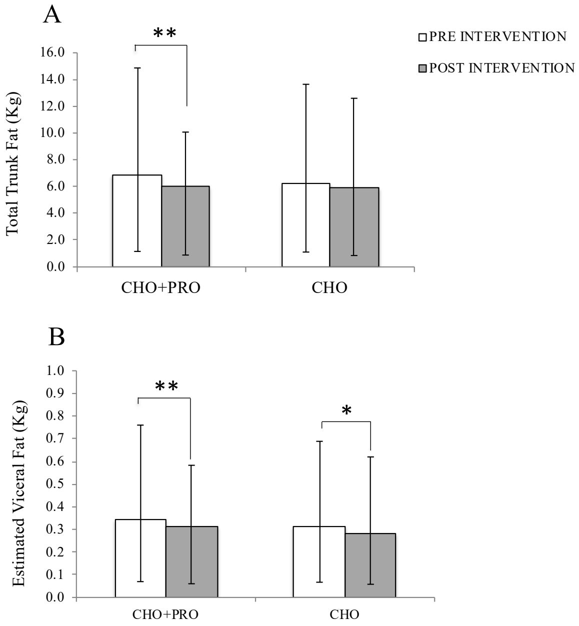

Figure 2. Observed changes in the total trunk fat (A) and estimated visceral fat (B).
CHO+PRO = participants ingesting orange juice mixed with beef and whey protein,
CHO = participants ingesting orange juice mixed with maltodextrin.
Data are presented as mean (95% CI). **p<0.01, *p<0.05; respect to pre-intervention values.

Training time distribution was as follows: 75–80% in Zone 1, ~5% in Zone 2, and 15–20% in Zone 3. The resulted training load using the ECOs methods described by Esteve-Lanao et al., (2017) was ~43%-7%-50% for Zone 1; Zone 2 and Zone 3 respectively.

No time, group or time x group interaction effects were determined for $\dot{V}O_{2peak}$, however, main time (F(1,23)=17.11, p=0.001, η_G^2=0.43) but no group or interaction effects were determined for MAS. Pairwise comparisons revealed that both groups significantly increased the speed at MAS (CHO+PRO p=0.001; CHO p=0.03).

Discussion

The present study shows that ingesting a 20 g post workout protein blend (beef and whey) mixed with orange juice over 10 weeks promoted positive changes in body composition, reduced body mass, total trunk fat and increased lean mass in endurance-trained runners. Despite the observed modification in the CHO+PRO group and the improved MAS determined in both groups (CHO+PRO and CHO), no significant differences between conditions were noticed at post-intervention.

Compared to CHO, the decrease in body mass in CHO+PRO was associated with a higher amount of fat mass loss (CHO+PRO: -1.02 ± 0.6 vs. CHO: -0.74 ± 1.3) alongside a superior increase of the whole-body lean mass (CHO+PRO: +0.57 ± 0.8 vs. CHO: +0.28 ± 1.0). Indeed, only the CHO+PRO group showed higher effect sizes to increase lower body limb lean and whole-body lean mass respectively (Table 3).

The observed results emphasize the positive effects of ingesting a protein supplement to preserve or promote muscle mass in endurance athletes (Doering et al., 2016; Naclerio et al., 2017). Maintaining appropriate levels of lower body limb lean mass in long distance runners has been associated with more efficient recovery, reduced overload related injuries and generally better training outcomes (Doering et al., 2016). Moreover, the ingestion of a post-workout admixture providing carbohydrates and 0.25 to 0.4 $g{\cdot}kg{\cdot}\text{body mass}^{-1}$ of high-quality protein has been shown to favor body net protein balance and support recovery after endurance exercises (Jager et al., 2017). Participants allocated to CHO+PRO were ingesting between 0.26 to 0.31 $g{\cdot}kg{\cdot}\text{body mass}^{-1}$ immediately post-workout or before breakfast during non-training days. The administered amount falls within the recommended protein intake to maximize muscle protein synthesis at rest (Areta et al., 2013) or to significantly improve muscle repair after exercise (Morton et al., 2015).

There was no apparent effect due to energy or macronutrient difference as an effect of the intervention. Thus, the only main difference between conditions was the composition of the post-workout supplement. According to the diet records, the amount of carbohydrates consumed by the two groups (CHO+PRO: 4.33±1.47; CHO: 4.20±0.87 $g{\cdot}kg^{-1}{\cdot}d^{-1}$, Table 2) was below the recommended dose of 5 to 7 $g{\cdot}kg^{-1}{\cdot}d^{-1}$ for endurance athletes (Thomas et al., 2016). The limited carbohydrate intake could have negatively influenced performance or induced loss of lean body mass. However, no negative effects on body composition or performance were observed for both treatments. When carbohydrates are provided below the required amount, a higher daily protein intake toward 2 $g{\cdot}kg^{-1}$ would be necessary to support metabolic adaptation including optimal glycogen replenishment and muscle remodeling (Thomas et al., 2016). Participants in both groups were consuming a relatively high amount of daily protein. Furthermore, no participant was ingesting less than 1.4 $g{\cdot}kg^{-1}{\cdot}d^{-1}$ which is well above than the minimum daily amount of protein (1.2 $g{\cdot}kg^{-1}{\cdot}d^{-1}$) recommended for endurance exercisers (Thomas et al., 2016). Additionally, only one participant in CHO+PRO and three in CHO ingested more than 1.65 $g{\cdot}kg^{-1}$ of protein which is the suggested average intake to satisfy the metabolic demands of endurance training (Kato et al., 2016). Our results seem to support the recommendation of ingesting high-quality protein-carbohydrate admixtures immediately after training for maintaining lean mass and reducing trunk fat (Kerksick et al., 2017). Although both CHO+PRO and CHO decreased whole body fat, only the CHO+PRO group significantly reduced total trunk fat (Table 3 and Figure 2A) and increased lower body lean mass (Table 3). The beneficial effect of ingesting high-quality protein supplements on body composition has been extensively reported in active or sedentary (Miller et al., 2014) overweight/obese (Arciero et al., 2014), as well as in physically active (Monteyne et al., 2018) or trained individuals (Morton et al., 2018; Taylor et al., 2016). Nonetheless, as visceral fat decreased in both conditions, it seems that regular exercise represents the main stimulus for mobilizing internal fat in normal weight trained athletes. The ingestion of animal protein, particularly whey, rather than vegetable protein has been associated with suppressed appetite, increased satiety (Miller et al., 2014), and favors protein synthesis which in turn would increase thermogenesis after ingesting high-protein meals (Acheson et al., 2011). Therefore, a hypothetically higher use of fat as the predominant fuel to support muscle-remodeling during the early recovery phase after ingesting a post-workout protein-carbohydrate admixture could be the cause of the more favorable changes in body composition observed in CHO+PRO compared to CHO. Moreover, recent evidences in rodents suggest that some components of whey protein such as Lactalbumin and Lactoferrin may increase postprandial lipolysis markers (Mobley et al., 2015), improve energy balance and decrease adiposity (Zapata et al., 2017) .

The present study is not without limitations; the diet was not strictly controlled but only recorded over 3 days before and after intervention. Although this approach has been extensively used, providing a pre-packed daily-meal scheme to participants would offer an ideal scenario to standardize and control their diet (Jeacocke and Burke, 2010). Although the observed trend to increase in lean mass for the CHO+PRO group could be explained by a gain in musculature, it is possible that non-muscle lean tissue in the trunk region made substantial contribution (Mitchell et al., 2017). Magnetic Resonance Imaging techniques would have been required to identify the contribution of skeletal muscle, viscera, and gut to the observed changes in lean mass indistinguishable with the use of DEXA as in the current study.

Considering the research design, the current findings support that the ingestion of a post-workout admixture providing protein from beef and whey mixed with orange juice represents a suitable alternative to improve body composition (trunk fat mass loss, increase whole and lower body limb lean mass) compared with the ingestion of carbohydrates alone. Nonetheless, no impact on performance has been observed.

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The authors declare that they have no conflicts of interest relevant to the content of this manuscript.

REFERENCES

Acheson, K. J., Blondel-Lubrano, A., Oguey-Araymon, S., Beaumont, M., Emady-Azar, S., Ammon-Zufferey, C., … Bovetto, L. (2011). Protein choices targeting thermogenesis and metabolism. *The American Journal of Clinical Nutrition*, *93*(3), 525–534. https://doi.org/10.3945/ajcn.110.005850

Arciero, P. J., Baur, D., Connelly, S., & Ormsbee, M. J. (2014). Timed-daily ingestion of whey protein and exercise training reduces visceral adipose tissue mass and improves insulin resistance: the PRISE study. *Journal of Applied Physiology (1985)*, *117*(1), 1–10. https://doi.org/10.1152/japplphysiol.00152.2014

Areta, J., Burke, L. M., Ross, M. L., Camera, D. M., West, D. D., Broad, E. M., … Coffey, V. G. (2013). Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. *The Journal of Physiology*, *591*(9), 2319–2331.

Castañeda, M. B., Levin, J. R., & Dunham, R. B. (1993). Using planned comparisons in management research: A case for the Bonferroni procedure. *Journal of Management*, *19*(3), 707–724. https://doi.org/org/10.1177/014920639301900311

Chernoff, R. (2004). Protein and older adults. *Journal of the American College of Nutrition*, *23*(6 Suppl), 627S–630S. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15640517

Cruzat, V. F., Krause, M., & Newsholme, P. (2014). Amino acid supplementation and impact on immune function in the context of exercise. *Journal of the International Society of Sports Nutrition*, *11*(1), 61. https://doi.org/10.1186/s12970-014-0061-8

D'Lugos, A. C., Luden, N. D., Faller, J. M., Akers, J. D., McKenzie, A. I., & Saunders, M. J. (2016). Supplemental Protein during Heavy Cycling Training and Recovery Impacts Skeletal Muscle and Heart Rate Responses but Not Performance. *Nutrients*, *8*(9). https://doi.org/10.3390/nu8090550

Doering, T. M., Reaburn, P. R., Phillips, S. M., & Jenkins, D. G. (2016). Postexercise Dietary Protein Strategies to Maximize Skeletal Muscle Repair and Remodeling in Masters Endurance Athletes: A Review. *International Journal of Sport Nutrition and Exercise Metabolism*, *26*(2), 168–178. https://doi.org/10.1123/ijsnem.2015-0102

Esteve-Lanao, J., Foster, C., Seiler, S., & Lucia, A. (2007). Impact of training intensity distribution on performance in endurance athletes. *Journal of Strength and Conditioning Research*, *21*(3), 943–949. https://doi.org/10.1519/R-19725.1

Esteve-Lanao, J., Moreno-Perez, D., Cardona, C. A., Larumbe-Zabala, E., Munoz, I., Selles, S., & Cejuela, R. (2017). Is Marathon Training Harder than the Ironman Training? An ECO-method Comparison. *Frontiers in Physiology*, *8*, 298. https://doi.org/10.3389/fphys.2017.00298

Jager, R., Kerksick, C. M., Campbell, B. I., Cribb, P. J., Wells, S. D., Skwiat, T. M., … Antonio, J. (2017). International Society of Sports Nutrition Position Stand: protein and exercise. *Journal of the International Society of Sports Nutrition*, *14*, 20. https://doi.org/10.1186/s12970-017-0177-8

Jeacocke, N. A., & Burke, L. M. (2010). Methods to standardize dietary intake before performance testing. *International Journal of Sport Nutrition and Exercise Metabolism*, *20*(2), 87–103. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20479482

Kato, H., Suzuki, K., Bannai, M., & Moore, D. R. (2016). Protein Requirements Are Elevated in Endurance Athletes after Exercise as Determined by the Indicator Amino Acid Oxidation Method. *PLoS One*, *11*(6), e0157406. https://doi.org/10.1371/journal.pone.0157406

Kerksick, C. M., Arent, S., Schoenfeld, B. J., Stout, J. R., Campbell, B., Wilborn, C. D., … Antonio, J. (2017). International society of sports nutrition position stand: nutrient timing. *Journal of the International Society of Sports Nutrition*, *14*, 33. https://doi.org/10.1186/s12970-017-0189-4

McLellan, T. M., Pasiakos, S. M., & Lieberman, H. R. (2014). Effects of protein in combination with carbohydrate supplements on acute or repeat endurance exercise performance: a systematic review. *Sports Medicine*, *44*(4), 535–550. https://doi.org/10.1007/s40279-013-0133-y

Miller, P. E., Alexander, D. D., & Perez, V. (2014). Effects of whey protein and resistance exercise on body composition: a meta-analysis of randomized controlled trials. *Journal of the American College of Nutrition*, *33*(2), 163–175. https://doi.org/10.1080/07315724.2013.875365

Mitchell, C. J., Milan, A. M., Mitchell, S. M., Zeng, N., Ramzan, F., Sharma, P., … Cameron-Smith, D. (2017). The effects of dietary protein intake on appendicular lean mass and muscle function in elderly men: a 10-wk randomized controlled trial. *The American Journal of Clinical Nutrition*, *106*(6), 1375–1383. https://doi.org/10.3945/ajcn.117.160325

Mobley, C. B., Fox, C. D., Ferguson, B. S., Pascoe, C. A., Healy, J. C., McAdam, J. S., … Roberts, M. D. (2015). Effects of protein type and composition on postprandial markers of skeletal muscle anabolism, adipose tissue lipolysis, and hypothalamic gene expression. *Journal of the International Society of Sports Nutrition*, *12*, 14. https://doi.org/10.1186/s12970-015-0076-9

Monteyne, A., Martin, A., Jackson, L., Corrigan, N., Stringer, E., Newey, J., … James, L. J. (2018). Whey protein consumption after resistance exercise reduces energy intake at a post-exercise meal. *European Journal of Nutrition*, *57*(2), 585–592. https://doi.org/10.1007/s00394-016-1344-4

Morton, R. W., McGlory, C., & Phillips, S. M. (2015). Nutritional interventions to augment resistance training-induced skeletal muscle hypertrophy. *Frontiers in Physiology*, *6*, 245. https://doi.org/10.3389/fphys.2015.00245

Morton, R. W., Murphy, K. T., McKellar, S. R., Schoenfeld, B. J., Henselmans, M., Helms, E., … Phillips, S. M. (2018). A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults. *British Journal of Sports Medicine*, *52*(6), 376–384. https://doi.org/10.1136/bjsports-2017-097608

Naclerio, F., & Larumbe-Zabala, E. (2016). Effects of Whey Protein Alone or as Part of a Multi-ingredient Formulation on Strength, Fat-Free Mass, or Lean Body Mass in Resistance-Trained Individuals: A Meta-analysis. *Sports Medicine*, *46*(1), 125–137. https://doi.org/10.1007/s40279-015-0403-y

Naclerio, F., Seijo, M., Larumbe-Zabala, E., Ashrafi, N., Christides, T., Karsten, B., & Nielsen, B. V. (2017). Effects of Supplementation with Beef or Whey Protein Versus Carbohydrate in Master Triathletes. *Journal of the American College of Nutrition*, *38*(8), 1–

9. https://doi.org/10.1080/07315724.2017.1335248

Phillips, S. M. (2012). Nutrient-rich meat proteins in offsetting age-related muscle loss. *Meat Science*, *92*(3), 174–178. https://doi.org/10.1016/j.meatsci.2012.04.027

Taylor, L. W., Wilborn, C., Roberts, M. D., White, A., & Dugan, K. (2016). Eight weeks of pre- and postexercise whey protein supplementation increases lean body mass and improves performance in Division III collegiate female basketball players. *Applied Physiology Nutrition and Metabolism*, *41*(3), 249–254. https://doi.org/10.1139/apnm-2015-0463

Thomas, T., Erdman, K. A., & Burke, L. M. (2016). American College of Sports Medicine Joint Position Statement: Nutrition and athletic performance. *Medicine and Science in Sports & Exercise*, *48*(3), 543–568. https://doi.org/10.1001/jama.1956.02970290016006

Zapata, R. C., Singh, A., Pezeshki, A., Nibber, T., & Chelikani, P. K. (2017). Whey Protein Components - Lactalbumin and Lactoferrin - Improve Energy Balance and Metabolism. *Scientific Reports*, *7*(1), 9917. https://doi.org/10.1038/s41598-017-09781-2