Effects of beef protein supplementation in male elite triathletes: a randomized, controlled, double-blind, cross-over study

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Abstract

Objective: Beef protein extracts are growing in popularity in recent years due to their purported anabolic effects as well as to their potential benefits on hematological variables. The present randomized, controlled, double-blind, cross-over study aimed to analyze the effects of beef protein supplementation on a group of male elite triathletes (Spanish National Team).

Methods: Six elite triathletes (age, 21 ± 3 years; VO_{2max}, 71.5 ± 3.0 $ml·kg·min^{-1}$) were randomly assigned to consume daily either 25 g of a beef supplement (BEEF) or an isoenergetic carbohydrates (CHO) supplement for 8 weeks, with both conditions being separated by a 5-week washout period. Outcomes, including blood analyses and anthropometrical measurements, were assessed before and after each 8-week intervention.

Results: No effects of supplement condition were observed on body mass nor on skinfold thicknesses, but BEEF induced significant and large benefits over CHO in the thigh cross-sectional area (3.02%, 95%CI=1.33 to 4.71 %; p=0.028, d=1.22). Contrary to CHO, BEEF presented a significant increase in *vastus lateralis* muscle thickness (p=0.46), but differences between conditions were not significant (p=0.173, d=0.87). Although a significantly more favorable testosterone-to-cortisol ratio (TCR) was observed for BEEF over CHO (37%, 95% CI=5 to 68 %; p=0.028, d=1.29), no significant differences were found for the hematological variables (i.e., iron, ferritin, red blood cell count, hemoglobin or hematocrit).

Conclusion: Beef protein supplementation seems to facilitate a more favorable anabolic environment (i.e., increased TCR and muscle mass) in male elite triathletes, with no impact on hematological variables.

Key words: nutrition; supplement; endurance; muscle mass; anabolism.

Introduction

Strong evidence supports the effectiveness of protein supplementation to increase muscle mass, strength and performance in healthy subjects (1–3). Endurance athletes can also benefit from protein supplementation (4,5). Given the high energy demands of endurance athletes, which can lead to the oxidation of muscle protein as a fuel (i.e., muscle catabolism), protein supplementation might prevent from muscle mass losses or even promote muscle mass gains (5). Moreover, some evidence suggests that protein supplementation might attenuate muscle damage and facilitate skeletal muscle mass remodeling in this population (6,7), which could potentially result in a greater tolerance to training loads and eventually facilitate training-induced adaptations (7). However, the anabolic effects of protein are not only affected by individual factors such as nutritional state, digestive capacity or the sensitivity of muscle anabolic pathways, but also by the source of protein intake (8).

Beef protein is gaining popularity in recent years due to its purported anabolic effects (9). Different studies have shown that beef protein intake stimulates muscle protein synthesis (10–12), especially when combined with physical exercise (13). Even though some studies have reported increases in muscle thickness or lean body mass after using beef protein supplementation compared to the ingestion of carbohydrate (14–16), such effects have not been observed by others (15–17). A recent meta-analysis suggested that beef protein supplementation might induce small albeit significant gains in muscle mass and lower-body muscle strength (18). However, if these results are also observed in endurance elite athletes remains to be elucidated.

Another potential benefit of beef is that, due to its higher content in heme-iron, it could potentially serve to improve iron status (18). Indeed, beef protein supplementation has been reported to enhance the iron status of master-age triathletes (15) and to increase hematocrit levels in collegiate distance runners (19), which is of potential clinical and athletic relevance for endurance athletes who are usually at a higher risk of iron deficiency which negatively influences performance (20). Protein supplementation might also benefit training-induced adaptations in endurance athletes.

Preliminary evidence supports a potential role of beef protein on the promotion of muscle anabolism – or at least prevention of catabolism – in endurance elite athletes along with an improvement in the hematological profile, which could favorably impact training-induced adaptations on physical performance (18). The aim of the present study was to compare the effects of beef protein supplementation (compared to a non-protein supplement composed of carbohydrates) on anthropometrical measures, hematological parameters and hormonal status in male elite triathletes.

Methods

Experimental design

The present study followed a randomized, crossover, controlled, double-blind design. Participants were randomly assigned to take either a beef protein (BEEF) or a carbohydrate supplement (CHO) for 8 weeks. Thereafter, a 5-week washout period was left, and participants were then assigned to the opposite condition. In order to prevent bias in the condition-order allocation, the participants were first matched based on their maximal oxygen uptake (VO_{2max}) and age, and then randomly assigned in a counterbalanced order to each condition using block randomization with a block size of 2.

Participants:

Male elite triathletes recruited from the Spanish national team volunteered to participate in the present study (descriptive data presented in the results section). All participants were healthy, highly trained (25–30 hours·week^{-1}), and competed at the international elite level (all of them participating in European or World triathlon cups, and including among others an under-23 duathlon World champion and a top 20 in Triathlon World series). Inclusion criteria were being over 18 years old, free from anemia, without musculoskeletal limitations or injuries, and agreeing not to ingest other nutritional supplements during the study. Participants taking iron supplements were excluded. The study was performed during the competitive season (February-July 2018). Participants were instructed to maintain their normal diet (apart from the supplement assigned) and training routine throughout the intervention. Participants and researchers involved in administering the supplements, conducting the assessments and supervising the intervention were blinded to the received condition. All the participants signed an informed consent form after the procedures had been explained in detail. The study was approved by the Local Ethics Committee (*University of Alcalá*, Madrid, Spain; CEI/HU/2018/13).

Nutritional intervention

Participants ingested daily a 25-g supplement of BEEF (100% All beef, Crown Sport Nutrition, Arnedo, Spain) or an isoenergetic CHO supplement. Both supplements (BEEF and CHO) were presented as vanilla-flavored powder to be diluted in ~300 ml of water. The diluted drinks were isoenergetic, similar in appearance, texture and taste, and dispensed in identical opaque sachets. The CHO supplement consisted of a mix of maltodextrin and oats, which were not completely homogenized to replicate the granulated texture of BEEF. This 'placebo' supplement was manufactured by an external laboratory (I.D.E.A.S. Naturalfoods S.L., Castellón, Spain), and the same flavor was applied to both supplements to ensure participants' blinding. The nutritional details of both supplements, which were analyzed in an external laboratory (I.D.E.A.S. Naturalfoods S.L., Castellón, Spain), are shown in **Table 1**. Supplements were ingested immediately after the afternoon training session (~8 pm) or in the morning, just before breakfast, on non-training days (1 day every 14 days of continuous training). Participants were not said which supplement they were taking at any moment during the study.

Table 1. Main nutritional characteristics of the supplements provided.

Nutrient	BEEF	CHO
Energy (kcal)	99.33	99.9
Carbohydrates (g)	<0.5	19.3
Lipids (g)	1.9	<0.5
Proteins (g)	20.5	2.0
Leucine (g)	1.65	0
Isoleucine (g)	0.94	0
Valine (g)	1.00	0
Iron (mg)	4.3	0
Heme-iron (mg)	4.1	0
Fiber	0	2.3

Supplements were isoenergetic and consisted of 25 g of beef protein or 27.1 g of carbohydrate powder, which were diluted in 300 ml of plain water. Abbreviations: BEEF, beef protein supplement; CHO, carbohydrate supplement.

Measurements

All outcome variables were assessed over two days on the week before and after each 8-week intervention, at approximately the same time of the day and under the same conditions (*i.e.,* the morning before the first training session, after an overnight fast). Participants were required to refrain from any hard exercise session 48 hours prior to the assessment sessions.

Nutritional assessment

Participants completed a 3-day food daily report (2 weekdays, and 1 weekend day) during the last week of each condition. Nutritional intake was then analyzed using a specific software (DietoPro,Valencia, Spain), and the mean intake of macronutrients and iron during the three days was computed.

Training control

All participants were trained by the same coach and support staff. Participants trained the three triathlon disciplines (i.e., swimming, cycling and running), and also included ~1-2 weekly sessions of resistance training. Coaches provided information about the weekly training volume (in hours) for each participant during each study phase.

Blood Samples

After a fasting period of 8-10 h, blood samples (two tubes of 5 and 8 mL each) were drawn from the antecubital vein and collected on vacutainer venous blood collection tubes (BD Vacutainer Blood Collection Tubes). The 5-ml tube (containing EDTA as anti-coagulant) was used for the analysis of complete blood count using a fully automated hematology analyzer (ABX Pentra 60CC, Horiba Medical, Montpellier, France). The second tube (containing silica to accelerate the clotting process) was used to determine the concentration of ferritin, cortisol, and testosterone after separating the serum from the clotted blood. The tube was inverted 5 times and the whole blood was allowed to stand for about 30 min at room temperature to facilitate the clotting process. Then, the tube was centrifuged at 4000 rpm for 10 min, and the resultant serum was aliquoted into labeled Eppendorf tubes. The serum concentration of ferritin, testosterone and cortisol was assessed through ELISA (Elecys 2010, Roche Diagnostics, Basel, Switzerland) using specific reagents (ferritin: 11820982, testosterone: 1776061, cortisol: 1875116, Roche Diagnostics).

Anthropometric Assessments

Body mass and height were assessed using a standard scale (Delta 707, Seca GmbH & Co. KG, Hamburg, Germany) and stadiometer (Harpender, Holtain Limited, Crymich, UK) according the methods described elsewhere (21). An expert anthropometrist (ISAK level 3), using a high precision caliper (Harpenden plicometer; John Bull British Indicators, England; constant pressure of 10 g/mm and precision of 0.2 mm) measured 9 skinfolds thicknesses (pectoral, suprailiac, supraspinal, abdominal, biceps, triceps, subscapular, front thigh and medial calf) of the right side of the body. The anthropometrist conducted three rounds of the aforementioned measurements (that is, three measurements were taken for each site) and the median was computed for the analysis. The sum of all skinfold thicknesses was used for analysis as a marker of subcutaneous fat (21). Thigh circumference was measured with the participant in standing position using conventional measuring tape (precision of 1 mm) at the midpoint between the greater trochanter of the right femur and the most superior point on the lateral border of the right tibia. The value was converted into cross-sectional area (CSA) by correcting the circumference value for the respective skinfold (22). The median of the three repeated measurements was considered for the analysis. A very high intraclass correlation coefficient (ICC) was found between the three measurements (ICC=0.99, 95% confidence interval [CI]=0.98-1.00). The same researcher conducted all the anthropometric measurements at pre- and post-intervention.

Muscular Structure

The muscle thickness of the *vastus lateralis* (VL) muscle was determined by means of ultrasonography (Acuson S2000, Siemens, Germany) with a 50 mm, 7.5 MHz, linear-array probe as explained elsewhere (23). Briefly, participants lied supine on an examination bed with the knee in full extension. To provide acoustic contact without depressing the dermal surface, the probe was coated with a water-soluble transmission gel (Aquasonic 100 Ultrasound Transmission gel). The transducer was placed at 50% of the femur length longitudinally to the thigh along the mid-sagittal axis of the muscle, carefully aligned to the fascicle plane. The same experienced researcher (blinded to participants' condition) took all images, and another blinded researcher performed all measurements using a specific software (ImageJ 1.42q, National Institute of Health, Maryland). The distance between superficial and deep aponeuroses was determined three times in the proximal, central and distal portion of the image, and the mean of these measures was computed for analysis (23). These procedures have previously proven reliable when performed by an experienced researcher (23). In our case, a very high reliability was found between the three measurements (ICC=0.91, 95% confidence interval [CI]=0.79-0.95). Measurements and pictures were taken after each assessment to ensure that the specific location of the probe was the same on all assessments for a given participant.

Statistical analysis

Data are shown as mean ± standard deviation. To deal with potential differences in initial values, we compared the relative change (post-intervention minus baseline, relative to baseline and expressed as a percentage) observed between conditions. Non-parametric tests were used given the small sample size analyzed. Differences within and between conditions were assessed using Wilcoxon signed-rank test. Differences between conditions are expressed along with 95%CI. The magnitude of the differences was assessed using standardized effect sizes (ES, Hedges' g). ES values were interpreted as trivial (<0.20), small (<0.60), moderate (<1.20) or large (>1.20) (24). The chances of finding differences between conditions were assessed with a specific spreadsheet (25) to make magnitude-based inferences as follows: <1%, almost certainly not; 1–5%, very unlikely; 5–25%, unlikely; 25–75%, possible; 75–95%, likely; 95–99%, very likely; and >99%, almost certain (24). If the chances of having better and poorer results were both ≥5%, the difference was considered unclear. The analyses were performed using SPSS version 23.0, IBM, NY and the significance level was set at 0.05.

Results

From the 10 eligible participants, six (age, 21 ± 3 years; weight, 66 ± 4 kg; height, VO_{2max}, 71.5 ± 3.0 $ml·kg·min^{-1}$) completed all aspects of the study (**Figure 1**). One triathlete could not be included in the study because he was diagnosed anemia, and the other three excluded participants changed from training group and had to move to another city. The 6 included participants maintained their normal competitive and training schedule and suffered no injuries. No supplement-related adverse effects or intolerances were reported. No differences between each 8-week phase were observed for any outcome when analyzed independently of the supplement provided (**Supplementary Table 1**).

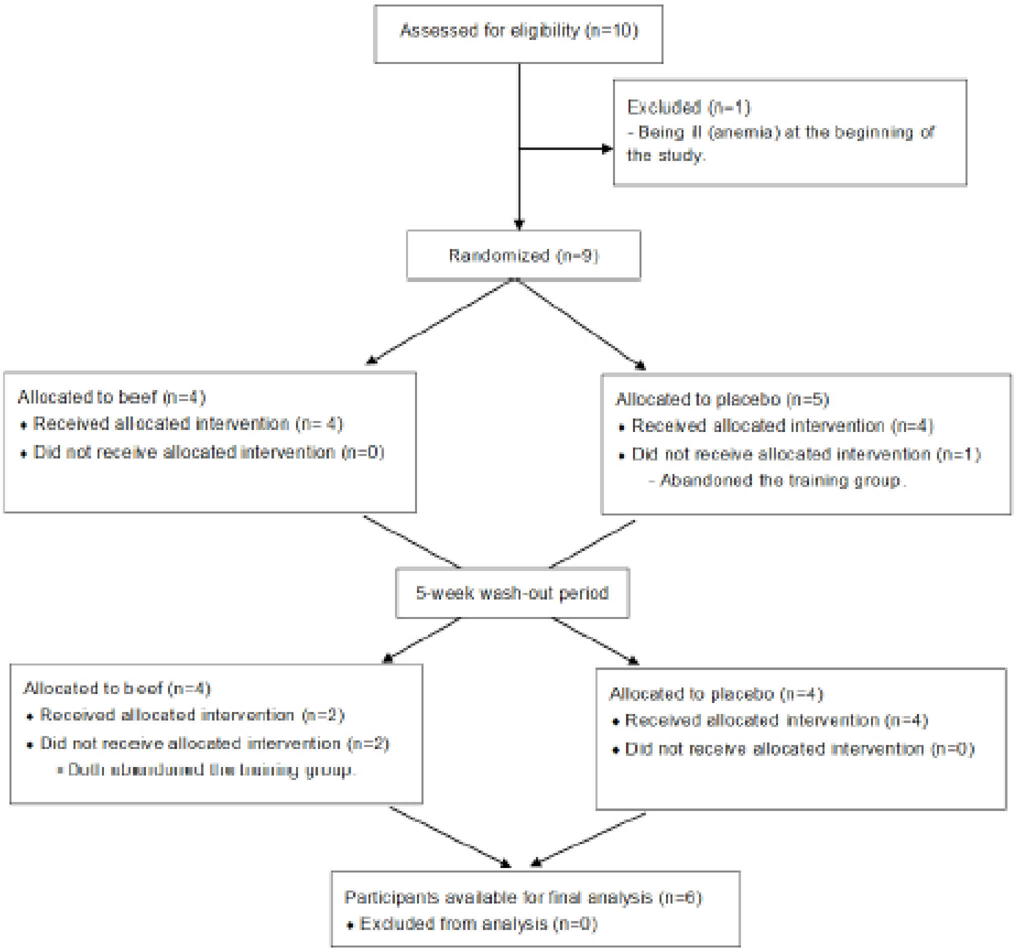

Figure 1. Flow chart of study participants.

No differences were found between conditions for training or nutritional variables. Nonetheless, it is worth noting that a close to statistically significant higher protein (p=0.075, ES=1.41) and lower carbohydrate (p=0.075, ES=0.72) intake observed in BEEF compared to CHO (**Table 2**). Regarding anthropometric measures (**Table 3**), no supplement effects were observed on body mass nor on skinfold thicknesses (both p>0.05). However, whereas the thigh CSA significantly decreased under the CHO condition (p=0.046, ES=-0.24), no change was observed for the BEEF condition (p=0.173, ES=0.25), which resulted in significant, very likely, and large differences between conditions (p=0.028, ES=1.22, **Figure 2**). Following a similar trend, a significant small increase in *vastus lateralis* muscle thickness was observed with BEEF (p=0.46, ES=0.56), but not with CHO (p=0.600, ES=-0.08). However, despite a moderate ES (ES=0.87), difference between conditions was unclear and statistically non-significant (p=0.173).

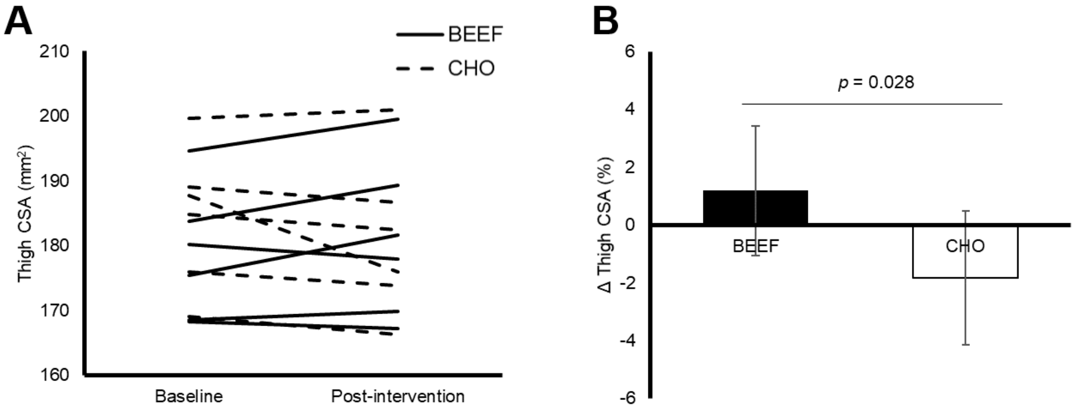

Figure 2. Individual (panel A) and mean delta change (panel B) in thigh cross-sectional area (CSA) after 8 weeks of supplementation with beef protein (BEEF) or carbohydrates (CHO).

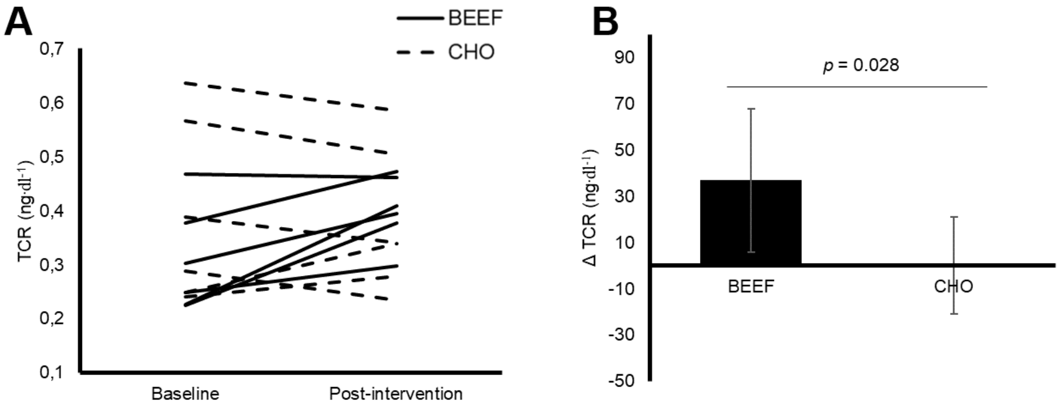

Figure 3. Individual (panel A) and mean delta change (panel B) in testosterone-to-cortisol ratio (TCR) after 8 weeks of supplementation with beef protein (BEEF) or carbohydrates (CHO).

Table 2. Differences in training and nutritional variables during eight weeks of supplementation with beef protein (BEEF) or carbohydrates (CHO).

	BEEF	CHO	p-value
Training variables			
Total volume (hours/week)	16.8 ± 3.8	17 ± 3.8	0.679
Swimming (sessions/week)	5.3 ± 0.5	5.6 ± 0.5	0.180
Cycling (sessions/week)	3.9 ± 1.0	3.6 ± 0.8	0.180
Running (sessions/week)	4.1 ± 1.2	4.1 ± 0.9	0.785
RT (sessions/week)	1.5 ± 0.6	1.8 ± 0.8	0.276
Competitions (n)	2.3 ± 1.0	3.7 ± 1.5	0.139
Nutritional variables			
Energy (kcal)	2892 ± 867	3130 ± 948	0.116
Protein (g/kg/day)	2.25 ± 0.22	1.89 ± 0.25	0.075
Fat (g/kg/day)	1.33 ± 0.52	1.17 ± 0.50	0.249
Carbohydrates (g/kg/day)	5.42 ± 1.96	7.06 ± 2.23	0.075
Iron (mg/day)	25.4 ± 10.1	26.2 ± 6.1	0.893

Data are mean ± SD. p-values were computed using Wilcoxon signed-rank tests. Abbreviations: RT, resistance training.

Table 3. Differences in anthropometrical variables after eight weeks of supplementation with beef protein (BEEF) or carbohydrates (CHO).

Variable	Group	Baseline	Post-intervention	Change (%)	Difference as a % (95% CI)	p-value	ES	MBI (+/trivial/-)
Body mass (kg)	BEEF	65.8 ± 4.0	65.9±4.0	0.23 ± 2.14	0.82 (-1.18, 2.82)	0.249	0.46	65/28/7 Unclear
	CHO	66.3 ± 3.9	65.9 ± 3.6	-0.59 ± 0.88				
Thigh CSA (mm^2)	BEEF	178 ± 10	181 ± 12	1.19 ± 2.25	3.02 (1.33, 4.71)	**0.028**	1.22	99/0/0 Very likely
	CHO	184 ± 11	181 ± 12*	-1.82 ± 2.32				
∑ skinfolds (mm)	BEEF	48.9 ± 10.4	46.6 ± 4.4	-2.66 ± 11.32	-4.84 (-18.57, 8.89)	0.463	-0.49	15/13/72 Unclear
	CHO	47.3 ± 6.2	48.2 ± 5.9	2.18 ± 6.08				
VL thickness (mm)	BEEF	24.9 ± 2.3	26.2 ± 2.0*	5.28 ± 6.20	5.92 (-6.09, 17.92)	0.173	0.87	80/11/9 Unclear
	CHO	26.2 ± 2.2	26.0 ± 2.3	-0.64 ± 6.42				

Data are mean ± SD. p-values were computed using Wilcoxon signed-rank test comparing the change (post *minus* baseline) observed in each group (beef vs CHO). Abbreviations: CI, confidence interval, CSA, cross-sectional area; ES, effect size; VL, vastus lateralis; MBI, magnitude-based inference. Significant differences compared to baseline: *p<0.05, computed with Wilcoxon signed-rank test.

With regards to blood markers of circulatory system and hormonal status (**Table 4**), BEEF elicited a significant moderate increase in the testosterone-to-cortisol ratio (p=0.046, ES=1.01), resulting in significant, very likely and large differences compared to CHO (p=0.028, ES=1.29, **Figure 3**).

This effect occurred along with a very close to significant and moderate ES to increase testosterone levels in BEEF (p=0.058, ES=0.42). Unclear and non-significant within- or between-condition effects were observed for any marker of iron status (i.e., iron, ferritin), red blood cell count, hemoglobin or hematocrit (all p>0.05, **Table 4**).

Table 4. Differences in biochemical/hematological variables after eight weeks of supplementation with beef protein (BEEF) or carbohydrates (CHO).

Variable	Group	Baseline	Post-intervention	Change (%)	Difference as a % (95% CI)	p-value	ES	MBI (+/trivial/-)
RBC (10^6/mm^3)	BEEF	5.1 ± 0.1	5.0 ± 0.3	-3.54 ± 4.96	-2.80 (-9.08, 3.48)	0.249	-0.64	11/11/79 Unclear
	CHO	5.0 ± 0.3	5.0 ± 0.3	-0.74 ± 2.91				
Hb (g/dl)	BEEF	15.1 ± 0.5	14.5 ± 0.6	-4.03 ± 4.17	-4.85 (-11.64, 1.94)	0.116	-1.06	5/7/89 Unclear
	CHO	14.6 ± 0.7	14.7 ± 0.9	0.82 ± 4.31				
HCT (%)	BEEF	46 ± 2	44 ± 2	-2.51 ± 4.59	-2.69 (-9.60, 4.21)	0.345	-0.61	12/14/74 Unclear
	CHO	44 ± 3	44 ± 2	0.19 ± 3.55				
Iron (μg/dl)	BEEF	108 ± 25	101 ± 27	-5.57 ± 23.40	-1.23 (-53.25, 50.78)	0.917	-0.04	31/33/36 Unclear
	CHO	120 ± 37	104 ± 20	-4.34 ± 38.52				
Ferritin (ng/ml)	BEEF	103 ± 37	102 ± 31	3.73 ± 23.64	-12.50 (-39.90, 14.90)	0.249	-0.48	7/21/72 Unclear
	CHO	96 ± 30	111 ± 38	16.23 ± 24.86				
Cortisol (μg /dl)	BEEF	18 ± 2	15 ± 2	-13.93 ± 20.25	-5.40 (-38.43, 27.63)	0.753	-0.24	22/27/51 Unclear
	CHO	16 ± 2	14 ± 1	-8.53 ± 21.77				
Testosterone (ng/dl)	BEEF	5.4 ± 1.2	6.0 ± 1.4	13.90 ± 14.55	22.80 (-14.87, 60.47)	0.173	1.12	85/9/6 Unclear
	CHO	5.9 ± 2.1	5.3 ± 2.0	-8.90 ± 23.73				
TCR (ng/dl)	BEEF	0.31 ± 0.10	0.40 ± 0.06*	37 ± 31	37 (5, 68)	**0.028**	1.29	98/1/1 Very likely
	CHO	0.40 ± 0.17	0.38 ± 0.14	0 ± 21				

Data are mean ± SD. p-values were computed using Wilcoxon signed-rank test comparing the change (post *minus* baseline) observed in each group (beef vs CHO). Abbreviations: ES, effect size; RBC, red blood cells; Hb, hemoglobin; HCT, hematocrit; TCR, testosterone/cortisol ratio; MBI, magnitude-based inference. Significant differences compared to baseline: *p<0.05, computed with Wilcoxon signed-rank test.

Discussion

The main finding of the present study is that 8 weeks of beef protein supplementation slightly increased daily protein intake and induced anabolic effects in male elite triathletes, as reflected by increases in muscle mass (i.e., thigh muscle area) and in the testosterone-to-cortisol ratio. In contrast, no benefits were observed on hematological variables related to iron status or red blood cell count.

Our finding of beef protein supplementation promoting increases in muscle mass is in line with previous reports. Meta-analytical evidence supports indeed the effectiveness of protein supplementation for increasing muscle mass and strength in healthy subjects/athletes (1,2), but most studies provided whey protein supplements. In this regard, a recent meta-analysis concluded that beef protein supplementation can also result in significant increases in lean body mass (18), as confirmed in the present study. It is worth highlighting that most research to date has focused on protein supplementation to optimize resistance training outcomes (16), and the evidence on its benefits for endurance athletes such as those studies here is scarcer (4). As summarized by Moore et al., prolonged endurance exercise induces the oxidation of amino acids as fuel (with disruption of muscle proteins), which can lead to muscle catabolism, especially during periods of negative energy intake (5). Indeed, endurance athletes present higher protein requirements than the general population, particularly when performing high training volumes (26). Protein supplementation might be therefore important to prevent muscle mass losses (i.e., catabolism) in endurance athletes (5).

In the present study we observed a decrease in thigh CSA when no protein supplement was administered, which might be reflective of a negative nitrogen balance. In contrast, beef protein supplementation induced a large increase on the thigh CSA together with a trend towards a greater increase on the vastus lateralis muscle thickness. Previous studies have also reported positive effects of whey protein to enhance muscle protein synthesis in endurance athletes (27,28), and particularly beef protein ingestion has also been associated with an increased muscle protein synthesis response in middle-aged and older adults (10–13). In agreement with the present findings, others studies reported positive effect of beef protein supplementation on the preservation of thigh muscle mass in master triathletes (15), also promoting increases in or lean body mass compared to a carbohydrate supplement in young subjects who trained both endurance and resistance training (14). Thus, these findings support that the previously reported effectiveness of beef protein supplementation to promote muscle mass gains can also be observed in elite endurance athletes.

The observed increase in testosterone-to-cortisol ratio might also be reflective of the anabolic effects of beef protein consumption. Both acute (29) and chronic (30) strenuous endurance exercise have been reported to elicit significant increases in cortisol levels together with reductions in testosterone. Although controversy exists, the testosterone-to-cortisol ratio is considered to be indicative of the anabolic/catabolic status, influencing protein synthesis and muscle metabolism (31). Thus, the observed increase in testosterone-to-cortisol ratio with beef protein supplementation can be potentially reflective of an enhanced muscle anabolism, which might have contributed to the observed increases in vastus lateralis thickness and the maintained thigh CSA. Moreover, marked decreases in the testosterone-to-cortisol ratio have been reported to be indicative of overtraining (32), and it can be therefore hypothesized that a higher testosterone-to-cortisol ratio might be potentially associated to a greater tolerance and assimilation of training loads.

In addition to the benefits on muscle mass, protein supplementation might also provide some other benefits in endurance athletes. It has been proposed that dietary protein should play a pivotal role in the diet of these athletes for enhancing recovery and eventually promoting greater exercise-induced adaptations (5). For instance, Huang et al. (6) observed lower levels of muscle damage markers (e.g., creatine kinase) along with improved endurance performance in marathon runners who ingested 33.5 g/d of whey protein compared to the ingestion of maltodextrin. Furthermore, whey protein supplementation decreased creatine kinase levels and improved performance compared to the intake of carbohydrates in top-class orienteering runners during a training camp (7). Thus, it could also be hypothesized that beef protein supplementation might also increase tolerance to exercise loads and facilitate training-induced adaptations. However, in the present study we did not assess performance-related outcomes, which would have provided greater insights into the potential benefits of beef protein supplementation for endurance athletes.

On the other hand, no benefits were observed on hematological variables, including iron status, hemoglobin and hematocrit. It has been reported that, compared to general population, endurance athletes might be at an increased risk of iron deficiency and reduced hemoglobin resulting in an impaired performance (20,33). It must be noted, however, that controversy exists on whether endurance athletes are certainly at increased risk of anemia or if it can be explained by a training-induced expansion of plasma volume (34). Previous preliminary evidence suggests that beef protein could provide benefits at hematological level due to its high content in heme-iron (18). Indeed, beef protein supplementation has been reported to increase the intake of heme-iron in female athletes, resulting in an increased hematocrit (19). Moreover, beef protein supplementation has been recently reported to provide additional benefits in the iron status (ferritin levels) of master-age triathletes compared to whey protein or a non-protein control group (15). The lack of differences observed between conditions for iron intake and hematological variables in the present study suggests that, at least in these individuals – who already presented a high iron intake at baseline (mean intake > 25 mg/day even during the CHO condition) –, beef protein supplementation might not promote further hematological changes. Although no supplement effects were observed in the present study, it is still unknown whether different results could have been found if a population with a worse iron status or at a greater risk of anemia (e.g., female athletes) had been included.

Some limitations must be noted. We analyzed a reduced number of participants due to the difficulties of conducting research with high-performance athletes such as those assessed here (i.e., elite triathletes competing at international level). Indeed, we initially aimed to recruit the whole group of male triathletes training at the Spanish High-Performance Center in Madrid (n=10). The drop outs and the eventual small sample size analyzed could have reduced statistical power, and thus these results should be confirmed in larger cohorts. Moreover, nutritional intake was registered with a self-reported food diary. Providing a prepared and prepacked diet to participants during the intervention would have offered an ideal scenario to standardize and control the influence of diet on the present results. Some of the measurement methods used here could also be optimized. For instance, the assessment of body composition by means of other methods such as dual-energy X-ray absorptiometry could have yielded more accurate results, and hormonal measurements were performed at a single time point (instead of several consecutive times), which does not take into account the pulsatile nature of their secretion. On the other hand, a period of five weeks was left between conditions, and although a longer time period could have served to ensure a proper wash-out, this enabled us to perform all measurements in the same mesocycle (competition period). A longer wash-out was unfeasible due to methodological constraints, as training loads would have greatly changed and athletes could even leave the training group. On the other hand, the fact of having performed a nutritional intervention in elite athletes and the cross-over design applied – which reduces biological variability compared to a parallel one – can be considered the major strengths of the study.

Conclusions

In summary, compared to carbohydrate, the ingestion of a beef protein powder supplement (25 g/d) over 8 weeks helped to maintain or increase lower limb muscle mass in male elite triathletes along with a more favorable anabolic environment, as reflected by the observed higher testosterone-to-cortisol ratio. Given the small sample size analyzed (n=6 in each condition), these results should be confirmed in larger cohorts.

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Authorship

PLV, ZM, FN and ELZ designed the study. PLV, ZM, FM and MG collected the data. PLV, ELZ and FN analyzed the data and drafted the manuscript. All authors revised the manuscript critically for important intellectual content, and gave final approval of the final version.

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