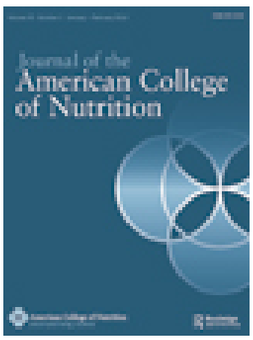

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Effects of Supplementation with Beef or Whey Protein Versus Carbohydrate in Master Triathletes

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ABSTRACT

Objective: The present study compares the effect of ingesting hydrolyzed beef protein, whey protein, and carbohydrate on performance, body composition (via plethysmography), muscular thickness, and blood indices of health, including ferritin concentrations, following a 10-week intervention program.

Methods: After being randomly assigned to one of the following groups—beef, whey, or carbohydrate—24 master-age (35–60 years old) male triathletes ($n = 8$ per treatment) ingested 20 g of supplement mixed with plain water once a day (immediately after training or before breakfast). All measurements were performed pre- and postinterventions.

Results: Only beef significantly reduced body mass ($p = 0.021$) along with a trend to preserve or increase thigh muscle mass (34.1 ± 6.1 vs 35.5 ± 7.4 mm). Both whey (38.4 ± 3.8 vs 36.9 ± 2.8 mm) and carbohydrate (36.0 ± 4.8 vs 34.1 ± 4.4 mm) interventions demonstrated a significantly ($p < 0.05$) decreased vastus medialis thickness Additionally, the beef condition produced a significant ($p < 0.05$) increase in ferritin concentrations (117 ± 78.3 vs 150.5 ± 82.8 ng/mL). No such changes were observed for the whey (149.1 ± 92.1 vs 138.5 ± 77.7 ng/mL) and carbohydrate (149.0 ± 41.3 vs 150.0 ± 48.1 ng/mL) groups. Furthermore, ferritin changes in the beef group were higher than the modification observed in whey ($p < 0.001$) and carbohydrate ($p = 0.025$) groups. No differences were found between whey and carbohydrate conditions ($p = 0.223$). No further changes were observed.

Conclusion: Ingesting a hydrolyzed beef protein beverage after workout or before breakfast (nontraining days) can be effective in preserving thigh muscle mass and in improving iron status in male master-age triathletes.

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Endurance athletes; diet; iron; ferritin; meat; maltodextrin; muscle thickness

Introduction

Endurance athletes have a higher tendency to develop both iron depletion and deficiency, which over time can cause anemia and impair training capacities while demonstrating a reduction in performance [1]. The reasons for the elevated iron demand in endurance athletes are an increased turnover and a loss through foot strike hemolysis, sweat, and gastrointestinal bleeding [1,2]. The rate of depletion of iron stores depends upon a balanced relationship between body iron loss and heme iron (the organic form of iron derived from animals) and nonheme iron (the inorganic form of iron derived from vegetable and cereal) intake [3].

Though the prevalence of iron depletion is higher in females than in males, current trends in reducing animal-based foods in favor of a higher proportion of vegetable sources could also negatively impact the iron stores in males performing serious endurance training regimens [1]. This is because absorption of the nonheme iron derived from plant-based sources is inhibited by many dietary factors, including phytates and polyphenols, which are also abundantly present in plant-based foods; nonheme iron absorption ranges from 2% to 10% of intake. In contrast, heme iron, present within hemoglobin or myoglobin molecules, is highly bioavailable [4]. The absorption of heme iron is affected by few dietary factors and its uptake can be as efficient as 40% [3]. However, heme iron has been estimated to only provide about 10% of all dietary iron in endurance athletes [4]. Compared to plant-derived nonheme iron, red meat is a rich source of heme iron with a higher bioavailability [1]. Thus, dietary modification has been suggested as a preferred strategy for ensuring adequate iron intake and maintenance of iron status and as the first line of action in the prevention of iron deficiency in athletes [4].

It is also well known that aerobic training diminishes amino acid utilization [5]. However, the oxidation capacity of amino acid is increased as a result of an improved activity of the limiting enzyme glutamate dehydrogenase [6]. Therefore, endurance athletes should increase their daily protein intake. Additionally, master endurance athletes who, due to age-related anabolic resistance, recover at a slower rate from training [7] should consume higher doses of high-quality proteins administered throughout the day and immediately after training [5]. Indeed, different nutritional strategies, including the consumption of high-quality protein sources, providing elevated amounts of micronutrients have been proposed as an effective nutritional

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countermeasure to optimize training adaptations, hasten recovery processes, and avoid training-related nutritional deficiencies after endurance training [5,8].

Both whey and beef are high-quality protein sources with an amino acid composition very similar to that found in skeletal muscle [9,10]. Although whey contains higher concentrations of branched-chain amino acids, specifically leucine, which is essential for supporting muscle protein synthesis after exercise [11], beef is also a source of iron, zinc, vitamin B12, and essential fatty acids [12]. Furthermore, it has been suggested that inflammation secondary to strenuous exercise leads to the release of hepcidin, a liver hormone that is a negative regulator of gut iron absorption. Because hepcidin would interfere with nonheme iron absorption after strenuous exercise, the consumption of iron supplements in this period would not be effective [13]. Consequently, foods with a high micronutrient density would be the best approach for maximizing recovery and providing a convenient amount of nutrients during the postworkout time. It can therefore be hypothesized that the postworkout ingestion of a high-quality protein beverage with a high proportion of essential amino acids and high micronutrient density of vitamin B, zinc, and heme iron will support recovery and prevent exercise-induced iron deficiency in endurance athletes. Therefore, interventions aiming to analyze training outcomes and hematological changes would be particularly relevant for endurance athletes. The aim of the current investigation therefore was to compare the effects of combining a 10-week endurance training program with one of the following commercially available products: (1) beef hydrolyzed protein powder (100% All Beef, Crown Sport Nutrition, La Rioja, Spain); (2) whey isolate (Isolac, Carbery); and (3) nonprotein, carbohydrate-only (maltodextrin), on performance, body composition, muscular thickness, and blood indices of health, including markers of iron deficiency such as serum ferritin, in master-age endurance triathletes.

Materials and methods

Participants

Thirty male master triathletes met the requirements to participate in the present study. Key criteria for inclusion were the following: (a) 35–60 years of age; (b) prestudy performance of regular endurance training for at least 2 years; (c) a normal health history; (d) free from musculoskeletal limitations; (e) agreement to not ingest any other nutritional supplements or nonprescription drugs/medication that can affect blood markers of health, including ferritin or hematocrit, as well as muscle growth and the ability to train intensely during the study; and (f) fluency in English. Key criteria used for exclusion were the following: (a) history of 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 improve strength and/or muscle mass, such as creatine, essential amino acids, whey protein, glutamine, dehydroepiandrosterone, multivitamin or iron supplement use within 8 weeks prior to the start of the study; (d) current use of tobacco products; and (e) the presence of any soft tissue or orthopedic limitations. Compliance was confirmed verbally and, prior to providing written consent, participants were informed of the potential risks and benefits of the investigation.

All experimental procedures were conducted in accordance with the Declaration of Helsinki and approved by the University Research Ethics Committee. As summarized in Fig. 1, after assessing for eligibility, 24 of the 30 recruited participants completed the study. The study was conducted during the Winter and Spring of 2016.

Experimental design

The present study involved a double-blind randomized controlled trial with 3 parallel groups and a between-participant design. The participants were randomly allocated into 3 equal-size treatment groups: beef protein, $n = 10$; whey protein, $n = 10$; or carbohydrate only (CHO), $n = 10$. Before and after a 10-week endurance training concomitant with either of the supplementary treatment periods, measurements of body composition, vastus medialis muscle thickness, peak oxygen consumption (VO_{2peak}), blood cell ($10^6/mm^3$) hemoglobin concentration (g/dl), hematocrit (%), mean corpuscular volume (mm^3), mean corpuscular hemoglobin mass (pg), mean corpuscular hemoglobin concentration (g/dl), red cell distribution width (%), and ferritin concentration (ng/mL) were determined. Following a preintervention endurance assessment, the participants were matched by their VO_{2peak} values, body composition, and age. In a double-blind fashion, the assignment of participants to treatments was performed by block randomization using a block size of 3. Presented as means (SD), the initial groups characteristics were as follows: Beef: age 47.0 (8.9) years, height 1.77 (0.07) m, body mass 78.2 (8.1) kg, VO_{2peak} 48.2 (5.4) ml/kg/min^{-1}; Whey: age 45.3 (8.9) years, height 1.79 (0.05) m, body mass 80.6 (13.2) kg, VO_{2peak} 46.4 (7.1) ml/kg/min^{-1}; CHO: age 46.2 (7.0) years, height 1.81 (0.05) m, body mass 77.9 (7.5) kg, VO_{2peak} 48.5 (6.9) ml/kg/min^{-1}.

Dietary (nutrition) monitoring

A qualified nutritionist collected information on participants' dietary habits and explained the correct procedures for recording dietary intakes. To determine energy and macronutrient contents, each participant's baseline diet (3 days, 2 weekdays, and 1 weekend day) was analyzed using Dietplan 6 software (Forestfield Software, UK). Participants were instructed to maintain their normal diet throughout the training period. In order to determine changes and evaluate differences caused by the supplementation protocol, diet composition was analyzed again during the last week of the intervention protocol.

Measurements

Body composition

Body mass and height were assessed on a standard scale and stadiometer according the methods described by Ross and Marfel-Jones [14]. Whole-body densitometry was assessed using air displacement via the Bod Pod (Life Measurements, Concord, CA) in accordance with the manufacturer's instructions as detailed elsewhere [15]. Briefly, the participants were tested wearing only tight-fitting clothing (swimsuit or undergarment) and an acrylic swim cap. Participants wore the exact

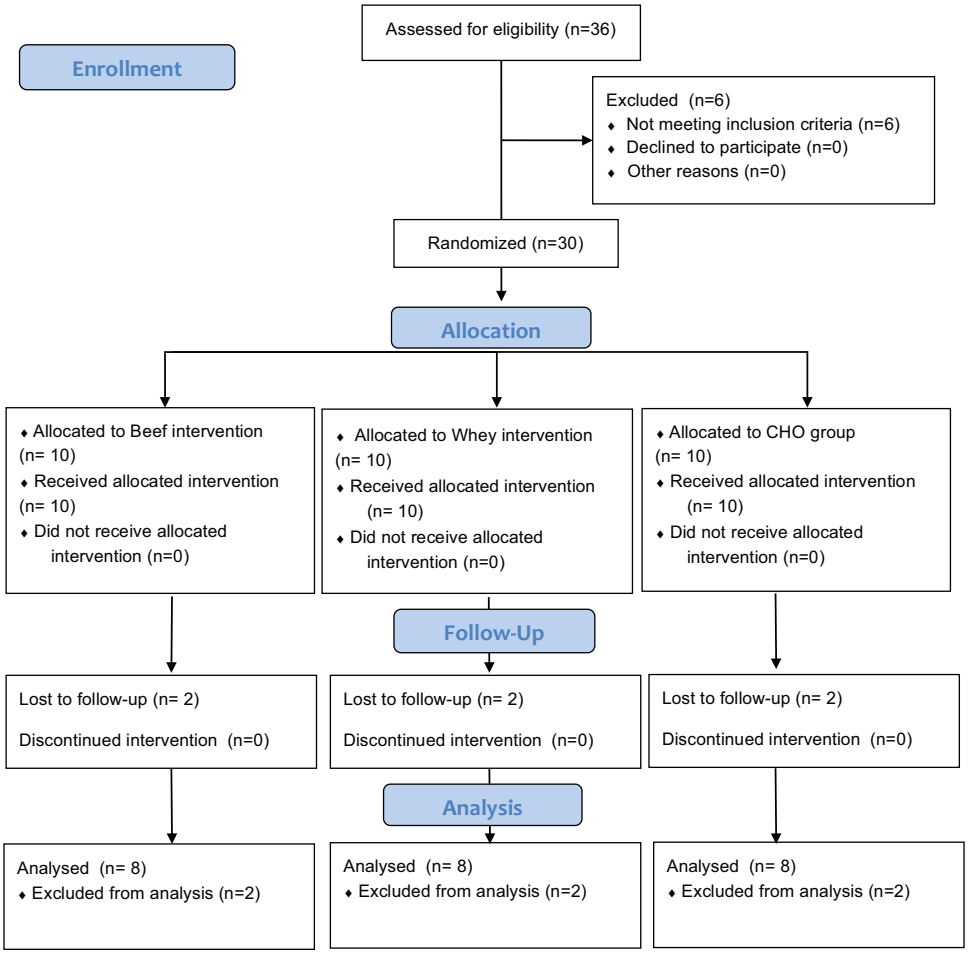

Figure 1. Flow diagram of participants throughout the course of the study.

same clothing for all testing. Thoracic gas volume was estimated using a predictive equation integral to the Bod Pod software. The calculated value for body density was used in the Siri equation [16] to estimate body composition. Body composition measurements were performed twice. If the agreement regarding percentage of body fat was within 0.05%, the 2 tests were averaged. If the 2 tests were outside the 0.05% agreement, a third test was performed and the average of the 3 measurements was used for all body composition variables.

Determination of peak oxygen consumption (VO_{2peak})

Following a standardized warmup, participants completed a progressive, incremental laboratory exercise test to exhaustion on a Cyclus2 ergometer (RBM Electronics, Leipzig, Germany). The test commenced at a work rate of 90 W. Thereafter, intensity increased at a step rate of 25 W every minute. Participants were instructed to maintain a cadence between 70 and 80 rpm^{-1} throughout the test. When cadence dropped by more than 10 rpm^{-1} for more than 10 s despite strong verbal encouragement, tests were terminated. Expired gases were collected continuously during the test using a Cortex MetaLyzer 3B gas analyzer (Cortex Biophysik, Leipzig, Germany). Additionally, heart rate (HR) was continuously monitored using a Polar Sporttester (Polar Electro, Jyväskylä, Finland). VO_{2peak} was calculated as the highest mean oxygen consumption over a 30-s period [17].

Muscle thickness

Right-side vastus medialis muscle thicknesses were measured in real time using a Diasus diagnostic ultrasound imaging unit (Dynamic Imaging, Livingston, UK) coupled to a 50-mm probe at a frequency of 7.5 MHz. During measurements, participants were lying supine with the right knee in an extended position. The probe was placed perpendicular to the skin surface and bone tissues at an 80% distance between the lateral condyle and greater trochanter of the femur [18]. To provide acoustic contact without depressing the dermal surface, the probe was coated with a water-soluble transmission gel (Aquasonic 100 Ultrasound Transmission gel). 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.

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 next test sessions, and the same operator performed all measurements. In order to avoid any swelling in the muscles that could interfere with the results, images were obtained at least 48 h before and after any exercise intervention.

Blood samples

After a fasting period of 8 h, participants arrived at the physiology laboratory on 2 separate occasions (one day before and one to 2 days after completion of the 10-week intervention period).

Two vacutainer venous blood collection tubes (BD Vacutainer Blood Collection Tubes) were used to collect 8 mL of venous blood from the antecubital vein. Containing EDTA as an anticoagulant, the first tube was inverted 8 times and immediately analyzed for complete blood count—that is, red blood cell concentration, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin mass, mean corpuscular hemoglobin concentration, and red cell distribution width—using a fully automated hematology analyzer (ABX Pentra 60C+, Horiba Medical, Montpellier, France). For the second tube (containing the additive silica to accelerate the clotting process), the serum was separated from clotted blood and used to determine serum ferritin concentrations. Before being centrifuged at 2400 rpm for 10 min, the clot-activating tube was inverted 5 times and the whole blood was allowed to stand for about 50 min at room temperature to facilitate the clotting process. The resultant serum was aliquoted into labeled Eppendorf tubes and stored at −80°C.

According to the manufacturer, all reagents for the biochemical indicators of iron status (1 × diluent M, 1 × wash buffer, 1 × biotinylated ferritin detector antibody, 1 × streptavidin–peroxidase conjugate for ferritin; 1 × diluent N) were equilibrated to room temperature (18–25°C). The serum samples were diluted (1:10) with 1 × Diluent M for ferritin. Samples were analyzed in duplicates using ELISA (Abcam, UK) for ferritin concentrations analysis. The calibration curve was constructed of 7 prepared ferritin standards (0.7 to 50 ng/mL). Serum samples were interpolated at a wavelength of 450 nm from the calibration standards using a 4-parameter logistic curve (My Assays, Ver. 2015). The mean intra-assay coefficient of variation for ferritin was 5.0%. The mean interassay coefficient of variation for ferritin was 8.4%.

Control of training

All participants were competitive master triathletes and at the time of the study had consistently trained between 6 to 10 h per week (4 to 7 training sessions per week) for the last 3 years.

During the progressive test, the values of HR, rate of perceived exertion (RPE) using a 15-point scale [20], oxygen consumption corresponding to the first (VT1) and second (VT2) ventilatory thresholds as well as the maximal heart rate (HR_{max}) were determined. Following the prescreening, participants committed to follow a training intervention period consisted of a 10-week polarized endurance training intensity distribution model. This model included 3 intensity zones delineated according to the VT2 localization and quantified using continuous heart rate registration and the associated RPE values determined during the progressive test. The polarized endurance training intensity distribution model involves significant proportions of both high- and low-intensity training and only a small proportion of moderate-intensity training. The intensity zones were calculated as zone 1, low intensity: ≤75% of VT2, ≤72% of HR_{max}, RPE 6 to 11; zone 2, moderate intensity: between 76% and 95% of VT2, 73% to 82% HR_{max}, RPE 12 to 14; and zone 3, high intensity: between 96% and 120% of VT2, 83% to 97% HR_{max}, RPE 15 to 18 [19]. Participants trained 4 to 6 sessions per week with a distribution of 75%–80% in zone 1, ∼5% in zone 2, and 15%–20% in zone 3. Participants controlled their training intensity by continuous HR registration and RPE producing during all training sessions. HR and RPE data files were checked weekly and used to quantify training intensity. In addition, participants were required to complete a training diary, recording average HR, training mode, and duration and distance of training sessions throughout the study. Training intensity distribution was quantified from heart rate and RPE to determine the percentage of training time spent in each of the 3 training zones for every individual training session. The average training time in each zone for all sessions was then determined. All participants performed their training during the afternoon (12:00 p.m. to 6:00 pm).

Dietary supplementation and control of intervention compliance

The 3 supplements under investigation were presented as 20 g sachets of vanilla-flavored powder diluted in ∼300 mL of plain water for each intake. The diluted drinks were similar in appearance, texture, and taste and were isoenergetic. The nutritional composition of each product and the amino acid profile of beef and whey proteins are shown in Table 1. Supplements were taken once a day for 10 weeks, for a total intake of 70 supplements. On training days, supplements were ingested just after training, whereas on nontraining days, supplements were self-administered in the morning before breakfast.

After completing the first assessment session, each participant was given a batch of one of the 3 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 during the intervention. Only

Table 1. Nutritional Composition of Drinks per Intake (20 g of Powder Plus ∼300 ml of Plain Water)

Nutrient	Beef	Whey	CHO
Energy value (kcal)	82	78	82
Carbohydrates (g)	0	0	20
Lipids (g)	1.54	0.3	0
Proteins (g)	16.4	18	0
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 EEA	6.82	8.91	—
Heme iron (mg)	16.27	—	—
Zinc (mg)	19.05	—	—
Folic acid (μg)	84.71	—	—
Vitamin B12 (μg)	3.26	—	—

CHO = carbohydrates, EEA = essential amino acids.

participants who completed the 70 days of supplementation intake with a minimum training frequency of 4 sessions per week (40 workouts in total) were included in the analysis. Due to non-study-related reasons, 6 participants (2 per group) dropped from the study. Consequently, 24 participants, 8 per group, successfully completed the study. Participants verbally confirmed that they maintained their habitual diet throughout the trial period.

Statistical analysis

A descriptive analysis was performed and subsequently Kolmogorov-Smirnov and Shapiro-Wilk tests were applied to assess normality. Sample characteristics at baseline were compared between conditions (beef vs whey vs CHO) using one-way analysis of variance (ANOVA). Changes in performance, body composition, muscle thickness, and blood indices were assessed using 3×2 repeated measures ANOVA to compare the effect of supplement conditions (beef vs whey vs CHO) over time (pre vs post). When the interaction between conditions and time was significant, in order to adjust for possible differences at enrollment, the percentage change was analyzed using analysis of covariance using the first assessment (pre) as a covariate. Bonferroni-adjusted post hoc analyses were performed when appropriate. Generalized eta squared (η^2_G) and Cohen's d values were reported to provide an estimate of standardized effect size (small $d = 0.2$, $\eta^2_G = 0.01$; moderate $d = 0.5$, $\eta^2_G = 0.06$; large $d = 0.8$, $\eta^2_G = 0.14$). The significance level was set to $p < 0.05$. Results are reported as means (SD) unless stated otherwise. Data analyses were performed with Stata 13.1 (StataCorp, College Station, TX).

Results

Table 2 shows the dietary monitoring results, presented as the average daily consumption of carbohydrate, protein, fat ($g \cdot kg^{-1} \cdot d^{-1}$), energy ($kcal \cdot kg^{-1} \cdot d^{-1}$), total iron ($mg \cdot d^{-1}$), heme iron ($mg \cdot d^{-1}$), and nonheme iron ($mg \cdot d^{-1}$) intake, before and after the intervention for the 3 treatment conditions.

At baseline, no between-group differences were observed in dietary variables. However, as a result of the nutritional intervention, the beef group, due to a significant increase in total iron intake ($p = 0.001$), increased the intake of heme iron ($p = 0.009$). Furthermore, the 2 protein treatment conditions (beef and whey) significantly increased protein intake ($p = 0.001$), and only the CHO group increased consumption of carbohydrates ($p = 0.005$). Additionally, only the CHO ($p = 0.008$) and whey ($p = 0.021$) groups produced significant increases in total energy intake. Despite the aforementioned changes from baseline intakes, no between-treatment differences were observed postintervention. No complaints about any negative symptoms (i.e., hypoglycemic reaction) or gastric-related discomfort due to the ingestion of the supplement during training and on nontraining days were reported.

At baseline, body composition and VO_{2peak} performance was equal between the groups. No significant differences were observed in all variables at test 1 (i.e., prior to study). Pre and post values for body composition, muscle thickness, and VO_{2peak} values are provided in Table 3, and blood variables are presented in Table 4.

Main time effects were observed for body mass (kg), fat mass (kg), fat mass (%), fat-free mass (kg), and fat-free mass (%). No interaction or between-group effects were identified. Pairwise comparison revealed a significant reduction in body mass ($p = 0.021$) for the beef group (Table 3). Significant interaction effects between supplement and time were found in vastus medialis thickness (Table 3) and ferritin concentrations (Table 4). Analysis of covariance revealed no significant time effects for vastus medialis thickness, $F(2,18) = 2.05$, $p = 0.158$, $\eta^2_G = 0.116$, but a significant increase in ferritin concentrations, $F(2,18) = 20.78$, $p < 0.001$, $\eta^2_G = 0.361$. Adjusted marginal means of the differences for each group were 4.55 ($p = 0.041$), −1.54 ($p = 0.515$), and −4.95 ($p = 0.021$) for beef, whey, and CHO groups respectively. However, no significant post hoc differences between groups were found.

Post hoc analysis revealed that the beef condition produced a higher ferritin concentration increase than whey ($p < 0.001$) and CHO ($p = 0.025$), and no differences were found between whey and CHO ($p = 0.223$) in this variable. No further differences were observed.

Discussion

The present study demonstrates that the ingestion of 20 g of hydrolyzed beef protein over 10 weeks preserved thigh muscle mass in master endurance athletes. Additionally, the ingestion of hydrolyzed beef protein resulted in an increase in serum ferritin levels, which reached similar values observed in the whey and CHO groups, which showed no changes after the intervention. No variation in performance or any of the measured blood parameters was observed for the 3 analyzed conditions. The decrease in body mass in the beef group is mainly due to a higher proportion of fat mass loss alongside a minor decrease of the fat-free mass. Furthermore, only the beef group showed no decrease in vastus medialis thickness, which reinforces the potential positive effect of this supplement to preserve muscle mass in endurance athletes [1]. Excessive decrease in muscle mass should be avoided in endurance master athletes because it can compromise performance [21]. Because the capacity to elevate the postworkout muscle protein synthesis to support recovery process decreases with age [22], the ingestion of a postworkout meal containing between 0.25 and 0.4 g·kg of high-quality protein has been proposed [5]; participants allocated in the whey and beef groups were ingesting ~0.22 g·kg immediately after exercise. The administered amount would not be considered enough to facilitate muscle repair and remodeling for the current participants [5], therefore limiting the potential benefit of the supplement on the muscular recovery process. Additionally, regularly physically active individuals should ingest more than 1.2 $g \cdot kg^{-1} \cdot d^{-1}$ of proteins daily [13]. According to the diet records, 5 participants (1 in beef, 2 in whey, and 2 in CHO) were ingesting less than 1.2 $g \cdot kg^{-1} \cdot d^{-1}$, with the remaining participants consuming more than 1.2 $g \cdot kg^{-1} \cdot d^{-1}$ and only 9 participants (3 in beef, 4 in whey, and 2 in CHO) reached the average requirement (1.65 $g \cdot kg^{-1}$) or recommended protein intake (1.83 $g \cdot kg^{-1} \cdot d^{-1}$) needed to satisfy the metabolic demands of endurance athletes [23]. Furthermore, the relatively lower amounts of CHO consumed by

Table 2. Descriptive Analysis of Participants' Diet Composition[a]

	Total Iron ($mg \cdot d^{-1}$)		Nonheme Iron ($mg \cdot d^{-1}$)		Heme Iron ($mg \cdot d^{-1}$)		Proteins ($g \cdot kg^{-1} \cdot d^{-1}$)		Carbohydrate ($g \cdot kg^{-1} \cdot d^{-1}$)		Fats ($g \cdot kg^{-1} \cdot d^{-1}$)		Energy ($kcal \cdot kg^{-1} \cdot d^{-1}$)	
Treatment	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Beef ($n = 8$)	11.83 (3.84)	14.56** (3.96)	8.09 (3.47)	8.43 (3.37)	3.74 (2.14)	5.70** (1.21)	1.29 (0.25)	1.52** (0.24)	3.36 (1.21)	3.46 (1.14)	1.07 (0.26)	1.09 (0.26)	28.58 (4.40)	30.54 (4.17)
Whey ($n = 8$)	15.02 (8.97)	15.72 (8.37)	11.66 (6.55)	11.65 (7.75)	3.36 (3.03)	4.07 (2.02)	1.48 (0.64)	1.72** (0.65)	3.53 (1.58)	3.68 (1.47)	1.39 (0.38)	1.40 (0.38)	30.34 (7.93)	35.10* (9.68)
CHO ($n = 8$)	14.03 (3.42)	15.21 (4.39)	10.36 (3.31)	10.40 (3.25)	3.67 (2.74)	4.85 (2.58)	1.30 (0.24)	1.40 (0.25)	2.98 (1.18)	3.23** (1.19)	1.35 (0.80)	1.34 (0.60)	25.84 (7.97)	31.44** (9.28)

CHO = carbohydrates.
[a]Pre- and postintervention values are presented as means (SD).
*$p < 0.05$ and **$p < 0.01$ from pre- to postintervention (last week of intervention).

Table 3. Descriptive Analysis of the Body Composition, Muscle Thickness, and Endurance Performance Variables[a]

	Beef ($n = 8$)		Whey ($n = 8$)		CHO ($n = 8$)		
Variables	Pre	Post	Pre	Post	Pre	Post	Repeated Measures ANOVA (3 groups × 2 times)
Body mass (kg)	78.9 (9.5)	77.3 (8.8)*	85.1 (11.6)	84.7 (13.5)	80.2 (7.1)	79.4 (6.4)	Time: $F(1,21) = 6.19$, $p = 0.021$, $\eta^2_G = 0.003$ Group: $F(2,21) = 1.05$, $p = 0.367$, $\eta^2_G = 0.09$ Group × Time: $F(2,21) = 0.9$, $p = 0.423$, $\eta^2_G = 0.001$
Fat mass (kg)	14.9 (3.9)	13.9 (4.7)	19.7 (8.4)	18.9 (10)	15.4 (4.9)	14.8 (5.0)	Time: $F(1,21) = 4.75$, $p = 0.041$, $\eta^2_G = 0.004$ Group: $F(2,21) = 1.35$, $p = 0.282$, $\eta^2_G = 0.112$ Group × Time: $F(2,21) = 0.11$, $p = 0.895$, $\eta^2_G = 0$
Fat mass (%)	18.9 (4.2)	17.9 (5.2)	22.5 (7.7)	21.4 (9.1)	19.2 (5.5)	18.4 (5.8)	Time: $F(1,21) = 5.55$, $p = 0.028$, $\eta^2_G = 0.006$ Group: $F(2,21) = 0.75$, $p = 0.486$, $\eta^2_G = 0.065$ Group × Time: $F(2,21) = 0.07$, $p = 0.934$, $\eta^2_G = 0$
Fat-free mass (kg)	64 (7.9)	63.4 (7.6)	65.4 (6.1)	65.8 (6.8)	64.7 (5.9)	64.7 (5.5)	Time: $F(1,21) = 0.25$, $p = 0.622$, $\eta^2_G = 0$ Group: $F(2,21) = 0.16$, $p = 0.853$, $\eta^2_G = 0.015$ Group × Time: $F(2,21) = 2.12$, $p = 0.145$, $\eta^2_G = 0.001$
Fat-free mass (%)	81.1 (4.2)	82.1 (5.2)	77.5 (7.7)	78.6 (9.1)	80.8 (5.5)	81.6 (5.8)	Time: $F(1,21) = 5.55$, $p = 0.028$, $\eta^2_G = 0.006$ Group: $F(2,21) = 0.75$, $p = 0.486$, $\eta^2_G = 0.065$ Group × Time: $F(2,21) = 0.07$, $p = 0.934$, $\eta^2_G = 0$
Vastus medialis thickness (mm)	34.1 (6.1)	35.5 (7.4)	38.4 (3.8)	36.9* (2.8)	36.0 (4.8)	34.1* (4.4)	Time: $F(1,21) = 2.49$, $p = 0.129$, $\eta^2_G = 0.005$ Group: $F(2,21) = 0.8$, $p = 0.461$, $\eta^2_G = 0.068$ Group × Time: $F(2,21) = 5.78$, $p = 0.010$, $\eta^2_G = 0.023$
Vo2peak (ml/kg/min)	48 (6.4)	46.4 (6.8)	45 (7.6)	46.8 (8.6)	50.9 (6.1)	48.9 (6.3)	Time: $F(1,21) = 0.69$, $p = 0.416$, $\eta^2_G = 0.002$ Group: $F(2,21) = 0.72$, $p = 0.496$, $\eta^2_G = 0.061$ Group × Time: $F(2,21) = 2.76$, $p = 0.086$, $\eta^2_G = 0.016$

CHO = carbohydrates, ANOVA = analysis of variance.
[a]Pre- and postintervention values are presented as means (SD).
*$p < 0.05$ with respect to preintervention values.

the 3 groups, ~3.5 g·kg^{-1}·d^{-1} (Table 2), falls notably below the recommended dose of 5 to 7 g for endurance athletes [13], which can negatively influence the loss of body mass as well as targeted performance changes.

Although there is no exact agreement on serum ferritin levels associated with iron depletion/deficiency, with various suggestions ranging from <10 to <35 ng/mL [13], for the present study, iron deficiency was considered as a serum ferritin concentration of <15 ng/ml, and anemia was defined as a hemoglobin concentration of <13 g/dl [24]. Even though at baseline or postintervention, none of the participants were iron deficient or anemic (Table 4), only the beef group demonstrated a significant increase in serum

Table 4. Descriptive Analysis of the Hematological Variables[a]

	Beef ($n = 8$)		Whey ($n = 8$)		CHO ($n = 8$)		
Variables	Pre	Post	Pre	Post	Pre	Post	Repeated Measures ANOVA (3 groups × 2 times)
Red blood cells (10^6/mm^3)	4.8 (0.4)	4.8 (0.3)	5 (0.3)	4.9 (0.3)	4.8 (0.4)	4.8 (0.3)	Time: $F(1,21) = 0.07$, $p = 0.794$, $\eta^2_G = 0.001$ Group: $F(2,21) = 1.07$, $p = 0.363$, $\eta^2_G = 0.07$ Group × Time: $F(2,21) = 0.32$, $p = 0.733$, $\eta^2_G = 0.008$
Hemoglobin (g/dl)	14.8 (0.8)	14.8 (1.1)	15.3 (1.1)	15 (1)	14.6 (1)	14.8 (1)	Time: $F(1,21) = 0.06$, $p = 0.813$, $\eta^2_G = 0.001$ Group: $F(2,21) = 0.69$, $p = 0.510$, $\eta^2_G = 0.048$ Group × Time: $F(1,21) = 0.45$, $p = 0.644$, $\eta^2_G = 0.01$
Hematocrit (%)	41.6 (2.6)	41.1 (2.8)	42.9 (3)	41.5 (2.7)	40.6 (3.2)	40.8 (2.4)	Time: $F(1,21) = 1.01$, $p = 0.327$, $\eta^2_G = 0.012$ Group: $F(2,21) = 0.82$, $p = 0.455$, $\eta^2_G = 0.055$ Group × Time: $F(2,21) = 0.6$, $p = 0.556$, $\eta^2_G = 0.015$
Mean corpuscular volume (mm^3)	86.3 (3.2)	85.7 (3.8)	85.6 (3.1)	85.6 (3.5)	84.9 (2.7)	85.3 (2.9)	Time: $F(1,21) = 0.04$, $p = 0.845$, $\eta^2_G = 0$ Group: $F(2,21) = 0.17$, $p = 0.845$, $\eta^2_G = 0.015$ Group × Time: $F(2,21) = 0.94$, $p = 0.407$, $\eta^2_G = 0.004$
Mean corpuscular hemoglobin (pg)	30.7 (1.1)	30.8 (1.4)	30.5 (1.3)	30.7 (1.2)	30.5 (1.1)	30.8 (1.3)	Time: $F(1,21) = 2.13$, $p = 0.159$, $\eta^2_G = 0.008$ Group: $F(2,21) = 0.06$, $p = 0.947$, $\eta^2_G = 0.005$ Group × Time: $F(2,21) = 0.21$, $p = 0.812$, $\eta^2_G = 0.002$
Mean corpuscular hemoglobin concentration (g/dl)	35.5 (0.5)	36 (0.7)	35.7 (0.6)	35.9 (0.7)	35.9 (0.7)	36.2 (0.8)	Time: $F(1,21) = 3.81$, $p = 0.064$, $\eta^2_G = 0.06$ Group: $F(2,21) = 0.69$, $p = 0.515$, $\eta^2_G = 0.041$ Group × Time: $F(2,21) = 0.39$, $p = 0.684$, $\eta^2_G = 0.013$
Red cell distribution width (%)	11.3 (0.8)	10.8 (1.2)	11.2 (0.9)	10.2 (1.1)	10.8 (1.1)	10.8 (0.9)	Time: $F(1,21) = 3.57$, $p = 0.073$, $\eta^2_G = 0.065$ Group: $F(2,21) = 0.47$, $p = 0.630$, $\eta^2_G = 0.026$ Group × Time: $F(2,21) = 1.14$, $p = 0.340$, $\eta^2_G = 0.043$
Ferritin (ng/mL)	117.5 (78.3)	150.5* (82.8)	149.1 (92.1)	138.5 (77.7)	149.0 (41.3)	150.0 (48.1)	Time: $F(1,21) = 2.55$, $p = 0.125$, $\eta^2_G = 0.004$ Group: $F(2,21) = 1.6$, $p = 0.226$, $\eta^2_G = 0.128$ Group × Time: $F(2,21) = 4.72$, $p = 0.020$, $\eta^2_G = 0.015$

CHO = carbohydrates, ANOVA = analysis of variance.
[a]Pre- and postintervention values are presented as means (SD).
*$p < 0.05$ with respect to baseline, whey, and CHO.

ferritin concentrations postintervention. However, at baseline, participants included in the beef group had nonsignificantly, F $(2,21) = 0.479$, $p = 0.626$, lowers levels of ferritin concentrations compared to the other groups. After the 10-week intervention, participants in the beef group reached ferritin levels similar to those observed in the whey and CHO groups. Iron intake for all groups before and during the intervention was notably above the recommended dosage for males endurance athletes (>8 mg·d^{-1}) [13]. Nonetheless, only the beef group demonstrated a significant increase in total and heme iron dietary intakes. Indeed, individual analysis demonstrated that all participants in the beef group increased their dietary heme iron ingestion as well as serum ferritin concentrations, whereas only 3 participants in the whey group and 1 participant in the CHO group demonstrated a trend of increasing serum ferritin concentrations during the intervention (supplementary material). Additionally, 2 participants in the beef treatment group showed low initial ferritin values (20.3 and 24.9 ng/mL), still within normal ranges, though regardless of group, no other participants demonstrated baseline values below 50 ng/mL. Because the absorption of dietary iron is increased with a compromised iron status [25], a markedly improved hematological index was expected in participants with very low baseline iron values. However, regardless of initial iron status, all participants in the beef group showed similar changes in ferritin concentrations.

Because the participants were trained endurance athletes with all hematological parameters within the normal ranges [26], their body composition, VO_{2peak} performance, and the majority of the blood markers may lack significant changes as an effect of the dietary supplementation. Due to its long-term consistency and maintenance with a regular training program [27], the metabolism of iron and protein, body composition, and performance are well established in this population. This may further explain the lack of any changes in performance and the measured blood markers with the exception of ferritin levels. The present investigation is novel because the supplementation effects were tested in master endurance athletes during the season in which they performed their regular training program integrated in their lifestyle routines. Despite the aforementioned concerns about a low carbohydrate dietary intake at baseline, participants were apparently well nourished and relatively well trained.

The convenience of additional iron intake in the form of hydrolyzed beef protein powder suggests that it may be a suitable alternative in the prevention of iron depletion through maintenance of iron stores in male endurance athletes during training periods. There was no significant difference between groups as an effect of the intervention in energy intake but, as highlighted, beef was the only group that increased total iron and specifically heme iron intakes.

Further research using larger groups of participants is required to confirm whether dietary interventions through a dietary change can significantly improve iron status and its potential impact on exercise performance. Nevertheless, considering the research design and sample size limitations, the current findings demonstrate the effects of hydrolyzed beef protein supplementation in preserving lower limb muscle mass and improving iron status. The lower levels of serum ferritin at the beginning of the study observed in the beef group do not permit further conclusions about the potential benefits of ingesting hydrolyzed beef protein powder for maintaining or increasing iron stores in endurance athletes.

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Author contributions

The study was designed by F.N. and E.L. Data were collected by M.S. and F.N. E.L., N.A., and F.N. analyzed the data. Interpretation and article preparation were undertaken by all authors. All authors approved the final version of the article.

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