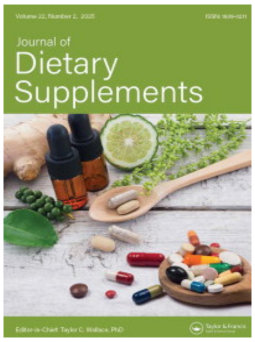

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Effect of a Multi-Ingredient Post-Workout Dietary Supplement on Body Composition and Muscle Strength – A Randomized Controlled Trial

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ABSTRACT

The aim of the current parallel randomized controlled trial was to compare the effects of ingesting a dietary supplement admixture providing carbohydrates, leucine-fortified whey protein, creatine, β-hydroxy-β-methylbutyrate, and vitamin D3 (Master Recovery 1:1, Crown Sport Nutrition, Spain), versus an isoenergetic carbohydrate-only comparator on body composition, muscle thickness, muscle strength, and performance over a 6-week resistance training program, performed three times per week, in aging, physically active individuals. Twenty participants (10 peri- and post-menopausal females and 10 males) completed the study after being randomly assigned to one of the following groups: post-workout multi-ingredient (PWS: $n = 10$, 52.0 ± 5 years, body mass 82.0 ± 18.0 kg) or a comparator (COM: $n = 10$, 51 ± 3 years, body mass 85.9 ± 17.0 kg). Treatment consisted of ingesting 60.0 g of the assigned supplement immediately after each workout. Compared to baseline, only PWS increased fat-free mass ($+1.34 \pm 1.2$ kg, $p = 0.003$), reduced fat mass (-1.09 ± 0.7 kg, $p < 0.001$), waist circumference (-2.5 ± 1.8 cm, $p < 0.001$), and waist-to-hip ratio (-0.03 ± 0.03 cm, $p = 0.007$). At post-intervention, waist circumference reduction was different between groups ($p = 0.02$, $d = 1.19$). Both treatments similarly improved vastus lateralis and elbow flexor thickness, medicine ball throw, and endurance performance. Although countermovement jump improved for both treatments, the PWS group showed a significantly higher performance increase compared to COM ($p < 0.01$, $d = 1.47$). Compared to ingesting carbohydrates only, the use of a targeted multi-ingredient promoted noticeable body composition outcomes and better vertical jump improvements with no further effects on hypertrophy, upper body, and endurance performance. The study was registered as a clinical trial at ClinicalTrials.gov (NCT05769088).

KEYWORDS
Aging; body composition; creatine; dietary supplement; muscle; muscle strength; resistance training; whey protein

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Introduction

Nutrition is universally acknowledged as one of the primary determinants impacting post-exercise recovery (Morton et al. 2015). Post-workout muti-ingredients are designed with the primary objective of engaging in the '3 R' paradigm (rehydration, refueling, and muscle repair) (Morton et al. 2015). Furthermore, recent scientific literature asserts that some amino-acid derivatives, such as creatine (Kerksick et al. 2018; Barnes 2023), and β-hydroxy-β-methylbutyrate (β-HMB) (Kerksick et al. 2018; Barnes 2023), have the potential to expedite post-exercise recovery and support optimal muscle function in athletes and physically active individuals *via* synergistic mechanisms. The proposed beneficial effects of post-workout admixtures rely on mitigating declines in physical performance after hard training sessions and promoting skeletal muscle mass accretion and remodeling (Nabuco et al. 2018; Naclerio et al. 2020). The claimed superior effects of multi-ingredients compared to the intake of each ingredient in isolation are based on their synergistic interaction or amplification effect once the included ingredients are ingested as a component of a special formulation (Liao et al. 2017). Nevertheless, the expected outcomes are based on the proven effects of each singular ingredient examined separately. For instance, whey protein is considered the highest quality protein due to its complete nutritional profile and rapid digestion kinetics (Boirie et al. 1997). Furthermore, its high protein quality is attributed to the rich array (>40%) of essential amino acids (EAA), particularly the branched-chain amino acids (BCAAs), including leucine (Phillips and Van Loon 2011).

In this regard, high-quality protein extracts have proven to maximize muscle protein synthesis and attenuate muscle protein breakdown during exercise and resting conditions (Naclerio and Larumbe-Zabala 2016). Additionally, adding of high-quality protein to the habitual diet favors metabolic health in older adults (Nabuco et al. 2019) and maximizes muscle mass and strength gains over long-term resistance training interventions (Huecker et al. 2019) in physically active middle-aged and older adults (Camargo et al. 2020). Moreover, β-HMB, a leucine metabolite, may also attenuate catabolism and exercise-induced muscle damage (Arazi et al. 2021; Kim and Kim 2022), favoring growth hormone production, tissue repair (Arazi et al. 2021) and muscular protein synthesis (Kraemer et al. 2015). Furthermore, creatine has been proven to promote muscle mass accretion and maximize strength and endurance outcomes in athletes and recreationally active individuals (Mills et al. 2020). Vitamin D is a cofactor associated with optimal muscular function and growth (Chiang et al. 2017), predominantly lower body strength (Beaudart et al. 2014) particularly in older individuals or those with vitamin D insufficiency. Indeed, adding Vitamin D3 to whey protein isolate enhanced skeletal mass index, strength, and anabolic markers in sarcopenic older adults (Bo et al. 2019). This may be attributed to the influence of Vitamin D receptors on human skeletal muscle (Zhang et al. 2019) although the specific critical link between vitamin D and muscle growth and remodeling remains unclear (Bouillon et al. 2022).

To the best of the authors' knowledge, there is still a paucity of research analyzing the convenience of regularly ingesting post-workout muti-ingredients to maximize exercise adaptation outcomes in physically active middle-aged and older adults. The aim of this study, therefore, was to compare the effects of ingesting either a commercially available post-workout multi-ingredient including, carbohydrates, Leucine-enriched whey protein, β-HMB, creatine monohydrate, and Vitamin D3 (Master Recovery 1:1, Crown Sports Nutrition, Spain) or an isoenergetic carbohydrate-only comparator (COM) on body composition (including muscular hypertrophy) and physical performance over a 6-week resistance training intervention. The primary outcomes were changes in fat mass, free mass, waist, and hip circumference along with vastus lateralis and elbow flexors muscular thickness. Secondary outcomes included changes in strength, power output, and muscular endurance. Furthermore, the post-workout global perceptual response, the total volume (kg lifted per session), and changes in endurance performance were considered exploratory outcomes. Based on previous literature we hypothesized that ingesting the above-described post-workout multi-ingredient instead of an isocaloric, only carbohydrate supplement will elicit higher training outcomes in physically active middle-aged and older (females and males) adults.

Material and methods

Experimental design

The current investigation is reported per the CONSORT criteria. The study was registered as a clinical trial at the US National Institutes of Health. https://www.clinicaltrials.gov (NCT05769088). Procedures were approved by the University of Greenwich Research Ethics Committee (approval reference number FREC-EHHS-21-2-23-03).

The intervention followed a double-blinded, randomized, controlled, and counterbalanced, parallel group design with a 1:1 allocation ratio. Following the inclusion criteria, familiarization, and baseline assessments, the participants were randomly allocated to receive either a post-workout multi-ingredient (PWS) or isoenergetic comparator (COM) composed of carbohydrates alone. Subsequently, the participants completed a standardized 6-week circuit-shaped resistance training program, including 3 workouts per week (18 total sessions).

Participants

Twenty-two recreationally active, middle-aged and older adults (12 peri- and post-menopausal females and 10 males; mean age: 54 ± 6 years) were initially recruited. Participants were recruited from the neighborhood and surrounding areas of the university campus, as well as from the university staff, through posters placed around campus facilities and close sport centers. Additional recruitment was conducted *via* word-of-mouth and internal university emails. The inclusion criteria were a minimum resistance training history of 6 months before the study onset and 45 years or older. Additionally, post- or peri-menopausal female participants, presenting a minimum of two symptoms of menopause onset (such as hot flushes, menstrual cycle alterations, and not menstruating for more than 1 year) (NICE 2023) were included. Participants were not eligible if they were sedentary or inactive, with no previous strength training experience, suffering from acute or chronic diseases (including advanced obesity, metabolic syndrome, long Covid-19, osteoporosis, or sarcopenia), following a medication prescription, or consuming supplements that could interfere with our research or affect exercise performance (i.e. creatine, protein amino-acids, NSAIDs, etc.). In accordance with the Declaration of Helsinki, all participants provided written informed consent.

To assess statistical power, we conducted a sensitivity analysis of the final sample size (PWS. $n = 10$ and COM, $n = 10$) to detect statistically significant differences between conditions in pre-post differences. Assuming a t-test model with two independent samples to compare adjusted means, 0.05 α error probability, and 0.80 power (1−β), it was determined that differences between groups could be detected with a Cohen's d above 1. As summarized in Figure 1, 22 participants were randomly allocated into one of the two intervention groups (PWS or COM). However, due to non-intervention-related reasons (lack of time to commit to the entirety of the protocol), two participants (one per group) dropped out of the study and eventually, 20 participants completed all aspects of the intervention protocol and were considered for the analysis. The final composition of the groups was equivalent at baseline: PWS ($n = 10$, 50% females) age 52.0 ± 5 years, 1.76 ± 0.1 m, 82.0 ± 18 kg; COM ($n = 10$, 50% females): age 51.0 ± 3 years, height 1.74 ± 0.1 m, body mass 85.0 ± 17 kg.

Procedures

Following confirmation of eligibility, enrollment, and before the baseline assessment, participants performed a 2-weekly familiarization program over 4 weeks (8 sessions) aimed at minimizing any potential learning effects with the assessment and training procedures. After the preintervention assessments, participants were matched by body mass and maximal isometric force. The assignment of participants to treatments was performed by block randomization using a block size of two and in a double-blind (PWS or COM) manner.

Assessments

Measurements were determined over two sessions. Day 1 included (i) muscle thickness using ultrasonography, (ii) waist and hip circumferences, and body composition *via* plethysmography (iii) vertical jump and (iv) medicine ball chest throw. Day 2 included the progressive cycling to exhaustion test. Before any testing session, participants were instructed to refrain from any vigorous activity. Environmental conditions at the sports science laboratory were controlled and maintained at a constant level during both testing and training sessions. Specifically, the mean ± SD air temperature, barometric pressure, and relative humidity were 19 ± 1.5 °C, 1020 ± 14 mBar, and 53.6 ± 5.7%, respectively.

Participants were assessed at the same time of day (±2 h) and on the same day of the week for both the baseline (t1) and post-intervention (t2) assessments. This study was conducted at the University of Greenwich (London, Avery Hill Campus). All assessments, training sessions, and supplement administration took place in the Exercise Physiology Laboratory and Strength Training Facility within this institution.

Body composition

Absolute (kg) and relative (percentage) fat mass and fat-free mass were assessed *via* air displacement plethysmography (BodPod®, Life Measurements, Concord, CA, USA)

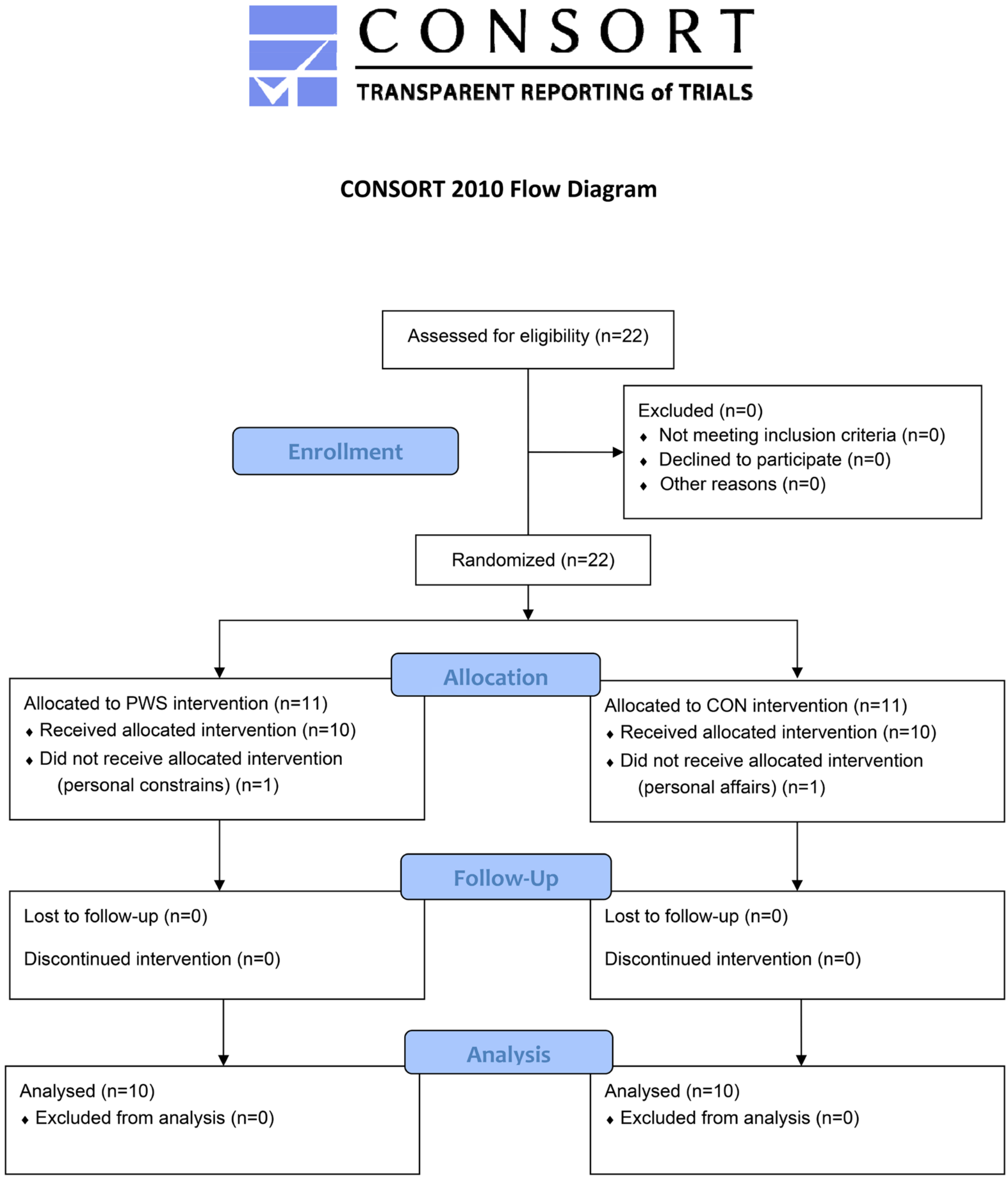

Figure 1. Participants CONSORT flow diagram.

following the methodology described elsewhere (Wenger 1993; Dempster and Aitkens 1995). Height was assessed in a stretched standing position to the nearest 0.01 m using a wall-mounted stadiometer (Seca GmbH, Hamburg, Germany) and body mass (BM) was corrected to the nearest 0.01 kg using a digital scale (Seca GmbH, Hamburg, Germany). Additionally, waist and hip circumferences were measured with a standard measuring tape, and the waist-to-hip ratio was calculated by dividing the waist by hip circumference. One trained researcher assessed all participants to eliminate inter-rater variability and ensure measurement consistency.

Muscle structure

Muscular architecture changes under relaxed and static conditions were evaluated using a real-time B-mode ultrasound imaging system (Philips Affiniti 70 Ultrasound, Philips Corporation, USA). At each marked site, an 18-5 MHz broadband linear-array transducer, along with water-soluble transmission gel (Aquasonic 100 Ultrasound Transmission gel), was positioned perpendicular to the skin surface and parallel to the longitudinal axis of the muscle, providing acoustic coupling during the test without depressing the dermal surface (Naclerio et al. 2019b). Following the methodology (Forrester 2004), outlined by Bradley and O'Donnell (2002), as described by Naclerio et al. (2019b), the same qualified and skilled researcher conducted all measurements using a standardized protocol. Muscle thickness (Th) of the elbow flexors (EF) at 80% of the humeral length, and the vastus lateralis (VL) at 60% of the femoral length were assessed in the dominant limbs. Muscle thickness was determined as the distance between the superficial and deep muscle aponeuroses for the VL, or between the superficial aponeurosis of the muscle and the muscle-bone boundary for the EF. Figure 2 illustrates examples of ultrasonography images depicting measurement sites for muscle architecture in EF and VL.

To measure EF thickness, participants were seated on a chair, with their torso straight and relaxed against the backrest. The assessed arm was maintained in a relaxed position at a 90° angle at the elbow joint on a bed, with the forearm in a relaxed pronated position. For VL thickness, participants were placed barefoot in a semi-recumbent and relaxed position on a bed set at 125°, with fully extended and relaxed knees and arms resting alongside the body. The EF assessment site was accurately located and marked at 80% of the distance between the coracoid process of the scapula and the medial epicondyle of the humerus. The VL assessment point was marked at 60% of the distance between the greater trochanter and the lateral condyle of the femur. To eliminate tissue distortion caused by excessive compression, the transducer was lightly rested on the skin surface, the ultrasound image on the screen was visually monitored, and participants were asked to provide verbal feedback on the pressure experienced on the skin.

Three images were obtained at each location, and the median of the measurements was calculated and used for the analysis. To ensure accurate replication of the measurement location, the position of the probe was recorded on acetate paper, and pre- and post-intervention images were compared based on identifiable markings (such as moles and small angiomas) on the skin surface as reference points. This process increased the reliability of repeated measures.

To prevent osmotic fluid shifts that could distort measurements of angle and thickness (Stasinaki et al. 2018), images were acquired at least 48 h after the last training session and prior to the physical performance tests. Intra-rater reliability of muscle thickness measurements was assessed by a single trained researcher, who performed measurements on the same scan, demonstrating excellent interclass correlation coefficients (ICC) (>0.90).

Vertical jump

A countermovement jump (CMJ) was performed according to the methodology described by Brown and Weir (2001). To eliminate the impact of arm-swing, the participants were

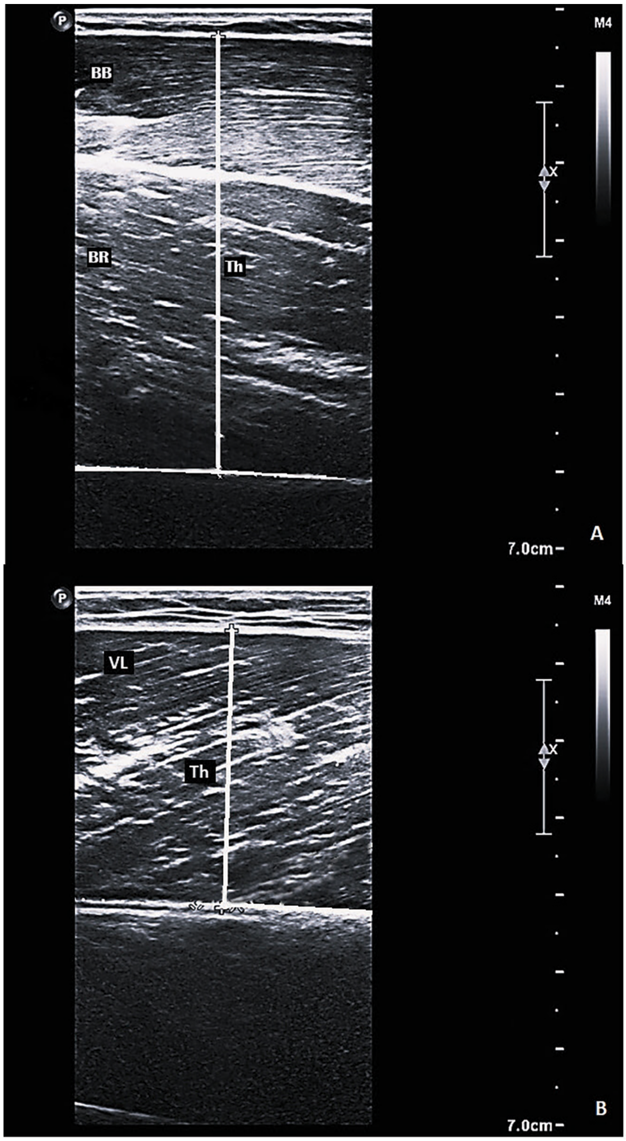

Figure 2. Sagittal ultrasound images of elbow flexors' (A) and vastus lateralis (B) muscle thickness.

required to maintain their hands on their hips for the entire action (Harman et al. 1990). A Kistler force platform (9287B, three-component force platform; Kistler, Hook, United Kingdom; dimensions: 900 × 600 × 100 mm) with a sampling rate of 2000 Hz was used to calculate the height from the difference, in meters (m), between maximum height of the center of mass (apex) and the last contact of the toe on the ground during the take-off. Based on the height, the best of three jumps was chosen for the analysis.

Seated medicine ball chest throw (SMBT)

Sitting on a chair placed against a wall, with the feet flat on the floor and separated at shoulder width, the participants threw the ball from their chest following the methodology defined by Harris et al. (2011). Based on the distance, the best of three attempts was chosen for the analysis. Males used a five-kg (circumference of 0.30 m), and females a three-kg (circumference of 0.21 m) medicine ball. A range from 0.97–0.99 ICC has been observed for this test in recreationally trained adults (Beckham et al. 2019).

Progressive cycling to exhaustion test

Following a standardized warm-up, participants completed a maximal incremental laboratory exercise test to exhaustion on a cyclo-ergometer (Lode Corival®). The test commenced at a work rate of 30 or 50 W (females and males, respectively) and increased 15 W every minute. The participants were encouraged to keep a constant cycling rate between 60 and 90 rpm while remaining in a sitting position. When cadence dropped by more than 10 rev·min^{-1} for more than 10 s despite strong verbal encouragement, tests were terminated. The test was designed to avoid long-term muscular fatigue, and every trial lasted <18 min. Expired gases were collected continuously during the test using a Cortex MetaLyzer 3B gas analyzer (Cortex Biophysik, Leipzig, Germany). The maximum heart rate (HRmax) and $\dot{V}O_2$peak (calculated as the highest mean oxygen consumption over a 30-s period [Karsten et al. 2015]) were determined and used for the analysis. Additionally, the oxidation and relative contributions of carbohydrates and fat across the test were estimated following the methodology described by Alkhatib et al. (2015). Fat oxidation (FAO) was estimated using specific equations (Alkhatib et al. 2015). Assuming minimal protein contribution during exercise the maximal fatty acid oxidation (FATmax) (g·min^{-1}) was determined from the progressive test as the highest amount of FAO averaged over 30 s. FATmax corresponding intensity was determined in watts (W) (Alkhatib 2010).

Resistance training (RT)

Training sessions were conducted on alternate days (i.e. Monday, Wednesday, and Friday). Each participant performed a supervised full-body resistance-training protocol involving a standardized warm-up followed by three circuits of one set of the following exercises: (i) box step-ups (ii) bench press, (iii) back squat with barbell, (iv) upright row, (v) deadlift, (vi) alternate lunges, (vii) shoulder press, and (viii) biceps curl. About 30-s rest between exercises and 2-min between circuits was allowed. As the workout aimed to create a high level of mechanical and muscular stress, a muscle hypertrophy training targeting 10–12 self-determined maximum repetitions (>70 to <75% 1RM) per set was designed (ACSM 2009). When participants were able to perform more than 12 repetitions per set, the load was increased between 2.5 and 5 kg (Steele et al. 2017). If fewer than 10 repetitions were completed, a rest period of ~10 s was allowed until the participants were able to reach the targeted number of repetitions per set. The time to complete the workouts was 43 ± 8 min. Additionally, the OMNI-RES scale (Robertson et al. 2003) was used to quantify the session rating of perceived exertion. To avoid easy or difficult elements toward the end of the sessions from skewing the overall rating of the exertion, the participants were asked to rate their session RPE by answering the question ‘How hard was your entire workout?’ 15 min after the completion of each resistance-training workout (Lodo et al. 2012).

Diet and supplementation protocol

Each participant completed a three-day food diary report (two weekdays and one weekend day) for three different time points: (i) first familiarization week, (ii) first interventional week, and (iii) last training week. The MyFitnessPal Inc.© (Version 2022, Texas, US) smartphone application was used to calculate the total (absolute and relative, in percentage) daily intake, and per-meal energy and macronutrient (proteins, carbohydrates, and fats) distribution (Evenepoel et al. 2020). Participants were instructed to maintain their normal diet throughout the intervention. They were asked to report any minimal change in food composition and size, ingestion of supplements, or compliance with the reported meals, including breakfast, lunch, pre- and post-workout food intake, and dinner. If any change had been detected (i.e. becoming vegetarian, restricting calories, fasting, taking additional nutritional supplements, etc.), that participant's data would have been excluded from the analysis.

During the 6-week training period (weeks 6 to 11), all participants consumed either one dose of the multi-ingredient (Crown Sport Nutrition, Spain) or an isoenergetic providing only maltodextrin beverage (Table 1).

The supplements were presented in analogous white sachets of chocolate-flavored powder to be dissolved in ~400 mL of room-temperature plain water and administered immediately after each workout session. No supplement was consumed during

Table 1. Nutritional composition of supplements per intake mixed with ~400 mL of plain water.

Description	Post-workout multi-ingredient (60 g dose)	Comparator (60 g dose)
Energy value (kcal)	204	203
Macronutrients		
Total Fats (g)	0.8g	0.6 g
Saturated	0.5 g	0 g
Total carbohydrates (g) of which	23 g	48 g
Saccharides	0.8 g	0 g
Protein	26 g *(21.5* g from whey	1.1 g
Composition		
Essential Amino Acids	21.500 mg	0 g
Aspartic Acid	2.07 g	0 g
Glutamic Acid	3.29 g	0 g
Alanine	0.95 g	0 g
Arginine	0.35 g	0 g
Cysteine + Cystine	0.41 g	0 g
Phenylalanine	0.52 g	0 g
Glycine	0.30 g	0 g
Histidine	0.27 g	0 g
Isoleucine	1.2 g	0 g
Leucine	1.87 g	0 g
Cystine + Methionine	0.81 g	0 g
Lysine	1.79 g	0 g
Methionine	0.40 g	0 g
Proline	1.18 g	0 g
Serine	0.91 g	0 g
Tyrosine	0.50 g	0 g
Threonine	1.31 g	0 g
Tryptophan	0.33 g	0 g
Valine	0.99 g	0 g
Phenylalanine + Tyrosine	1.02 g	0 g
Creatine	5 g	0 g
HMB calcium	1.8 g	0 g
Vitamin D3	1000 IU	0 IU
Sodium	0.2 g	0.03 g

non-exercising days. Therefore, each participant ingested a total of 18 doses. Even though the drinks were similar in appearance, texture, and taste, they were provided in identical black and opaque bottle shakers to maximize the double-blinded procedure. In order to avoid any interference with digestion, absorption or effects of the assigned supplement, the participants were instructed to avoid caffeine-containing substances or food intake 3 h pre- and 2 h post-workout during training days.

Statistical analysis

A descriptive analysis was conducted, and subsequently, the Shapiro-Wilk test was utilized to evaluate the normality of the data. Baseline sample characteristics were compared between conditions using Analysis of Covariance (ANCOVA), where sex served as the covariate. Changes from pre- to post-treatment were assessed using a 2 (times) × 2 (treatments) repeated-measures ANCOVA, again employing sex as the covariate. One-sample t-tests of the pre-to-post differences in each outcome variable were conducted for each treatment condition. To assess the magnitude of the differences from the baseline outcome, confidence intervals (CIs) of the differences were calculated and plotted, with statistically significant intervals being those that did not cross zero. Furthermore, mean differences between treatment conditions were evaluated with a one-way ANCOVA and Bonferroni-adjusted post hoc analysis was performed for pair-wise comparisons in all ANCOVA models. Eta squared (η^2) and Cohen's d values were reported to provide estimates of the standardized effect size (small $d = 0.2$, $\eta^2 = 0.01$; moderate $d = 0.5$, $\eta^2 = 0.06$; and large $d = 0.8$, $\eta^2 = 0.14$). The significance level was set to $p < 0.05$. Results are presented as mean ± standard deviation unless otherwise specified. The Statistical Package for the Social Sciences (SPSS for Windows, version 28.0.1.1 Windows; SPSS, Inc., Chicago, IL, USA) was employed for all statistical analyses.

Results

No differences between groups were observed at baseline or as a result of the nutritional intervention regarding energy, carbohydrate, and protein intake (Table 2). Additionally, no participants reported negative symptoms related to supplement ingestion, such as hypoglycemic reactions or gastric discomfort.

Table 3 describes the mean and standard deviation values along with the absolute changes [95% CI] in body composition (body mass, fat mass, fat-free mass, waist circumference and waist-to-hip ratio), muscle thickness (EF and VL), and performance (CMJ, seated chest medicine ball throw and total volume lifted during workouts, $\dot{V}O_2$ peak and FATmax intensity) for the two analyzed groups.

No significant differences between groups were observed at pre-intervention for any of the analyzed variables at baseline (all $p > 0.05$).

Changes in body composition and muscle thickness

Significant reductions of absolute (kg) and percentage of fat mass along with increases of fat-free mass were only observed for the PWS group. Moreover, waist circumference

Table 2. Descriptive analysis of the participants' diet composition.

Macronutrients	Post-MTN pre ($n = 10$)	Post-MTN post ($n = 10$)	COMP pre ($n = 10$)	COMP post ($n = 10$)
Proteins				
$g{\cdot}d^{-1}$	89.1 ± 27	88.6 ± 27	88.7 ± 26	89.1 ± 26
$g{\cdot}kg^{-1}{\cdot}d^{-1}$	1.09 ± 0.3	1.07 ± 0.2	1.04 ± 0.3	1.04 ± 0.3
% of total energy	15.9 ± 3.9	15.7 ± 3.6	15.6 ± 3.2	15.6 ± 3.3
Carbohydrates				
$g{\cdot}d^{-1}$	249.3 ± 75	253.2 ± 79	248.8 ± 78	249.4 ± 78
$g{\cdot}kg^{-1}{\cdot}d^{-1}$	3.04 ± 1.3	3.06 ± 1.4	2.93 ± 1.2	2.92 ± 1.2
% of total energy	44.4 ± 11.8	44.9 ± 12	43.9 ± 11.6	43.7 ± 11.5
Fats				
$g{\cdot}d^{-1}$	99.1 ± 29	98.6 ± 29	102 ± 33	103 ± 32
$g{\cdot}kg^{-1}{\cdot}d^{-1}$	1.21 ± 0.4	1.19 ± 0.3	1.2 ± 0.5	1.21 ± 0.4
% of total energy	39.7 ± 10.4	39.4 ± 10.1	40.5 ± 11.1	40.6 ± 11
Energy				
Total daily energy	2245 ± 467	2254 ± 461	2268 ± 479	2281 ± 472
$kcal{\cdot}kg^{-1}{\cdot}d^{-1}$	27.4 ± 5.6	27.3 ± 5.7	26.7 ± 6.1	26.7 ± 6

Notes: Values are presented as mean ± standard deviation

and waist-to-hip ratio significantly diminished in the PWS (−2.5 ± 1.8, $d = 0.9$ and −0.03 ± 0.03 $d = 0.6$ respectively) but not for the COM group. Indeed, when adjusted values were considered, a significantly higher waist circumference reduction ($p = 0.02$) favoring the PWS treatment with an effect size ($d = 1.19$) large enough to identify differences between groups was observed.

Both groups significantly enhanced VL (PWS: +2.4 ± 0.5 mm; COM: 2.1 ± 0.5 mm) and EF (PWS: +3.6 ± 0.6 mm; COM: +1.6 ± 0.6 mm) muscle thickness, with no differences between groups.

Changes in performance

Compared to baseline, both PWS and COM groups significantly increased CMJ height (PWS: 2.87 ± 1.1 $d = 1.1$; COM: 1.12 ± 1.2, $d = 0.8$) and SMBT (PWS: 0.27 ± 0.2; $d = 1.2$ COM: 0.2 ± 0.2, $d = 0.9$). When adjusted values were considered, a more pronounced significant improvement ($p = 0.005$) and substantial effect size ($d = 1.47$) favoring the PWS group. Nonetheless, no differences between groups were determined for SMBT at post-intervention.

The total lifted workout volume significantly increased for both groups, with no differences between them when adjusted values were considered.

Exploratory variables

Both groups significantly improved VO_2 peak and FATmax intensity with no significant differences between them when adjusted values were considered.

The average scores on the Global Rating of Perceived Effort (RPE), assessed by the OMNI-RES (0–10) scale did not reveal any statistically significant disparities between the PWS (8.3 ± 0.8) and COM (8.1 ± 0.9) conditions. Additionally, no between-sexes differences were identified for any of the variables analyzed, both groups similarly responded to treatment-induced changes in body composition and performance.

Table 3. Mean (M) ± standard deviation (SD) of the pre- and post-values and the changes M ± SD [95% CI] of the analyzed variables for the two intervention groups.

	Post-workout multi-ingredient ($n = 10$)			Comparator ($n = 10$)			Between-groups comparisons	
Variables	Pre	Post	Changes [95% CI]	Pre	Post	Changes [95% CI]	*p* value	ES
Body mass (kg)	82 ± 17.9	82.6 ± 18.5	0.59 ± 1.3 [−1.48, 0.29]	84.9 ± 17.6	85.4 ± 17.6	0.45 ± 1.4 [−1.34, 0.44]	0.81	0.11
Fat mass (%)	32.1 ± 6.4	30.7 ± 6.6	−1.38 ± 0.7 [−1.94, −0.82]**	31.9 ± 6.6	31.3 ± 6.7	−0.52 ± 1 [−1.08, 0.04][t]	0.07	0.90
Fat mass (kg)	26.7 ± 8.6	25.6 ± 8.7	−1.09 ± 0.7 [−1.64, −0.54]**	27.3 ± 8.9	26.9 ± 8.6	−0.34 ± 1 [−0.89, 0.21]	0.10	0.80
Fat-free mass (%)	67.9 ± 6.4	69.3 ± 6.6	1.38 ± 0.7 [0.82, 1.94]**	68.2 ± 6.6	68.7 ± 6.7	0.52 ± 1 [−0.04, 1.08][t]	0.07	0.90
Fat-free mass (kg)	55.4 ± 11.6	56.8 ± 12.4	1.34 ± 1.2 [0.54, 2.15]**	57.7 ± 11.8	58.5 ± 12.2	0.79 ± 1.2 [−0.02, 1.60][t]	0.25	0.56
Waist circumference (cm)	88.2 ± 12.9	85.7 ± 12.5	−2.5 ± 1.8 [−1.41, −3.59]**	89.2 ± 12.3	88.8 ± 12.8	−0.40 ± 1.5 [−1.49, 0.69]	0.02	1.19
Waist-to-hip ratio	0.85 ± 0.08	0.82 ± 0.08	−0.03 ± 0.03 [−0.04, −0.01]**	0.85 ± 0.11	0.84 ± 0.11	−0.01 ± 0.02 [−0.03, 0.01]	0.20	0.62
Vastus lateralis muscle thickness (cm)	2.54 ± 0.5	2.79 ± 0.5	0.24 ± 0.1 [0.17, 0.32]**	2.61 ± 0.4	2.82 ± 0.5	0.21 ± 0.1 [0.14, 0.39]**	0.40	0.40
Elbow flexors muscle thickness (cm)	3.93 ± 0.7	4.3 ± 0.8	0.36 ± 0.2 [0.24, 0.49]**	4.2 ± 0.7	4.36 ± 0.7	0.16 ± 0.2 [0.03, 0.28]*	0.16	0.75
CMJ (cm)	27.6 ± 6.4	30.5 ± 6.4	2.87 ± 1.1 [2.12, 3.62]**	27.2 ± 5.3	28.3 ± 5.6	1.12 ± 1.2 [0.38, 1.87]**	0.01	1.47
Seated chest medicine ball throw (m)	3.54 ± 0.4	3.82 ± 0.3	0.27 ± 0.2 [0.15, 0.40]**	3.82 ± 0.4	4.02 ± 0.4	0.2 ± 0.2 [0.07, 0.33]**	0.99	0.01
Total volume lifted (kg)	10022 ± 2904	13687 ± 3791	3664 ± 1693 [2693, 4637]**	10973 ± 2678	14412 ± 3411	3439 ± 1189 [2468, 4411]**	0.48	0.34
VO_2 peak ($mL \cdot kg^{-1} \cdot min^{-1}$)	36.8 ± 8.2	40.1 ± 8.3	3.3 ± 2.5 [1.61, 4.99]**	40.2 ± 6.4	42.6 ± 5.7	2.4 ± 2.6 [0.71, 4.09]**	0.73	0.16
FATmax intensity (Watts)	70 ± 31	80 ± 25	10.1 ± 9.2 [3.41, 16.79]**	63 ± 18	73 ± 15	9.6 ± 10.9 [2.91, 16.29]*	0.55	0.28

*$p \leq 0.05$, **$p \leq 0.01$, [t]$p \leq 0.10$ respect to baseline levels; ES is the standardized effect size presented as Cohen's *d*.

Discussion

Results of the present study suggest that ingesting a post-workout supplement, including carbohydrates, whey protein, creatine monohydrate, β-HMB and vitamin D, promoted noticeable body composition outcomes, not observed when consuming an isocaloric-only carbohydrate supplement. Nonetheless, except for the lower body impulsive force, where the PWS group reached a higher post-intervention performance than the COM, no differences between groups, were determined in supporting hypertrophy, upper body impulsive force, and endurance performance enhancements. Furthermore, no impact of the type of supplement was observed on workout volume (kg lifted) or the experienced global rating of perceived effort.

No significant change in energy or macronutrient intake was detected for either group (Table 2). Consequently, the primary distinction between treatments relied on the composition of the supplements. The analysis of dietary patterns indicated that all participants regardless of the group consumed ~3 $g{\cdot}kg^{-1}$ of BM of carbohydrate (~44% of calories) slightly higher than 1 $g{\cdot}kg^{-1}$ of BM of protein (~15% of calories) and ~1.2 g kg^{-1} of BM of fat (~40% of calories). These figures are below the recommended daily intake for carbohydrates (4–7 $g{\cdot}kg^{-1}$ of BM) and proteins (~1.6 $g{\cdot}kg^{-1}$ of BM) (Morton et al. 2018) while slightly above the recommended fat intake (30% of the calories) (Thomas et al. 2016) for physically active individuals looking for hypertrophy (Thomas et al. 2016).

Our results align with previous studies indicating the significance of specialized nutrition on body composition (Bonilla et al. 2021). The observed fat mass reduction experienced by the PWS group underscores the impact of protein-containing post-workout meals on fat metabolism. The ingestion of whey protein has been associated with suppressed appetite, increased satiety and favors protein synthesis, which in turn would increase post-exercise thermogenesis (Acheson et al. 2011). Therefore, a hypothetically increased use of fat as the predominant fuel to support muscle remodeling and recovery could be the cause of the significant fat mass reduction observed in PWS but not in the COM group. Moreover, it has been suggested that lactalbumin and lactoferrin found in whey protein may increase postprandial lipolysis markers, improve energy balance, and decrease adiposity (Naclerio et al. 2019a). On the other hand, an increased post-workout protein intake could have been particularly impactful in stimulating post-exercise muscle protein synthesis in our participants who consumed lower daily protein intake than the recommended to support muscle mass accretion (Stokes et al. 2018; Van Vliet et al. 2018). Although both groups similarly enhanced VL and EF thickness, a remarkably significant increase in FFM was observed only for the PWS group ($p = 0.007$, $d = 1.10$), while only a trend toward significance ($p = 0.064$, $d = 0.65$) was noted for the COM group. Furthermore, it is worth noticing, that although no statistical differences between groups was identified at post-intervention, a large effect size ($d = 0.90$), favoring the PWS treatment was determined.

The relative amount of carbohydrate (~0.29 $g{\cdot}kg^{-1}$), protein (0.33 $g{\cdot}kg^{-1}$) and creatine monohydrate (0.06 $g{\cdot}kg^{-1}$) included in the post-workout multi-ingredient, administered to our participants was within the recommended effective doses to maximize muscle mass accretion (Cooper et al. 2012; Naclerio et al. 2020). Nonetheless, it seems that for middle-aged physically active participants ingesting a multi-ingredient supplement three times per week was not enough to induce significantly superior outcomes compared to the ingestion of only carbohydrates, at least over a 6-week training period. In summary, our results seem to support the notion that while specific nutrient compositions (reduced carbohydrate in favor of protein content while maintaining a similar energy consumption) may promote fat loss, body mass gain may also need an appropriate energy intake combined with resistance training to facilitate quality weight gain characterized primarily by FFM increase (Slater et al. 2019).

On the other hand, when considering performance outcomes, it is worth highlighting that the PWS group showed greater CMJ performance increases than the COM. However, such a difference between treatments was not seen for the medicine ball throw. In this regard, the applied training routine imposed a higher training volume on lower body muscles involved in knee and hip extension and therefore contributed to vertical jump-like action during four exercises (parallel squat, hang clean; and alternate lunges) whilst the upper body muscle action during shoulder flexion and elbow extension was mainly activated in only one exercise (bench press). These differences in the training overload may have impacted the observed results, particularly when considering that the most prominent resistance-training adaptations and nutritional support benefits are likely to occur in the most heavily utilized muscle groups (Bernárdez-Vázquez et al. 2022). Additionally, it seems that for middle-aged physically active individuals, a 6-week resistance-training program, whether combined with the ingestion of a multi-ingredient or carbohydrates alone (Table 1), could be similarly effective in promoting endurance capacity with no differentiated effects on the global perception of effort. These results suggest that while targeted muscle hypertrophy and strength gains are influenced by specific training loads and muscle utilization, broader endurance improvements can be achieved through consistent circuit-based resistance training and nutritional support, regardless of the specific supplement used.

Our investigation may be predisposed to several limitations. Firstly, despite the employment of a validated application (MyFitnessPal Inc.©) for recording nutritional intake, the self-reporting method employed might introduce measurement inaccuracies. A more standardized methodology would have entailed participants weighing their food prior to consumption. Secondly, the non-individualization of the supplement dosage resulted in varying relative amounts of proteins (0.24 to 0.43 $g{\cdot}kg^{-1}$ of BM), carbohydrates (0.21 to 0.38 $g{\cdot}kg^{-1}$ of BM) and creatine (0.05 to 0.08 $g{\cdot}kg^{-1}$ of BM) among participants, potentially affording broader benefits to those with lighter body mass. However, subsequent statistical analysis examining individual variances in muscle mass accrual did not identify any notable differences between participants. Additionally, because our study was designed as a highly ecological intervention, avoiding diet modifications, the participants were asked to maintain their usual caffeine intake (avoiding caffeine or energetic intake 3 h pre- and 2 h post-workout during training days) so that the impact of integrating postworkout supplementation to the habitual diet could be assessed. Lastly, the included peri- and post-menopausal women were not complemented by any additional blood tests beyond a symptomatology questionnaire, which might have nuanced the results across different stages of menopause. Despite this, statistical assessments to discern variations across menopausal statuses did not demonstrate disparities for any investigated parameter. The potential for observing more pronounced differences between supplements could likely be enhanced with longer interventions using larger sample sizes. Nevertheless, given the study's ecological validity, we adhered to the dosages typically recommended by manufacturers.

Future research should endeavor to delineate the specific contributions of each constituent within multi-ingredient supplements to elucidate their singular and synergistic impacts on performance and body composition. Further exploration into the timing, dosage, and formulation of these supplements, in the context of diverse exercise modalities, promises to deepen our understanding of their effectiveness and mechanisms of action particularly considering the widespread commercial/consumer use of such supplementation.

Conclusion

Compared to ingesting a post-workout-only carbohydrate beverage, a multi-ingredient providing carbohydrates, whey protein, and creatine enriched with L-leucine, β-HMB, and Vitamin D3, promoted fat mass decrease and fat-free mass increase, along with noticeable differences in waist circumference reduction and higher vertical jump performance enhancements. Nonetheless, no differences between supplements were observed in muscle thickness, upper body impulsive force, endurance performance, workout volume, or the experienced global rating of perceived exertion.

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