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Pre-workout multi-ingredients or carbohydrate alone promote similar resistance training outcomes in middle-aged adults: a double-blind, randomized controlled trial

Joel Puente-Fernández[a], Eneko Larumbe-Zabala[b], Justin Roberts[c] and Fernando Naclerio[a]

[a]Institute for Lifecourse Development, School of Human Sciences, Centre for Exercise Activity and Rehabilitation, University of Greenwich, Eltham, UK; [b]Canary Islands Health Research Institute Foundation (FCIISC), La Laguna, Tenerife, Spain; [c]Cambridge Centre for Sport and Exercise Sciences, School of Psychology and Sport Science, Anglia Ruskin University, Cambridge, UK

ABSTRACT

Background: Pre-workout multi-ingredients are designed to enhance energy levels and acutely increase exercise performance. This study compared the effectiveness of ingesting an admixture providing caffeinated ingredients and plant-based protein enriched with amino acids versus carbohydrates alone on body composition, hypertrophy and physical performance.

Methods: Forty-three middle-aged physically active individuals (26 peri- and post-menopausal females and 17 males) completed the study after being randomly assigned to one of the following intervention groups: pre-workout (PREW $n = 24$, 54 ± 4 years, body mass 77.6 ± 16.0 kg) or a carbohydrate-only comparator (COMP $n = 19$, 52 ± 4 years, body mass 80.6 ± 16.0 kg). Measurement of fat and fat-free mass (via plethysmography), waist and hip circumferences, muscle thickness (via ultrasound), strength, power output and muscle endurance performance were collected before and after a 6-week resistance training programme performed 3 times per week. Treatment consisted of ingesting 30g of the assigned supplement, mixed with 400mL of plain water, 15min before each workout session.

Results: Both groups significantly ($p < 0.05$) reduced fat mass (mean ± SD) (PREW −1.4 ± 1.6 kg; COMP −1.0 ± 1.5 kg), increased fat-free mass (PREW +0.9 ± 1.1 kg; COMP + 0.8 ± 0.9 kg); vastus lateralis (PREW +0.2 ± 0.2 cm; COMP + 0.1 ± 0.6 cm), and elbow flexors (PREW +0.5 ± 0.3 cm; COMP + 0.4 ± 0.2 cm) muscle thickness. Only the PREW group showed a significant reduction in the waist circumference (−1.8 ± 1.8 cm, $p < 0.01$). However, no significant differences between groups (PREW vs. COMP) were identified at post-intervention. Isometric force, countermovement jump, medicine ball throw, and upper and lower body muscle endurance

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CONTACT Fernando Naclerio f.j.naclerio@gre.ac.uk School of Human Sciences, University of Greenwich, Avery Hill Campus, Sparrows Farm (Office SF112B), Sparrows Lane Eltham, SE9 2BT, UK

performance improved ($p < 0.05$) for both treatments, with no difference between groups.
Conclusion: Except for waist circumference reduction, ingesting a pre-workout vegan protein-based caffeinated supplement promotes no further resistance training benefits in middle-aged individuals.

1. Introduction

Multi-ingredient pre-workout formulations (PREW) represent a wide category of dietary supplements with purported efficacy in enhancing physical performance [1]. Previous interventions demonstrated valuable effects of PREW on exercise outcomes including strength [2], power output [1,3], muscular endurance [4] and gaining muscle mass [5–7]. In this context, caffeine has arisen as the predominant ingredient due to its thermogenic [8] and ergogenic effect on both endurance [1,9,10], and strength [11] performance. For instance, the co-administration of caffeine with yerba mate containing caffeoyl derivatives such as chlorogenic acid, phytosterols, and saponins, promoted fat metabolism [12], increased fatty acid oxidation, and reduced the perception of effort during low-intensity endurance exercise [13].

In addition to caffeinated substances, some commercially available PREW include high-quality proteins, such as whey fortified with amino acids (AA) (e.g. L-leucine, L-arginine, L-tyrosine, or L-taurine) or their derivatives (e.g. citrulline-malate, betaine, or L-carnitine) that may act synergistically with caffeine to enhance muscular efficiency and extend the onset of fatigue [14,15]. For example, L-carnitine is a conditionally essential AA derivative that plays a role in fatty acid metabolism. It may promote vasodilation and improve oxygen supply to the working muscles [16]. L-arginine, a conditionally essential AA in adults, serves as a precursor for creatine, a component of the body's energy metabolism [17]. L-citrulline is a non-essential and non-proteogenic AA which promotes vasodilation, rate of oxidative ATP production and phosphocreatine recovery after high-intensity exercise [18].

Recent studies have reported beneficial effects of L-citrulline or citrulline-malate supplementation in maximizing strength, power output, and muscle endurance in both recreationally active and trained athletes [19–21]. Furthermore, combining L-arginine and L-citrulline has been proposed as an effective nutritional intervention to promote endogenous synthesis of nitric oxide (NO) which may optimize the removal of metabolic waste products, attenuate fatigue [22], and enhance physical performance [23]. The ingestion of L-tyrosine has been proposed to improve prolonged submaximal exercise in the heat, along with lower perceived exertion [24]. L-taurine supplementation has also been associated with improved muscular endurance [25]. Betaine, a glycine aminoacidic derivative, favors muscle blood flow by elevating the levels of NO and promoting fluid and thermal homeostasis [6,26]. Individually, or in combination, it is therefore feasible that such nutrients may have beneficial effects when consumed within a PREW formula.

Furthermore, carbohydrate (CHO) administration before workouts is widely accepted as a key dietary strategy to ensure the availability of circulating glucose to support limited muscle fuel stores during intense and prolonged steady or intermittent exercise [27–29].

Indeed, PREW including caffeine and CHO have been reported to promote faster intestinal absorption and increased exogenous CHO oxidation rates during exercise [30]. However, divergent conclusions concerning the advantages of PREW supplementation in healthy middle-aged and older adults have also been noted in the literature [31–34]. Currently, there is a paucity of research regarding the use of PREW supplements to maximize exercise training adaptations in middle-aged and older physically active adults [35].

The aim of this study therefore was to compare the effectiveness of combining a 6-week resistance training (RT) programme with a commercially available PREW including caffeinated ingredients and plant-based protein extracts (Crown Sport Nutrition, Spain) vs. an isocaloric, carbohydrate-only supplement comparator (COMP) on body composition, muscle thickness, and physical performance in middle-aged, healthy, physically active individuals. Additionally, considering that females and males may show distinct benefits from physical exercise [36], exhibit differential molecular responses [37], and training-induced adaptations to resistance exercise programmes [38], we also explored differences in the intervention-induced outcomes among female and male participants. Based on the literature, we hypothesized that ingesting PREW over a 6-week RT programme would maximize fat loss, fat-free mass gain, and muscular hypertrophy, along with a more favorable performance enhancement effect than COMP alone.

2. Material and methods

2.1. Experimental design

The intervention followed a double-blinded, randomized, parallel-group controlled trial design. Ethical approval was granted by FREC-EHHS-21–2–23–03. Following the inclusion criteria, familiarization period, and baseline assessments, participants were randomly allocated to receive either a vegan protein-based multi-ingredient supplement (PREW) or an isocaloric, carbohydrate-only comparator (COMP). Primary outcomes were changes in body composition (fat mass, fat-free mass and waist circumference) and muscle thickness from vastus lateralis and elbow flexor. Changes in isometric strength, vertical jump, medicine ball throw, and 30-second continued repetition tests in sit-and-stand and bench press exercises were considered secondary outcomes. Additionally, the 15-to-20-minute post-workout global rating of perceived exertion (S-RPE) from the OMNI-RES (0–10) scale was considered an exploratory variable. All tests were performed at baseline and follow-up at 6 weeks.

2.2. Participants

Forty-four healthy and recreationally active, middle-aged, and older adults (26 peri- and post-menopausal females and 17 males; age: 53 ± 5 years) were initially recruited. The inclusion criteria required participants to have a minimum regular resistance training history of 6 months before the beginning of the study and to be 45 years of age or older. Female participants were additionally required to be post- or peri-menopausal, exhibiting at least two symptoms of menopause onset, such as hot flushes, menstrual cycle alterations, and not menstruating for more than 1 year [39]. Participants were not eligible if

suffering from acute illness or chronic diseases (including obesity [BMI ≥30 kg/m^2] [40], metabolic syndrome, long COVID-19, osteoporosis, or sarcopenia), following a medication prescription, or consuming supplements or medications that could interfere with our research or affecting exercise performance (i.e. creatine, protein amino-acids, NSAIDs, etc.).

All participants confirmed verbal compliance prior to providing written informed consent. All experimental procedures were conducted in accordance with the Declaration of Helsinki and registered with ClinicalTrials.gov, U.S. National Institutes of Health (Identifier: NCT05769088).

To assess the statistical power of the study, we conducted a sensitivity analysis of the final sample size (PREW, $N = 22$ and COMP, $n = 19$) 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 ($1 - \beta$), it was determined that differences between conditions could be detected with a Cohen's d above 0.89.

2.3. Procedures

After inclusion and before the baseline assessment, participants performed eight sessions of familiarization on alternate days, aimed at minimizing any potential learning effects of the training procedures. Following the initial assessment, participants were matched by body mass and isometric strength. Assignment of participants to treatments was performed by block randomization using a block size of two and in a double-blind (PREW or COMP) manner.

All pre- and post-intervention assessments were conducted in a single session conducted the week before and separately after the 6-week intervention period, 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 sessions 48 hours before the assessments. Furthermore, participants abstained from consuming food or beverages for 3 hours before the assessment sessions and from ingesting energy sources within 2 hours before the workouts. The pre-and post-intervention evaluation encompassed the following components in the specified order: (i) body composition, (ii) muscle thickness, and (iii) physical performance.

2.3.1. Assessments

2.3.1.1. Body composition. Body mass (BM) and height were assessed according to the methods described by Ross et al. [41]. Height was measured in a stretched standing position to the nearest 0.01 m using a wall-mounted stadiometer (Seca GmbH, Hamburg, Germany), and BM was corrected to the nearest 0.01 kg using a digital scale (Seca GmbH, Hamburg, Germany). Whole body densitometry using air displacement via the Bod Pod (Life Measurements, Concord, CA) was used in accordance with the manufacturer's instructions for the assessment of body composition measures as detailed elsewhere (e.g. avoid strenuous exercise for at least 24 hours, no eating or drinking for at least 2 hours prior to the assessment) [42].

2.3.1.1.1. Waist and hip circumferences. The circumferences of the waist and hip were assessed using a stretch-resistant measuring tape and following the methodologies

described by Stewart et al. [43]. Subsequently, the waist-to-hip ratio was calculated by dividing the waist circumference by the hip circumference. To minimize inter-rater variability and ensure measurement consistency, a single trained researcher performed all assessments.

2.3.2. Muscle thickness

Muscular thickness 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 [44]. Following the methodology outlined by Bradley and O'Donnell [45], as described by Naclerio et al. [46], the same qualified and skilled researcher conducted all measurements using a standardized protocol. Muscle thickness of the elbow flexors (EF) and the vastus lateralis (VL) 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 1 illustrates examples of ultrasonography images depicting measurement sites for muscle architecture in EF and VL.

To measure the thickness of the EF, 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

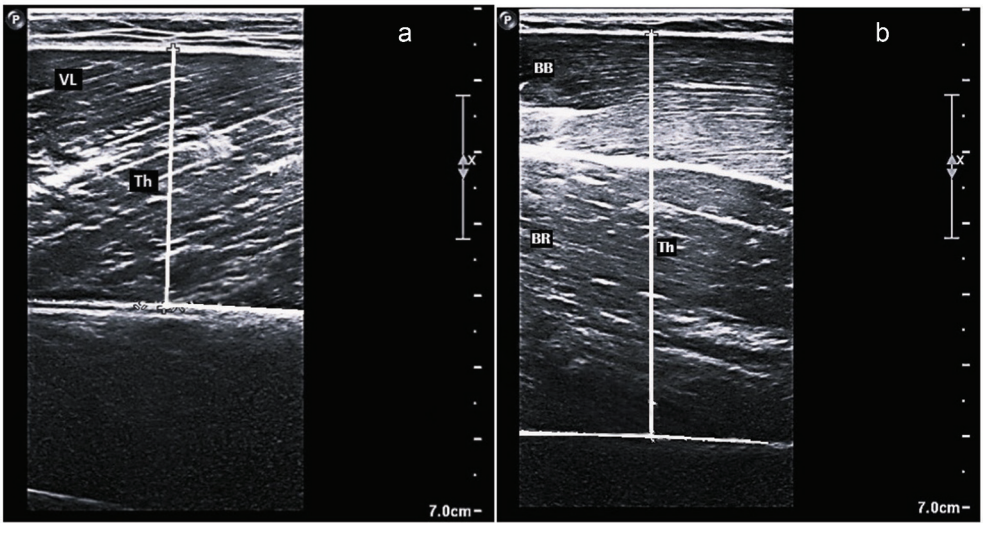

Figure 1. Sagittal ultrasound images of elbow flexors' (a) and vastus lateralis (b) muscle thickness.

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 [47], images were acquired at least 48 hours 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 >0.90 intraclass correlation coefficient (ICC).

2.3.3. Isometric mid-thigh pull test

A T.K.K. 5402 dynamometer (Takei Scientific Instruments Co. Ltd., Niigata, Japan) with a base of 31.5 X 31.5 cm, equipped with a chain (51 cm), and a latissimus pulldown bar (120 cm; Perform Better, United Kingdom), was used to evaluate full-body maximal isometric force (MIF) [48]. Participants started in a standing position, on the foot grips, adjusting the chain length to position the bar slightly above their knees and gripped the bar without using straps. Before pulling, they were instructed to maintain tension on the chain to avoid jerking movements. Subsequently, participants exerted maximum force while pulling upwards [49]. Three attempts of 5 seconds with a 30-second rest between each attempt were performed. The highest recorded value in kg of force (kgF) was selected for further analysis. Additionally, >0.90 ICC was observed for this outcome.

2.3.4. Vertical jump

A countermovement jump (CMJ) was executed following the methodology described by Brown and Weir [50]. To eliminate the influence of arm-swing on the final result, participants were required to maintain their hands on their hips throughout the jumping action [51]. A Kistler force platform (9287B, three-component force platform; Kistler, Hook, United Kingdom; dimensions: $900 \times 600 \times 100$ mm) with a sampling rate of 2000 hz was used to calculate the jump height in centimeters (cm). The height was determined as the difference between the 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. The ICC was superior to 0.90.

2.3.5. Chest medicine ball throw (CMBT)

Participants were seated on a chair placed against a wall with the feet flat on the floor and positioned shoulder-width apart. Following the methodology defined by Harris et al. [52], participants performed a chest throw with a medicine ball (5 kg for males and 3 kg for females). Based on the distance achieved, the best of three attempts was chosen for the

analysis. A range from 0.97–0.99 ICC has been observed for this test in recreationally trained adults [53].

2.3.6. 30-seconds bench press (BP) and squat (SQ)

The BP exercise was performed using free weights with an individually tailored load to approximate sixteen maximum repetitions. Participants were instructed to perform all possible repetitions in 30 seconds. They commenced the exercise in a supine position on a flat bench, with their elbows fully extended, and were instructed to lower the barbell toward the chest before engaging in the concentric phase.

The squat (SQ) exercise was performed with no external overload. Participants began seated on an individually adjusted bench, so that the posterior thighs were parallel to the ground, back straight, feet positioned parallel at shoulder width apart, and toes angled slightly outwards. Arms were crossed at the wrists and held against the chest. The participants were instructed to stand up and sit as many times as possible in 30 seconds.

For both exercises, the total number of completed repetitions was considered for the analysis. One qualified instructor monitored the appropriate execution of both BP (arms' range of motion and bar path from the chest to the end position with elbows completely extended) and SQ (ensuring the back was straight, avoiding additional impulse from the arms and thighs, and returning to parallel position at the end of the descent phase). The ICC was higher than 0.90 for both tests.

2.4. Training and control of the intervention compliance

Training sessions were conducted on alternate days (i.e. Monday, Wednesday, and Friday). Each participant engaged in a supervised full-body resistance-training protocol, which included a standardized warm-up of about 12 minutes followed by three circuits of one set of the following exercises: (i) box step-ups (ii) bench press, (iii) sit-and-stand from the box, (iv) bent-over row, (v) deadlift, (vi) alternate lunges, (vii) shoulder press, and (viii) leg extension. Approximately 30-sec rest between exercises and 3 minutes between circuits were allowed. The aim of the workout was to induce a high level of mechanical and metabolic stress, focusing on muscle endurance training with a target of 16 self-determined [54] maximum repetitions per set [55]. When participants were able to perform more than 16 repetitions per set, the load was slightly increased between 2.5 to 5 kg. If fewer than 16 repetitions were completed, a rest period of approximately 10 seconds was allowed until the participants were able to reach the targeted number of repetitions per set. The duration of the workouts was 49 ± 8 minutes. Furthermore, the S-RPE was measured after 15 to 20 min of having completed each workout session. The participants rated their global perception of effort on the OMNI-RES scale [56] by answering the question "How hard was your entire workout?" [57].

2.5. Supplementation protocol

The two products were presented in analogous white sachets of citric-flavored powder to be dissolved in ~400 mL of room-temperature plain water and dispensed in identical 500-mL black and opaque bottle shakers. The diluted isoenergetic drinks were similar in appearance, texture, and taste. Both supplements were ingested on training days, within

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

Description	Multi-Ingredient (30 g dose)	Comparator (30 g dose)
Energy value (kcal)	60	60
Macronutrients		
Total carbohydrates from maltodextrin (g)	5 g	15 g
Fats	0 g	–
Total proteins included added amino acids (g)	9 g	–
Amino acids and other ingredients		
L-Leucine (g)	3	–
L-Isoleucine (g)	1.5	–
L-Valine (g)	1.5	–
L-Lysine (g)	2.7	–
L-Arginine Base (g)	2.5	–
L-Methionine (g)	0.7	–
L-Phenylalanine (g)	1.1	–
Taurine (g)	1	–
L-Threonine (g)	1.2	–
L-Tryptophan (g)	0.3	–
Tyrosine (g)	1	–
Citrulline Malate	2.5	–
Betaine (HCl)	2	–
Acetyl-L-Carnitine	1.3	–
Caffeine (mg)	406	–
Yerba Mate (2% in Caffeine)	300	–

15 minutes before each workout session. No supplement was consumed during non-exercising days. The nutritional composition of each product is presented in Table 1.

2.6. Dietary monitoring

Each participant's baseline diet (3 days, 2 weekdays, and 1 weekend day) was analyzed using MyFitnessPal Inc.© (Version 2022, Texas, US) smartphone application [58]. Participants were instructed to maintain their normal diet throughout the intervention. They were asked to report any minimal change regarding 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. To evaluate differences caused by the supplementation protocol, the diet was analyzed again during the last week of the intervention.

3. Statistical analysis

A descriptive analysis was performed and subsequently the Shapiro – Francia tests were applied to assess normality. Sample characteristics at baseline were compared between groups using an independent means Student's T-test. All pre- and post-intervention data were summarized and reported as mean ± standard deviation unless stated otherwise. Raw changes in all outcome variables were calculated by subtracting pre- from post-assessment values. Under the assumption that both treatment groups would promote changes from baseline values due to the

common training intervention and that the amount of change would also be dependent on each participant's baseline performance levels, one-way analysis of covariance (ANCOVA) models were used to compare differences in raw change between groups, using the pre-assessment values as covariates. A one-sample t-test of the pre-to-post differences in each outcome variable was performed 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. Confidence intervals not crossing zero were considered statistically significant. Eta squared (η^2) and Cohen's d standardized effect sizes of the adjusted differences between intervention groups were calculated from the ANCOVA F tests and compared to common benchmarks [59] (small $\eta^2 = 0.01$, $d = 0.2$; moderate $\eta^2 = 0.06$, $d = 0.5$; and large $\eta^2 = 0.14$, $d = 0.8$). The significance level was set to $p \leq 0.05$. Results are reported as mean ± standard deviation unless stated otherwise. All statistics were performed using the Statistical Package for the Social Sciences (SPSS for Windows, version 28.0.1.1 Inc., Chicago, IL, USA).

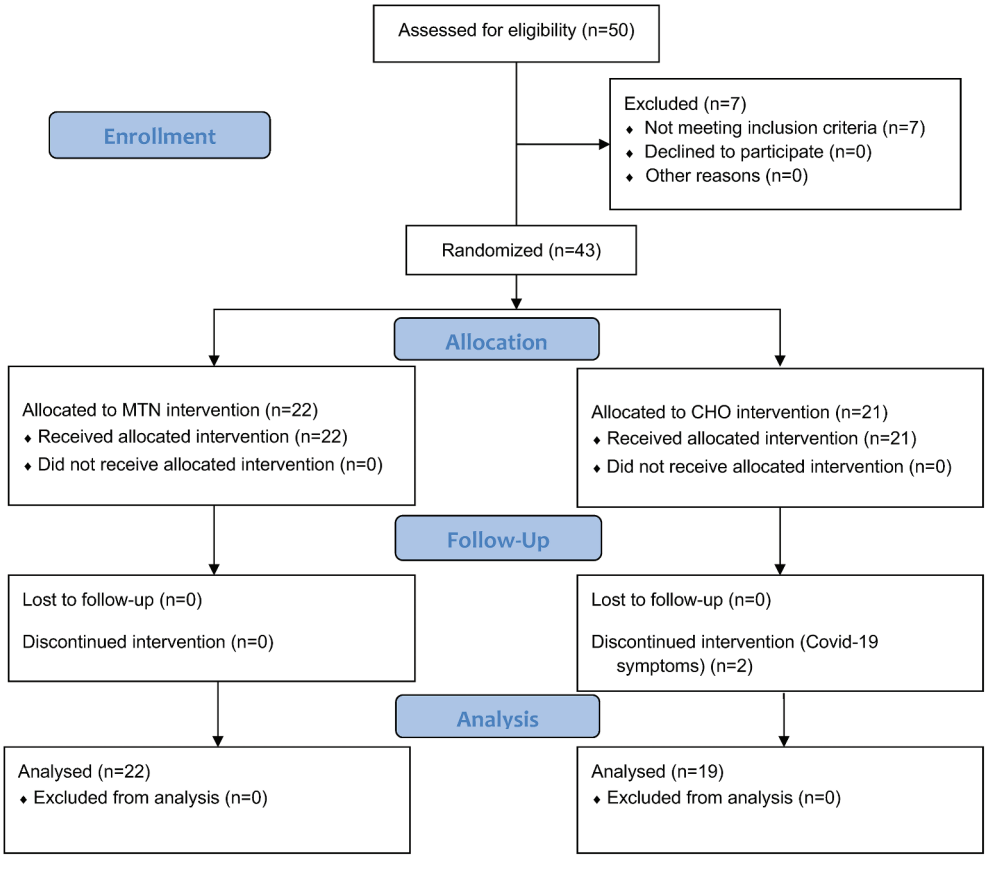

Figure 2. Participants CONSORT flow diagram.

4. Results

As summarized in Figure 2, forty-one of the initially recruited 44 participants completed all aspects of the study (Figure 2). Groups characteristics were equivalent at baseline: PREW [$n = 22$, 13 females (59%) and 9 males]: age 54 ± 4 years, height 1.72 ± 0.10 m, body mass 77.6 ± 16.0 kg, isometric strength 128 ± 49 kg; COMP [$n = 19$, 11 females (58%) and 8 males]: age 52 ± 4 years, height 1.72 ± 0.10 m, body mass 80.6 ± 16.0 kg, isometric strength 126 ± 33 kg.

Table 2 shows the dietary monitoring results, determined before and after the intervention.

No differences between groups were either found at baseline or as a result of the nutritional intervention for energy, carbohydrates, protein and fat intake. No complaints about any negative symptoms (i.e. hypoglycemic reaction) or gastric discomfort due to the ingestion of supplements were reported.

Table 3 describes the mean and standard deviation values along with the observed absolute changes [95% CI] in body composition (BM, waist circumference, hip circumference, waist-to-hip ratio, fat mass and fat-free mass), muscle thickness (EF and VL), and performance (CMJ, seated chest medicine ball throw, midthigh pull isometric strength and 30-s sit to stand and bench press muscular endurance tests) for each of the intervention groups. No significant differences were observed at pre-intervention in any of the analyzed dependent variables.

Even though both groups showed a significant reduction of body fat (percentage and absolute [kg])) along with a concomitant increase of fat-free mass (percentage and absolute [kg]) a significant reduction of the waist circumference and waist-to-hip ratio was observed only for the PREW group. However, no further difference between treatments was identified when the adjusted values were analyzed post-intervention (Figure 3).

A non-significant trend effect was observed for FM in kg ($F(1,39) = 2.79$, $p = 0.104$, $\eta 2 = 0.065$) and percentage ($F(1,39) = 3.723$, $p = 0.061$, $\eta 2 = 0.086$) values. Additionally, no significant between-groups difference emerged upon adjustment

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

Macronutrients	PREW pre ($n = 22$)	PREW post ($n = 22$)	COMP pre ($n = 19$)	COMP post ($n = 19$)
Proteins				
$g \cdot d^{-1}$	88.5 ± 29.3	87.5 ± 28.9	88.7 ± 28.3	89.5 ± 29
$g \cdot kg^{-1} \cdot d^{-1}$	1.14 ± 0.3	1.13 ± 0.3	1.1 ± 0.3	1.11 ± 0.3
% of total energy	15.9 ± 3.9	15.6 ± 3.6	14.5 ± 3.2	14.8 ± 3.3
Carbohydrates				
$g \cdot d^{-1}$	247 ± 78.6	255.42 ± 79	249.9 ± 78.1	244.4 ± 77.8
$g \cdot kg^{-1} \cdot d^{-1}$	3.2 ± 1.4	3.3 ± 1.6	3.1 ± 1.3	3 ± 1.2
% of total energy	44.3 ± 11.8	45.5 ± 12	40.7 ± 11.6	40.3 ± 11.5
Fats				
$g \cdot d^{-1}$	98.7 ± 29.7	97.1 ± 29.4	122.1 ± 32.3	120.9 ± 31.8
$g \cdot kg^{-1} \cdot d^{-1}$	1.3 ± 0.4	1.3 ± 0.5	1.5 ± 0.7	1.5 ± 0.7
% of total energy	39.8 ± 10.4	38.9 ± 10.1	44.8 ± 11.1	44.9 ± 11
Energy				
Total daily energy	2231 ± 479	2246 ± 467	2453 ± 489	2426 ± 477
$Kcal \cdot kg^{-1} \cdot d^{-1}$	28.8 ± 5.9	29 ± 5.7	30.4 ± 6.2	30.1 ± 6

Values are presented as mean ± standard deviation.

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

	Multi-Ingredient ($n = 24$)			Comparator ($n = 19$)			Between-Groups Comparisons	
Variable	Pre	Post	Changes [95% CI]	Pre	Post	Changes [95% CI]	p value	ES
Body Mass (kg)	77.6 ± 16	77.4 ± 15	−0.25 ± 1.6 [−0.98,0.49]	80.6 ± 16	80.6 ± 16	0.08 ± 1.8 [−0.78,0.90]	0.38	−0.285
Waist Circumference (cm)	85 ± 10.9	83.2 ± 10.5	−1.8 ± 1.8 [−2.65,-0.94]**	88 ± 13.7	87.3 ± 12.8	−1.16 ± 2.5 [−1.68,0.27]	0.10	−0.546
Hip Circumference (cm)	101.2 ± 6.7	100.3 ± 6.9	−0.81 ± 2.4 [−1.94,0.31]	105 ± 8.9	104.1 ± 9.2	−0.61 ± 2.9 [−2.19,0.37]	0.70	−0.130
Waist-to-hip ratio	0.84 ± 0.08	0.83 ± 0.08	−0.01 ± 0.02 [−0.02, 0.01]**	0.84 ± 0.12	0.84 ± 0.11	−0.005 ± 0.03 [−0.01,0.01]	0.38	−0.278
Fat Mass (%)	31.5 ± 9.3	30.1 ± 9	−1.41 ± 1.6 [−2.09,-0.74]**	34.5 ± 9.2	33.5 ± 9	−1.01 ± 1.5 [−1.78,-0.25]*	0.20	−0.419
Fat Mass (kg)	24.4 ± 8.2	23.2 ± 7.7	−1.15 ± 1.5 [−1.82,-0.47]**	28.4 ± 11.2	27.5 ± 11	−0.88 ± 1.6 [−1.66,-0.12]*	0.30	−0.343
Fat-Free Mass (%)	68.5 ± 9.3	69.9 ± 9	1.41 ± 1.6 [0.74,2.09]**	65.5 ± 9.2	66.5 ± 9	1.01 ± 1.5 [0.25,1.78]*	0.20	0.419
Fat-Free Mass (kg)	53.2 ± 13.5	54.1 ± 13.2	0.91 ± 1.1 [0.48,1.34]**	53.5 ± 9.6	54.2 ± 9.5	0.76 ± 0.9 [0.25,1.23]**	0.67	0.113
Vastus Lateralis Muscle Thickness (cm)	2.64 ± 0.5	2.8 ± 0.5	0.16 ± 0.2 [0.05,0.27]**	2.65 ± 0.3	2.78 ± 0.4	0.13 ± 0.6 [0.04,0.21]*	0.75	0.099
Elbow Flexors Muscle Thickness (cm)	3.6 ± 0.8	4.1 ± 0.8	0.51 ± 0.3 [0.39,0.63]**	3.6 ± 0.7	4.1 ± 0.7	0.42 ± 0.2 [0.2,0.56]**	0.58	0.176
CMJ (cm)	25.5 ± 6	26.8 ± 6	1.33 ± 1.7 [0.70,1.95]**	24.3 ± 5	25.3 ± 5	0.98 ± 1.8 [0.14,1.29]*	0.29	0.342
Seated Chest Medicine Ball Throw (m)	3.49 ± 0.6	3.67 ± 0.6	0.18 ± 0.16 [0.11,0.26]**	3.61 ± 0.4	3.71 ± 0.4	0.1 ± 0.2 [0.02,0.19]*	0.21	0.401
Maximal Isometric Mid-Thigh Pull (kg)	128.2 ± 49	137.8 ± 46	55 ± 24 [−14.83,124.83]	126 ± 33	129.9 ± 30	2.47 ± 12 [−76.97,81.91]	0.33	0.313
30-s Sit-to-Stand Repetitions (n)	24.3 ± 5	27.3 ± 5	3 ± 2.1 [1.8,4.2]**	24.7 ± 6	27.8 ± 5	3.1 ± 2.3 [1.8,4.4]**	0.79	0.084
30-s Bench Press Repetitions (n)	23.2 ± 5.1	27.8 ± 5.1	4.6 ± 2.1 [3.61,5.58]**	25.7 ± 4.2	29.8 ± 3.5	4.1 ± 2.4 [3.05,5.16]**	0.98	0.008
Total Volume Lifted (kg)	9222 ± 2733	13303 ± 3784	4082 ± 1928 [3273,4890]**	9677 ± 2280	13496 ± 3849	3669 ± 2079 [2749,4589]**	0.42	0.173

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

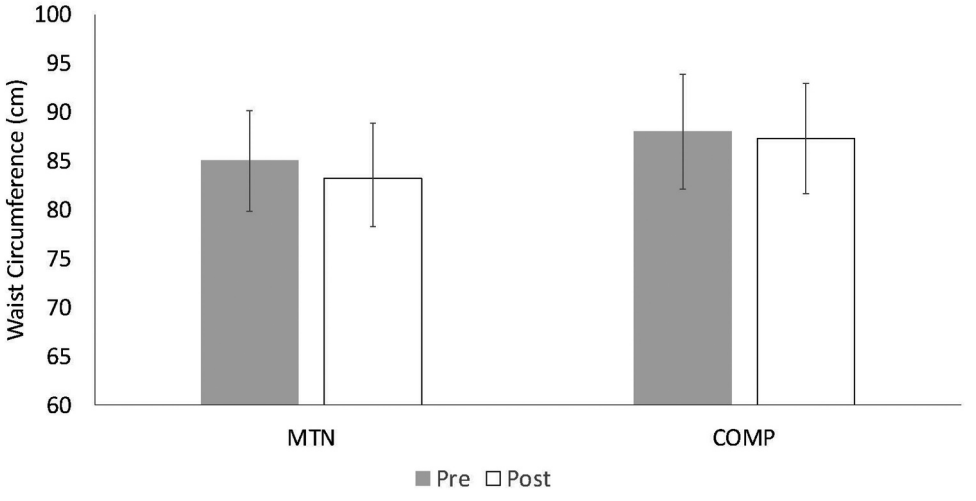

Figure 3. Estimated marginal means and 95% confidence intervals of adjusted changes in waist circumference. Analysis of covariance (ANCOVA) model was used to compare differences in raw change between groups, using the pre-assessment values as covariates. $^{**}p < 0.01$; from the baseline values. PREW=Preworkout, COMP= comparator (only carbohydrate) treatment.

for pre-intervention values for FM in kg ($p = 0.302$, d = –0.343) and percentage ($p = 0.201$, d = –0.42) values.

While no significant absolute FFM change was evident when adjusted from baseline values ($p = 0.48$), a significant increase from pre- to post-intervention in VL thickness was observed (F (1,39) = 6.52, $p = 0.015$, η2 = 0.132). Remarkably, both supplement protocols yielded similar hypertrophy outcomes ($p = 0.754$). Similarly, a significant difference of larger magnitude was observed from pre- to post-values for EF muscle thickness (F (1,39) = 21.25, $p < 0.001$, η2 = 0.288). However, no significant differences were noted between conditions ($p = 0.578$).

Both the PREW and COMP groups demonstrated significant absolute and adjusted increases in CMJ (F (1,39) = 6.48, $p < 0.015$, η2 = 0.128). However, no significant difference emerged between conditions when values were adjusted for baseline results ($p = 0.287$), despite the PREW group showing larger CMJ increases (1.55 vs 0.99 cm). Similarly, CMBT exhibited a trend toward a significant increase from pre- to post-values (F (1,39) = 3.07, $p < 0.088$, η2 = 0.066). Nonetheless, no significant difference between conditions was observed ($p = 0.212$, d = 0.4), although PREW yielded a larger increase than COMP (18.9 vs. 12.0 cm, respectively).

Both the 30-second sit-to-stand and 30-second bench press tests significantly improved under both conditions from pre- to post-intervention results (F (1,39) = 8.92, $p = 0.005$, η2 = 0.194, and F (1,39) = 6.33, $p = 0.016$, η2 = 0.143, respectively). However, no significant difference was observed between groups when the average mean difference adjusted by pre-values was considered.

The total volume (kg) lifted during workouts increased significantly under both conditions ($p < 0.001$) with no differences ($p > 0.05$) between groups.

4.1. Exploratory variables

The average S-RPE scores did not reveal any statistically significant disparities between groups (PREW: 7.79 ± 0.50 vs. COMP: 7.94 ± 0.60). Additionally, no effect of sex (all $p > 0.05$) was observed for any of the analyzed variables.

5. Discussion

Results of the present study suggest that ingesting a pre-workout caffeinated vegan protein-based multi-ingredient providing 12 g of EAA, and 406 mg of caffeine promoted similar body composition and performance outcomes to an isoenergetic carbohydrate-only supplement in middle-aged physically active female and male adults. The waist circumference was the only variable showing a significant reduction in the PREW group (Figure 3). Based on these findings, and within the limitations of our study procedures, except for the observed reduction of waist circumference, we must reject our hypothesis asserting that compared to a carbohydrate-only isocaloric comparator, a caffeinated protein-based PREW might further stimulate fat loss and optimize more favorable body composition and performance outcomes.

No significant energy or macronutrient consumption changes were observed from pre- to post-intervention for any group (Table 3). Both groups ingested an acceptable amount and distribution range of macronutrients across the study [60,61]. Therefore, there were no limitations due to insufficient energy, protein, carbohydrate and fat for any of the groups.

The multi-ingredient admixture used in our study included 406 mg of anhydrous caffeine (4.9 ± 2 mg·kg^{-1}) and 300 mg of yerba mate extract with 2% of caffeine (~6 mg of caffeine) (~0.01 mg·kg^{-1}). Thus, the resulting mean relative dose of caffeine per intake was ~4.9 mg·kg^{-1}, which was within the range of recommended moderate doses (3 to 6 mg·kg^{-1}) related to ergogenic effects for resistance training [62]. Nonetheless, it is worth noticing that the non-interaction effects between the PREW and COMP at post-intervention agree with previous investigations reporting no performance benefits from the pre-workout ingestion of 3 mg·kg^{-1} of caffeine in habitual caffeine consumers [63].

Previous acute studies conducted in middle-aged adults reported beneficial effects of caffeinated PREW containing protein and fortified with amino acids to maximize resistance training outcomes [15]. Similar to the current study, the participants ingested a PREW providing ~5.2 mg·kg^{-1} of caffeine, 0.21 g·kg^{-1} of carbohydrates with a high proportion of isomaltulose (a slow-release disaccharide), and 0.12 g·kg^{-1} of protein along with citrulline-malate, L-leucine, L-tyrosine, L-taurine, and betaine. The proposed attenuation of effects due to habitual caffeine intake could have been the cause of discrepancies between the current 6-week intervention study and acute trial designs. Even though regular caffeine consumption does not diminish acute performance benefits on muscular function [64], the scientific consensus on its ergogenic effects remains divided, particularly concerning long-term adaptations, and individual and genetic variability in caffeine metabolism [65]. In our study, both groups significantly improved CMJ, CMBT, and both lower and upper body muscle endurance tests with no significant difference between them (Table 3). These results are supported by the meta-analysis by

[66], suggesting that the long-term ergogenic effects of caffeine supplementation, particularly for upper body (e.g. medicine ball throw), may not significantly differ from placebo.

The variability in responses to caffeine supplementation across different studies highlights the complexity of its ergogenic properties. While acute caffeine intake has been shown to enhance performance in specific contexts, the translation of these benefits to long-term training adaptations remains uncertain [67]. Our research supports the notion that, over a 6-week RT regimen, PREW supplementation including caffeine does not confer additional advantages to enhance muscle function and promote body composition outcomes beyond those achieved through regular training alone. Indeed, for middle-aged recreationally trained adults, performing a regular resistance exercise programme seems to be the most important aspect impacting the observed training adaptations [68]. Along those lines, unlike previous studies reporting a significant effect of pre-workout supplementation to reduce the perceptual response to endurance [13], and strength [69] exercise bouts, we did not identify differences in the global perceptual response between groups, even during the initial workout sessions.

Our study is not without limitations. Firstly, although diet composition was registered with a self-reported food diary and analyzed with a validated application (MyFitnessPal Inc.©), 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 observed results. Secondly, most of the participants had a daily caffeine intake of 3–5 coffees or teas (100–300 mg of caffeine), which could have impacted the observed results. However, 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 hours pre- and 2 hours post-workout during training days) so that the impact of integrating PREW supplement to the habitual nutritional habits could be assessed. Although there are concerns regarding the variability of individual responses to different protocols of caffeine ingestion, including form of administration (e.g. coffee, capsules, etc.) and timing [70], it is worth highlighting that the participants in our study ingested the PREW supplement 15 minutes before performing a ~ 65-minute workout (including the 12-minute warm-up). Caffeine is rapidly absorbed by the body when consumed in coffee, powders, or capsules, appearing in the blood within 5–15 minutes and peaking between 40 and 80 minutes post-ingestion [71]. Considering this, the window for observing caffeine's ergogenic effects in the context of the current study covered the beginning of the workout and likely peaked during the latter half of the session, when physical fatigue was more pronounced, and performance began to decline. Thirdly, considering that the most prominent resistance training adaptations and nutritional support benefits are likely to occur in the most heavily utilized muscle groups [72], the low training volume (only one exercise) directly impacting the elbow flexors may have limited the observed adaptations on this muscular group. Lastly, we aimed to include peri- and post-menopausal women but no additional blood tests were performed aside from the symptoms questionnaire [73], which might potentially influence the results between early peri-menopausal and post-menopausal women. Future longer investigations using larger sample sizes are needed to comprehensively understand the long-term effects of combining pre-workout supplementation with resistance training in middle-aged female and male individuals.

6. Conclusion

Despite observing a significant reduction in waist circumference for the PREW treatment group, compared to the ingestion of only carbohydrates, a pre-workout vegan protein-based caffeinated admixture did not maximize body composition and performance outcomes in middle-aged individuals engaged in a resistance training programme over 6 weeks.

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ORCID

Justin Roberts http://orcid.org/0000-0002-3169-2041
Fernando Naclerio http://orcid.org/0000-0001-7405-4894

Author contributions

F.N. and J.P.-F. contributed to the design of the work, literature search, acquisition, analysis, and interpretation of the data and drafting the manuscript. E.L.-Z. contributed to the conception and design of the work, JR revised the methodology, contributed to the conception and design of the work. All authors revised the manuscript critically for important intellectual content and gave final approval of the final version. All authors have read and agreed to the published version of the manuscript

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