EFFECTS OF A MULTI-INGREDIENT BEVERAGE ON RECOVERY OF CONTRACTILE PROPERTIES, PERFORMANCE, AND MUSCLE SORENESS AFTER HARD RESISTANCE TRAINING SESSIONS

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ABSTRACT

Carbohydrate-protein–based supplements have been proposed for maximizing post-exercise recovery**.** This study compared the effects of post-workout supplementation ingesting a multi-ingredient (MTN) vs. carbohydrate alone (CHO) on the recovery of muscle function and perceived muscle soreness (DOMS) after hard resistance workouts. In a double-blinded, crossover design, 10 resistance trained males (26.9±7.4 years) performed two identical 5-day intervention periods while ingesting either MTN or CHO. The participants performed one workout per day during the first three days. thereafter, they were assessed 1-h, 24-h and 48-h after the completion of the third workout-session. Primary outcome was tensiomyography [muscle displacement (Dm), contraction time (Tc), and contraction velocity (Vc)] of the vastus medialis (VM) and biceps femoris long head (BFLH). Secondary outcomes were performance and DOMS. At 24-h, both conditions decreased ($p<0.05$) Dm (MTN -1.71±1.8, CHO -1.58±1.46 mm) and Vc (MTN -0.03±0.03, CHO 0.03±0.04 $m{\cdot}s^{-1}$) in VM. At 48-h all tensiomyography variables were recovered under the MTN while remained depressed ($p<0.01$) in CHO (VM, Dm 1.61±1.60, Vc -0.04±0.04 $m{\cdot}s^{-1}$; BFLH, Dm 1.54±1.52, Vc -0.02±0.02 $m{\cdot}s^{-1}$). Vertical jump performance decreased in CHO, but not in MTN. Although both conditions decreased upper body strength and power at 1-h, values returned to baseline in 24-h for MTM while needed 48-h in CHO. DOMS similarly increased at both 24-h and 48-h in both conditions. Compared to the ingestion of only carbohydrates, post-workout multi-ingredient supplementation seems to hasten recovery of muscular contractile properties and performance without attenuating DOMS after hard resistance workouts.

Keywords: Supplement; protein-carbohydrate admixture; tensiomyography; recovery; strength; DOMS.

INTRODUCTION

Different nutritional strategies involving the use of multi-ingredient preparations providing micronutrients (e.g. minerals, vitamins, probiotics) and macronutrients (e.g. carbohydrate, protein, or fats) have been investigated for their potential recovery-enhancing effect following hard workout sessions (7,21). Indeed, recent researches have confirmed the positive effect of post-workout admixtures providing high-quality, rapidly digestible protein mainly from animal sources (36) such as whey (10) or beef (37) to maximally stimulate muscle protein synthesis and improving recovery between training sessions in athletes (2). Different studies have investigated the effects of post-workout supplementation on recovery using standard assessments of maximal strength, mechanical power, muscular endurance (6,26) and the time course of muscle soreness measured over 48-h (30), 72-h (3) until 120-h (31) after a strenuous exercise bout. Ratamess et al. reported improvements in strength and upper body power after 4 weeks of combining amino-acid supplementation with high volume resistance training compared with placebo (26). Also, Hoffman et al. reported higher enhancement performance effect determined 24-h and 48-h after training in athletes ingesting a pre and post workout blend protein including 2 g of carbohydrates vs. the ingestion of maltodextrin (6). On the other hand, using a cross over study, Rindom et al. reported no differences in ingesting protein from whey or collagen to accelerate the regaining of exercise performance or attenuate muscle soreness over 48-h after performing 4 high-intensity resistance training sessions (30).

To the best of the authors' knowledge, no investigation has analyzed the impact of post-exercise nutrition on the muscle function recovery time course integrating measurements of involuntary contractile properties, via tensiomyography (TMG), and performance by the assessment of strength and power exercises. In this context, TMG is a sensitive non-invasive method for estimating in vivo, contractile and mechanical

properties of individual muscles through the simple measurement of the muscle belly radial deformation in response to an electrical stimulus (14). Because no physical effort is required by participants being evaluated (29), TMG has been used to objectively estimate the fatigued muscle responses to different active or passive recovery strategies (29). TMG uses evoked muscular activity to estimate muscle function independently on voluntary drives, motivation, or the influence of technique when performing specific sports-exercise involving multiple muscle groups (14). Nonetheless, in order to estimate the time course of recovery in athletic population within an applicable contextual scenario, the TMG parameters should be integrated with other measurements of in vivo human performance (maximal strength, mechanical power, etc.) and muscular disruption (14). Consequently, well-controlled investigations examining the effect of post-workout supplementation on recovery from hard training sessions, analyzing changes in the contractile properties of the fatigued muscles, exercise performance and indicators of muscle disruption are warranted. The aim of the present study was, therefore, to investigate the effects of a multi-ingredient admixture containing carbohydrate and high-quality protein (Recovery Crown® Sport Nutrition, Spain) on the recovery of muscular function following hard resistance training sessions. The primary outcome was the estimation of changes in muscular contractile properties measured by TMG. Secondary outcomes included changes in performance as well as on the perception of muscular soreness due to its impact in limiting further exercise performance following hard workout sessions. We hypothesized that supplementing with admixtures combining high-quality protein and carbohydrate will accelerate the recovery of muscular contractile properties, hasten performance regain and attenuate delayed onset of muscle soreness (DOMS), compared with the ingestion of carbohydrate alone.

METHODS

Experimental Approach to the Problem

The investigation was conducted as a double blinded, randomized, crossover within-participant comparison design with two 1-week intervention periods separated by 2-weeks for a wash out, recovery period. Following inclusion, familiarization, baseline assessments, and a 5-day recovery period, the participants were randomly allocated to receive either a multi-ingredient (MTN) or maltodextrin (CHO). Thereafter, the participants underwent the first 5-day training and testing intervention followed by a 2-week washout period, and then switched to the other nutritional treatment for continuation with the second 5-day identical second training and testing intervention (Figure 1).

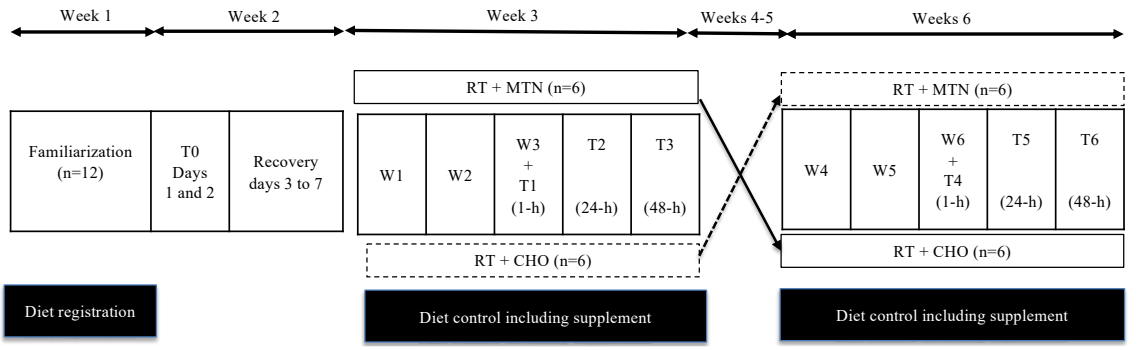

Figure 1. Schematic overview of the study design. The overall study period consisted of six consecutive weeks: (i) Week 1: Familiarization (ii) Week 2: Pre-tests (T0) involving day 1 (for body mass, height and tensiomyography assessments); day 2 (for baseline muscle soreness, vertical jump, upper body strength and power assessments) and 5 days for recovery (iii) Week 3: First intervention period composed of 3 workouts (W1, W2 and W3) and 3 assessment sessions performed at 1-h (T1), 24-h (T2) and 48-h (T3) after the completion of the last (3rd) workout (iv) Week 4: First week of recovery/washout (v) Week 5: Second week of recovery (washout period) (vi) Week 6: Second intervention period composed of 3 workouts (W4, W5 and W6) and 3 assessment sessions performed at 1-h (T4), 24-h (T5) and 48-h (T6) after the completion of the last (6th) workout. **MTN**: multi-ingredient condition, **CHO**: carbohydrate condition.

Subjects

Twelve resistance-trained college male participants (mean ± standard deviation; age: 26.9 ± 7.4 years; body mass: 74.45 ± 8.36 kg; height: 178.5 ± 4.6 cm, body mass index 23.4 ± 2.5 kg/m^2) with a minimum of 1-year experience performing high-intensity resistance exercise 2–3 times per week prior to inclusion volunteered to take part in the present

study. All participants received detailed written and oral information regarding the purpose and the possible risks of procedures and gave their written consent to participate. All experimental procedures were conducted in accordance with the Declaration of Helsinki and approved by the University Research Ethics Committee. Exclusion criteria comprised: (i) participating in competition lifting sports, e.g., weightlifting, powerlifting, and bodybuilding or > 3 resistance exercise training sessions per week within 6 months prior to inclusion in the study (to avoid elite or sub elite strength athletes); (ii) a history of musculoskeletal pain or injuries; neurological or metabolic disorders and (iii) use of dietary supplements or prescription medicine that would potentially affect muscle recovery or function (i.e., protein supplements, antioxidant supplements, NSAIDs, and angiotensin converting enzyme inhibitors).

Sample size estimations were calculated based on the main primary outcome measures (TMG markers, Dm and Vc) determined in the of the VM. This muscle was chosen due to its high activation during squatting movements (4), which was the prevalent muscular action for the conducted resistance exercise routine (see supplementary material). Within-subject correlation for MTN condition in Dm for VM was r = 0.39, and the effect size of the pre to 24-h differences were d =1.45. Assuming an α-error probability of 0.05, it was determined that our final sample size (n=10) achieved 95% statistical power.

Procedures

Familiarization period: After being considered eligible for the study, the participants completed three sessions of familiarization (week 1) aimed to explain the training protocol, exercise techniques, and the assessment procedures.

Assessments: After completing the familiarization, the participants underwent the following assessment schedule:

(i) Two-day pre-test. On day 1 (T0, first test session) the participants reported to the laboratory in postprandial state (i.e., approximately 2 h since last meal) and were assessed for body mass and height. Thereafter, a tensiomyography assessment on vastus medialis (VM) and biceps femoris long head (BFLH) was conducted. On day 2 (T0, second test session), the participants were assessed for the perceived muscle soreness (baseline) followed by a standardized 10 min warm up before performing the vertical jump, upper body strength and power assessments.

(ii) After completing the pre-test and following a 5-day recovery period (week 2, Figure 1), the participants underwent the first 5-day intervention period, including the intake of either MTN or CHO. The participants performed 3 hard consecutive workouts (one workout per day) during the first three days (workout 1, workout 2 and workout 3). Thereafter, the participants were assessed at 1-h (test 1), 24-h (test 2) and 48-h (test 3) after the completion of the third workout-session (week 3, Figure 1).

(iii) Once the first 1-week of training and testing was completed, the participants had a 2-week recovery phase (weeks 4 and 5, Figure 1). During this period the volunteers were instructed to maintain their habitual activity levels and dietary habits.

(iv) After the 2-week wash out phase, the participants completed a second 5-day intervention phase including 3 more consecutive workouts (one workout per day) performed during the first 3 days of the week (workout 4, workout 5 and workout 6) and the three assessments sessions conducted at 1-h (test 4), 24-h (test 5) and 48-h (test 6) after completing the last (6^{th}) workout (week 6, Figure 1). In this second intervention period the participants ingested the opposite supplement compared to the one administered in the first training and testing phase.

All performance tests (vertical jump, upper body strength and power) were repeated at 1-h (test 1 and 4), 24-h (test 2 and 5), and 48-h (test 3 and 6) after the final (third or sixth) exercise bout in each intervention period (weeks 3 and 6), while lower limb muscle soreness and tensiomyography from VM and the BFLH were measured at 24-h and 48-h after completing each of the 3-day training period and before starting the performance tests (Figure 1). All participants were instructed to refrain from strenuous physical activity for 48-h before the first baseline tests and before the first 5-day training and testing intervention.

Tensiomyography Measuring Protocol: A TMG portable device (TMG Measurement System, 146 TMG-BMC Ltd., Ljubljana, Slovenia with a maximal stimulation output of 110 $mA \cdot ms^{-1}$) was used to measure the contractile properties of the VM and BFLH at the dominant limb (12,28). These muscles were chosen due to their meaningful activation during 6 of the 8 closed kinetic chains exercises included in the training protocol (explained below) (4,5). Additionally, previous studies using tensiomyographic measurements have demonstrated the validity and reliability of this method for assessing the contractile properties in both vastus medialis and biceps femoris (32).

All measurements were collected by the same trained researcher, and obtained at rest, in supine position for the VM, and in prone position for BFLH. Both positions held a knee joint angle of 40° relative to the anatomical position of 0° (knee joint fully extended). For the supine position, a supporting pad was used to maintain the knee in a comfortable position, and for the prone position a pad was placed under the lower shin and the feet dangled over the edge of the bed (see supplementary material, Figures S1 and S2). All the measurement procedures were accomplished according to methodology described by Rey et al. (2012b) and none of the participants reported discomfort during

electrical stimulation. Both electrodes were placed symmetric to the sensor and 55 mm apart, with the positive electrode in proximal and the negative electrode in distal position to the proximal muscle insertion. The sensor (digital displacement transducer) was set perpendicular to the muscle belly. The anatomical location of the electrodes and sensor were marked and kept constant during the complete experimental period. The assessed variables were: maximal radial displacement of the muscle belly (Dm), contraction time between 10 and 90% Dm (Tc), and mean velocity (Vc) that was calculated by dividing the Dm by the sum of the Tc and the delayed time (Td) (12). These 3 variables have demonstrated high levels of accuracy, reliability and sensitivity to reflect changes in the neuromuscular function by tensiomyography analysis (15,24,28). Furthermore, as it is not uncommon for Tc and Dm to alter disproportionately to one another, changes in Tc independent from Dm can be driven by an alteration in the rate of contraction, as measured by Vc (14).

Muscle contractile properties were analyzed during a twitch contraction evoked by individual maximal electrical stimulation over the muscle belly of 1 millisecond duration. Peak muscle twitch was identified by a plateau in displacement curves that, despite an increased stimulation amplitude, did not result in greater muscle displacement (14). Maximal electrical stimulation and maximal muscle belly displacement were found by progressively increasing the electric current by ≥ 10 to 20 mA for each stimulation, starting at 30 mA. A resting time of 10-second between consecutive measurements was prescribed to minimize the effects of fatigue and potentiation (24). The maximal response was achieved around 100 mA in all the cases. For the aim of the present investigation, a decrease of Dm, a longer Tc, and loss of Vc was associated with a delayed recovery of the muscle contractile properties (14).

The intraclass correlation coefficient (ICC) scores (95%) for TMG variables using

for the present study ranged from 0.89 to 0.92, similar to those reported in previous investigations (29).

Performance measures

Vertical Jump: Countermovement Jump was performed on a Kistler force platform (9287B, 3 component force platform; Kistler, Hook, United Kingdom; dimensions: 900 x 600 x 100 mm) with a sampling rate of 2000 Hz where vertical forces were recorded. From standing erect position, participants descended to a self-selected depth and immediately jumped upward as high as possible. To exclude the influence of arm swing, subjects were instructed to keep their hands-on hips. Participants performed 3 consecutive jumps. Jump height was calculated 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 takeoff. Based on the height, the best of the 3 jumps was chosen for the analysis (19).

Upper Body Strength and Power: The 1RM value for the bench press exercise (BP) using free weights was determined according to the methodology described by McGuigan (2016). Additionally, the maximal upper body power was measured for the BP exercise using 50% of the previously determined 1RM value. Participants were required to perform 5 repetitions with a maximal possible velocity and using a correct technique. Mechanical power was estimated from the repetition that produced the maximal average power (calculated during the concentric phase of the BP exercise). A recently validated (11) portable single optoelectronic infrared camera system with a fixed sampling frequency of 500 Hz was used to track a retroreflective strip placed at the center of the bar during the five BP repetitions. The device was connected to a computer through a USB interface and the proprietary software Velowin 1.6.314 (Deportec, Spain). All data were filtered using a low pass 10 Hz cut-off filter prior to calculating the displacement and

velocity and consequently estimating the average force (Newtons) and the mechanical power (watts) achieved during the BP performed with 50% of the previously determined 1RM. The test-retest reliability coefficients (ICCs), coefficient of variation (CV) and standard error of measurement (SEM) for the 1RM BP and BP power at 50% were, respectively: ICC 0.95, CV 2.1%, SEM 3.12; and ICC 0.90, CV 2.5%, SEM 23.08.

Delayed Onset Muscle Soreness (DOMS): Muscle soreness in anterior and posterior thigh (lower limb) was evaluated at pre-intervention, immediately after the familiarization period during the pre-test sessions (T0), and at both post 24 and post 48 h, of completing workout 3, before commencing test 2 and test 3, respectively. Participants were asked to perform the standardized warm-up movements during T0 (slow squat movement without external overload walk and slight jogging). The participants then evaluated lower extremity muscle soreness on a visual analogue scale (VAS) of 100 mm going from no pain at all (0 mm) to worst possible pain (100 mm) as described elsewhere (1). In order to maintain consistency, the same researcher conducted the assessment of DOMS.

Exercise Protocol: During each workout session, the participants performed a supervised full-body resistance-training protocol. Training sessions were carried out late in the afternoon or early evening. After a warm-up, the participants performed a total of 3 circuits involving 1 set of the following exercises: (i) alternate box set ups (ii) hang clean; (iii) bench press; (iv) parallel squat using free weights; (v) upright row; (vi) alternate lunges; (vii) deadlift; (viii) squat on an isoinertial fly-wheel concentric-eccentric machine. As the workout was aimed to create a high level of mechanical and metabolic stress, a muscle endurance oriented workout (>15 repetitions per set) was designed (27). Accordingly, every set involved 16 self-determined maximum repetitions (33) (> 40 to < 60% 1RM) (27) using the heaviest possible load. Experienced strength and conditioning

coaches monitored all training sessions to ensure participants' compliance with the training protocol. 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 minimum rest period of 15 sec was introduced until the participants were able to perform 16 repetitions per set. A ~30 sec rest period was permitted between exercises. Recovery between circuits was 3 minutes. The time to complete each singular circuit was 8.5 ± 0.75 min. All the participants completed all lifts for each exercise. The average time to complete the workouts was 45 min, including the warm-up. All workout sessions (W1–W3 and W4-W6) were identical (i.e., identical total number of repetitions per exercise and inter-set recovery periods).

Control of Dietary Habits and Supplementation Protocol: A research nutritionist collected dietary habits and explained the proper procedures for recording dietary intake. Each participant completed a 3-day food diary report (two weekdays, and one weekend day). The food diary report was then analyzed using Dietplan 6 (Forestfield Software, UK) to determine energy and macronutrient content. Participants were instructed to maintain their habitual reported diet throughout the study, including the washout period. Additionally, 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, post-workout food intake after supplementation and dinner. If any change in diet patterns were reported or identified, the participants were dropped from the study.

During weeks 3 and 6, all the participants consumed either one single 59 g dose of a commercially available post-workout supplement (Recovery, from Crown Sport Nutrition, Spain) providing 222 kcal including 37 g of carbohydrates, 8.6 g of whey isolate protein, 7.4 g of beef hydrolysate protein, 0.8 g of fat and 2 g of glutamine, or an

isoenergetic, non-protein, 59 g dose of maltodextrin (contrast condition, CHO). Supplements were mixed with 250 ml of water within 10 min after completing every workout or testing session. No supplementation was consumed on non-exercising days (weekend and weeks 4 and 5). The complete description of supplements' nutritional values including the amino-acid constituents of the multi-ingredient is shown in Table 1.

Table 1. Nutritional composition of supplements per intake (59 g of powder plus 250 ml of plain water)

Nutrient	**MTN**	**CHO**
Energy value (kcal)	222	222
Carbohydrates (g)	38	55
Fat (g)	0.78	0
Proteins and added amino-acids (g)	18	
Alanine (g)	0.95	-
Arginine (g)	0.34	-
Aspartic acid (g)	1.92	-
Cysteine + Cistina (g)	0.43	-
Glutamic acid (g)	5.28	-
Glycine (g)	0.27	-
Histidine (g)	0.28	-
Isoleucine (g)	1.31	-
Leucine (g)	1.79	-
Lysine (g)	1.76	-
Methionine (g)	0.39	-
L-Ornithine	0.02	
Phenylalanine (g)	0.51	-
Proline (g)	1.09	-
Serine (g)	0.90	-
L-Taurine	0.02	
Threonine (g)	1.28	-
Tryptophan (g)	0.29	-
Tyrosine (g)	0.48	-
Valine (g)	1.20	-
Total EAA (g)	**8.81**	**-**
Heme Iron (mg)	1.26	**-**
Zinc (mg)	1.47	**-**
Potassium (mg)	134.52	**-**
Magnesium (mg)	19.50	**-**
Selenium (µg)	1.80	**-**
Calcium (mg)	37.49	**-**
Folic Acid (µg)	6.27	**-**
Niacin (mg)	8.15	-
Vitamin B 6 (mg)	0.03	**-**
Vitamin B 12 (µg)	0.24	**-**

Notes: EAA: essential amino acids; MTN supplement admixture including carbohydrates, proteins from beef and whey and glutamine, CHO: supplement providing only maltodextrin.

Statistical Analysis

A descriptive analysis was performed and subsequently the Kolmogorov-Smirnov and Shapiro-Francia tests were applied to assess normality. Raw changes in all outcome variables were calculated by subtracting pre from post assessment values, without adjusting for pre values, since the same subjects performed under both conditions acting as their own controls. In order to assess the magnitude of the differences from baseline, confidence intervals (CI) of the differences were calculated and plotted. Those CIs not crossing zero were considered statistically significant from the baseline performance. Additionally, two-tailed one sample student's tests were used to test for a null effect hypothesis. Before testing the main hypothesis, the possible treatment order effect was checked using a 2 (order: MTN-CHO vs. CHO-MTN) × 2 (conditions: MTN vs. CHO) Analysis of Variance (ANOVA). A 2 (conditions: MTN vs. CHO) × 3 (times: post 1-h, 24-h and 48-h) repeated measures ANOVA was used to compare differences between conditions and post workout measurements in raw change of vertical jump, upper body strength and power. As TMG and DOMS were assessed at 24-h and 48-h after completing the last training session, a 2 (conditions: MTN vs. CHO) by 2 (times: 24-h and 48-h) repeated measures ANOVA was used. Differences over time were compared using Bonferroni-adjusted pairwise comparisons when appropriate. Generalized eta squared (η_G^2) and Cohen´s *d* values were reported to provide an estimate of standardized effect size (small d=0.2, η_G^2=0.01; moderate d=0.5, η_G^2=0.06; and large d=0.8, η_G^2=0.14) Significance level was set at 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 20.0; SPSS, Inc., Chicago, IL, USA).

RESULTS

Due to reasons not related to the intervention, two participants dropped out of the study. Consequently, ten participants successfully completed all the workouts and testing sessions under both analyzed conditions.

No treatment order effect was observed for any of the analyzed variables (TMG markers, Dm, Tc and Vc) for both VM and BFLH, performance (vertical jump, 1RM BP and power) and DOMS measured at 24 or 48-h.

Diet Analysis: Table 2 shows the daily consumption of carbohydrate, protein, fat (grams) and energy (kcal) without including and including the two post-workout supplements.

Table 2. Descriptive analysis of the participant's diet composition, including and not including post - workout supplementation

Macronutrients	No supplementation (n=10)	With MTN (n=10)	With CHO (n=10)
Proteins			
$g \cdot d^{-1}$	123.5 ± 11.1	139.6 ± 11.1* $^{\delta}$	123.5 ± 11.1
$g \cdot kg^{-1} \cdot d^{-1}$	1.7 ± 0.2	1.9 ± 0.3* $^{\delta}$	1.7 ± 0.2
% of total energy	22.8 ± 3.8	23.3 ± 1.9 $^{\delta}$	20.6 ± 1.8
Carbohydrate			
$g \cdot d^{-1}$	261.4 ± 40.4	299.1 ± 40.4*	317.36 ± 40.4*
$g \cdot kg^{-1} \cdot d^{-1}$	3.5 ± 0.4	4.0 ± 0.4*	4.3 ± 0.4*
% of total energy	47.7 ± 5.6	49.7 ± 4.3	52.7 ± 4.2*
Fats			
$g \cdot d^{-1}$	70.8 ± 12.13	71.59 ± 12.1	70.8 ± 12.1
$g \cdot kg^{-1} \cdot d^{-1}$	0.97 ± 0.2	0.98 ± 0.2	0.97 ± 0.2
% of total energy	29.4 ± 5.0	27.0 ± 3.9*	26.7 ± 3.9
Energy			
Total daily energy	2236.4 ± 204.5	2464.4 ± 204.5*	2466.0 ± 204.5*
$Kcal \cdot kg^{-1} \cdot d^{-1}$	30.3 ± 3.4	33.4 ± 3.7*	33.4 ± 3.7*

Notes: values are presented as mean ± standard deviation.
*$p<0.01$ respect to diet without post workout supplementation.
$^{\delta}$ $p<0.01$ from diet with MTN supplementation compared to diet with CHO supplementation.

The ingestion of the multi-ingredient supplement (MTN) determined significant increases in total daily protein and carbohydrate intake. Meanwhile, the ingestion of 59 g of maltodextrin significantly increased the total daily carbohydrate compared to the recorded habitual diet.

Tensiomyography: The measured absolute values of the TMG (Dm, Tc and Vc) variables are presented as supplementary material in Table S1, Figures S3 (VN) and S4 (BFLH).

Table 3 describes the changes measured at 24-h and 48-h after performing the last workout session of the 3 TMG analyzed variables, including the 95% CI for two conditions.

At 24-h after the last workout, the skeletal muscle contractile properties showed very similar changes in the two (MTN and CHO) compared conditions. Both Dm and Vc significantly decreased in VM. No other significant changes were determined.

At 48-h after the last workout, the skeletal muscle contractile properties reached very similar

values compared to baseline in MTN while remained significantly depressed under CHO. Both Dm and Vc were significantly lower than baseline for both VM and BFLH.

Despite under the MTN condition, the participants seemed to speed up the recovery time to regain the skeletal muscle contractile properties, no significant differences between conditions were determined at 24-h and 48-h. Nonetheless, it is worth highlighting the moderate effect sizes observed at 48-h under the CHO condition to produce lower values of Dm and Vc in the VM along with a longer Tc in BFLH (Table 3).

Table 3. Mean (M) ± standard deviation (SD) and 95% CI of the differences measured at 24 and 48 h for the tensiomyography variables in the two assessed conditions

Conditions	**MTN (n=10)**		**CHO (n=10)**		**ANOVA Repeated Measures (2 times x 2 conditions)**	**Conditions comparisons**	
Variables	**Post 24-h**	**Post 48-h**	**Post 24-h**	**Post 48-h**		**24 h**	**48 h**
Vastus Medialis							
Dm (mm)	-1.71 ± 1.18** § [-1.55, -0.86]	-0.37 ± 0.96 [-1.06, 0.32]	-1.58 ± 1.46** [-2.63, -0.53]	-1.61 ± 1.60** [-2.75, -0.46]	Condition: F(1,9)=2.22; p=0.17; η_G^2 =0.08 Time: F(1,9)=7.36; p=0.02; η_G^2 =0.10 Time x Condition: F(1,9)=3.74; p=0.09;η_G^2=0.11	p=0.76 ES=0.10	p=0.067 ES=0.66
Tc (ms)	-0.32 ± 2.45 [-2.07, 1.43]	-0.19 ± 1.14 [-1.01, 0.63]	-1.06 ± 1.72 [-2.29, 0.17]	0.12 ± 1.90 [-1.24, 1.48]	Condition: F(1,9)=0.17; p=0.69; η_G^2 =0.01 Time: F(1,9)=1.99; p=0.19; η_G^2=0.05 Time x Condition: F(1,9)=1.15; p=0.31; η_G^2=0.04	p=0.41 ES=0.28	p=0.59 ES=0.18
Vc ($m \cdot s^{-1}$)	-0.03 ± 0.03** § [-0.05, -0.01]	-0.01 ± 0.02 [-0.02, 0.01]	-0.03 ± 0.04* [-0.05, 0.00]	-0.04 ± 0.04* [-0.06, -0.01]	Condition: F(1,9)=1.91; p=0.2; η_G^2=0.00 Time: F(1,9)=2.44; p=0.15; η_G^2=0.00 Time x Condition: F(1,9)=3.95; p=0.08;η_G^2=0.01	p=0.59 ES=0.18	p=0.074 ES=0.66
Biceps Femoris Long Head							
Dm (mm)	-1.20 ± 1.91^T [-2.57, 0.17]	-0.51 ± 2.87 [-2.57, 1.54]	-1.32 ± 2.32^T [-2.97, 0.34]	-1.54 ± 1.52** [-2.63, -0.46]	Condition: F(1,9)=0.72; p=0.42; η_G^2=0.02 Time: F(1,9)=0.26; p=0.62; η_G^2=0.00 Time x Condition: F(1,9)=1.61; p=0.24;η_G^2=0.01	p=0.88 ES=0.05	p=0.18 ES=0.45
Tc (ms)	-2.82 ± 8.45 [-8.87, 3.22]	-0.19 ± 5.12 [-6.00, 5.61]	-3.20 ± 7.94 [-8.88, 2.47]	-3.31 ± 7.45 [-8.67, 2.06]	Condition: F(1,9)=3.1; p=0.11; η_G^2=0.01 Time: F(1,9)=1.47; p=0.26; η_G^2=0.01 Time x Condition: F(1,9)=1.12; p=0.32; =0.01	p=0.88 ES=0.07	p=0.08 ES=0.62
Vc ($m \cdot s^{-1}$)	-0.01 ± 0.03 [-0.04, 0.01]	-0.01 ± 0.03 [-0.04, 0.02]	-0.02 ± 0.03 [-0.04, 0.01]	-0.02 ± 0.02* [-0.04, -0.00]	Condition: F(1,9)=0.62; p=0.45; η_G^2=0.03 Time: F(1,9)=0.02; p=0.89; η_G^2=0.01 Time x Condition: F(1,9)=0.56; p=0.48;=0.00	p=0.68 ES=0.09	p=0.28 ES=0.37

Notes: **p < 0.01, *p < 0.05 and ^{T}p <0.10 respect to baseline values; § p < 0.01, respect to 48 h values; ES is the standardized effect size presented as Cohen`s

Performance measures: The measured absolute values of the performance variables (vertical jump, upper body strength and power), are presented as supplementary material in Table S2 and Figures S5.

Vertical Jump Height (m): Significant performance reduction compared to baseline was observed for the CHO at 1-h (-0.06 ± 0.02 m, p=0.001), 24-h (-0.03 ± 0.02 m, p=0.001) and 48-h (-0.02 ± 0.025 m, p=0.043) after workout (Figure 2A).

Significant effects for ANOVA interaction (F[2,18]=6.35, p=0.01, η_G^2=0.14), time

(F[2,18]=6.72, p=0.01, η_G^2=0.14), and condition (F[1,9]=13.58, p=0.01, η_G^2=0.21) were observed when comparing the differences measured at 1-h, 24-h and 48-h after workout.

At the three after workout assessed time points, lower jump performance was observed only under the CHO condition (1-h, p=0.001, d=2.41; 24-h, p=0.011, d=1.01 and 48-h p=0.005, d=1.24) while no significant changes were determined for the MTN condition (Figure 2A). Furthermore, 1-h post workout, under CHO the participants jumped significantly lower (p=0.003, d=1.21) compared to the MTN condition. No further differences were observed between the three compared time points (1-h, 24-h and 48-h after workout) or conditions.

Upper Body Strength: At 1-h after workout, significant reduction in strength was observed for both CHO (-2.4 ± 3.4 kg, p=0.048, d=0.68) and MTN (-5.5 ± 3.5 kg, p=0.001, d=1.49, Figure 2B).

At 24-h, significant lower strength performance (-7.7 ± 4.0 kg, p=0.001, d=1.83) was identified only for the CHO condition (Figure 2B).

At 48-h, no significant differences compared to baseline were determined for the both conditions.

A significant interaction (time x condition) effect was determined between the differences calculated at 1-h, 24-h and 48-h after the last workout (F[2,18]=9.53, p=0.002, η_G^2=0.22). However, no main time (F[2,18]=2.01, p=0.16, η_G^2=0.04) nor condition (F[1,9]=4.18, p=0.07, η_G^2=0.02) effects were observed.

Under the CHO condition, a decreased level of strength was determined at 24 h with respect to the measured at both 1-h (p=0.021, d=0.89) and 48-h (CHO, p=0.045, d=0.74). On the other hand, under MTN, the lowest 1 RM values observed at 1-h were significantly lower than those observed at both 24-h (p=0.012, d=1.00) and 48-h h (MTN, p=0.021, d=0.88) post workout. Furthermore, under CHO the participants showed lower strength performance compared to the MTN condition (p=0.003, d=1.23) at 24-h after workout. No further differences were observed

between the three compared time points (1 h, 24 h and 48 h after the last workout) or conditions (Figure 2B).

Upper Body Power: Significant performance reduction compared to baseline was observed both under CHO (-28.4 ± 14.6 watts, p=0.001, d=1.84) and MTN (-19.9 ± 24.0 watts, p=0.025, d=0.78) 1-h after training. Additionally, CHO showed a significant lower performance at 48-h (-22.4 ± 21.8 watts, p=0.007, d=0.97) compared to baseline (Figure 2C).

A significant interaction (time x condition) effect was determined between the differences calculated at 1-h, 24-h and 48-h after the last workout (F[2,18]=4.44, p=0.03, η_G^2=0.02). Although a significant condition effect (F[1,9]=35.31, p=0.001, η_G^2=0.11) was determined, no main effect for time (F[2,18]=1.98, p=0.17, η_G^2=0.06) was found.

Under the MTN condition, the participants produced a similar level of mechanical power performance compared to baseline at 24-h and 48-h post workout. Conversely, under CHO the participants showed similarly low values of mechanical power over the three-time points. These values were significantly inferior to those measured during the MTN condition at 24-h (p=0.005, d=1.11) and 48-h (p=0.001, d=2.02) after exercise (Figure 2C). No further differences were observed between the three compared time points (1-h, 24-h and 48-h after the last workout) or conditions.

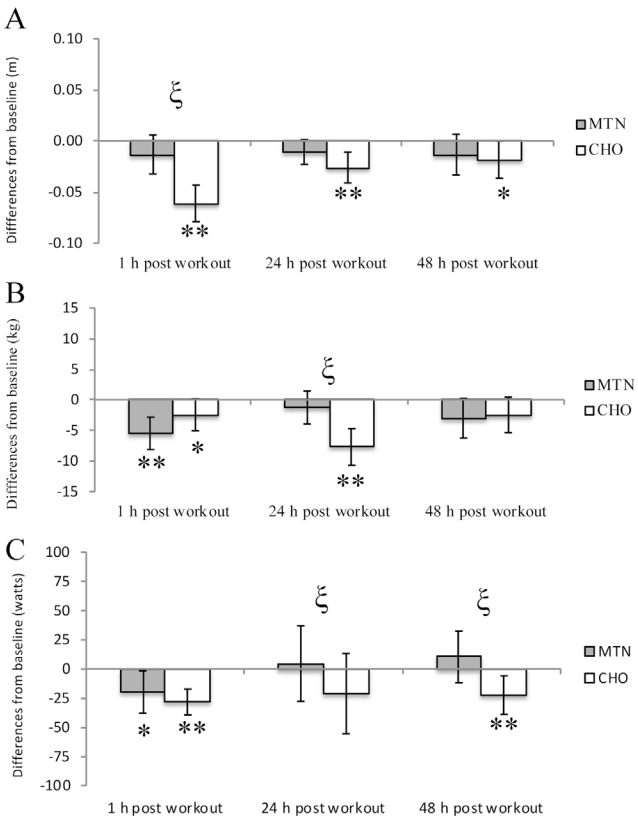

Figure 2. Estimated marginal means and 95% confidence intervals of differences in vertical jump (A) weight lifted in one maximum repetition in the bench press exercise (B) and mechanical power produced with 50% of the maximum lifting weigh in the bench press exercise (C).* $p < 0.05$; ** $p < 0.01$ from the baseline values $^{\xi}$ $p < 0.01$ between conditions. MTN: multi-ingredient condition, CHO: carbohydrate condition.

Delayed Onset of Muscle Soreness (DOMS): Main effects for time (F[1,9]=5.34, p=0.046, η_G^2=0.09) and condition (F[1,9]=38.38, p=0.001, η_G^2=0.27) were determined. However, no interaction time x condition effect was observed (F[1,9]=0.67, p=0.43, η_G^2=0.00).

Both conditions showed significant increases in the delayed muscle soreness at both 24-h (p<0.001) and 48-h (p<0.001) after completing the last training session (Figure 3).

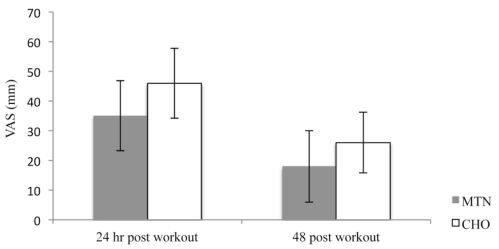

Figure 3. Estimated marginal means and 95% confidence intervals of the delayed muscle soreness (DOMS) measured at 24 and 48 h after the last training workout. MTN: multi-ingredient condition, CHO: carbohydrate condition, VAS: Visual Analogue Scale.

DISCUSSION

Results from the present study support the post-workout ingestion of multi-ingredient supplements providing carbohydrate, protein and a small amount of fat as a beneficial alternative to ingesting only carbohydrates to accelerate the recovery of the involuntary muscular contractile properties estimated by TMG after a series of consecutive hard resistance workouts. Furthermore, ingesting the multi-ingredient provided some beneficial effects on hastening the regain in strength and power performance. Based on these findings, and within the confines of the study procedures, we accept our research hypothesis asserting that post-workout supplementation with multi-ingredients may accelerate the restoration of muscle contractile properties and improve performance recovery times compared to the intake of only carbohydrates. However, as both conditions similarly increased DOMS measured at both 24-h and 48-h, we cannot accept the hypothesis supporting the superior benefit of multi-ingredients compared to carbohydrate in attenuating muscle soreness perception.

Our results reinforce previous similar studies indicating that supplementation with high quality protein (6) or amino acids (26) may optimize the recovery of exercise performance following resistance workouts.

An innovation of our investigation was the use of TMG to evaluate the effects of MTN vs. CHO on recovering contractile muscle properties after performing a series of hard resistance workouts. A reduced Dm is interpreted as an increase in muscle belly stiffness (29) while both Tc and Vc have been considered indicative of muscle fatigue rate (14). Overall, results from table 3 show a similar pattern of changes in the TMG variables under the two tested conditions at 24-h post workout. Even though no differences between conditions were determined at any of the three time points, when participants followed the MTN supplementation, Dm, and Vc recovered after 48-h but they did not recover under the CHO condition. Indeed, compared to the MTN, moderate effect size to produce lower Dm and Vc in VM or longer Tc in BFLH at 48-h were determined for the CHO

condition (Table 3). Although the disruption of the contractile properties seems to be more pronounced in VM than in BFLH, the pattern of response was similar for both analyzed muscles. These results indicate that over 48-h, ingesting a post workout multi-ingredient supplement optimizes the time to recover the contractile capacity of VM and BFLH. The impaired contractile properties observed in both conditions after 24-h could be associated with a reduced excitation-contraction coupling efficiency, impaired conducting properties of membrane potentials, and disrupted muscle cell structures (24,25). Whether these disruptive events may also be detrimental to strength and power performance is still unclear (25). In our study, the decrease of Dm and Vc observed in VM at 24-h in both MTN and CHO treatments corresponded with significant decreases of jump performance in the CHO but not in the MTN condition. Indeed, ingestion of the MTN allowed a full recovery of the muscular contractile properties, as measured by the TMG, after 48-h. a different scenario was observed under the CHO condition where almost all TMG variables remained depressed for both analyzed muscles (Table 3). Some studies showed improved responses when supplements combining carbohydrates with high-quality protein are ingested after workout (13,22). However, whether this post-exercise feeding strategy can ameliorate recovery, accelerating the restoration of muscular function from strength training, is still under debate (23). In our study, the MTN provided 38 g of carbohydrate, ranged from 0.44 to 0.61 $g \cdot kg^{-1}$, and 18 g of protein, ranged from 0.21 to 0.30 $g \cdot kg^{-1}$. Although the amount of carbohydrate was lower than the recommended 1 to 1.2 $g \cdot kg^{-1}$ for optimizing glycogen restoration during the post-training period (35), the addition of protein in a ratio of 2:1 for the CHO/protein relationship may have compensated the suboptimal administration of carbohydrates to still obtain an optimal glycogen restoration. Nonetheless, it is worth highlighting that the performed routine required maximum efforts to accomplish each exercise-set, it involved a moderate volume workout (20) including 3 sets per exercise (24 total sets) and consequently it is unlikely that this workout could have induced a meaningful depletion of muscle glycogen stores.

Regarding the post exercise muscle remodeling process, the amount of protein included in the MTN falls within the accepted doses (0.18 to 0.30 $g \cdot kg^{-1}$) to further stimulate muscle protein synthesis in young individuals (18). Although the amount of Leucine included in the MTN (~1.8 g) was lower than the proposed ~3 g dose to optimally drive protein synthesis after exercise in young males, in our participants, this amount could have still been effective in promoting muscle protein synthesis after workout. Indeed, Leucine modulates distinct steps of translation initiation and protein synthesis directly in skeletal muscle through different signalling pathways including the activation of the mechanistic target of rapamycin complex 1 (mTORC1) (38).

Although no analysis of nutrient availability (e.g. plasma amino acids concentration) was performed after ingesting the supplements, it is likely that in well-nourished individuals consuming an overall daily protein intake of ~1.9 g/kg/d, which is close to the upper limit of 2 $g \cdot kg \cdot d^{-1}$ recommended for optimizing exercise training-induced adaptations (8), combining carbohydrates and protein with other essential nutrients (e.g. iron, zinc, vitamin B12 or the essential fatty acid included in the beef protein) configures an attractive nutritional strategy for maximizing recovery from resistance training.

Our participants performed 3 consecutive hard resistance workouts using a circuit training routine involving 1 weightlifting, 5 lower body, and 2 upper body exercises. Additionally, the last exercise (squat on an isoinertial flywheel) was intentionally included to create a high level of quadriceps eccentric activation for inducing DOMS. Although the repeated bout effect adaption (23) to subsequent exercises involving high eccentric component might also have reduced the perceived DOMS in the second part of the study, the participants showed similar significant increases in DOMS under both conditions. However, it is worth noting that despite no ANOVA interaction effects between times and conditions were determined, a large effect size (d=0.78) favoring lower level of DOMS in MTN vs. CHO was identified (Figure 3). The decline in muscle contractile capacities and physical performance, along with the intensification of muscle soreness

are associated with the exercise-induced fatigue after tasks involving a high eccentric component. Additionally, the decrease in jump, upper body strength, and power followed a similar pattern of response to the TMG variables. For instance, when participants consumed the carbohydrate drink, they exhibited a larger reduction of the jump performance at all time points. Similarly, the values of BP strength and power measured at 24-h and 48-h respectively under CHO were lower than those determined under the MTN condition (Figure 2).

Our study is not without limitations. The diet was not controlled, but only recorded over 3 days. Although this approach has been extensively used, providing a prepared and pre-packed diet to participants during the intervention or during the days before a performance trial would offer an ideal scenario to standardize and control their diet (9). The supplementation protocol considered the absolute dose recommended by the manufacturer. Future studies should consider individualized doses based on the amount of carbohydrate and protein administered in terms of body mass or fat-free mass. Additionally, due to limited resources and the participants' restricted time for being controlled during the post exercise period, additional biochemical measurements (e.g. plasma aminoacidic or markers of muscle damage) to estimate the availability of nutrients derived from supplements or their impact to attenuate the disruption of the muscle membrane were not conducted. Furthermore, as the performed training routine involved 5 exercises for lower body, 1 combined weightlifting movement and only 2 for the upper body, the skeletal muscle properties were measured only on the lower body. In support of our design, and in order to reach the maximal possible concentric and eccentric activation of both leg extensors (e.g. vastus medialis) and flexors (e.g. biceps femoris long head), the squat exercise using an isoinertial fly-wheel device was included (17). Nonetheless, other investigations involving different exercises and set configurations assessing upper body contractile capacities are needed. Furthermore, analyzing the electromyographic signal of the main activated muscles involved in the voluntary exercises (bench press and vertical jump) could have provided a better insight on how the two compared nutritional

strategies influenced the rate to recover the performance capacity. Lately, only male participants volunteered to participate in the present study. Due to differences in protein metabolism between men and women (34) other similar studies including female participants need to be conducted. Indeed, our results should not be generalized to females or other age groups beyond those used in the present study.

In conclusion, the present investigation advocates for the ingestion of post-workout multi-ingredients providing carbohydrate, protein and a small amount of fat for accelerating the recovery of muscular function after a series of hard resistance training sessions in recreationally trained males.

PRACTICAL APPLICATIONS

Collectively our results support the post-workout administration of multi-nutrient admixtures to rapidly provide energy and essential nutrients including amino acids to the working muscle for maximizing recovery from resistance training. Strength and conditioning coaches can consider the ingestion of post-workout supplement providing ~0.45 to 0.60 $g \cdot kg^{-1}$ of carbohydrates, 0.20 to 0.30 $g \cdot kg^{-1}$ of high-quality protein (2:1 ratio of CHO/Protein) and a small amount of fat for optimizing recovery in athletes conducting consecutive hard workouts sessions.

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