

Ingesting a Post-Workout Vegan-Protein Multi-Ingredient Expedites Recovery After Resistance Training in Trained Young Males

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Abstract

Post workout multi-ingredient admixtures are commonly used to maximize recovery after exercise. The present double-blind, cross-over study compared the acute effects of ingesting a protein-vegan multi-ingredient (VGMT) vs. maltodextrin (MALT) on indices of muscle function. Ten trained males, (26.8 ± 1.9 years) performed two identical, 3-day resistance training periods (one workout-session per day) while receiving either VGMT or MALT (10 minutes after the completion of each workout). Following a baseline evaluation, we conducted assessments at, 1-h, 24-h and 48-h after the 3-day training period. Primary outcome included the evoked tensiomyography contraction velocity (V_c) of vastus medialis (VM), biceps femoris long head (BFLH) and anterior deltoids (AD). Secondary outcomes involved strength and power performance while the other tensiomyography variables [muscle displacement (D_m), contraction time (T_c)] were considered as exploratory. After 1-h, all the tensiomyography variables measured at VM and BFLH were similarly depressed in both treatments. Only MALT showed a significantly lower V_c ($-0.02 \text{ m}\cdot\text{s}^{-1}$, 95% CI, $-0.04, -0.01$) in the AD. After 24-h, the VGMT treatment normalized all tensiomyography values. Conversely, impaired scores were observed in V_c for the VM ($-0.03 \text{ m}\cdot\text{s}^{-1}$, 95% CI, $-0.06, -0.01$) and BFLH ($-0.02 \text{ m}\cdot\text{s}^{-1}$, 95% CI, $-0.05, 0.01$) in the MALT treatment. Particularly, the V_c in VM was lower ($p=0.043$) in MALT compared to VGMT. Overall, both treatments required 48-h to regain their performance capacity; however, VGMT produced better vertical jump and squat performance at 24-h vs. MALT. Compared to MALT, a vegan-protein multi-ingredient appears to hasten the recovery of muscular function over a 24-h period.

Keywords: Multi-nutrient supplement; tensiomyography; muscular contractile properties: strength; power.

Introduction

One of the most influential factors affecting recovery from resistance exercises is nutrition (Morton et al. 2015), whereby different post-workout feeding strategies often include multi-ingredients admixtures composed by carbohydrate, protein from different sources (Naclerio et al. 2014). The enrichment of such formulae with aminoacidic and associated derivatives, e.g. β -hydroxy β -methylbutyrate (β -HMB), has also been proposed to accelerate recovery between hard training sessions (Rindom et al. 2016; Naclerio et al. 2020). Most of these admixtures include high-quality animal-based protein which, compared to plant-based sources, contain higher proportions of essential amino acids (EAA), which better stimulate muscle protein synthesis (Phillips 2016).

Previous research has confirmed the positive effect of post-workout protein admixtures from animal sources to speed-up recovery after hard resistance training sessions in athletes. Hoffman et al. (2010) reported higher enhancement performance effects, determined 24 and 48 hours after a lower body resistance training programme in well trained strength and power participants, ingesting a preworkout and post-workout collagen, whey and casein protein admixture, including 2 g of carbohydrates and fortified with branched chain amino acids vs. the ingestion of maltodextrin. Rindom et al. (2016) reported no differences in ingesting protein from whey or collagen to accelerate the regaining of exercise performance after four high-intensity consecutive resistance training workouts. Furthermore, in young resistance-trained males, the post-exercise consumption of a complex milk-based protein beverage was more effective than only ingesting carbohydrates to attenuate the overall performance, estimated by the post-workout assessment of agility, push-ups, and sprints (Lynch 2013). More recently, Naclerio et al. (2020) suggested that compared to carbohydrate alone, a post-workout multi-ingredient providing carbohydrate,

and protein from whey and beef, optimised the regain of strength and power performance, favouring the recovery of muscular function estimated by tensiomyography (TMG) following three consecutive resistance training sessions.

Although the proportion of EAA is lower in vegetable sources, when equivalent amounts of EAA (e.g. leucine) are ingested, whether by the ingestion of multiple plant-based protein sources (Trommelen et al. 2019) or by a blend admixture fortified with EAA, similar effects to optimise exercise-induced outcomes (e.g. increase of post-exercise muscle protein synthesis and promotion of post-workout tissue remodelling and recovery), are observed between plant and animal proteins (Joy et al. 2013). Consequently, in order to obtain comparable anabolic effects from plant and animal protein-based foods, higher doses of vegetable-rich protein food should be ingested. However, consuming large amounts of protein plant-sources will concomitantly increase nitrogen, energy intake, amino acid oxidations and ureagenesis (van Vliet et al. 2015). One alternative for enhancing the effectiveness of plant-based protein to support post-exercise recovery and training-outcomes is the adhesion of key EAA such as Leucine. Herein, we compared the effects of a vegetable protein-based multi-ingredient containing carbohydrate, fat and enriched with amino acids (V-PRO Recovery ST Crown® Sport Nutrition, Spain) vs. the ingestion of an isocaloric only carbohydrate supplement, on the recovery of muscular function following hard resistance training sessions. The primary outcomes included changes in muscular contractile properties estimated by the evoked mean contraction velocity (V_c) using TMG. Secondary outcomes included changes in strength and power performance. Furthermore, the other TMG variables [muscle displacement (D_m), contraction time (T_c)] needed for determining the V_c were considered as exploratory. We hypothesized that compared to the ingestion of carbohydrate alone, a vegan protein-based multi-ingredient will speed up

recovery of muscular function after hard resistance training sessions. As the most relevant factor to improve recovery after the completion of high-intensity training sessions is the energetic content of the supplement rather than the nutritional composition (McLellan et al. 2014), the inclusion of another treatment receiving other protein sources (e.g. whey) or the comparison with a non-caloric condition was considered not necessary for the aim of the present study.

Material and Methods

Experimental Design

We performed a double-blinded, randomized, controlled crossover design with two 1-week intervention periods separated by a 2-week washout period. After the completion of three sessions of familiarization with the training and testing protocols (week 1), the participants performed the baseline assessments followed by a 5-day recovery period to be then randomly allocated to either a multi-ingredient (VGMT) or a maltodextrin (MALT) treatment. Thereafter, the participants underwent the first 3-day training period followed by 3 testing sessions conducted at 1-h, 24 and 48-h. Afterwards, a 2-week washout period was completed before switching to the other treatment for continuation with the second identical 3 daily training sessions and testing phase (Figure 1).

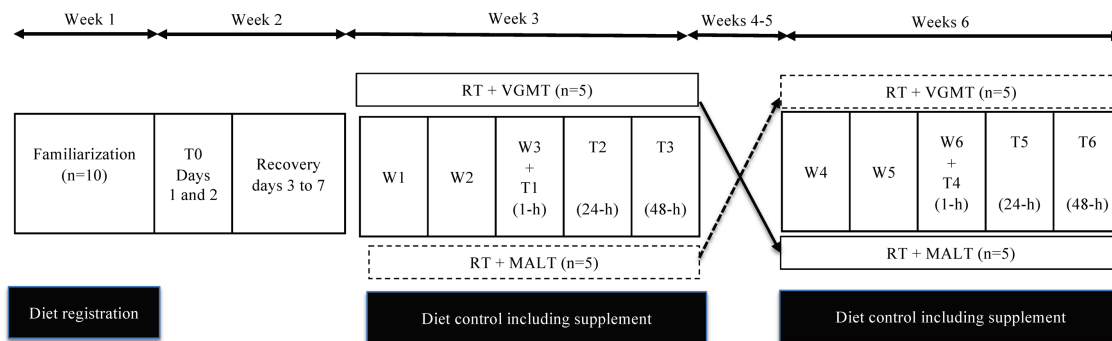


Figure 1. Schematic overview of the study design. The overall intervention involved six consecutive weeks. 1st week: Familiarization, 2nd week: Two days for pre-tests (T0) followed by a 5-day recovery period, 3rd week: First training (W1, W2 and W3) and testing (T1, T2 and T3) period, 4th and 5th weeks: Recovery/washout, 6th week: second training (W4, W5 and W6) and testing (T4, T5 and T6) period. VGMT: multi-ingredient treatment, MALT: maltodextrin treatment.

Participants

Ten resistance-trained male [mean \pm standard deviation (SD); age: 26.8 ± 1.9 years; body mass: 80.0 ± 13.1 kg; height: 176.6 ± 5.5 cm, body mass index 25.6 ± 4.0 kg·m⁻²) with a minimum of 1-year experience in resistance training, volunteered to take part in the study. Exclusion criteria included: (i) competing in weightlifting, powerlifting, and bodybuilding or performing >5 resistance workouts/week 6 months prior the study (to avoid elite strength athletes); (ii) a history of musculoskeletal injuries; neurological or metabolic disorders and (iii) use of dietary supplements or prescription medicine that would affect recovery (i.e., protein amino-acids supplements, NSAIDs, etc.). All participants provided written informed consent in accordance with the Declaration of Helsinki. Procedures were approved by the University Research Ethics committee (FES-FREC-18-03.04.14).

To determine the appropriate sample size, an interim analysis was performed once 6 participants completed the study. The analysis was conducted based on the primary outcome measure [the resulting Vc of the vastus medialis (VM) using TMG]. The VM rather than biceps femoris long head (BFLH) or anterior deltoids (AD) was considered as

the main outcome due to its high activation during squatting movements (Escamilla 2001), which was the prevalent action for the implemented routine composed by 8 exercises, with 6 requiring squatting actions. Assuming an α -error of 0.05, for the resulted effect size of $d = 1.8$ calculated between two dependent means determined at pre and 1-h post-workout, the required sample size of $n = 7$ was estimated to achieve $> 80\%$ statistical power.

Assessments

Participants underwent the following assessment schedule after familiarization:

- (i) Day 1 (T0, first session): 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 VM, BFLH and AD was conducted.
- (ii) Day 2 (T0, second session: Assessments of vertical jump (VJ); 5 kg overhead medicine ball throw (5-kg-MBT) and one repetition maximum (1RM) in bench press (BP) and parallel squat (SQ) were performed.
- (iii) After the baseline assessment and a 5-day recovery period (week 2), the participants underwent the first 3-day training period (week 3), including the intake of either VGMT or MALT. The participants performed 3 hard consecutive workouts (one workout per day) during the first three days followed by assessments conducted at 1-h, 24-h and 48-h after the completion of the third workout-session.
- (iv) A 2-week washout phase (weeks 4 and 5) was conducted before the participants completed an identical second 3-day training phase (week 6) followed by the three assessment sessions conducted at the same time points as in week 2 (Figure 1). In this occasion the participants ingested the other supplement compared to the one administered previously.

All participants were instructed to refrain from strenuous physical activity for 48-h before the first baseline tests and prior to both training and testing phases.

Tensiomyography: A TMG portable device (TMG Measurement System, 146 TMG-BMC Ltd., Ljubljana, Slovenia with a maximal stimulation output of 110 mA·ms⁻¹) was used to measure the contractile properties of the VM, BFLH and AD at the dominant limb (Rey et al. 2012a; Loturco et al. 2016). Measurements were collected by the same trained researcher, following the methodology described by Rey et al. (2012b) and obtained at rest, in supine position for the VM, prone position for BFLH and sitting position for the AD. Changes in the evoked muscular contractile properties were estimated by analysing the following variables: (i) maximal radial displacement of the muscle belly (Dm); (ii) contraction time between 10 and 90% of Dm (Tc); and (iii) mean contraction velocity (Vc) calculated by dividing the Dm by the sum of the Tc and the delayed time (Td) (Loturco et al. 2016). These 3 variables have demonstrated high levels of accuracy, reliability and sensitivity to reflect changes in the neuromuscular function by tensiomyography analysis (Martín-Rodríguez et al. 2017). 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 (Macgregor et al. 2018).

The intraclass correlation coefficient (ICC) at their 95% confidence interval (CI) for TMG variables ranged from 0.88 to 0.91, similar to those reported in previous investigations (Rey et al. 2012a).

Vertical Jump: Countermovement Jump was performed according to the methodology described by Brown and Weir (2001). 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 was used to calculate the height from the difference,

in meters (m), between maximum height of the centre of mass (apex) and the last contact of the toe on the ground during the take-off. Based on the height, the best of the 3 jumps was chosen for the analysis.

Medicine Ball Throw: Three overhead throws, were performed with a 5-kg medicine ball (circumference 0.30 m) using the methodology described by Viitasalo (1988). Based on the distance, the best of the 3 attempts was chosen for the analysis.

Strength: The 1RM value for the BP and SQ using free weights was determined according to the methodology described by McGuigan (2016).

Training

Late in the afternoon (4 to 6 pm), the participants performed a supervised full-body resistance-training protocol involving a standardized warm-up followed by three circuits of 1 set of the following exercises: (i) vertical jump (ii) hang clean; (iii) bench press; (iv) parallel squat; (v) upright row; (vi) alternate lunges; (vii) deadlift; (viii) alternate box set ups. A ~30-sec rest period was allowed between exercises and 3 min between circuits. As the workout was aimed to create a high level of mechanical and metabolic stress, a muscle endurance training (>15 repetitions per set) was designed (American College of Sports Medicine 2009). Accordingly, every set involved 16 self-determined maximum repetitions (Steele et al. 2017) (>40 to <60% 1RM) using the heaviest possible load (American College of Sports Medicine 2009). When participants were able to perform more than 16 repetitions per set, the load was increased from 2.5 to a maximum of 5 kg. If fewer than 16 repetitions were completed, a minimum rest period of 15-sec was introduced until the participants were able to reach the targeted number of repetitions per set. The time to complete the workouts was ~45-min.

Diet and Supplementation

Each participant completed a 3-day food diary report (two weekdays, and one weekend day). The Food Processor Software (Version 11.4.70, UK) was used to calculate energy nutritional composition of the reported diets. Participants were instructed to maintain their habitual diet throughout the study. If any change in diet patterns were identified, the participants were dropped from the study.

During the 3-day training periods (weeks 3 and 6), all the participants consumed either one single 70 g dose of a commercially available post-workout supplement (V-PRO Recovery ST, Crown Sport Nutrition, Spain) or an isoenergetic, non-protein, 66.2 g dose of maltodextrin (see Table 1). Supplements were mixed with ~250 ml of water and administered within 10 min after completing the three workout-sessions. No supplementation was consumed after the assessment sessions or on non-exercising days (weekends and weeks 4 and 5).

Table 1. Nutritional composition of supplements per intake mixing with ~250 ml of plain water

Nutrient	VGMT (70 g)	MALT (66.2 g)
Energy value (kcal)	273	258
Carbohydrates (g)	31	63
Fat (g)	1.9	0
Proteins included added amino acids (g)	~30	-
Added Amino Acids		
HMB calcium (g)	1.8	-
L-Carnitine L-tartrate (g)	1.5	-
N-Acetyl-L-cysteine (g)	0.4	-
L-Glutamine (g)	2	
L-Leucine (g)	1	
L-Lysine (g)	1.2	
L-Taurine (g)	0.5	
Final Product Aminogram		
Alanine (g)	1.3	-
Arginine (g)	2.0	-
Aspartic acid (g)	2.2	-
Cysteine + Cistin (g)	0.75	-
Glutamic acid (g)	6.3	-
Glycine (g)	1.0	-
Histidine (g)	0.56	-
Isoleucine (g)	1.1	-
L-Leucine (g)	3.0	-
Lysine (g)	2.1	-
Methionine (g)	1.3	-
Phenylalanine (g)	1.4	-
Proline (g)	1.1	-
Serine (g)	1.2	-
L-Taurine	0.5	
Threonine (g)	0.91	-
Tryptophan (g)	0.25	-
Tyrosine (g)	1.2	-
Valine (g)	1.4	-
Total EAA (g)	12.2	-

Notes: EAA: essential amino acids; VGMT supplement admixture including carbohydrates, proteins from rice, fat and added amino acids or derivatives, MALT: maltodextrin.

Statistical Analysis

Descriptive analyses were performed and Shapiro-Francia tests were applied to assess normality. Before testing the main hypothesis, the possible treatment order effect and the effectiveness of the washout phase to rule out any carryover effect was checked. For all the analysed variables, a preliminary test using the sum of all values obtained for each participant at 1-h, 24-h and 48-h in the two periods was calculated and compared across the two sequenced conditions. We used an independent means Student's t-test to compare the values measured in the 5 participants who started with VGMT vs. the results determined in the other 5 who started with MALT (Wellek and Blettner 2012).

Changes in all outcome variables were calculated by subtracting pre from post assessment values, without adjusting for pre values, since the same participants performed under both treatments acting as their own controls. In order to assess the magnitude of the differences from baseline, the 95% CI of the differences were calculated and plotted. Those CIs not crossing zero were considered statistically significant from baseline. Additionally, two-tailed one sample student's tests were used to test for a null effect hypothesis. A 2 (treatments: VGMT vs. MALT) \times 3 (times: post 1-h, 24-h and 48-h) repeated measures analysis of variance (ANOVA) was used to compare differences between treatments and post-workout measurements in raw change of tensiomyography and performance variables. Differences over time were compared using Bonferroni-adjusted pairwise comparisons when appropriate. Eta squared (η^2) and Cohen's d values were reported to provide an estimate of standardized effect size (small $\eta^2 = 0.01$, $d = 0.2$; moderate $\eta^2 = 0.06$, $d = 0.5$ and large $\eta^2 = 0.14$, $d=0.8$). Significance level was set at 0.05. Results are reported as mean \pm standard deviation (SD) 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

No carryover effect was observed for the main TMG outcomes variable (V_c) in the 3 analysed muscles (VM $p = 0.51$; BFLH, $p = 0.553$ and AD, $p = 0.221$) or for the performance tests (VJ, $p = 0.383$; 5-kg-MBT, $p = 0.211$; 1RM BP, $p = 0.476$ and SQ, $p = 0.142$) and the exploratory variables (all $p > 0.05$).

Diet Analysis: Table 2 shows the daily consumption of macronutrients (grams) and energy (kcal) including and not 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 VGMT (n=10)	With MALT (n=10)
Proteins			
g \cdot d $^{-1}$	137.3 \pm 17.7	165.3 \pm 17.7* $^{\delta}$	137.3 \pm 17.7
g \cdot kg $^{-1}$ \cdot d $^{-1}$	1.8 \pm 0.1	2.2 \pm 0.2* $^{\delta}$	1.8 \pm 0.1
% of total energy	24.6 \pm 2.1	26.1 \pm 2.1 $^{\delta}$	24.6 \pm 2.1
Carbohydrate			
g \cdot d $^{-1}$	276.5 \pm 34.4	307.5 \pm 34.4*	339.5 \pm 34.4*
g \cdot kg $^{-1}$ \cdot d $^{-1}$	3.7 \pm 0.6	4.1 \pm 0.7*	4.5 \pm 0.7*
% of total energy	49.4 \pm 3.4	49.4 \pm 3.0	54.6 \pm 3.3*
Fats			
g \cdot d $^{-1}$	65.0 \pm 17.5	66.9 \pm 17.5	65.0 \pm 17.5
g \cdot kg $^{-1}$ \cdot d $^{-1}$	0.85 \pm 0.2	0.88 \pm 0.2	0.85 \pm 0.2
% of total energy	26.0 \pm 4.2	24.0 \pm 3.9* $^{\delta}$	26.0 \pm 4.2
Energy			
Total daily energy	2301.7 \pm 314.8	2561.3 \pm 314.8*	2560.0 \pm 314.8*
Kcal \cdot kg $^{-1}$ \cdot d $^{-1}$	30.3 \pm 4.3	33.8 \pm 4.6*	33.8 \pm 4.6*

Notes: values are presented as mean \pm standard deviation

* $p < 0.01$ respect to diet without post workout supplementation

$^{\delta}$ $p < 0.01$ from diet with VGMT supplementation compared to diet with MALT supplementation

Overall, the ingestion of VGMT increased both protein and carbohydrate intake, while adding 66.2 g of maltodextrin increased the daily carbohydrate intake alone. VGMT and MALT significantly increased energy intake.

Tensiomyography: Table 3 describes the changes measured at the three assessed time points of the all TMG variables for the two compared treatments.

Table 3. Mean (M) \pm standard deviation (SD) and 95% CI of the differences measured at 1, 24 and 48 h for the tensiomyography variables in the two assessed treatments

Muscles	Conditions	VGMT (n=10)			MALT (n=10)			ANOVA Repeated Measures (2 times x 2 treatments)
	Variables	Post 1-h	Post 24-h	Post 48-h	Post 1-h	Post 24-h	Post 48-h	
Vastus Medialis	Vc (m·s ⁻¹)	-0.06 \pm 0.03** § ^a [-0.08, -0.04]	-0.01 \pm 0.03 [-0.03, 0.01]	-0.01 \pm 0.03 [-0.02, 0.02]	-0.05 \pm 0.04** § [-0.08, -0.02]	-0.03 \pm 0.03* [-0.06, -0.01]	-0.02 \pm 0.05 [-0.05, 0.01]	Time: F(2,18)=8.09; p=0.03; η^2 =0.20 Condition: F(1,9)=1.63; p=0.23; η^2 =0.01 Time x Condition: (2,18)=2.71; p=0.09 η^2 =0.06
	Dm (mm)	-2.49 \pm 1.60** § ^a [-3.63, -1.34]	-0.62 \pm 1.62 [-1.78, 0.54]	0.01 \pm 0.92 [-0.65, 0.66]	-2.29 \pm 2.26** § [-3.90, -0.68]	-1.43 \pm 1.22** [-2.30, -0.55]	-0.69 \pm 2.27 [-2.31, 0.94]	Time: F(2,18)=9.52; p=0.01 η^2 =0.21 Condition: F(1,9)=1.45; p=0.26; η^2 =0.02 Time x Condition: F(2,18)=1.06; p=0.37; η^2 =0.02
	Tc (ms)	4.69 \pm 5.81* [0.52, 8.85]	0.84 \pm 4.94 [-2.70, 4.38]	1.90 \pm 4.49 [-1.31, 5.11]	1.28 \pm 3.58 [-1.28, 3.84]	2.80 \pm 5.78 [-1.34, 6.93]	3.39 \pm 3.89* [0.61, 6.18]	Time: F(2,18)=0.38; p=0.69; η^2 =0.01 Condition: F(1,9)=0.01; p=0.99; η^2 =0.01 Time x Condition: F(2,18)=4.32; p=0.03; η^2 =0.07
Biceps Femoris Long Head	Vc (m·s ⁻¹)	-0.03 \pm 0.04* [-0.06, -0.01]	-0.02 \pm 0.03 [-0.04, 0.01]	-0.03 \pm 0.04 [-0.05, 0.01]	-0.03 \pm 0.04* [-0.06, -0.01]	-0.02 \pm 0.02* [-0.03, -0.01]	-0.02 \pm 0.03 [-0.05, 0.01]	Time: F(1,18)=0.43; p=0.66; η^2 =0.02 Condition: F(1,9)=0.92; p=0.36; η^2 =0.01 Time x Condition: F(2,18)=0.10; p=0.91; η^2 =0.01
	Dm (mm)	-1.55 \pm 2.29 [-3.18, 0.08]	-0.78 \pm 2.16 [-2.32, 0.77]	-1.39 \pm 2.48 [-3.17, 0.39]	-1.43 \pm 2.77 [-3.42, 0.55]	-0.53 \pm 1.73 [-1.77, 0.71]	-0.49 \pm 1.69 [-1.70, 0.72]	Time: F(2,18)=1.06; p=0.37; η^2 =0.03 Condition: F(1,9)=1.87; p=0.20; η^2 =0.01 Time x Condition: F(2,18)=0.61; p=0.55; η^2 =0.01
	Tc (ms)	2.15 \pm 5.83 [-2.02, 6.32]	4.16 \pm 7.91 [-1.59, 9.81]	3.63 \pm 13.63 [-6.42, 13.38]	0.87 \pm 7.03§ [-4.16, 5.89]	3.98 \pm 10.35 [-3.42, 11.39]	5.08 \pm 6.69* [0.30, 9.87]	Time: F(2,18)=0.98; p=0.40; η^2 =0.02 Condition: F(1,9)=0.01; p=1.00; η^2 =0.01 Time x Condition: F(2,18)=0.20; p=0.82; η^2 =0.01
Anterior Deltoids	Vc (m·s ⁻¹)	-0.01 \pm 0.03 [-0.03, 0.01]	-0.01 \pm 0.05 [-0.04, 0.03]	-0.01 \pm 0.04 [-0.04, 0.03]	-0.02 \pm 0.03* [-0.04, -0.01]	-0.01 \pm 0.03 [-0.04, 0.01]	-0.01 \pm 0.03 [-0.04, 0.01]	Time: F(2,18)=0.59; p=0.56; η^2 =0.01 Condition: F(1,9)=3.61; p=0.09; η^2 =0.03 Time x Condition: F(2,18)=0.08; p=0.92; η^2 =0.01
	Dm (mm)	-0.36 \pm 1.04 [-1.11, 0.39]	-0.22 \pm 1.75 [-1.47, 1.04]	-0.11 \pm 1.49 [-0.95, 1.17]	-0.82 \pm 1.13* [-1.63, -0.02]	-0.60 \pm 1.31 [-1.65, 0.34]	-0.22 \pm 1.74 [-1.47, 1.03]	Time: F(2,18)=1.18; p=0.33; η^2 =0.03 Condition: F(1,9)=7.20; p=0.03; η^2 =0.05 Time x Condition: F(2,18)=0.06; p=0.94; η^2 =0.01
	Tc (ms)	-0.76 \pm 2.46 [-2.52, 0.99]	-0.97 \pm 3.53 [-3.50, 1.56]	1.41 \pm 4.77 [-2.01, 4.82]	-1.44 \pm 1.95* [-2.84, 0.05]	-0.89 \pm 5.21 [-4.62, 2.84]	1.02 \pm 6.66 [-3.74, 5.79]	Time: F(2,18)=2.33; p=0.13; η^2 =0.06 Condition: F(1,9)=0.10; p=0.76; η^2 =0.01 Time x Condition: F(2,18)=0.07; p=0.94; η^2 =0.01

Notes: **p < 0.01, *p < 0.05 respect to baseline values; § p < 0.01, 1-h vs. 24 h^a p < 0.01, 1 h vs 48 h.

VGMT= vegan multi-ingredient; MALT= maltodextrin; Vc= evoked mean contraction velocity, Dm= evoked muscle displacement Tc= evoked contraction time

After 1-h both treatments similarly impaired the TMG variables in VM and BFLH, however, MALT, but not VGMT showed depressed TMG values for AD.

At 24-h, only MALT produced significantly slower Vc in both VM ($p = 0.011$) and BFLH ($p = 0.010$) along with longer Dm in VM ($p = 0.001$). Although no interaction effect was observed for the Vc measured at VM, due the close to significant F tests and moderate effect size ($\eta^2 = 0.06$), in order to reduce the risk of committing a type II error, Bonferroni-adjusted pairwise comparisons were conducted (Castañeda et al. 1993). We found that the Vc (primary outcome) of the VM measured in the MALT condition was significantly ($p = 0.043$, $d = 0.75$) lower than the observed when participants performed under the VGMT condition. This effect suggests a faster recovery of the involuntary muscular function when the participants ingested the multi-ingredient.

At 48-h, the Vc for the three analysed muscles is normalized for both VGMT and MALT, showing no differences to baseline or between conditions. Nonetheless, longer Tc for both VM and BFLH were determined in MALT.

Vertical Jump: Significant ($p < 0.01$) performance reduction compared to baseline was observed at 1-h and 24-h but not at 48-h for VGMT and MALT treatments (Figure 2A). Significant time ($F[2,18] = 6.34$, $p = 0.01$, $\eta^2 = 0.38$), but not for treatment or interaction effects were observed when comparing the differences measured at 1-h, 24-h and 48-h.

Significant ($p < 0.05$, $d > 0.8$) lower performance were observed for both VGMT and MALT at 1-h and 24-h vs. 48-h. At 24-h participants jumped significantly lower ($p = 0.039$, $d = 0.75$) under MALT vs. the VGMT treatment (Figure 2A).

Medicine Ball Throw: Significant ($p < 0.05$) performance reductions vs. baseline were observed after 1-h for VGMT and MALT (Figure 2B). Significant time ($F[2,18] = 6.59$, $p = 0.01$, $\eta^2 = 0.27$), but not for treatment or interaction effects were observed between differences calculated at 1-h, 24-h and 48-h. No differences between the three-time points (1-h, 24-h and 48-h) were determined in MALT. Under VGMT performance was significantly attenuated ($p < 0.05$, $d < 0.80$)

as recovery periods progressed from 1-h to 48-h (Figure 2B).

Strength: Significant reductions vs. baseline ($p < 0.05$, $d > 0.70$) were observed at 1-h and 24-h in both treatments for BP and SQ (Figure 2C and 2D). Significant time (BP: $F[2,18] = 20.09$, $p = 0.01$, $\eta^2 = 0.63$; SQ: $F[2,18] = 21.60$, $p = 0.01$, $\eta^2 = 0.67$), but not for treatment or interaction effects were observed when comparing the differences calculated at 1-h, 24-h and 48-h. In overall, strength was significantly recovered ($p < 0.05$, $d < 0.80$) over 48-h in both treatments (Figure 2C and 2D). At 24-h, the strength loss in SQ was significantly higher ($p = 0.041$, $d = 0.72$) in MALT vs. VGMT (Figure 2D).

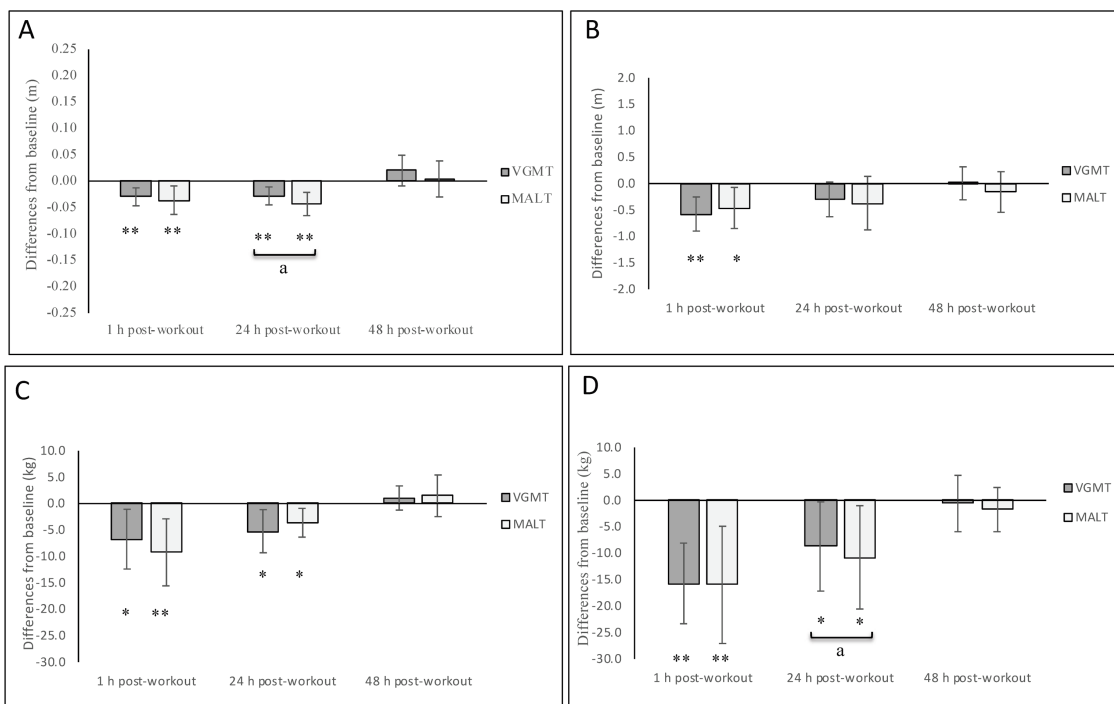


Figure 2. Estimated marginal means and 95% confidence intervals of differences in vertical jump (A) and medicine ball throw (B) weight lifted in one maximum repetition in the bench press (C) and squat (D). * $p < 0.05$; ** $p < 0.01$ from the baseline values ^a $p < 0.05$ between treatments. VGMT, multi-ingredient treatment, MALT, maltodextrin treatment.

Discussion

Our results suggest that the VGMT treatment vs. MALT expedites the recovery of the lower body involuntary muscular function within 24-h of performing three days of successive hard resistance workouts. No between-group differences were observed after a 48-h recovery period.

At 24-h of having completed the last workout session, the VGMT attenuated the impairment of the primary outcome measure (V_c) in both VM and BFLH. Even though no statistical differences between treatments were identified at any of three-time points (1-h, 24-h, and 48-h post-training) for secondary outcomes, when differences from the baseline measures are sought, the VGMT seems to produce a more favourable effect to speed up recovery after 24-h (Figures 2A and 2D). Furthermore, given the inverse relationship between V_c and muscle fatigue (Macgregor et al. 2018), the higher (normalized) values of V_c measured under VGMT vs. MALT for both VM and BFLH are suggesting a possible advantage of the multi-nutrient to speed up recovery over a 24-h period.

The impaired lower body contractile properties observed in both treatments at 1-h post-workout could be associated with peripheral mechanistic fatigue events such as a reduced excitation-contraction coupling efficiency, impaired membrane potentials, and disrupted muscle cell structures (de Paula Simola et al. 2015; Raeder et al. 2016).

The significant loss of the VJ and SQ performance in both treatments corresponded with depressed values of V_c and D_m observed in VM at 24-h in MALT but not under the VGMT condition which indeed seems to fully recover the evoked VM muscle function after 24-h. In fact, the participants needed 48-h to normalize the lower body TMG values (Table 3) and to re-establish the baseline performance in VJ and SQ under the MALT treatment (Figure 2, A and D). Conversely, no differences between conditions were observed in the TMG variables determined for the AD nor for the 5-kg-MBT. Nonetheless, immediately after workout, participants consuming maltodextrin exhibited a significant impairment in V_c and D_m measured in AD (Table 3) despite no concomitant differences between treatments in 5-kg-MBT were identified (Figure 2B). The reason for the observed results could be attributed to the low volume of upper body training (only two exercises) that, maybe was not hard enough to significantly reduce the upper body muscular function.

The VGMT provided similar amounts of carbohydrate (31 g) and protein (30 g), ranged from ~ 0.30 to $0.5 \text{ g}\cdot\text{kg}^{-1}$ for both macronutrients. Although the amount of carbohydrate was lower than the

recommended 1 to 1.2 g·kg⁻¹ for optimising glycogen restoration after exhaustive training sessions (Thomas et al. 2016), the inclusion of protein in a ratio of 1:1 for the carbohydrate/protein relationship and the added amino acids may have compensated the suboptimal administration of carbohydrates. Nonetheless, it is worth highlighting that the implemented moderate volume routine with 16 repetitions to failure sets (24 total sets) unlikely may have induced a meaningful depletion of muscle glycogen stores.

Regarding the post-exercise muscle remodelling process, the amount of protein included in the VGMT falls well above the accepted doses (0.18 to 0.30 g·kg⁻¹) to further stimulate muscle protein synthesis in young individuals (Morton et al. 2015). Furthermore, the amount of EAA and Leucine included in the VGMT was 163 (117 to 203) mg·kg⁻¹ and 40 (30 to 50) mg·kg⁻¹ respectively. These doses are similar to those used in previous studies to maximally stimulate muscle protein synthesis under exercise treatments in young males (Naclerio et al. 2017, 2019; Trommelen et al. 2019).

Our results reinforce the notion that post-workout feeding with fast absorptive rate carbohydrates, such as maltodextrin, complete protein vegetable source providing enough amount of EAA, (L-Leucine), may speed up recovery after resistance training workouts. Furthermore, the addition of nutrients associated with a more favourable anabolic response such as L-glutamine (Coqueiro et al. 2019), L-lysine (van Vliet et al. 2015), HMB (Wilkinson et al. 2013), L-Carnitine, L-tartrate (Spiering et al. 2008) and N-acetyl-cysteine (Silva et al. 2008) could accelerate the recovery under the VGMT treatment.

The present study had several limitations that must be considered when attempting to draw evidence-based inferences. First, although our interim analysis suggested that 10 participants were enough to answer our hypotheses, the moderate η^2 values with non-significant p values observed for the interaction effect (time × condition) of the Vc at VM (primary outcome measure) could increase the risk of type 2 error. Nonetheless, the presented Cohen's *d* values reduces the risk of misinterpretation of the observed results that need to be confirmed in future studies. Furthermore, as

a third animal-based protein admixture (e.g. whey or beef) condition was not considered for the present design, future studies are necessary that are aimed at comparing acute and long-term effects of post-workout multi-ingredient supplements, differentiated by the protein sources (vegan vs. animal). Even though dietary records were recorded the participants diets were not fully controlled outside of the supplement routine. Although this approach has been extensively used, providing a pre-packed diet to participants before and during the intervention would have offered a more accurate scenario to control their diet (Jeacocke and Burke 2010). The supplementation protocol considered the absolute dose recommended by the manufacturer. Future studies should consider individualized doses based on the participants' body mass or fat-free mass. Lastly, as we only examined male participants, given the differences in protein metabolism observed between sexes (Tarnopolsky 2000) our findings cannot be generalized to females.

In conclusion, the present investigation advocates for the ingestion of a vegan protein-based multi-ingredient providing similar amounts of carbohydrates and proteins ($\sim 0.30\text{-}0.50\text{ g}\cdot\text{kg}^{-1}$, 1:1 ratio) fortified with amino-acid and derivatives, instead of carbohydrates alone, for accelerating the recovery of muscular function after a series of hard resistance training sessions in recreationally trained males.

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Authorship: FN and ELZ designed the study. MS, FN and JP-F collected the data. FN, ELZ and MS analysed the data FN, ELZ and CPE drafted the manuscript. All authors revised the manuscript critically for important intellectual content and gave final approval of the final version.

References

- American College of Sports Medicine (2009) American College of Sports Medicine position stand: progression models in resistance training for healthy adults. *Med Sci Sport Exe* 41:687-708. doi: 10.1249/MSS.0b013e3181915670
- Brown LE, Weir JP (2001) ASEP procedures recommendation I: Accurate assessment of muscular strength. *JEPonline* 4:1-21
- Castañeda MB, Levin JR, Dunham RB (1993) Using planned comparisons in management research: A case for the Bonferroni procedure. *J Manage* 19:707-724. doi: 10.1177/014920639301900311
- Coqueiro AY, Rogero MM, Tirapegui J (2019) Glutamine as an anti-fatigue amino acid in sports nutrition. *Nutrients* 11:863. doi: 10.3390/nu11040863
- de Paula Simola RA, Harms N, Raeder C, et al (2015) Assessment of neuromuscular function after different strength training protocols using tensiomyography. *J Strength Cond Res* 29:1339-1348. doi: 10.1519/JSC.0000000000000768
- Escamilla RF (2001) Knee biomechanics of the dynamic squat exercise. *Med Sci Sport Exerc* 33:127-141
- Hoffman JR, Ratamess NA, Tranchina CP, et al (2010) Effect of a proprietary protein supplement on recovery indices following resistance exercise in strength/power athletes. *Amino Acids* 38:771-778. doi: 10.1007/s00726-009-0283-2
- Jeacocke NA, Burke LM (2010) Methods to standardize dietary intake before performance testing. *Int J Sport Nutr Exerc Metab* 20:87-103
- Joy JM, Lowery RP, Wilson JM, et al (2013) The effects of 8 weeks of whey or rice protein supplementation on body composition and exercise performance. *Nutr J* 12:86
- Loturco I, Pereira LA, Kobal R, et al (2016) Muscle Contraction Velocity: A Suitable Approach to Analyze the Functional Adaptations in Elite Soccer Players. *J Sport Sci Med* 15:483-491
- Lynch S (2013) The differential effects of a complex protein drink versus isocaloric carbohydrate drink on performance indices following high-intensity resistance training: A two arm crossover design. *J Int Soc Sports Nutr* 12:31. doi: 10.1186/1550-2783-10-31
- Macgregor LJ, Hunter AM, Orizio C, et al (2018) Assessment of Skeletal Muscle Contractile Properties by Radial Displacement: The Case for Tensiomyography. *Sport Med* 48:1607-1620. doi: 10.1007/s40279-018-0912-6
- Martín-Rodríguez S, Loturco I, Hunter AM, et al (2017) Reliability and measurement error of tensiomyography to assess mechanical muscle function: A systematic review. *J. Strength Cond. Res.* 31:3524-3536
- McGuigan M (2016) Administration, scoring and interpretation of selected tests. In: Haff GG, Tripplet NT (eds) *Essentials of Strength and Conditioning*. Human Kinetics Champaign, IL, pp 259-316.
- McLellan TM, Pasiakos SM, Lieberman HR (2014) Effects of protein in combination with carbohydrate supplements on acute or repeat endurance exercise performance: a systematic review. *Sport Med* 44:535-550. doi: 10.1007/s40279-013-0133-y
- Morton RW, McGlory C, Phillips SM (2015) Nutritional interventions to augment resistance training-induced skeletal muscle hypertrophy. *Front Physiol* 6:245. doi: 10.3389/fphys.2015.00245
- Naclerio F, Larumbe-Zabala E, Cooper K, Seijo M (2020) Effects of a multi-ingredient beverage on recovery of contractile properties, performance, and muscle soreness after hard resistance

training sessions. *J Strength Cond Res*. doi: 10.1519/JSC.0000000000003397

- Naclerio F, Larumbe-Zabala E, Cooper R, et al (2014) Effect of a multi-ingredient supplement on intermittent sprint performance, fatigue perception, muscle damage and immunosuppression in recreational athletes. *J Int Soc Sports Nutr* 11:. doi: 10.1186/1550-2783-11-S1-P8
- Naclerio F, Larumbe-Zabala E, Larrosa M, et al (2019) Intake of Animal Protein Blend Plus Carbohydrate Improves Body Composition with no Impact on Performance in Endurance Athletes. *Int J Sport Nutr Exerc Metab* 29:474–480. doi: 10.1123/ijsnem.2018-0359
- Naclerio F, Seijo-Bujia M, Larumbe-Zabala E, Earnest CP (2017) Carbohydrates Alone or Mixing With Beef or Whey Protein Promote Similar Training Outcomes in Resistance Training Males: A Double-Blind, Randomized Controlled Clinical Trial. *Int J Sport Nutr Exerc Metab* 27:408–420. doi: 10.1123/ijsnem.2017-0003
- Phillips SM (2016) The impact of protein quality on the promotion of resistance exercise-induced changes in muscle mass. *Nutr Metab* 13:64. doi: 10.1186/s12986-016-0124-8
- Raeder C, Wiewelhove T, Simola RÁDP, et al (2016) Assessment of Fatigue and Recovery in Male and Female Athletes After 6 Days of Intensified Strength Training. *J Strength Cond Res* 30:3412–3427. doi: 10.1519/JSC.0000000000001427
- Rey E, Lago-Penas C, Lago-Ballesteros J (2012a) Tensiomyography of selected lower-limb muscles in professional soccer players. *J Electromyogr Kinesiol* 22:866–872. doi: 10.1016/j.jelekin.2012.06.003
- Rey E, Lago-Penas C, Lago-Ballesteros J, Casais L (2012b) The effect of recovery strategies on contractile properties using tensiomyography and perceived muscle soreness in professional soccer players. *J Strength Cond Res* 26:3081–3088. doi: 10.1519/JSC.0b013e3182470d33
- Rindom E, Nielsen MH, Kececi K, et al (2016) Effect of protein quality on recovery after intense resistance training. *Eur J Appl Physiol* 116:2225–2236. doi: 10.1007/s00421-016-3477-9
- Silva LA, Silveira PCL, Pinho CA, et al (2008) N-acetylcysteine supplementation and oxidative damage and inflammatory response after eccentric exercise. *Int J Sport Nutr Exerc Metab* 18:379–388. doi: 10.1123/ijsnem.18.4.379
- Spiering BA, Kraemer WJ, Hatfield DL, et al (2008) Effects Of L-Carnitine L-Tartrate Supplementation On Muscle Oxygenation Responses To Resistance Exercise. *J Strength Cond Res*. 22:1130–1135
- Steele J, Fisher J, Giessing J, Gentil P (2017) Clarity in reporting terminology and definitions of set endpoints in resistance training. *Muscle Nerve* 56:368–374. doi: 10.1002/mus.25557
- Tarnopolsky MA (2000) Gender differences in substrate metabolism during endurance exercise. *Can J Appl Physiol* 25:312–327
- Thomas T, Erdman KA, Burke LM (2016) American College of Sports Medicine Joint Position Statement: Nutrition and athletic performance. *Med Sci Sport Exerc* 48:543–68. doi: 10.1001/jama.1956.02970290016006
- Trommelen J, Betz MW, van Loon LJC (2019) The Muscle Protein Synthetic Response to Meal Ingestion Following Resistance-Type Exercise. *Sport Med* 49:185–197. doi: 10.1007/s40279-019-01053-5
- van Vliet S, Burd NA, van Loon LJ (2015) The Skeletal Muscle Anabolic Response to Plant- versus Animal-Based Protein Consumption. *J Nutr* 14:981–991. doi: 10.3945/jn.114.204305
- Viitasalo JT (1988) Evaluation of explosive strength for young and adult athletes. *Res Q Exerc Sport*

59:9–13. doi: 10.1080/02701367.1988.10605467

Wellek S, Blettner M (2012) On the Proper Use of the Crossover Design in Clinical Trials. *Dtsch Arzteblatt Online* 109:276–281. doi: 10.3238/arztebl.2012.0276

Wilkinson DJ, Hossain T, Hill DS, et al (2013) Effects of leucine and its metabolite β -hydroxy- β -methylbutyrate on human skeletal muscle protein metabolism. *J Physiol* 591:2911–2923. doi: 10.1113/jphysiol.2013.253203

