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PHYSIOLOGY AND NUTRITION

Whey protein supplementation does not accelerate recovery from a single bout of eccentric exercise

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ABSTRACT

The current double blind, randomized, placebo-controlled trial with two parallel groups aimed to assess the impact of whey protein supplementation on recovery of muscle function and muscle soreness following eccentric exercise. During a 9-day period, forty recreationally active males received twice daily supplementation with either whey protein (PRO; 60 g/day) or an iso-energetic amount of carbohydrate (CON). Muscle function and soreness were assessed before, and 0, 3, 24, 48, and 72 h after performing 100 drop jumps. Recovery of isometric maximal voluntary contraction (MVC) did not significantly differ between groups (timextreatment, $P = 0.56$). In contrast, the recovery of isokinetic MVC at $90°{\cdot}s^{-1}$ was faster in CON as opposed to PRO (timextreatment interaction, $P = 0.044$). Recovery of isokinetic MVC at $180°{\cdot}s^{-1}$ was also faster in CON as opposed to PRO (timextreatment interaction, $P = 0.011$). Recovery of countermovement jump performance did not differ between groups (timextreatment interaction, $P = 0.52$). Muscle soreness, CK and CRP showed a transient increase over time ($P < 0.001$), with no differences between groups. In conclusion, whey protein supplementation does not accelerate recovery of muscle function or attenuate muscle soreness and inflammation during 3 days of recovery from a single bout of eccentric exercise.

ARTICLE HISTORY
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KEYWORDS
Eccentric exercise; recovery; muscle function; muscle soreness; protein supplementation; carbohydrates

Introduction

Unaccustomed eccentric exercise results in microtrauma to myofibers and the surrounding extracellular matrix. This microtrauma, or muscle damage, is characterized by a transient disruption of sarcomeres and sarcolemma resulting from an increase of tension during lengthening muscle actions (Hyldahl & Hubal, 2014). This muscle damage is further exacerbated when exercise is performed with higher forces and a greater range of motion (Owens et al., 2019).

Exercise-induced muscle damage (EIMD) typically results in increased pain and soreness, a decline in muscle function (force generation), decreased range of motion of the affected limb and reduced training quality, that may persist for several days after exercise (Clarkson & Hubal, 2002). In addition, EIMD is characterized by an efflux of myocellular enzymes and proteins that enter the bloodstream after disruption of the cytoskeleton and sarcolemma (Hyldahl & Hubal, 2014; Peake et al., 2017). Signalling proteins, such as chemokines, are hereby released into the circulation, activating an inflammatory response which sets off a cascade of events that acts on muscle nociceptors that stimulate soreness (Hyldahl & Hubal, 2014).

The intake of ample amounts of protein is generally advocated as a strategy to facilitate recovery following exercise (Thomas et al., 2016). This can be attributed to the crucial role of protein in the regulation of skeletal muscle remodelling, i.e. stimulation of post-exercise muscle protein synthesis and greater gains in muscle strength and muscle hypertrophy following prolonged resistance exercise training (Burd et al., 2009; Cermak et al., 2012). Nevertheless, evidence for the role of protein supplementation in recovery of muscle function following exercise is less evident. While some studies report accelerated recovery of muscle function and an attenuated rise in markers of muscle damage and soreness (Abbott et al., 2019; Buckley et al., 2010; Cockburn et al., 2008, 2010; Cooke et al., 2010; Davies et al., 2018; Ives et al., 2017; Nosaka et al., 2006), others observed no benefits of peri-exercise protein supplementation for recovery of muscle function, muscle damage, and soreness (Blacker, Williams, Fallowfield, Bilzon & Willems, 2010; Eddens et al., 2017; Green et al., 2008; Wojcik et al., 2001). Consequently, two systematic reviews concluded that the level of evidence to support a role for protein supplementation in recovery of muscle function and soreness after muscle-damaging exercise is rather limited (Davies et al., 2018; Pasiakos et al., 2014). In this regard, it should be noted that many previous studies were characterized by low sample sizes, lack of (information on) dietary control or dietary standardization, and limited assessment of muscle function and performance. Clearly, there still is need for well-designed studies to assess the proposed benefits of protein supplementation on post-exercise recovery. Therefore, the primary aim of the

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Supplemental data for this article can be accessed online here.

present study was to assess whether protein supplementation during the days before and after a single bout of strenuous eccentric exercise accelerates recovery of muscle function. Furthermore, we aimed to examine whether protein supplementation reduces muscle soreness and/or attenuates the rise in circulating plasma markers of muscle damage and inflammation during recovery from exercise. We hypothesized that daily supplementation with 60 g of whey protein as opposed to an isoenergetic carbohydrate control accelerates recovery of muscle function and muscle soreness from a single bout of strenuous eccentric exercise.

Materials and methods

Study design

The present study was a double blind, randomized, placebo-controlled trial with a parallel group design. Participants were allocated to a 9-day experimental period, with daily supplementation of either whey protein (PRO) or an isoenergetic carbohydrate control (CON). On day 5 of the experimental period, participants were subjected to a bout of eccentric exercise, after which recovery of muscle function was monitored during the remaining period. All testing procedures were conducted at the sport and research centre of HAN University of Applied Sciences in Nijmegen, the Netherlands, between March 2019 and August 2019. The study was approved by the Independent Review Board Nijmegen Medical Ethical Committee the Netherlands, and conformed to the standards for the use of human participants as outlined in the most recent version of the Helsinki Declaration. The study was registered at the Netherlands Trial Registry (www.trialregister.nl) as NL7550.

Participants and screening

Forty recreationally active (≤5 h exercise per week), non-obese (≥18.5 and ≤27.5 kg/m^2), young (≥18 and ≤35 y) males were recruited through advertising on the university campus and local newspapers. Exclusion criteria were participation in structural exercise with a major eccentric component (e.g., soccer, basketball, trail running, etc.), medication use, lactose intolerance, any history of medical or surgical events that may affect the study outcomes, and following a specific diet (e.g., weight loss, ketogenic, vegan). After checking in- and exclusion criteria by email or telephone, participants were invited to our lab for a screening visit. During this visit, participants were informed about the nature and possible risks of the experimental procedures before their written informed consent was obtained. Hereafter, eligibility was verified by a health and lifestyle questionnaire and participants were familiarized with all exercise testing procedures. Participants' body mass and height were determined by a digital scale and a mobile stadiometer, respectively (Seca 770 and Seca 213i, Hamburg, Germany). In addition, whole-body and regional body composition were measured by dual-energy x-ray absorptiometry (Horizon W, Hologic, MA, USA) and determined using the system's software package (Apex version 5.6.0.5) using the classic calibration algorithm. A schematic overview of participant flow is presented as a supplemental figure (Supplemental Digital Content 1, CONSORT flow diagram).

Randomization and blinding

Participants were allocated randomly in a 1:1 ratio to either the CON or PRO group, stratified for lean mass (<65 vs >65 kg). A computer-generated randomization list was made by an independent researcher and shared with the supplement manufacturer. Allocation concealment was ensured by the manufacturer of the supplements, who labelled all protein and placebo supplements according to the participant number. The researchers responsible for screening allocated each eligible participant to the next available number on entry into the trial. All study personnel and participants were blinded to treatment assignment for the duration of the study. The randomization list was revealed to the researchers once recruitment, data collection, and data entry were completed and checked, and the data set was locked.

Intervention

Supplementation

During the 9-day experimental period, participants received either a whey protein concentrate supplement or an isoenergetic carbohydrate control. The daily supplemental dose of the whey protein supplement contained 1221 KJ (287 kcal), 58.5 g protein, 3.8 g fat and 5.0 g carbohydrate, whereas the isoenergetic carbohydrate (maltodextrin) control contained 1226 KJ (288 kcal), ≤0,1 g protein, 0 g fat and 72 g carbohydrates. The whey protein and carbohydrate placebo, both supplied by FrieslandCampina, were provided as powder in blinded jars with measuring scoops and a blender bottle. The daily dose (75 g powder) was distributed over two servings. The first serving (25 g of powder, i.e. 19.5 g of protein) had to be consumed mid-morning between breakfast and lunch, whereas the second serving (50 g of powder, i.e. 39 g of protein) had to be consumed ~1 h before sleep. This supplementation strategy was chosen based on recent research that identified the morning (Gillen et al., 2017), and pre-sleep period (Trommelen & van Loon, 2016) as strategic protein supplementation periods for athletes. Participants were required to mix each 25 g of powder with 250 mL of water. The resulting whey protein and carbohydrate control drink could not be discerned by taste (vanilla flavoured), smell, texture, or colour. Compliance with the supplementation regimen was checked by daily online questionnaires and during each visit.

Muscle-damaging exercise

Muscle damage was induced by 100 drop jumps, adapted from a protocol previously shown to be effective in causing muscle damage (Clifford et al., 2016). In the current study, participants were equipped with a 5 kg weighted vest and performed 10 sets of 10 drop jumps from a 60 cm platform with both hands on their hips, landing in a squat position (~90° knee angle) immediately followed by a maximal vertical jump. Each set of 10 drop jumps had to be performed within 90 s, followed by an inter-set rest of 90 s. During execution of the drop jumps, participants were verbally encouraged to perform each jump

with maximal effort. At the end of the protocol, ratings of perceived exertion (RPE) were obtained using a 10-point RPE scale.

Study procedures

An overview of study procedures is presented in Figure 1. At least one week before commencing the experimental period, participants visited our lab (visit 1; screening) to practice the drop jump technique, and to become completely familiarized with all exercise testing procedures. A second visit was planned before the experimental period to provide participants with the test products. On day 5 of the experimental period, participants reported to the laboratory (visit 3) in an overnight fasted state at ~08.00 h. After checking health and compliance and conducting pre-exercise measurements (PRE) participants performed the eccentric exercise bout, followed by post-exercise measurements at 0 (POST) and 3 h following exercise. After a lunch provided at the laboratory, participants resumed their daily life. At visit 4 (+24 h), 5 (+48 h) and 6 (+72 h) participants arrived at the laboratory at ~09.00 h in the fed state. After checking health and compliance, all outcome measurements were repeated.

During the 9-day experimental period, participants were encouraged to maintain their habitual diet, except for several dietary restrictions to prevent the intake of excessive amounts of protein. In this regard, participants were allowed to ingest a daily maximum of 2 glasses of milk or other drinkable dairy products, 1 serving of cheese, 1 bowl of yoghurt or comparable dairy products, 1 serving of meat product on sandwich during breakfast or lunch, and 1 portion of meat with dinner. Besides the dietary restrictions, participants were asked to consume an identical breakfast before visit 4, 5, and 6.

Furthermore, the use of prescription and over-the-counter medication to suppress pain or inflammation was prohibited from 1 day before the eccentric exercise bout day until 4 days post-exercise. During the intervention period participants were asked to refrain from any vigorous physical activity (e.g., only walking or cycling for transportation means) and to abstain from alcohol 24 h prior to all testing and caffeine 5 h prior to testing. During the whole study, participants kept an online diary to record information on compliance with product intake and study guidelines, and the occurrence of adverse events.

Study outcomes

Maximal voluntary contraction

Muscle function was assessed by isometric (knee extensors) and isokinetic (knee extensors and flexors) strength testing of the upper right leg on a dynamometer (Humac Norm Isokinetic Extremity System, CSMI, Stoughton MA, USA). Participants were seated in an upright position with the back-chair seat set to an angle of 85°. To minimize any extraneous body movements, participants were fastened to the chair and lever arm of the dynamometer by torso, thigh and shin straps. The dynamometer was adjusted so that the femoral epicondyle was in line with the axis of rotation of the lever arm. Three maximal voluntary isometric knee extensions were performed for a duration of 4 s at a knee angle of 60º with 1-min rest intervals between successive attempts. Subsequently, 5 reciprocal maximal voluntary isokinetic knee extensions and flexions (concentric muscle actions of the quadriceps and hamstrings) were determined at angular velocities of 90 and 180°·s^{-1}. For each maximal strength test, the single best contraction was used for analyses. Participants were instructed and verbally encouraged to execute each contraction with maximal force and were given verbal feedback on the number of repetitions. Between each different test, participants rested for 90 s. All isokinetic tests were performed over an 90º range of motion with the knee fully extended being 0º. Analyses were performed over a 10–75º range of motion to dismiss any end-range deceleration. Data were generated using the Humac Norm system's software package and MATLAB (Mathworks, Natick, MA). Dynamometry of the upper leg has been shown to be a highly reliable method to assess muscle function in a time series (Morton et al., 2005).

Vertical jump height

Countermovement jump (CMJ) height was used as a more ecological valid measurement of muscle function and was calculated from flight time with an optoelectrical measurement system (OptoJump, Microgate, Bolzano, Italy). Participants performed 5 maximal countermovement jumps (1 min inter-set rest), with their hands on the hips, by descending rapidly into a ~ 90° knee angle before a maximal vertical bilateral take-off. Participants were verbally encouraged to perform each attempt with maximal force, without verbal feedback on the height of

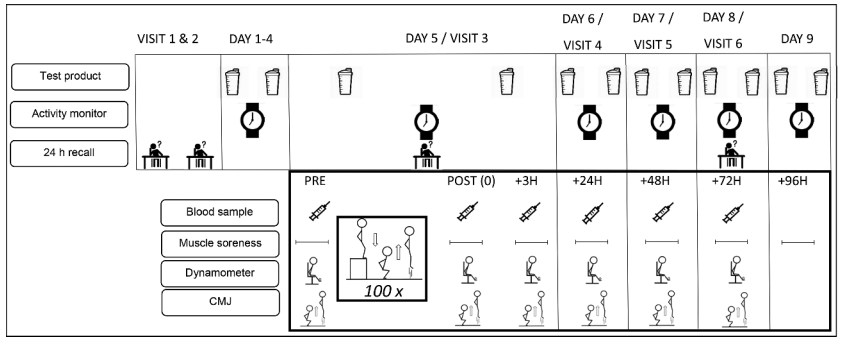

Figure 1. Overview of study procedures. Thirty-nine participants completed a 9-day experimental period, comprising twice daily supplementation with either placebo (n = 19) or whey protein (n = 20). Measurements of muscle function, muscle soreness and blood markers of muscle damage, inflammation and stress response were conducted before, and 0, 3, 24, 48, and 72 h after performing 100 drop jumps on day 5 of the supplementation period.

each jump. The single best vertical jump height performance was used for analysis. CMJ tests using flight time have been shown to be a highly reliable test for the measurement of jump height in physically active men (Cormack et al., 2008; Moir et al., 2004).

Muscle soreness

Muscle soreness was measured by both a Visual Analogue Scale (VAS), measuring current muscle soreness, and a retrospective pain questionnaire measuring muscle soreness over the preceding 24 h period. For the acute measurement participants were asked to hold a squat (90° knee angle) for 3 s. Subsequently, the participants rated their perceived muscle soreness (legs only) on a visual analogue scale (VAS), which consists of a line from 0 mm (no muscle soreness) to 100 mm (worst imaginable muscle soreness). For the measurement of muscle soreness over the preceding 24-h period, we used a 7-point (Likert) retrospective pain questionnaire for muscle soreness (Vickers, 2001) to evaluate retrospective perceived pain during daily life activities with 0 "a complete absence of muscle pain" and 6 "a severe muscle pain that limits my ability to move". The retrospective pain questionnaire was completed at 24, 48, 72, and 96 h post-exercise.

Blood markers of muscle damage, inflammation and stress response

Blood samples for measurement of creatine kinase (CK; pre, 0, 1, 3, 24, 48, 72 h), c-reactive protein (CRP; pre, 0, 1, 24, 48, 72 h) and cortisol (pre, 0, 3, 24, 48, 72 h) concentrations were drawn from an antecubital vein. Plasma glucose, insulin, and amino acid concentrations were assessed only pre-exercise and directly post-exercise (0 h). Blood samples were collected in EDTA-containing tubes and centrifuged at 1000 *g* for 10 min. Aliquots of plasma were immediately stored at − 80°C until further analysis.

Plasma CK concentrations were measured enzymatically by use of the Atellica®CH Creatine Kinase assay using the Atellica®CH analyser (Siemens Healthcare Diagnostics, ref 11,097,640). Plasma CRP concentrations were measured turbidimetrically by use of the Atellica®CH C-Reactive Protein_2 assay using the Atellica®CH analyser (Siemens Healthcare Diagnostics, ref 11,097,631). Glucose was measured by use of an enzymatic method with hexokinase on Cobas C system (Roche Diagnostics, ref 05168791190). Insulin was measured by use of an Electric-Chemiluminiscence Immuno Assay (ECLIA) on Cobas C system (Roche Diagnostics, ref. 12017547122). Cortisol was measured by use of an Electric-Chemiluminiscence Immuno Assay (ECLIA) on LIAISON®XL system (DiaSorin, ref. LIASON®Cortisol). Free amino acids in serum were derivatized using the EZ:faast amino acid kit (Phenomenex) and quantified by LC-MS analysis performed on a TSQ Quantis Triple Quadrupole MS (Thermo Fisher Scientific, SanJose, USA) and a LC system (Thermo Fisher Scientific Vanquish UHPLC).

Dietary intake and physical activity

Dietary intake was assessed on 4 occasions by a web-based 24-h recall system (Compl-eat, Wageningen University, Wageningen, The Netherlands), as described previously (Wardenaar et al., 2015). The recalls were completed during visit 1 and visit 2 (before the experimental period), and during visit 3 and 6 (during the experimental period), in the presence of a trained dietitian. Physical activity was assessed for 24 h per day throughout the 9-day experimental protocol using a wrist-worn physical activity monitor (GT9X Link, Actigraph, Pensacola, FL, USA). The physical activity monitor was worn on the non-dominant wrist, sampling frequency was 30 Hz and data were stored in 10 s epochs. Physical activity data were used to assess the percentage of time spent sedentary or during light, moderate, or vigorous physical activity, according to the Freedson classification (Freedson et al., 1998).

Sample size calculation and data analysis

A sample size calculation was performed with the GLIMMPSE software, for repeated measures designs (https://glimmpse.samplesizeshop.org). Sample size was calculated based on previous studies evaluating the effect of dietary interventions on muscle function (MVC) using a similar design (Buchwald-Werner et al., 2018; Howatson et al., 2012). With 6 measurement time points (pre, 0, 3, 24, 48, 72 h) and a 0.8 power to detect a significant difference ($P < 0.05$, two-sided), a total sample size of 32 participants was calculated for the primary outcome MVC. Given the greater risk for a type I error due to the multiple components of MVC, we planned to complete the study with 40 participants. Randomized participants withdrawn from participation before the start of the experimental period were replaced by new participants.

Prior to hypothesis testing, data were examined for normality. Non–normally distributed variables were logarithmically transformed before analysis. Baseline characteristics were compared by independent sample t-tests. The primary analyses were conducted on participants who completed the study per protocol. The effect of protein supplementation on recovery was assessed by using mixed model ANOVA, with time as within factor (6; pre, 0, 3, 24, 48 and 72 h post-exercise) and treatment (CON vs PRO) as between factor. Statistical significance was set at $P < 0.05$. Data are presented as mean±SD, or as otherwise indicated. All analyses were performed using SPSS 25.0 (IBM Corp., Armonk, NY, USA).

Results

Participants

The experimental period was completed by 40 out of 48 participants initially allocated to the experimental conditions. Eight participants did not start the experimental period due to illness, medication use, not meeting study procedures, or personal reasons. The analyses were conducted with 39 participants (CON $n = 19$; PRO $n = 20$), due to a protocol deviation (medication use) by one participant (Supplemental Digital Content 1, CONSORT Flow Diagram). No differences between participants' characteristics (Table 1) or any of the outcome variables were detected at baseline.

Table 1. Participants' characteristics.

	Control (n = 19)	Protein (n = 20)	P value
Age (y)	23 ± 4	24 ± 4	0.54
Body mass (kg)	78.3 ± 5.4	75.2 ± 6.9	0.40
Height (cm)	184 ± 6	182 ± 8	0.60
BMI (kg/m^2)	23.2 ± 1.3	22.5 ± 1.9	0.18
Lean mass (kg)	58.5 ± 4.8	56.9 ± 4.3	0.30
Fat mass (kg)	15.4 ± 4.1	13.9 ± 3.8	0.26
Fat mass (%)	20.0 ± 4.9	18.7 ± 3.9	0.37

Data are presented as mean±SD. BMI: body mass index.

Intervention

Compliance with the test products was 100% in both groups. Participants' blinding was confirmed through an exit survey. In this regard, 68 and 40% of the participants in the CON and PRO group guessed the intervention correctly, whereas 16 and 45% guessed the intervention incorrectly, and 16 and 15% replied with "I don't know", for CON and PRO, respectively (X^2: $P = 0.12$). Ratings of perceived exertion after 100 drop jumps were not different between CON (6 ± 2) and PRO (7 ± 2) ($P = 0.33$). Plasma BCAA concentrations assessed during visit 3 increased from 560 ± 106 µmol/L in the fasted state to 1078 ± 181 µmol/L at ~1 h following ingestion of whey protein, while a decrease (from 525 ± 106 to 490 ± 96 µmol/L) was observed in the carbohydrate control group (time x treatment interaction, $P < 0.001$). Plasma glucose concentrations increased following ingestion of the supplement, with no differences between groups (CON: from 5.4 ± 0.4 to 6.0 ± 0.9 mmol/L; PRO: from 5.3 ± 0.3 to 5.9 ± 0.6 mmol/L; time x treatment interaction, P = 1.000). However, insulin concentrations increased to a greater extent in PRO when compared to CON (PRO: from 7.8 ± 2.7 to 20.6 ± 11.6 mU/L; CON: from 9.9 ± 4.0 to 15.2 ± 5.7 mU/L; time x treatment interaction, P = 0.002).

Maximal voluntary contraction

The impact of the exercise bout on MVC in the CON and PRO group is presented in Figure 2(a-c) as relative change, while absolute changes are shown as supplemental Figure (Supplemental Digital Content 2, Absolute Change in Muscle Function). After an initial decline of ~15% from pre- to 0 h post-exercise (CON: from 278 ± 49 to 241 ± 60 N·m; PRO: from 265 ± 54 to 219 ± 43 N·m), isometric MVC of the knee extensors recovered gradually over the 72 h period following exercise, with no differences between treatments (time x treatment, P = 0.56; Figure 2(a)). In contrast, after an initial decline of ~14% (CON: from 185 ± 31 to 160 ± 41 N·m; PRO: from 180 ± 34 to 155 ± 34 N·m), recovery of isokinetic MVC of the knee extensors at 90°·s^{-1} was attenuated in PRO when compared with CON (time x treatment interaction, P = 0.044; Figure 2(b)). After an initial decline of ~13% (CON: from 134 ± 27 to 118 ± 28 N·m; PRO from 129 ± 31 to 110 ± 31 N·m), also recovery of isokinetic MVC of the knee extensors at 180°·s^{-1} was attenuated in PRO when compared with CON (time x treatment interaction, P = 0.011; Figure 2(c)). In contrast to the knee extensors, MVC of the knee flexors at 90 and 180°·s-^{1} was not affected by the drop jump protocol ($P > 0.40$).

Vertical jump height

CMJ performance showed an initial decline from 33.2 ± 5.5 to 30.9 ± 7.5 cm (–7 ± 9%) and 33.8 ± 4.5 to 30.7 ± 4.8 cm (–9 ± 6%) in the CON and PRO group, respectively. CMJ performance recovered gradually over the 72 h period following exercise, with no differences between treatments (time x treatment interaction, P = 0.52; Figure 2(d)).

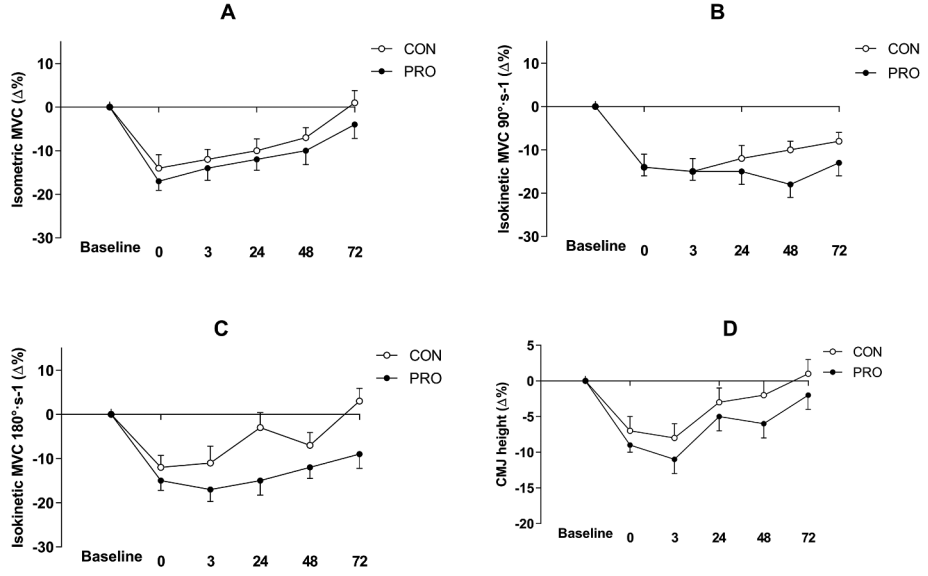

Figure 2. Relative change in muscle function from baseline at 0, 3, 24, 48 and 72 h post-exercise in CON (n = 19) and PRO (n = 20). Data are presented as mean±SEM. Panel A, Isometric maximal voluntary contraction (MVC) of the knee extensors. Mixed-model design ANOVA indicated no differences in recovery between groups (time x treatment, P = 0.56). Panel B, Isokinetic MVC of the knee extensors at 90°·s^{-1}. Mixed-model design ANOVA indicated a significant difference in recovery between CON vs. PRO (time x treatment interaction, P = 0.044). Panel C, Isokinetic MVC of the knee extensors at 180°·s^{-1}. Mixed-model design ANOVA indicated a significant difference between CON vs. PRO (time x treatment interaction, P = 0.011). Panel D, Countermovement jump performance (CMJ). Mixed-model design ANOVA indicated no differences in recovery between groups (time x treatment, P = 0.52).

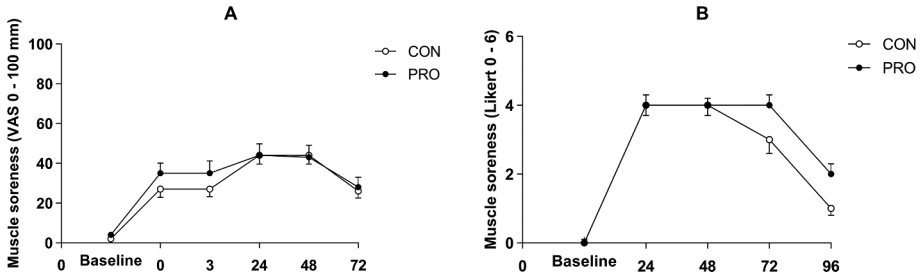

Figure 3. Muscle soreness at baseline and 0, 3, 24, 48 and 72 h post-exercise in CON (n = 19) and PRO (n = 20). Data are presented as mean±SEM. Panel A, Muscle soreness assessed by Visual Analogue Scale during a 3-second squat hold at 90º knee angle. Mixed-model design ANOVA indicated a significant increase in muscle soreness (time effect, P < 0.001), with no differences between groups (time x treatment, P = 0.28). Panel B, Muscle soreness assessed by retrospective pain questionnaire. Mixed-model design ANOVA indicated an increased muscle soreness following exercise (P < 0.001), with no differences between groups (time x treatment, P = 0.69).

Muscle soreness

The impact of the exercise bout on muscle soreness in the CON and PRO group is presented in Figure 3(a,b). Muscle soreness as assessed by VAS increased immediately following exercise, peaked at 24 h post-exercise, and remained moderately elevated throughout the remaining post-exercise period, with no differences between groups (time x treatment, P = 0.28). In line, the 7-point retrospective pain questionnaire for muscle soreness increased following exercise and remained elevated throughout the entire recovery period (P < 0.001), with no differences between groups over time (time x treatment interaction, P = 0.69).

Blood markers of muscle damage, inflammation and stress response

The effects of the exercise bout on CK, CRP and cortisol in the CON and PRO group are presented in Figure 4(a-c), respectively. CK concentrations peaked at 24 h post-exercise (CON: from 140 ± 88 to 586 ± 387 U·L; PRO: from 137 ± 92 to 594 ± 389 U·L) and remained elevated during recovery, with no differences between groups (time x treatment interaction, P = 0.88). CRP concentrations peaked at 24 h post-exercise (CON: from 769 ± 865 to 1637 ± 1108 mg·L; PRO: from 886 ± 1468 to 1752 ± 2165 mg·L) and returned to baseline values in 48 to 72 h, with no differences between groups (time x treatment interaction, P = 0.57). Cortisol levels decreased in the first 3 h post-exercise (CON: from 459 ± 87 to 210 ± 52 nmol/L; PRO: from 456 ± 102 to 233 ± 68 nmol/L), followed by a gradual return to baseline values over 72 h, with no differences between groups (time x treatment interaction, P = 0.92).

Dietary intake and physical activity

Table 2 presents the dietary intake of the participants before and during the 9-day experimental period. Total protein intake decreased from 1.2 ± 0.4 to 1.0 ± 0.2 g/kg/body mass in CON and increased from 1.1 ± 0.3 to 1.7 ± 0.3 g/kg/body mass in PRO (time x treatment interaction, P < 0.001). Total carbohydrate intake increased from 3.4 ± 1.5 to 4.4 ± 1.4 g/kg/body mass in CON, whereas no changes were observed in PRO (from 3.3 ± 1.0 to 3.1 ± 1.1 g/kg/body mass (time x treatment interaction, P = 0.001). There were no changes in energy intake over time (time effect P = 0.16) or differences between groups over time (time x treatment interaction, P = 0.95). With respect to habitual physical activity, no changes were observed over time (P ≥ 0.06 for all intensity zones) or between groups over time (time x treatment interaction, P ≥ 0.15 for all intensity zones) (Supplemental Digital Content 3, Habitual Physical Activity Level).

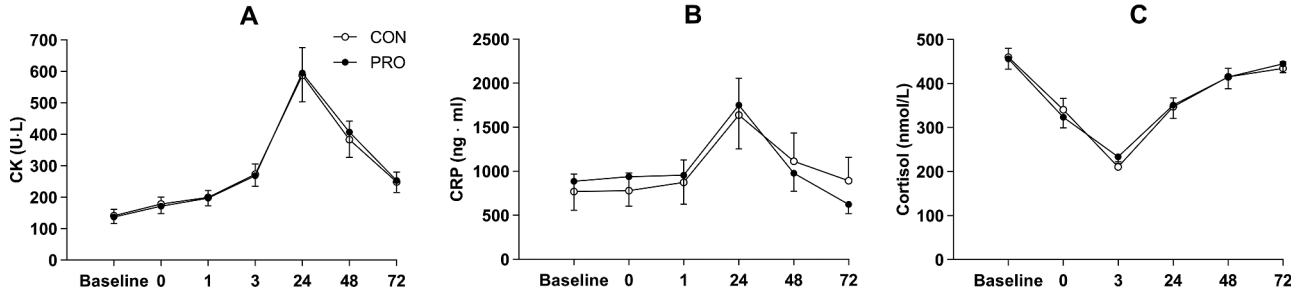

Figure 4. Blood markers of muscle damage, inflammation and stress response at baseline and 0, 3, 24, 48 and 72 h post-exercise in CON (n = 19) and PRO (n = 20). Data are presented as mean±SEM. Panel A, Creatine kinase (CK). Mixed-model design ANOVA indicated a significant increase in creatine kinase concentrations (time effect, P < 0.001), with no differences between groups (time x treatment, P = 0.88). Panel B, C-Reactive Protein (CRP). Mixed-model design ANOVA indicated a significant increase in CRP concentrations (time effect, P < 0.001), with no differences between groups (time x treatment, P = 0.57). Panel C, Cortisol. Mixed-model design ANOVA indicated a significant increase in cortisol concentrations (time effect, P < 0.001), with no differences between groups (time x treatment, P = 0.92).

Table 2. Participants' dietary intake before and during the experimental period in healthy young males receiving either placebo or protein supplementation.

	Control (n = 19)		Protein (n = 20)		P value	
	Before	During	Before	During	Time	Time x treatment
Energy (MJ)[a]	9.6 ± 3.5	9.9 ± 2.6	8.9 ± 2.9	9.4 ± 2.5	0.16	0.95
Energy (Kcal)[a]	2285 ± 832	2357 ± 625	2130 ± 681	2249 ± 608	0.16	0.95
Protein (En%)[a]	18 ± 6	13 ± 3	16 ± 3	24 ± 3	0.11	<0.001
Protein absolute (g)[a]	93 ± 29	75 ± 14	82 ± 23	129 ± 19	0.002	<0.001
Protein relative (g/kg/body mass)[a]	1.2 ± 0.4	1.0 ± 0.2	1.1 ± 0.3	1.7 ± 0.3	0.002	<0.001
Protein relative excluding supplement (g/kg/body mass)[a]	1.2 ± 0.4	1.0 ± 0.2	1.1 ± 0.3	0.9 ± 0.3	<0.001	0.67
Carbohydrate (En%)	47 ± 6	59 ± 4	48 ± 6	43 ± 5	0.02	<0.001
Carbohydrate absolute (g)[a]	265 ± 107	343 ± 100	247 ± 69	240 ± 80	0.02	0.003
Carbohydrate relative (g/kg/body mass)[a]	3.4 ± 1.5	4.4 ± 1.4	3.3 ± 1.0	3.1 ± 1.1	0.06	0.001
Carbohydrate relative excluding supplement (g/kg/body mass)[a]	3.4 ± 1.5	3.5 ± 1.4	3.3 ± 1.0	3.2 ± 1.1	0.86	0.37
Fat (En%)[a]	28 ± 8	26 ± 5	31 ± 7	32 ± 5	0.68	0.41
Fat absolute (g)[a]	76 ± 35	68 ± 23	78 ± 33	78 ± 26	0.90	0.66
Fat relative (g/kg/body mass)[a]	1.0 ± 0.5	0.9 ± 0.3	1.0 ± 0.4	1.1 ± 0.4	0.90	0.66

Data are presented as mean±SD.
[a]Non-normal distributed data were logarithmically transformed prior to analysis.
No significant differences between groups were observed at baseline.

Discussion

In the present study, we assessed the impact of protein supplementation on recovery from eccentric exercise. The eccentric exercise protocol resulted in a transient loss of muscle function that was accompanied by an increase in muscle soreness and blood markers related to muscle damage and inflammation. Daily supplementation with whey protein did not accelerate recovery of muscle function or alleviate muscle soreness and inflammation over 72 h of recovery following a single bout of eccentric exercise.

The drop jump protocol employed in the current study successfully induced muscle damage, as indicated by an initial ~15% decline in isometric and isokinetic muscle function, followed by a gradual recovery over the 72 h post-exercise period. This response is similar to other research using a drop jump protocol (Clifford et al., 2016), and comparable with downhill running or repeated sprinting protocols (Brown et al., 2018; Green et al., 2008). Next, to dynamometry, we also used CMJ height to assess muscle function in a more ecologically valid manner. The greatest decline in CMJ height (~8–10%) was observed at 3 h post-exercise, with performance returning to baseline values at 72 h post-exercise.

Although the drop jump protocol successfully induced muscle damage, no benefits of protein supplementation on subsequent recovery of muscle function were observed. In fact, the carbohydrate control intervention was associated with a faster post-exercise recovery compared with protein supplementation for two of the four measures of muscle function. This finding was rather unexpected as some studies have reported benefits (Cockburn et al., 2008, 2010; Cooke et al., 2010; Howatson et al., 2012) and other reported no impact of protein supplementation on recovery of muscle function (Brown et al., 2018; Eddens et al., 2017; Green et al., 2008; Nosaka et al., 2006). This discrepancy may be, at least partly, explained by differences in the applied study design. Most studies that observed a benefit of protein supplementation used a non-energetic placebo as a reference (Cockburn et al., 2008, 2010; Howatson et al., 2012), whereas the majority of studies that used an isoenergetic placebo as a reference reported no benefits of protein supplementation on recovery of muscle function (Brown et al., 2018; Eddens et al., 2017; Wojcik et al., 2001). These data indicate that increasing energy content, rather than protein *per se*, may support post-exercise recovery of muscle function. Furthermore, differences in exercise testing protocol may also contribute to the apparent discrepancy. The present study employed an extensive assessment of muscle function, comprising both isometric and isokinetic MVC testing, complemented with the assessment of CMJ height. The rate of recovery of muscle function appeared to be different for the specific muscle function outcomes, with the difference between the protein and carbohydrate control treatment being more pronounced for the isokinetic MVCs. This suggests that relevant differences in recovery between carbohydrates and protein may be missed when only isometric strength measures would be applied. Also, the muscle groups targeted during the assessment of isokinetic MVC may also contribute to the discrepancy in study results. We targeted the knee extensors at 90 and 180°·s^{-1}, whereas the work by Cockburn et al. (2008) targeted the knee flexors at an angular velocity of 60°·s^{-1}.

Regardless of the differences in study design between our study and previous studies, our results show that protein supplementation does not accelerate recovery from a bout of eccentric exercise. In contrast, we found that carbohydrate supplementation accelerated recovery when compared to protein supplementation. Although this finding is difficult to explain, it is tempting to speculate on the benefits of carbohydrate supplementation in the recovery of muscle function. Koopman et al. (2006) demonstrated that glycogen stores in type 1 and type 2 muscle fibres can be lowered by ~20 and ~40%, respectively, after performing 8 sets of leg press and 8 sets of leg extensions at 75% of the participants' 1 repetition maximum (1RM). The exercise protocol used in the present study may be considered even more demanding, potentially resulting in even greater decreases in type II muscle fibre glycogen content in the quadriceps. As glycogen lowering exercise is associated with a decline in isokinetic strength (Jacobs et al., 1981), muscle glycogen repletion may be a key factor in determining the time to recover from high-intensity eccentric exercise. This view is further reinforced by the finding that glycogen resynthesis is impaired after eccentric exercise (Costill et al., 1990; Widrick et al., 1992), thereby providing a physiological rationale for carbohydrate supplementation to

accelerate recovery of muscle function following muscle-damaging exercise.

Along with a transient decline in muscle function following eccentric exercise, we observed an increase in muscle soreness, which peaked at 24 to 48 h following exercise. Although some studies reported benefits of protein supplementation for muscle soreness (Abbott et al., 2019; Cockburn et al., 2010; Howatson et al., 2012), we found no evidence for alleviation of muscle soreness in the protein group compared to the carbohydrate group, which is consistent with most previous work (Brown et al., 2018; Buckley et al., 2010; Cockburn et al., 2008; Eddens et al., 2017). Furthermore, muscle-damaging exercise has been associated with a CK response, that could be attenuated by protein supplementation (Cockburn et al., 2008). However, despite a clear CK response following exercise, which peaked at ~600 IU at 24 h post-exercise, we found no differences between the protein and control group. In addition, muscle-damaging exercise is associated with an inflammatory response. This was evidenced in our study by a clear post-exercise CRP response, peaking 24 h after exercise. The time-course of CRP following exercise corresponds with the literature (Eddens et al., 2017). However, the CRP response was shown not to be influenced by protein supplementation.

Current guidelines for protein intake in athletes are mainly based on studies investigating the impact of protein supplementation on post-exercise muscle protein synthesis and muscle hypertrophy after resistance exercise training (Morton et al., 2018; Trommelen et al., 2019). Based upon findings of these studies, guidelines on the quality, dose, and timing of protein ingestion have been developed to optimize muscle adaptations and recovery (Thomas et al., 2016). In line with these guidelines, participants in the current study consumed ~20 g of high-quality protein (whey) peri-exercise and ~40 g of protein prior to sleep, resulting in a total daily protein intake of ~1.7 g/kg/body mass. Hence, the total daily dose is comparable (Cockburn et al., 2008) or even higher than previous studies (Brown et al., 2018; Eddens et al., 2017). Although the protein intake in the current study conformed to the current guidelines, post-exercise recovery of muscle function seemed to be faster in the carbohydrate control group compared with the protein treatment. This observation is incongruent with the impact of protein vs carbohydrate supplementation on muscle protein synthesis (Burd et al., 2009; Trommelen et al., 2019) and long-term adaptation to exercise training (Cermak et al., 2012). This supports the view that acute measures of muscle anabolism cannot be simply translated to recovery of muscle function (Pasiakos et al., 2014). In fact, our findings indicate an important role for carbohydrates in the recovery from eccentric exercise, when considering muscle function rather than muscle protein synthesis as primary outcome. Consequently, provision of ample amounts of carbohydrates should be considered in situations where rapid recovery of muscle function is paramount.

The novelty of the current study includes an extensive assessment of physiological and biochemical variables associated with post-exercise recovery, along with a strategic protein supplementation strategy, and proper dietary assessment and standardization procedures. Nonetheless, we should acknowledge some limitations. Given the between-subject variation in the assessment of muscle function (Hubal et al., 2007), a within-subject design (crossover) might be preferred to assess the impact of short-term nutritional interventions. However, due to the well-known repeated bout effect (McHugh, 2003), a prolonged washout period would be required, which may also increase the variation within subjects. As a consequence, we opted for a parallel group design. Furthermore, to compensate for the increase in energy intake due to protein supplementation, we selected an isoenergetic carbohydrate placebo. Consequently, it is unclear whether the current findings can be attributed to an increase in carbohydrate intake in the control group, or an increase in protein intake in the protein group. However, it seems highly unlikely that protein supplementation impaired recovery following eccentric exercise. Finally, we included a relatively homogenous population of recreationally active, healthy young males, who were not accustomed to eccentric exercise. As a result, we should be cautious to generalize our findings to other populations, such as females and well-trained or elite athletes.

In conclusion, whey protein supplementation does not accelerate recovery of muscle function or alleviate muscle soreness and inflammation over 72 h of recovery following a single bout of eccentric exercise in healthy young males.

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Disclosure statement

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Author contributions

AFMK, AMHH, and JWvD designed the study. LH, JDB, and JK collected data. EGF-V and PAMJS were responsible for the project logistics and administration. LH and JWvD conducted statistical analysis, and results were interpreted and discussed by all authors. LH wrote the initial draft. JWvD and LJCvL edited the manuscript.

All authors approved the final version of the manuscript prior to submission. LH and JWvD are accountable for data accuracy and integrity.

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