

Effect of protein quality on recovery after intense resistance training

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Abstract

Purpose The present study investigated the effects of high- versus low-quality protein supplementation on the regain of exercise performance during recovery from a period of high-intensity resistance training.

Methods In a diet-controlled crossover study, 12 resistance-trained participants performed two identical training periods, with each training period including four sessions of high-intensity resistance exercise during 5 days, while receiving either high- or low-quality protein. Prior to and at 3, 24 and 48 h after the training periods, performance was evaluated in knee extensor and flexor isometric maximal voluntary contraction (MVC), counter-movement jumping height (CMJ), and peak and mean anaerobic power. In addition, prior to and at 48 h after the training periods, performance in time-to-exhaustion at 70 % of VO_{2max} (TTE) was evaluated.

Results After the intense training periods, decrements in the order of 4–24 % were observed for MVC_{ext} , CMJ, mean anaerobic power, and TTE. In particular for TTE, this decrement in exercise performance did not attain full recovery at 48 h post-exercise. The regain of exercise performance was not dictated by type of protein supplement.

Conclusion The regain of muscle strength as well as anaerobic or aerobic performances were not markedly influenced by the type of protein supplement.

Keywords Athletic performance · Humans · Whey protein · Collagen protein · Protein quality · Strength training

Abbreviations

BM	Body mass
BCAA	Branched chain amino acid
CP	Collagen protein
CMJ	Counter-movement jumping height
EAA	Essential amino acid
FI	Fatigue index
MVC	Isometric maximal voluntary contraction
mTORC1	Mammalian target of rapamycin complex 1
NSAIDs	Non-steroidal anti-inflammatory drugs
RM	Repetition maximum
RPM	Repetitions per minute
VO_{2max}	Peak oxygen uptake
TTE	Time-to-exhaustion
VAS	Visual analog scale
WP	Whey protein

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Introduction

Duration of required recovery following intense exercise is essential for professional as well as recreational athletes, as it constitutes a constraint to the frequency of exercise bouts at a given exercise intensity. In accordance, the post-exercise recovery period following one or several sessions of high-intensity resistance exercise is often associated with temporary (few hours to several days/weeks) decrements

in physical performance, increased general fatigue, and local muscle soreness (Hoffman et al. 2010; Ratamess et al. 2003; Farup et al. 2015; Proske and Allen 2005). Therefore, strategies to accelerate recovery following exercise to increase the number of high-intensity exercise bouts and enhance performance are desirable. A number of factors may affect recovery and acute performance, including, e.g., sleep pattern, hydration level, and dietary intake (Beck et al. 2015).

In relation to dietary intake, several micronutrients (e.g., antioxidants, probiotics, and vitamins) as well as macronutrients (e.g., carbohydrate, protein, branched chain amino acids [BCAAs], and omega 3 fatty acids) have been investigated for their potential recovery-enhancing effect following intense exercise (Pasiakos et al. 2014; Michailidis et al. 2013; Tipton 2015; Beck et al. 2015). As for protein ingestion following resistance exercise, this is known to amplify the increase in fractional muscle protein synthesis rate resulting in a positive net protein balance (Biolo et al. 1997; Tipton et al. 1999; Wolfe 2006; Atherton and Smith 2012). Furthermore, high-quality protein sources, such as whey protein derived from dairy products, has been shown to be superior in stimulating muscle protein synthesis compared with low-quality protein, such as soy protein (Wilkinson et al. 2007; Hartman et al. 2007). The superiority of dairy compared with soy protein likely relates to the higher delivery of essential amino acids (EAAs) and BCAAs to the skeletal muscle cells (Volek et al. 2013; Hulmi et al. 2010). For instance, earlier reports have found EAAs (and in particular the BCAAs) to be sufficient to robustly increase muscle protein synthesis (Tipton et al. 1999). Moreover, the BCAA, leucine, has been demonstrated to be a direct stimulator of mTORC1, the nodal point in translation initiation, with concomitant increases in muscle protein synthesis (Moberg et al. 2014; Anthony et al. 2000; Churchward-Venne et al. 2012, 2014).

While the effect of EAAs/BCAAs on muscle protein synthesis and accretion of muscle mass is well described, the potential influence on acute recovery following resistance exercise remains less investigated (Pasiakos et al. 2014). However, augmented protein synthesis could potentially accelerate regeneration of myocellular protein structures exposed to tear from prior high-intensity resistance exercise training (Hylldahl and Hubal 2014). The effect of whey protein supplementation on the recovery of muscle force production and attenuated muscle soreness following single-bout high-intensity eccentric exercise has been recently investigated, with some studies showing signs of recovery-enhancing effects (Kirby et al. 2012; Jackman et al. 2010; Buckley et al. 2010), while others do not (Farup et al. 2014).

A few studies have indicated a positive effect of protein supplementation or high amounts of dietary protein on

recovery of performance after a short period of endurance (Hansen et al. 2015; Witard et al. 2011, 2014) or resistance (Hoffman et al. 2010; Ratamess et al. 2003) training. Among studies using resistance exercise training, most of these have not directly assessed muscle performance or only included measures of maximal muscle force production. Such studies are, therefore, largely unable to evaluate the recovery of performances dependent on the anaerobic as well as the aerobic energy systems. However, performance reliant on anaerobic and aerobic metabolism may be severely compromised in the recovery period following high-intensity resistance exercise. For instance, skeletal muscle glucose uptake, signaling for glucose uptake, and glycogen synthesis have previously been reported to be attenuated after high-intensity exercise (Asp et al. 1995, 1996). Furthermore, a recurring limitation in several of the abovementioned studies is a lack of dietary control, in particular in relation to dietary protein intake, which may markedly confound the interpretation of the results (Ratamess et al. 2003; Hoffman et al. 2010; Sharp and Pearson 2010). In fact, the previous studies have highlighted the importance of an overall positive nitrogen balance, when assessing the effect of protein supplementation on regain of performance decrements following strenuous exercise, thereby underlining the importance of dietary control (Nelson et al. 2012).

Consequently, well-controlled studies examining the effect of high-quality protein supplementation during a period of intense resistance exercise training on acute aerobic and anaerobic performance measures are warranted. The aim of this study was, therefore, to investigate the effects of high- versus low-quality (based on BCAA content) protein on recovery of diverse performance measures following a period of intense resistance training. We hypothesized that supplementation of high-quality protein (whey) would accelerate recovery of muscle strength, anaerobic power, and aerobic capacity, compared with supplementation of low-quality protein (collagen).

Methods

Participants

Twelve young healthy men (mean \pm standard deviation; age: 24.6 ± 2.1 years; weight: 79.4 ± 9.3 kg; height: 183.5 ± 5.5 cm) were included in this study. Physical activity level, assessed by questionnaire, revealed that subjects were accustomed to resistance training (recreationally training high-intensity resistance exercise 2–3 times per week for at least 1 year prior to inclusion).

All participants received detailed written and oral information of the purpose and the possible risks of procedures

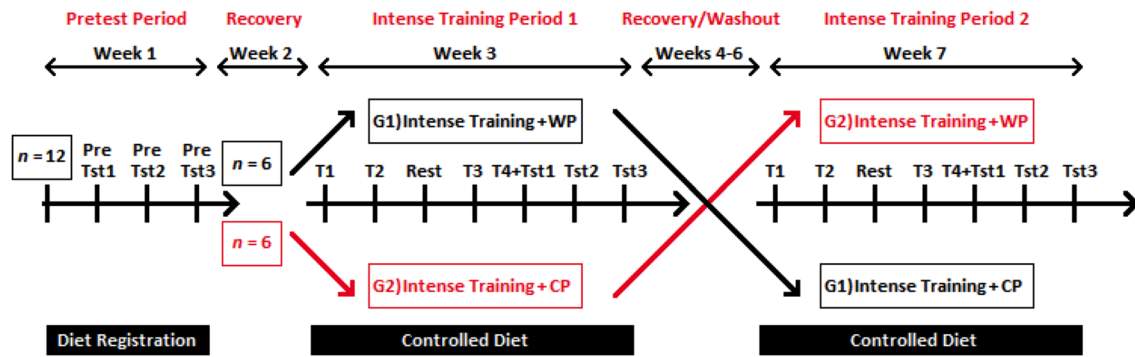


Fig. 1 Schematic overview of the study design. The overall study period consisted of seven consecutive weeks. Pre-tests (Pre Tst1–Pre Tst3) were made in week 1, week 2 was a recovery week, and week 3 and 7 were the two intense training periods consisting of

four identical training sessions (T1–T4) and three post-test sessions (Tst1–Tst3). In weeks 4–6, a 3-week recovery/washout period was completed

and gave their written consent to participate. The study was approved by The Central Denmark Region Committees on Health Research Ethics (Ref. No. 1-10-72-145-15) and was carried out in accordance with the declaration of Helsinki. Exclusion criteria comprised: (1) 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); (2) a history of musculoskeletal pain or injuries; and (3) 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).

Study design

The study design was conducted as a double blinded, randomized, crossover study (Fig. 1). Following inclusion, the participants were randomly allocated to receive either whey protein (WP) or collagen protein (CP) in the first training period, whereafter the participants switched to the other protein type in the second training period.

The study was initiated with a pre-test week, followed by 1 week of recovery. This was then followed by the first week of intense resistance training, including intake of either WP or CP. Next, the participants had a second recovery period (3 weeks), during which the participants were instructed to maintain their habitual activity levels. After the recovery period, the second week of intense resistance training was completed, including intake of the WP or CP (opposite to the type of protein ingested in the first training week). Following the final resistance exercise bout in each week, three performance tests (Tst1–3, described below) were completed to assess recovery 3, 24, and 48 h following the last training session.

In the pre-test period, all participants underwent 3 days of performance tests to determine baseline values for various aerobic and anaerobic performance variables. All participants were instructed to refrain from strenuous physical activity for 48 h before the first baseline tests and before the first training days in each of the intense training periods. On day 1 (Pre Tst1), the participants reported to the laboratory in the postprandial state (i.e., approximately 2 h since last meal) and rested for 20 min. After this, an incremental maximal oxygen consumption-test on a cycling ergometer (SRM-ergometer, Ingenieurbuero Schoberer GmbH, Juelich, Germany) was performed. On day 2 (Pre Tst2), dynamic strength was tested via three repetition maximum (3RM) strength tests in each of the five different exercises (the same strength exercises as conducted during training weeks). Finally, on day 3 (Pre Tst3), the participants underwent testing of maximal isometric strength (maximal voluntary contractions, MVC) for the knee extensors and knee flexors, counter-movement jump (CMJ), Wingate 30 s all-out anaerobic test, and an aerobic time-to-exhaustion (TTE) test at 70 % of $\text{VO}_{2\text{max}}$. Adequate rest was given between each test. MVC, CMJ, and Wingate 30 s all-out tests were all repeated at 3 (Tst1), 24 (Tst2), and 48 h (Tst3) after the final (fourth) exercise bout in each of the training weeks (Fig. 1). TTE was only conducted at 48 h after the final exercise bout of each training week.

During the final 3 days of the pre-test period, the participants were asked to weigh and report all ingested energy-containing foods and drinks. In the intense training weeks, all participants received food, which for each participant was identical on intense training week one and two. Thirty minutes before and immediately after each exercise session, the participants ingested the WP or CP supplement. On non-exercising days (rest day and Tst2), the participants ingested a supplement serving corresponding to the same absolute time point as on exercise days.

Table 1 Amino-acid distribution

Amino acids	% AA in whey	% AA in collagen	g AA/whey sup.	g AA/collagen sup.
Alanine	5.3	8.1	1.5	2.3
Arginine	2.6	8.4	0.7	2.4
Aspartic acid	11.6	6.6	3.2	1.9
Cystein + cysteine	2.5	–	0.7	–
Glutamic acid	18.3	12.4	5.1	3.5
Glycine	1.9	20.6	0.5	5.8
Histidine*	1.9	0.8	0.5	0.2
Hydroxylysine	–	1.2	–	0.3
Hydroxyproline	–	11.4	–	3.2
Isoleucine*	6.5	1.5	1.8	0.4
Leucine*	11	2.9	3.1	0.8
Lysine*	9.5	3.4	2.6	1.0
Methionine*	2.3	0.6	0.6	0.2
Phenylalanine*	3.3	2.1	0.9	0.6
Proline	6.3	11.5	1.8	3.2
Serine	5.4	3.4	1.5	1.0
Threonine*	7.6	1.9	2.1	0.5
Thryptophane*	1.9	–	0.5	–
Tyrosine	3	0.5	0.8	0.1
Valine*	6	2.4	1.7	0.7
Total EAA	50	15.6	13.9	4.4
Total BCAA	23.5	6.8	6.5	1.9

Essential amino acids are denoted by *. Branched Chain amino acids are denoted by **

Exercise protocol

During each workout session, the participants performed a supervised full-body resistance training program consisting of five exercises: (1) five sets of Leg press, (2) four sets of Bench press, (3) four sets of Pull down, (4) three sets of isolated Knee flexion, and (5) three sets of isolated Knee extension. After a brief warm-up, the participants began the exercises in the listed order. Work load was 15RM, with inter-set recovery set to 90 s and with 5 min of recovery between exercises. A set was completed when reaching 12 repetitions or as many fewer repetitions as possible before reaching volitional fatigue. All workout sessions (T1–4) were identical (i.e., identical work load and inter-set recovery periods). The number of repetitions was registered during the sessions.

Diet and supplementation

Participants recorded all energy-containing food and drinks on the final 3 days of the pre-test period, while maintaining normal habitual food intake during the recording period. Participants received a login to an online food registration software program (Madlog.dk Aps, Kolding, Denmark) and

received verbal and written information on the usage of the program before the recording period. Dietary distribution of macronutrients and intake of total energy were analyzed following the recording period and were used in conjunction with guidelines from the Danish Health Authority to compose a standardized diet for the training periods (Norden 2012). Prior to initiating each training period, the participants reported to the laboratory to receive foods for the entire week, with the amounts based on the estimates of the daily energy expenditure of the participants (ten Haaf and Weijs 2014) and validated by the diet registration. During the intense training periods, the participants received 1.4 g protein/kg bodyweight through the standardized diet (excluding the protein supplementation), which is in line with recommendations for protein intake among athletes (Phillips 2012).

Added to this, each supplementary drink contained 25 g of protein, increasing the daily protein intake to 2.0 g protein/kg bodyweight. The amino-acid constituents of the whey protein (Arla Foods Ingredients Group P/S, Viby J., Denmark) and the collagen protein (Rousselot Gelatinas do Brasil S.A, Brazil) are shown in Table 1. These data were provided by the suppliers and relied on to be in accordance with the EU regulation concerning the methods of

sampling and analysis for the official control of feed [Regulation TCOTEC-C (2009) Commission Regulation (EC) No 152/2009. European Commission, Brussels].

Maximal aerobic capacity

To establish the exact work rate for the time-to-exhaustion test, resting oxygen consumption, submaximal oxygen consumption (VO_2) at various submaximal work rates, as well as maximal oxygen consumption ($\text{VO}_{2\text{max}}$) were measured and the corresponding work rate was noted. A linear regression curve was then calculated for each participant to calculate a target Watt corresponding to 70 % of $\text{VO}_{2\text{max}}$.

Exercise VO_2 measures were collected while exercising on the cycle ergometer. The settings of the bike ergometer were recorded for each participant to make sure that the settings were identical for each test at baseline, and at follow-up. oxygen consumption, CO_2 production, and ventilation rate were measured continuously and were recorded as averages of 30-s intervals (Oxycon Pro, Erich Jaeger GmbH, Hoechberg, Germany).

Resting VO_2 was measured at the end of a 15 min of rest period. Submaximal VO_2 measures were then collected at the end of a 5-min workout at 50, 100, and 150 W, respectively. After a brief break, participants performed an incremental bike exercise test until exhaustion to assess $\text{VO}_{2\text{max}}$. Participants began exercising at 150 W with the work increasing 30 W every 60 s until exhaustion. The participants were verbally encouraged to exercise for as long as possible. $\text{VO}_{2\text{max}}$ was defined as the highest attainable level of VO_2 during the incremental test (30 s averaging interval).

Maximal voluntary contraction

Isometric MVC of the knee extensors and knee flexors was examined in an isokinetic dynamometer (Humac Norm, CSMI, Stoughton, USA). The participants were seated with 90° hip flexion and restraining straps crossing the torso, hips, and thighs. The transverse axis of the knee was aligned with the axis of the dynamometer. The dominant leg was attached to the dynamometer arm, while the contralateral leg was placed behind a stabilization bar. The participants were instructed to grab the chair handles. The dynamometer was adjusted individually, so that the contact point between the leg and the dynamometer arm was 3 cm proximal to the malleolus medialis. The settings of the chair were recorded for each participant to ensure identical settings at baseline and at follow-up.

The MVC was measured by four maximal isometric knee extensor contractions at 70° knee flexion (0° equivalent to full extension) interspaced by 1 min of recovery, followed by four isometric knee flexor contractions at

40° knee flexion interspaced by 1 min of recovery. The researchers applied strong verbal encouragement and online visual feedback during the contractions. The highest peak torque of the four attempts was used for further analysis.

Counter-movement jump

Counter-movement jump (CMJ) was performed on a jumping mat (Speedmat, Swiftperformance, Australia) to assess maximal jumping height (i.e., maximal power output during the jump). Participants were given four attempts interspaced by 1 min of recovery. The best of the four attempts was used for further analysis.

Repetition maximum assessment

To estimate the intensity needed in the training period, a 3RM test was conducted in each of the five exercises; (1) leg press; (2) bench press; (3) pull down; (4) knee flexion; and (5) knee extension. From the 3RM, a 15RM was calculated using Brzycki's equation (Brzycki 1993). The 3RM test was chosen over a 15RM test to avoid the risk of developing metabolic fatigue during the test when multiple attempts were needed. This ensured optimal performance in each attempt.

After a brief warm-up, the participants performed a set of three repetitions in the respective exercise. The initial load was determined by asking the participants (i.e., all participants were experienced in the exercises). If the participant could complete the three repetitions, more loading was applied (approximately 5–10 % dependent on exercise). This procedure was repeated until the true 3RM was found. Recovery periods between attempts were three min.

Anaerobic performance

To assess each participant's anaerobic power and capacity, a 30-s all-out sprint test (Wingate test) was performed on the same bike ergometer as the incremental test. The isokinetic mode of the cycle ergometer was used with an upper limit for cadence at 120 rpm, i.e., when reaching 120-rpm additional resistance was added by the ergometer to increase power output.

After a 5-min warm-up at a low intensity at 100 rpm, participants accelerated up to 120 rpm and after a 10-s count-down, the 30-s all-out sprint test was initiated. The participants were verbally encouraged to exercise as forcefully as possible throughout the test. Both the peak power and mean power from the test were used for further analysis. To measure the level of fatigue during the anaerobic test, the fatigue index (FI) was calculated (Laurent et al. 2007). FI was

determined as the percentage difference between maximal and minimum power outputs during the 30-s all-out sprint.

Aerobic endurance performance

The participants performed the TTE on the same bike ergometer as the incremental test at a constant Watt load corresponding to 70 % of the predetermined $\text{VO}_{2\text{max}}$. The participants were verbally encouraged to exercise until volitional exhaustion. The test was terminated when participants could no longer maintain a cadence at the predetermined Watt load. Inability to maintain cadence was defined as a decrease in rpm by 10 % for more than 20 s. Ad libitum water was available during the test.

Muscle soreness

Muscle soreness in the lower extremity was evaluated before commencing each workout and each test session in the intense training periods. Participants refrained from strenuous physical activity for 48 h before the first evaluation (T1). Participants were asked to perform three functional movements (rising from and sitting down in a chair, walk up a flight of stairs, and walk down a flight of stairs). The participants then evaluated lower extremity muscle soreness on a visual analog scale (VAS) of 100 mm going from no pain at all (0 mm) to worst possible pain (100 mm), previously described (Bijur et al. 2001).

Statistical analyses

We expected a within-subject difference of 8–12 % between the two conditions for the primary outcomes, MVC, and TTE (Farup et al. 2014; Kirby et al. 2012; Hansen et al. 2015), and a within-subject standard deviation (SD) of 8 %. Using a power of 0.8 and alpha level of 0.05, this indicated that a sample size of 10–12 participants was sufficient to detect the expected changes.

Data were first checked for normality of distribution and equal variance. Statistical analyses were performed using the Stata (Stata v 14, StataCorp LP, College Station, TX, USA). Differences in total repetitions and time-to-exhaustion were determined using a paired *t* test. The interaction and main effects of time (pre, 3, 24 and 48 h) and/or group (WP and CP) on dependent variables (VAS, muscle strength, CMJ height and anaerobic power) were assessed using a mixed-effect two-way analysis of variance with repeated measures using subject and subject \times time as random effects. Linear comparison post-hoc analysis was used to evaluate pairwise differences within each dependent variable. Results are expressed as mean \pm standard error of the mean or SD in brackets (only for descriptive baseline data). Alpha level was set to ≤ 0.05 .

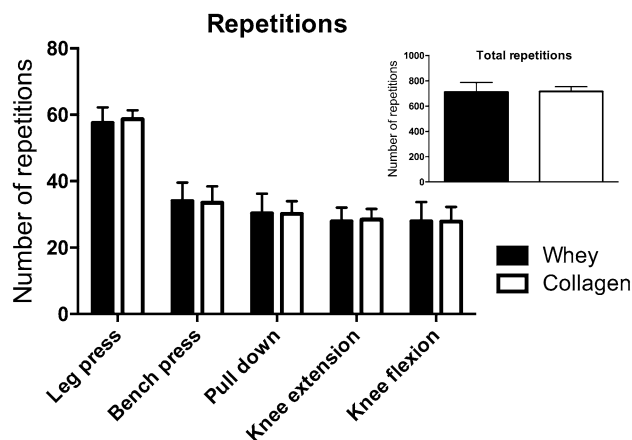


Fig. 2 Repetitions. Repetitions per training session and total repetitions during the entire training period for whey and collagen protein groups. Data are presented as mean and standard deviation

Results

Baseline characteristics

Participants achieved a $\text{VO}_{2\text{max}}$ of 4398.2 (354.5) ml O_2/min or 55.8 (5.5) ml $\text{O}_2/\text{kg}/\text{min}$ in the incremental bike test. Participants 3RMs were as follows: 219 (39) kg in the leg press; 90 (18) kg in the bench press; 89 (12) kg in the pull down; 64 (6) kg in the knee flexion; and 111 (13) kg in the knee extension.

Average daily habitual food intake during the 3 days of diet registration consisted of a total energy consumption of 3102.4 (405.7) kcal, with energy contribution from macronutrients distributed as follows: 19.4 (2.5) % from protein; 47.8 (6.4) % from carbohydrate; and 32.9 (6.0) % from fat. Participant's macronutrient intake (relative to body mass) was 1.8 (0.3) g/kg for protein, 4.7 (1.3) g/kg for carbohydrate, and 1.4 (0.4) g/kg for fat.

Training compliance, workload, and diet during training weeks

All participants completed the eight training sessions, equivalent to 100 % compliance. The mean number of repetitions per training session as well as total repetitions for the entire training period did not differ between WP and CP groups (see Fig. 2).

Average daily food intake (without protein supplementation) during training weeks consisted of a total energy content of 2835.7 (333.7) kcal. Within recommended range of the nutritional guidelines (Norden 2012), energy contribution from macronutrients was standardized as follows: 15 % from protein; 55 % from carbohydrate; and 30 % from fat. When expressed per body mass (BM), the participants ingested 1.4 g/kg BM for protein, 5.0 g/kg BM for

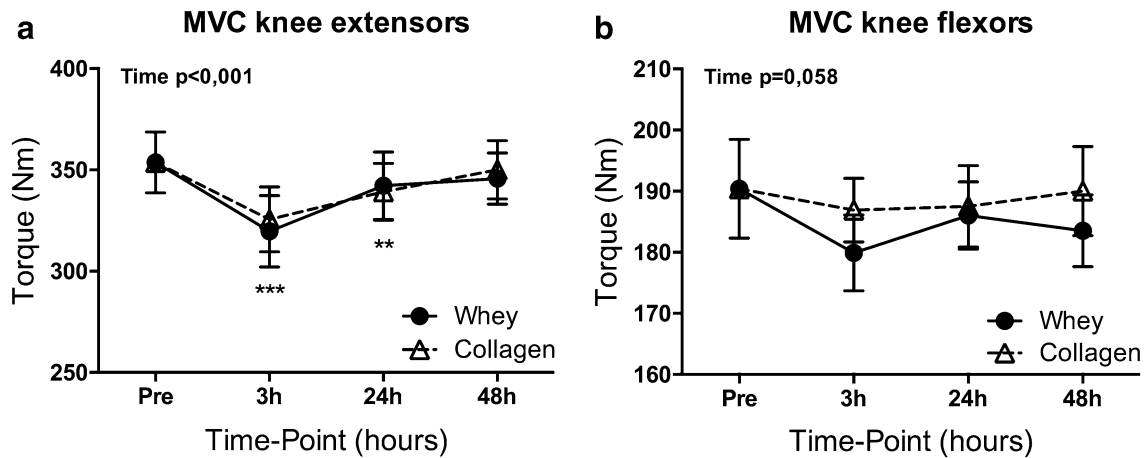


Fig. 3 Maximal voluntary contraction. Summary of changes in maximal voluntary isometric contractions at **a** 70° knee angle for knee extensors and **b** 40° knee angle for knee flexors at 3, 24, and 48 h following the final exercise bout for whey or collagen protein groups.

Data are shown as mean change and standard error of the mean (*error bars*). Overall effect of time is shown in *upper left corner*. Significant difference from pre-exercise is denoted by * $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$

carbohydrate, and 2.6 g/kg BM for fat during the training weeks. When including protein supplementation, the participant’s total energy intake was 3059.7 (333.7) kcal. The total protein intake was 2.0 (0.1) g/kg BM.

Maximal voluntary contraction

For MVC_{ext} , a time effect was observed ($P < 0.001$, Fig. 3a). Post-hoc analysis revealed a 10 ± 2 and 8 ± 2 % ($P < 0.001$) decrease in MVC_{ext} at 3 h and 3 ± 2 and 4 ± 2 % ($P < 0.01$) decrease at 24 h following the final exercise bout for WP and CP groups, respectively. At 48 h, MVC_{ext} had returned to baseline in both groups. No differences between groups were observed at any time points.

For MVC_{flex} no time effect was observed; however, a tendency towards a time effect was revealed ($P = 0.058$, Fig. 3b). No differences between groups were observed at any time points.

Counter-movement jump

A significant time x group interaction was observed for CMJ height ($P < 0.05$, Fig. 4).

For WP group, post-hoc analysis revealed a 4 ± 2 % ($P < 0.01$) decrease in CMJ height at 3 h, 4 ± 2 % ($P < 0.01$) at 24 h and 3 ± 2 % ($P < 0.05$) at 48 h following the final exercise bout. For CP group, post-hoc analysis revealed 6 ± 1 % ($P < 0.001$) decrease in CMJ height at 3 h and 4 ± 2 % ($P < 0.01$) decrease in CMJ height at 24 h following the final exercise bout. By 48 h, CMJ height had returned to baseline value for CP group. Difference between groups was observed at 3 h following the final

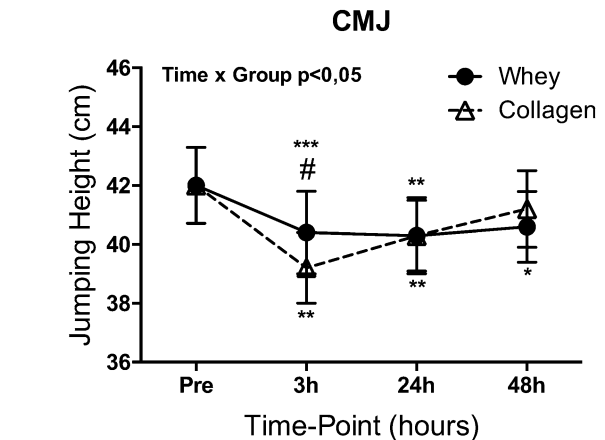


Fig. 4 Counter-movement jump. Summary of changes in jumping height at 3, 24, and 48 h following the final exercise bout for whey or collagen protein groups. Data are shown as mean change and standard error of the mean (*error bars*). Group \times time interaction is shown in *upper left corner*. Significant differences between groups are denoted by # $P < 0.05$. Significant difference from pre-exercise is denoted by * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ (*upper* is CP and *lower* is WP)

exercise bout, with WP displaying an attenuated loss of CMJ height compared with the CP group ($P < 0.05$).

Anaerobic performance

For peak anaerobic power, no time effect was observed; however, a tendency towards a time effect was noted ($P = 0.058$, Fig. 5a). No difference between groups was observed at any time points.

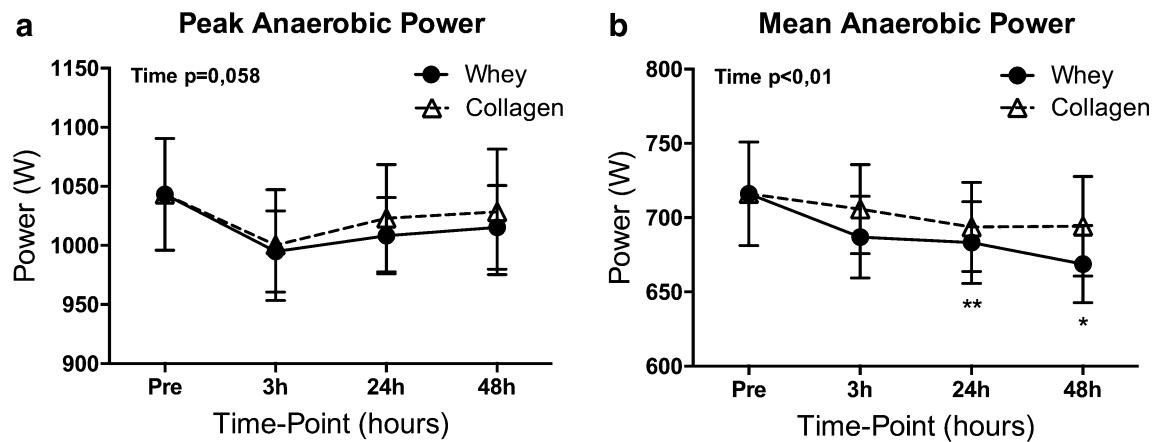


Fig. 5 Anaerobic power. Summary of changes in **a** peak anaerobic power and **b** mean anaerobic power during a 30-s all-out bike sprint at 3, 24, and 48 h following the final exercise bout for whey or collagen protein groups. Data are shown as mean change and stand-

ard error of the mean (*error bars*). Overall effect of time is shown in *upper left corner*. Significant difference from pre-exercise are denoted by * $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$

For mean anaerobic power, a time effect was observed ($P < 0.01$, Fig. 5b). Post-hoc analysis revealed no difference between baseline and 3 h, however, a 4 ± 2 and 3 ± 2 % ($P < 0.001$) decrease from baseline in mean anaerobic power was observed at 24 h and a 6 ± 2 and 3 ± 1 % ($P < 0.01$) decrease in mean anaerobic power at 48 h for WP and CP groups, respectively. No differences between groups were observed at any time points.

For FI, no significant change was observed for group \times time interaction or time alone.

Aerobic endurance performance

Time-to-exhaustion at 70 % of VO_{2max} decreased from 48.3 ± 3.1 min at baseline to 37.5 ± 4.8 and 36.8 ± 4.3 min (24 ± 7 and 24 ± 6 %) at 48 h following the final exercise bout for WP and CP groups, respectively ($P < 0.001$, Fig. 6). No group differences were observed ($P = 0.84$).

Muscle soreness

For muscle soreness, evaluated by VAS during both chair squats and walking up or down stairs, a time effect was observed for all three measures ($P < 0.001$, Fig. 7a–c). For VAS_{chair} , post-hoc analysis revealed an increase in muscle soreness from baseline to all other time points ($P < 0.001$) except at 48 h following the final exercise bout at which time the muscle soreness had returned to baseline levels. For VAS_{up} and VAS_{down} , post-hoc analysis revealed an increase in muscle soreness from baseline to T2, T3, T4, and 3 h, ($P < 0.001$) as well as 24 h ($P < 0.01$) following the final exercise bout. No differences between groups were observed at any time points.

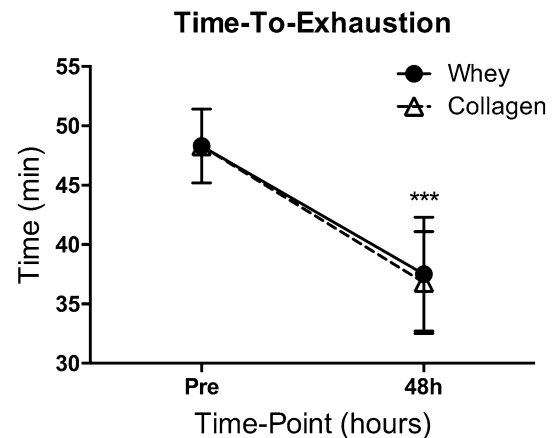


Fig. 6 Time-to-exhaustion. Summary of changes in time-to-exhaustion in cycling at 70 % VO_{2max} at 48 h following the final exercise bout for whey or collagen protein groups. Data are shown as mean change and standard error of the mean (*error bars*). Significant difference from pre-exercise is denoted by * $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$

Discussion

In this study, we evaluated the effect of high-quality whey protein versus low-quality collagen protein supplementation on the regain of divergent exercise performance parameters following an intensive period of resistance training. By this approach, we found that maximal muscle force production, as well as performances reliant on anaerobic and aerobic energy systems, was all impaired during the immediate 48 h of recovery. However, contrary to our hypothesis, high-quality whey protein supplementation did not accelerate the regain of exercise performance compared

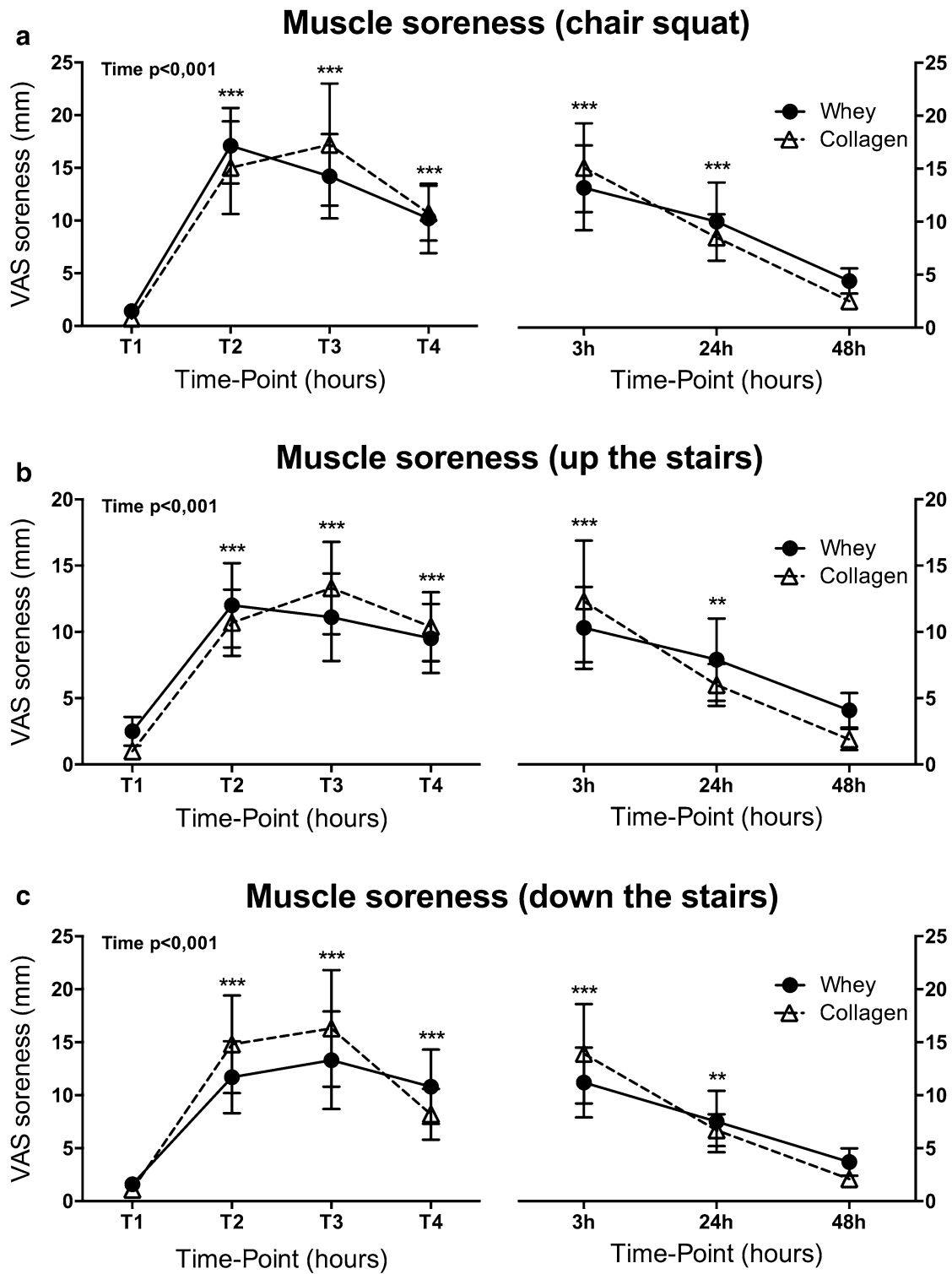


Fig. 7 Muscle soreness. Summary of changes in subjective muscle soreness [visual analog scale (VAS), in mm] during **a** chair squats, **b** walking up, and **c** walking down a flight of stairs before each identical training session (T1–T4) and at 3, 24, and 48 h following the final exercise bout for whey or collagen protein groups. Data are

shown as mean change and standard error of the mean (*error bars*). Overall effect of time is shown in *upper left corner*. Significant difference from pre-exercise is denoted by * $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$

with low-quality collagen protein. While the decrement in jumping performance (CMJ) was attenuated during immediate recovery in the WP compared with the CP, this finding was not supported by MVC or anaerobic power data, indicating that this may be a trivial or random finding.

Earlier studies have indicated that supplementation with whey protein or BCAAs may influence the regain of exercise performance and attenuate muscle soreness following a short period of intense resistance training (Hoffman et al. 2010; Ratamess et al. 2003; Sharp and Pearson 2010) or endurance exercise training (Hansen et al. 2015). In accordance, in training-accustomed participants, Ratamess et al. (2003) found improvements in 1RM muscle strength and ballistic bench-press peak power after 4 weeks of high-volume resistance training when receiving amino-acid supplementation compared with placebo. Similarly, Hoffman et al. (2010) found improvements in the number of squat repetitions at 24 and 48 h post-exercise in strength/power athletes receiving whey protein compared with placebo. These results indicate an improved recovery when ingesting protein high in BCAAs and leucine, which may relate to enhanced post-exercise muscle protein synthesis to support repair of damaged cytoskeletal or myofibrillar structures (Nelson et al. 2012; Atherton and Smith 2012). As for the latter, no studies have, to our knowledge, assessed if muscle protein turnover (i.e., increased net protein synthesis) can explain a potential positive effect of protein ingestion on recovery of exercise performance. In contrast to these studies, we observed no effect of supplementation with different types of protein on muscle strength, anaerobic power, or aerobic capacity. For CMJ, we observed an attenuated decrement in jumping height 3 h following the final exercise bout, however, this was not supported by findings in MVC or anaerobic power performances. Thus, although a similar attenuated decrement in jumping height has been reported earlier (Skillen et al. 2008), our results indicate that this may be a random finding.

We were not able to include a non-protein-supplement control group in this study. Therefore, we cannot exclude the possibility that the EAA and BCAA content in the collagen supplement and the provided controlled diet were enough to support a positive net muscle protein balance and thus enhance recovery following exercise. Previous studies have demonstrated the importance of maintaining a positive nitrogen balance for recovery after strenuous exercise (Nelson et al. 2012; Thomson et al. 2011). Accordingly, supplementing with additional BCAAs, when in a negative nitrogen balance, has been reported to lead to better recovery of cycling performance compared with carbohydrate (Thomson et al. 2011). In contrast, additional BCAAs seemingly do not accelerate recovery of cycling performance, when in a positive nitrogen balance (Nelson et al. 2012). In relation to this study, we speculate if the BCAA content from the

dietary protein was sufficient to provide a positive nitrogen balance, which may explain why the type of protein supplementation did not enhance recovery. Consequently, our findings question the importance of additional protein supplementation for recovery enhancement when in a state of positive nitrogen balance mediated through a normal dietary protein intake.

A limitation to most previous studies is the focus on maximal muscle force to evaluate the recovery, while the evaluation of performance measures relying on anaerobic glycolysis or aerobic metabolism has been neglected. However, such performance measures may be important as high-intensity resistance exercise (e.g., eccentric exercise) has been shown to induce a temporary impairment in glucose tolerance (Green et al. 2010), which may be attributable to changes in sarcolemma properties. Supporting such a notion, GLUT-4 protein levels has previously been observed to decrease following eccentric exercise (Asp et al. 1996; Asp et al. 1995), which may negatively affect skeletal muscle glucose metabolism. Collectively, glycogen re-synthesis may constitute a limiting factor for the regain of glycogen-dependent anaerobic and aerobic performances. Based on these findings, we speculated if glycogen re-synthesis could be impaired following an intense period of resistance training and that this would negatively influence anaerobic power and TTE performance. Our findings on TTE support that this speculation, as a substantial decrement in TTE performance, was observed at 48 h, at which time point which the muscle strength (MVC) had returned to baseline levels. These findings support that different mechanisms are responsible for the decrements in TTE versus MVC performances. While we did not directly measure muscle glucose uptake or glycogen synthesis, we speculate that the post-training decrease in the TTE performance relates to an impaired glucose uptake as reported following eccentric exercise (Green et al. 2010). Therefore, although the resistance exercise is not expected to deplete the muscle glycogen stores and participants ingested 5-g carbohydrate/kg BW during the training period, our results indicate that the glycogen availability may have been impaired following the period of intense resistance exercise. Notably, in our case, the whey protein was not superior in attenuating the decrement in TTE performance compared with collagen protein, despite a higher content of BCAAs.

Importantly, in contrast to most previous studies (Sharp and Pearson 2010; Ratamess et al. 2003; Hoffman et al. 2010; Kirby et al. 2012), we strictly controlled the subjects diet and in particular dietary protein intake during the training weeks. Moreover, our crossover design enabled us to ensure that each subject received the same amount of carbohydrate, fat, and protein per kg bodyweight during the two training weeks from exactly the same food sources, which practically eliminated the diet of the participants

as a potential confounder. For the same reason, we chose collagen protein as the placebo supplementation instead of carbohydrate. By this approach, we ensured that participants ingested exactly the same amount of nitrogen, while ingesting different amounts of BCAA, which is known to positively stimulate muscle protein synthesis and lead to muscular hypertrophy (Hartman et al. 2007; Wilkinson et al. 2007). As this study did not include measures of muscle protein synthesis or glucose metabolism, we are unable to directly relate these to the regain of aerobic exercise performance. Future studies should seek to include such measures.

As this study aimed to investigate the effect of protein quality, we did not include additional carbohydrate or other compounds (e.g., beta-hydroxy-beta-methylbutyrate; HMB). Therefore, we cannot exclude that other macronutrients or metabolites from amino acids may have recovery-enhancing effects as recently indicated by Kraemer et al. (2015). For instance, enhancement in glycogen re-synthesis could potentially augment performance in TTE, which was substantially impaired in this study.

In summary, strength-, anaerobic- and aerobic performance was markedly reduced following a period of high-intensity resistance training. However, high-quality whey protein supplementation did not exert noteworthy improvements in the regain of exercise performance compared with low-quality collagen protein.

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Compliance with ethical standards

Conflict of interest None declared by the authors.

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