



## Original paper

# Supplementation with a whey protein hydrolysate enhances recovery of muscle force-generating capacity following eccentric exercise

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## Abstract

There is evidence that protein hydrolysates can speed tissue repair following damage and may therefore be useful for accelerating recovery from exercise induced muscle damage. The potential for a hydrolysate (WPI<sub>HD</sub>) of whey protein isolate (WPI) to speed recovery following eccentric exercise was evaluated by assessing effects on recovery of peak isometric torque (PIT). In a double-blind randomised parallel trial, 28 sedentary males had muscle soreness (MS), serum creatine kinase (CK) activity, plasma TNF $\alpha$ , and PIT assessed at baseline and after 100 maximal eccentric contractions (ECC) of their knee extensors. Participants then consumed 250 ml of flavoured water (FW;  $n = 11$ ), or FW containing 25 g WPI ( $n = 11$ ) or 25 g WPI<sub>HD</sub> ( $n = 6$ ) and the assessments were repeated 1, 2, 6 and 24 h later. PIT decreased  $\sim 23\%$  following ECC, remained suppressed in FW and WPI, but recovered fully in WPI<sub>HD</sub> by 6 h ( $P = 0.006$ , treatment  $\times$  time interaction). MS increased following ECC ( $P < 0.001$  for time), and remained elevated with no difference between groups ( $P = 0.61$ ). TNF $\alpha$  and CK did not change ( $P > 0.45$ ). WPI<sub>HD</sub> may be a useful supplement for assisting athletes to recover from fatiguing eccentric exercise.

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## 1. Introduction

The high mechanical forces experienced by skeletal muscle tissue during intense exercise, particularly eccentric exercise, can induce muscle damage which is followed by an acute inflammatory response, the development of pain, and a loss of muscle force-generating capacity.<sup>1,2</sup>

Recent evidence suggests that protein hydrolysates can accelerate the repair of damaged tissue,<sup>3</sup> with 8 weeks of supplementation with a collagen protein hydrolysate almost doubling the rate of healing of pressure ulcers compared with a placebo.<sup>3</sup> Given that some of the loss of force-generating

capacity of skeletal muscle following eccentric exercise is associated with tissue damage, and protein hydrolysates may accelerate the repair of damaged tissue,<sup>3</sup> it is possible that protein hydrolysates may be useful for speeding recovery of force-generating capacity following eccentric exercise. The purpose of this study was to determine whether supplementation with a hydrolysed form of whey protein isolate (WPI<sub>HD</sub>) could speed recovery of muscle force-generating capacity following eccentric exercise.

## 2. Methods

Forty-three sedentary males aged 18–30 years were recruited by public advertisement. The study used a randomised, double-blind, placebo-controlled, parallel design. Volunteers reported to the laboratory after an overnight fast. Assessments of the peak isometric torque (PIT) of the knee

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extensor muscles of the right leg, as well as muscle soreness, serum creatine kinase activity (CK, marker of muscle damage) and plasma TNF $\alpha$  concentrations (marker of inflammation), were assessed prior to the performance of 100 maximal eccentric contractions (ECCs) of the knee extensors of the right leg, immediately after ECC, and at 1, 2, 6 and 24 h post-ECC. Following the assessments immediately after the completion of ECC, volunteers were randomly allocated to consume 250 ml of a flavoured water placebo ( $n = 14$ , FW) or 250 ml of FW containing 25 g of whey protein isolate (WPI;  $n = 14$ ), or 25 g of WPI<sub>HD</sub> ( $n = 15$ ), and then consumed the same supplement again immediately after the assessments at 6 h, and 2 h prior to the assessments at 24 h.

Volunteers were excluded if they participated in regular physical activity more than once per week for the purpose of improving or maintaining their physical fitness; had undertaken resistance training of the quadricep muscles during the previous 3 months; had a knee, quadricep or other musculoskeletal or medical problem which might have interfered with their ability to perform the required exercise; had experienced delayed onset muscle soreness in their quadricep muscles during the previous 3 months, or had any previous allergic or sensitivity response to dairy proteins. Volunteers were instructed not to take over-the-counter medication, “cold & flu” treatments, analgesics, aspirin or other anti-inflammatory preparations for at least 7 days prior to the study, and to abstain from alcohol for 48 h prior to the study.

Ethical approval was obtained from the Human Research Ethics Committee of the University of South Australia. Volunteers provided written informed consent prior to participation. The study sponsor (MG Nutritionals) did not have the right to disapprove publication of this study.

Muscle fatigue and muscle damage were induced through the performance of 100 maximal ECC of the knee extensor muscles of the right leg on a Kin-Com isokinetic dynamometer (Chattecx, Tennessee, USA). Each volunteer performed 10 warm-up repetitions at low resistance, followed by 100 maximal ECC at an angular velocity of  $40^\circ \text{ s}^{-1}$  through an  $80^\circ$  range of motion. Volunteers performed a maximal isometric contraction against the dynamometer lever arm for approximately 1 s before the initiation of each ECC, and with continued maximal effort, resisted the forced lengthening of their knee extensors. The knee extensors were relaxed at the end of each ECC, and during the recovery phase the relaxed leg was returned to the starting position by the experimenter.

Isometric strength was determined from the PIT achieved during the best of three maximal isometric contractions of the knee extensors with the knee flexed at an angle of  $90^\circ$ . Body position, approximate axis of rotation of the knee joint and dynamometer lever arm length were consistent for each volunteer, and verbal encouragement to achieve a maximal effort was given during all contractions.

Of the 43 volunteers who entered the study, 15 failed to demonstrate any reduction in PIT after the 100 ECC (prior to consuming any supplement) indicating that they had not exerted a maximal effort (FW,  $n = 3$ ; WPI,  $n = 3$ ; WPI<sub>HD</sub>,

$n = 9$ ). The data for these non-compliant volunteers were excluded, leaving data from 28 volunteers for analysis (FW,  $n = 11$ ; WPI,  $n = 11$ ; WPI<sub>HD</sub>,  $n = 6$ ).

Muscle soreness was evaluated using a 100 mm visual analogue scale (VAS).<sup>4</sup> The VAS consisted of a 100 mm horizontal line with anchor points consisting of “no soreness” on the left to the “worst soreness possible” on the right hand end. Volunteers were seated and requested to extend the knee of the right leg (so the leg was horizontal) while a 5-kg mass was suspended from the ankle. The volunteers placed a mark at the point on the VAS corresponding to their perception of the soreness in the quadricep muscles of the leg. The extent of the muscle soreness was quantified using the measured distance (in mm) from the left hand end of the continuum to the mark made by the volunteer.

Blood samples (10 ml) were collected by venepuncture for assessment of serum CK and plasma TNF $\alpha$  concentrations. Serum CK was measured in duplicate using a zero order kinetic assay method (Olympus CK-NAC, Olympus Diagnostic Systems, Middlesex, UK) on an automated analyzer (Olympus AU5400, Olympus Diagnostic Systems, Middlesex, UK). Plasma TNF $\alpha$  was measured in triplicate by ELISA using a commercial kit (R&D Systems, Minneapolis, USA).

Volunteers were randomly allocated to consume 250 ml of FW (control) or 250 ml of FW containing 25 g of WPI (NutraPro, MG Nutritionals, Brunswick, Australia) or 25 g of WPI<sub>HD</sub> (NutraBoost XR, MG Nutritionals, Brunswick, Australia). The FW consisted of 250 ml of water to which 7.5 g of vanilla flavouring (IFF Flavouring, Melbourne, Australia) and 2.5 g of skim milk powder (MG Nutritionals, Brunswick, Australia) was added. The WPI and WPI<sub>HD</sub> consisted of 250 ml of water to which 25 g of the appropriate protein, 1.25 g of skim milk powder and 3.75 g of vanilla flavouring had been added. The supplements were consumed within a two min period immediately after the assessments that followed ECC, and again immediately after the assessments at 6 h, and 2 h prior to the assessments at 24 h.

Baseline parameters for the three supplement groups were compared using one-way analysis of variance (ANOVA). Two-way ANOVA with repeated measures was used to determine the effects of the supplements on the dependent measures over time. Where ANOVA demonstrated a significant main effect post hoc analysis was conducted to identify differences between means using Tukey’s test. Statistical significance was set at an  $\alpha$ -level of  $P < 0.05$ . All data are shown as mean  $\pm$  S.E.M.

### 3. Results

There was no difference in the maximum isometric torque developed between groups at baseline (WPI  $165.5 \pm 21.6$  nm, WPI<sub>HD</sub>  $186.8 \pm 45.7$  nm, FW  $146.0 \pm 10.4$  nm,  $P = 0.53$ ). PIT decreased immediately post-ECC in all treatment groups ( $\sim 23\%$  reduction; Fig. 1). PIT improved rapidly following supplementation with WPI<sub>HD</sub> ( $P < 0.02$  for post-

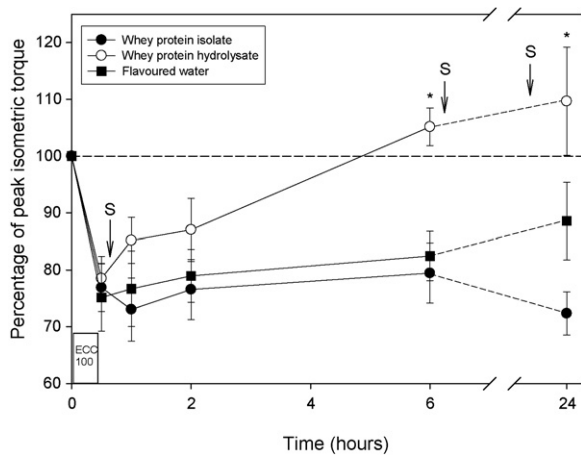


Fig. 1. Percentage of peak isometric torque of the knee extensors prior to and after the performance of 100 maximal eccentric contractions followed by supplementation with a flavoured water placebo, whey protein isolate, or hydrolysed whey protein isolate. Values are mean  $\pm$  S.E.M. ECC100: 100 maximal eccentric contractions of the knee extensors. S: dose of supplement consumed. \*Significantly different from immediately post-ECC100 for hydrolysed whey protein isolate ( $P < 0.02$ ).

ECC compared with 6 and 24 h from post hoc testing) compared with FW and WPI ( $P = 0.006$ ; treatment  $\times$  time interaction from ANOVA;  $P < 0.02$  for post-ECC compared with 6 and 24 h from post hoc testing), such that PIT had returned to baseline values by 6 h, while PIT in FW and WPI remained suppressed for the duration of the 24 h study period ( $P > 0.74$  for post-ECC compared with 1, 2, 6 and 24 h from post hoc testing).

At baseline, there was no difference in muscle soreness between treatment groups ( $P = 0.19$ , Fig. 2). Muscle soreness increased following ECC ( $P < 0.001$  for time), reaching  $27.3 \pm 3.3\%$  of maximum possible soreness immediately after ECC, and remained elevated for the duration of the

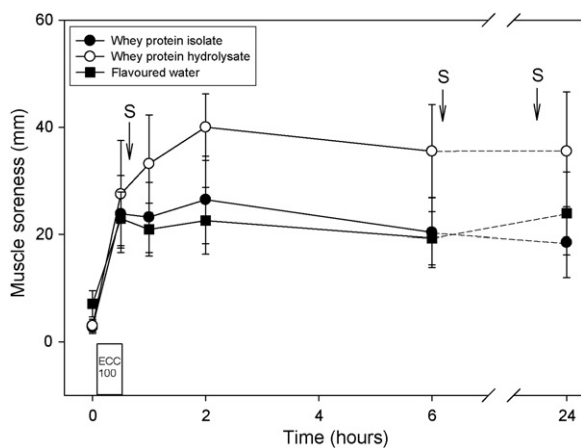


Fig. 2. Knee extensor muscle soreness assessed using a 100-mm visual analogue scale prior to and after the performance of 100 maximal eccentric contractions followed by supplementation with a flavoured water placebo, whey protein isolate, or hydrolysed whey protein isolate. Values are mean  $\pm$  S.E.M. ECC100: 100 maximal eccentric contractions of the knee extensors. S: dose of supplement consumed.

study period with no difference between treatments ( $P = 0.61$ , treatment  $\times$  time interaction).

There were no differences in serum CK activity between groups at baseline ( $P = 0.29$ ; Table 1), and serum CK did not change ( $P = 0.46$  for time,  $P = 0.43$  for treatment  $\times$  time). Similarly, there were no differences in plasma TNF $\alpha$  concentration between treatment groups at baseline ( $P = 0.72$ , Table 1), and TNF $\alpha$  did not change ( $P = 0.93$  for time,  $P = 0.58$  for treatment  $\times$  time).

#### 4. Discussion

The main finding of this study was that the consumption of a single dose of an hydrolysate of whey protein isolate resulted in a more rapid recovery of muscle force-generating capacity following eccentric exercise compared with a flavoured water placebo or a non-hydrolysed form of the same whey protein isolate. Indeed, the effect of this hydrolysate was such that complete recovery of muscle force-generating capacity had been achieved by 6 h post-supplementation.

Eccentric exercise of the kind performed in the present study results in muscle damage and prolonged impairment of muscle force-generating capacity.<sup>1</sup> A recent study by Byrne and Eston<sup>5</sup> demonstrated a reduction in PIT of the knee extensors of  $\sim 30\%$  following 100 maximal ECCs, which was similar to the  $\sim 23\%$  reduction observed in the present study. However, Byrne and Eston<sup>5</sup> reported that peak torque remained suppressed after 7 days. While peak torque remained suppressed for the whole of the 24 h study period in the WPI and FW groups in the present study, torque-generating capacity had completely recovered by 6 h post-exercise in the volunteers who consumed WPI<sub>HD</sub>. The mechanism underlying the rapid recovery of muscle function following the consumption of WPI<sub>HD</sub> was not apparent from the data collected during the present study, but part of the recovery might have been attributable to the supplement stimulating repair of damaged skeletal muscle tissue. A number of indirect markers of muscle damage and inflammation were assessed in the present study, including muscle soreness, serum CK, and plasma TNF $\alpha$  concentrations, but there was no evidence of any effect of the hydrolysate on these markers. The lack of any significant increase in serum CK (marker of muscle damage) might be explained by the short timeframe over which the study was conducted, since serum CK activity can take up to 48 h to increase to any significant extent following eccentric exercise.<sup>6</sup> It is likely that the large variation in the CK response between volunteers also contributed to the lack of ability to detect a significant change since, while there was overall a mean increase in CK levels by 24 h post-ECC in all treatment groups, this was not statistically significant, suggesting a lack of statistical power to detect any significant changes. The lack of any effect of ECC on plasma TNF $\alpha$ , might have been due to the relatively short duration of the exercise protocol employed, since the major

Table 1

Markers of muscle damage and inflammation in sedentary males prior to and following the performance of 100 maximal eccentric contractions of the knee extensor muscles followed by supplementation with flavoured water, or flavoured water containing whey protein isolate or hydrolysed whey protein isolate

Biomarker	Treatment	Pre-exercise	Immediately post-exercise	1 h post-exercise	2 h post-exercise	6 h post-exercise	24 h post-exercise
Serum creatine kinase activity (IU l <sup>-1</sup> )	WPI <sub>HD</sub> (n=6)	187.3 ± 53.9	164.4 ± 24.7	173.0 ± 26.3	191.0 ± 58.0	180.8 ± 32.1	386.8 ± 170.1
	WPI (n=11)	129.6 ± 19.1	134.3 ± 19.8	111.22 ± 21.4	145.44 ± 22.4	193.5 ± 38.1	316.4 ± 107.3
	FW (n=11)	129.6 ± 13.1	131.2 ± 12.6	127.6 ± 10.9	140.1 ± 13.4	162.9 ± 14.9	196.5 ± 25.4
Plasma tumor necrosis factor-α concentration (pg ml <sup>-1</sup> )	WPI <sub>HD</sub> (n=6)	1.36 ± 0.13	1.21 ± 0.14	1.29 ± 0.16	1.27 ± 0.20	1.18 ± 0.16	1.46 ± 0.11
	WPI (n=11)	1.39 ± 0.31	1.61 ± 0.36	1.39 ± 0.29	1.53 ± 0.40	1.62 ± 0.41	1.47 ± 0.37
	FW (n=11)	1.13 ± 0.16	1.39 ± 0.23	1.60 ± 0.39	1.29 ± 0.19	1.43 ± 0.14	1.47 ± 0.14

Values are mean ± S.E.M. WPI<sub>HD</sub>: hydrolysate of whey protein isolate. WPI: whey protein isolate. FW: flavoured water placebo.

ity of studies reporting elevations in TNFα concentrations have employed longer duration exercise interventions (i.e. >2 h) to induce muscle damage.<sup>7</sup> However, the initial development of muscle soreness, and its persistence throughout the study period in all volunteers, including those who consumed the hydrolysate, suggests that some level of muscle damage and/or inflammation may have been present in all treatment groups, but this did not manifest in changes in circulating markers of inflammation or muscle damage within the timeframe of the protocol. Future studies should investigate the effect of this hydrolysate on markers of muscle damage and inflammation following ECC over a longer time frame.

This study provided preliminary evidence that a novel hydrolysate of whey protein isolate provided a rapid recovery of muscle force-generating capacity following eccentric exercise. Future studies should investigate the mechanism by which this particular hydrolysate was able to elicit an improvement in the recovery of muscle force-generation.

## Disclosure

MK Rowney was an employee of MG Nutritionals Pty Ltd. at the time of this study.

## Practical implications

- This protein hydrolysate can accelerate recovery from exercise induced muscle damage.
- A single dose of this hydrolysate of whey protein isolate resulted in a more rapid recovery of muscle force-generating capacity following eccentric exercise compared

with a flavoured water placebo or a non-hydrolysed form of the same whey protein isolate.

- The effect of this hydrolysate was achievement of complete recovery of muscle force-generating capacity by 6 h post-supplementation.

## Acknowledgements

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