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**Whey protein hydrolysate supplementation accelerates recovery from exercise-induced muscle damage in females.**

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## **Abstract**

A number of different forms of protein and their analogues have been investigated for their efficacy in ameliorating exercise-induced muscle damage (EIMD) and recovery. Preliminary data regarding whey protein hydrolysate (WPH) supplementation are promising. However, its efficacy beyond acute eccentric/resistance exercise bouts or longer-term training programmes are limited and all investigations have been conducted in male or mixed-sex groups. This study sought to elucidate whether the benefits of WPH previously reported can be demonstrated in females following repeated-sprint exercise. Twenty physically active females were assigned to consume two doses of 70 ml WPH or isoenergetic carbohydrate (CHO) for 4 days post EIMD. Measures of muscle soreness, limb girth, flexibility, muscle function and creatine kinase were collected pre, immediately post, and 24, 48 and 72 h post-exercise. Time effects were observed for all variables ( $p < 0.05$ ) except limb girth; indicative of EIMD. Flexibility improved beyond baseline measures following WPH by 72 h, but had failed to recover in the CHO group ( $p = 0.011$ ). Reactive strength index was higher throughout recovery in the WPH group compared to CHO ( $p = 0.016$ ). Reductions in creatine kinase were greater following WPH compared to CHO at 48 h post EIMD ( $p = 0.031$ ). The findings suggest that four day supplementation of WPH is beneficial for reducing symptoms of EIMD and improving recovery of muscle function in physically active females.

**Key words** creatine kinase, reactive strength index, hamstring flexibility, repeated sprint

## **Introduction**

Exercise has been shown to increase protein turnover and amino acid oxidation (Evans, 1991) and this might be exacerbated in exercise-induced muscle damage (EIMD) paradigms given the structural damage to skeletal muscle that might occur. Indeed, rates of muscle protein synthesis (MPS) and muscle protein breakdown (MPB) are increased following unaccustomed, muscle-damaging exercise, and while this has been suggested to be unrelated to the muscle contraction performed (Phillips, Tipton, Aarsland, Wolf, & Wolfe, 1997), others suggest that MPS are greater following eccentric compared to concentric contractions (Eliasson et al., 2006; Moore, Phillips, Babraj, Smith, & Rennie, 2005); perhaps mediated through a combination of greater tension and stretching of the muscle (Eliasson et al., 2006). However, at least in the fasted state there is a negative net muscle protein balance which does not become positive post-exercise if not compensated for through protein availability (Kumar, Atherton, Smith, & Rennie, 2009; Phillips et al., 1997; Pitkanen et al., 2003). Consequently, protein intake might provide the required amino acids necessary for improving protein balance, which is crucial for repairing damaged structural proteins (Saunders, 2007; Tipton, 2008), and thus attenuating the negative symptoms associated with muscle damage.

Of contemporary interest is supplementation with hydrolysed proteins. These supplements are pre-digested proteins that are partially broken-down when exposed to heat, enzymes, or acids; producing large quantities of shorter chain peptides. As such, it is recognised that protein hydrolysates are more readily digested and absorbed, and increase circulating amino acid concentrations more rapidly than 'intact' proteins (Koopman et al., 2009; Manninen, 2004; Morifuji et al., 2010; Silk et al., 1979). Recently, the efficacy of whey protein hydrolysate (WPH) supplementation on reducing markers of muscle damage and accelerating recovery has received attention in the literature. The evidence for WPH in combination with carbohydrate are encouraging; with reported decreases in systemic indices of muscle damage (Hansen,

Bangsbo, Jensen, Bibby, & Madsen, 2015; Lollo et al., 2014), increases in satellite cell proliferation (Farup et al., 2014), alterations in signalling associated with muscle protein turnover (Rahbek, Farup, de Paoli, & Vissing, 2015), and accelerated physical (Cooke, Rybalka, Stathis, Cribb, & Hayes, 2010; Hansen et al., 2015) and psychological (Hansen et al., 2015) recovery. Data also appear to suggest that when consumed in isolation, there is greater benefit of WPH over other forms of whey to reduce symptoms of EIMD with both acute (Buckley et al., 2010) and more long-term (Lollo et al., 2014) supplementation strategies.

Preliminary data regarding WPH supplementation are promising, however, presently, no study has examined effects following an acute bout of repeated-sprint exercise and all investigations exploring the influence of WPH on EIMD and recovery have been conducted with male or mixed sex groups (Buckley et al., 2010; Cooke et al., 2010; Farup et al., 2014; Hansen et al., 2015; Lollo et al., 2014; Rahbek et al., 2015). Although there have been no reported sex differences in the basal and post-exercise rates of MPS and MPB (Fujita, Rasmussen, Bell, Cadenas, & Volpi, 2007; Miller et al., 2006), the literature examining the differences in the susceptibility to EIMD between men and women remains equivocal (Dannecker et al., 2012; Enns & Tiidus, 2010). Certainly, more research in females is warranted, and female exercisers would benefit from a practical nutritional intervention to improve recovery; from a single bout of exercise, and during intensified training periods, where recovery times may be limited. Therefore, the aim of this investigation was to examine the efficacy of WPH gel supplementation on physiological and functional recovery following a bout of exercise designed to cause temporary muscle damage in females. It was hypothesised that indices of EIMD would be attenuated by the consumption of the WPH gel.

## **Materials and methods**

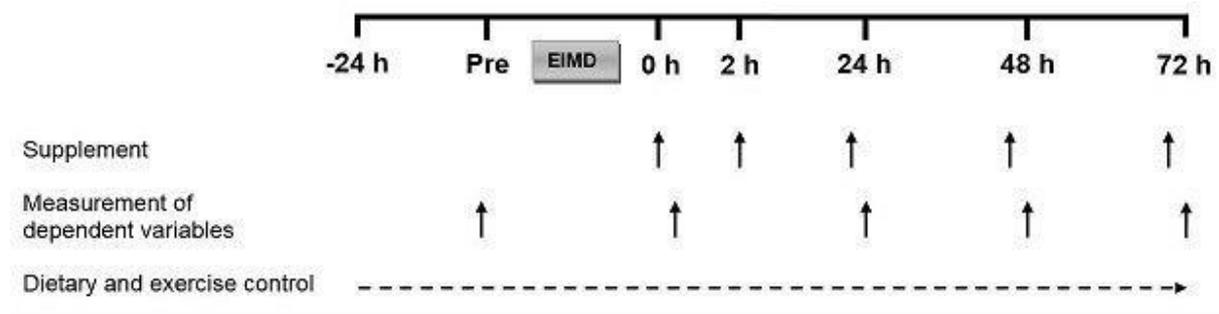
### **Participants**

Twenty physically active females (mean  $\pm$  SD age  $20 \pm 1$  y; stature  $165.9 \pm 5.6$  cm; body mass  $61.8 \pm 7.9$  kg) from a university dance team volunteered to participate and provided written informed consent. Participants were required to complete a menstrual cycle questionnaire, which identified the contraceptive use of participants; eight were using an oral combination pill (all monophasic), six were using a progesterone only pill/implant/injection, and six were normally menstruating. All testing took place during the early/mid luteal phase or where applicable in the 14 days prior to a withdrawal bleed. For 24 h prior to, and throughout the testing period, participants were required to refrain from strenuous exercise, and any anti-inflammatory drugs or alternative treatments, and dietary intake was controlled. The study received ethical approval from the Faculty of Health and Life Sciences Ethics Committee at the University of Northumbria.

### **Experimental protocol**

Using a randomised, double-blind design, participants were allocated to a whey protein hydrolysate group (WPH) or an isoenergetic carbohydrate group (CHO) and these groups were matched and counterbalanced for muscle function (maximum voluntary isometric contraction). Participants were provided with standardised meals 24 h prior to initial testing and were fasted for  $\geq 10$  h except for water, which was consumed *ad libitum*. On arrival at the laboratory, baseline measures of dependent variables were recorded and participants subsequently completed the exercise protocol designed to induce muscle damage. After a 2 min rest, participants consumed a dose of the WPH or CHO supplement within 10 min and baseline measures were repeated. Participants consumed a standardised breakfast meal and a supplement was provided to be consumed 2 h post-exercise. Baseline measures were then

repeated following an overnight fast at the same time of day ( $\pm 1$  h to account for diurnal variation) for the following 3 days after the exercise; 24, 48, and 72 h post damaging exercise. During this time, all food was provided and participants were required to consume two bolus 20 g doses of WPH or CHO each day. Please refer to Figure 1 for an illustration of the study design.



### Dietary control

Food intake was controlled throughout all trial periods; breakfast, lunch, evening meals as well as regular snacks were provided (please refer to Table 1 for an example of the food provided each day). This ensured that sufficient amounts of carbohydrate ( $5-7 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) (Burke, Loucks, & Broad, 2006) and protein ( $1.2-1.7 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) (Tipton & Wolfe, 2004) recommended for athletic populations were met by all participants (Table 2). In addition, quantities of carbohydrate thought to saturate muscle glycogen resynthesis ( $1-1.2 \text{ g}\cdot\text{kg}^{-1}$ ) and quantities of protein thought to support MPS ( $0.25-0.3 \text{ g}\cdot\text{kg}^{-1}$ ) (Thomas, Erdman, & Burke, 2016) were consumed within 45-60 min of exercise. No changes in body mass were observed between the initial testing day (day 1;  $64.8 \pm 7.5 \text{ kg}$  and  $58.7 \pm 7.3 \text{ kg}$  for WPH and CHO, respectively) and the final testing day (day 4;  $65.1 \pm 7.1 \text{ kg}$  and  $58.9 \pm 7.6 \text{ kg}$  for WPH and CHO, respectively) in both treatment groups (both  $p > 0.05$ ), demonstrating that participants were likely in energy balance.

Table 1. Standardised daily meal plan for participants over the four-day data collection period.

<u>Meal</u>	<u>Food and drink provided</u>
<u>Breakfast</u>	<u>2 x white bread, toasted, with butter and strawberry jam</u> <u>1 x glass of milk</u>
<u>Lunch<sup>1</sup></u>	<u>1 x sandwich or salad</u> <u>1 x packet of crisps</u> <u>1 x fruit smoothie</u>
<u>Evening Meal<sup>1</sup></u>	<u>1 x curry or chilli</u>
<u>Snacks</u>	<u>1 x banana</u> <u>1 x cereal bar</u> <u>1 x packet of jelly sweets</u> <u>1 x yoghurt</u>

<sup>1</sup>The meals did not deviate from this standardised plan, however specific foods and flavours provided during lunch and the evening meal were altered each day to ensure a varied diet and to avoid monotony.

Table 2. Daily dietary intake of participants over the four-day data collection period<sup>1</sup>, mean  $\pm$  SD.

<u>Variable</u>		<u>Excluding Supplements</u>		<u>Including Supplements</u>	
		<u>WPH</u>	<u>CHO</u>	<u>WPH</u>	<u>CHO</u>
<u>Energy</u>	<u>kcal</u>	<u>2066 <math>\pm</math> 108</u>	<u>2019 <math>\pm</math> 183</u>	<u>2220 <math>\pm</math> 108</u>	<u>2173 <math>\pm</math> 183</u>
	<u>MJ</u>	<u>8.6 <math>\pm</math> 0.5</u>	<u>8.4 <math>\pm</math> 0.8</u>	<u>9.3 <math>\pm</math> 0.5</u>	<u>9.1 <math>\pm</math> 0.8</u>
<u>Carbohydrate</u>	<u>g<math>\cdot</math>kg<sup>-1</sup></u>	<u>5.0 <math>\pm</math> 0.7</u>	<u>5.5 <math>\pm</math> 0.9</u>	<u>5.0 <math>\pm</math> 0.7*</u>	<u>6.2 <math>\pm</math> 1.0*</u>
	<u>%TEI</u>	<u>61 <math>\pm</math> 3</u>	<u>63 <math>\pm</math> 2</u>	<u>58 <math>\pm</math> 3*</u>	<u>66 <math>\pm</math> 2*</u>
<u>Protein</u>	<u>g<math>\cdot</math>kg<sup>-1</sup></u>	<u>1.2 <math>\pm</math> 0.2</u>	<u>1.3 <math>\pm</math> 0.2</u>	<u>1.8 <math>\pm</math> 0.2*</u>	<u>1.3 <math>\pm</math> 0.2*</u>
	<u>%TEI</u>	<u>15 <math>\pm</math> 1</u>	<u>15 <math>\pm</math> 1</u>	<u>21 <math>\pm</math> 1*</u>	<u>14 <math>\pm</math> 1*</u>
<u>Fat</u>	<u>g<math>\cdot</math>kg<sup>-1</sup></u>	<u>0.9 <math>\pm</math> 0.1</u>	<u>0.9 <math>\pm</math> 0.2</u>	<u>0.9 <math>\pm</math> 0.1</u>	<u>0.9 <math>\pm</math> 0.2</u>
	<u>%TEI</u>	<u>25 <math>\pm</math> 3</u>	<u>24 <math>\pm</math> 1</u>	<u>23 <math>\pm</math> 3</u>	<u>23 <math>\pm</math> 1</u>

<sup>1</sup>As determined using dietary analysis software (Nutritics Ltd, Swords, Ireland). WPH, whey protein hydrolysate group ( $n = 10$ ); CHO, carbohydrate group ( $n = 10$ ); %TEI, percentage of total energy intake. \*denotes significant difference between groups ( $p < 0.05$ ).

## Supplementation

The nutritional composition of the supplements is presented in Table 3.

Table 3. Nutritional composition of the supplements per serving.

	<u>WPH</u>	<u>CHO</u>
<u>Serving size (mL)</u>	<u>78</u>	<u>78</u>
<u>Energy (kcal)</u>	<u>88</u>	<u>88</u>
<u>Energy (kJ)</u>	<u>368</u>	<u>368</u>
<u>Protein (g)<sup>1</sup></u>	<u>20</u>	<u>0</u>
<u>Carbohydrate (g)</u>	<u>1.8</u>	<u>21.8</u>
<u>Fat (g)</u>	<u>0.1</u>	<u>0.1</u>

<sup>1</sup>According to manufactures' information, WPH contains 7 g BCAA. WPH, whey protein hydrolysate gel; CHO, carbohydrate gel.

Post EIMD, participants consumed a daily dose of two 20g bolus amounts of the WPH or CHO in gel form. On the day of muscle-damaging exercise, these doses were consumed immediately post and 2 h post-exercise. For the following two days, these doses were consumed 30-60 min prior to subsequent morning visits and prior to their evening meal, and a final supplement was consumed prior to final measurements at 72 h post-exercise. This is based on recent work

demonstrating an effect when WPH is consumed for three days following EIMD (Farup et al., 2014; Rahbek et al., 2015). Both WPH and CHO gels were lemon flavoured, isovolumetric and isocaloric, and were microbiologically screened and Informed Sport tested. Supplements were provided in identical packaging (Science in Sport Ltd, Farringdon, London) and subsequently labelled in a double-blind manner.

### **Exercise protocol**

Prior to baseline measurement of muscle function and prior to exercise, participants completed a standardised warm up (Glaister, Howatson, Abraham, et al., 2008; Glaister et al., 2007; Glaister, Howatson, Pattison, & McInnes, 2008). Participants were also given 5 min to perform any personal stretches and prepare themselves for measurement of muscle function and the exercise protocol.

Participants completed a repeated-sprint protocol described previously (Howatson & Milak, 2009). This comprises 15 x 30 m sprints (each separated by 60 s rest) with a rapid 10 m deceleration phase. This damage model has been demonstrated to induce muscle damage previously (Howatson & Milak, 2009; Keane, Salicki, Goodall, Thomas, & Howatson, 2015). Rate of perceived exertion (RPE; (Borg, 1982)) and heart rate (HR; Model RS-400, Polar, Kempele, Finland) were collected after each sprint effort. The 15 x 30 m sprint times were also recorded to determine total sprint time, mean sprint time, and rate of fatigue using the following formula (Fitzsimons, Dawson, Ward, & Wilkinson, 1993):

Fatigue index (%) = (100 x [total sprint time/ideal sprint time]) - 100, in which total sprint time = sum of sprint times from all sprints, and ideal sprint time = the number of sprints x fastest sprint time.

## **Dependant variables**

### ***Muscle soreness***

Subjective delayed onset of muscle soreness (DOMS) was measured using a 200 mm visual analogue scale with 'no soreness' and 'unbearably sore' anchored at each end of the scale. On each occasion, participants were required to complete a 90° squat with hands on their hips, and upon standing, to indicate on the line the level of perceived active lower limb soreness felt. Pain pressure threshold (PPT) was measured with a digital algometer with a connecting 1.0 cm<sup>2</sup> flat, circular rubber disc (Model FDX, Wagner Instruments, Greenwich, USA). Three muscle locations were determined; the rectus femoris (RF), the vastus lateralis (VL), and medial head of the gastrocnemius (GM) (Clifford, Bell, West, Howatson, & Stevenson, 2016). All measurements were taken on the right side of the participant and were marked with permanent marker to ensure accuracy on consecutive days (Vatine, Shapira, Magora, Adler, & Magora, 1993). To determine PPT, participants were asked to verbally indicate when the pressure applied to the muscle while supine (at an approximate rate of 5 N·s<sup>-1</sup>) became uncomfortable. Intra-trial and inter-trial percentage coefficient of variation (%CV) was < 8% for all locations.

### ***Limb girth***

Limb girth was measured as an indirect marker of inflammatory swelling and oedema (Smith, 1991; van Someren, Edwards, & Howatson, 2005). An anthropometric tape measure (Bodycare Products, Warwickshire, United Kingdom) was used to determine girths at the calf (measured at its largest girth at baseline) and mid-thigh (located as midway between the inguinal fold and the superior border of the patella) of the right leg. These locations on the skin were marked with permanent marker on the initial day of testing to ensure consistency in measurement on subsequent days. Calf and mid-thigh girth intra-examiner %CVs were < 1%.

### ***Hamstring stiffness and flexibility***

The sit and reach test was used to measure hamstring stiffness and flexibility. Participants were required to sit with their knees fully extended and feet together against the sit and reach box; the heel position in line with the 15 cm position on the box. With one hand placed over the other, participants were instructed to slowly reach forward along the measuring board to avoid rapid or forceful movements. They were asked to stretch as far as possible (but not to the point of pain) and to hold their 'best stretch' for approximately 2 s (American College of Sports Medicine, 2013). The score of this final position was recorded to the nearest 0.5 cm. Intra-trial and inter-trial %CV was < 5%.

### ***Muscle function***

Participants completed three countermovement jumps (CMJ) and three drop jumps (for measurement of reactive strength index (RSI)) using a light timing system (Optojump, Microgate, Bolzano, Italy), keeping their hands on their hips throughout. For CMJ, participants were asked to squat down (bending at the knee, hip and ankle while keeping their heels on the floor and their back straight) with their feet shoulder width apart and to jump vertically and maximally. For RSI (the jump height (cm) ÷ contact time (s) of each drop jump), participants were asked to drop from a 30 cm box and upon landing to perform a two-footed jump maximally with minimum contact time. Legs were kept straight while jumping; only bending once the feet contacted the ground. Each jump effort was separated by 60 s of rest, and the peak CMJ and RSI was used for analysis. Intra-trial and inter-trial %CV was both < 4% and < 12% for CMJ and RSI respectively.

Maximum voluntary isometric contraction (MVC) of the right knee extensors was measured using a strain gauge (MIE Digital Myometer, MIE Medical Research Ltd, Leeds, UK). While in a seated position, the strain gauge load cell was wrapped immediately above the malleoli (a

layer of padding was in place to avoid participant discomfort) and attached securely to a plinth on a purpose-built chair at the same height. The knee joint angle was standardised at 90<sup>0</sup> of flexion using a goniometer and confirmed before each contraction. Participants received a verbal countdown of 3 s before extending their knee ‘as fast and as hard as possible’ (Sahaly, Vandewalle, Driss, & Monod, 2001) and to do this for approximately 3 s. Participants completed three MVCs with 30 s rest between each effort and the peak force was used for analysis. Intra-trial and inter-trial %CV was < 4%.

Sprint time of a maximal effort 30 m sprint was recorded. The sprint was initiated from a line 30 cm behind the start line to prevent false triggering of the timing gates (Brower telemetric timers, Brower timing systems, Draper, USA). Both intra-trial and inter-trial %CV was < 2%.

### ***Blood sampling and analysis***

Blood samples (10 mL) were collected via venepuncture from the antecubital fossa area into serum gel vacutainers. After allowing samples to rest at room temperature for a minimum of 20 min, samples were centrifuged for 15 min (4°C) at 3000 RCF in order to obtain serum. The aliquots were stored at -80°C for later analysis of total CK. Due to difficulties with blood sampling, data for a single time point was missing out of a total of 100. Serum total CK concentrations were determined spectrophotometrically using an automated system (Roche Modular, Roche Diagnostics, Burgess Hill, UK). The inter-assay and intra-assay %CV were both < 2%.

### **Statistical analysis**

To account for inter-individual variability, all dependant variables except for DOMS and CK are expressed as a percentage change relative to pre muscle damage values. Statistical software (IBM Statistical Package for Social Sciences (SPSS) V22 IBM, Armonk, USA) was used for

inferential analysis and statistical significance was accepted at the  $p \leq 0.05$  level *a priori*. Two-way group (2; WPH vs CHO) x time (5; pre, and 0, 24, 48 and 72 h post EIMD) repeated measures analysis of variance were performed for each dependent variable. Violations of assumptions were corrected and Least Significant Difference test (LSD) for adjustment for multiple comparisons was used to analyse significant main effects. Independent samples *t* tests were conducted on peak HR, peak RPE, fatigue, and total and mean sprint time to examine differences in exercise intensity during the repeated sprint protocol between groups. Where appropriate, Cohen's D effect sizes (ES) were calculated with the magnitude of effects considered small (0.2), medium (0.5) and large ( $> 0.8$ ).

## **Results**

Independent samples *t* tests determined no differences between WPH and CHO groups for total sprint time, mean sprint time, fatigue, peak HR, and peak RPE during the repeated sprint protocol, thereby providing evidence that the exercise intensity was similar between groups. All dependent variable data not illustrated in figures are presented in Table 4.

Table 4. Values for dependent variables in response to muscle-damaging exercise, mean  $\pm$  SD.

<u>Variable</u>	<u>Group</u>	<u>Time post muscle-damaging exercise (h)</u>				
		<u>Pre</u>	<u>0</u>	<u>24</u>	<u>48</u>	<u>72</u>
<u>DOMS, mm</u>	<u>WPH</u>	<u>0.0 <math>\pm</math> 0.0</u>	<u>16.8 <math>\pm</math> 19.9<sup>†</sup></u>	<u>47.6 <math>\pm</math> 26.7<sup>†</sup></u>	<u>56.7 <math>\pm</math> 17.8<sup>†</sup></u>	<u>19.4 <math>\pm</math> 13.2<sup>†</sup></u>
	<u>CHO</u>	<u>1.0 <math>\pm</math> 2.5</u>	<u>13.0 <math>\pm</math> 20.1</u>	<u>65.0 <math>\pm</math> 49.0<sup>†</sup></u>	<u>71.2 <math>\pm</math> 45.0<sup>†</sup></u>	<u>37.1 <math>\pm</math> 27.4<sup>†</sup></u>
<u>RF PPT, % (N)</u>	<u>WPH</u>	<u>100 <math>\pm</math> 0</u>	<u>102.5 <math>\pm</math> 13.0</u>	<u>89.9 <math>\pm</math> 16.6</u>	<u>98.9 <math>\pm</math> 14.7</u>	<u>120.5 <math>\pm</math> 23.2<sup>†</sup></u>
		<u>(61.1 <math>\pm</math> 18.2)</u>	<u>(63.8 <math>\pm</math> 25.0)</u>	<u>(56.1 <math>\pm</math> 23.7)</u>	<u>(62.0 <math>\pm</math> 24.8)</u>	<u>(75.3 <math>\pm</math> 30.8)</u>
	<u>CHO</u>	<u>100 <math>\pm</math> 0</u>	<u>102.3 <math>\pm</math> 11.2</u>	<u>97.4 <math>\pm</math> 30.6</u>	<u>104.1 <math>\pm</math> 29.2</u>	<u>123.4 <math>\pm</math> 36.1<sup>†</sup></u>
		<u>(52.6 <math>\pm</math> 14.7)</u>	<u>(53.8 <math>\pm</math> 15.3)</u>	<u>(51.7 <math>\pm</math> 23.8)</u>	<u>(55.4 <math>\pm</math> 23.0)</u>	<u>(65.6 <math>\pm</math> 26.7)</u>
<u>VL PPT, % (N)</u>	<u>WPH</u>	<u>100 <math>\pm</math> 0</u>	<u>101.5 <math>\pm</math> 12.0</u>	<u>87.4 <math>\pm</math> 15.7</u>	<u>95.5 <math>\pm</math> 20.7</u>	<u>119.5 <math>\pm</math> 18.2<sup>†</sup></u>
		<u>(61.0 <math>\pm</math> 17.5)</u>	<u>(61.9 <math>\pm</math> 20.2)</u>	<u>(53.9 <math>\pm</math> 20.4)</u>	<u>(59.1 <math>\pm</math> 23.9)</u>	<u>(73.7 <math>\pm</math> 26.8)</u>
	<u>CHO</u>	<u>100 <math>\pm</math> 0</u>	<u>99.5 <math>\pm</math> 12.5</u>	<u>98.2 <math>\pm</math> 25.9</u>	<u>100.7 <math>\pm</math> 33.0</u>	<u>120.8 <math>\pm</math> 37.2</u>
		<u>(50.9 <math>\pm</math> 15.6)</u>	<u>(50.3 <math>\pm</math> 15.2)</u>	<u>(48.7 <math>\pm</math> 16.6)</u>	<u>(50.9 <math>\pm</math> 21.6)</u>	<u>(60.8 <math>\pm</math> 24.8)</u>

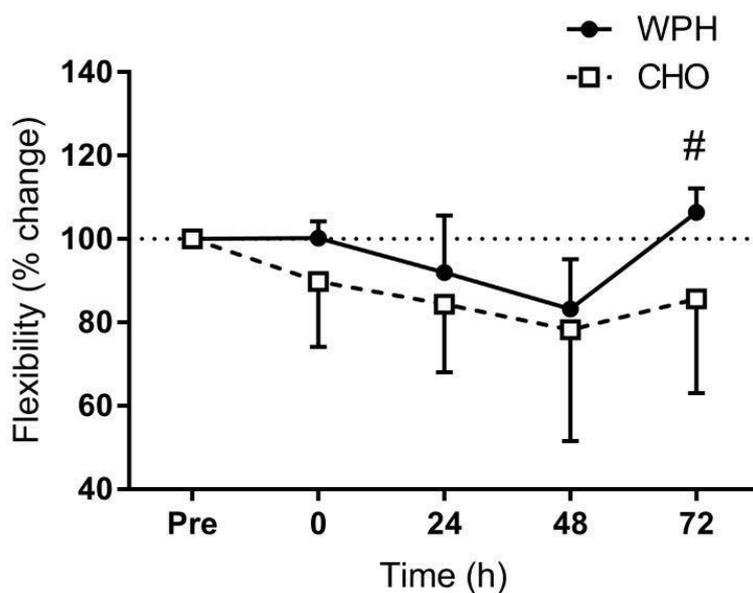
<b><u>GM PPT, % (N)</u></b>	<b><u>WPH</u></b>	<u>100 ± 0</u>	<u>101.1 ± 15.6</u>	<u>94.1 ± 16.6</u>	<u>106.9 ± 15.1</u>	<u>125.9 ± 22.5<sup>†</sup></u>
		<u>(60.6 ± 20.4)</u>	<u>(61.1 ± 23.5)</u>	<u>(57.2 ± 22.2)</u>	<u>(64.1 ± 21.7)</u>	<u>(74.2 ± 23.0)</u>
	<b><u>CHO</u></b>	<u>100 ± 0</u>	<u>97.3 ± 15.5</u>	<u>94.6 ± 26.3</u>	<u>101.9 ± 28.8</u>	<u>116.0 ± 28.5</u>
		<u>(48.6 ± 17.8)</u>	<u>(47.2 ± 17.5)</u>	<u>(45.7 ± 21.0)</u>	<u>(48.3 ± 17.8)</u>	<u>(56.0 ± 24.4)</u>
<b><u>Thigh girth, % (cm)</u></b>	<b><u>WPH</u></b>	<u>100 ± 0</u>	<u>100.3 ± 0.8</u>	<u>100.1 ± 0.6</u>	<u>99.8 ± 1.2</u>	<u>99.7 ± 1.0</u>
		<u>(51.9 ± 4.4)</u>	<u>(52.1 ± 4.4)</u>	<u>(52.0 ± 4.3)</u>	<u>(51.8 ± 4.0)</u>	<u>(51.7 ± 4.2)</u>
	<b><u>CHO</u></b>	<u>100 ± 0</u>	<u>99.9 ± 0.7</u>	<u>100.2 ± 0.8</u>	<u>100.2 ± 0.8</u>	<u>100.6 ± 0.5</u>
		<u>(48.9 ± 3.5)</u>	<u>(48.8 ± 3.3)</u>	<u>(48.8 ± 3.2)</u>	<u>(48.8 ± 3.2)</u>	<u>(48.8 ± 3.3)</u>
<b><u>Calf girth, % (cm)</u></b>	<b><u>WPH</u></b>	<u>100 ± 0</u>	<u>99.9 ± 0.4</u>	<u>99.6 ± 0.5</u>	<u>99.8 ± 0.6</u>	<u>99.9 ± 0.8</u>
		<u>(36.9 ± 1.8)</u>	<u>(36.8 ± 1.7)</u>	<u>(36.7 ± 1.7)</u>	<u>(36.8 ± 1.7)</u>	<u>(36.8 ± 1.7)</u>
	<b><u>CHO</u></b>	<u>100 ± 0</u>	<u>99.6 ± 0.5</u>	<u>99.7 ± 1.1</u>	<u>99.7 ± 0.6</u>	<u>100.0 ± 0.9</u>
		<u>(35.0 ± 2.8)</u>	<u>(34.9 ± 2.7)</u>	<u>(34.9 ± 2.7)</u>	<u>(34.9 ± 2.8)</u>	<u>(35.0 ± 2.9)</u>
<b><u>CMJ, % (cm)</u></b>	<b><u>WPH</u></b>	<u>100 ± 0</u>	<u>86.7 ± 8.4<sup>†</sup></u>	<u>94.2 ± 8.3</u>	<u>92.2 ± 4.2<sup>†</sup></u>	<u>95.2 ± 7.1</u>
		<u>(26.8 ± 4.4)</u>	<u>(23.2 ± 4.5)</u>	<u>(25.3 ± 5.5)</u>	<u>(24.6 ± 4.1)</u>	<u>(25.6 ± 5.3)</u>
	<b><u>CHO</u></b>	<u>100 ± 0</u>	<u>88.1 ± 6.9<sup>†</sup></u>	<u>87.4 ± 10.0<sup>†</sup></u>	<u>89.7 ± 9.3<sup>†</sup></u>	<u>94.5 ± 11.1</u>

		<u>(24.3 ± 2.8)</u>	<u>(21.3 ± 2.0)</u>	<u>(21.1 ± 2.6)</u>	<u>(21.7 ± 2.8)</u>	<u>(22.9 ± 3.2)</u>
<b><u>MVC, % (N)</u></b>	<b><u>WPH</u></b>	<u>100 ± 0</u>	<u>91.6 ± 8.2<sup>‡</sup></u>	<u>89.4 ± 10.3<sup>‡</sup></u>	<u>89.5 ± 8.5<sup>‡</sup></u>	<u>95.0 ± 9.9</u>
		<u>(445.0 ± 69.9)</u>	<u>(409.4 ± 80.3)</u>	<u>(398.2 ± 75.1)</u>	<u>(399.8 ± 79.2)</u>	<u>(423.7 ± 84.0)</u>
	<b><u>CHO</u></b>	<u>100 ± 0</u>	<u>84.6 ± 7.0<sup>‡</sup></u>	<u>87.5 ± 9.2<sup>‡</sup></u>	<u>88.1 ± 8.3<sup>‡</sup></u>	<u>89.6 ± 11.5<sup>‡</sup></u>
		<u>(400.4 ± 66.6)</u>	<u>(399.2 ± 68.8)</u>	<u>(349.5 ± 61.2)</u>	<u>(353.2 ± 70.5)</u>	<u>(356.7 ± 62.9)</u>
<b><u>30 m sprint time, %</u></b>	<b><u>WPH</u></b>	<u>100 ± 0</u>	<u>102.7 ± 4.5</u>	<u>101.8 ± 3.5</u>	<u>101.2 ± 2.8</u>	<u>99.7 ± 3.4</u>
<b><u>(s)</u></b>		<u>(5.31 ± 0.34)</u>	<u>(5.45 ± 0.38)</u>	<u>(5.40 ± 0.37)</u>	<u>(5.37 ± 0.38)</u>	<u>(5.29 ± 0.36)</u>
	<b><u>CHO</u></b>	<u>100 ± 0</u>	<u>102.7 ± 4.7</u>	<u>102.7 ± 4.4<sup>‡</sup></u>	<u>100.6 ± 7.3</u>	<u>100.7 ± 5.5</u>
		<u>(5.36 ± 0.26)</u>	<u>(5.50 ± 0.34)</u>	<u>(5.50 ± 0.30)</u>	<u>(5.38 ± 0.38)</u>	<u>(5.39 ± 0.30)</u>

WPH, whey protein hydrolysate group (n = 10); CHO, carbohydrate group (n = 10); %, % change from pre-exercise (Pre); DOMS, delayed onset muscle soreness; RF, rectus femoris; VL, vastus lateralis; GM, medial head of the gastrocnemius; PPT, pain pressure threshold; CMJ, countermovement jump; MVC, maximal voluntary isometric contraction. <sup>‡</sup>denotes significant difference from pre-exercise value (p < 0.05).

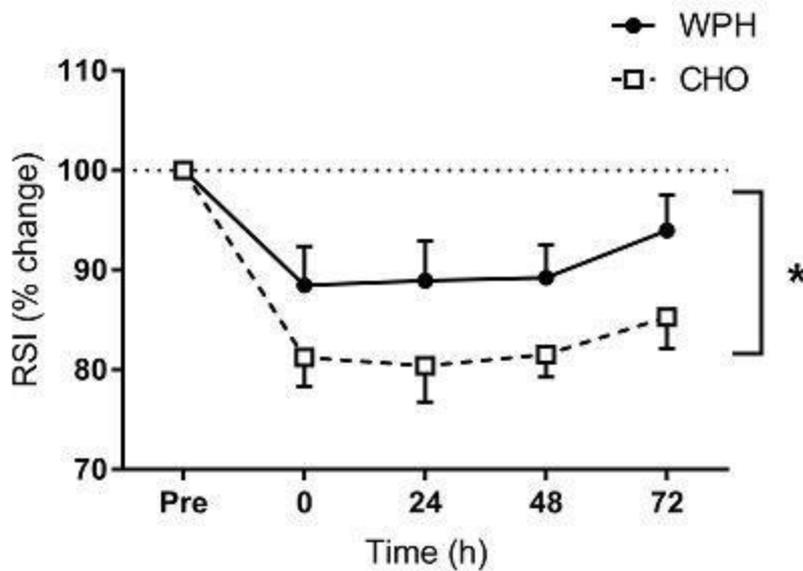
Delayed onset muscle soreness increased immediately post-exercise and remained elevated throughout recovery in both groups ( $p < 0.001$ ), peaking at 48 h post-exercise; with no group differences or interaction effects. At all three locations (RF, VL and GM), there was a main effect of time for PPT percentage change (all  $p \leq 0.001$ ), which reached lowest levels at 24 h and then increased throughout recovery. There were no group differences and no interaction effects for PPT.

Thigh and calf girths were unaffected post-exercise and there were no group differences or interaction effects. Flexibility was reduced throughout recovery ( $p < 0.001$ ), with lowest levels observed at 48 h post-exercise in both groups (Figure 2),

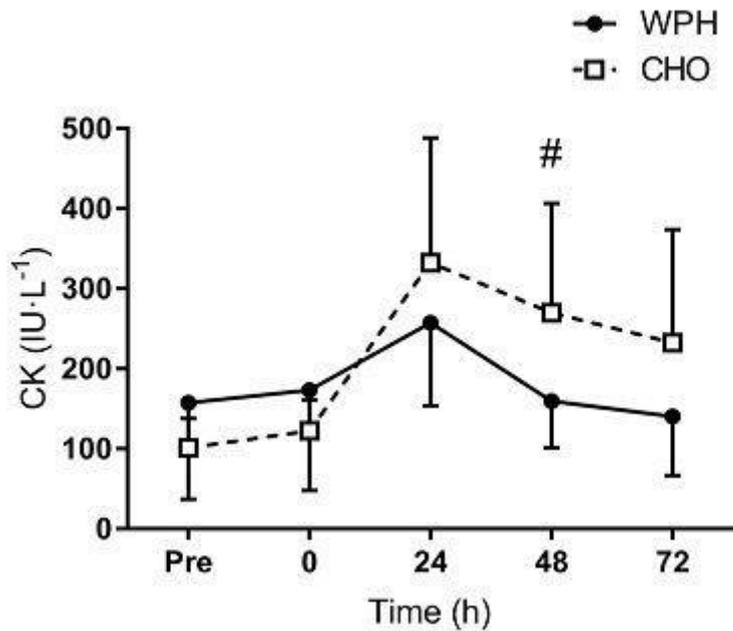


and no main effect of group ( $p = 0.104$ ). However, there was an interaction effect ( $p = 0.050$ ), where flexibility was improved beyond baseline measures at 72 h in the WPH group, but had failed to recover in the CHO group ( $p = 0.011$ , ES = 1.3).

All measures of muscle function were reduced post-exercise and progressively recovered throughout recovery ( $p < 0.001$  for CMJ, RSI and MVC; and  $p = 0.016$  for 30 m sprint time). While recovery of these measures appeared to accelerate with WPH, a group effect was only evident with RSI ( $p = 0.016$ , ES = 0.6) (Figure 3).



Both groups experienced an increase in circulating total CK ( $p < 0.001$ ), which peaked 24 h post-exercise and remained elevated throughout recovery. There were no main effects of group ( $p = 0.408$ ). However, there was an interaction effect ( $p = 0.002$ ) and reductions in CK were greater following WPH consumption at 48 h compared to CHO ( $p = 0.031$ , ES = -1.1); where CK remained elevated throughout the 72 h recovery period (Figure 4).



## Discussion

This investigation examined the effect of whey protein hydrolysate (WPH) supplementation on exercise recovery following EIMD in females. This study demonstrated for the first time that WPH reduces circulating CK, attenuates the decline in RSI, and accelerates recovery of hamstring flexibility compared to isocaloric CHO supplementation following repeated-sprint exercise in females.

While not all measures improved, this study is in agreement with a number of investigations reporting accelerated recovery of muscle function following EIMD with ingestion of WPH (Buckley et al., 2010; Cooke et al., 2010; Hansen et al., 2015); although some have demonstrated no effect (Farup et al., 2014; Rahbek et al., 2015), or a detrimental effect (Lollo et al., 2014). Indeed, one study observed that isometric muscle force recovered beyond baseline values by 6 h post EIMD after a single 25 g dose of WPH, while it remained suppressed with isoproteic whey protein isolate and non-caloric placebo supplementation (Buckley et al., 2010).

The predominant mechanism thought to be responsible for the role of WPH in accelerating recovery is through the provision and increased availability of amino acids; vital for regeneration and/or *de novo* synthesis of protein and the repair of damaged contractile elements of the muscle fibres (Biolo, Tipton, Klein, & Wolfe, 1997). Indeed, though not directly measured in the present investigation, WPH supplementation may be superior compared to other forms of protein in this regard, as plasma concentrations of amino acids and dipeptides (and therefore their bioavailability) are greater following ingestion of protein hydrolysates compared to non-hydrolysed proteins (Koopman et al., 2009; Morifuji et al., 2010; Power, Hallihan, & Jakeman, 2009; Tang, Moore, Kujbida, Tarnopolsky, & Phillips, 2009). Importantly, while global MPS is increased with dietary protein intake, this includes an increase in myofibrillar protein synthesis observed at rest (Brodsky et al., 2004), and following resistance (Moore et al., 2009), endurance (Breen et al., 2011), concurrent (Camera et al., 2015), and repeated sprint cycling exercise (Coffey et al., 2011). An increase in myofibrillar protein synthesis with WPH ingestion may contribute to repair and remodeling of damaged myofibrils following EIMD. Perhaps a potential acceleration of myofibrillar repair may explain the observed improvement in hamstring flexibility and the reduction in CK at 48 h post EIMD with WPH supplementation reported in the present study.

In addition, more compliant muscles are thought to be capable of storing more elastic energy (Brughelli & Cronin, 2007), therefore performance during activities utilising the stretch shortening cycle (such as drop jumps for measurement of RSI) might be improved. However, reductions in CK and improvements in flexibility were only evident at 48 h and 72 h post exercise, respectively; while reductions in RSI were attenuated throughout recovery. Notwithstanding, no other measures of muscle function were effected by WPH supplementation. Therefore, the role of accelerated myofibrillar repair in attenuating increases

in CK and reductions in RSI, and accelerating recovery of flexibility with WPH supplementation remains speculative and warrants further investigation.

A strength of the present investigation was the dietary control employed throughout testing periods. The participants either achieved the recommended 1.2-1.7 g·kg<sup>-1</sup>·day<sup>-1</sup> of protein (Tipton & Wolfe, 2004) (CHO group; 1.3 ± 0.2 g·kg<sup>-1</sup>·day<sup>-1</sup>) or a protein-rich diet (WPH group; 1.8 ± 0.2 g·kg<sup>-1</sup>·day<sup>-1</sup>). Some argue that as long as recommended levels of protein are achieved, further supplementation might be unnecessary in trained populations (Rennie & Tipton, 2000; Tipton, 2008). Despite this, a number of well-controlled studies have demonstrated that additional WPH (Hansen et al., 2015; Lollo et al., 2014) and BCAA (Coombes & McNaughton, 2000; Howatson et al., 2012; Jackman, Witard, Jeukendrup, & Tipton, 2010) supplementation is beneficial in attenuating EIMD, in spite of participants consuming recommended protein intakes. In the present investigation, since both groups were provided with sufficient intakes of macronutrients, and the daily diet and supplements were isocaloric, the attenuated reductions in muscle function and lower CK can be attributed to the additional protein provided by the WPH. Therefore, at least following strenuous exercise in females, this study lends support for the use of additional protein beyond recommended levels to reduce muscle damage and accelerate recovery.

This study did not measure nitrogen balance, signaling enzymes associated with protein turnover, nor rates of MPS and MPB. Therefore, it was not possible to identify specific mechanisms which might have been responsible for the attenuated muscle damage response and accelerated recovery from EIMD with WPH compared with isocaloric CHO. Moreover, besides the provision of amino acids, there may be other mechanisms by which WPH influences recovery from EIMD. For instance, protein hydrolysate has been reported to exhibit antioxidant properties (Peng, Xiong, & Kong, 2009), which might contribute to reducing muscle damage by attenuating the oxidative stress response associated with strenuous exercise.

Moreover, WPH dipeptides have also been shown to increase glucose uptake in isolated skeletal muscle (Morifuji, Koga, Kawanaka, & Higuchi, 2009). While not measured in the present investigation, such effects of WPH might certainly have contributed to the present findings. The intervention in the present study also involved ingestion of WPH immediately post EIMD, and throughout the recovery period; therefore, it is difficult to identify whether ingestion close to the exercise bout is important. Interestingly, while RSI was significantly higher with WPH supplementation compared to an isocaloric CHO throughout recovery, the decline in RSI immediately post-exercise and ingestion of the first supplement was not different between groups ( $11.5 \pm 12.4$  and  $18.8 \pm 9.2\%$  in WPH and CHO groups, respectively; independent *t* test;  $p = 0.155$ ). In addition, the interaction effects observed in measures of CK and flexibility were evident at 48 h and 72 h post EIMD, respectively. Intuitively, for optimal recovery amino acids should be ingested both immediately post and in the days of recovery post-exercise where MPS is thought to persist (Miller et al., 2005; Phillips et al., 1997). However, the present study did not investigate the influence of supplementation timing and more research is warranted to establish optimal supplementation strategies.

The main findings of this study were that four days of WPH supplementation improved recovery of muscle function (evidenced by improved RSI and flexibility) compared to isocaloric CHO supplementation, and that this was likely attributable to a reduction in muscle damage (evidenced by reduced CK). Though not directly measured, it is also likely that an increased delivery of amino acids with WPH supplementation was responsible for accelerating the repair of damaged skeletal muscle and thus its force generating capacity. While the observed improvements are arguably modest, acceleration in recovery of muscle function is of relevance to exercising females, and therefore is an important consequence of WPH supplementation. Indeed, these data support previous research demonstrating that protein intakes beyond recommended levels can ameliorate recovery from EIMD. This research adds

to the existing body of knowledge by demonstrating the application of WPH supplementation in female populations to improve recovery following strenuous exercise.

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### **Figure captions**

Fig 1. Schematic of testing protocol illustrating time-points where the supplements were consumed and measures of dependent variables taken. Diet and exercise was controlled for 24 h prior to exercise-induced muscle damage (EIMD) and for the duration of data collection.

Fig 2. Hamstring stiffness and flexibility measured using the sit and reach test post exercise-induced muscle damage in the whey protein hydrolysate (WPH) (n = 10) and carbohydrate

(CHO) (n = 10) groups. Values presented as mean  $\pm$  SD. #denotes significantly higher at 72 h in WPH group. Significance at  $p < 0.05$ .

Fig 3. Reactive strength index (RSI) post exercise-induced muscle damage in the whey protein hydrolysate (WPH) (n = 10) and carbohydrate (CHO) (n = 10) groups. Values presented as mean  $\pm$  SD. \*denotes significantly higher RSI in WPH group. Significance at  $p < 0.05$ .

Fig 4. Total creatine kinase (CK) post exercise-induced muscle damage in the whey protein hydrolysate (WPH) (n = 10) and carbohydrate (CHO) (n = 10) groups. Values presented as mean  $\pm$  SD. #denotes significantly greater reductions at 48 h in WPH group. Significance at  $p < 0.05$ .