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

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23 **Abstract**

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

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

42

43

44 **Introduction**

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

60 Of contemporary interest is supplementation with hydrolysed proteins. These supplements
61 are pre-digested proteins that are partially broken-down when exposed to heat, enzymes, or
62 acids; producing large quantities of shorter chain peptides. As such, it is recognised that
63 protein hydrolysates are more readily digested and absorbed, and increase circulating amino
64 acid concentrations more rapidly than 'intact' proteins (Koopman et al., 2009; Manninen,
65 2004; Morifuji et al., 2010; Silk et al., 1979). Recently, the efficacy of whey protein
66 hydrolysate (WPH) supplementation on reducing markers of muscle damage and accelerating
67 recovery has received attention in the literature. The evidence for WPH in combination with
68 carbohydrate are encouraging; with reported decreases in systemic indices of muscle damage

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

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

92

93 **Materials and methods**

94 **Participants**

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

106 **Experimental protocol**

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

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

122 **Dietary control**

123 Food intake was controlled throughout all trial periods; breakfast, lunch, evening meals as
124 well as regular snacks were provided (please refer to Table 1 for an example of the food
125 provided each day). This ensured that sufficient amounts of carbohydrate ($5-7 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$)
126 (Burke, Loucks, & Broad, 2006) and protein ($1.2-1.7 \text{ g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$) (Tipton & Wolfe, 2004)
127 recommended for athletic populations were met by all participants (Table 2). In addition,
128 quantities of carbohydrate thought to saturate muscle glycogen resynthesis ($1-1.2 \text{ g}\cdot\text{kg}^{-1}$) and
129 quantities of protein thought to support MPS ($0.25-0.3 \text{ g}\cdot\text{kg}^{-1}$) (Thomas, Erdman, & Burke,
130 2016) were consumed within 45-60 min of exercise. No changes in body mass were observed
131 between the initial testing day (day 1; 64.8 ± 7.5 kg and 58.7 ± 7.3 kg for WPH and CHO,
132 respectively) and the final testing day (day 4; 65.1 ± 7.1 kg and 58.9 ± 7.6 kg for WPH and
133 CHO, respectively) in both treatment groups (both $p > 0.05$), demonstrating that participants
134 were likely in energy balance.

135 **Supplementation**

136 The nutritional composition of the supplements is presented in Table 3. Post EIMD,
137 participants consumed a daily dose of two 20g bolus amounts of the WPH or CHO in gel
138 form. On the day of muscle-damaging exercise, these doses were consumed immediately post
139 and 2 h post-exercise. For the following two days, these doses were consumed 30-60 min
140 prior to subsequent morning visits and prior to their evening meal, and a final supplement

141 was consumed prior to final measurements at 72 h post-exercise. This is based on recent
142 work demonstrating an effect when WPH is consumed for three days following EIMD (Farup
143 et al., 2014; Rahbek et al., 2015). Both WPH and CHO gels were lemon flavoured,
144 isovolumetric and isocaloric, and were microbiologically screened and Informed Sport tested.
145 Supplements were provided in identical packaging (Science in Sport Ltd, Farringdon,
146 London) and subsequently labelled in a double-blind manner.

147 **Exercise protocol**

148 Prior to baseline measurement of muscle function and prior to exercise, participants
149 completed a standardised warm up (Glaister, Howatson, Abraham, et al., 2008; Glaister et al.,
150 2007; Glaister, Howatson, Pattison, & McInnes, 2008). Participants were also given 5 min to
151 perform any personal stretches and prepare themselves for measurement of muscle function
152 and the exercise protocol.

153 Participants completed a repeated-sprint protocol described previously (Howatson & Milak,
154 2009). This comprises 15 x 30 m sprints (each separated by 60 s rest) with a rapid 10 m
155 deceleration phase. This damage model has been demonstrated to induce muscle damage
156 previously (Howatson & Milak, 2009; Keane, Salicki, Goodall, Thomas, & Howatson, 2015).
157 Rate of perceived exertion (RPE; (Borg, 1982)) and heart rate (HR; Model RS-400, Polar,
158 Kempele, Finland) were collected after each sprint effort. The 15 x 30 m sprint times were
159 also recorded to determine total sprint time, mean sprint time, and rate of fatigue using the
160 following formula (Fitzsimons, Dawson, Ward, & Wilkinson, 1993):

161 $\text{Fatigue index (\%)} = (100 \times [\text{total sprint time/ideal sprint time}]) - 100$, in which total sprint
162 time = sum of sprint times from all sprints, and ideal sprint time = the number of sprints x
163 fastest sprint time.

164 **Dependant variables**

165 ***Muscle soreness***

166 Subjective delayed onset of muscle soreness (DOMS) was measured using a 200 mm visual
167 analogue scale with ‘no soreness’ and ‘unbearably sore’ anchored at each end of the scale.
168 On each occasion, participants were required to complete a 90⁰ squat with hands on their
169 hips, and upon standing, to indicate on the line the level of perceived active lower limb
170 soreness felt. Pain pressure threshold (PPT) was measured with a digital algometer with a
171 connecting 1.0 cm² flat, circular rubber disc (Model FDX, Wagner Instruments, Greenwich,
172 USA). Three muscle locations were determined; the rectus femoris (RF), the vastus lateralis
173 (VL), and medial head of the gastrocnemius (GM) (Clifford, Bell, West, Howatson, &
174 Stevenson, 2016). All measurements were taken on the right side of the participant and were
175 marked with permanent marker to ensure accuracy on consecutive days (Vatine, Shapira,
176 Magora, Adler, & Magora, 1993). To determine PPT, participants were asked to verbally
177 indicate when the pressure applied to the muscle while supine (at an approximate rate of 5
178 N·s⁻¹) became uncomfortable. Intra-trial and inter-trial percentage coefficient of variation
179 (%CV) was < 8% for all locations.

180 ***Limb girth***

181 Limb girth was measured as an indirect marker of inflammatory swelling and oedema (Smith,
182 1991; van Someren, Edwards, & Howatson, 2005). An anthropometric tape measure
183 (Bodycare Products, Warwickshire, United Kingdom) was used to determine girths at the calf
184 (measured at its largest girth at baseline) and mid-thigh (located as midway between the
185 inguinal fold and the superior border of the patella) of the right leg. These locations on the
186 skin were marked with permanent marker on the initial day of testing to ensure consistency in
187 measurement on subsequent days. Calf and mid-thigh girth intra-examiner %CVs were < 1%.

188 ***Hamstring stiffness and flexibility***

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

197 ***Muscle function***

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

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

212 (a layer of padding was in place to avoid participant discomfort) and attached securely to a
213 plinth on a purpose-built chair at the same height. The knee joint angle was standardised at
214 90⁰ of flexion using a goniometer and confirmed before each contraction. Participants
215 received a verbal countdown of 3 s before extending their knee ‘as fast and as hard as
216 possible’ (Sahaly, Vandewalle, Driss, & Monod, 2001) and to do this for approximately 3 s.
217 Participants completed three MVCs with 30 s rest between each effort and the peak force was
218 used for analysis. Intra-trial and inter-trial %CV was < 4%.

219 Sprint time of a maximal effort 30 m sprint was recorded. The sprint was initiated from a line
220 30 cm behind the start line to prevent false triggering of the timing gates (Brower telemetric
221 timers, Brower timing systems, Draper, USA). Both intra-trial and inter-trial %CV was < 2%.

222 ***Blood sampling and analysis***

223 Blood samples (10 mL) were collected via venepuncture from the antecubital fossa area into
224 serum gel vacutainers. After allowing samples to rest at room temperature for a minimum of
225 20 min, samples were centrifuged for 15 min (4°C) at 3000 RCF in order to obtain serum.
226 The aliquots were stored at -80°C for later analysis of total CK. Due to difficulties with blood
227 sampling, data for a single time point was missing out of a total of 100. Serum total CK
228 concentrations were determined spectrophotometrically using an automated system (Roche
229 Modular, Roche Diagnostics, Burgess Hill, UK). The inter-assay and intra-assay %CV were
230 both < 2%.

231 **Statistical analysis**

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

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

244 **Results**

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

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

255 Thigh and calf girths were unaffected post-exercise and there were no group differences or
256 interaction effects. Flexibility was reduced throughout recovery ($p < 0.001$), with lowest
257 levels observed at 48 h post-exercise in both groups (Figure 2), and no main effect of group
258 ($p = 0.104$). However, there was an interaction effect ($p = 0.050$), where flexibility was

259 improved beyond baseline measures at 72 h in the WPH group, but had failed to recover in
260 the CHO group ($p = 0.011$, ES = 1.3).

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

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

270 **Discussion**

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

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

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

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

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

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

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

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

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

357 protein intakes beyond recommended levels can ameliorate recovery from EIMD. This
358 research adds to the existing body of knowledge by demonstrating the application of WPH
359 supplementation in female populations to improve recovery following strenuous exercise.

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362 protein hydrolysate and carbohydrate supplements used in the present study. All other
363 elements of the study were funded by Northumbria University, UK. The company supplying
364 the supplements had no role in the study design, data collection and analysis, decision to
365 publish, or preparation of the manuscript.

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549

550 **Figure captions**

551 Fig 1. Schematic of testing protocol illustrating time-points where the supplements were
552 consumed and measures of dependent variables taken. Diet and exercise was controlled for
553 24 h prior to exercise-induced muscle damage (EIMD) and for the duration of data collection.

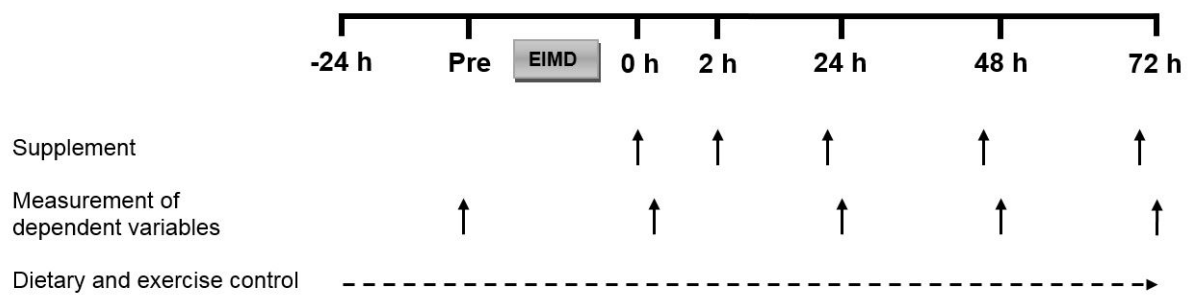
554 Fig 2. Hamstring stiffness and flexibility measured using the sit and reach test post exercise-
555 induced muscle damage in the whey protein hydrolysate (WPH) (n = 10) and carbohydrate
556 (CHO) (n = 10) groups. Values presented as mean \pm SD. #denotes significantly higher at 72 h
557 in WPH group. Significance at $p < 0.05$.

558 Fig 3. Reactive strength index (RSI) post exercise-induced muscle damage in the whey
559 protein hydrolysate (WPH) (n = 10) and carbohydrate (CHO) (n = 10) groups. Values
560 presented as mean \pm SD. *denotes significantly higher RSI in WPH group. Significance at $p <$
561 0.05.

562 Fig 4. Total creatine kinase (CK) post exercise-induced muscle damage in the whey protein
563 hydrolysate (WPH) (n = 10) and carbohydrate (CHO) (n = 10) groups. Values presented as
564 mean \pm SD. #denotes significantly greater reductions at 48 h in WPH group. Significance at
565 $p < 0.05$.

566

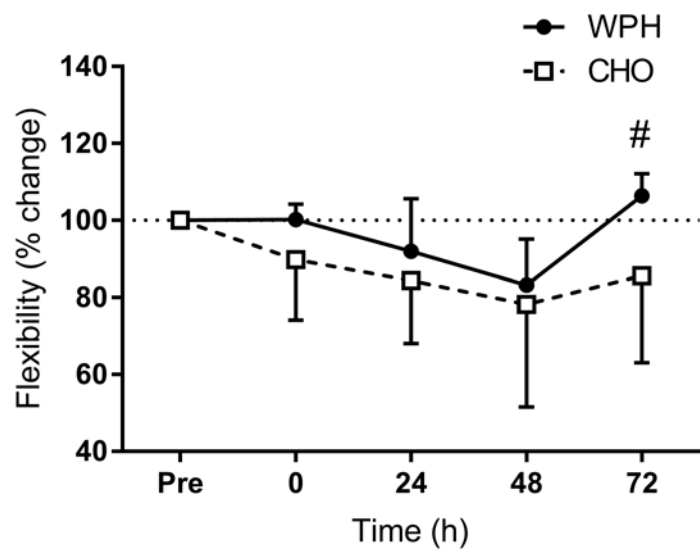
567 Figure 1



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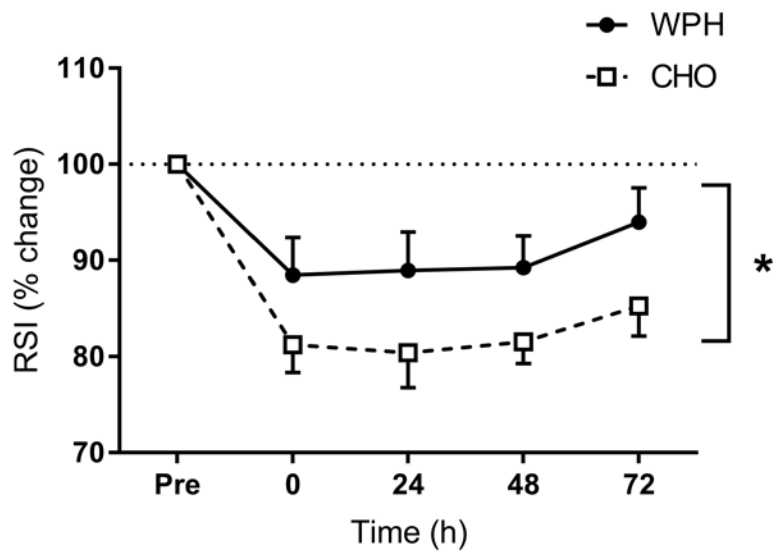
570 Figure 2.



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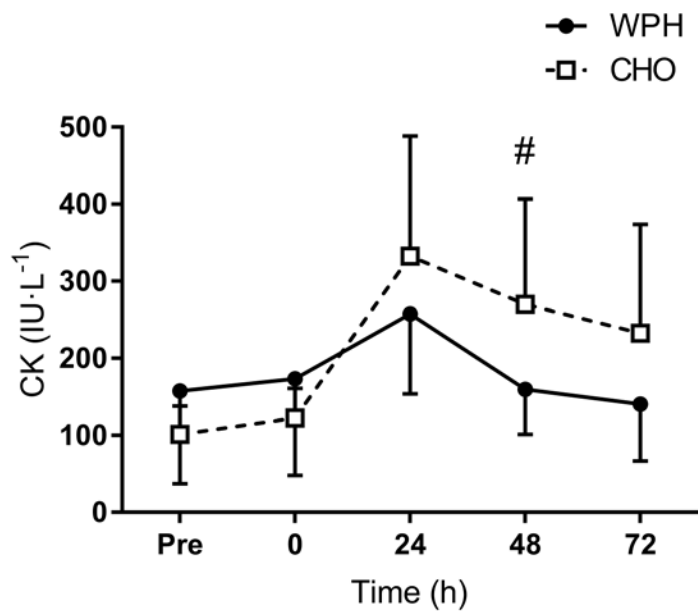


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576 Figure 4.

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

Meal	Food and drink provided
Breakfast	2 x white bread, toasted, with butter and strawberry jam 1 x glass of milk
Lunch¹	1 x sandwich or salad 1 x packet of crisps 1 x fruit smoothie
Evening Meal¹	1 x curry or chilli
Snacks	1 x banana 1 x cereal bar 1 x packet of jelly sweets 1 x yoghurt

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

585

586 Table 2. Daily dietary intake of participants over the four-day data collection period¹, mean ±
 587 SD.

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

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

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 595 protein hydrolysate group (*n* = 10); CHO, carbohydrate group (*n* = 10); %TEI, percentage of
 596 total energy intake. * denotes significant difference between groups (*p* < 0.05).

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

Variable	Group	Time post muscle-damaging exercise (h)				
		Pre	0	24	48	72
DOMS, mm	WPH	0.0 \pm 0.0	16.8 \pm 19.9 [†]	47.6 \pm 26.7 [†]	56.7 \pm 17.8 [†]	19.4 \pm 13.2 [†]
	CHO	1.0 \pm 2.5	13.0 \pm 20.1	65.0 \pm 49.0 [†]	71.2 \pm 45.0 [†]	37.1 \pm 27.4 [†]
RF PPT, % (N)	WPH	100 \pm 0 (61.1 \pm 18.2)	102.5 \pm 13.0 (63.8 \pm 25.0)	89.9 \pm 16.6 (56.1 \pm 23.7)	98.9 \pm 14.7 (62.0 \pm 24.8)	120.5 \pm 23.2 [†] (75.3 \pm 30.8)
	CHO	100 \pm 0 (52.6 \pm 14.7)	102.3 \pm 11.2 (53.8 \pm 15.3)	97.4 \pm 30.6 (51.7 \pm 23.8)	104.1 \pm 29.2 (55.4 \pm 23.0)	123.4 \pm 36.1 [†] (65.6 \pm 26.7)
VL PPT, % (N)	WPH	100 \pm 0 (61.0 \pm 17.5)	101.5 \pm 12.0 (61.9 \pm 20.2)	87.4 \pm 15.7 (53.9 \pm 20.4)	95.5 \pm 20.7 (59.1 \pm 23.9)	119.5 \pm 18.2 [†] (73.7 \pm 26.8)
	CHO	100 \pm 0 (50.9 \pm 15.6)	99.5 \pm 12.5 (50.3 \pm 15.2)	98.2 \pm 25.9 (48.7 \pm 16.6)	100.7 \pm 33.0 (50.9 \pm 21.6)	120.8 \pm 37.2 (60.8 \pm 24.8)
GM PPT, % (N)	WPH	100 \pm 0	101.1 \pm 15.6	94.1 \pm 16.6	106.9 \pm 15.1	125.9 \pm 22.5 [†]

		(60.6 ± 20.4)	(61.1 ± 23.5)	(57.2 ± 22.2)	(64.1 ± 21.7)	(74.2 ± 23.0)
	CHO	100 ± 0	97.3 ± 15.5	94.6 ± 26.3	101.9 ± 28.8	116.0 ± 28.5
		(48.6 ± 17.8)	(47.2 ± 17.5)	(45.7 ± 21.0)	(48.3 ± 17.8)	(56.0 ± 24.4)
Thigh girth, % (cm)	WPH	100 ± 0	100.3 ± 0.8	100.1 ± 0.6	99.8 ± 1.2	99.7 ± 1.0
		(51.9 ± 4.4)	(52.1 ± 4.4)	(52.0 ± 4.3)	(51.8 ± 4.0)	(51.7 ± 4.2)
	CHO	100 ± 0	99.9 ± 0.7	100.2 ± 0.8	100.2 ± 0.8	100.6 ± 0.5
		(48.9 ± 3.5)	(48.8 ± 3.3)	(48.8 ± 3.2)	(48.8 ± 3.2)	(48.8 ± 3.3)
Calf girth, % (cm)	WPH	100 ± 0	99.9 ± 0.4	99.6 ± 0.5	99.8 ± 0.6	99.9 ± 0.8
		(36.9 ± 1.8)	(36.8 ± 1.7)	(36.7 ± 1.7)	(36.8 ± 1.7)	(36.8 ± 1.7)
	CHO	100 ± 0	99.6 ± 0.5	99.7 ± 1.1	99.7 ± 0.6	100.0 ± 0.9
		(35.0 ± 2.8)	(34.9 ± 2.7)	(34.9 ± 2.7)	(34.9 ± 2.8)	(35.0 ± 2.9)
CMJ, % (cm)	WPH	100 ± 0	86.7 ± 8.4 [‡]	94.2 ± 8.3	92.2 ± 4.2 [‡]	95.2 ± 7.1
		(26.8 ± 4.4)	(23.2 ± 4.5)	(25.3 ± 5.5)	(24.6 ± 4.1)	(25.6 ± 5.3)
	CHO	100 ± 0	88.1 ± 6.9 [‡]	87.4 ± 10.0 [‡]	89.7 ± 9.3 [‡]	94.5 ± 11.1
		(24.3 ± 2.8)	(21.3 ± 2.0)	(21.1 ± 2.6)	(21.7 ± 2.8)	(22.9 ± 3.2)

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

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

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