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1 **Title:** The effects of a high protein diet on markers of muscle damage following exercise in
2 active older adults: a randomized, controlled trial

3

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26 **Abstract**

27 **Purpose:** This study examined whether a higher protein diet following strenuous exercise can
28 alter markers of muscle damage and inflammation in older adults. **Methods:** Using a double-
29 blind, independent group's design, 10 males and 8 females (age, 57 ± 4 years; mass, 72.3 ± 5.6
30 kg; height, 1.7 ± 6.5 m) were supplied with a higher protein (HP; $2.50 \text{ g}\cdot\text{kg}\cdot\text{day}^{-1}$) or moderate
31 protein (MP; $1.25 \text{ g}\cdot\text{kg}\cdot\text{day}^{-1}$) diet for 48 h after 140 squats with 25% of their body mass.
32 Maximal isometric voluntary contractions (MIVC), muscle soreness, creatine kinase (CK),
33 brief assessment of mood adapted (BAM+), and inflammatory markers were measured pre, 24
34 and 48 h post-exercise. **Results:** MIVC decreased post-exercise ($P = 0.001$, $\eta^2: 0.421$) but did
35 not differ between groups ($P = 0.822$, $\eta^2: 0.012$). Muscle soreness peaked at 24 h post in MP
36 (44 ± 30 mm) and 48 h post in HP (70 ± 46 mm) ($P = 0.005$; $\eta^2: 0.282$); however, no group
37 differences were found ($P = 0.585$; $\eta^2: 0.083$). Monocytes and lymphocytes significantly
38 decreased post-exercise and eosinophils increased 24 h post ($P < 0.05$) but neutrophils, CK,
39 interleukin-6, c-reactive protein, monocyte-chemotactic protein-1 and BAM+ were unchanged
40 by exercise or the intervention ($P > 0.05$). **Conclusion:** In conclusion, $2.50 \text{ g}\cdot\text{kg}\cdot\text{day}^{-1}$ of
41 protein is not more effective than $1.25 \text{ g}\cdot\text{kg}\cdot\text{day}^{-1}$ for attenuating indirect markers of muscle
42 damage and inflammation following strenuous exercise in older adults.

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45 **Key words:** High intensity exercise; whey protein; immunity.

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55 **Introduction**

56 High intensity exercise, especially that encompassing repetitive eccentric-muscle contractions,
57 often leads to muscle soreness, inflammation and a drop in neuromuscular function that can
58 persist for several days (Hyldahl & Hubal, 2014; Warren, Ingalls, Lowe, & Armstrong, 2002).
59 These symptoms are thought to be the result of disruption to the excitation-contraction coupling
60 process and/or ultrastructural damage to muscle fibres and the surrounding extracellular matrix
61 (Hyldahl & Hubal, 2014; Warren et al., 2002).

62 There are several factors that influence the magnitude of force loss and muscle soreness
63 following exercise, including the type, volume, intensity and novelty of the bout (Hyldahl &
64 Hubal, 2014). An important, but only more recently considered factor, is age; indeed, it has
65 been shown older individuals (≥ 50 years of age) recover from exercise at a slower rate than
66 their younger counterparts (Brisswalter & Nosaka, 2013; Doering, Jenkins, et al., 2016;
67 Easthope et al., 2010). The reasons for this are likely multi-factorial, but one recent study
68 suggested that the so called ‘anabolic resistance’ associated with old age, characterized by an
69 impaired muscle protein synthetic response, is likely to be an important factor. Indeed, Doering
70 et al., (Doering, Reaburn, Borges, Cox, & Jenkins, 2017) found that in response to 20 g of whey
71 protein, myofibril fractional synthetic rate (FSR) was $\sim 12\%$ lower in older (~ 53 years old)
72 versus younger (~ 27 years old) adults in the 3 days following a bout of muscle-damaging
73 exercise. These findings were associated with poorer performance during a cycling time trial
74 10 h after the exercise bout, suggesting that the recovery of muscle force was slower in the
75 older adults. It was speculated that this could be due to an age related impairment in mammalian
76 target of rapamycin complex 1 and/or satellite cell activation, possibly driven by immune
77 senescence or “inflammageing”, the age-related phenomenon characterised by a persistent
78 elevation in systemic immune markers such as interleukin-6 and c-reactive protein (Calder et
79 al., 2017; Doering et al., 2017; Doering, Reaburn, Phillips, & Jenkins, 2016).

80 In addition to ensuring adequate energy intake (Minor, Heusinger, Melanson, Hamilton, &
81 Miller, 2012), one way to overcome the age-related decrease in MPS is to consume higher
82 amounts of dietary protein following exercise. Studies have shown that older adults require
83 higher amounts of protein (≥ 0.40 g·kg⁻¹) than younger adults (0.20-0.25 g·kg⁻¹) to maximally
84 stimulate MPS (Katsanos, Kobayashi, Sheffield-Moore, Aarsland, & Wolfe, 2006; Moore et
85 al., 2015). This suggests that increasing post-exercise protein intake is a potential strategy for

86 enhancing acute functional recovery and attenuating markers of exercise induced muscle
87 damage (EIMD) in older adults.

88 To date, this has only been explored by one study (Doering et al., 2017). In this trial, when 8
89 masters triathletes (~53 years old) consumed 3 meals containing 0.60 g·kg⁻¹ as opposed to 0.30
90 g·kg⁻¹ of protein every 2 h following 30 minutes of downhill running, they reported less fatigue
91 and were 5% stronger when re-tested 8 hours later. However, it is unclear whether higher
92 protein intake would have expedited recovery in the 48-h following the exercise task, which is
93 when markers of EIMD like creatine kinase (CK) and muscle soreness (DOMS) tend to be
94 greatest (Clarkson & Sayers, 1999). Such changes could have important implications for
95 muscle regeneration in older adults, which is shown to be impaired in the days following
96 strenuous eccentric exercise or muscle injury (Lovering & Brooks, 2014). Moreover, prolonged
97 impairments in functional capacity could not only hinder exercise performance but could also
98 affect tasks for daily living or deter older adults from performing exercise (Lovering & Brooks,
99 2014). Thus, interventions that could help to manage these symptoms in the days following
100 exercise are desirable. Furthermore, Doering et al. (2017) did not measure changes in
101 inflammation. Yet, one of the mechanisms by which increasing dietary protein intake might
102 support muscle remodelling is by attenuating the acute inflammatory response associated with
103 strenuous exercise (Kato et al., 2016; Kerasioti et al., 2013; Rowlands et al., 2016).

104 Consequently, the aim of this study was to assess whether a higher protein intake (2.50
105 g·kg·day⁻¹ or 0.50 g·kg⁻¹ per meal) for 2 days following strenuous exercise could attenuate
106 inflammation and markers of muscle damage in recreationally trained adults over the age of
107 50. We hypothesized that a higher protein intake would lessen muscle soreness and
108 inflammation following the exercise bout, and muscle function would be restored quicker in
109 the subsequent 48 h.

110

111 **Methods**

112 *Participants*

113 Eighteen male (n = 10) and female (n = 8) physically active ≥50-year olds volunteered for this
114 study (see Table 1 for physical characteristics). They were recruited by contacting local sports
115 via email and social media. All participants were required to be performing ≥3 h per week of
116 training for an endurance sport (running, swimming, rowing, cycling) to be eligible for this

117 study. This was to ensure they would be able to complete the exercise task. None of the
118 participants were competing at a national level or higher and all participants verbally confirmed
119 they were not accustomed to the strenuous squatting exercise used in this study. Based on a
120 similarly designed study (Bell, Stevenson, Davison, & Howatson, 2016), we calculated (using
121 G*Power) that at 80% power, and an α of 0.05, at least 8 volunteers were required to detect a
122 group difference of 10% in our primary outcome MIVC (7 SD units) post-exercise.

123 Participants completed a medical screening questionnaire and were excluded if they had a food
124 allergy, had, or were using anti-inflammatory medications (within 1 month of participation),
125 had received hormone replacement medications, had a previous history of cardiovascular or
126 renal disease, or any other contraindication to the study procedures. Participants were required
127 to avoid putative recovery interventions (e.g., massage) throughout the testing period.
128 Institutional ethical approval was granted by the Newcastle University Ethics Committee; all
129 participants read a participant information sheet before providing written informed consent
130 prior to participation.

131 *Experimental design*

132 In a double blind, placebo-controlled, parallel groups design, participants were randomized to
133 1 of 2 experimental treatment arms: a higher protein group (HP) or a moderate protein group
134 (MP). Participants were randomly stratified using sex and maximal isometric voluntary
135 contraction (MIVC) scores as blocking factors. These scores were collected at a familiarisation
136 session completed ≥ 5 days prior to the main trials. To ensure blinding, the diets were prepared
137 and prescribed by a registered sport nutritionist who was not involved in data collection.
138 Participants were also not informed which diet they were receiving and were falsely led to
139 believe that the differences in protein intake between the two diets was solely from the
140 maltodextrin and whey protein supplements they received.

141 On the day of and the two days following the main trials, participants consumed a standardized
142 breakfast (Oat and Honey cereal bar, Nature Valley, UK; energy, 192 kcal; carbohydrate, 27.1
143 g; fat, 7.2 g; protein, 3.4 g) 30 minutes prior to performing the baseline measures (08:00 –
144 09:00). Water before testing was allowed ad-libitum. The baseline measures were collected in
145 the following order: muscle soreness, Brief Assessment of Mood adapted (BAM+), a venous
146 blood sample, and MIVC. Immediately following these measures, participants performed 140
147 weighted squats to induce muscle-damage. They were then provided with all meals and

148 supplements for the following two days. Participants were instructed to avoid intense exercise
149 in the 48-h leading up to the main trials and until all testing was completed.

150 *Muscle damaging exercise protocol*

151 To induce muscle damage, participants performed a total of 140 squats while wearing a vest
152 containing 25% of their body mass (kg). The squats were performed as 7 sets of 20 repetitions,
153 separated by 2 minutes of passive recovery. Participants were required to squat down to an
154 angle equivalent to 90° of knee flexion for each repetition. This protocol was adapted from a
155 previous study that found 140 squats, without additional weight, induced significant muscle
156 damage in untrained young adults (Shimomura et al., 2010). The additional weight added in
157 the present study was to try and augment muscle damage.

158 *Dietary Intervention*

159 In the 48h post muscle damaging exercise, participants were provided with all of their food and
160 fluids. Participants were allowed to consume water or non-caloric drinks ad libitum throughout
161 this period, but all other foods and beverages were prohibited. Each feed post-testing (5 in
162 total), was formulated to contain either 0.50 g·kg·BM⁻¹ (HP diet) or 0.25·g·kg⁻¹ (MP diet) of
163 protein, corresponding to 2.50 or 1.25 g·kg·BM·day⁻¹ of protein, respectively. Participants had
164 one feed immediately post-exercise and the further 4 feeds every 3 h (see Supplementary File
165 for further details). The protein amounts were based on the current per meal recommendations
166 for athletic populations (Moore et al., 2015). The daily energy macronutrient composition of
167 the two diets is provided in Table 1. Further details on the diets are provided in a Supplementary
168 File.

169 *Maximal isometric voluntary contraction*

170 As described previously (Clifford et al., 2017), MIVC was measured with a portable strain
171 gauge (MIE Medical Research Ltd., Leeds, UK). Participants were seated upright and had a
172 perspex gauze attached to a force transducer strapped to their ankle. After a countdown,
173 participants were instructed to maximally extended their right knee flexor and hold for a 3
174 second contraction. The peak value (N) from 3 maximal contractions (separated by a 60 s rest
175 period) was used for analysis. The inter-day CV for this measure and procedure is 3.9% in our
176 lab.

177

178 *Muscle soreness*

179 Lower limb muscle soreness was measured subjectively with a 200 mm visual analogue scale
180 (Clifford et al., 2017). Participants performed a squat to a 90-degree knee angle and drew a
181 vertical line on a visual analogue scale labelled with ‘no soreness’ (0 mm) at one end and
182 ‘unbearably painful’ at the other (200 mm). The line placement was measured with a ruler and
183 recorded.

184 *Brief assessment of mood adapted*

185 The BAM+ is a measure of performance readiness and was scored by marking a vertical line
186 on a 100 mm VAS between “not at all” and “extremely”. The scores were calculated by
187 subtracting the 4 positively associated questions by the 6 negatively associated questions. A
188 full list of the included questions is available in Shearer et al. (Shearer et al., 2017).

189 *Blood sampling*

190 Venous blood samples were collected via venepuncture. At all 3 time points (0, 24 and 48 h
191 post-exercise), blood was drawn into a 10 ml vacutainer for serum and a 10- and 4-ml
192 vacutainer coated with di-potassium ethylene diamine tetra-acetic acid (EDTA). The 4 ml
193 EDTA vacutainer was transported to a local hospital for analysis of full blood counts. The
194 remaining tubes were centrifuged at 3000 rpm (4 °C) for 10 minutes to separate the supernatant,
195 which were subsequently aspirated into aliquots and stored in a -80° freezer for later analysis.

196 *Blood analysis*

197 Full blood cell counts were assessed with an automated haematology system (Sysmex XE-
198 2100, Illinois, US). CV for this analysis is <10%. Creatine kinase (CK) and high sensitivity C-
199 reactive protein (hs-CRP) was measured in serum using an automated system based on an
200 electrochemiluminescence method (Roche Modular, Roche Diagnostics, UK). CV for this
201 analysis was <5%. Plasma interleukin-6 (IL-6), interleukin-1 β (IL-1 β) and monocyte
202 chemoattractant protein (MCP-1) were measured using commercially available ELISA kits (R
203 and D systems, MN, US). Because ~25% of the samples were below the detectable limit for
204 IL-1 β analysis results are not reported for this marker. CV for IL-6 and MCP-1 were 15 and
205 5%, respectively.

206 *Statistical Analysis*

207 Data were analysed using SPSS (Version 24, SPSS, Armonk, NY). All data are expressed as
208 means \pm standard deviation (SD); an α level of $P < 0.05$ was accepted to be statistically
209 significant. Baseline values of muscle function, age, height, body mass and energy intake were
210 assessed for group differences using an independent samples t-test. Between group differences
211 in activity levels, carbohydrate, fat and protein intakes were analysed with Mann–Whitney U
212 non-parametric test because they were not normally distributed ($P < 0.05$ on the Shapiro-Wilk
213 test). Dependent variables were analysed with a mixed model analysis of variance (ANOVA)
214 with two group levels (HP and MP) and three repeated measures time-points (0, 24 and 48 h
215 post-exercise). Because leukocytes and eosinophils were significantly different between groups
216 at 0 h these variables analysed as percentage change from baseline. Muscle soreness, IL-6,
217 MCP-1 and eosinophils were not normally distributed and therefore logged transformed prior
218 to analysis. If the ANOVA indicated a significant effect, post-hoc tests with Bonferroni
219 corrections were performed to locate the specific differences. Where sphericity was
220 significantly violated, Greenhouse-Geisser adjustments were used. Partial-eta² (η^2) effect
221 size statistics were considered small (0.01–0.06), medium (0.06–0.14) or large (≥ 0.14)
222 changes.

223 **Results**

224 There were no differences in the participant's physical characteristics, activity levels and
225 energy intake between the two groups ($P > 0.05$; Table 1). However, as expected, fat and
226 carbohydrate intake were lower and protein intake higher in the HP group ($P < 0.05$; Table 1).

227 MIVC were lower following muscle damaging exercise in both groups (time effect; $P = 0.001$,
228 η^2 : 0.421; Figure 1A) but no interaction effects were present ($P = 0.822$, η^2 : 0.012). BAM+
229 reduced after exercise (time effect; $P = 0.049$; η^2 : 0.172; Figure 1C); however, there was no
230 interaction effect ($P = 0.363$; η^2 : 0.058). Muscle soreness increased in the days following
231 exercise, peaking at 24 h post in the MP group and 48 h post in the HP group (time effect; $P =$
232 0.005 ; η^2 : 0.282); however, no interaction effects were found ($P = 0.585$; η^2 : 0.083; Figure
233 1B).

234 Monocytes and lymphocytes were decreased in the days after exercise, and eosinophils
235 increased 24 h post, but total leukocyte count, neutrophils and basophils remained unchanged
236 pre to post-exercise (Table 2). There were no group differences in any of the haematological
237 markers (Table 2). CK did not increase after exercise (time effect $P = 0.359$, η^2 : 0.062) and
238 there were no group differences at any time point (interaction effect; $P = 0.779$, η^2 : 0.006;

239 Figure 1D). hs-CRP displayed no time ($P = 0.783$, $\eta^2: 0.015$) or interaction effects ($P = 0.905$,
240 $\eta^2: 0.006$; Figure 1F), neither did IL-6 (time: $P = 0.497$, $\eta^2: 0.039$; interaction: $P = 0.159$,
241 $\eta^2: 0.133$; Figure 1E) or MCP-1 (time: $P = 0.772$, $\eta^2: 0.009$; interaction: $P = 0.685$, η^2 :
242 0.016 ; Figure 1G).

243 **Discussion**

244 In contrast to our hypothesis, a higher protein diet for 2 days following strenuous exercise was
245 no more effective than a moderate protein diet for attenuating inflammation and markers of
246 EIMD in active older adults.

247 Only one other study has examined the effects of high protein intake on recovery from
248 strenuous exercise in older adults. In contrast to the present study, they found feeding high
249 amounts of dietary protein in the post-exercise period enhanced the recovery of muscle function
250 in 8 master's triathletes (Doering et al., 2017) The reason for the disparate findings between
251 the current and previous study is not overtly clear but it could be related to the amount and
252 timing of protein intake and/or when the measures were collected. For example, Doering and
253 colleagues fed their participants higher amounts of protein ($0.60 \text{ g}\cdot\text{kg}^{-1}$ vs. $0.50 \text{ g}\cdot\text{kg}^{-1}$) in the
254 post-exercise period but did not monitor recovery for longer than 8 h post-exercise. By contrast,
255 in the present study, the dietary control and collection of outcome measures continued for 48
256 h post-exercise. As such, it could be that; 1) the $0.50 \text{ g}\cdot\text{kg}^{-1}$ of protein we provided at each
257 feed was not sufficient to affect myofibrillar recovery processes/inflammation in our
258 participants or that; 2) higher than the recommended amounts of protein are only beneficial
259 when recovery times are short (e.g., ≤ 8 h). The fact that we did not measure markers of EIMD
260 at 8 h post-exercise to compare with Doering et al. (2017) is an acknowledged limitation of this
261 study. Clearly, more studies are needed to determine if higher than recommended protein
262 intakes can expedite recovery in older active adults.

263 Although there was a decrease in MIVC and an increase in muscle soreness following exercise,
264 none of the other markers typically associated with EIMD — including the pro-inflammatory
265 markers (e.g., neutrophils, IL-6, MCP-1) and CK, were significantly altered 24 and 48 h
266 following the exercise bout. CK was also not altered in a previous study that used an identical
267 protocol in untrained participants but without the added weight (Shimomura et al., 2010). This
268 study observed less muscle soreness than we did, but greater decrements in muscle function,
269 possibly due to the fact the participants were sedentary. This would suggest that the exercise
270 bout, while novel to the participants and encompassing a large number of eccentric muscle

271 contractions, only induced mild muscle-damage in our participants and, therefore, despite their
272 age, the systemic inflammatory response was minor (Paulsen, Mikkelsen, Raastad, & Peake,
273 2012). It is possible that the minor changes in the markers of muscle-damage limited our ability
274 to detect small group differences or rendered the high protein diet less effective. With regards
275 to the latter point, it would be reasonable to assume that any intervention aiming to influence
276 recovery processes after exercise would be more effective if the symptoms of muscle damage
277 are marked and prolonged. Perhaps if the participants were less physically active or over the
278 age of 65, which is when impairments in muscle regeneration accelerate further (Cruz-Jentoft
279 et al., 2010; Kamandulis et al., 2017), muscle damage and inflammation would have been
280 greater. With that said, we did anticipate that we would see larger changes in these markers,
281 given that Doering et al. (Doering, Jenkins, et al., 2016) found MPS to be lower in trained
282 triathletes of similar age to our volunteers, and other studies have found markers of muscle
283 damage to be exacerbated in adults 50 - 65 years of age (Lavender & Nosaka, 2006; Ploutz-
284 Snyder, Giamis, Formikell, & Rosenbaum, 2001).

285 It should also be highlighted that the magnitude of changes to neuromuscular and soreness
286 variables observed in this study are akin to those we have seen after competitive events such
287 as a marathon (Clifford et al., 2017) or soccer match (Abbott, Brett, Cockburn, & Clifford,
288 2019). As such, the changes in muscle function and muscle soreness in the present study are
289 likely a better reflection of the changes observed after more ecologically valid forms of
290 exercise, than the changes observed after most lab-based exercise protocols that typically result
291 in severe myofibrillar disruption, evoking symptoms that last for several weeks (Paulsen et al.,
292 2012).

293 It is important to note that, in general, the benefits of increasing dietary protein on acute muscle
294 function recovery remains equivocal, irrespective of age. Indeed, a systematic review of the
295 literature suggested that while, in theory, increasing protein intake to augment MPS should
296 enhance myofibrillar remodelling and, ostensibly, the recovery of muscle function, there is
297 little high-quality research to support this assumption (Pasiakos, Lieberman, & McLellan,
298 2014). Indeed, it has been proposed by others that the turnover of intramuscular proteins is
299 probably too slow to significantly influence the acute restoration of muscle contractile function
300 following strenuous exercise (Farup et al., 2014; Owens, Twist, Cobley, Howatson, & Close,
301 2019). With that said, ingesting whey protein post-exercise, as in this study, can also attenuate
302 inflammation (Kato et al., 2016; Kerasioti et al., 2013; Rowlands et al., 2016) and increase
303 muscle satellite cell activity (Farup et al., 2014), both of which might positively influence acute

304 functional recovery following exercise (Owens et al., 2019). Thus, an increase in MPS and
305 protein turnover are unlikely to be the only mechanisms by which dietary protein could
306 ameliorate symptoms of muscle damage. Future studies examining the effects of protein intake
307 of recovery, irrespective of age, should aim to control pre and post-exercise dietary intake, but
308 also, where possible, take measures of MPS, satellite cell activation and inflammation
309 alongside measures of functional recovery like isometric strength and muscle soreness.

310 A limitation of this study is that, due to ethical constraints, we did not measure MPS to see if
311 the HP diet augmented muscle FSR in the 48-h following exercise. However, as summarised
312 by Moore and colleagues, the fact that several studies show higher amounts of protein (≥ 0.40
313 $\text{g}\cdot\text{kg}\cdot\text{meal}^{-1}$) optimises MPS in older adults, lends support to this assertion (Moore et al., 2015).
314 Due to funding constraints we also limited our observations to 48 h post-exercise when some
315 variables had not completely returned to baseline. We suggest that future studies continue
316 monitoring recovery for 72 – 96 h post-exercise or until markers are restored to baseline levels
317 to ensure they do not miss any differences that might arise during the later stages of recovery.
318 Similarly, by not taking blood samples < 24 h post exercise, we likely missed peak increases in
319 the inflammatory cytokines measured and acknowledge this is a limitation of the current study.
320 A key strength of this study is the strict dietary control, a design aspect often neglected in
321 protein and exercise recovery research, and probably a key reason for the equivocal findings to
322 date (Pasiakos et al., 2014). Nonetheless, we acknowledge that not standardizing diet in the 48
323 h prior to exercise could have influenced the findings and recommend future studies take this
324 into consideration.

325 **Conclusion**

326 In conclusion, a higher protein diet ($2.5 \text{ g}\cdot\text{kg}\cdot\text{day}^{-1}$) for 2 days did not attenuate markers of
327 muscle damage or inflammation following unaccustomed exercise in older (~ 57 years) active
328 adults. This could be due to the fact muscle damage was only mild. Future studies should utilise
329 exercise protocols that elicit greater levels of muscle damage.

330 **Decelerations**

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332 Funding for the cytokine assay kits was provided by the British Association of Sport and
333 Exercise Sciences (BASES) from a grant that was awarded to Tom Clifford. The organisation
334 had no input on the design, analysis and interpretation of the results.

335 **Conflict of interest**

336 The authors declare no conflicts of interest.

337 **Author contributions**

338 The study was designed by TC, EH and EJS; data were collected and analysed by TC, EH,
339 KBD, GT, JS and KS; data interpretation and manuscript preparation were undertaken by TC,
340 JS, EH, EJS, KBD, GT, KS. All authors approved the final version of the paper.

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Table 1. Participant's physical characteristics and daily dietary intakes in the 48 h following muscle damaging exercise.

	HP	MP
Physical characteristics		
Sex (no. M/F)	5/4	5/4
Age (years)	57 ± 4	56 ± 4
Mass (kg)	73.6 ± 10.8	71.0 ± 9.3
Height (m)	1.73 ± 7.1	1.73 ± 5.9
Activity levels (h·wk ⁻¹)	8.5 ± 4.7	7.7 ± 2.5
MIVC (N)	385 ± 124	408 ± 151
Dietary intake		
Energy		
Kcal·day ⁻¹	2464.85 ± 321.01	2425.86 ± 266.45
Kcal·kg·day ⁻¹	33.55 ± 0.58	34.34 ± 0.85
Protein*		
g·day ⁻¹	184.05 ± 26.90	88.69 ± 57
g·kg·day ⁻¹	2.50 ± 0.00	1.25 ± 0.00
Carbohydrate*		
g·day ⁻¹	284.60 ± 41.60	308.83 ± 40.28
g·kg·day ⁻¹	3.86 ± 7.02	4.35 ± 9.81
Fat*		
g·day ⁻¹	29.27 ± 2.68	52.52 ± 3.93
g·kg·day ⁻¹	0.40 ± 0.23	0.74 ± 0.49

455 HP, higher protein; MP, moderate protein; MIVC, maximal isometric contractions; *between group
456 difference (P < 0.05). Values are means ± SDs. n = 9 per group. M = male, F = female.

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464 **Table 2.** Heamotological markers pre (0 h), 24 and 48 h post-exercise in the high protein (HP) and moderate protein (MP) groups.
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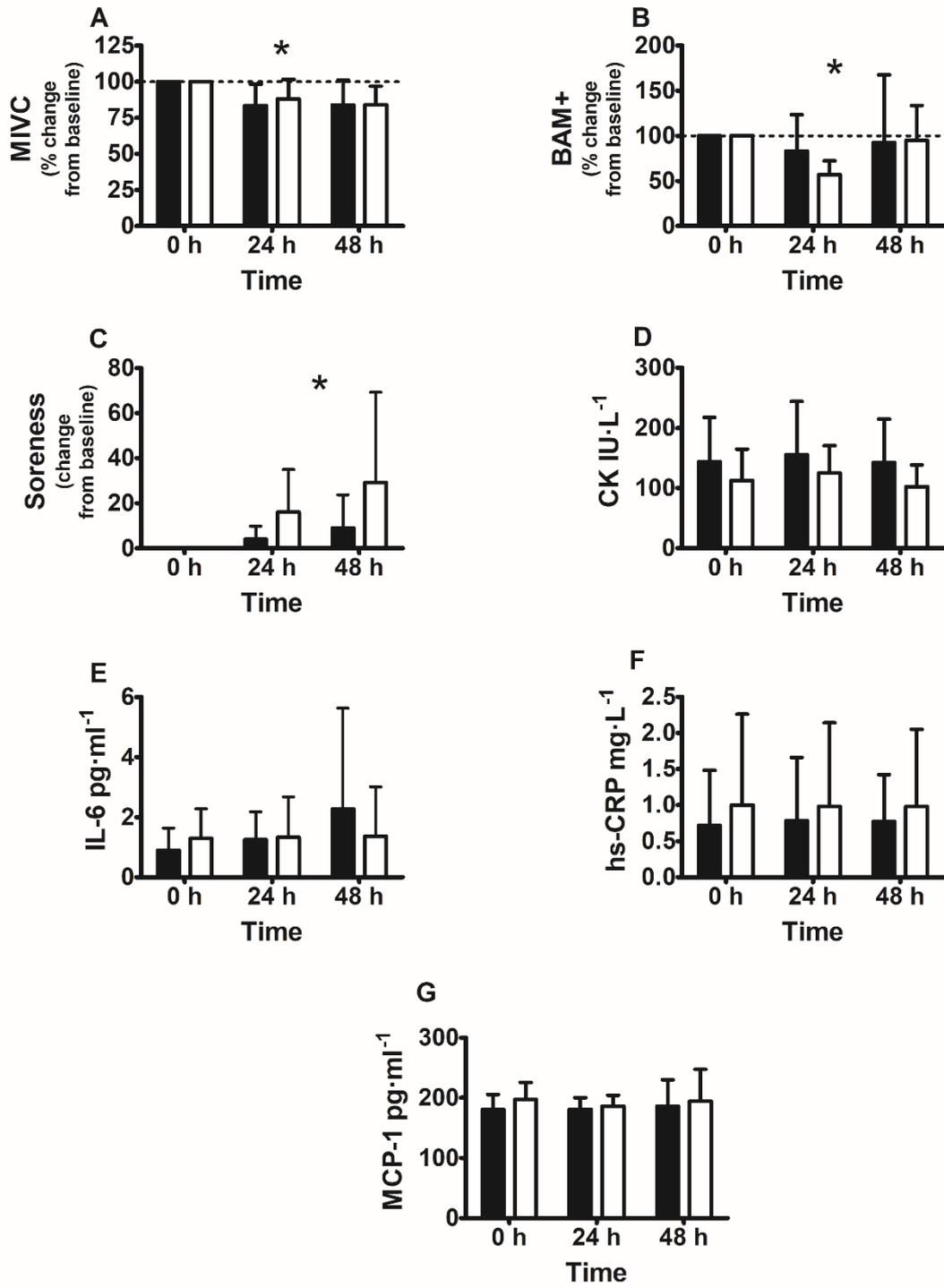
	0 h	+24 h	+48 h	<i>P</i> time effect*	<i>P</i> interaction effect*
Leukocytes (10^{*9} Cells·L⁻¹)					
HP	5.67 ± 0.90	5.71 ± 1.15	5.41 ± 0.78	0.322 (0.068)	0.710 (0.20)
MP	4.54 ± 1.09	4.60 ± 1.15	4.54 ± 1.21		
Neutrophils (10^{*9} Cells·L⁻¹)					
HP	3.18 ± 0.85	3.38 ± 1.09	3.19 ± 0.86	0.349 (0.60)	0.600 (0.24)
MP	2.46 ± 0.96	2.64 ± 0.95	2.65 ± 1.07		
Lymphocytes (10^{*9} Cells·L⁻¹)					
HP	1.74 ± 0.41	1.59 ± 0.35**	1.52 ± 0.29**	0.001 (0.408)	0.486 (0.044)
MP	1.47 ± 0.12	1.40 ± 0.10**	1.33 ± 0.10**		
Monocytes (10^{*9} Cells·L⁻¹)					
HP	0.50 ± 0.11	0.30 ± 0.23**	0.46 ± 0.11	0.001 (0.601)	0.381 (0.053)
MP	0.45 ± 0.13	0.15 ± 0.14**	0.39 ± 0.12		
Eosonphils (10^{*9} Cells·L⁻¹)					
HP	0.21 ± 0.08	0.39 ± 0.10**	0.20 ± 0.10	0.001 (0.693)	0.133 (0.129)
MP	0.13 ± 0.07	0.38 ± 0.12**	0.14 ± 0.07		
Basophils (10^{*9} Cells·L⁻¹)					
HP	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.668 (0.025)	0.668 (0.025)
MP	0.04 ± 0.01	0.03 ± 0.02	0.04 ± 0.02		

466 *Number in parenthesis is η^2 effect sizes. **Different to baseline ($P < 0.05$). $n = 9$ per group.
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469 **Figure 1:** Changes in maximal isometric voluntary contractions (MIVC), Brief Assessment of
470 Mood Adapted (BAM+), muscle soreness, creatine kinase (CK), interleukin-6 (IL-6), high-
471 sensitivity C-reactive protein (hs-CRP) and monocyte chemotactic protein-1 (MCP-1) pre-
472 exercise (0 h), 24 and 48 h post exercise after a high protein (HP) or moderate protein diet
473 (MP). *Denotes time effect, $P < 0.05$. MIVC, BAM+ and muscle soreness are presented as
474 change from baseline for illustrative purposes.

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MP HP



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481 **Online supplementary material**

482 Both diets provided a small breakfast before testing, followed by 3 meals and two liquid
483 boluses. Each feed post-testing (5 in total), was formulated to contain either $0.50 \text{ g}\cdot\text{kg}\cdot\text{BM}^{-1}$
484 (HP diet) or $0.25 \text{ g}\cdot\text{kg}^{-1}$ (MP diet) of protein, corresponding to 2.50 or $1.25 \text{ g}\cdot\text{kg}\cdot\text{BM}\cdot\text{day}^{-1}$ of
485 protein, respectively. The protein amounts were based on the current per meal
486 recommendations for athletic populations (Moore et al., 2015). For example, it is currently
487 recommended that $0.25 \text{ g}\cdot\text{kg}^{-1}$ of protein is required at each meal to optimise MPS in healthy
488 younger adults (Moore, 2015). This amount was then doubled for the experimental HP diet,
489 ensuring that post-exercise and each subsequent feed provided the $\geq 0.40 \text{ g}\cdot\text{kg}\cdot\text{BM}^{-1}$ postulated
490 to optimise daily MPS in older athletes (Doering, Reaburn, Phillips, & Jenkins, 2016; Moore
491 et al., 2015). The food stuffs provided were identical for both diets (tuna, chicken, pasta, cous
492 cous, mayonnaise, whey protein and maltodextrin) with the exception of additional olive oil in
493 the MP diet to match them for energy content.

494 To match the HP and MP diets for energy, the MP diet contained more carbohydrates and fats
495 (see Table 1 in manuscript). The specific foods were the same, but the ratios of each were
496 altered to get the desired energy intake. The overall energy content of the diet was
497 individualised for each participant and calculated to cover the energy needs for a moderate
498 level of activity using the Harris-Benedict equation (Harris & Benedict, 1918). To ensure an
499 even distribution of protein intake throughout the day — and therefore facilitate optimal
500 conditions for MPS (Areta et al., 2013), participants were instructed to consume each bolus 2
501 - 4 h apart. The foods were the same for each diet, and therefore the amino acid quality and
502 distribution was the same in each condition. Compliance with the diet was confirmed verbally
503 at each visit.

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