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Human Kinetics
ORIGINAL RESEARCH

The Effects of a High-Protein Diet on Markers of Muscle Damage Following Exercise in Active Older Adults: A Randomized, Controlled Trial

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Purpose: This study examined whether a higher protein diet following strenuous exercise can alter markers of muscle damage and inflammation in older adults. ***Methods:*** Using a double-blind, independent group design, 10 males and eight females (age 57 ± 4 years; mass 72.3 ± 5.6 kg; height 1.7 ± 6.5 m) were supplied with a higher protein (2.50 g·kg^{-1}·day^{-1}) or moderate protein (1.25 g·kg^{-1}·day^{-1}) diet for 48 hr after 140 squats with 25% of their body mass. Maximal isometric voluntary contractions, muscle soreness, creatine kinase, Brief Assessment of Mood Adapted, and inflammatory markers were measured preexercise, and 24 hr and 48 hr postexercise. ***Results:*** The maximal isometric voluntary contractions decreased postexercise ($p = .001$, $\eta_p^2 = .421$), but did not differ between groups ($p = .822$, $\eta_p^2 = .012$). Muscle soreness peaked at 24 hr post in moderate protein (44 ± 30 mm) and 48 hr post in higher protein (70 ± 46 mm; $p = .005$; $\eta_p^2 = .282$); however, no group differences were found ($p = .585$; $\eta_p^2 = .083$). Monocytes and lymphocytes significantly decreased postexercise, and eosinophils increased 24 hr postexercise ($p < 0.05$), but neutrophils, creatine kinase, interleukin-6, C-reactive protein, monocyte chemotactic protein-1, and Brief Assessment of Mood Adapted were unchanged by exercise or the intervention ($p > .05$). ***Conclusion:*** In conclusion, 2.50 g·kg^{-1}·day^{-1} of protein is not more effective than 1.25 g·kg^{-1}·day^{-1} for attenuating indirect markers of muscle damage and inflammation following strenuous exercise in older adults.

Keywords: high-intensity exercise, immunity, whey protein

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

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

In addition to ensuring adequate energy intake (Minor et al., 2012), one way to overcome the age-related decrease in muscle protein synthesis (MPS) is to consume higher amounts of dietary protein following exercise. Studies have shown that older adults require higher amounts of protein (≥0.40 g/kg) than younger adults (0.20–0.25 g/kg) to maximally stimulate MPS (Katsanos et al., 2006; Moore et al., 2015). This suggests that increasing postexercise protein intake is a potential strategy for enhancing acute functional recovery and attenuating markers of exercise-induced muscle damage (EIMD) in older adults.

To date, this has only been explored by one study (Doering et al., 2017). In this trial, when eight master triathletes (~53 years old) consumed three isocaloric meals containing 0.60 g/kg as opposed to 0.30 g/kg of protein every 2 hr following 30 min of downhill running, they reported less fatigue and were 5% stronger when retested 8 hr later. However, it is unclear whether higher protein intake would have expedited recovery in the 48 hr following the exercise task, which is when markers of EIMD like creatine kinase (CK) and muscle soreness (delayed onset muscle soreness)

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tend to be greatest (Clarkson & Sayers, 1999). Such changes could have important implications for muscle regeneration in older adults, which is shown to be impaired in the days following strenuous eccentric exercise or muscle injury (Lovering & Brooks, 2014). Moreover, prolonged impairments in functional capacity could not only hinder exercise performance but could also affect tasks for daily living or deter older adults from performing exercise (Lovering & Brooks, 2014). Thus, interventions that could help to manage these symptoms in the days following exercise are desirable. Furthermore, Doering et al. (2017) did not measure changes in inflammation. Yet, one of the mechanisms by which increasing dietary protein intake might support muscle remodeling is by attenuating the acute inflammatory response associated with strenuous exercise (Kato et al., 2016; Kerasioti et al., 2013; Rowlands et al., 2016).

Consequently, the aim of this study was to assess whether a higher protein intake (2.50 $g \cdot kg^{-1} \cdot day^{-1}$ or 0.50 g/kg per meal) for 2 days following strenuous exercise could attenuate inflammation and markers of muscle damage in recreationally trained adults over the age of 50 years. We hypothesized that a higher protein intake would lessen muscle soreness and inflammation following the exercise bout and muscle function would be restored quicker in the subsequent 48 hr.

Methods

Participants

Eighteen physically active ≥50 years olds (see Table 1 for physical characteristics) took part in this study. They were recruited by contacting local sports via e-mail and social media. All participants were required to be performing ≥3 hr per week of training for an endurance sport (running, swimming, rowing, and cycling) to be eligible for this study. This was to ensure they would be able to complete the exercise task. None of the participants were competing at a national level or higher, and all participants verbally confirmed that they were not accustomed to the strenuous squatting exercise used in this study. The participants were required to avoid putative recovery interventions (e.g., massage) throughout the testing period. Based on a similarly designed study (Bell et al., 2016), we calculated (using G*Power, Autenzell, Germany) that, at 80% power and an α of .05, at least eight volunteers were required to detect a group difference of 10% in our primary outcome MIVC (7 *SD* units) postexercise.

The participants completed a medical screening questionnaire and were excluded if they had a food allergy, had used or were using anti-inflammatory medications (within 1 month of participation), had received hormone replacement medications, had a previous history of cardiovascular or renal disease, or any other contraindication to the study procedures. Institutional ethical approval was granted by the Newcastle University Ethics Committee; all participants read a participant information sheet before providing written informed consent prior to participation.

Experimental Design

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

Table 1 Participants' Physical Characteristics and Daily Dietary Intakes in the 48 hr Following Muscle Damaging Exercise ($n = 9$ Per Group)

Variable	HP	MP
Physical characteristics		
Sex (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 (hr/week)	8.5 ± 4.7	7.7 ± 2.5
MIVC (N)	385 ± 124	408 ± 151
Dietary intake		
Energy		
Kcal/day	2,464.85 ± 321.01	2,425.86 ± 266.45
$Kcal \cdot kg^{-1} \cdot day^{-1}$	33.55 ± 0.58	34.34 ± 0.85
Protein*		
g/day	184.05 ± 26.90	88.69 ± 57
$g \cdot kg^{-1} \cdot day^{-1}$	2.50 ± 0.00	1.25 ± 0.00
Carbohydrate*		
g/day	284.60 ± 41.60	308.83 ± 40.28
$g \cdot kg^{-1} \cdot day^{-1}$	3.86 ± 7.02	4.35 ± 9.81
Fat*		
g/day	29.27 ± 2.68	52.52 ± 3.93
$g \cdot kg^{-1} \cdot day^{-1}$	0.40 ± 0.23	0.74 ± 0.49

Note. HP = higher protein; MP = moderate protein; MIVC = maximal isometric voluntary contractions; M = male; F = female. Values are presented as mean ± *SD*.
*Between-group difference ($p < .05$).

On the day of and the 2 days following the main trials, the participants consumed a standardized breakfast (Oat and Honey cereal bar; Nature Valley, Minneapolis, USA; energy 192 kcal; carbohydrate 27.1 g; fat 7.2 g; protein 3.4 g) 30 min prior to performing the baseline measures (08:00–09:00 a.m.). Water before testing was allowed ad libitum. The baseline measures were collected in the following order: muscle soreness, Brief Assessment of Mood Adapted, a venous blood sample, and MIVC. Immediately following these measures, the participants performed 140 weighted squats to induce muscle damage. They were then provided with all meals and supplements for the following 2 days. The participants were instructed to avoid intense exercise in the 48 hr leading up to the main trials and until all testing was completed.

Muscle Damaging Exercise Protocol

To induce muscle damage, the participants performed a total of 140 squats while wearing a vest containing 25% of their body mass (in kilograms). The squats were performed as seven sets of 20 repetitions, separated by 2 min of passive recovery. The participants were required to squat down to an angle equivalent to 90° of knee flexion for each repetition. This protocol was adapted from a previous

study that found 140 squats, without additional weight, induced significant muscle damage in untrained young adults (Shimomura et al., 2010). The additional weight was added in the present study to try and augment muscle damage.

Dietary Intervention

In the 48 hr post the muscle-damaging exercise, the participants were provided with all of their food and fluids. The participants were allowed to consume water or noncaloric drinks ad libitum throughout this period, but all other foods and beverages were prohibited. Each feed posttesting (five in total) was formulated to contain either 0.50 g/kg·BM^{-1} (HP diet) or 0.25·g/kg (MP diet) of protein, corresponding to 2.50 or 1.25 g·kg^{-1}·day^{-1} of protein, respectively. The participants had one feed immediately postexercise and the additional four feeds every 3 hr (see Supplementary Material [available online] for further details). The protein amounts were based on the current per meal recommendations for athletic populations (Moore et al., 2015). The daily energy macronutrient composition of the two diets is provided in Table 1. Further details on the diets are provided in a Supplementary Material.

Maximal Isometric Voluntary Contraction

As described previously (Clifford et al., 2017), MIVC was measured with a portable strain gauge (MIE Medical Research Ltd., Leeds, United Kingdom). The participants were seated upright and had a perspex gauze attached to a force transducer strapped to their ankle. After a countdown, the participants were instructed to maximally extend their right knee flexor and hold for a 3-s contraction. The peak value (N) from three maximal contractions (separated by a 60-s rest period) was used for analysis. The interday coefficient of varation (CV) for this measure and procedure is 3.9% in our lab.

Muscle Soreness

Lower limb muscle soreness was measured subjectively with a 200-mm visual analog scale (Clifford et al., 2017). The participants performed a squat to a 90° knee angle and drew a vertical line on a visual analog scale labeled with "no soreness" (0 mm) at one end and "unbearably painful" at the other (200 mm). The line placement was measured with a ruler and recorded.

Brief Assessment of Mood Adapted

The Brief Assessment of Mood Adapted is a measure of performance readiness and was scored by marking a vertical line on a 100-mm visual analog scale between "not at all" and "extremely." The scores were calculated by subtracting the four positively associated questions by the six negatively associated questions. A full list of the included questions is available in Shearer et al. (2017).

Blood Sampling

Venous blood samples were collected via venepuncture. At all three time points (0, 24, and 48 hr postexercise), blood was drawn into a 10-ml vacutainer for serum and a 10- and 4-ml vacutainer coated with dipotassium ethylene diamine tetraacetic acid. The 4-ml vacutainer coated with dipotassium ethylene diamine tetraacetic acid was transported to a local hospital for the analysis of full blood counts. The remaining tubes were centrifuged at 3,000 revolutions per minute (4 °C) for 10 min to separate the supernatant, which were subsequently aspirated into aliquots and stored in a −80° freezer for later analysis.

Blood Analysis

Full blood cell counts were assessed with an automated hematology system (Sysmex XE-2100, Sysmex, Norderstedt, Germany). The CV for this analysis is <10%. CK and high-sensitivity C-reactive protein was measured in serum using an automated system based on an electrochemiluminescence method (Roche Modular; Roche Diagnostics Ltd., Basel, Switzerland). The CV for this analysis was <5%. Plasma interluekin-6, interleukin-1β (IL-1β), and monocyte chemoattractant protein (MCP-1) were measured using commercially available ELISA kits (R&D Systems, Inc., Minneapolis, MN). Because ~25% of the samples were below the detectable limit for IL-1β analysis, the results are not reported for this marker. The CVs for IL-6 and MCP-1 were 15% and 5%, respectively.

Statistical Analysis

The data were analyzed using SPSS (version 24.0, SPSS, Armonk, NY). All data are expressed as mean ± *SD*; an α level of $p < .05$ was accepted to be statistically significant. The baseline values of muscle function, age, height, body mass, and energy intake were assessed for group differences using an independent samples *t* test. Between-group differences in activity levels, carbohydrate, fat, and protein intakes were analyzed with a Mann–Whitney *U* nonparametric test because they were not normally distributed ($p < .05$ on the Shapiro–Wilk's test). Dependent variables were analyzed with a mixed-model analysis of variance with two group levels (HP and MP) and three repeated-measures time points (0, 24, and 48 hr postexercise). Because leukocytes and eosinophils were significantly different between groups at 0 hr, these variables were analyzed as a percentage change from the baseline. Muscle soreness, IL-6, MCP-1, and eosinophils were not normally distributed and, therefore, were logged-transformed prior to analysis. If the analysis of variance indicated a significant effect, post hoc tests with Bonferroni corrections were performed to locate the specific differences. Where sphericity was significantly violated, Greenhouse–Geisser adjustments were used. Partial-eta squared (η_p^2) effect size statistics were considered small (.01–.06), medium (.06–.14), or large (≥.14) changes.

Results

There were no differences in the participants' physical characteristics, activity levels, and energy intake between the two groups ($p > .05$; Table 1). However, as expected, fat and carbohydrate intake were lower, and protein intake was higher in the HP group ($p < .05$; Table 1).

The MIVC were lower following muscle-damaging exercise in both groups (time effect; $p = .001$, $\eta_p^2 = .421$; Figure 1a), but no interaction effects were present ($p = .822$, $\eta_p^2 = .012$). The Brief Assessment of Mood Adapted was reduced after exercise (time effect; $p = .049$, $\eta_p^2 = .172$; Figure 1c); however, there was no interaction effect ($p = .363$, $\eta_p^2 = .058$). Muscle soreness increased in the days following exercise, peaking at 24 hr postexercise in the MP group and 48 hr postexercise in the HP group (time effect; $p = .005$, $\eta_p^2 = .282$); however, no interaction effects were found ($p = .585$, $\eta_p^2 = .083$) (Figure 1b).

Monocytes and lymphocytes were decreased in the days after exercise, and eosinophils increased 24 hr postexercise, but the total leukocyte count, neutrophils, and basophils remained unchanged

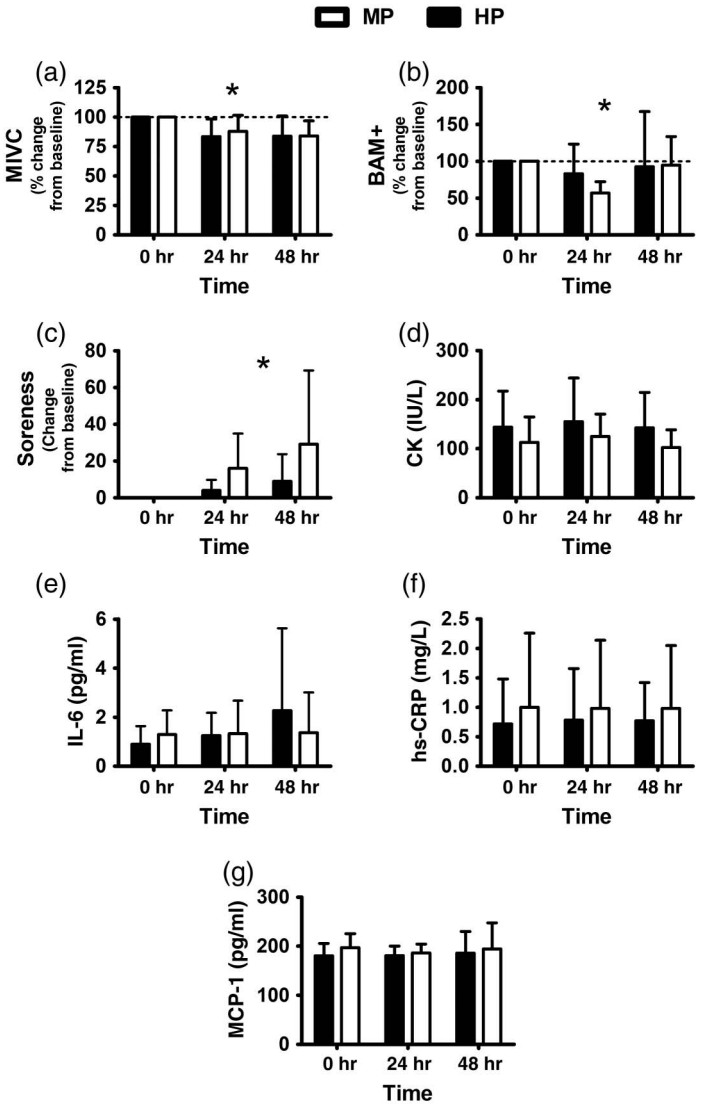

Figure 1 — Changes in (a) MIVC, (b) BAM+, (c) muscle soreness, (d) CK, (e) IL-6, (f) hs-CRP, and (g) MCP-1 preexercise (0 hr), 24 hr, and 48 hr postexercise after an HP or MP diet. MIVC, BAM+, and muscle soreness are presented as change from the baseline for illustrative purposes. MIVC = maximal isometric voluntary contractions; BAM+ = Brief Assessment of Mood Adapted; CK = creatine kinase; IL-6 = interleukin-6; hs-CRP = high-sensitivity C-reactive protein; MCP-1 = monocyte chemotactic protein-1; HP = high protein; MP = moderate protein. *Denotes time effect $p < .05$.

pre- to postexercise (Table 2). There were no group differences in any of the hematological markers (Table 2). CK did not increase after exercise (time effect; $p = .359$, $\eta_p^2 = .062$), and there were no group differences at any time point (interaction effect; $p = .779$, $\eta_p^2 = .006$; Figure 1d). High-sensitivity C-reactive protein displayed no time ($p = .783$, $\eta_p^2 = .015$) or interaction effects ($p = .905$, $\eta_p^2 = .006$; Figure 1f), nor did IL-6 (time: $p = .497$, $\eta_p^2 = .039$; interaction: $p = .159$, $\eta_p^2 = .133$; Figure 1e) or MCP-1 (time: $p = .772$, $\eta_p^2 = .009$; interaction: $p = .685$, $\eta_p^2 = .016$; Figure 1g).

Discussion

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

Only one other study has examined the effects of high protein intake on recovery from strenuous exercise in older adults. In contrast to the present study, they found that feeding high amounts of dietary protein in the postexercise period enhanced the recovery of muscle function in eight master's triathletes (Doering et al., 2017). The reason for the disparate findings between the current and previous study is not overtly clear, but it could be related to the amount and timing of protein intake and/or when the measures were collected. For example, Doering et al. fed their participants higher amounts of protein (0.60 vs. 0.50 g/kg) in the postexercise period, but did not monitor recovery for longer than 8 hr postexercise. By contrast, in the present study, the dietary control and collection of outcome measures continued for 48 hr postexercise. As such, it could be that (a) the 0.50 g/kg of protein we provided at each feed was not sufficient to affect myofibrillar recovery processes/inflammation in our participants or that (b) higher than the recommended amounts of protein are only beneficial when recovery times are short (e.g., ≤8 hr). The fact that we did not measure markers of EIMD at 8 hr postexercise to compare with Doering et al. (2017) is an acknowledged limitation of this study. Clearly, more studies are needed to determine whether higher than recommended protein intakes can expedite recovery in older active adults.

Although there was a decrease in MIVC and an increase in muscle soreness following exercise, none of the other markers typically associated with EIMD—including the pro-inflammatory markers (e.g., neutrophils, IL-6, MCP-1) and CK—were significantly altered 24 and 48 hr following the exercise bout. CK was also not altered in a previous study that used an identical protocol in untrained participants but without the added weight (Shimomura et al., 2010). This study observed less muscle soreness than we did, but greater decrements in muscle function, possibly due to the fact the participants were sedentary. This would suggest that the exercise bout, while novel to the participants and encompassing a large number of eccentric muscle contractions, only induced mild muscle damage in our participants and, therefore, despite their age, the systemic inflammatory response was minor (Paulsen et al., 2012). It is possible that the minor changes in the markers of muscle damage limited our ability to detect small group differences or rendered the high protein diet less effective. With regard to the latter point, it would be reasonable to assume that any intervention aiming to influence recovery processes after exercise would be more effective if the symptoms of muscle damage are marked and prolonged. Perhaps if the participants were less physically active or over the age of 65 years, which is when impairments in muscle regeneration accelerate further (Cruz-Jentoft et al., 2010; Kamandulis et al., 2017), muscle damage and inflammation would have been greater. With that said, we did anticipate that we would see larger changes in these markers, given that Doering et al. (2016) found MPS to be lower in trained triathletes of similar age to our volunteers, and other studies have found markers of muscle damage to be exacerbated in adults 50–65 years of age (Lavender & Nosaka, 2006; Ploutz-Snyder et al., 2001).

It should also be highlighted that the magnitude of changes to neuromuscular and soreness variables observed in this study are akin to those we have seen after competitive events, such as a marathon (Clifford et al., 2017) or soccer match (Abbott et al., 2019). As such, the changes in muscle function and muscle soreness in the present study are likely a better reflection of the changes observed after more ecologically valid forms of exercise

Table 2 Heamotological Markers Preexercise (0 hr), 24 hr, and 48 hr Postexercise in the HP and MP Groups ($n = 9$ Per Group)

Variable	0 hr	+24 hr	+48 hr	p time effect[a]	p interaction effect[a]
Leukocytes ($10^9 \times$ cells/L)					
HP	5.67 ± 0.90	5.71 ± 1.15	5.41 ± 0.78	.322 (.068)	.710 (.20)
MP	4.54 ± 1.09	4.60 ± 1.15	4.54 ± 1.21		
Neutrophils ($10^9 \times$ cells/L)					
HP	3.18 ± 0.85	3.38 ± 1.09	3.19 ± 0.86	.349 (.60)	.600 (.24)
MP	2.46 ± 0.96	2.64 ± 0.95	2.65 ± 1.07		
Lymphocytes ($10^9 \times$ cells/L)					
HP	1.74 ± 0.41	1.59 ± 0.35*	1.52 ± 0.29*	.001 (.408)	.486 (.044)
MP	1.47 ± 0.12	1.40 ± 0.10*	1.33 ± 0.10*		
Monocytes ($10^9 \times$ cells/L)					
HP	0.50 ± 0.11	0.30 ± 0.23*	0.46 ± 0.11	.001 (.601)	.381 (.053)
MP	0.45 ± 0.13	0.15 ± 0.14*	0.39 ± 0.12		
Eosonphils ($10^9 \times$ cells/L)					
HP	0.21 ± 0.08	0.39 ± 0.10*	0.20 ± 0.10	.001 (.693)	.133 (.129)
MP	0.13 ± 0.07	0.38 ± 0.12*	0.14 ± 0.07		
Basophils ($10^9 \times$ cells/L)					
HP	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	.668 (.025)	.668 (.025)
MP	0.04 ± 0.01	0.03 ± 0.02	0.04 ± 0.02		

Note. HP = higher protein; MP = moderate protein.
[a]Number in parenthesis is η_p^2 effect size.
*Different from baseline ($p < .05$).

than the changes observed after most lab-based exercise protocols that typically result in severe myofibrillar disruption, evoking symptoms that last for several weeks (Paulsen et al., 2012).

It is important to note that, in general, the benefits of increasing dietary protein on acute muscle function recovery remains equivocal, irrespective of age. Indeed, a systematic review of the literature suggested that while, in theory, increasing protein intake to augment MPS should enhance myofibrillar remodeling and, ostensibly, the recovery of muscle function, there is little high-quality research to support this assumption (Pasiakos et al., 2014). Indeed, it has been proposed by others that the turnover of intramuscular proteins is probably too slow to significantly influence the acute restoration of muscle contractile function following strenuous exercise (Farup et al., 2014; Owens et al., 2019). With that said, ingesting whey protein postexercise, as in this study, can also attenuate inflammation (Kato et al., 2016; Kerasioti et al., 2013; Rowlands et al., 2016) and increase muscle satellite cell activity (Farup et al., 2014), both of which might positively influence acute functional recovery following exercise (Owens et al., 2019). Thus, an increase in MPS and protein turnover are unlikely to be the only mechanisms by which dietary protein could ameliorate symptoms of muscle damage. Future studies examining the effects of protein intake of recovery, irrespective of age, should aim to control pre- and postexercise dietary intake, but also, where possible, take measures of MPS, satellite cell activation, and inflammation alongside measures of functional recovery, like isometric strength and muscle soreness.

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

Conclusion

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

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Stevenson; the data were collected and analyzed by T. Clifford, E.J. Hayes, K. Bowden Davies, G. Taylor, J.H. Scragg, and K. Smith; the data interpretation and manuscript preparation were undertaken by T. Clifford, J.H. Scragg, E.J. Hayes, E.J. Stevenson, K. Bowden Davies, G. Taylor, and K. Smith. All authors approved the final version of the paper.

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Supplementary Material

Further information on the two trial diets

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

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