Milk Protein Ingestion does not Enhance Recovery from Muscle-Damaging Resistance Exercise in Untrained Males and Females: A Randomised Controlled Trial

Alice G. Pearson[1,2], Lindsay S. Macnaughton[1,2], Karen Hind[2]

[1]Department of Sport and Exercise Sciences, Durham University, United Kingdom

[2]Wolfson Research Institute for Health and Wellbeing, Durham University, United Kingdom

Corresponding author: Alice G Pearson; alice.g.pearson@durham.ac.uk

Abstract

Milk-based proteins are a common choice of post-exercise nutrition to enhance exercise recovery and adaptation. Peri-exercise milk protein ingestion may attenuate exercise-induced muscle damage (EIMD), which is a particular risk to untrained individuals. However, most research has been conducted with males and due to potential sex differences in EIMD, research with both sexes is required. This parallel-group randomised controlled trial examined the impact of milk-protein ingestion on recovery from EIMD. Untrained males and females performed a single bout of leg-based resistance exercise and consumed a milk protein (MILK-PRO: n = 4 male, n = 8 female) or isoenergetic control (CON: n = 4 male, n = 8 female) supplement over 4 days post-exercise (17 doses total). Maximum strength was assessed ≥3 wk pre- and 72 and 168 h post- exercise and measures of leg circumference, range of motion, muscle soreness, pressure-pain threshold (PPT), and serum creatine kinase concentration ([CK]) were conducted pre and immediately, 24, 48, 72, and 168 h post-exercise. Resistance exercise induced mild muscle damage that was not attenuated with MILK-PRO relative to CON. Peak increases in [CK] and reductions in PPT were greater in males compared with females. Changes in other markers were comparable between sexes. We conclude that moderate resistance exercise in naïve individuals induces muscle damage without compromising muscle strength. We support sex differences in EIMD and emphasise the need for further research with both sexes. Milk protein ingestion was not beneficial for recovery from EIMD, thus alternative management strategies should be investigated.

This trial was prospectively registered at ClinicalTrials.gov PRS (protocol ID: 290580A).

Introduction

Milk-based foods provide a rich source of carbohydrate, micronutrients, and a complete amino acid profile, and therefore, could optimise exercise recovery (James et al., 2019). Cow's milk stimulates comparable rates of muscle glycogen resynthesis (Ferguson-Stegall et al., 2011) and rehydration (Seery and Jakeman, 2016) following cycling exercise compared with carbohydrate ingestion. Due to its essential amino acid content, particularly leucine (Rafiq et al., 2016), milk protein is a common choice of post-exercise nutrition to stimulate muscle protein synthesis (MPS) (Wilkinson et al., 2007). The amount of protein required to maximally stimulate MPS rates after leg-based exercise (20 g) (Witard et al., 2014, Moore et al., 2009) is conveniently achieved with the ingestion of ~555 mL of cow's milk or as little as 170 g of dairy yoghurt. By frequently elevating MPS rates, regular milk protein consumption alongside resistance training can promote exercise training adaptations (Hartman et al., 2007, Josse et al., 2010). Therefore, milk protein may offer an ideal nutritional aid to optimise several domains of post-exercise recovery.

One exercise recovery component that may be modulated by milk protein consumption is exercise-induced muscle damage (EIMD). EIMD is a consequence of unaccustomed or eccentric muscle contractions (Staublr, 1989) and is therefore a particular risk to individuals naïve to resistance exercise. EIMD is characterised by a temporary reduction in skeletal muscle functional capacity; the release of intramuscular proteins into the circulation; and perceived feelings of muscle soreness (Allen, 2001, Pyne, 1994, Warren et al., 1999, Clarkson and Hubal, 2002), which may prolong exercise recovery and limit future training quality. Acute, severe muscle damage can hinder chronic muscle adaptations to exercise (Damas et al., 2016b, Eriksson et al., 2006, Foley et al., 1999, Lauritzen et al., 2009), although, whether mild muscle damage is a prerequisite for exercise adaptation by preparing the muscle for future hypertrophy warrants investigation (Damas et al., 2018). Experimental models of EIMD typically incorporate extreme exercise stimuli to maximise damage and do not reflect the magnitude of muscle damage that occurs during habitual training (i.e., milder). Therefore, examination of mild muscle damage induced by physiologically relevant exercise protocols is warranted.

The impact of ingested milk protein on the management of EIMD is equivocal. Cockburn and colleagues examined EIMD following unilateral leg flexions in trained males with post-exercise milk consumption, proving 34 g of protein, which attenuated the decrement in maximal strength and rise in serum creatine kinase concentration ([CK]) compared with

carbohydrate ingestion (Cockburn et al., 2008). Subsequent studies demonstrated that consuming half the quantity of milk (17 g of protein) is sufficient to attenuate symptoms of EIMD (Cockburn et al., 2012; 2013). While supporting data have been provided (Draganidis et al., 2017, Norikazu et al., 2013), others report no differences in muscle soreness, maximal voluntary contraction (MVC), or [CK] with the ingestion of milk protein versus carbohydrate beverages (Rankin et al., 2015, Wojcik et al., 2001, Gee et al., 2019). Further, the influence of milk protein ingestion timing in relation to exercise is unclear (Cockburn et al., 2010). Therefore, the optimal amount, timing, and source of milk protein to ingest in relation to exercise for the management of EIMD is currently uncertain.

Most research has been conducted with males, despite several reports of sex differences in EIMD (Sewright et al., 2008, Minahan et al., 2015b, Kerksick et al., 2008, Fernandez-Gonzalo et al., 2014). Females can experience attenuated EIMD relative to males, potentially driven by an oestrogen-mediated stabilisation of myofibre membranes (Tiidus, 2003). Maintaining membrane stability aids calcium homeostasis and reduces the loss of intramuscular enzymes into the circulation (Duncan and Jackson, 1987) and to this end, females have demonstrated lower post-exercise elevations in [CK] compared with males (Sewright et al., 2008, Minahan et al., 2015b). Only one study has examined EIMD responses to milk protein ingestion in trained, young adults of both sexes (Rankin et al., 2015). Milk was ingested following maximal eccentric knee flexion exercise and was compared with an isoenergetic carbohydrate beverage. Females experienced a beneficial effect of milk consumption on the change in peak torque and passive muscle soreness for 72 h post-exercise, while it was unclear whether milk consumption was beneficial in males. Evidently, data extrapolated from males is not commensurate with females and therefore, further research conducted with both sexes into the management of EIMD is warranted. To address this knowledge gap, the present study aimed to investigate the impact of milk protein ingestion on recovery from muscle damaging resistance exercise in untrained males and females.

Methods

Experimental design

A CONSORT flow diagram of the study procedure is presented in *Figure 1* and the study design is presented in *Figure 2*. A randomised, single-blind, parallel group trial examined the impact of milk protein ingestion on indirect markers of muscle damage during 7 days of post-exercise recovery. Following initial eligibility screening, participants attended two familiarisation sessions to assess maximal leg strength and body composition. Participants were then equally randomised by drawing a folded piece of paper labelled 'A' or 'B' from an opaque envelope to a milk protein (MILK-PRO: n = 8 females, n = 4 males) or control (CON: n = 8 females, n = 4 males) group. A three-week period separated the familiarisation and muscle damage exercise sessions to reduce the influence of repeated-bout effects and to standardise menstrual cycle phase in females. Participants consumed one dose of their allocated supplement pre-exercise and 16 doses over 5 days post-exercise. Venous blood samples and measures of limb circumference, range of motion (ROM), and muscle soreness were obtained pre, immediately post, and 24, 48, 72, and 168 h post-exercise. Maximal leg strength was measured at +72 and +168 h. All study procedures were conducted in the Human Performance Laboratory, Truscott Imaging Suite, and Fitness Centre at Durham University. The study was approved by the Tyne and Wear South NHS Research Ethics Committee (21/NE/0073) and the Department of Sport and Exercise Sciences Research Ethics Sub-Committee, Durham University, and all participants provided signed informed consent. This trial was prospectively registered at ClinicalTrials.gov PRS (protocol ID: 290580A).

Insert Figure 1 here.

Insert Figure 2 here.

Participants

A statistical power analysis was conducted using G*Power 3.1 to determine the study sample size. The power calculation was based on a similar study by Cockburn et al. (2008), which reported a significant difference in the change in isokinetic MVC from baseline between milk protein and carbohydrate groups of 25%. The calculation revealed that 5 participants per group (20 total) were required to have 80% power to detect significant between-group differences when using a dependent *t*-test with 0.05 two-sided significance level. Therefore, 24 participants were required to allow for 20% dropout.

Twenty-five healthy, untrained participants volunteered and were eligible for this study, and 24 completed the study (females: n = 16; age 23.9 ± 4.7 y; 44% White, 44% Asian, 12% other, males: n = 8; age 26.1 ± 5.6 y; 63% White, 37% other). Two participants failed to attend one laboratory visit (+168 h) due to illness. An additional 6 participants (n = 4 female, n = 2 male; age 27.3 ± 5.0 y) volunteered to receive repeat body composition assessments and were included in the DXA precision error assessment. Participants met the following inclusion criteria: free from musculoskeletal disorders and injury; do not habitually (twice per week for previous one-month period) consume nutritional supplements, ergogenic aids, or non-steroidal anti-inflammatory drugs; do not frequently engage in therapies that may alleviate muscle damage (e.g., massage, cryotherapy); and have not performed resistance or eccentric exercise during the previous 6 months. Female participants were naturally menstruating (self-reported regular menstrual cycle during the previous 12 months) or used hormonal contraceptives (n = 2 MILK-PRO, n = 2 CON: combined pill, progesterone-only pill, or Depo-Provera injection) and not pregnant.

Baseline assessments and familiarisation

Participants completed an online health and readiness to exercise questionnaire. For naturally menstruating females, all baseline and experimental measurements were conducted during the late follicular phase of the menstrual cycle (days 5-11). Cycle phase was estimated from self-reported data on timing and duration of menses, and sessions were conducted as close as feasible following the last day of menses. For males, and female users of hormonal contraceptives, measurements were conducted at any time due to the inability to estimate pseudo cycle phase (i.e., due to absence of withdrawal bleed).

Maximal strength was assessed with a 1RM test (Baechle and Earle, 2008) at -28 d, which served as a familiarisation session, and repeated at -25 d to confirm 1RM (McCurdy et al., 2004, Ritti-Dias et al., 2011). Participants were demonstrated the correct form for using the leg extension and leg curl exercise machines (Versa leg extension/leg curl, Matrix, Wisconsin, USA) before completing a warm-up set with a light load (10 repetitions, easily performed). The exercise load was progressively increased by 10-20% for each successive single full repetition attempt, with a 3 min inter-set rest period. Following a failed attempt, the exercise load was reduced by 5-10% until 1RM was established. This protocol was completed using the leg extension and then leg curl machine with 5 min rest between exercises. The test-retest reliability of the 1RM protocol is good to excellent (median ICC = 0.97; CV = 4.2%), independent of sex, age, and training experience (Grgic et al., 2020).

Body composition was assessed using dual energy X-ray absorptiometry (DXA) (Lunar iDXA, GE Healthcare, Madison, WI) at -25 d. Participants followed a standardised pre-scan protocol (Nana et al., 2015). Participants were measured wearing minimal, metal-free clothing (e.g., t-shirt and shorts) and with jewellery removed. Body mass was measured to the nearest 100 g and stature to the nearest mm (Seca Weighting and Measuring Systems, Birmingham, UK). Participants were positioned centrally and supine on the DXA scan bed with hands in a mid-prone position and head in the frankfort plane. Two scans were completed after re-positioning to enable calculation of precision error. Total body mass, lean body mass (LBM), fat mass, and body fat percentage (BF%) were derived. Precision error (coefficient of variation; CV% (Root Mean Square SD)) was 0.98% (0.152 g•cm^{-2}) and 0.41% (0.169 g•cm^{-2}) for the assessments of fat mass and LBM, respectively.

Physical activity and dietary control

Participants recorded habitual activity and dietary intake for 3 random days during the 3-week wash-out period (baseline dietary intake). Three hours before attending the laboratory for the experimental trials (+0-72 and +168 h), participants were guided to consume a standardised breakfast (238 kcal, 8 g protein, 45 g carbohydrate, 3 g fat) alongside their supplement. Besides the prescribed breakfast and supplement, participants consumed their habitual diet, which was assessed with 24-hour dietary recalls at each visit. Participants were instructed to abstain from the use of non-steroidal anti-inflammatory drugs, strenuous exercise, engagement in massage or cryotherapy, and the consumption of alcohol, additional protein supplements to those provided, vitamin and mineral supplements, and ergogenic aids during the study period. Dietary intake was analysed using Nutritics software (Nutritics, Dublin, Republic of Ireland).

Supplementation

The experimental supplement was a milk-protein based yoghurt (MILK-PRO; Arla Foods Ltd, Skyr Icelandic style yoghurt, strawberry) and the control intervention was an isoenergetic oat-based yoghurt (CON; Oatly AB, Oatgurt, strawberry) (*Table 1*). Participants consumed 4 doses per day of their allocated supplement at ~4 h intervals during +0-72 h and one dose on the morning of +168 h (17 doses in total). Although participants were informed that the study was investigating the impact of milk-protein on EIMD, the supplements were indistinguishable in taste, texture, and appearance and were provided by the lead researcher in plastic pots that were devoid of product information and labelled ‘A’ or ‘B’ to ensure participant blinding.

Insert Table 1 here.

Experimental protocol

Resistance exercise

Resistance exercise sessions were supervised. The protocol was performed on the leg extension followed by the leg curl machine, separated by 5 min rest. Participants performed a warm-up of 10 repetitions at 50% of their pre-determined 1RM. Three sets of each exercise (2 min inter-set rest) were completed at 80% 1RM to volitional failure. Lifting tempo was targeted as 1 and 2 s for concentric and eccentric phases of muscle contraction, respectively. Strong verbal encouragement was given throughout to all participants.

Muscle damage markers

Participants attended the laboratory at approximately the same time each day. The following assessments were conducted in the same order during each visit (pre, post, +24, +48, +72, +168 h). Blood samples were collected from an antecubital vein of the forearm using standard venepuncture techniques into three reagent-free vacutainers (10 mL). Limb circumference was measured using a standard anthropometric measuring tape at the mid-, lower-, and upper-quartile points of the trochanterion-tibiale lateral site with the participant in a standing position. The mean value of these 3 sites was used for analysis. Knee joint ROM was calculated as the difference between the relaxed and flexed knee joint angle, as measured using a standard goniometer with the participant supine. Muscle soreness was rated separately for the quadricep, hamstring, glute, and calf muscles using a 10-point visual rating scale (VRS) ranging from '0 - not sore at all' to '10 - extremely sore' while performing a bodyweight squat. Mean soreness was calculated from these values. Pressure-pain threshold (PPT) was assessed using a computerised pressure algometer (Medoc, AlgoMed, Ramat Yishai, Israel) with the participant supine. The probe head (1 cm^2) of the algometer was placed at the mid-, lower-, and upper-quartile points of the trochanterion-tibiale lateral site and increasing pressure was applied until the participant verbally indicated pain. The mean value of these 3 sites was used for analysis. At +72 and +168 h, the 1RM test was repeated. 1RM was not assessed at 0-48 h as it would not align with habitual training practices to perform a maximal strength test between exercise bouts, and it is advised that untrained individuals perform resistance exercise 2-3 $d \bullet wk^{-1}$ (i.e., ~2 d rest between sessions) (ACSM, 2009).

Serum preparation and analysis

Whole-blood samples were left at room temperature for 30 min before being stored on ice (maximum 2 h) and then centrifuged at 4°C with 1100 g force for 15 min. Serum samples were transferred into 1 mL microcentrifuge tubes and stored at -80°C until analysis. Diluted samples (×200 dilution factor) were measured in triplicate for [CK] using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Abcam plc, Cambridge, UK) and sample optical density was measured using a microplate reader at a wavelength of 450 nm. Any outliers within the triplicate measures were excluded prior to calculating the mean of the values for statistical analyses. The intra-assay precision of this ELISA was 20.7%.

Statistical analyses

Statistical analyses were conducted using IBM SPSS (version 25, SPSS Inc., Chicago, IL). Shapiro-Wilk and Levene's tests were used to assess statistical assumptions and equality of variances between groups, respectively. Data that violated the assumptions were analysed with the equivalent non-parametric test. Data were analysed with mixed analysis of variance/Kruskal Wallis and Friedman's tests and data that violated the Mauchly's test of sphericity were corrected with Huynh-Feldt. Any significant interactions were analysed using independent *t*-tests/Mann-Whitney U for between-group comparisons and paired *t*-tests/Wilcoxon signed-rank for within-group comparison. Bonferroni corrections were used to correct for multiple comparisons. To examine sex differences, MILK-PRO and CON data were pooled, and female users of hormonal contraceptives were excluded from analyses. Hedges' *g* effect sizes (ES) with confidence intervals were calculated using the standardised mean difference between males and females in the change from baseline to each post-exercise time-point for EIMD markers. Statistical significance was set at $p < 0.05$. Confidence intervals assume 95% confidence in the range of the mean. Data are reported as mean ± standard deviation (SD) unless otherwise stated.

Results

All participants received the supplement they were allocated at baseline (59% failed to identify their supplement) and reportedly consumed all doses. Baseline body composition data are presented in *Table 2*. There were no significant differences in body composition between MILK-PRO and CON, although males had significantly lower BF% and greater stature and LBM than females ($p < 0.01$).

Insert Table 2 here.

Dietary intake

Table 3 displays dietary intake data. There were no significant differences between MILK-PRO and CON in baseline dietary intake. There were significant group*time interactions for absolute ($F_{1,18}$ = 30.32, p < 0.001) and relative ($F_{1,18}$ = 26.76, p < 0.001) protein intake, such that intake increased in MILK-PRO (60.7 g and 1.03 g•d^{-1}•kgBM^{-1}, respectively) and was unchanged in CON. There was a significant group*time interaction for absolute, but not relative, CHO intake ($F_{1,18}$ = 5.69, p = 0.028), such that during supplementation, the increase from baseline was significantly greater for CON than MILK-PRO. There were no significant sex*time interactions.

Insert Table 3 here.

Resistance Exercise

Table 4 describes the muscle damage exercise bout. There were no significant differences between MILK-PRO and CON for exercise outcomes. Exercise load and volume were higher in males compared with females for both exercises (all p < 0.05).

Insert Table 4 here.

One-repetition maximum

There were no significant differences between MILK-PRO and CON for 1RM or the change in 1RM at any time-point (*Figure 3, Table S1*). There were significant effects of time for MILK-PRO, such that relative to baseline (the highest value of -28 and -25 d), 1RM was greater at +168 h for leg extension and +72 and +168 h for leg curl. 1RM did not change relative to baseline in CON. Absolute 1RM was higher in males than females at all time-points (p < 0.01), though the relative peak change in 1RM was indifferent between sexes (*Figure 3 inserts*). When normalised to body mass, leg extension 1RM remained higher in males than females at -25 d (1.5 ± 0.2 vs 1.1 ± 0.2 kg•kgBM^{-1}; p = 0.002) but was comparable at other time-points and when normalised to LBM. Leg curl 1RM remained higher in males at -28 d, +72 h, and +168 h when normalised both to body mass and LBM ($p \leq 0.042$). ES at +72 and +168 h significantly favoured females for leg extension and males for leg curl (*Figure 6*).

Insert Figure 3 here.

Muscle soreness

There were no significant differences between MILK-PRO and CON in muscle soreness VRS score or the change from pre-exercise in VRS score at any time-point (*Table S1*). Relative to pre, muscle soreness was significantly elevated for 48 h in MILK-PRO and 72 h in CON

(*Figure 4.a*). Relative to pre-exercise, muscle soreness remained significantly elevated at +48 h in females and +72 h in males, although the pre-peak change was indifferent between sexes (p = 0.669; *Figure 4.a insert*). ES revealed that females had smaller increases in soreness at +24-168 h compared with males (*Figure 6*).

There was a significant main effect of time (p = 0.007) but no group*time interaction ($F_{3,59}$ = 0.73, p = 0.538) for PPT (*Figure 4.b*). Peak reductions in PPT from pre-exercise were comparable between MILK-PRO and CON (*Table S1*). There was a significant sex*time interaction ($F_{3,48}$ = 3.11, p = 0.037). In females, PPT did not significantly change following exercise and so reductions in PPT were significantly greater in males compared with females for 72 h post-exercise (*Table S1*). ES indicated smaller decreases in PPT in females relative to males at all time-points (*Figure 6*).

Leg circumference

There were no significant group*time ($F_{3,63}$ = 1.98, p = 0.123) or sex*time ($F_{3,47}$ = 0.69, p = 0.448) interactions for leg circumference. Leg circumference significantly increased relative to pre-exercise at post in CON and at all time-points in MILK-PRO (*Figure 4.c*). The change in leg circumference from pre-exercise was greater in MILK-PRO than CON at +72 h and comparable between males and females at all time-points (*Table S1*). Based on ES, females experienced lesser increases in leg circumference than males at all time-points (*Figure 6*).

Range of motion

There were no significant group*time ($F_{5,100}$ = 1.27, p = 0.285) or sex*time ($F_{5,85}$ = 0.97, p = 0.439) interactions for ROM (*Figure 4.d*) and the pre-peak change in ROM were comparable between MILK-PRO and CON and between males and females (*Table S1*). ES indicate that the decrease in ROM was greater in females at post and males at +24-168 h (*Figure 6*).

Insert Figure 4 here.

Creatine kinase

One outlying participant was identified (1287% increase from pre to +72 h). Removal of these data from the analysis did not impact significance levels and so presented data include this participant. There were no significant differences in [CK] between MILK-PRO and CON at any time-point (*Figure 5*). The rise in [CK] at +72 h relative to pre-exercise was significantly greater in MILK-PRO compared with CON (p = 0.031), although the pre-peak increase was indifferent between groups (*Table S1*). Compared with females, males had greater pre-post and

pre-peak [CK] elevations (*Figure 5 insert*). Based on ES, females experienced smaller [CK] elevations at all time-points (*Figure 6*).

Insert Figure 5 here.

Insert Figure 6 here.

Discussion

This study is the first to investigate the impact of milk protein ingestion on recovery from resistance exercise-induced muscle damage in untrained males and females. We demonstrate that a single bout of habitual-type leg-based resistance exercise induces mild muscle damage without impairing maximal strength. In contrast with previous research (Cockburn et al., 2008, Draganidis et al., 2017, Rankin et al., 2015, Cockburn et al., 2010), we report no attenuative impact of milk protein ingestion on EIMD. Exercise elevated [CK], reduced pain threshold, and prolonged muscle soreness more so in males than females, although changes in muscle swelling, flexibility, and strength were comparable between sexes. Overall, this study demonstrates that milk protein is not an effective nutritional strategy to mitigate mild resistance EIMD and some symptoms of EIMD are attenuated in females relative to males.

Experimental models of EIMD typically aim to maximise muscle damage via extreme and unrealistic exercise protocols. Such protocols include the performance of many (≥10) sets of one exercise (Burnley et al., 2010, Draganidis et al., 2017, Wojcik et al., 2001), prolonged duration of continuous contractions (Nosaka et al., 2002), or exclusively eccentric contractions (Dale et al., 2015, Farup et al., 2014, Ives et al., 2017) which does not reflect habitual resistance training (ACSM, 2009). These exercise protocols induce severe muscle damage with ~50-fold increases in plasma [CK] (Nosaka and Newton, 2002) and sustained (7 days) strength decrements (Farup et al., 2014, Byrne and Eston, 2002). While these study designs allow for proof of concept and easier identification of EIMD management strategies, they lack ecological validity. Accordingly, the present study involved realistic exercise and demonstrated that a mildly stressful stimulus induces muscle damage, marked by elevated muscle soreness, swelling, [CK], and reduced flexibility, without compromising muscle strength.

Contradicting the present study, others have reported significant strength declines in untrained males (Farup et al., 2014, Dale et al., 2015, Ives et al., 2017, Wojcik et al., 2001) and females (Brown et al., 1997, Hicks et al., 2016, Paschalis et al., 2013) following leg-based resistance

exercise, which is deemed the best indicator of EIMD (Paulsen et al., 2012, Warren et al., 1999, Damas et al., 2016a). Nonetheless, peak strength loss occurs immediately post-exercise (Clarkson et al., 1992, Clarkson and Hubal, 2002) and here, strength assessments were not conducted until 72 and 168 h post-exercise to examine the impact of muscle damage on maximal strength at the time the muscle group would generally be re-exercised (i.e., after ≥2 d rest); thus, any strength deficits may have been restored. Therefore, in the present study, maximal strength changes do not best indicate the magnitude of EIMD.

Leg extension and curl 1RM increased between baseline assessments, which is expected for those naïve to resistance exercise (McCurdy et al., 2004, Ritti-Dias et al., 2011). Initial strength gains in response to new exercise stimuli involve a learning effect, such as improvements in form and posture during movement. Hence in the present study, the participants' familiarity and confidence in performing the exercise may have driven strength improvements from -28 to -25 d. In addition, exercise adaptation occurs at a neural level to promote early-stage strength gains (Gabriel et al., 2006). Although the presence of neurological adaptation cannot be confirmed in the current study, others have provided evidence in untrained young adults of increased motor unit firing rate between the first and second bout of leg extension exercise, concurrent with a 16% improvement in maximal force production (Kamen and Knight, 2004). Maximal strength increased further after the damaging exercise bout compared with baseline in MILK-PRO, however this change was within the measurement error for the 1RM test (Grgic et al., 2020) and cannot be deemed a true increase. This suggests that first, one familiarisation session was sufficient for participants to achieve a reliable 1RM and second, that muscle damage was not so severe that maximal strength decrements occurred. Therefore, our findings indicate that in untrained males and females mild muscle damage does not hinder the early-stage neurological adaptation required to prepare the muscle for future morphological adaptation.

We found that ingested milk protein did not attenuate EIMD at the dose provided. Exercise increased muscle soreness VRS ~2-fold in both MILK-PRO and CON, thus conflicting Rankin et al. (2015), in which trained males and females benefited from ingested milk protein (17 g) for reducing passive and active muscle soreness 72 h following leg-based resistance exercise. The small increase in muscle soreness rating in the current study may have limited the ability to detect meaningful between-group differences. Nonetheless, other studies reported comparable muscle soreness between protein and control groups despite ~5-fold (Gee et al., 2019) and ~10-fold (Cockburn et al., 2008) increases. Therefore, the present outcomes might

379 contrast Rankin et al. (2015) due to participant training status, as the current study presents
380 novel findings on untrained individuals. Previous studies conducted with trained males have
381 produced equivocal data. Cockburn and colleagues found no effect of milk protein relative to
382 carbohydrate or water ingestion for reducing post-exercise muscle soreness (Cockburn et al.,
383 2013; 2012; 2008), whereas soreness was lowered by a milk-based protein-carbohydrate
384 beverage consumed pre- or post-exercise (Cockburn et al., 2010). These studies included a
385 single peri-exercise dose of milk protein, unlike the present study which provided multiple
386 supplement doses over several days. Comparably, Draganidis et al. (2017) provided milk
387 protein concentrate for 8 days post-exercise, which more rapidly alleviated muscle soreness
388 relative to carbohydrate. However, milk protein did not attenuate soreness until 4-5 days post-
389 exercise (Draganidis et al., 2017) and it is therefore plausible that the present study may have
390 observed differences in soreness between MILK-PRO and CON had measures been taken at
391 these time-points.

392 One possible explanation for why in this study, unlike others (Rankin et al., 2015, Draganidis
393 et al., 2017, Cockburn et al., 2010), milk protein supplementation did not attenuate muscle
394 soreness relates to participant blinding. In these previous studies, participants were aware of
395 their treatment condition and so bias may have arisen within subjective measures if participants
396 believed the ingested protein should alleviate their muscle soreness. The present study
397 overcame this limitation and was single-blinded by providing a low-protein yoghurt, similar in
398 taste and appearance to the high-protein yoghurt, as opposed to water or a carbohydrate control
399 beverage as used previously (Rankin et al., 2015, Draganidis et al., 2017, Cockburn et al.,
400 2010). By this means, our findings may more accurately represent the impact of milk protein
401 consumption on muscle soreness.

402 An unexpected finding is the tendency for [CK] to rise more following exercise in MILK-PRO
403 than CON and this difference reached statistical significance at +72 h. At this time-point, leg
404 circumference was also increased more in MILK-PRO than CON, which may suggest that CK
405 influx into the extracellular space induced muscle swelling. Notwithstanding, peak changes
406 both in [CK] and leg circumference were comparable between supplement groups, opposing
407 previous findings in males in which ingested milk protein limited post-exercise [CK] elevations
408 relative to ingested carbohydrate (Rankin et al., 2015, Draganidis et al., 2017). While the
409 greater CK response at +72 h in MILK-PRO is difficult to explain, one explanation is that
410 resting and post-exercise [CK] is highly variable and some individuals, termed high responders,
411 experience significantly greater exercise-induced increases in [CK] compared to others, i.e.,

low responders (Clarkson and Ebbeling, 1988, Nosaka and Clarkson, 1996, Damas et al., 2016a). Here, two males and two females out of the five high responders (participants in the upper-quartile for [CK] change) at +72 h were in MILK-PRO. These participants were not high-responders to other EIMD outcomes and did not possess distinguishable characteristics from other participants. Thus, it seems the heightened [CK] peak at +72 h in MILK-PRO versus CON is simply due to inter-individual variability and highlights the limitation of using [CK] to judge EIMD severity.

Despite disagreement (Morawetz et al., 2020, Dannecker et al., 2012, Sayers and Clarkson, 2001, Hubal and Clarkson, 2009), EIMD can be attenuated in females (Kerksick et al., 2008, Minahan et al., 2015a, Sewright et al., 2008, Fernandez-Gonzalo et al., 2014), which is attributed to protective effects of oestrogen on myofibre integrity (Tiidus, 2003). The present study conducted measurements in females during the late-follicular phase of the menstrual cycle when plasma oestrogen concentration is elevated, albeit not peaked (Owen, 1975). Oestrogen maintains myofibre membrane permeability following tissue injury (Tiidus, 2003, Bär et al., 1988) and hence might explain why peak elevations in post-exercise [CK] were attenuated in females compared with males, akin to previous observations (Minahan et al., 2015a, Fernandez-Gonzalo et al., 2014, Hicks et al., 2016). However, this association is speculative here, as neither oestrogen concentration nor ultrastructural myofibre damage were measured.

Sex differences were also identified for subjective symptoms of EIMD. In females, pain threshold was unaffected by exercise and muscle soreness was recovered by +48 h whereas in males, PPT was significantly reduced and soreness was sustained until +72 h. Exercise-induced muscle soreness is potentially driven by metabolic events and disruption of the extracellular matrix (Stauber et al., 1990). Following an exercise-induced increase in myofibre membrane permeability, the calcium-mediated mast cell degranulation and subsequent histamine release sensitises nociceptors and increases pain sensations (Marchettini et al., 1996, Stauber et al., 1990). The current study supports these mechanisms as relative to females, males experienced elevated CK leakage – indicative of weakened membrane integrity – concurrent with prolonged muscle soreness and lower pain threshold. Meanwhile, mechanically-driven changes in muscle strength and flexibility were indifferent between sexes. Therefore, our findings indicate a female protection against EIMD, perhaps specific to metabolically-induced muscle damage and mediated by oestrogen.

Compared with females, males possessed greater maximal strength and consequently, during the muscle damaging exercise bout utilised a higher absolute exercise load and performed a greater work volume. In males, performing a higher volume of elbow flexor exercise has been associated with larger increases in muscle soreness, swelling, [CK], and decrements in strength and ROM than a lower volume of load-equated exercise (Howatson et al., 2007, Nosaka et al., 2001). However, when exercise load is not matched between comparator groups – as was the case here between males and females – higher compared with lower exercise volumes are not always linked to severer muscle damage (Arazi and Asadi, 2018, Draganidis et al., 2013). Therefore, the sex differences in muscle soreness and [CK] identified in the present study could be mediated by dissimilarities in exercise volume, oestrogen status, or other undetermined factors, though nonetheless highlight the need for further sex-comparative and female-focussed research.

Limitations

The following study limitations should be considered when interpreting and implementing its outcomes. First, only indirect EIMD markers were used, which are variable, subjective, and cannot objectively quantify myofibre damage, unlike direct assessments such as muscle biopsy sampling. Nevertheless, biopsy procedures themself can cause tissue damage (Malm et al., 2000). Second, serum oestrogen was not measured and so menstrual cycle phase cannot be confirmed, nor can it be inferred whether oestrogen impacted EIMD and sex differences. Further, four females used hormonal contraceptives, which can impact EIMD (Carter et al., 2001, Minahan et al., 2015b). These females were removed from between-sex analyses and, by chance, evenly distributed between supplement groups and so outcomes should be unaffected. Third, dietary intake was not fully controlled, albeit dietary intake data indicated no unexpected changes from baseline. However, dietary intake was not assessed at +96-144 h and any notable changes in dietary intake during this time relative to +0-72 h could have impacted the +168 h assessments. Although, participants were instructed to maintain their habitual diet throughout the study. Fourth, the ELISA test used to determine [CK] had low intra-assay precision (CV = 20.7%) – likely due to pipetting and washing techniques – thus reducing the accuracy of [CK] data. Finally, sample sizes were uneven between males and females, which could have reduced statistical power and increased type I error risk (Rusticus and Lovato, 2014).

Conclusion

We have demonstrated that peri-exercise milk protein ingestion is not beneficial for recovery from muscle damage induced by an ecologically-valid resistance exercise bout. Nonetheless, milk protein may still be consumed to enhance other elements of post-exercise recovery and future studies should investigate alternative management strategies for mild EIMD. Our findings revealed that, despite mild muscle damage, post-exercise maximal strength can be preserved, allowing the continuation of high-quality exercise. As such, experimental models of EIMD should include exercise protocols that mimic habitual training to increase the application of study outcomes to real-world settings. Furthermore, this study shows that females experience attenuated muscle damage responses to unaccustomed exercise relative to males, highlighting the need for further research conducted with both sexes.

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AP, LM, and KH designed the study; AP conducted data collection; AP conducted data analysis; all authors wrote and approved the final manuscript. We would like to thank Dr Paul Chazot for his assistance with serum analysis.

Statements and Declarations

The authors declare no conflicts of interest.

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Data Availability

Data generated or analysed during this study are provided in full within the published article. Raw data are available from the corresponding author upon reasonable request.

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