

1 **The effect of milk on recovery from repeat-sprint cycling in female team-sport athletes**

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22

23 Abstract

24 The consumption of milk post-eccentric exercise attenuates the effects of muscle damage in
25 team-sport athletes. However, participation in team sport involves both concentric-eccentric
26 loading and metabolic stress. Therefore, the aim of this study was to investigate the effects of
27 post-exercise milk consumption on recovery from a cycling protocol designed to simulate the
28 metabolic demands of team sport. Ten female team-sport athletes participated in a
29 randomised cross-over investigation. Upon completion of the protocol participants consumed
30 500ml of milk (MILK) or 500ml of an energy-matched carbohydrate (CHO) drink. Muscle
31 function (peak torque, rate of force development (RFD), countermovement jump (CMJ), 20m
32 sprint), muscle soreness and tiredness, serum creatine kinase (CK), (high-sensitivity C-
33 reactive protein (hsCRP) and measures of oxidative stress (protein carbonyls (PC) and
34 GSH:GSSG (oxidized glutathione:reduced glutathione) ratio) were determined pre-, 24h, 48h
35 and 72h post-exercise. MILK had a *possible beneficial* effect in attenuating losses in peak
36 torque ($180^{\circ}/s$) from baseline to 24h ($3.2\pm 7.8\%$ v $-6.2\pm 7.5\%$, MILK v CHO) and a *possible*
37 *beneficial* effect in minimising soreness (baseline-48h; baseline-72h) and tiredness (baseline-
38 24h; baseline-72h). There was no change in oxidative stress following the exercise protocol,
39 though a *likely benefit* of milk was observed for GSH:GSSH ratio at baseline-24h ($0.369 \times/\div$
40 $1.89, 1.103 \times/\div 3.96$, MILK v CHO). MILK had an *unclear* effect on all other variables.
41 Consumption of 500ml milk post-repeat sprint cycling had little to no benefit in minimising
42 losses in peak torque, or minimising increases in soreness and tiredness and had no effect on
43 serum markers of muscle damage and inflammation. **Key words:** MUSCLE DAMAGE,
44 RECOVERY, PROTEIN METABOLISM, FEMALE ATHLETE, TEAM SPORT

45 **Introduction**

46 Participation in exercise is known to result in both mechanical and metabolic stress.
47 Mechanical stress, primarily consequential to eccentric muscle actions, results in
48 morphological changes in the muscle (Lauritzen et al. 2009), with early damage occurring
49 immediately following the muscle insult (Friden et al. 1983) and further damage evident 1-3
50 days after the exercise stress (Clarkson and Sayers 1999). Metabolic stress is evident by
51 increases in metabolic flux through glycolytic and oxidative pathways, and increases in blood
52 lactate, inflammation, oxidative stress, and markers of oxidative damage in the post-exercise
53 period (Powers et al. 2011). Such stresses lead to a subsequent decline in muscle function and
54 increases in muscle soreness which hinders the ability to perform exercise. In order to
55 facilitate a faster recovery and maintain subsequent training volumes, intensity and
56 performance, athletes employ a range of recovery strategies (Howatson and van Someren
57 2008).

58 Nutritional interventions are one such strategy and there is evidence that the consumption of
59 milk following eccentric exercise can attenuate decreases in muscle function in males
60 (Cockburn et al. 2008, 2010, 2012) and females (Rankin et al. 2015) and limit increases in
61 muscle soreness and creatine kinase, perhaps by enhancing protein synthesis post-exercise or
62 limiting protein degradation. However, these investigations employed an eccentric exercise
63 protocol on an isolated muscle group resulting in higher levels of mechanical stress and lower
64 levels of metabolic stress than is normally observed following exercise participation (Silva et
65 al. 2013). Metabolic stress leads to diverse physiological processes and may lead to different
66 effects on muscle performance and markers of muscle damage. No studies have examined the
67 effects of milk on recovery from exercise that primarily induces a metabolic rather than
68 mechanical stress.

69 Milk is a source of carbohydrate and anti-oxidants including Vitamin E, Vitamin A and
70 glutathione (Haug et al. 2007) which are effective in reducing lipid peroxidation (Finaud et al.
71 2006). Moreover, milk is a source of whey protein that contains cysteine and methionine
72 amino acids which are necessary for glutathione (GSH) production thus enhancing the anti-
73 oxidant defence system (Madureira et al. 2007; Elia et al. 2006; Marshall 2004). Previous
74 animal research concluded that supplementation with whey protein over a six week period
75 prevented oxidative stress induced by heavy exercise (Elia et al. 2006). Still, these studies
76 have examined long term supplementation with whey and not a single post-exercise intake
77 nor its impact on muscle function or soreness

78 In addition, unlike many other carbohydrate-protein recovery drinks, milk is an excellent
79 source of casein. It has been suggested that milk's antioxidant capacity comes mainly from its
80 casein fractions (Zulueta et al. 2009) due to the high amounts of antioxidant amino acids,
81 including tyrosine, tryptophan, histidine, lysine and methionine present in casein (Rival et al.
82 2001). However, no studies have specifically examined casein's effects on oxidative stress
83 following exercise.

84 Some previous research has shown that carbohydrate or carbohydrate-protein mixtures do not
85 reduce exercise-induced oxidative stress following endurance exercise (Karolkiewicz et al.
86 2001; McAnulty et al 2003; Goldfarb et al. 2009). However, McAnulty et al. (2007) reported
87 lower oxidative stress levels when carbohydrate was consumed during both continuous
88 cycling and cycling with intermittent rest periods. Furthermore, Kerasioti et al. (2012)
89 reported that the consumption of a carbohydrate-protein cake immediately post-exercise (2h
90 steady-state cycling) and at three further 1-hour intervals before a second exercise bout,
91 reduced lipid peroxidation. The disparate results from these investigations may be because of
92 different exercise modes and varied methods for the determination of oxidative stress and
93 oxidative damage. Furthermore, these investigations focused on immediate recovery (1-24h),

94 and did not investigate the effect of the observed stress on subsequent muscle function and
95 soreness, which is of significance to the training athlete.

96 Participation in a team sport results in muscle damage, inflammation and oxidative stress
97 (Ascensão et al. 2008; Fatouros et al. 2010; Silva et al. 2013; Marin et al. 2011) and a
98 subsequent decline in performance (Silva et al. 2013). As previous research has identified
99 milk as being beneficial for recovery from isolated eccentric exercise, investigating the effect
100 of milk on recovery from metabolic stress may provide insight in to the mechanisms by
101 which milk might be beneficial for team sport athletes, and also offer an understanding of the
102 relationships between metabolic stress, oxidative stress, inflammation and performance.

103 Therefore the aim of this study is to investigate the effect of milk on recovery from metabolic
104 stress in team sport participants utilising a protocol designed to simulate the isolated
105 metabolic demands of team sport. We hypothesise that post-exercise consumption of milk
106 will attenuate decreases in muscle function and markers of muscle damage and oxidative
107 stress.

108 **Materials and Methods**

109 *Participants*

110 Ten female team sport (camogie and ladies gaelic football) athletes (mean age 22.1 ± 1.8 y)
111 were recruited to take part in this study. Mean (\pm SD) height and mass was 162.7 ± 9.0 cm
112 and 61.9 ± 8.1 kg, respectively. All participants completed a medical health screening
113 questionnaire and were excluded from the study if they met any of the following criteria:
114 intolerance to dairy or lactose products, lower limb or back injury in the previous three
115 months, surgery in the previous 6 months, known coronary disease, uncontrolled metabolic
116 disorder or respiratory disease, pregnancy or post-partum. All participants were provided

117 with verbal and written briefings, following which written informed consent was recorded.
118 Ethical Approval was provided by the London Sport Institute Ethics Sub-Committee at
119 Middlesex University, and the Institute of Technology Carlow, where the data collection took
120 place.

121 *Study design*

122 The study utilised a randomised cross-over design, with participants attending a
123 familiarisation session plus two blocks of four days of testing. All participants completed the
124 trials during two consecutive follicular phases of their menstrual cycle as in previous
125 investigations utilising female participants (Dannecker et al. 2013; Rankin et al. 2015). All
126 testing took place at the same time of day following an overnight fast and 24h abstinence
127 from alcohol, caffeine and exercise. Briefly, the first laboratory visits comprised of
128 familiarisation with initial measurements of muscle function (peak torque, rate of force
129 development (RFD), countermovement jump height (CMJ), 20m sprint time), and
130 familiarisation with an intermittent cycling protocol (standardised warm-up, five 2-min
131 blocks of the protocol and one 15s maximal sprint) following which participants were
132 randomly assigned to their group order.

133 Between 3 and 5 days later participants completed baseline measures of muscle function,
134 soreness and provided a blood sample for analysis of muscle damage (CK), inflammation
135 (hsCRP) and oxidative stress (Protein Carbonyls and GSG:GSSH ratio). This was followed
136 by completion of an intermittent cycling protocol lasting approximately 60min designed to
137 induce metabolic damage. Immediately upon completion of the protocol participants
138 consumed 500ml of milk (MILK) or 500ml of an energy-matched carbohydrate drink (CHO)
139 and were instructed not to consume any other fluid or food for a period of two hours.
140 Participants returned to the laboratory to repeat the baseline measures at 2h (blood sample

141 only), 24h, 48h and 72h post-exercise. Participants were requested to refrain from any other
142 strenuous activity for the duration of the study, and from treating the symptoms of muscle
143 soreness and tiredness with interventions such as massage, cryotherapy, nutritional
144 supplements and non-steroidal anti-inflammatory drugs.

145 *Nutritional Intervention and Dietary Control*

146 Immediately following the cycling protocol participants were provided with either 500ml of
147 milk (Avonmore 1% Light, Glanbia, Kilkenny, Ireland) or 500ml of an energy-matched
148 carbohydrate solution, which was consumed within 30min. Macronutrient composition (per
149 500ml) of the milk was as follows: Energy 910kJ/215kcal, Protein 17.0g, Carbohydrate 25.5g,
150 Fat 5.0g. A volume of 500ml was chosen based on previous research (Cockburn et al., 2010,
151 2012). From an applied perspective, it was felt that 500ml was an easily consumed volume
152 and that consumption of larger volumes may lead to stomach fullness and discomfort. The
153 energy-matched carbohydrate solution consisted of glucose (52.6g) mixed with water and a
154 commercially available orange flavoured fruit cordial (Nutritional information per 100ml of
155 fruit cordial: Energy 37kJ/9kcal, Protein 0.2g, Carbohydrate 0.8g, Sodium Trace; MiWadi,
156 Dublin, Ireland).

157 In order to maintain dietary control, participants completed a food diary for 24h prior to
158 baseline testing and for the subsequent 3 days, and were asked to repeat the same diet for the
159 second block of testing. Each participant was provided with a weighing scale and measuring
160 jug for the duration of the study and was instructed to follow their usual eating habits before
161 and during the investigation.

162

163 *Metabolic stress protocol*

164 Metabolic stress was induced by the completion of the Cycling Intermittent Sprint Protocol
165 (CISP, Hayes et al. 2013) designed to simulate the metabolic demands of a repeat sprint sport.
166 The protocol was modified slightly to include additional sprints (4x15s) which has been
167 reported to result in oxidative stress (Jówko et al. 2014).

168 Thus the exercise protocol employed for the current study comprised of a standardised warm-
169 up (5 min at 95 W and two 30-s bouts at 120 W with 30-s rest in between) followed by 2 x
170 [14 x 2min bouts of exercise comprising of 10s of passive rest, 5s of maximal sprinting and
171 105s of active recovery, with a 15s maximal sprint followed by 1min active recovery taking
172 place after the 7th and 14th 2min bout](Figure 1). The exercise bouts were separated by a
173 10min ‘half-time’ period during which the participants were permitted to dismount the
174 ergometer and walk around in order to reduce venous pooling in the lower extremities, and
175 minimise feelings of light-headedness and nausea. The total time for each ‘half’ was 30min
176 and 30s, thereby simulating the ~30min halves of ladies gaelic football and camogie, sports
177 from which the majority of participants were drawn. Power output, cadence, speed, HR and
178 RPE were recorded throughout the exercise bout. Participants returned to complete the
179 second cycling trial during the follicular phase of their next menstrual cycle, following which
180 the drink not consumed on the first trial was ingested.

181 *Blood sampling*

182 On each day, prior to any other measures and following a 10min rest, a blood sample was
183 collected by venepuncture from a forearm vein in ethylenediaminetetraacetic acid (EDTA),
184 heparin and serum separator (SST) tubes. The samples were then centrifuged, aliquoted and
185 stored at -80°C for later analysis of creatine kinase (CK), GSSG:GSH (oxidized
186 glutathione:reduced glutathione) ratio, protein carbonyls (PC) and high sensitivity C-reactive
187 protein (hsCRP). A blood sample was also collected 2h post completion of the exercise

188 protocol. Total serum CK and hsCRP were measured using high sensitivity procedures
189 (Roche Cobas 6000 chemistry module c501, Hoffmann-La Roche, Basel, Switzerland). PC
190 were determined using a commercially available kit (Abcam, Cambridge, UK) utilising a
191 methodology based on that described by Levine et al (1990). Briefly samples were
192 derivatised initially with dinitrophenylhydrazine (DNPH) producing functional groups into
193 the oxidized protein which were detected by spectrophotometry. The samples were washed to
194 remove excess DNPH and the oxidised carbonyls were solubilised with guanidine prior to
195 their detection by measuring absorbance at 375 nm. Finally, the protein content in each
196 sample was measured so that the carbonyl content was expressed in terms of nmol/mg of total
197 protein. The intra- and inter-assay variation for this kit is <7% and <10%, respectively. For
198 the determination of GSH:GSSG using a commercially available kit (Abcam, Cambridge,
199 UK) samples were first deproteinised by centrifugation through a 10kDa spin column. Thiol
200 Green Indicator Reaction mix was then added which becomes strongly fluorescent upon
201 reacting directly with glutathione. Samples were incubated prior to the measurement of
202 fluorescence. Intra- and inter- assay CVs for this procedure are <15%.

203

204 *Muscle Soreness and Muscle Tiredness*

205 Active muscle soreness during squatting to approximately 90° knee flexion was measured on
206 a visual analogue scale (VAS), with participants rating their level of soreness on a scale of 0
207 (no soreness) to 10 (as bad as it could be), as in previous research (Rankin et al. 2015). A
208 similar VAS was used to measure muscle tiredness, with 0 indicating no tiredness, and 10
209 indicating as tired as could be.

210 *Peak Torque*

211 To determine peak torque (Nm), following a standardised warm-up, participants completed
212 three dominant leg maximal effort knee extension repetitions at 60°/s and 180°/s, with 60s
213 recovery between speeds on a Biodex System 3 Isokinetic dynamometer (Biodex Medical
214 System, NY, USA). All participants were instructed to give maximal effort and to complete
215 full range of motion at the knee for each repetition. Interclass correlations for this protocol at
216 IT Carlow are 0.83-0.94.

217 *Rate of force development*

218 Rate of force development (RFD) was determined over the first 200ms of an isometric
219 contraction, according to previous studies (Aagard et al. 2002). Briefly, two maximal 5s
220 isometric contractions of the dominant leg quadriceps were performed on the same isokinetic
221 dynamometer used for isokinetic peak torque measurements, with the knee fixed at an angle
222 of 70° (0° = full extension). Participants were instructed to contract and ‘push away as fast
223 and forcefully as possible’. Any repetitions that showed a countermovement (a visible drop in
224 the force signal) were excluded; otherwise RFD was determined from the repetition with the
225 highest torque measurement. RFD was calculated over the time interval of 0-200ms
226 ($\Delta\text{torque}/\Delta\text{time}$) relative to the onset of contraction, which was defined as the time point
227 when the torque generated exceeded the baseline by $>7.5\text{Nm}$ (Aagard et al. 2002).

228 *Countermovement Jump Height*

229 Countermovement jump height was measured in cm using an Optojump optical measurement
230 system (Microgate, Bolzano, Italy). Participants completed three trials employing standard
231 countermovement jump technique, where they were instructed to flex their knees to
232 approximately 90° and immediately jump for maximal height. Jump height was calculated
233 from flight time and the highest recorded jump was used for analysis.

234 *Sprint performance*

235 Twenty metre sprint performance, from a standing start 20 cm behind the start line, was
236 recorded using timing gates (Microgate Racetime 2, Bolzano, Italy). Participants were
237 instructed to sprint through the timing gates as fast as possible and completed three sprints
238 with a rest time of 120s between sprints. Each participant's best time from three trials was
239 used for analysis.

240 *Data analysis*

241 Data was analysed by making probabilistic magnitude based inferences about the true values
242 of outcomes as described by Batterham and Hopkins (2006). By defining the smallest
243 practical or biological effect, the probability of a worthwhile effect with inferential
244 descriptions is possible. Within-group effects over time were determined using a published
245 spreadsheet (Hopkins 2006) and the effect of MILK versus CHO was analysed using a
246 second spreadsheet for analysis of a controlled trial (Hopkins 2006). Comparisons were made
247 between baseline and 2h, 24h, 48h, and 72h post-exercise. Data for peak torque,
248 countermovement jump and 20m sprint were log-transformed to overcome heteroscedastic
249 error (Nevill and Lane 2007). Muscle soreness values were not log-transformed because of
250 interval scaling (Nevill and Lane 2007). Means of log-transformed data were then back
251 transformed to provide mean percentage change and percentage SD. Serum markers are
252 however, reported as factors because of large percentage changes (Hopkins 2003). The
253 smallest worthwhile effect was the smallest Cohen change in the mean: 0.2 times the
254 between-subject SD for the baseline values of all participants (Batterham and Hopkins 2006).
255 Chances of benefit and harm were assessed qualitatively as follows: <1% almost certainly
256 none, 1-5% very unlikely, 5-25% unlikely, 25-75% possibly, 75-95% likely, 95-99% very
257 likely, >99% almost certainly (Hopkins 2002).

258 **Results**259 **Preliminary Data**

260 Analysis of participants' food diaries (Nutritics Professional Diet Analysis, Nutritics Ltd.,
261 Dublin, Ireland) indicated that there were no differences in energy, carbohydrate, protein or
262 fat intake between trials ($P>0.05$). Analysis of the repeat-sprint exercise bouts revealed no
263 significant differences ($P>0.05$) between trials on performance or physiological measures
264 (Table 1).

265 **Within-group effects**

266 Analysis of within-group effects revealed post-exercise decreases in peak torque, CMJ, sprint
267 performance and RFD, increases in serum CK and hsCRP and in muscle soreness and
268 tiredness. No change was observed in PC or GSH:GSSG ratio. Mean effects \pm 90%CI, with
269 qualitative inferences, are presented in Table 2.

270

271 **Between-group effects**272 *Peak torque*

273 Baseline knee extension peak torque values of the dominant leg at 60°/s for the milk
274 condition and the carbohydrate condition were 143.1 ± 25.5 Nm and 141.1 ± 23.9 Nm
275 respectively. Percentage changes in peak torque at 60°/s for dominant knee extension can be
276 seen in Figure 2. With a Smallest Worthwhile Effect (SWE) of 3.8%, changes in the peak
277 torque of the dominant leg at 60°/s between baseline and 24h for the milk condition and
278 carbohydrate condition were $-3.9 \pm 4.5\%$ and $-3.1 \pm 4.3\%$ respectively, a trivial effect for

279 CHO. Unclear outcomes for the comparison of MILK versus CHO were found at baseline-
 280 48h (-6.4 ± 12.2 % and -4.3 ± 4.4 %) and baseline-72h (-4.4 ± 9.5 % and -2.4 ± 8.5 %).

281

282 At baseline the peak torque values at $180^\circ/\text{s}$ for the dominant leg of participants for MILK
 283 and CHO were 97.7 ± 16.6 Nm and 95.5 ± 16.0 Nm respectively. Changes in the peak torque
 284 of the dominant leg at $180^\circ/\text{s}$ between baseline and 24h for MILK and CHO (SWE: 3.7%)
 285 were -3.2 ± 7.8 %, and -6.2 ± 7.5 % respectively, a possible benefit of milk. There was an
 286 unclear outcome for MILK (-5.0 ± 12.7 %) versus CHO (-3.1 ± 8.9 %) at baseline-48h. A
 287 possible negative effect was observed for MILK (-7.3 ± 12.7 %) compared to CHO ($-0.8 \pm$
 288 9.2%) at baseline-72h.

289 *Rate of Force Development*

290 Immediately prior to the repeat-sprint cycling exercise the mean rate of force development
 291 was 433.1 ± 159.1 $\text{Nm}\cdot\text{s}^{-1}$ and 405.6 ± 106.1 $\text{Nm}\cdot\text{s}^{-1}$ for the MILK and CHO conditions
 292 respectively. The SWE was 9.4% and unclear outcomes for MILK versus CHO were found at
 293 baseline-24h (-5.5 ± 25.3 % v -10.5 ± 22.0 %), baseline-48h (-1.9 ± 16.4 % v -2.7 ± 20.3 %)
 294 and baseline-72h (-0.5 ± 30.7 % v $6.3 \pm 28.7\%$).

295 *20m sprint*

296 Baseline 20m sprint times for the milk condition and carbohydrate condition were 3.58 ± 0.12
 297 s and 3.59 ± 0.11 s respectively and the SWE was determined as 0.7%. Unclear outcomes
 298 were found for MILK versus CHO at baseline-24h (1.8 ± 2.2 % and 1.0 ± 1.3 %), baseline-
 299 48h (1.0 ± 2.8 % and 0.6 ± 2.5 %) and baseline-72h (0.2 ± 2.2 % and -0.4 ± 1.2 %).

300 *Countermovement jump performance*

301 Immediately prior to the cycling exercise the mean countermovement jump heights of the
302 milk and carbohydrate conditions were 28.9 ± 2.9 cm and 28.8 ± 2.4 cm respectively, with a
303 SWE of 1.9%. Unclear outcomes for MILK versus CHO were found at all time points,
304 baseline-24h (-1.6 ± 4.7 % v -2.0 ± 3.1 %), baseline-48h (-1.4 ± 4.6 % and -1.9 ± 5.4 %) and
305 baseline-72h (-0.2 ± 4.1 % v -0.0 ± 4.7 %). A summary of the statistical analysis for Peak
306 Torque, RFD (0-200ms) of the dominant leg, 20m sprint performance and countermovement
307 jump performance can be seen in Table 3.

308

309 *Soreness*

310 The exercise protocol resulted in a small increase in muscle soreness for both conditions,
311 peaking at 24h for MILK and 48h for CHO (Figure 3). By 72h soreness had almost returned
312 to zero. A comparison of changes in soreness for the period baseline-24h indicated an unclear
313 outcome for MILK (7.0 ± 8.2 %) compared to CHO (12.0 ± 15.5 %). A possible benefit of
314 MILK versus CHO was seen at baseline-48h (4.0 ± 7.0 % v 14.0 ± 21.7 %) and baseline-72h
315 (1.0 ± 3.2 % v 8.0 ± 10.2 %)

316 *Tiredness*

317 Muscle tiredness increased over time for both conditions but by 72h tiredness had almost
318 returned to zero. A comparison of changes in muscle tiredness from baseline to 24h indicated
319 a possible benefit for the consumption of MILK (18.0 ± 11.4 %) compared to the
320 consumption of CHO (24.0 ± 15.8 %). An unclear outcome was observed from baseline to
321 48h (16.0 ± 12.6 % versus 24.0 ± 20.7 % for MILK and CHO conditions respectively).
322 Changes in tiredness from baseline-72h showed a possible benefit of MILK (1.0 ± 3.2 %)

323 compared to CHO (9.0 ± 13.7 %). A summary of the statistical analysis for muscle soreness
324 and muscle tiredness can be seen in Table 3.

325 *Creatine Kinase*

326 Serum CK values prior to exercise were 155.0 ± 75.4 U/l and 138.0 ± 62.6 U/l for the milk
327 and carbohydrate conditions respectively. Unclear outcomes for the comparison of MILK
328 versus CHO were found at baseline-2h ($1.52 \times/\div 1.27$ v $1.41 \times/\div 1.17$), baseline-24h (1.30
329 $\times/\div 1.55$ v $1.26 \times/\div 1.65$), baseline-48h ($1.03 \times/\div 1.57$ v $1.14 \times/\div 1.57$) and baseline-72h (0.99
330 $\times/\div 1.56$ and $0.97 \times/\div 1.76$).

331 *hsCRP*

332 Baseline hsCRP values immediately prior to exercise were 1.16 ± 0.91 mg/l and 1.38 ± 0.99
333 mg/l for the milk and carbohydrate conditions respectively. Unclear outcomes were observed
334 for MILK versus CHO at baseline-2h ($1.00 \times/\div 1.07$ v $1.02 \times/\div 1.17$), baseline-24h ($2.09 \times/\div$
335 1.75 v $1.42 \times/\div 2.03$), baseline-48h ($1.59 \times/\div 1.50$ v $1.15 \times/\div 1.98$) and baseline-72h (1.25
336 $\times/\div 1.73$ v $0.925 \times/\div 1.99$).

337 *Protein Carbonyls (PC)*

338 Protein Carbonyl values prior to exercise were 0.54 ± 0.14 nmol/mg/protein for the MILK
339 group and 0.49 ± 0.36 nmol/mg/protein for the CHO group. Unclear outcomes were observed
340 for MILK versus CHO; baseline-2h ($1.10 \times/\div 1.54$ v $0.88 \times/\div 2.36$), baseline-24h ($1.23 \times/\div$
341 1.82 v $0.85 \times/\div 2.72$), baseline-48h ($1.33 \times/\div 1.23$ v $0.76 \times/\div 1.99$) and baseline-72h ($0.88 \times/\div$
342 1.46 and $0.957 \times/\div 3.04$).

343 *GSH:GSSH ratio*

344 Prior to the cycling exercise the mean GSH:GSSG ratio of the milk and carbohydrate
345 conditions were 0.57 ± 0.27 and 0.98 ± 0.92 respectively. An unclear outcome for MILK
346 versus CHO was found at baseline-2h ($0.871 \times/\div 1.99$ v $1.014 \times/\div 8.52$). A likely benefit of
347 MILK was observed at baseline-24h ($0.369 \times/\div 1.89$ v $1.103 \times/\div 3.96$). Unclear outcomes
348 were found at other timepoints: baseline-48h ($0.827 \times/\div 3.15$ and $1.105 \times/\div 8.800$) and
349 baseline-72h ($0.751 \times/\div 6.39$ v $0.686 \times/\div 7.72$). A summary of the statistical analysis for
350 Creatine Kinase, hs-CRP, PC and GSH:GSSG ratio can be seen in Table 3.

351 Discussion

352 Repeat-sprint cycling resulted in decrements in muscle function and increases in perceptions
353 of soreness and tiredness, and serum markers of muscle damage and inflammation. However,
354 no increase in oxidative stress was observed. The results indicate that the consumption of
355 500ml of milk post-intermittent sprint cycling exercise had minimal effect on recovery of
356 muscle function, inflammation and markers of muscle damage. While a possible benefit of
357 milk was observed for peak torque at $180^\circ/\text{s}$ at 24h post-exercise, no effect was seen on any
358 other muscle function variable. A possible negative effect for MILK was noted at 72h post-
359 exercise for peak torque at $180^\circ/\text{s}$. The consumption of milk attenuated increases in muscle
360 soreness and tiredness over 72h post-exercise, compared to the consumption of a volume and
361 energy matched carbohydrate drink. Markers of oxidative stress did not increase following
362 the exercise protocol.

363 A possible benefit for MILK was observed for Peak Torque at $180^\circ/\text{s}$ at 24h post exercise, but
364 at no other time and for no other muscle function variable, CK or hsCRP. The lack of effect
365 of milk on muscle function, inflammation and CK is likely because of the nature of the
366 exercise protocol. Previous research has found that the consumption of milk following
367 eccentrically loaded exercise had a beneficial effect in attenuating decreases in peak torque,

368 sprint performance and reactive strength index (Cockburn et al. 2008, 2010, 2012; Rankin et
369 al. 2015). Cycling employs concentric muscle actions (Bijker et al. 2002), resulting in
370 metabolic rather than mechanical stress, and may explain the low level of muscle damage
371 observed in this study. This lower level of damage is reflected in the smaller decreases in
372 muscle performance observed across all variables (3.98%) compared to previous studies
373 involving eccentric exercise with female team-sport athletes (6.95%, Rankin et al. 2015).
374 Serum CK levels were elevated during the recovery period, peaking 24h post-exercise, and
375 returning to baseline values by 72h with no difference between interventions. Mean peak CK
376 values (225.6 ± 72.3 and 193.6 ± 61.0 IU/l for MILK and CHO respectively) were lower
377 than observed for female athletes in investigations employing eccentric exercise ($8873.0 \pm$
378 13306.0 and 11697.7 ± 8423.3 IU/l, MILK and CHO respectively, Rankin et al. 2015),
379 though similar to other cycling studies investigating recovery interventions (Bell et al. 2014;
380 Jowko et al. 2014). In summary, the observed levels of muscle damage were low, losses in
381 muscle function were small and milk had no effect on minimising these effects.

382 It is possible that an increase in protein synthesis or a decrease in protein breakdown
383 following the mechanical stress of eccentric loading in previous studies is enhanced with the
384 intake of milk post-exercise, a mechanism not observed following isolated metabolic stress in
385 this study. It is conceivable that the nature of the exercise (cycling) may have stimulated
386 mitochondrial protein synthesis rather than myofibrillar protein synthesis. Wilkinson et al.
387 (2008) noted a 67% increase in myofibrillar FSR following a resistance exercise session but
388 no change following an endurance (cycling) session while Dumke et al (2009) reported
389 stimulation of genes associated with mitochondrial biogenesis following cycling, an effect
390 reported by others following high intensity sprint cycling protocols and training (Little et al,
391 2010; Granata et al., 2016; MacInnis et al, 2017). In the current study the measurements of
392 muscle function over the recovery period (peak torque, RFD, CMJ, sprint performance) were

393 not dependent on mitochondrial function and therefore any benefit of milk intake may not
394 have been apparent from the measures chosen. It is possible that a larger intake of milk may
395 have produced different outcomes. The amount of protein provided by the 500ml of milk was
396 17g which provided a mean relative intake of 0.27 g/kg, though given the range in body mass,
397 the range in relative intake was 0.23-0.32 g/kg. The lower relative intake of protein may have
398 been insufficient to maximally stimulate protein synthesis and attenuate muscle function
399 losses. Nevertheless, Cockburn et al (2012) reported that 500ml of milk consumed post-
400 exercise was as effective as 1000ml in attenuating the negative effects of EIMD. Further
401 research is warranted to extrapolate this further.

402 Exercise causes a systemic inflammatory response with increases in cytokine and leucocyte
403 activity (Febbraio and Pedersen 2005). hsCRP is an acute phase protein and its synthesis in
404 the liver is triggered as an inflammatory response following increase cytokine secretion, most
405 notably IL-6. Mean absolute hsCRP values were very similar in both trials, suggesting similar
406 inflammatory responses. Not surprisingly, given that cycling is concentric in nature, the
407 hsCRP response to the exercise protocol was considerably lower than that observed following
408 resistance exercise (Bowtell et al. 2011) and marathon running (Clifford et al. 2016),
409 suggesting that the magnitude of the inflammatory response is significantly influenced by the
410 mode of exercise. The values, however, are comparable to that reported previously in cycling
411 research (Roengrit et al. 2014) with peak hsCRP observed at 24h post-exercise, returning
412 towards baseline values by 72h post-exercise. The magnitude of change was not different
413 between the conditions, indicating that post-exercise consumption of milk did not modulate
414 the inflammatory response after metabolic exercise. There is considerable disagreement in the
415 literature regarding the effects of carbohydrate and carbohydrate-protein intake on
416 inflammatory measures. For example, while Kerasioti et al. (2013) observed a decrease in
417 inflammatory markers (IL-6, CRP) following the ingestion of a carbohydrate-protein cake,

418 Cosio-Lima et al. (2012) reported no differences in inflammatory responses (TNF- α , IL-6)
419 following the ingestion of carbohydrate versus carbohydrate-protein solutions. Such
420 contradictory results are probably reflective of the variation in exercise protocols employed,
421 the inflammatory markers measured or the specific composition of the nutritional
422 interventions. It is important to note that inhibition of post-exercise inflammation is not
423 always an aim of post-exercise nutritional intervention, as prevention of exercise-induced
424 inflammation may inhibit muscular adaptation to exercise (Mastaloudis et al, 2004).

425 Interestingly, even though post-exercise muscle soreness and tiredness ratings were low
426 compared to those following eccentric exercise, post-exercise milk consumption had a
427 positive effect in attenuating increases in muscle soreness and tiredness over the 72h post-
428 exercise period. This is somewhat unexpected given that there were no differences in muscle
429 function or inflammation levels between the groups. Inflammation is a proposed mechanism
430 for the manifestation of muscle soreness (MacIntyre et al. 1995). Increased histamines and
431 bradykinins sensitise nociceptors and increase the sensation of pain (Malm 2001). Oedema
432 may also contribute to the sensation of soreness, with swelling exerting increased osmotic
433 pressure within the fibres and further sensitising nociceptors (Malm 2001; Clarkson and
434 Hubal 2002). The disparity of results observed in this study may indicate different pathways
435 for the recovery of muscle function and perception of soreness and tiredness which may or
436 may not involve the inflammatory response. It is possible that hsCRP may not be reflective of
437 the total cytokine activity in the post-exercise period and that measurement of other cytokines
438 such as TNF- α or IL-6 may have provided greater insight. Nonetheless, because this was not
439 a blind study it is plausible that information bias may have occurred through knowledge of
440 the nature of the allocated intervention (Booth et al. 1992), where participants may have been
441 aware of previously published research highlighting potential benefits of milk for recovery,
442 thus leading to a perception of attenuated soreness and tiredness.

443 Surprisingly, the intermittent sprint protocol utilised in this study did not result in an increase
444 in oxidative stress as indicated by PC and GSH:GSSG ratio, despite the considerable physical
445 demand imposed by the exercise. This is in contrast with previous research that reported
446 increases in post-exercise oxidative stress following sprint cycling (Jowko et al. 2014),
447 though in agreement with Bloomer et al. (2007) and Farney et al. (2012). There are a number
448 of possible reasons for this observation. Firstly, only selective biomarkers of oxidative stress
449 were measured; the inclusion of additional markers or alternative assay techniques may have
450 indicated an increase in oxidative stress. Both PC and GSH:GSSG have been identified as
451 useful indicators of protein oxidation and redox disturbance respectively (Powers et al. 2010).
452 Protein Carbonyls are produced when ROS attack amino acids and thus levels increase when
453 oxidative stress levels increase. GSH is an antioxidant used in the reduction of lipid
454 hydroperoxides generating oxidised glutathione (GSSG). When cells are exposed to oxidative
455 stress levels of GSSG increase and the ratio of GSSG to GSH increases. Nevertheless the
456 validity of many measures of oxidative stress has been questioned, with poor correlations
457 between different markers of oxidative stress and between different methods for measuring
458 individual markers (Finkler et al. 2014). Secondly, oxidative stress may have occurred in
459 muscle tissue but was not detected in the serum samples as has been reported in animal
460 research (You et al. 2005). Thirdly, a lack of observed oxidative stress may be because the
461 participants were trained team-sport players, well-accustomed to the metabolic demands of
462 repeat-sprinting all be it with a different exercise mode. This training may have resulted in
463 adaptations that resulted in minimal change in PC and GSH:GSSG ratio over the duration of
464 this study. Lending support to this idea Bloomer et al. (2006, 2012) reported no increase in
465 oxidative stress in trained individuals following sprint cycling, and Bogdanis et al. (2013)
466 reported attenuated oxidative stress responses following high intensity interval training.
467 Finally, it is possible that a lack of oxidative stress may be because of the gender of the

468 participants. Previous research has indicated that females experience lower levels of oxidative
469 stress than males (Ide et al. 2002) and that following exercise markers of oxidative stress
470 return to baseline quicker than males (Mastaloudis et al. 2004). This lower level of oxidative
471 stress may be because of smaller muscle mass, leading to lower levels of mitochondrial flux
472 and lower ROS production (Ide et al. 2002). Additionally, Tiidus (1995, 2003) proposed that
473 estrogen may act as an antioxidant against lipid peroxidation of the cell membrane during
474 exercise. Estrogens possess a hydroxyl group on their A (phenolic) ring in a similar structure
475 to tocopherol (Vitamin E) which is a known antioxidant (Ayres et al. 1998; Persky et al.
476 2000). Acting in a similar way to tocopherol, estrogen may donate hydrogen atoms leading to
477 a termination of peroxidation chain reactions (Tiidus 1995; Ayres et al. 1998; Persky et al.
478 2000). Future investigations examining the effect of milk on oxidative stress should carefully
479 consider exercise protocol design, training status and gender of the participants.

480 The majority of investigations examining the effects of nutritional interventions on recovery
481 from metabolic stress employed a protocol that required participants to consume the
482 nutritional substance over a prolonged period of time. For example, Samaras et al. (2014)
483 observed an increase in glutathione levels when athletes were supplemented with a protein-
484 carbohydrate bar for two months. The only investigation examining the effects of post-
485 exercise consumption of a protein-carbohydrate supplement on oxidative stress markers was
486 Kerasioti et al. (2012). While they observed a reduction in Thiobarbituric Acid Reactive
487 Substances (TBARS) with the intake of an experimental cake post-2hours of cycling, there
488 was no effect on subsequent time-trial performance. It is thus likely that the timing of
489 antioxidant supplementation is an important factor that could influence exercise-induced
490 oxidative stress (Goldfarb et al. 2009), and that post exercise intake of antioxidants does not
491 have a positive effect on oxidative stress markers and may even result in a pro-oxidant
492 response (Nieman et al. 2002). Thus it is unlikely that one-off post-exercise consumption of

493 nutritional products, as in the current study, will have any effect on recovery from oxidative
494 stress. In this study the beverage was consumed only after the exercise bout, as this is a
495 regular practice of team sport athletes.

496 In conclusion, the consumption of 500ml of milk post non-eccentric exercise had minimal
497 effect on the attenuation of losses in muscle function and no effect on increases in markers of
498 muscle damage and inflammation following repeat-sprint cycling, though consumption of
499 milk did reduce perceptions of soreness and tiredness. Speculatively, the benefit of milk for
500 athletic recovery is likely to be greatest following activities that have an eccentric component.
501 Further research is warranted in this area to investigate the effects of milk on recovery from
502 exercise that induces high levels of oxidative stress. However, recently it has been suggested
503 that minimising oxidative stress may inhibit adaptation to exercise (Paulsen et al. 2014;
504 Buresh and Berg 2015; Pingitore et al. 2015). From this perspective future research
505 examining the effect of nutritional interventions on oxidative stress should carefully consider
506 the practical application of outcomes. Consideration could be given to examining prolonged
507 intake of milk prior to exercise performance and during the recovery period and the effect of
508 milk on recovery from simulated or actual sport performance.

509

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753 **Table 1.** Comparison of performance and physiological variables for cycling trials prior to
 754 Milk and Carbohydrate consumption

Variable	1 st half		2 nd half		Total	
	MILK	CHO	MILK	CHO	MILK	CHO
Average power (W)	117.1± 15.7	112.0± 14.4	109.3± 14.8	105.3± 16.1	113.2± 14.8	108.7± 14.6
Peak power (W)	695.4± 135.6	661.3± 124.1	638.6± 135.4	636.0± 129.4	667.0± 129.4	648.7± 130.3
Power/mass (W/kg)	2.0 ± 0.3	1.8 ± 0.2	1.8 ± 0.2	1.7 ± 0.2	1.9 ± 0.2	1.8 ± 0.2
Estimated EE (kcal)	333.3± 24.2	325.5± 25.2	307.7± 27.6	308.9± 37.3	320.5± 24.4	317.2± 26.7
Average cadence (rpm)	70.1 ± 3.9	67.7 ± 4.7	68.6 ± 4.2	66.5 ± 4.5	69.4 ± 4.0	67.1 ± 4.1
Peak cadence (rpm)	120.1± 12.3	117.8± 9.1	118.7± 13.0	115.0± 9.8	119.4± 11.3	116.4± 8.8
Rev count	2255.2± 124.1	2207.8± 128.6	2128.0± 136.6	2121.7± 215.7	2191.6± 112.4	2164.8± 130.6
Average speed (kmh)	27.7 ± 1.5	27.3 ± 1.4	27.2 ± 1.5	26.6 ± 1.7	27.5 ± 1.4	27.0 ± 1.4
Distance covered (km)	14.5 ± 0.5	14.5 ± 1.1	14.0 ± 0.8	13.9 ± 0.8	14.2 ± 0.6	14.1 ± 0.9
Average pace (min:ss/km)	1:58± 0:06	2:01± 0:07	2:00± 0:05	2:02± 0:08	2:00± 0:06	2:01± 0:06
HR (bpm)	171.7± 10.9	169.9± 8.2	168.9± 9.7	167.5± 5.8	170.3± 10.1	168.7± 6.5
RPE	11.8 ± 1.6	11.9 ± 2.1	13.8 ± 1.8	14.1 ± 2.8	12.8 ± 1.4	13.0 ± 2.3

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762 **Table 2.** Within-group effects over time for dependent variables

Variable	Timeframe	Mean effect \pm 90% CI	Qualitative inference
Peak Torque 60°/s Extension			
MILK	B-24	-4.0 \pm 2.7	Possibly lower
	B-48	-7.1 \pm 7.4	Likely lower
	B-72	-4.9 \pm 5.6	Possibly lower
CHO	B-24	-3.2 \pm 2.5	Possibly lower
	B-48	-4.4 \pm 2.5	Possibly lower
	B-72	-2.7 \pm 4.7	Possibly lower
Peak Torque 180°/s Extension			
MILK	B-24	-3.4 \pm 4.5	Possibly lower
	B-48	-5.8 \pm 8.1	Possibly lower
	B-72	-2.5 \pm 1.7	Unclear
CHO	B-24	-6.5 \pm 4.4	Possibly lower
	B-48	-3.5 \pm 5.1	Likely lower
	B-72	-1.1 \pm 5.3	Unclear
CMJ			
MILK	B-24	-1.7 \pm 2.7	Possibly lower
	B-48	-1.5 \pm 2.6	Possibly lower
	B-72	0.2 \pm 2.4	Unclear
CHO	B-24	-2.0 \pm 1.8	Possibly lower
	B-48	-2.0 \pm 3.2	Possibly lower
	B-72	0.1 \pm 2.8	Unclear
20m sprint			
MILK	B-24	-1.8 \pm 1.2	Likely lower
	B-48	-0.9 \pm 1.6	Possibly lower
	B-72	0.2 \pm 1.3	Unclear
CHO	B-24	-1.0 \pm 0.7	Likely lower
	B-48	-0.5 \pm 1.5	Unclear
	B-72	-0.4 \pm 0.7	Unclear
RFD			
MILK	B-24	-8.5 \pm 14.6	Possibly lower
	B-48	0.8 \pm 8.9	Likely trivial
	B-72	-3.9 \pm 18.1	Unclear
CHO	B-24	-12.7 \pm 11.9	Likely lower
	B-48	-4.9 \pm 12.9	Unclear
	B-72	3.3 \pm 15.0	Unclear
Creatine Kinase			
MILK	B-24	1.376 \times/\div 1.283	Likely increase
	B-48	1.165 \times/\div 1.401	Unclear
	B-72	1.096 \times/\div 1.323	Unclear
CHO	B-24	1.461 \times/\div 1.273	Very likely increase
	B-48	1.283 \times/\div 1.274	Likely increase
	B-72	1.083 \times/\div 1.508	Unclear

hsCRP			
MILK	B-24	2.163 x/÷ 1.353	Most likely increase
	B-48	1.685 x/÷ 1.259	Mostlylikely increase
	B-72	1.487 x/÷ 1.358	Likely increase
CHO	B-24	1.735 x/÷ 1.375	Very likely increase
	B-48	1.437 x/÷ 1.405	Likely increase
	B-72	1.087 x/÷ 1.582	Unclear
Soreness			
MILK	B-24	0.7 ± 0.5	Likely increase
	B-48	0.3 ± 0.4	Likely trivial
	B-72	0.1 ± 0.2	Most likely trivial
CHO	B-24	1.2 ± 0.9	Likely increase
	B-48	1.3 ± 1.3	Likely increase
	B-72	0.8 ± 0.9	Possible increase
Tiredness			
MILK	B-24	1.8 ± 0.7	Most likely increase
	B-48	1.5 ± 0.8	Very likely increase
	B-72	0.1 ± 0.2	Most likely trivial
CHO	B-24	2.4 ± 0.9	Most likely increase
	B-48	2.3 ± 1.3	Very likely increase
	B-72	0.9 ± 0.8	Likely increase
PC			
MILK	B-24	1.111 x/÷ 1.449	Unclear
	B-48	1.081 x/÷ 1.296	Unclear
		0.843 x/÷ 1.269	Unclear
CHO	B-24	0.940 x/÷ 1.925	Unclear
	B-48	0.761 x/÷ 1.929	Unclear
	B-72	0.716 x/÷ 1.727	Unclear
GSH : GSSG			
MILK	B-24	0.370 x/÷ 1.532	Unclear
	B-48	0.750 x/÷ 2.381	Unclear
	B-72	0.650 x/÷ 3.394	Unclear
CHO	B-24	1.356 x/÷ 3.910	Unclear
	B-48	1.105 x/÷ 7.949	Unclear
	B-72	0.657 x/÷ 5.040	Unclear

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771 **Table 3.** Effects on Peak Torque, RFD, sprint performance, CMJ, soreness, tiredness, CK,
 772 hsCRP, PC and GSH:GSSG ratio following repeat sprint cycling exercise

Variable	Time Frame	Mean effect ^a ± 90% CI ^b	Qualitative Inference
Peak Torque 60°/s Dominant leg	B – 24h	-0.3 ± 3.2	Likely trivial
	B – 48h	-2.4 ± 8.0	Unclear
	B – 72h	2.0 ± 6.2	Unclear
Peak Torque 180°/s Dominant leg	B – 24h	3.3 ± 6.5	Possibly beneficial
	B – 48h	-2.5 ± 9.5	Unclear
	B – 72h	-7.1 ± 8.9	Possibly negative
RFD (0-200ms) Dominant leg	B – 24h	2.0 ± 6.2	Unclear
	B – 48h	6.3 ± 16.4	Unclear
	B – 72h	-4.4 ± 21.0	Unclear
20m Sprint performance	B – 24h	0.8 ± 1.4	Unclear
	B – 48h	0.4 ± 2.1	Unclear
	B – 72h	0.6 ± 4.0	Unclear
Countermovement jump performance	B – 24h	0.3 ± 3.2	Unclear
	B – 48h	0.5 ± 4.0	Unclear
	B – 72h	-0.1 ± 3.5	Unclear
Muscle soreness	B – 24h	-0.4 ± 1.0	Unclear
	B – 48h	-1.0 ± 1.3	Possibly beneficial
	B – 72h	-0.7 ± 1.0	Possibly beneficial
Muscle Tiredness	B – 24h	-0.6 ± 1.1	Possibly beneficial
	B – 48h	-0.8 ± 1.3	Unclear
	B – 72h	-0.8 ± 0.8	Possibly beneficial
CK	B – 2h	1.075 x/÷ 1.185	Unclear
	B – 24h	1.025 x/÷ 1.516	Unclear
	B – 48h	0.906 x/÷ 1.495	Unclear
	B – 72h	1.013 x/÷ 1.591	Unclear
hs-CRP	B – 2h	0.983 x/÷ 1.111	Unclear
	B – 24h	1.475 x/÷ 1.720	Unclear
	B – 48h	1.377 x/÷ 1.593	Unclear
	B – 72h	1.347 x/÷ 1.696	Unclear
PC	B – 2h	1.241 x/÷ 1.783	Unclear
	B – 24h	1.445 x/÷ 2.087	Unclear
	B – 48h	1.749 x/÷ 1.984	Unclear
	B – 72h	0.920 x/÷ 2.157	Unclear
GSH:GSSG ratio	B – 2h	0.859 x/÷ 4.433	Unclear
	B – 24h	0.334 x/÷ 3.228	Likely Positive
	B – 48h	0.749 x/÷ 8.474	Unclear
	B – 72h	1.095 x/÷ 5.337	Unclear

773 Qualitative Inference represents the likelihood that the true value will have the observed
 774 magnitude

775 ^a Mean effect refers to MILK minus CHO

776 ^b ± 90% CI: add and subtract this number to the mean effect to obtain the 90% confidence
 777 intervals for the true difference

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Figure Captions

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Figure 1. Schematic of the Exercise Protocol

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Figure 2. Peak torque at 60°/s for dominant knee extension in response to repeat sprint

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cycling exercise for MILK (n=10) and CHO (n=10). Values are presented as means \pm SD.

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Figure 3. Muscle soreness in response to repeat sprint cycling exercise for MILK (n=10)

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and CHO (n=10). Values are presented as means \pm SD.

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Figure 1

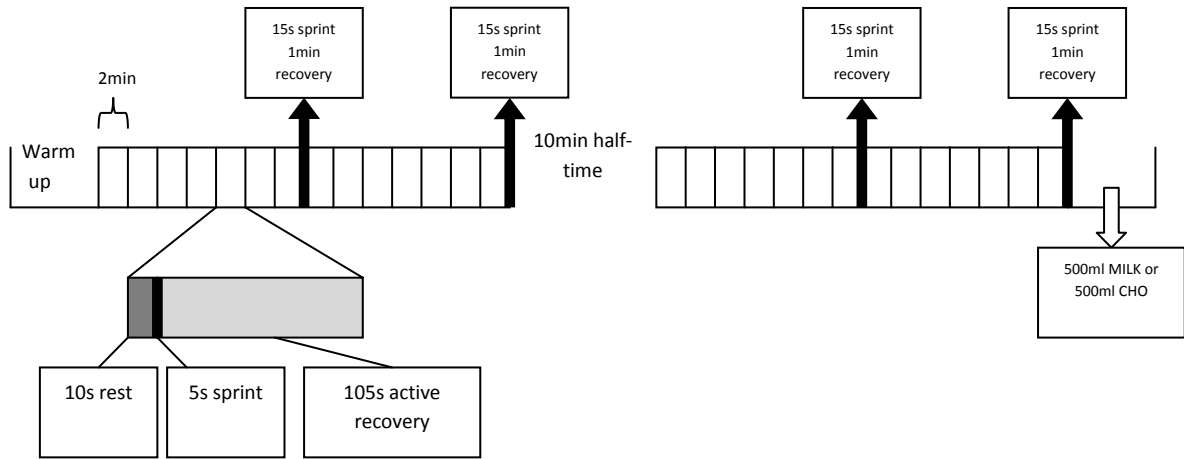


Figure 2

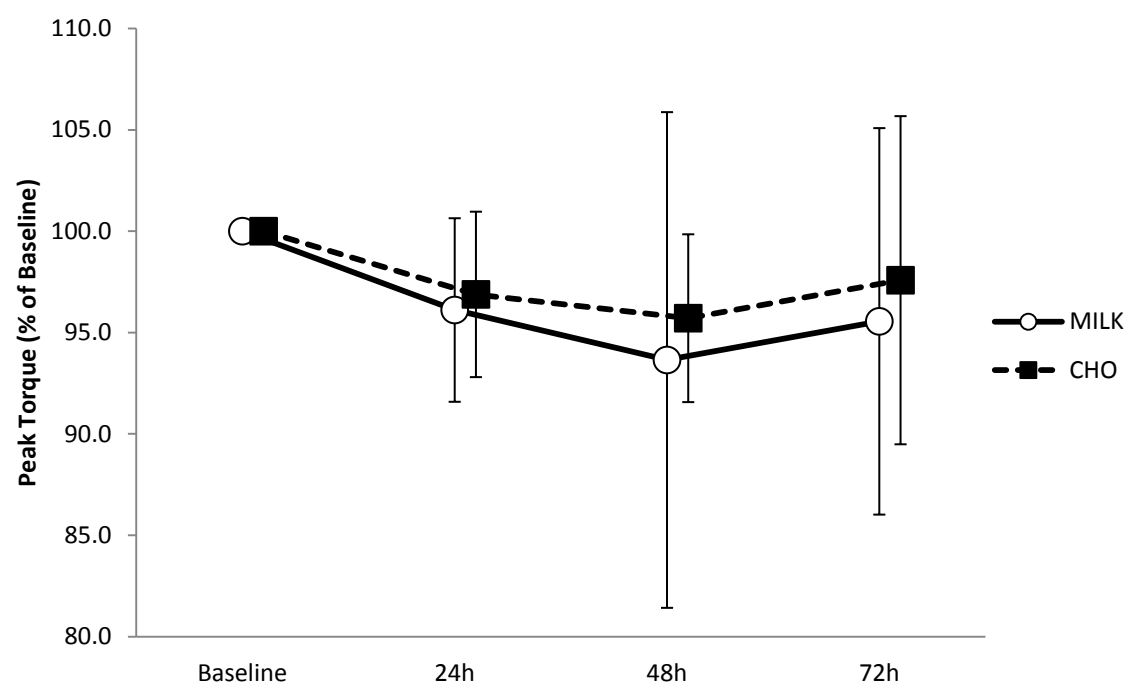


Figure 3

