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 Montmorency tart cherry juice does not reduce markers of muscle soreness, function and inflammation following professional male rugby League match-play

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Article

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1   **Montmorency tart cherry juice does not reduce markers of muscle soreness, function**

2   **and inflammation following professional male Rugby League match-play.**

3

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25 **Abstract**

26 Rugby League (RL) match-play causes muscle damage, inflammation and symptoms of

27 fatigue. To facilitate recovery, nutritional interventions are often employed, including

28 Montmorency cherry juice (MC). We assessed the effects of MC on recovery following RL

29 match-play in eleven male professional RL players who played in two matches (7-days apart)

30 with MC or placebo (PLB) supplemented for 5-days pre-match, match day and 2-days post-

31 match. Blood was collected 48h pre-match, half-time, within 30-mins of full-time and 48h

32 post-match to assess Interleukin concentrations (IL-6, -8 -10). Self-reported sleep, fatigue,

33 mood, stress, and muscle-soreness were assessed 24h pre and 24h and 48h post-matches with

34 muscle function assessed 48h pre and 48h post-match. No differences in distance covered

35 (6334±1944 Vs 6596±1776 m) and total collisions (28±11 Vs 29±13) were observed between

36 both matches. There was a small albeit significant increase in IL-6, -8 and -10 concentrations

37 pre to post-match in both PLB (IL-6: 0.83±0.92 Vs 2.91±1.40, IL-8: 2.16±1.22 Vs 3.91±1.61

38 and IL-10: 2.51±2.14 Vs 0.61±0.50 pg·mL^{-1}) and MC groups (IL-6: 0.53±0.53 Vs 2.24±1.73,

39 IL-8: 1.85±0.96 Vs 3.46±1.12 and IL-10: 0.48±0.50 Vs 2.54±2.10 pg·mL^{-1}), although there

40 were no significant differences between groups ($P<0.05$). Likewise, there was a small but

41 significant increase in muscle soreness ($P=0.01$) alongside a reduction in CMJ ($P=0.003$) with

42 no significant differences between groups. No significant changes in sleep, fatigue or mood

43 ($P>0.05$) were observed pre to post-match or between groups. These data suggest MC does

44 not affect the modest changes observed in cytokine responses and markers of recovery from

45 professional RL match-play.

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47 Word Count: 250

48

Introduction

Rugby League (RL) is a collision-based team sport requiring players to perform high-intensity activity whilst experiencing repeated intermittent high-impact collisions (Austin & Kelly, 2014; Gabbett, Polley, Dwyer, Kearney, & Corvo, 2014; Twist et al., 2014). Given that players possess high levels of fat-free mass to improve strength and power (Morehen, Routledge, Twist, Morton, & Close, 2015) combined with the intense physical demands (Lindsay et al., 2016; Takarada, 2003; Twist & Sykes, 2011), players are regularly exposed to muscle damage with limited recovery days between matches (McLean, Coutts, Kelly, McGuigan, & Cormack, 2010). Reported feelings of soreness peak at 24h post-match, and can remain elevated for up to four days post-match (McLean et al., 2010; Twist, Waldron, Highton, Burt, & Daniels, 2012) persisting throughout the entire playing season (Fletcher et al., 2016). Considering players strive to commence each match as physically ready as possible, it is crucial to identify strategies that may help to facilitate the alleviation of post-match muscle damage and soreness.

In recent years, the relationship between the production of pro- and anti-inflammatory agents, produced in skeletal muscle (myokines) and in the circulation (cytokines), has received great attention following exercise-induced tissue injury (Hennigar & Pasiakos, 2017). Interleukins are a group of cytokines that elicit a wide variety of immunomodulatory functions in cells and tissues including cell proliferation, maturation, migration and adhesion. Following both endurance and intensive exercise (Suzuki, 2018), IL-8 is secreted into the circulation during the pro-inflammatory phase (Nieman et al., 2003) and if injury to skeletal muscle has occurred, signifcant elevations in circulating IL-6 are regarded as a signal that the recovery has begun (Chan McGee, Watt, Hargreaves, & Fabbraio, 2004; Fischer, 2006). Subsequently, during the anti-inflammatory phase, IL-10 acts in a compensatory mechanism, dampening the pro-inflammatory response with research showing significant increases in IL-10 after high-

74 intensity exercise (Suzuki, Tominaga, Ruhee, & Ma, 2020). Whilst research in team sport

75 athletes has shown increases in both IL-8 (Bell et al., 2016) and IL-10 (Nieman et al., 2004),

76 both these studies were performed during controlled laboratory enviroments using exercise

77 modalities which does not reflect the true responses that may be elicited from live team sport

78 collison based match-play. Despite the importance of assessing cytokines in response to

79 disruptions sustained to skeletal muscle, to date studies have only assessed IL-6 (Cunniffe et

80 al., 2011; Cunniffe et al., 2010) following rugby match-play and not IL-8 or IL-10. In the only

81 study to date assessing IL-6 in rugby players, IL-6 was only measured at pre- and post-match

82 time points in rugby union (RU) players (Cunniffe et al., 2011; Cunniffe et al., 2010), with no

83 assessment at half-time or during the recovery days post-match, which is a period of the week

84 whereby rugby players report with persistent upper and lower body soreness all season

85 (Fletcher et al., 2016). Therefore, assessing IL-6, IL-8 and IL-10 on a training day before a

86 match, at half-time, full-time and on a recovery training day may help to assess the magnitude

87 of damage and inflammation caused from the demands of RL match-play.

88

89 Considering players experience both exercise-induced muscle damage (EIMD) and impact-

90 induced muscle damage (IIMD) following match-play (Naughton, Miller, & Slater, 2017), the

91 development of individual recovery strategies are crucial. Post-exercise nutrition has received

92 considerable attention, in particular foods or food components thought to possess anti-

93 inflammatory properties such as polyphenols (Owens, Twist, Cobley, Howatson, & Close,

94 2018). One of the most researched polyphenols, in terms of its effects on EIMD, is the

95 Montmorency cherries (*Prunus Cerasus*), which have shown promising evidence to reduce

96 soreness in laboratory-based trials (Bell, Stevenson, Davison, & Howatson, 2016; Bowtell,

97 Sumners, Dyer, Fox, & Mileva, 2011; Quinlan & Hill, 2019) and following marathon running

98 (Howatson et al., 2010). However, such observations have been made following restrictions of

99   polyphenols prior to the intervention and therefore do not necessarily reflect nutritional intakes

100   that are typically consumed from team sport athletes including rugby players (Bradley et al.,

101   2015; Morehen et al., 2016).

102

103   Currently, there is no data which has investigated the impact of professional RL matches on

104   changes in IL concentrations. Similarly, no study has assessed the effects of MC on reducing

105   blood markers of inflammation from contact sports like rugby, where match-play demands

106   include both EIMD and IIMD, despite the supplement being regularly consumed in such

107   environments. It is therefore crucial that real world studies, with appropriate dietary controls

108   reflective of applied practice, (i.e. not following polyphenol deplete diets) are now performed

109   to assess the true benefit of MC supplementation on muscle damage (Close, Kasper, & Morton,

110   2019) and to further understand the efficacy of spending limited budgets on such supplements

111   which could be directed elsewhere such as improving the general food provision or employing

112   sport nutritionists. To this end, the aim of the present study was to assess the impact of RL

113   match-play on markers of inflammation and the effectiveness of ingesting MC prior to and

114   following competitive match-play on markers of muscle soreness, markers of inflammation

115   and functional measures. We hypothesised that rugby match-play would increase markers of

116   inflammation, increase muscle soreness and decrease markers of muscle function whilst

117   ingestion of MC would have no beneficial effect on markers of recovery compared with a

118   placebo.

119 **Methods**

120 **Participants**

121 Eleven male academy level, professional RL players (mean ± SD; age 18 ± 1 years, body mass

122 92.2 ± 8.6 kg, height 182 ± 0.04 cm) from a European Super League rugby club volunteered

123 to take part in this study. Players were the starting players who were most likely to play in both

124 matches and represented all positional groups (five forwards and six backs). Players were from

125 the same club and were injury and illness free throughout the study period. Ethical approval

126 was granted by the Liverpool John Moores University ethics committee (H17/SPS/020).

127

128 **Experimental Design**

129 A schematic representation of the overall study design is reported in Figure 1. A familiarisation

130 week was initially implemented to ensure players and coaching staff were comfortable with

131 researchers collecting data, including venous blood samples, during normal training and match-

132 day time points including the half-time and full-time period. Following this, players followed

133 a single-blind randomised cross-over design study over two consecutive weeks (termed week-

134 one and -two), including two consecutive scheduled matches (one home and one away fixture)

135 during the 2017/2018 season. A single-blind design was used given the club requested that the

136 lead researcher personally made and administered all the drinks to reduce the risk of

137 supplement contamination. Although the within subject cross-over design may be influenced

138 by the repeated bout effect (RBE), previous work with professional rugby players has shown

139 persistent increases in reported muscle soreness throughout a whole season (Fletcher et al.,

140 2016) demonstrating no RBE being present in rugby players. Players continued with normal

141 in-season training which based upon the minutes trained and coaches training plan was the

142 same for both study weeks, although it must be stressed that given these were academy players

143 no GPS data were available to confirm this. Players were instructed to adhere to and follow

144 normal habitual nutritional intakes the day before both matches concomitant with professional

145 rugby players (e.g. a polyphenol-void diet was not enforced), including match-day and in-game

146 fuelling strategies (Bradley et al., 2016). All players self-reported that they adhered to their

147 habitual match-day fuelling strategy for both matches which was prescribed to them by the

148 club nutritionist. This included players consuming 6g per kg body mass of carbohydrate the

149 day before the game with ~60g/h of exogenous carbohydrates during each match in line with

150 traditional sport nutrition guidelines (Baker, Rollo, Stein, & Jeukendrup, 2015).

151

152 During week one six players consumed the Montmorency cherry supplement (MC) and five

153 players consumed the placebo (PLB) which was then reversed in week two. To ensure there

154 were no positional differences, week one involved three forwards and three backs receiving the

155 MC with two forwards and three backs receiving the PLB, and this was reversed for week two.

156 Muscle soreness, subjective wellness including sleep, fatigue, stress and countermovement

157 jump (CMJ) and drop jump (DJ) performance were measured before and after matches in

158 weeks one and two. Match-play demands were recorded via Global Positioning System (GPS)

159 in all players. Blood samples were collected 48h pre-match (due to club logistics this was

160 deemed the best chance of collecting a genuine baseline sample) at half-time of the matches,

161 full-time (within 30 minutes of the match finishing) and 48h post-match.

162

163 **Supplementation**

164 Using an online random number generator, players were randomly assigned to either a MC or

165 PLB group. The MC supplements were prepared by mixing two 30 mL dosages (Per 30 mL:

166 102kcal, 25g carbohydrate, 0g fat, 1g protein, 320mg anthocyanins) with two 100mL bottles

167 of water, and stored in a refrigerator prior to consumption. The MC was a batch tested,

168 commercially available MC concentrate (CherryActive[TM], Sunbury, UK) which was consumed

169 well before the use-by date. The PLB supplement was a commercially available fruit cordial,

170 mixed with water and maltodextrin (Science in Sport, Nelson, UK) into two separate bottles,

171 to match for energy and carbohydrate content with the MC (10 kcal, 25g carbohydrate, 0g fat,

172 0g protein). Pilot work (n=7) in our laboratory confirmed that the masking was effective

173 although we did not perform exit interviews on the players to assess if they could identify the

174 MC drink. Two MC or two PLB supplements were prepared for each player, each day, into

175 separate bottles and then sealed by the club's sports nutritionist off-site in order to maintain the

176 single blind design to follow previously published administration protocols (Bell et al., 2016;

177 Howatson et al., 2010). The MC or PLB supplements were provided to the players at the

178 training facility along with instructions detailing the dosing schedule (one bottle in the morning

179 and one bottle in the evening, seven consecutive days, five days before, match day, and two

180 days after the match). Each day the players were reminded to consume each bottle by using

181 cellular contact (WhatsApp messages) from the club nutritionist and all players reported 100%

182 compliance to both MC and PLB supplementation.

183

184 **Match Analysis**

185 To allow measurement and recording of match movement demands, all players were fitted with

186 a micro-technology device (Optimeye S5, Catapult Innovation, Melbourne, Australia). These

187 were simultaneously activated at pitch-side before kick-off, to enable acquisition of satellite

188 signals. Match duration, relative and absolute number of collisions, distance covered, and high-

189 speed running were recorded. Collisions experienced were determined via accelerometer and

190 gyroscope data provided in G force. For a collision to be registered, the player maintained a

191 nonvertical position classified as leaning forward by more than 60°, backward by more than

192 30° or leaning left or right by more than 45° for 1 second. During each match, players either

193 played the full duration of the game or were substituted, for tactical reasons, by the head coach

194 given that these were competitive in-season league fixtures.

195

196 **Whole blood sampling**

197 Whole blood samples (10mL) were drawn from a superficial vein in the antecubital fossa of

198 the forearm using standard venepuncture techniques (Vacutainer Systems, Becton, Dickinson).

199 Blood samples were collected at the training facility 48h before (the last time blood could be

200 drawn before the match, therefore acting as baseline) and 48h after each fixture as players

201 arrived for normal training commitments (16:30-17:00h) and stored on ice for ~2h before being

202 transported back to the laboratory for serum separation. On match day, blood samples were

203 collected in the changing room at each respective rugby club. This occurred during the normal

204 half-time interval (~10 min) and within 30 min of each match finishing. All blood samples

205 were successfully obtained within the allocated time frame, enabled by six researchers being

206 present in the changing room at each match. Blood samples were stored on ice and transported

207 back to the laboratory where they were centrifuged at 1500g for 15min at 4°C before duplicate

208 aliquots of serum were stored at -80°C for later analyses.

209

210 **Preparation of Human Soluble Protein Flex Set Assay**

211 Commercially available Cytometric Bead Array (CBA) Human Soluble Protein Master Buffer

212 Kits and individual Human Flex Sets for IL-1, -2, -4, -6, -8 and -10 (BD Biosciences^{TM}, San

213 Diego, CA) were used, according to manufacturer's instructions. Briefly, a series of standards

214 ranging from 0 - 2,500 pg·mL^{-1} were prepared by serial dilution and the theoretical detection

215 limits of the manufacturer (determined by evaluating the estimated result of the average

216 multiplex fluorescent immunoassay [MFI] of the negative control [0 pg/mL, n=30] + 2 standard

217 deviations - IL-1; 1.0, IL-2; 11.2, IL-4; 1.4, IL-6; 1.6, IL-8; 1.2 and IL-10; 0.13 pg·mL^{-1}) were

218   extended, in order that any values with an MFI below the detection limit of zero standard were

219   considered below the detection limits of the kits. Reported intra-assay coefficient of variations

220   (CV) were as follows: IL-1; 3, IL-2; 5, IL-4; 3, IL-6; 2, IL-8; 2 and IL-10; 4% with samples

221   run in duplicate (2% CV). The capture beads for all proteins analysed were mixed with wash

222   buffer, vortexed centrifuged at 200g for 5min., prior to the supernatant being removed and

223   beads resuspended with in Capture Bead Diluent according to manufacturer's instructions,

224   before being vortex mixed and incubated for 15min at room temperature (RT), prior to samples

225   and standards being incubated with beads for 1hr at RT with mixing. PE-detection reagents for

226   all analytes were pooled, resuspended in assay diluent and added to the standards and samples

227   plus capture beads for 2h at RT with mixing (in the dark) prior to centrifugation at 200g for

228   5min at RT. The supernatant was aspirated and beads were resuspended with wash buffer and

229   analysed using the BD FACSCalibur^{TM} supported by Cell Quest Pro Software (both Becton

230   Dickinson, Franklin Lakes, NJ, USA), with 2000 events captured per analyte per sample. Data

231   were uploaded from Cell Quest Pro and Filtered using FCS Filter^{TM} and analysed using FCAP

232   array software (Hungary Software Ltd, for BD Biosciences, San Jose CA, USA).

233

234   Countermovement Jump (CMJ) and Drop Jump (DJ) performance

235   At 48h before and 48h after each match players performed a series of CMJ's and DJ's wearing

236   trainers. Flight time was recorded based on previous recommendations (Cormack, Newton,

237   McGuigan, & Doyle, 2008) and research in rugby players (Oxendale, Twist, Daniels, &

238   Highton, 2016). Flight times (the difference between take-off and landing time) were measured

239   using two photoelectric parallel bars (Optojump^{TM}, Microgate, Bolzano, Italy) as previously

240   described (Oxendale, Twist, Daniels, & Highton, 2016). During the DJ protocol, a 30cm box

241   was placed in front of the Optojump^{TM} bars and players were instructed to step on to the box

242   and to keep both hands on their hips throughout testing. Players performed three jumps with

243 the maximum flight time (Twist et al., 2012) and minimal contact time used as an index of the

244 maximal rate of force development and reactive strength index (RSI) (Twist & Eston, 2007)

245 used for analysis. All players were familiar with jump procedures as part of the club's regular

246 monitoring process with reliability for these measurements demonstrating a CV of 2.3% and

247 2.7% respectively during one week of familiarisation.

248

249 **Self-reported Subjective Wellness**

250 At 24h pre-match, 24h and 48h post-match, participants provided a rating of perceived sleep

251 quality, fatigue, muscle soreness, mood and stress using a 1-5 Likert scale which has been

252 adapted from others (McLean et al., 2010) and previously used with RL players (Twist et al.,

253 2012). Higher values were indicative of a positive response to the question. Similar scales have

254 been shown to possess strong reliability and validity (De Vries & Van Heck, 2003).

255 Participants were familiar with this procedure as part of their habitual club monitoring

256 processes and were instructed to complete the scales on their own to reduce influence from

257 other players.

258

259 **Statistical analysis**

260 Match characteristics, subjective wellness, jump performance and cytokine data are presented

261 as separate means (±SD) for both MC and PLB treatment conditions. Changes were analysed

262 using a two-way repeated measure general linear model (GLM) where the within factors was

263 time (48h pre-match, half-time, full-time and 48h post-match) and condition (MC and PLB).

264 The tests of within subjects effects provided values for Mauchly's test for sphericity. If this

265 was violated, then a Greenhouse-Geisser correction was used. The difference between means

266 were tested at a significance level of $P<0.05$.

267 **Results**

268 **Match characteristics**

269 There were no significant differences in absolute distance covered (6334±1924 vs 6596±177

270 m, P=0.75), relative distance covered (72.6±4.8 vs 79.3±5.5 m.min^{-1}, P=0.009), total collisions

271 (28±11 vs 29±13, P=0.89), high speed running (4457±1315 vs 4286±1532 m, P=0.78) and

272 playing duration (67:10±19:7 vs 67:10±19:3 min, P=0.99, between the two matches. Seven

273 players played the full 80 min in both matches, with a mean difference of 9 minutes between

274 matches for the remaining 4 players.

275

276 **Cytokine Responses**

277 Data for interleukin -1, -2, and -4 were all below the known theoretical detection limits of the

278 assays from the manufacturer and therefore these data are not reported.

279

280 **Interleukin-6**

281 There was no significant treatment by time interactions ($F_{1.43,\ 14.37}$ = 1.00, P=0.365) or any

282 significant main effect of supplementation ($F_{1,\ 10,}$ = 2.14, P=0.173). There was a significant

283 main effect of time for IL-6 concentrations (Figure 2A) following match-play ($F_{1.44,\ 14.46}$ =

284 15.71, P<0.001). IL-6 was significantly greater at full-time (2.58±1.57 pg·ml^{-1}) compared with

285 48h pre-match (0.68±0.75 pg·ml^{-1}; P=0.006: 95% CI for differences = 0.54 to 3.25 pg·ml^{-1})

286 and 48h post-match (0.85±0.51 pg·ml^{-1}; P=0.004: 95% CI for differences = 0.56 to 2.90 pg·ml^{-}

287 ^{1}) although no significant difference was seen compared with half-time (1.49±0.89 pg·ml^{-1};

288 P=0.166: -0.30 to 2.47 pg·ml^{-1}).

289

290 **Interleukin-8**

291 There was no significant treatment by time interactions ($F_{3.00, 30.00}$, = 0.79, P=0.510) or any

292 significant main effect of supplementation ($F_{1, 10}$, = 0.51, P=0.493). There was a significant

293 main effect of time for IL-8 concentrations (Figure 2B) following match-play ($F_{3.00, 30.00}$ =

294 17.69, P<0.001). IL-8 was significantly greater at full-time (3.68±1.38 pg·ml^{-1}) compared with

295 48h pre-match (2.01±1.08 pg·ml^{-1}; P<0.001: 95% CI for differences = 0.82 to 2.54 pg·ml^{-1}),

296 half-time (2.55±1.24 pg·ml^{-1}; P=0.009: 95% CI for differences = 0.27 to 1.99 pg·ml^{-1}) and 48h

297 post-match (2.38±1.02 pg·ml^{-1}; P=0.004: 95% CI for differences = 0.41 to 2.19 pg·ml^{-1}).

298

299 **Interleukin-10**

300 There was no significant treatment by time interactions ($F_{1.32, 13.026}$, = 0.01, P=0.827) or any

301 significant main effect of supplementation ($F_{1, 10}$, = 0.08, P=0.777). There was a significant

302 main effect of time for IL-10 concentrations (Figure 2C) following match-play ($F_{1.40, 13.99}$ =

303 8.32, P=0.007). IL-10 concentrations were greater at full-time (2.52±2.07 pg·ml^{-1}) compared

304 with 48h pre-match (0.54±0.49 pg·ml^{-1}; P=0.059: 95% CI for differences = -0.06 to 4.02 pg·ml^{-1}

305 ^{1}) half-time (1.01±0.92 pg·ml^{-1}; P=0.100: 95% CI for differences = -0.21 to 3.24 pg·ml^{-1}) and

306 48h post-match (0.63±0.55 pg·ml^{-1}; P=0.070: 95% CI for differences = -0.12 to 3.91 pg·ml^{-1}).

307

308 **Self-reported Subjective Wellness**

309 There was no significant difference between pre-match and 24h post-match sleep (P=1.00 and

310 P=0.86), fatigue (P=0.26 and P=0.33), mood (P=0.71 and P=0.92) and stress (P=0.71 and

311 P=0.83) in both PLB and MC groups respectively (Table 1). Similarly, there were no

312 significant difference between pre-match and 48h post-match sleep (P=0.40 and P=0.52),

313 fatigue (P=0.27 and P=0.86), mood (P=0.80 and P=0.54) and stress (P=0.26 and P=0.14).

314 However, at 24h post-match there was a significant increase in players self-reported muscle

315 soreness when compared to pre-match in both PLB (P=0.03) and MC groups (P=0.01) although

316   at 48h post-match self-reported muscle soreness showed no difference to pre-match in both

317   PLB (P=0.25) and MC groups (P=0.90).

318

319   **CMJ and DJ performance**

320   There was a significant decrease in both PLB (P=0.004) and MC (P=0.007) countermovement

321   flight time from 24h pre-match (PLB: 0.53±0.03 and MC: 0.54±0.02 s) to 48h post-match

322   (PLB: 0.51±0.03 and MC: 0.51±0.02 s). Similarly, there was a decrease in PLB (2.16±0.34

323   m·s^{-1}) and MC (2.17±0.33 m·s^{-1}) drop jump performance from 24h pre-match to 48h post-match

324   (PLB: 2.05±0.40 m·s^{-1} and MC: 2.06±0.41 m·s^{-1}) although this was not statistically significant

325   (P=0.228 and P=0.893, respectively). Data are shown in Table 1.

Discussion

326

327 The aim of the present study was to investigate the effects of RL match-play on circulating

328 inflammatory markers and to assess the efficacy of MC on markers of muscle soreness and

329 recovery following rugby match-play. To this end, using flow cytometry, we measured

330 concentrations of IL-6, IL-8 and IL-10 collected at 48h pre-match, half-time, full-time and 48h

331 post-match from eleven male professional rugby players over two successive in-season RL

332 matches. Following professional RL match-play we report small, yet significant, increases in

333 IL-6, IL-8 and IL-10 at half-time and full-time compared with 48h pre-match along with small

334 but significant reduction in CMJ and increases in soreness. However, we report for the first

335 time in collision sport athletes, that supplementation with MC caused no improvements in

336 reducing inflammatory markers, soreness, sleep or muscle function compared with a placebo.

337 These data contradict the assumption that reductions in inflammation and improvements in

338 recovery are possible following the ingestion of MC following rugby match-play.

339

340 To investigate the efficacy of MC it was important to assess the magnitude of inflammation,

341 soreness and loss of muscle function that is caused from RL match-play. Previous research has

342 shown increases in IL-6 concentration following match-play in professional RU players

343 (Cunniffe et al., 2011; Cunniffe et al., 2010). We confirm and extend these findings by showing

344 increases in IL-6, IL-8 and IL-10 concentrations following competitive RL match-play at half-

345 time and full-time in RL players. Interestingly, although previous research has shown increases

346 in IL-8 from other team sport athletes (Bell et al., 2016), this was performed in a controlled

347 laboratory environment and following a simulated football exercise test, and as such, we show

348 for the first time in academy RL players, significant elevations in IL-8 following RL match-

349 play. Similarly, we provide novel data in RL players showing significant increases in the anti-

350 inflammatory cytokine IL-10 post-match, which has previously only been shown following

351   laboratory-controlled resistance training and yet to be investigated in team sport athletes

352   (Nieman et al., 2004).

353

354   Our findings confirm and extend recent work in academy soccer (Abbott, Brashill, Brett, &

355   Clifford, 2019) and rugby (Kupusarevic, McShane, & Clifford, 2019) players who have

356   reported no effects of MC on markers of muscle soreness and function although we

357   demonstrate no effects of MC on IL-6, -8 and -10. Our findings are however in contrast with

358   others who have shown beneficial effects of MC on reducing markers of inflammation and

359   soreness following laboratory trials (Bell et al., 2016; Bowtell et al., 2011), and recreational

360   marathon running (Howatson et al., 2010). A possible explanation as to why we found no

361   effects of MC could be because the analgesic effects of polyphenols may only exert beneficial

362   effects with exercise modalities that invoke EIMD without the additional IIMD. Indeed,

363   research collected from full time professional RU players shows resting metabolic rates

364   significantly increased by as much as ~1000 calories, the morning after rugby match-play, due

365   to collisions and damage (Hudson, Cole, Morton, Stewart, & Close, 2020). With this in mind,

366   supplementation of cherry juice may still be beneficial in less trained athletes and/or following

367   much higher amounts of damage beyond what is seen in a typical competitive academy RL

368   game. Another possible explanation for the disparity between the present study and previous

369   literature on MC supplementation may be the fact that players in our study were not asked to

370   restrict any polyphenol rich foods or beverages prior to, or during, the intervention period

371   unlike previous research (Bell, Walshe, Davison, Stevenson, & Howatson, 2015; Bell et al.,

372   2016; Bowtell et al., 2011). It is possible that the benefits of MC are only reported when

373   replenishing deficient concentrations of polyphenols following a restriction period, rather than

374   the added benefit of enhanced polyphenol concentrations. To this end, if players follow diets

375   rich in fruits and vegetables then the use of such supplements may not be needed, however

376   there may still be a benefit for those players who do not have a polyphenol rich diet.

377

378   A limitation of the present study was that players' dietary polyphenol intakes were not

379   measured and it is therefore possible that a wide range of undetected polyphenols were

380   consumed by the players. Additionally, we did not batch test the cherry juice for polyphenol

381   content before being administered to players although we did ensure it was stored according to

382   the manufacturers instructions. It is therefore possible that the MC juice may have less

383   polyphenols than stated on the label and/or players may have had a wide range of baseline

384   polyphenols from their own habitual diets. Although this may be deemed a limitation, the

385   present study was designed for maximal ecological validity (Close et al., 2019) and therefore

386   attempted to replicate real-world consumption of this product. Furthermore, given the structure

387   of the game weeks, only five days were given as a washout period. Whilst this may appear

388   short, polyphenols have been shown to have short half-lives (1-2 h) (Kay, Mazza, & Holub,

389   2005) and as such there would have been no hang-over effects from the supplementation.

390   Moreover, although it has been suggested in animal models that some polyphenol metabolites

391   may accumulate and store in target tissues (Manach, Scalbert, Morand, Rémésy, & Jiménez,

392   2004) this has not been shown in humans. Future studies, however, may wish to consider a

393   longer wash-out period and/or directly measuring plasma polyphenols. We also acknowledge

394   that the potential increase in IL-6 as a result of match-play might be due to regulation of liver

395   glycogenolysis and glucose output. However, all players consumed ~60g/h of carbohydrates

396   during exercise, a feeding strategy that is known to suppress IL-6 concentrations (Hennigar &

397   Pasiakos, 2017). Furthermore, examination of individual data demonstrates that the forward

398   players who had the highest collisions presented with the highest circulating IL-6

399   concentrations compared with the backs who performed more extensive running further

400 supporting the hypothesis that the increase in IL-6 was damage mediated. Although others have

401 assessed other biochemical indices related to inflammation such as myoglobin and high-

402 sensitivity C-reactive protein following damaging simulation interventions (Naughton, Miller,

403 & Slater, 2018), future work should now assess these inflammation indices following live

404 match play. Although previous research has shown benefits of MC juice on recovery indices

405 (soreness and muscle function) in the 24h-48h following exercise (Bell et al., 2016; Bowtell et

406 al., 2011), unfortunately we were unable to collect blood at 24h post-match and may have

407 missed this opportunity to show potential benefits of MC juice in this study. As such, future

408 work should now assess a portfolio of inflammatory markers from pre-match to post-match

409 and into the subsequent recovery days following live rugby match-play. Finally, although we

410 show small changes in muscle function, future work should now assess this in a larger group

411 of players to better understand the effects of recovery interventions on rugby players.

412

413 This study has several practical applications with immediate translation. Firstly, following RL-

414 match-play that caused small changes in IL concentrations from baseline to post-match,

415 consuming MC alongside habitual nutritional intakes showed no beneficial effects for reducing

416 markers of inflammation, muscle soreness and functional performance. The data in this study

417 therefore question the efficacy of such supplements in RL where budgets are often a key

418 consideration, and players may want to consider adopt a food-first approach when consuming

419 polyphenols in their diet. Secondly, given the data shows *medium* to *large* correlations between

420 total collisions and self-reported muscle soreness scores and increases in IL's, practitioners

421 working with athletes in sports that involves both exercise- and impact-induced muscle damage

422 may consider the efficacy of simply asking players to report how they feel as the best proxy

423 marker of damage and inflammation - although this clearly requires an honest assessment from

424 the athlete.

425

426   In conclusion, the present study has assessed inflammatory markers following RL match-play

427   whilst also assessing the effectiveness of MC consumption in professional RL players.

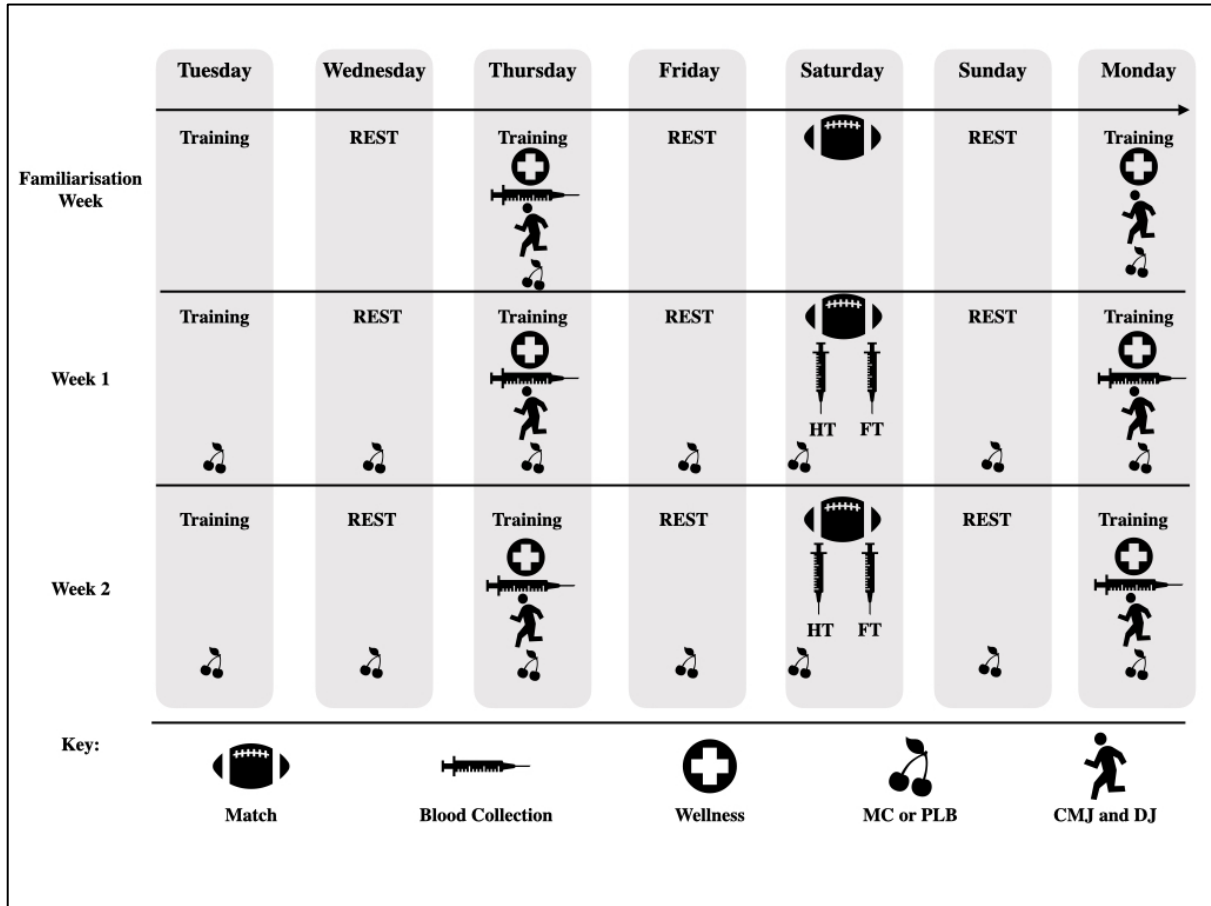428   Following match-play, we report small but significant increases in IL's, muscle soreness and

429   small reductions in CMJ compared with baseline, however we show no beneficial effects of

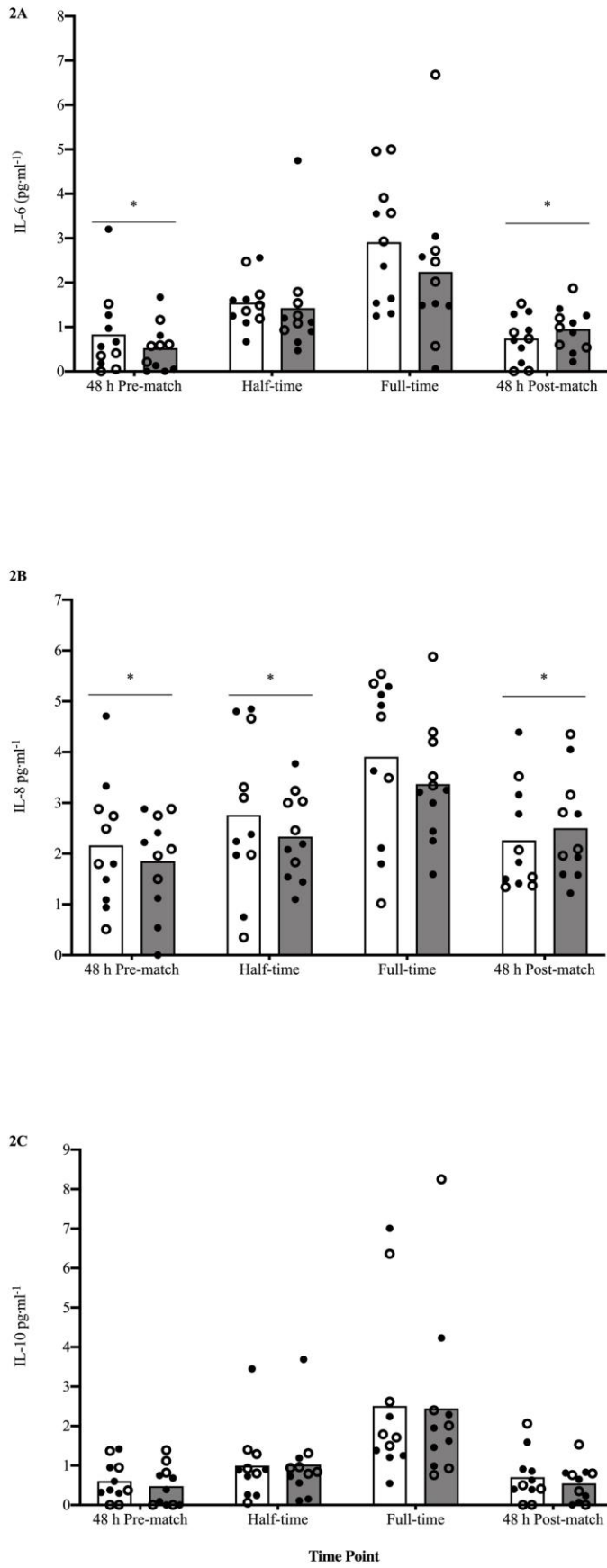430   MC on markers of recovery in professional academy rugby players.

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435 Figure 1.
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440
441   Figure 2 A, B and C

442
443 **Table 1.**
444

	24 h pre-match		24 h post-match		48 h post-match	
	PLB	MC	PLB	MC	PLB	MC
Sleep quality (AU)	3.5 ± 0.7	3.4 ± 0.4	3.5 ± 0.8	3.9 ± 0.9	3.7 ± 0.6	3.6 ±0.5
Fatigue (AU)	3.7 ± 0.7	3.6 ± 0.6	3.3 ± 0.8	3.3 ± 0.3	3.4 ± 0.7	3.5 ± 0.4
Muscle soreness (AU)	3.3 ± 0.7	3.4 ± 0.4	*2.6 ± 0.8	2.6 ± 0.5	3.0 ± 0.8	3.2 ± 0.9
Mood (AU)	3.5 ± 0.7	3.4 ± 0.5	3.4 ± 0.5	3.4 ± 0.7	3.6 ± 0.8	3.6 ± 0.6
Stress (AU)	3.5 ± 0.7	3.5 ± 0.9	3.4 ± 0.5	3.4 ± 0.3	3.9 ± 0.8	3.8 ± 0.6
CMJ (s)	0.53 ± 0.03	0.54 ± 0.04	-	-	*0.51 ± 0.03	*0.52 ± 0.10
DJ ($m^.s^{-1}$)	2.16 ± 0.34	2.17 ± 0.32	-	-	2.05 ± 0.40	2.06 ± 0.33

445
446 *denotes significant difference from pre-match value ($P<0.05$). PLB = Placebo, MC =
447 Montmorency Cherry Juice, CMJ = Countermovement jump, DJ = Drop jump.
448
449
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451

452 **Figure 1.** Schematic showing the overall study design. A familiarisation week is followed by

453 a two-week intervention period which included two matches, blood collection, subjective

454 wellness scores and jump performance. HT = Half-time, FT = Full-time, MC = Montmorency

455 cherry juice, PLB = Placebo, CMJ = Countermovement Jump, DJ = Drop jump.

456

457 **Figure 2.** Interleukin-6 (2A), -8 (2B) and -10 (2C) concentration at 48h pre-match, half-time,

458 full-time and 48h post-professional rugby league match-play. White bars represent placebo

459 group, grey bars represent Montmorency cherry juice group. Open circles represent forward

460 players, closed circles represent back players. [*]denotes significant difference between 48h

461 pre-match, half-time and 48h post-match compared to full-time values ($P<0.05$).

462

463 **Table 1.** Differences in subjective markers at 24 h and 48 h post-match and CMJ and DJ

464 performance 48 h post-match in comparison with baseline, Mean ± SD.

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