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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Abstract

Rugby League (RL) match-play causes muscle damage, inflammation and symptoms of fatigue. To facilitate recovery, nutritional interventions are often employed, including Montmorency cherry juice (MC). We assessed the effects of MC on recovery following RL match-play in eleven male professional RL players who played in two matches (7-days apart) with MC or placebo (PLB) supplemented for 5-days pre-match, match day and 2-days post-match. Blood was collected 48h pre-match, half-time, within 30-mins of full-time and 48h post-match to assess Interleukin concentrations (IL-6, -8 -10). Self-reported sleep, fatigue, mood, stress, and muscle-soreness were assessed 24h pre and 24h and 48h post-matches with muscle function assessed 48h pre and 48h post-match. No differences in distance covered (6334±1944 Vs 6596±1776 m) and total collisions (28±11 Vs 29±13) were observed between both matches. There was a small albeit significant increase in IL-6, -8 and -10 concentrations 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 and IL-10: 2.51±2.14 Vs 0.61±0.50 $pg{\cdot}mL^{-1}$) and MC groups (IL-6: 0.53±0.53 Vs 2.24±1.73, IL-8: 1.85±0.96 Vs 3.46±1.12 and IL-10: 0.48±0.50 Vs 2.54±2.10 $pg{\cdot}mL^{-1}$), although there were no significant differences between groups ($P<0.05$). Likewise, there was a small but significant increase in muscle soreness ($P=0.01$) alongside a reduction in CMJ ($P=0.003$) with no significant differences between groups. No significant changes in sleep, fatigue or mood ($P>0.05$) were observed pre to post-match or between groups. These data suggest MC does not affect the modest changes observed in cytokine responses and markers of recovery from professional RL match-play.

Word Count: 250

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-

intensity exercise (Suzuki, Tominaga, Ruhee, & Ma, 2020). Whilst research in team sport athletes has shown increases in both IL-8 (Bell et al., 2016) and IL-10 (Nieman et al., 2004), both these studies were performed during controlled laboratory enviroments using exercise modalities which does not reflect the true responses that may be elicited from live team sport collison based match-play. Despite the importance of assessing cytokines in response to disruptions sustained to skeletal muscle, to date studies have only assessed IL-6 (Cunniffe et al., 2011; Cunniffe et al., 2010) following rugby match-play and not IL-8 or IL-10. In the only study to date assessing IL-6 in rugby players, IL-6 was only measured at pre- and post-match time points in rugby union (RU) players (Cunniffe et al., 2011; Cunniffe et al., 2010), with no assessment at half-time or during the recovery days post-match, which is a period of the week whereby rugby players report with persistent upper and lower body soreness all season (Fletcher et al., 2016). Therefore, assessing IL-6, IL-8 and IL-10 on a training day before a match, at half-time, full-time and on a recovery training day may help to assess the magnitude of damage and inflammation caused from the demands of RL match-play.

Considering players experience both exercise-induced muscle damage (EIMD) and impact-induced muscle damage (IIMD) following match-play (Naughton, Miller, & Slater, 2017), the development of individual recovery strategies are crucial. Post-exercise nutrition has received considerable attention, in particular foods or food components thought to possess anti-inflammatory properties such as polyphenols (Owens, Twist, Cobley, Howatson, & Close, 2018). One of the most researched polyphenols, in terms of its effects on EIMD, is the Montmorency cherries (*Prunus Cerasus*), which have shown promising evidence to reduce soreness in laboratory-based trials (Bell, Stevenson, Davison, & Howatson, 2016; Bowtell, Sumners, Dyer, Fox, & Mileva, 2011; Quinlan & Hill, 2019) and following marathon running (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.

Methods

Participants

Eleven male academy level, professional RL players (mean ± SD; age 18 ± 1 years, body mass 92.2 ± 8.6 kg, height 182 ± 0.04 cm) from a European Super League rugby club volunteered to take part in this study. Players were the starting players who were most likely to play in both matches and represented all positional groups (five forwards and six backs). Players were from the same club and were injury and illness free throughout the study period. Ethical approval was granted by the Liverpool John Moores University ethics committee (H17/SPS/020).

Experimental Design

A schematic representation of the overall study design is reported in Figure 1. A familiarisation week was initially implemented to ensure players and coaching staff were comfortable with researchers collecting data, including venous blood samples, during normal training and match-day time points including the half-time and full-time period. Following this, players followed a single-blind randomised cross-over design study over two consecutive weeks (termed week-one and -two), including two consecutive scheduled matches (one home and one away fixture) during the 2017/2018 season. A single-blind design was used given the club requested that the lead researcher personally made and administered all the drinks to reduce the risk of supplement contamination. Although the within subject cross-over design may be influenced by the repeated bout effect (RBE), previous work with professional rugby players has shown persistent increases in reported muscle soreness throughout a whole season (Fletcher et al., 2016) demonstrating no RBE being present in rugby players. Players continued with normal in-season training which based upon the minutes trained and coaches training plan was the same for both study weeks, although it must be stressed that given these were academy players no GPS data were available to confirm this. Players were instructed to adhere to and follow

normal habitual nutritional intakes the day before both matches concomitant with professional rugby players (e.g. a polyphenol-void diet was not enforced), including match-day and in-game fuelling strategies (Bradley et al., 2016). All players self-reported that they adhered to their habitual match-day fuelling strategy for both matches which was prescribed to them by the club nutritionist. This included players consuming 6g per kg body mass of carbohydrate the day before the game with ~60g/h of exogenous carbohydrates during each match in line with traditional sport nutrition guidelines (Baker, Rollo, Stein, & Jeukendrup, 2015).

During week one six players consumed the Montmorency cherry supplement (MC) and five players consumed the placebo (PLB) which was then reversed in week two. To ensure there were no positional differences, week one involved three forwards and three backs receiving the MC with two forwards and three backs receiving the PLB, and this was reversed for week two. Muscle soreness, subjective wellness including sleep, fatigue, stress and countermovement jump (CMJ) and drop jump (DJ) performance were measured before and after matches in weeks one and two. Match-play demands were recorded via Global Positioning System (GPS) in all players. Blood samples were collected 48h pre-match (due to club logistics this was deemed the best chance of collecting a genuine baseline sample) at half-time of the matches, full-time (within 30 minutes of the match finishing) and 48h post-match.

Supplementation

Using an online random number generator, players were randomly assigned to either a MC or PLB group. The MC supplements were prepared by mixing two 30 mL dosages (Per 30 mL: 102kcal, 25g carbohydrate, 0g fat, 1g protein, 320mg anthocyanins) with two 100mL bottles of water, and stored in a refrigerator prior to consumption. The MC was a batch tested, commercially available MC concentrate (CherryActive^{TM}, Sunbury, UK) which was consumed

well before the use-by date. The PLB supplement was a commercially available fruit cordial, mixed with water and maltodextrin (Science in Sport, Nelson, UK) into two separate bottles, to match for energy and carbohydrate content with the MC (10 kcal, 25g carbohydrate, 0g fat, 0g protein). Pilot work (n=7) in our laboratory confirmed that the masking was effective although we did not perform exit interviews on the players to assess if they could identify the MC drink. Two MC or two PLB supplements were prepared for each player, each day, into separate bottles and then sealed by the club's sports nutritionist off-site in order to maintain the single blind design to follow previously published administration protocols (Bell et al., 2016; Howatson et al., 2010). The MC or PLB supplements were provided to the players at the training facility along with instructions detailing the dosing schedule (one bottle in the morning and one bottle in the evening, seven consecutive days, five days before, match day, and two days after the match). Each day the players were reminded to consume each bottle by using cellular contact (WhatsApp messages) from the club nutritionist and all players reported 100% compliance to both MC and PLB supplementation.

Match Analysis

To allow measurement and recording of match movement demands, all players were fitted with a micro-technology device (Optimeye S5, Catapult Innovation, Melbourne, Australia). These were simultaneously activated at pitch-side before kick-off, to enable acquisition of satellite signals. Match duration, relative and absolute number of collisions, distance covered, and high-speed running were recorded. Collisions experienced were determined via accelerometer and gyroscope data provided in G force. For a collision to be registered, the player maintained a nonvertical position classified as leaning forward by more than 60°, backward by more than 30° or leaning left or right by more than 45° for 1 second. During each match, players either

played the full duration of the game or were substituted, for tactical reasons, by the head coach given that these were competitive in-season league fixtures.

Whole blood sampling

Whole blood samples (10mL) were drawn from a superficial vein in the antecubital fossa of the forearm using standard venepuncture techniques (Vacutainer Systems, Becton, Dickinson). Blood samples were collected at the training facility 48h before (the last time blood could be drawn before the match, therefore acting as baseline) and 48h after each fixture as players arrived for normal training commitments (16:30-17:00h) and stored on ice for ~2h before being transported back to the laboratory for serum separation. On match day, blood samples were collected in the changing room at each respective rugby club. This occurred during the normal half-time interval (~10 min) and within 30 min of each match finishing. All blood samples were successfully obtained within the allocated time frame, enabled by six researchers being present in the changing room at each match. Blood samples were stored on ice and transported back to the laboratory where they were centrifuged at 1500g for 15min at 4°C before duplicate aliquots of serum were stored at -80°C for later analyses.

Preparation of Human Soluble Protein Flex Set Assay

Commercially available Cytometric Bead Array (CBA) Human Soluble Protein Master Buffer Kits and individual Human Flex Sets for IL-1, -2, -4, -6, -8 and -10 (BD Biosciences^{TM}, San Diego, CA) were used, according to manufacturer's instructions. Briefly, a series of standards ranging from 0 - 2,500 $pg{\cdot}mL^{-1}$ were prepared by serial dilution and the theoretical detection limits of the manufacturer (determined by evaluating the estimated result of the average multiplex fluorescent immunoassay [MFI] of the negative control [0 pg/mL, n=30] + 2 standard 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 $pg{\cdot}mL^{-1}$) were

extended, in order that any values with an MFI below the detection limit of zero standard were considered below the detection limits of the kits. Reported intra-assay coefficient of variations (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 run in duplicate (2% CV). The capture beads for all proteins analysed were mixed with wash buffer, vortexed centrifuged at 200g for 5min., prior to the supernatant being removed and beads resuspended with in Capture Bead Diluent according to manufacturer's instructions, before being vortex mixed and incubated for 15min at room temperature (RT), prior to samples and standards being incubated with beads for 1hr at RT with mixing. PE-detection reagents for all analytes were pooled, resuspended in assay diluent and added to the standards and samples plus capture beads for 2h at RT with mixing (in the dark) prior to centrifugation at 200g for 5min at RT. The supernatant was aspirated and beads were resuspended with wash buffer and analysed using the BD FACSCalibur™ supported by Cell Quest Pro Software (both Becton Dickinson, Franklin Lakes, NJ, USA), with 2000 events captured per analyte per sample. Data were uploaded from Cell Quest Pro and Filtered using FCS Filter™ and analysed using FCAP array software (Hungary Software Ltd, for BD Biosciences, San Jose CA, USA).

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

At 48h before and 48h after each match players performed a series of CMJ's and DJ's wearing trainers. Flight time was recorded based on previous recommendations (Cormack, Newton, McGuigan, & Doyle, 2008) and research in rugby players (Oxendale, Twist, Daniels, & Highton, 2016). Flight times (the difference between take-off and landing time) were measured using two photoelectric parallel bars (Optojump™, Microgate, Bolzano, Italy) as previously described (Oxendale, Twist, Daniels, & Highton, 2016). During the DJ protocol, a 30cm box was placed in front of the Optojump™ bars and players were instructed to step on to the box and to keep both hands on their hips throughout testing. Players performed three jumps with

the maximum flight time (Twist et al., 2012) and minimal contact time used as an index of the maximal rate of force development and reactive strength index (RSI) (Twist & Eston, 2007) used for analysis. All players were familiar with jump procedures as part of the club's regular monitoring process with reliability for these measurements demonstrating a CV of 2.3% and 2.7% respectively during one week of familiarisation.

Self-reported Subjective Wellness

At 24h pre-match, 24h and 48h post-match, participants provided a rating of perceived sleep quality, fatigue, muscle soreness, mood and stress using a 1-5 Likert scale which has been adapted from others (McLean et al., 2010) and previously used with RL players (Twist et al., 2012). Higher values were indicative of a positive response to the question. Similar scales have been shown to possess strong reliability and validity (De Vries & Van Heck, 2003). Participants were familiar with this procedure as part of their habitual club monitoring processes and were instructed to complete the scales on their own to reduce influence from other players.

Statistical analysis

Match characteristics, subjective wellness, jump performance and cytokine data are presented as separate means (±SD) for both MC and PLB treatment conditions. Changes were analysed using a two-way repeated measure general linear model (GLM) where the within factors was time (48h pre-match, half-time, full-time and 48h post-match) and condition (MC and PLB). The tests of within subjects effects provided values for Mauchly's test for sphericity. If this was violated, then a Greenhouse-Geisser correction was used. The difference between means were tested at a significance level of $P<0.05$.

Results

Match characteristics

There were no significant differences in absolute distance covered (6334±1924 vs 6596±177 m, P=0.75), relative distance covered (72.6±4.8 vs 79.3±5.5 m.min^{-1}, P=0.009), total collisions (28±11 vs 29±13, P=0.89), high speed running (4457±1315 vs 4286±1532 m, P=0.78) and playing duration (67:10±19:7 vs 67:10±19:3 min, P=0.99, between the two matches. Seven players played the full 80 min in both matches, with a mean difference of 9 minutes between matches for the remaining 4 players.

Cytokine Responses

Data for interleukin -1, -2, and -4 were all below the known theoretical detection limits of the assays from the manufacturer and therefore these data are not reported.

Interleukin-6

There was no significant treatment by time interactions ($F_{1.43,\ 14.37}$ = 1.00, P=0.365) or any significant main effect of supplementation ($F_{1,\ 10,}$ = 2.14, P=0.173). There was a significant main effect of time for IL-6 concentrations (Figure 2A) following match-play ($F_{1.44,\ 14.46}$ = 15.71, P<0.001). IL-6 was significantly greater at full-time (2.58±1.57 pg·ml^{-1}) compared with 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}) 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^{-1}) although no significant difference was seen compared with half-time (1.49±0.89 pg·ml^{-1}; P=0.166: -0.30 to 2.47 pg·ml^{-1}).

Interleukin-8

There was no significant treatment by time interactions ($F_{3.00, 30.00,} = 0.79$, $P=0.510$) or any significant main effect of supplementation ($F_{1, 10,} = 0.51$, $P=0.493$). There was a significant main effect of time for IL-8 concentrations (Figure 2B) following match-play ($F_{3.00, 30.00} = 17.69$, $P<0.001$). IL-8 was significantly greater at full-time (3.68±1.38 pg·ml^{-1}) compared with 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}), 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 post-match (2.38±1.02 pg·ml^{-1}; $P=0.004$: 95% CI for differences = 0.41 to 2.19 pg·ml^{-1}).

Interleukin-10

There was no significant treatment by time interactions ($F_{1.32, 13.026,} = 0.01$, $P=0.827$) or any significant main effect of supplementation ($F_{1, 10,} = 0.08$, $P=0.777$). There was a significant main effect of time for IL-10 concentrations (Figure 2C) following match-play ($F_{1.40, 13.99} = 8.32$, $P=0.007$). IL-10 concentrations were greater at full-time (2.52±2.07 pg·ml^{-1}) compared 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}) 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 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}).

Self-reported Subjective Wellness

There was no significant difference between pre-match and 24h post-match sleep ($P=1.00$ and $P=0.86$), fatigue ($P=0.26$ and $P=0.33$), mood ($P=0.71$ and $P=0.92$) and stress ($P=0.71$ and $P=0.83$) in both PLB and MC groups respectively (Table 1). Similarly, there were no significant difference between pre-match and 48h post-match sleep ($P=0.40$ and $P=0.52$), fatigue ($P=0.27$ and $P=0.86$), mood ($P=0.80$ and $P=0.54$) and stress ($P=0.26$ and $P=0.14$). However, at 24h post-match there was a significant increase in players self-reported muscle 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 $m \cdot s^{-1}$) and MC (2.17±0.33 $m \cdot s^{-1}$) drop jump performance from 24h pre-match to 48h post-match
324 (PLB: 2.05±0.40 $m \cdot s^{-1}$ and MC: 2.06±0.41 $m \cdot s^{-1}$) although this was not statistically significant
325 (P=0.228 and P=0.893, respectively). Data are shown in Table 1.

Discussion

The aim of the present study was to investigate the effects of RL match-play on circulating inflammatory markers and to assess the efficacy of MC on markers of muscle soreness and recovery following rugby match-play. To this end, using flow cytometry, we measured concentrations of IL-6, IL-8 and IL-10 collected at 48h pre-match, half-time, full-time and 48h post-match from eleven male professional rugby players over two successive in-season RL matches. Following professional RL match-play we report small, yet significant, increases in IL-6, IL-8 and IL-10 at half-time and full-time compared with 48h pre-match along with small but significant reduction in CMJ and increases in soreness. However, we report for the first time in collision sport athletes, that supplementation with MC caused no improvements in reducing inflammatory markers, soreness, sleep or muscle function compared with a placebo. These data contradict the assumption that reductions in inflammation and improvements in recovery are possible following the ingestion of MC following rugby match-play.

To investigate the efficacy of MC it was important to assess the magnitude of inflammation, soreness and loss of muscle function that is caused from RL match-play. Previous research has shown increases in IL-6 concentration following match-play in professional RU players (Cunniffe et al., 2011; Cunniffe et al., 2010). We confirm and extend these findings by showing increases in IL-6, IL-8 and IL-10 concentrations following competitive RL match-play at half-time and full-time in RL players. Interestingly, although previous research has shown increases in IL-8 from other team sport athletes (Bell et al., 2016), this was performed in a controlled laboratory environment and following a simulated football exercise test, and as such, we show for the first time in academy RL players, significant elevations in IL-8 following RL match-play. Similarly, we provide novel data in RL players showing significant increases in the anti-inflammatory cytokine IL-10 post-match, which has previously only been shown following

laboratory-controlled resistance training and yet to be investigated in team sport athletes (Nieman et al., 2004).

Our findings confirm and extend recent work in academy soccer (Abbott, Brashill, Brett, & Clifford, 2019) and rugby (Kupusarevic, McShane, & Clifford, 2019) players who have reported no effects of MC on markers of muscle soreness and function although we demonstrate no effects of MC on IL-6, -8 and -10. Our findings are however in contrast with others who have shown beneficial effects of MC on reducing markers of inflammation and soreness following laboratory trials (Bell et al., 2016; Bowtell et al., 2011), and recreational marathon running (Howatson et al., 2010). A possible explanation as to why we found no effects of MC could be because the analgesic effects of polyphenols may only exert beneficial effects with exercise modalities that invoke EIMD without the additional IIMD. Indeed, research collected from full time professional RU players shows resting metabolic rates significantly increased by as much as ~1000 calories, the morning after rugby match-play, due to collisions and damage (Hudson, Cole, Morton, Stewart, & Close, 2020). With this in mind, supplementation of cherry juice may still be beneficial in less trained athletes and/or following much higher amounts of damage beyond what is seen in a typical competitive academy RL game. Another possible explanation for the disparity between the present study and previous literature on MC supplementation may be the fact that players in our study were not asked to restrict any polyphenol rich foods or beverages prior to, or during, the intervention period unlike previous research (Bell, Walshe, Davison, Stevenson, & Howatson, 2015; Bell et al., 2016; Bowtell et al., 2011). It is possible that the benefits of MC are only reported when replenishing deficient concentrations of polyphenols following a restriction period, rather than the added benefit of enhanced polyphenol concentrations. To this end, if players follow diets

rich in fruits and vegetables then the use of such supplements may not be needed, however there may still be a benefit for those players who do not have a polyphenol rich diet.

A limitation of the present study was that players' dietary polyphenol intakes were not measured and it is therefore possible that a wide range of undetected polyphenols were consumed by the players. Additionally, we did not batch test the cherry juice for polyphenol content before being administered to players although we did ensure it was stored according to the manufacturers instructions. It is therefore possible that the MC juice may have less polyphenols than stated on the label and/or players may have had a wide range of baseline polyphenols from their own habitual diets. Although this may be deemed a limitation, the present study was designed for maximal ecological validity (Close et al., 2019) and therefore attempted to replicate real-world consumption of this product. Furthermore, given the structure of the game weeks, only five days were given as a washout period. Whilst this may appear short, polyphenols have been shown to have short half-lives (1-2 h) (Kay, Mazza, & Holub, 2005) and as such there would have been no hang-over effects from the supplementation. Moreover, although it has been suggested in animal models that some polyphenol metabolites may accumulate and store in target tissues (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004) this has not been shown in humans. Future studies, however, may wish to consider a longer wash-out period and/or directly measuring plasma polyphenols. We also acknowledge that the potential increase in IL-6 as a result of match-play might be due to regulation of liver glycogenolysis and glucose output. However, all players consumed ~60g/h of carbohydrates during exercise, a feeding strategy that is known to suppress IL-6 concentrations (Hennigar & Pasiakos, 2017). Furthermore, examination of individual data demonstrates that the forward players who had the highest collisions presented with the highest circulating IL-6 concentrations compared with the backs who performed more extensive running further

supporting the hypothesis that the increase in IL-6 was damage mediated. Although others have assessed other biochemical indices related to inflammation such as myoglobin and high-sensitivity C-reactive protein following damaging simulation interventions (Naughton, Miller, & Slater, 2018), future work should now assess these inflammation indices following live match play. Although previous research has shown benefits of MC juice on recovery indices (soreness and muscle function) in the 24h-48h following exercise (Bell et al., 2016; Bowtell et al., 2011), unfortunately we were unable to collect blood at 24h post-match and may have missed this opportunity to show potential benefits of MC juice in this study. As such, future work should now assess a portfolio of inflammatory markers from pre-match to post-match and into the subsequent recovery days following live rugby match-play. Finally, although we show small changes in muscle function, future work should now assess this in a larger group of players to better understand the effects of recovery interventions on rugby players.

This study has several practical applications with immediate translation. Firstly, following RL-match-play that caused small changes in IL concentrations from baseline to post-match, consuming MC alongside habitual nutritional intakes showed no beneficial effects for reducing markers of inflammation, muscle soreness and functional performance. The data in this study therefore question the efficacy of such supplements in RL where budgets are often a key consideration, and players may want to consider adopt a food-first approach when consuming polyphenols in their diet. Secondly, given the data shows *medium* to *large* correlations between total collisions and self-reported muscle soreness scores and increases in IL's, practitioners working with athletes in sports that involves both exercise- and impact-induced muscle damage may consider the efficacy of simply asking players to report how they feel as the best proxy marker of damage and inflammation - although this clearly requires an honest assessment from 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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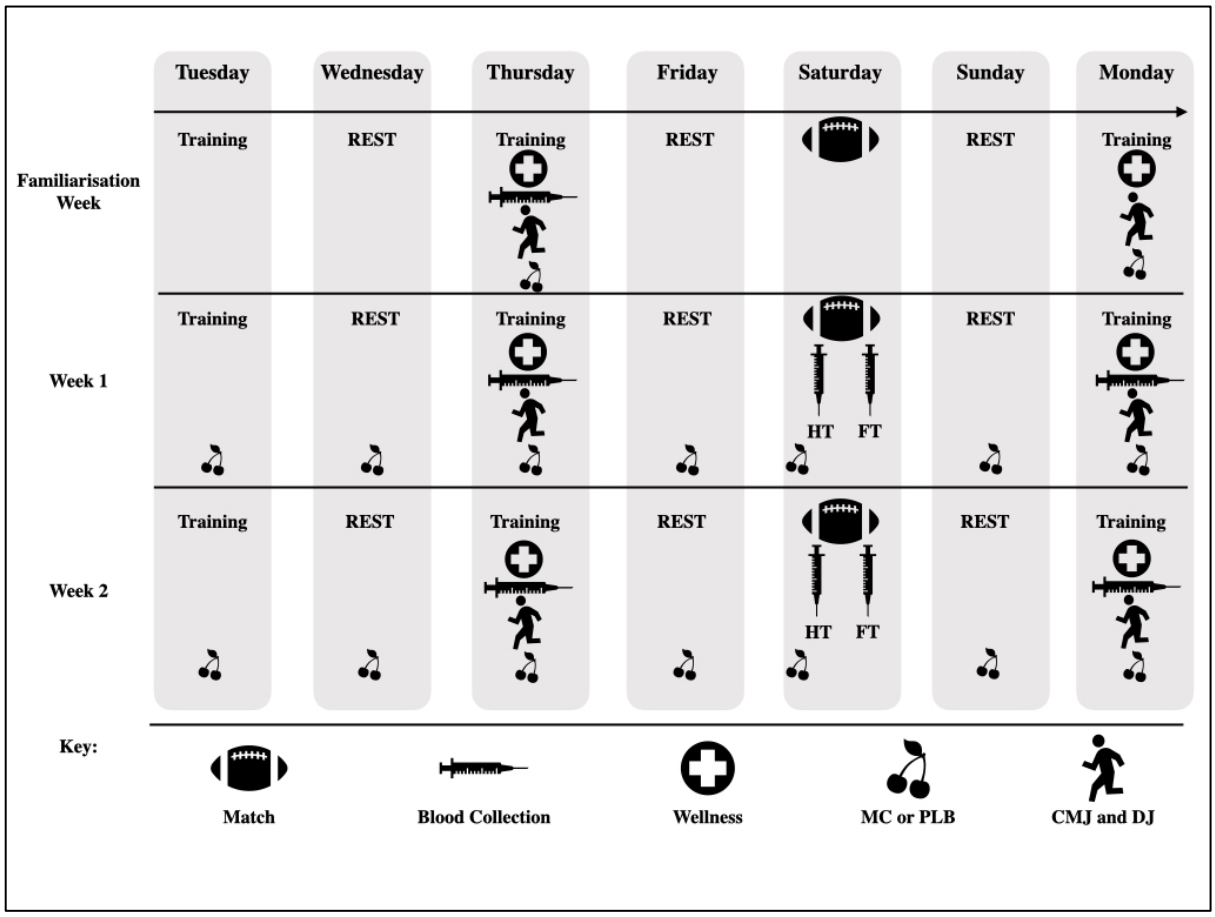
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Familiarisation Week
Training
REST
Training
REST
Training
REST
Training
Week 1
Training
REST
Training
REST
HT FT
REST
Training
Week 2
Training
REST
Training
REST
HT FT
REST
Training
Key:
Match
Blood Collection
Wellness
MC or PLB
CMJ and DJ

Figure 1.

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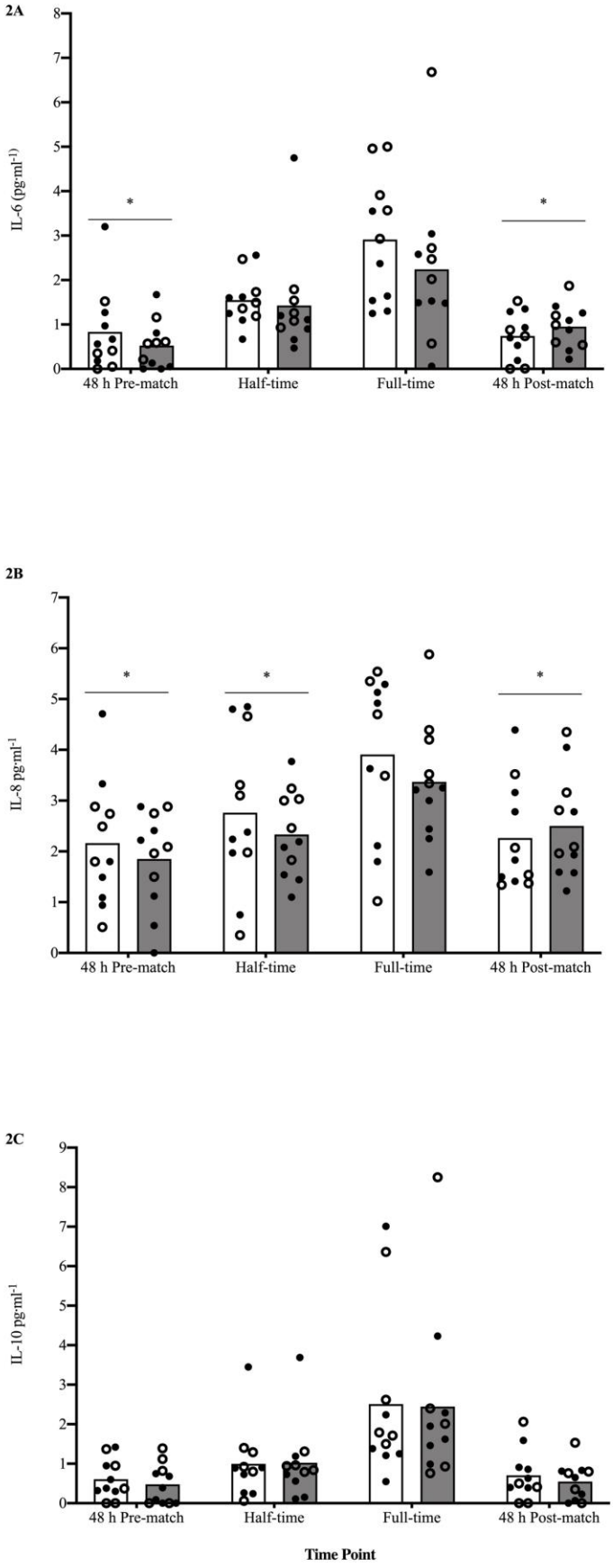

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441 Figure 2 A, B and C

Table 1.

	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 ($m \cdot s^{-1}$)	2.16 ± 0.34	2.17 ± 0.32	-	-	2.05 ± 0.40	2.06 ± 0.33

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

Figure 1. Schematic showing the overall study design. A familiarisation week is followed by a two-week intervention period which included two matches, blood collection, subjective wellness scores and jump performance. HT = Half-time, FT = Full-time, MC = Montmorency cherry juice, PLB = Placebo, CMJ = Countermovement Jump, DJ = Drop jump.

Figure 2. Interleukin-6 (2A), -8 (2B) and -10 (2C) concentration at 48h pre-match, half-time, full-time and 48h post-professional rugby league match-play. White bars represent placebo group, grey bars represent Montmorency cherry juice group. Open circles represent forward players, closed circles represent back players. *denotes significant difference between 48h pre-match, half-time and 48h post-match compared to full-time values ($P<0.05$).

Table 1. Differences in subjective markers at 24 h and 48 h post-match and CMJ and DJ performance 48 h post-match in comparison with baseline, Mean ± SD.

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