Effect of drinking rate on the retention of water or milk following exercise-induced dehydration.

Journal:	*International Journal of Sport Nutrition & Exercise Metabolism*
Manuscript ID	IJSNEM.2019-0176.R1
Manuscript Type:	Original Research
Keywords:	nutrition, rehydration, fluid balance, hypohydration

Accepted author manuscript version reprinted, by permission, from *International Journal of Sport Nutrition and Exercise Metabolism*, 2020, 30 (2): 128-138, https://doi.org/10.1123/ijsnem.2019-0176.

Introduction

Individuals typically do not consume enough fluid during exercise to counteract sweat losses, producing a post-exercise state of body water deficit (i.e. dehydration) (Garth & Burke, 2013). As a result, individuals are encouraged to drink fluid during recovery to reinstate total body water balance prior to recommencing physical activity (Evans et al., 2017; Sawka et al., 2007). However, rapidly consuming large volumes of hypotonic fluid has the potential to reduce plasma osmolality (POSM), resulting in increased urinary output (i.e. "fluid induced diuresis"), potentially delaying a return to euhydration (Mitchell et al., 1994; Robertson, 1974). Hence, there is considerable scientific interest in understanding factors that enhance fluid retention and assist with rehydration after exercise.

When consumed without food and matched for volume, nutrient dense beverages (e.g. milk and milk-based beverages) appear to promote greater fluid retention compared to water and carbohydrate-electrolyte solutions (Desbrow et al., 2014; Seery & Jakeman, 2016; Shirreffs et al., 2007; Watson et al., 2008). The effectiveness of milk as a rehydration solution has been attributed to a number of its constituents (i.e. sodium (Merson et al., 2008; Shirreffs & Maughan, 1998), carbohydrate (Osterberg et al., 2009), and protein (Hobson & James, 2015; James et al., 2014; James et al., 2012)), which are believed to delay gastric emptying and/or attenuate changes in P_{OSM}, reducing the degree of fluid induced diuresis (Calbet & MacLean, 1997; Clayton et al., 2014; Murray et al., 1999; Vist & Maughan, 1995).

Typically, post-exercise rehydration studies control drinking rate by prescribing fixed volumes of beverages within standardised time periods. In contrast, active individuals may consume fluids at different rates, which is likely to influence nutrient delivery and consequently, fluid retention. To date, only two studies have investigated the influence of drinking rate on fluid recovery (Jones et al., 2010; Kovacs et al., 2002). The initial investigation failed to detect differences in fluid retention when a carbohydrate-electrolyte beverage was consumed over 3 h (79±6%) compared to 5 h (82±5%) following exercise-induced dehydration (3.0% body mass (BM) loss). In contrast, Jones et al., (2010) reported significantly greater retention when water was consumed over a 4 h (75±12%) compared to a 1 h

Commented [LB1]: added

Commented [LB2]: revised

Commented [LB3]: added

(55±18%) drinking period following an exercise-induced 2.0% BM loss. The explanation for the equivocal findings may relate to the subtle differences in the drinking rates and/or the use of beverages with different nutrient profiles (and hence osmolalities). Furthermore, when consumed *ad libitum*, individuals typically ingest the largest volume of fluid within the first 30 min following exercise (Baguley et al., 2016). To date, the effect of drinking rate on the retention of fluid from beverages with contrasting nutrient profiles has not been systematically examined. In addition, no previous investigation has compared a conservative drink pattern to a rapid ingestion rate (e.g. large volumes consumed in ~30 min), which may reflect the actual behavior of individuals following exercise.

Therefore, the aim of the current study was to investigate the effect of rapid vs slower drinking rates on fluid retention using beverages with contrasting nutrient profiles (milk vs. water). It was hypothesized that the fluid retained from the consumption of a nutrient dense beverage would be unaffected by drinking rate; and that slower intake of a hypotonic beverage would enhance subsequent fluid retention.

Methods

Overview of study designs

This investigation was intended to systematically explore the effect of drinking rate on subsequent fluid recovery. The investigation was conducted in two parts, with the results from Part A used to inform the design of Part B. Part A explored the impact of drinking rates of different beverages (milk and water) on fluid retention. In Part B, further exploration of different drinking rates was performed. In addition, the trials were conducted in separate laboratories (Part A - Australia, Part B - Scotland). All participants were fully informed of the nature and possible risks of the investigations before providing written informed consent. The investigation was approved by the Griffith University and University of Stirling's Human Ethics Committees and the procedures were conducted in accordance with the principles outlined by the declaration of Helsinki.

Participant characteristics

In Part A thirteen healthy males volunteered to take part. However, one participant was unable to continue with the study after completing the first trial for reasons unrelated to the study (i.e. work commitments). Consequently, twelve male participants (age: 23.5±5.3 y; height: 179±6 cm; BM: 77.3±9.6 kg; maximal oxygen consumption ($VO2_{peak}$): 43.1±6.4 mL·kg^{-1}·h^{-1} (Mean±SD)) completed four experimental trials.

Commented [LB4]: revised

In Part B fourteen healthy participants volunteered to take part. However, one participant withdrew from the study due to external factors and one participant's data was excluded because they could not achieve the required level of dehydration. Consequently, twelve (9 males and 3 females) participants (age: 28.3±6.3 y; height: 176±11 cm; BM 74.0±10.2 kg; $VO2_{peak}$: 50.6±7.6mL·kg^{-1}·h^{-1}) completed four experimental trials.

Study designs

A schematic representation of the experimental protocols is displayed in Figure 1. Both parts utilised a repeated-measures experimental design, involving 4 experimental trials; each separated by a minimum of 5 d. For all trials, participants lost ~2.0% BM through intermittent cycle exercise before cooling down and beginning a rehydration period in which different treatment interventions were examined (Part A, water or milk ingested over 30 or 90 min; Part B, water ingested over 15, 45, or 90 min with either the 15 or 45 min trial repeated). An incomplete Latin square design was used to counterbalance the order of treatments.

Preliminary requirements

Participants undertook an incremental test to exhaustion on a cycle ergometer. The protocol began at 100 W, and increased in 50 W increments every 2.5 min until volitional exhaustion, with participant's breath sampled continuously via a calibrated gas analysis system (Part A: Medgraphics Ultima, USA; Part B: Servomex Group Ltd, United Kingdom). The test was used to determine $VO2_{peak}$ and maximum heart rate (HR_{max}), with these values used to guide the prescription of exercise intensity for the experimental trials.

Participants were instructed to abstain from caffeine (12 h), alcohol (24 h) and moderate to strenuous exercise (12 h) before all trials. During the 24 h period preceding the first trial, individuals completed a food and beverage diary. They were also instructed to drink 500 mL of water at least 2 h before arrival at the laboratory (to assist with hydration) and abstain from all food and fluid (excluding water) after 21:00 h. Individuals were then instructed to repeat these behaviors prior to all subsequent experimental trials.

Experimental procedures

Participants arrived at the laboratory between 05:30 and 08:00 h and verbally acknowledged compliance to the pre-experimental conditions. A urine sample was taken for determination of hydration status (Part A: urine specific gravity (U_{SG}) (Palette Digital Refractometer, ATAGO, USA) and Part B: urine osmolality (U_{OSM}) (Löser Osmometer, Camlab, UK). If participants recorded a U_{SG} ≥1.024 (Sommerfield et al., 2016) or U_{OSM} of >700 mOsm·kg^{-1} (Sawka et al., 2007) they were considered hypohydrated. In Part A, hypohydrated participants were required to consume 600 mL of plain water over 5 min, before providing a second urine sample 30-60 min later. If this urine sample achieved the thresholds for euhydration the participants continued with the trial (this practice was then replicated on all subsequent trials). If the threshold value was not reached within the 60 min period the trial was rescheduled. Participants then rested in a seated position for 5 min prior to venepuncture of a forearm vein. Following this initial blood collection, participants were provided with a standardised breakfast in a quantity relative to BM (20 kJ·kg-1 and 1 g CHO·kg-1) that consisted of raisin toast, strawberry jam and fruit juice (200 mL), before completing a questionnaire on GI subjective symptoms, voiding their bladder and obtaining a baseline nude BM measurement (Part A: A&D Company Ltd, Tokyo, Japan, to nearest 20 g; Part B: Marsden, Rotherham, United Kingdom, to nearest 10 g).

Commented [LB5]: revised

Exercise-induced dehydration

After completing a brief standardised warm up, participants began cycling in a warm environment (Part A: 25.2±0.8 ºC and 84±11% RH, Part B: 26.4±0.7ºC and 38±5% RH). Individuals commenced exercise at a workload corresponding to ~65% of HR_{max}. Intensity was recorded by an investigator and replicated on all subsequent trials. Following 50 min of cycling, participants BM was measured. A BM loss of <1.8% from baseline required participants to continue exercising in 10 min bouts until a BM loss ≥1.8% was achieved. Following exercise, dehydrated participants rested in a seated position for 15 min prior to having a cool shower. Afterwards, participants dried themselves thoroughly, before a cannula was inserted into a forearm vein and a blood sample obtained. Participants then emptied their bladder and provided a urine sample before a final nude BM measure was recorded to determine total fluid loss (30 min post-exercise).

Post-exercise fluid replacement

In Part A, water or low fat cow's milk (Maleny Dairies, Queensland, Australia; 210 kJ Energy, 5.3 g CHO, 4.0 g Protein, 1.4 g Fat, 48 mg $Na^{+}\cdot 100\ mL^{-1}$) were ingested in a quantity equal to 100% of the volume of sweat lost during exercise. The fluid volume was ingested in six equal aliquots spread evenly over either a 30 or 90 min period, resulting in the beverage treatments: Water 30 min (W30), Water 90 min (W90), Milk 30 min (M30), and Milk 90 min (M90). Participants were instructed to consume each aliquot at an even pace over 5 or 15 min according to the relevant drinking rate. In Part B, water in a quantity equal to 100% of the volume of sweat lost during exercise was ingested. The volume was provided in three aliquots spaced evenly over either a 15, 45 or 90 min drinking period, resulting in the following beverage treatments: Water 15 min (DR15); Water 45 min (DR45); and Water 90 min (DR90). To assess within individual variation, participants in part B repeated either the DR15 or DR45 trial. To assess inter-site variation W90 (Part A) was compared to DR90 (Part B).

A 3 h rehydration monitoring period (from the commencement of drinking) was applied to all trials. Observations were made every hour and included measures of nude BM, urine and plasma measures of hydration status. In addition, subjective measures of bloatedness, fullness and thirst were recorded. All measurements were obtained while participants remained seated.

Body mass and fluid retention

BM change (estimate of fluid loss) was calculated by subtracting the post-exercise BM (post-void) from the pre-exercise BM. Net BM change was calculated by subtracting the 3 h BM measurement from the pre-exercise BM. Percent fluid retention at the conclusion of the observation period was calculated by the following equation:

$$\text{Fluid Retained (\%)} = 100 \text{ x } \frac{\text{(Total beverage ingested (g)} - \text{Total urine output (g))}}{\text{Total beverage ingested (g)}}$$

Urine and blood collection, storage and analysis

Additional urine sampling was performed at pre-exercise, post-exercise (immediately pre-drinking), immediately post-drinking and then at 120 min and 180 min after the start of drinking. At each of these urine collection points, participants completely voided their bladder into an empty container for subsequent measures of urine volume. Total urine loss was calculated from the accumulated urine output in the period from the commencement of drinking until the end of the observation period. A sample of urine was retained for determination of urine osmolality. Blood sampling was performed at pre-exercise, post-exercise (immediately pre-drinking), immediately post-drinking and then at 120 min and 180 min after the start of drinking for the determination of P_{OSM}. Participants remained seated prior to a 5 mL blood sample being drawn from an antecubital vein. All samples were collected into EDTA pre-treated vacutainers and centrifuged at room temperature for 10 min at ~1350×g. Plasma was analysed in duplicate on a calibrated freezing-point depression osmometer (Part A: Osmomat 030, Germany and Part B: Löser osmometer, Camlab, UK). Cannulas were kept patent by flushing sterile saline (2 mL of 0.9% NaCl; Becton Dickson, NJ, USA) on completion of each sample (with an equivalent volume of blood initially discarded before collection of subsequent samples).

Commented [LB6]: revised

Subjective measures

Subjective ratings of bloatedness, fullness and thirst were recorded on separate 100 mm visual analog scales, with 0 mm representing ‘not at all’ and 100 mm representing ‘extremely’. Scales were administered via a computerized modifiable software program (Marsh-Richard et al., 2009).

Statistical analyses

Statistical analyses were performed using SPSS Statistics for Windows, Version 22 (SPSS Inc., IBM, Chicago, IL). All measures were examined for normality and sphericity using the Shapiro-Wilk test (p>0.05) and Mauchly's test (p>0.05), respectively. Where assumptions of sphericity in repeated-measures analyses were violated, the Greenhouse-Geisser statistic was applied. One-way repeated-measures analysis of variance (ANOVA) were performed to verify that pre-trial conditions and exercise-induced fluid loss did not differ across trials. For Part A, a three-factor (i.e. Beverage x Rate x Time) repeated-measures ANOVA was used to compare main outcomes; two-factor (i.e. Beverage x Rate) repeated-measures ANOVA were conducted to compare total fluid retention and net BM changes across treatments. Pairwise comparison (Bonferroni) were performed where significant main effects were present. For Part B, two-factor (i.e. Rate x Time) repeated-measures ANOVA were used to compare outcomes between the different beverage ingestion rates. Paired *t*-tests or Wilcoxon tests were used where appropriate to conduct post-hoc comparisons on significant interaction effects. An adjusted-alpha (i.e. p=0.05 divided by the number of tests performed) was used to account for multiple comparisons. The test-retest reliability was calculated as a coefficient of variation (CV%) using the traditional method and any difference in responses between sites was assessed using an unpaired *t*-test. Statistical significance was accepted at p<0.05. All data are reported as Mean±SD, unless stated as Mean±SEM.

Results

Standardisation procedures

All participants reported compliance with the standardisation procedures in the 24 h prior to arriving at the laboratory. In Part A, two participants were administered water (600 mL) due to a pre-exercise USG ≥1.024 on Trial 1; this practice was repeated on all subsequent trials to ensure consistency. The remaining participants had a U_{SG}<1.024 at the commencement of each trial. Exercise duration and pre-exercise values for BM, U_{SG} and P_{OSM} were similar across all treatments, and did not differ significantly by trial order (p>0.05). Exercise-induced BM loss differed significantly (p<0.01) by trial order (Trial 1:

Commented [LB7]: revised

1.54±0.26 kg; Trial 2: 1.44±0.28 kg; Trial 3: 1.41±0.31 kg; Trial 4: 1.38±0.32 kg); however, counterbalancing ensured that mass loss was similar across treatment conditions (Table 1).

In Part B, exercise duration and pre-exercise values for BM, U_{OSM}, $P_{OSM,}$ and exercise induced BM loss were similar across all treatments (Table 1); and did not differ significantly by trial order (p>0.05).

Urine output and fluid retention

In Part A, cumulative urine output was greater with water than with milk at 120 min (398±190 vs. 139±44 g) and 180 min (592±248 vs. 224±70 g) after the start of drinking (p<0.01; Figure 2A). A significant effect of beverage was observed on fluid retention (W30: 56.5±16.1%; W90: 59.7±19.9%; M30: 82.9±6%; M90: 84.9±7%) with the proportion of ingested fluid retained lower with water than milk (58.1±15.6 vs. 83.9±6.1%, p<0.01). No other significant differences were observed in either analysis.

In Part B, a similar cumulative urine output response was observed when water was ingested at DR15, DR45 and DR90 rates. Three hours after the start of the drinking period, cumulative urine output was lower for the DR90 trial (602±183 g) compared to the DR45 (750±373 g) and DR15 (754±230 g) trials, but this did not reach statistical significance (p>0.05). The mean difference (95% CI) between DR15 and DR90 was 7.4(1.2-13.6)%, equivalent to 152 (43-260) mL (Figure 2B). Fluid retention was significantly higher (p<0.05) on the DR90 trial (57.1±12.9%) compared to the DR15 trial (49.7±11.0%), but these trials were not different (p>0.05) to DR45 (51.6±19.8%).

Net fluid balance

In Part A, all experimental trials concluded with participants in a state of negative net fluid balance 180 min after the ingestion period started (Part A: W30: -0.68±0.31 L; W90: -0.61±0.25 L; M30: -0.27±0.07 L, M90: -0.28±0.08 L; Figure 3A). Post hoc comparisons revealed that milk ingestion led to less negative fluid balance compared to water at 120 min (-0.40±0.19 vs. -0.14±0.04 L, p=0.001) and 180 min (-0.64±0.27 vs. -0.28±0.07 L, p<0.001) after drinking started. Fluid balance was also less negative immediately post-drinking for the 30 min compared to the 90 min drinking trials (-0.14±0.08 vs. 0.04±0.03 L, $p<0.001$), since participants had less time to produce urine on these trials.

In Part B, all experimental trials concluded with participants in a state of negative net fluid balance (DR15: -0.75±0.23 L; DR45: -0.75±0.37 L; DR90: -0.60±0.18 L; Figure 3B). No differences were observed between trials.

Plasma osmolality

In Part A, the consumption of water decreased P_{OSM} compared to milk at the cessation of drinking (291±4 vs. 298±5 $mOsm \cdot kg^{-1}$, $p<0.001$), but this effect was not evident by 180 min (Water: 290±2 $mOsm \cdot kg^{-1}$; Milk: 293±4 $mOsm \cdot kg^{-1}$, $p=0.033$). P_{OSM} did not differ significantly as a result of the fluid ingestion rate at any point ($p>0.05$).

In Part B, a drinking rate by time interaction was not evident for P_{OSM}. Plasma osmolality 180 minutes after start of drink ingestion did not differ significantly as a result of the fluid ingestion rate (DR15: 304±2 $mOsm \cdot kg^{-1}$; DR45: 302±3 $mOsm \cdot kg^{-1}$; DR90: 303±5 $mOsm \cdot kg^{-1}$, $p>0.05$).

Subjective measures

In Part A, analysis for bloatedness, fullness, and thirst ratings identified a significant effect of time on each variable ($p<0.01$). A significant effect of beverage was also observed for fullness ($p=0.022$). For bloatedness and fullness there were significant time x beverage interaction effects (bloatedness: $p=0.014$; fullness $p<0.01$). Post hoc comparisons revealed that the 30 min drinking protocol increased feelings of bloatedness ($p<0.01$) and decreased feelings of thirst ($p<0.01$) immediately after drinking compared to the 90 min protocol. The consumption of milk increased feelings of fullness immediately after drinking ($p<0.01$) and at 120 min ($p<0.01$), compared to the consumption of water. No other significant differences were observed.

In Part B, perceived bloatedness and fullness were significantly higher immediately after drinking on the DR15 trials compared to the DR45 and DR90 drinking rates ($p<0.01$), but were not different at subsequent time points up to 180 min. No other significant differences were observed at any other time point (Figure 4).

Reliability and inter-lab repeatability

The CV% of test re-test reliability between duplicate trials on DR15 and DR45 ingestion rates (Part B) was 17%. Data from repeated trials was not significantly different (Table 2). The fluid retention on 90 min water rate trials (Part A: W90 and Part B: DR90) was not significantly different between testing sites (W90: 59.7±19.9%; DR90: 57.1±12.9%, p=0.73).

Discussion

This two-part study explored the effect of drinking rate on fluid retention of different beverages following exercise-induced dehydration. In keeping with our hypothesis, Part A observed that drinking milk resulted in greater fluid retention than water during a 3 h recovery period. This effect was not influenced by drinking rate (i.e. 30 vs. 90 min). Consequently, Part B assessed retention of water consumed over alternative drinking rates (i.e. 15 vs. 45 vs. 90 min), as well as the day-to-day variation in post-exercise fluid retention. Part B, indicated that the 15 min drinking protocol led to a significant reduction in fluid retention compared to the 90 min drinking protocol. However, the magnitude of the effect was within the CV% of the repeated trials (17%). Thus, findings from this study suggest the influence of drinking rate on post-exercise fluid recovery is small and that the nutrient composition of a beverage has a more pronounced impact on fluid retention than the beverage ingestion rate.

Only two studies have previously investigated the influence of drinking rate on fluid recovery (Jones et al., 2010; Kovacs et al., 2002). Results from these studies are contradictory, with only one investigation (Jones et al., 2010) identifying an influence of drinking rate on fluid retention. Jones et al., (2010) had participants ingest water at 1.61 $L \cdot h^{-1}$ vs. 0.40 $L \cdot h^{-1}$. Kovacs et al., (2002) had participants ingest a carbohydrate-electrolyte sports drink at a maximum rate of 1.32 $L \cdot h^{-1}$ in the first hour, with an average rate over 3 hours of 0.77 $L \cdot h^{-1}$ and compared this to fluid retention with a slow drinking rate of 0.53 $L \cdot h^{-1}$ over 5 h. These fluid consumption patterns are slower than those observed when individuals drink *ad* *libitum* post-exercise (e.g. with drinking rates in the first 30 min exceeding 2 L·h^{-1}, Baguley et al., 2016). The present study attempted to assess drinking rates across a broader range (5.84 L·h^{-1} (1.46 L in 15 min) to 0.95 L·h^{-1} (1.42 L in 90 min)) to elucidate effects on fluid retention. We observed little impact of contrasting drinking rates on fluid retention with water. In fact, the only difference noted in Part B (DR15 vs. DR90) was within the CV% of the method.

The current findings suggest that the nutrient profile of different beverages have a greater impact on fluid retention than ingestion rate. Indeed, when consumed exclusively and matched for volume, milk beverages promote greater fluid retention than water at rest (Maughan et al., 2016) and during the post-exercise period (Seery & Jakeman, 2016; Shirreffs et al., 2007; Watson et al., 2008). These effects may be mediated by the composition of milk (whey/casein protein), electrolyte content and insulin response to carbohydrate/protein delivery. In addition, the electrolyte content of milk (Shirreffs et al., 2007) and insulin mediated impacts on renal water transport (Magaldi et al., 1994) both have the potential to enhance fluid retention.

In a practical sense post-exercise, athletes typically consume fluids ad libitum and the beverage choice, drinking rate and total volume consumed are determined by many factors, including prior exercise (intensity, duration and type), environmental conditions, thirst, palatability, gastrointestinal tolerance, drink availability, exercise commitments and other, unrelated dietary goals (Minehan et al., 2002; Passe et al., 2000). The rapid consumption of large volumes of milk or water during the immediate post-exercise period may be poorly tolerated by some individuals. However, the range of subjective responses to our most rapid drinking rates highlights individual differences in tolerance. For those who drink beverages rapidly in the immediate post-exercise period, the rates examined in the present study do not appear to compromise fluid retention when a fixed volume is provided and may facilitate the consumption of other fluids after completing a "prescribed" volume of a beverage. Conversely, it is not known whether rapid beverage ingestion compromises subsequent voluntary fluid consumption in ad libitum drinking scenarios due to an action on thirst response mediated via the gut-brain axis (Zimmerman et al., 2019).

Commented [LB8]: Revised

Several methodological limitations require acknowledgement. Firstly, this study did not employ a direct measure of gastric emptying. Hence, while greater fluid retention was achieved during Milk trials, the distribution of the retained fluid (e.g. within the GI tract (as potentially indicated by higher "fullness" ratings) as opposed to vascular space) and therefore physiological relevance of this fluid retention remains unknown. In addition, the recovery period for this study (3 h from the start of drinking) was shorter than previous work in this area (typically ≥4 h), which may have resulted in small volumes of uncaptured fluid losses in response to the differences in drinking strategy. The decision to shorten the duration of the observation was based on a number of factors; (1) the relatively small volumes of urine seen beyond 90 min following the cessation of drinking in our previous study (Desbrow et al 2014), (2) the smaller volume of fluid being ingested (100% vs. 150% fluid replacement), (3) the practical relevance of 4 h observation, given that many individuals are likely to eat/drink within this period of time and (4) our previous study (Maughan et al., 2016) demonstrated the pattern of response in cumulative urine output and calculated hydration index to ingested drinks was observed to be similar at 2 h post-drinking and 4 h post-drinking.

Commented [LB9]: Added

Conclusion

This study suggests that drinking more rapidly does not compromise post-exercise fluid retention following moderate intensity exercise in recreationally active participants. This observation was consistent between different testing sites and across different drinking rates. Laboratory informed findings suggest that beverage composition is more influential than fluid ingestion rate in determining post-exercise fluid retention.

Acknowledgments

The authors declare no conflicts of interest.

B. Desbrow and S. D. R. Galloway conceived the project. B. Desbrow, S. D. R. Galloway, G. R. Cox, C. Irwin, N. Rodriguez-Sanchez, D. McCartney, P. Rodriguez-Giustiniani and L. Sayer developed the overall research plan. L. Sayer, D. McCartney, C. Irwin, N. Rodriguez-Sanchez and P. Rodriguez-

Giustiniani conducted the research and analysed the samples. B. Desbrow, S. D. R. Galloway, C. Irwin, N. Rodriguez-Sanchez, D. McCartney and L. Sayer performed the statistical analysis. B. Desbrow, S. D. R. Galloway, G. R. Cox, C. Irwin, N. Rodriguez-Sanchez, D. McCartney, P. Rodriguez-Giustiniani and L. Sayer wrote the paper. All the authors approved the final version of the paper.

References

Baguley, B. J., Zilujko, J., Leveritt, M. D., Desbrow, B., & Irwin, C. (2016). The effect of ad libitum consumption of a milk-based liquid meal supplement vs. a traditional sports drink on fluid balance after exercise. *International Journal of Sport Nutrition and Exercise Metabolism, 26*(4), 347-355.

Baker, L. B., & Jeukendrup, A. E. (2014). Optimal composition of fluid-replacement beverages. *Comprehensive Physiology*. *4*(2), 575-620.

Borg, G. A. (1973). Perceived exertion: a note on "history" and methods. *Medicine and Science in Sports, 5*(2), 90-93.

Calbet, J., & MacLean, D. (1997). Role of caloric content on gastric emptying in humans. *The Journal of Physiology, 498*(2), 553-559.

Clayton, D. J., Evans, G. H., & James, L. J. (2014). Effect of drink carbohydrate content on postexercise gastric emptying, rehydration, and the calculation of net fluid balance. *International Journal of Sport Nutrition and Exercise Metabolism*, *24*(1), 79-89.

Desbrow, B., Jansen, S., Barrett, A., Leveritt, M. D., & Irwin, C. (2014). Comparing the rehydration potential of different milk-based drinks to a carbohydrate–electrolyte beverage. *Applied Physiology, Nutrition, and Metabolism, 39*(12), 1366-1372.

Evans, G. H., James, L. J., Shirreffs, S. M., & Maughan, R. J. (2017). Optimizing the restoration and maintenance of fluid balance after exercise-induced dehydration. *Journal of Applied Physiology*, *122*(4), 945-951.

Evans, G. H., Shirreffs, S. M., & Maughan, R. J. (2009). Postexercise rehydration in man: the effects of carbohydrate content and osmolality of drinks ingested ad libitum. *Applied Physiology, Nutrition,* *and Metabolism, 34*(4), 785-793.

Garth, A. K., & Burke, L. M. (2013). What do athletes drink during competitive sporting activities? *Sports Medicine, 43*(7), 539-564.

Goulet, E. D., Lamontagne-Lacasse, M., Gigou, P.-Y., Kenefick, R. W., Ely, B. R., & Cheuvront, S. (2010). Pre-exercise Hypohydration Effects On Jumping Ability And Muscle Strength, Endurance And Anaerobic Capacity: A Meta-analysis: 1681. *Medicine & Science in Sports & Exercise, 42*(5), 362.

Hobson, R., & James, L. (2015). The addition of whey protein to a carbohydrate–electrolyte drink does not influence post-exercise rehydration. *Journal of Sports Sciences, 33*(1), 77-84.

James, L. J., Gingell, R., & Evans, G. H. (2012). Whey protein addition to a carbohydrate-electrolyte rehydration solution ingested after exercise in the heat. *Journal of Athletic Training, 47*(1), 61-66.

James, L. J., Mattin, L., Aldiss, P., Adebishi, R., & Hobson, R. M. (2014). Effect of whey protein isolate on rehydration after exercise. *Amino Acids, 46*(5), 1217-1224.

Jones, E. J., Graham, J., Newcomb, T., & Frischman, N. (2010). Effects of Bolus vs. Metered Rehydration Rates on Fluid Retention and Hydration Efficiency using 150% Fluid Replacement: 2290. *Medicine & Science in Sports & Exercise, 42*(5), 575.

Kovacs, E. M., Schmahl, R. M., Senden, J. M., & Brouns, F. (2002). Effect of high and low rates of fluid intake on post-exercise rehydration. *International Journal of Sport Nutrition and Exercise Metabolism, 12*(1), 14-23.

Magaldi, A., Cesar, K., & Yano, Y. (1994). Effect of insulin on water and urea transport in the inner medullary collecting duct. *American Journal of Physiology-Renal Physiology, 266*(3), F394-F399.

Marsh-Richard, D. M., Hatzis, E. S., Mathias, C. W., Venditti, N., & Dougherty, D. M. (2009). Adaptive Visual Analog Scales (AVAS): a modifiable software program for the creation, administration, and scoring of visual analog scales. *Behavior Research Methods, 41*(1), 99-106.

Maughan, R. J., Watson, P., Cordery, P. A., Walsh, N. P., Oliver, S. J., Dolci, A., Galloway, S. D. (2016). A randomized trial to assess the potential of different beverages to affect hydration status:

development of a beverage hydration index. *The American Journal of Clinical Nutrition, 103*(3), 717-723.

Merson, S. J., Maughan, R. J., & Shirreffs, S. M. (2008). Rehydration with drinks differing in sodium concentration and recovery from moderate exercise-induced hypohydration in man. *European Journal of Applied Physiology, 103*(5), 585.

Minehan, M. R., Riley, M. D., & Burke, L. M. (2002). Effect of flavor and awareness of kilojoule content of drinks on preference and fluid balance in team sports. *International Journal of Sport Nutrition and Exercise Metabolism, 12*(1), 81-92.

Mitchell, J. B., Grandjean, P. W., Pizza, F. X., Starling, R. D., & Holtz, R. W. (1994). The effect of volume ingested on rehydration and gastric emptying following exercise-induced dehydration. *Medicine and Science in Sports and Exercise, 26*(9), 1135-1143.

Murray, R., Bartoli, W., Stofan, J., Horn, M., & Eddy, D. (1999). A comparison of the gastric emptying characteristics of selected sports drinks. *International Journal of Sport Nutrition, 9*(3), 263-274.

Osterberg, K. L., Pallardy, S. E., Johnson, R. J., & Horswill, C. A. (2009). Carbohydrate exerts a mild influence on fluid retention following exercise-induced dehydration. *Journal of Applied Physiology, 108*(2), 245-250.

Passe, D., Horn, M., & Murray, R. (2000). Impact of beverage acceptability on fluid intake during exercise. *Appetite, 35*(3), 219-229.

Robertson, G. L. (1974). Vasopressin in osmotic regulation in man. *Annual Review of Medicine, 25*(1), 315-322.

Savoie, F.A., Kenefick, R. W., Ely, B. R., Cheuvront, S. N., & Goulet, E. D. (2015). Effect of hypohydration on muscle endurance, strength, anaerobic power and capacity and vertical jumping ability: a meta-analysis. *Sports Medicine, 45*(8), 1207-1227.

Sawka, M. N., Burke, L. M., Eichner, E. R., Maughan, R. J., Montain, S. J., & Stachenfeld, N. S. (2007). American College of Sports Medicine position stand. Exercise and fluid replacement. *Medicine and Science in Sports and Exercise, 39*(2), 377-390.

Seery, S., & Jakeman, P. (2016). A metered intake of milk following exercise and thermal dehydration restores whole-body net fluid balance better than a carbohydrate–electrolyte solution or water in healthy young men. *British Journal of Nutrition, 116*(6), 1013-1021.

Shirreffs, S. M., & Maughan, R. J. (1998). Volume repletion after exercise-induced volume depletion in humans: replacement of water and sodium losses. *American Journal of Physiology-Renal Physiology, 274*(5), F868-F875.

Shirreffs, S. M., Watson, P., & Maughan, R. J. (2007). Milk as an effective post-exercise rehydration drink. *British Journal of Nutrition, 98*(1), 173-180.

Sommerfield, L. M., McAnulty, S. R., McBride, J. M., Zwetsloot, J. J., Austin, M. D., Mehlhorn, J. D., Utter, A. C. (2016). Validity of Urine specific gravity when compared to plasma osmolality as a measure of hydration status in male and female NCAA collegiate athletes. *Journal of strength and conditioning research/National Strength & Conditioning Association, 30*(8), 2219.

Vist, G. E., & Maughan, R. J. (1995). The effect of osmolality and carbohydrate content on the rate of gastric emptying of liquids in man. *The Journal of Physiology, 486*(2), 523-531.

Watson, P., Love, T. D., Maughan, R. J., & Shirreffs, S. M. (2008). A comparison of the effects of milk and a carbohydrate-electrolyte drink on the restoration of fluid balance and exercise capacity in a hot, humid environment. *European Journal of Applied Physiology, 104*(4), 633-642.

Zimmerman, C. A., Huey, E. L., Ahn, J. S., Beutler, L. R., Tan, C. L., Kosar, S., Madisen, L. (2019). A gut-to-brain signal of fluid osmolarity controls thirst satiation. *Nature*, *568*(7750), 98-102

Figure legends and footnotes

Figure 1. Schematic of experimental protocol investigating the effect of drinking rate on fluid retention following exercise.

Figure 2. Cumulative urine output before and after the test drink ingestion equal to the volume of sweat lost during exercise. A = Part A (Water or Milk ingested over 30 or 90 min, Water 30 (W30); Water 90 (W90); Milk 30 (M30); and Milk 90 (M90)), B = Part B (Water ingested over 15 (DR15), 45 (DR45) or 90 (DR90) mins). *a*, milk significantly different to water. Values are Mean±SD.

Figure 3. Net fluid balance responses before and after the test drink ingestion equal to the volume of sweat lost during exercise. A = Part A (Water or Milk ingested over 30 or 90 min, Water 30 (W30); Water 90 (W90); Milk 30 (M30); and Milk 90 (M90)), B = Part B (Water ingested over 15 (DR15), 45 (DR45) or 90 (DR90) mins). *a*, milk significantly different to water; *b*, rapid drinking significantly different to metered drinking. Values are Mean±SD.

Figure 4. Subjective gastrointestinal ratings of bloatedness, fullness and thirst before and after test drink ingestion equal to the volume of sweat lost during exercise. Part A = Panels A, B and C and Part B = Panels D, E and F. *a*, milk significantly different to water; *b*, rapid drinking significantly different to metered drinking; *c*, fast ingestion rate significantly different to slow ingestion rate. Values are Mean±SEM, where 0 represents 'not at all' and 100 represents 'extremely much' for each subjective feeling.

2

409 Table 1. Pre-trial conditions and impact of exercise-induced dehydration

Part A	**W30**	**W90**	**M30**	**M90**	***p*-value**
Pre-Ex U_{SG}	1.015±0.006	1.015±0.007	1.013±0.005	1.014±0.005	0.35
Pre-Ex P_{OSM} (mOsm·kg^{-1})	290±4	292±5	290±6	289±5	0.67
Pre-Ex BM (kg)	77.10±9.67	77.27±9.78	76.77±9.73	76.57±9.52	0.28
Ex Duration (min)	70±14	70±13	70±13	70±12	0.86
BM Loss (kg)	1.46±0.28	1.42±0.30	1.43±0.32	1.46±0.29	0.79
BM Loss (%)	1.9±0.3	1.9±0.4	1.9±0.4	1.9±0.3	0.82
Part B	**DR15**	**DR45**	**DR90**		***p*-value**
Pre-Ex U_{OSM}	477±218	474±178	443±185		0.76
Pre-Ex P_{OSM} (mOsm·kg^{-1})	303±5	302±3	302± 5		0.36
Pre-Ex BM (kg)	71.60±9.90	71.54± 10.15	71.31± 10.08		0.39
Ex Duration (min)	79±12	81±13	80± 11		0.62
BM Loss (kg)	1.46±0.35	1.51± 0.33	1.45±0.32		0.30
BM Loss (%)	2.0± 0.4	2.1±0.2	2.0±0.3		0.61

410 BM: Body mass; Ex: Exercise; P_{OSM}: Plasma osmolality; U_{SG}: Urine specific gravity; U_{OSM}: Urine osmolality.
411 Values are Mean±SD.

413 Table 2. Test-retest trial data (Part B: pooled from DR15 and DR45)

	Initial Trial	Repeat Trial	*p-value*
Pre-Trial Conditions			
Pre-Ex U_{OSM}	483±197	479±197	0.30
Pre-Ex P_{OSM} (mOsm·kg^{-1})	307±5	307±7	0.81
Pre-Ex BM (kg)	72.56±11.10	72.38±10.94	0.30
Ex Duration (min)	80.0±13.5	80.8±13.1	0.34
BM Loss (kg)	1.43±0.32	1.39±0.39	0.62
Fluid Retention Data			
Cumulative urine output (g)	792± 280	704±175	0.07
U_{OSM} 180 min after drinking started (mOsm·kg^{-1})	297±75	281±127	0.69
P_{OSM} 180 min after drinking started (mOsm·kg^{-1})	303±4	302±4	0.38
Fluid retention (%)	52.8±7.0	55.0±7.5	0.21

414 Values are mean±SD.

415

416

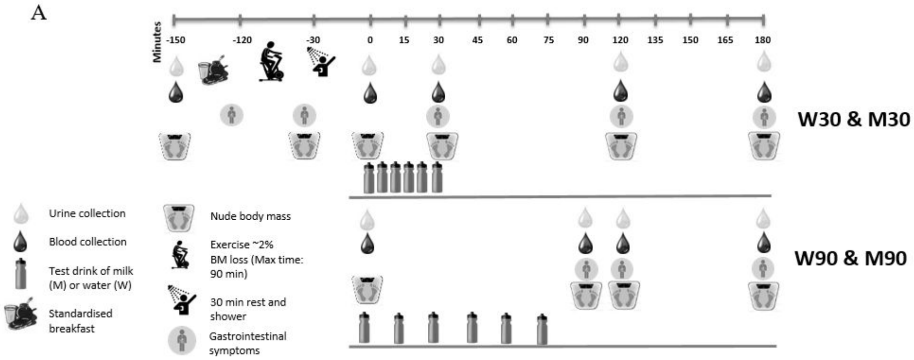
417

418

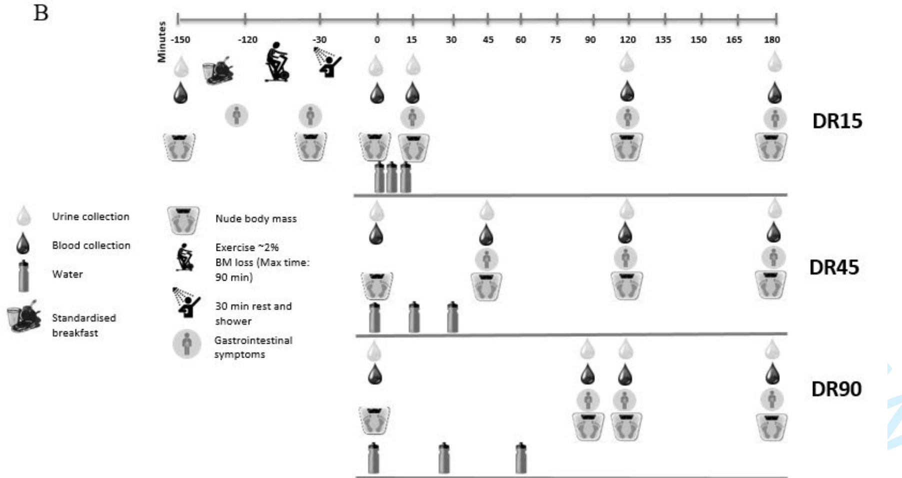
4

Figure 1

A

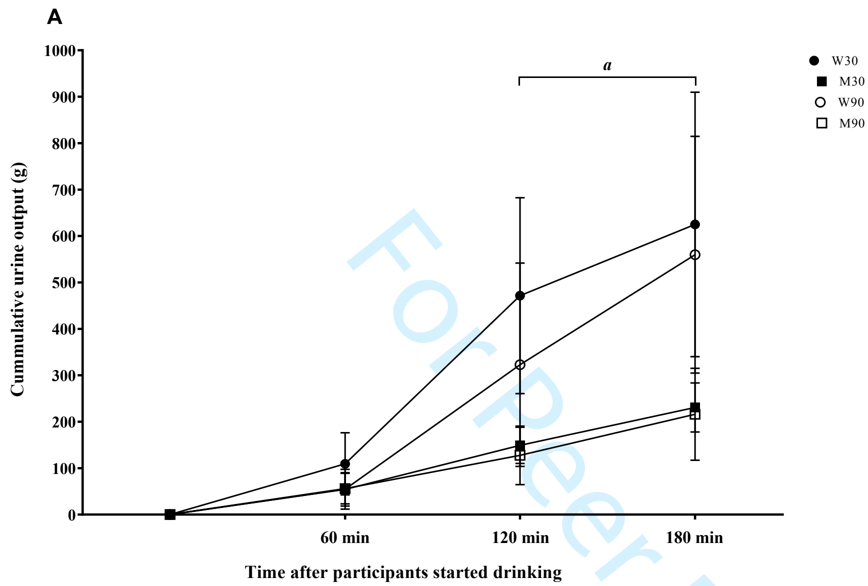

Minutes
-150
-120
-30
0
15
30
45
60
75
90
120
135
150
165
180
W30 & M30
W90 & M90
Urine collection
Blood collection
Test drink of milk (M) or water (W)
Standardised breakfast
Nude body mass
Exercise ~2% BM loss (Max time: 90 min)
30 min rest and shower
Gastrointestinal symptoms

B


Minutes
-150
-120
-30
0
15
30
45
60
75
90
120
135
150
165
180
DR15
DR45
DR90
Urine collection
Blood collection
Water
Standardised breakfast
Nude body mass
Exercise ~2% BM loss (Max time: 90 min)
30 min rest and shower
Gastrointestinal symptoms

5

Figure 2


A
a
W30
M30
W90
M90
Cummulative urine output (g)
1000
900
800
700
600
500
400
300
200
100
0
60 min
120 min
180 min
Time after participants started drinking

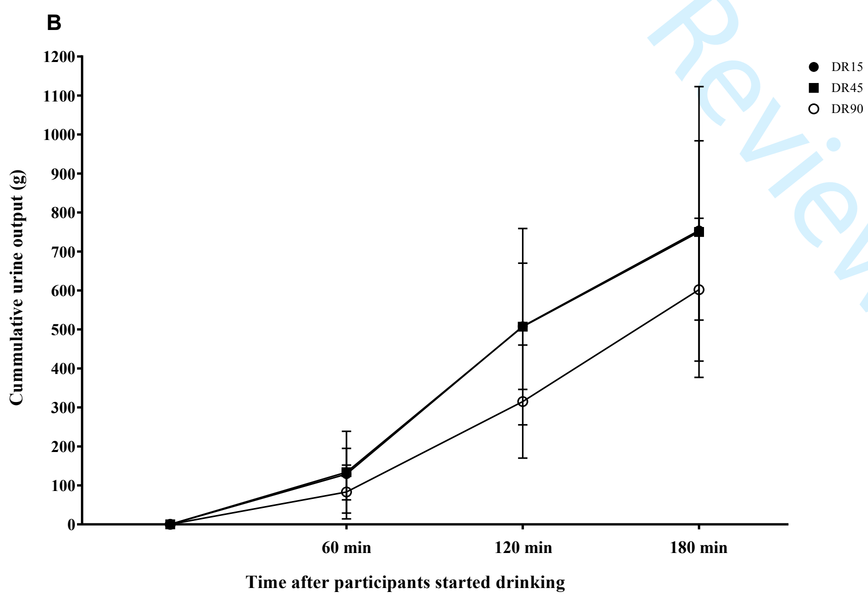
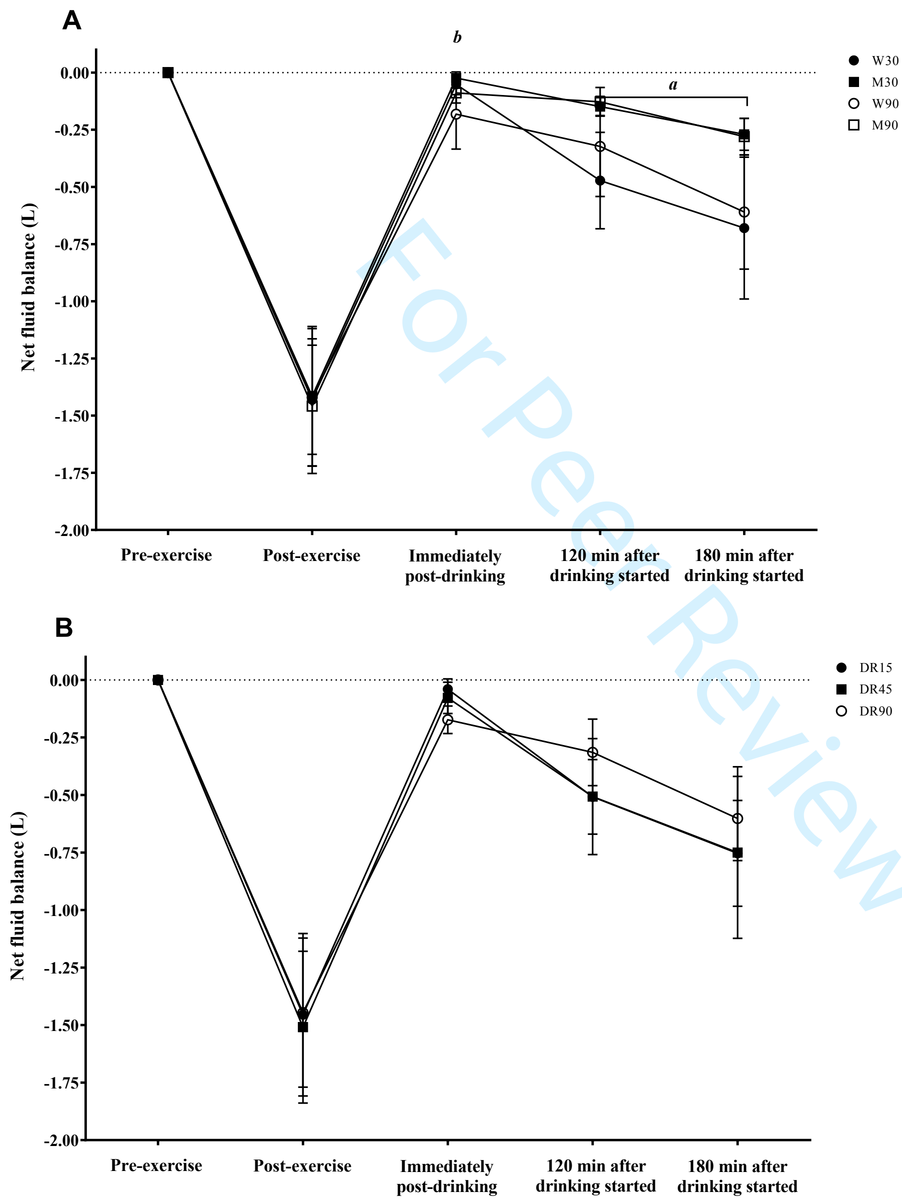

B
DR15
DR45
DR90
Cummulative urine output (g)
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
60 min
120 min
180 min
Time after participants started drinking

Figure 3


A
b
a
W30
M30
W90
M90
Net fluid balance (L)
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
-2.00
Pre-exercise
Post-exercise
Immediately post-drinking
120 min after drinking started
180 min after drinking started
B
DR15
DR45
DR90
Net fluid balance (L)
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
-2.00
Pre-exercise
Post-exercise
Immediately post-drinking
120 min after drinking started
180 min after drinking started

7

Figure 4

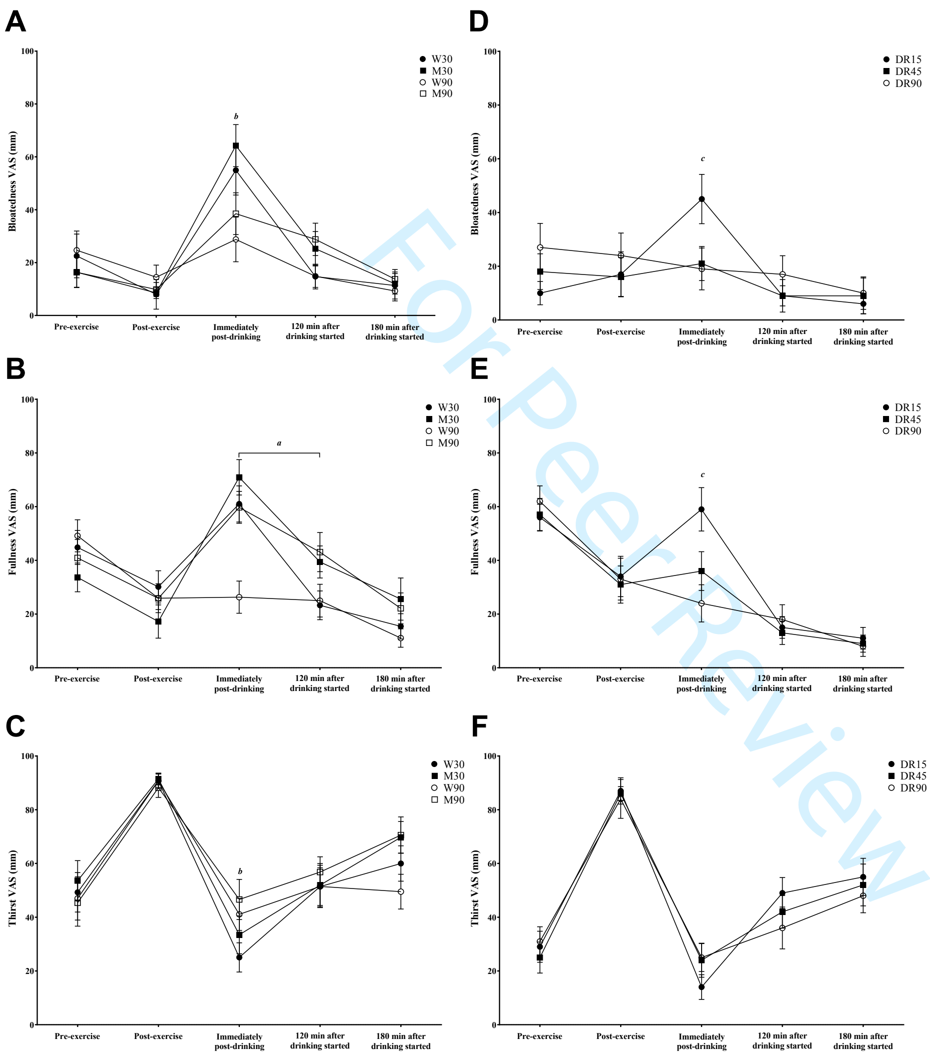

A
B
C
D
E
F
W30
M30
W90
M90
DR15
DR45
DR90
Bloatedness VAS (mm)
Fullness VAS (mm)
Thirst VAS (mm)
Pre-exercise
Post-exercise
Immediately post-drinking
120 min after drinking started
180 min after drinking started
a
b
c

Figure 1

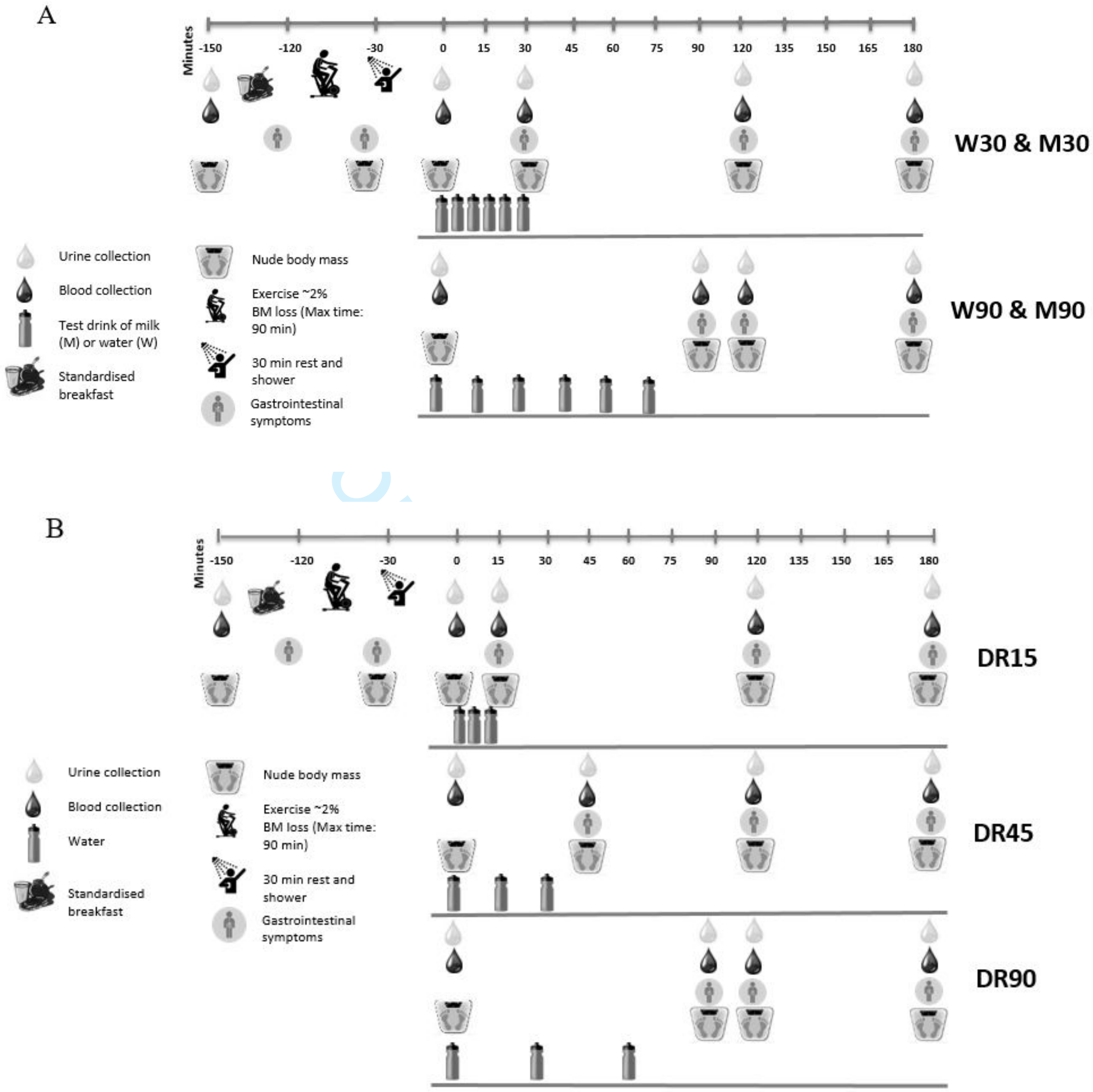

A
Minutes
-150
-120
-30
0
15
30
45
60
75
90
120
135
150
165
180
W30 & M30
W90 & M90
Urine collection
Blood collection
Test drink of milk (M) or water (W)
Standardised breakfast
Nude body mass
Exercise ~2% BM loss (Max time: 90 min)
30 min rest and shower
Gastrointestinal symptoms
B
DR15
DR45
DR90
Water

Figure 2

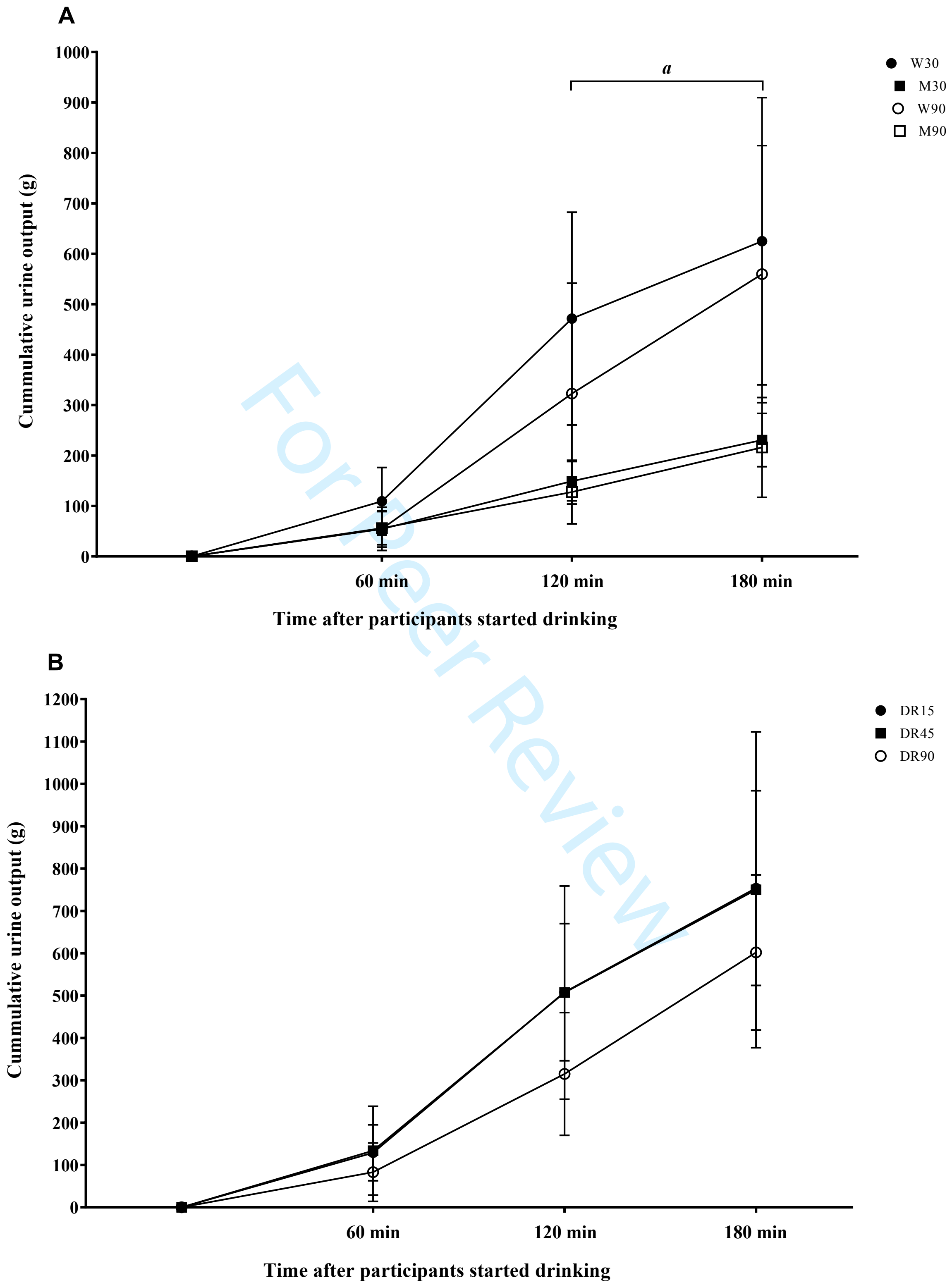

A
a
W30
M30
W90
M90
Cummulative urine output (g)
0
100
200
300
400
500
600
700
800
900
1000
60 min
120 min
180 min
Time after participants started drinking
B
DR15
DR45
DR90
Cummulative urine output (g)
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
60 min
120 min
180 min
Time after participants started drinking

Figure 3

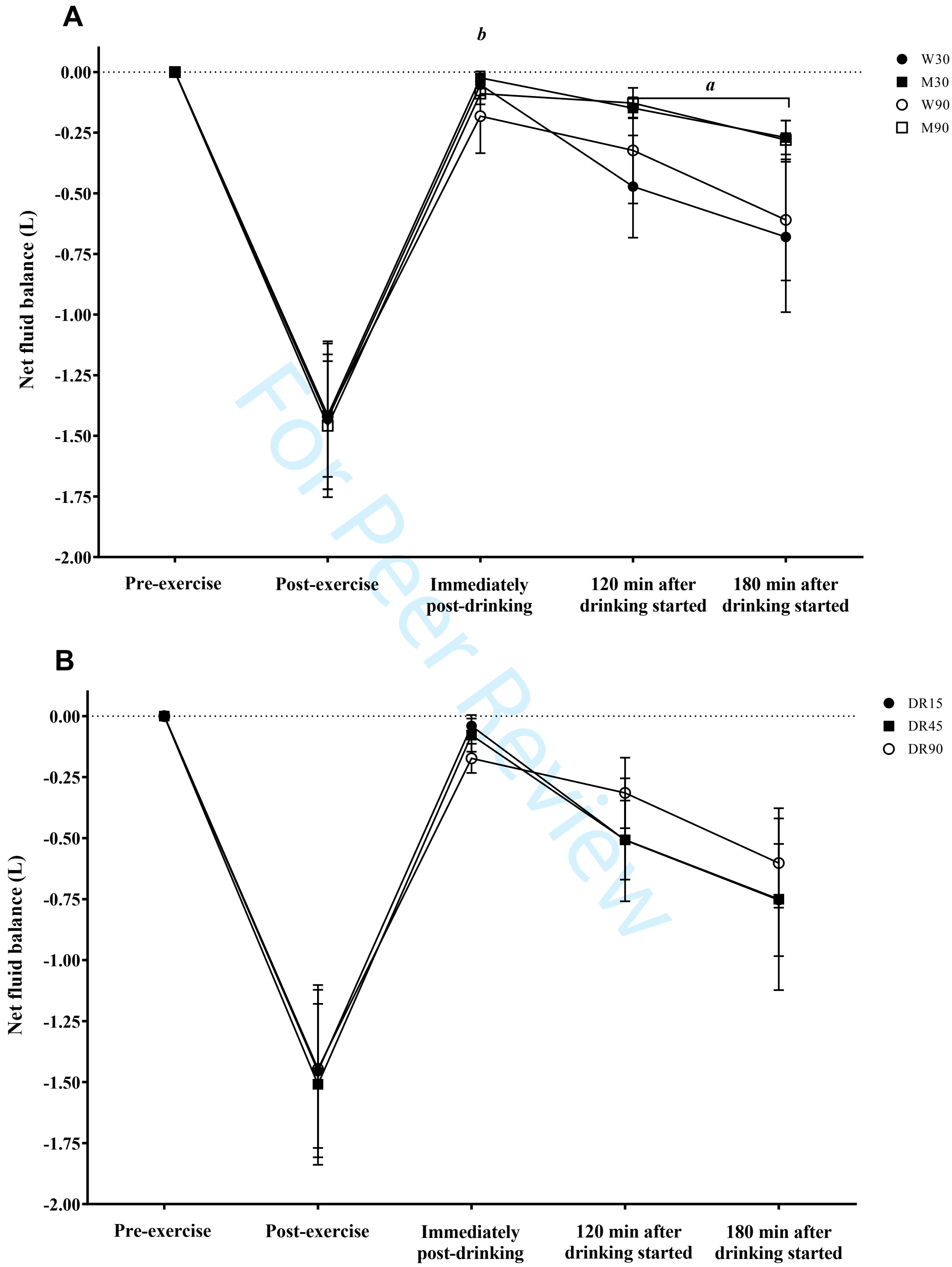

A
b
a
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
-2.00
Net fluid balance (L)
W30
M30
W90
M90
Pre-exercise
Post-exercise
Immediately post-drinking
120 min after drinking started
180 min after drinking started
B
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
-1.50
-1.75
-2.00
Net fluid balance (L)
DR15
DR45
DR90
Pre-exercise
Post-exercise
Immediately post-drinking
120 min after drinking started
180 min after drinking started

Figure 4

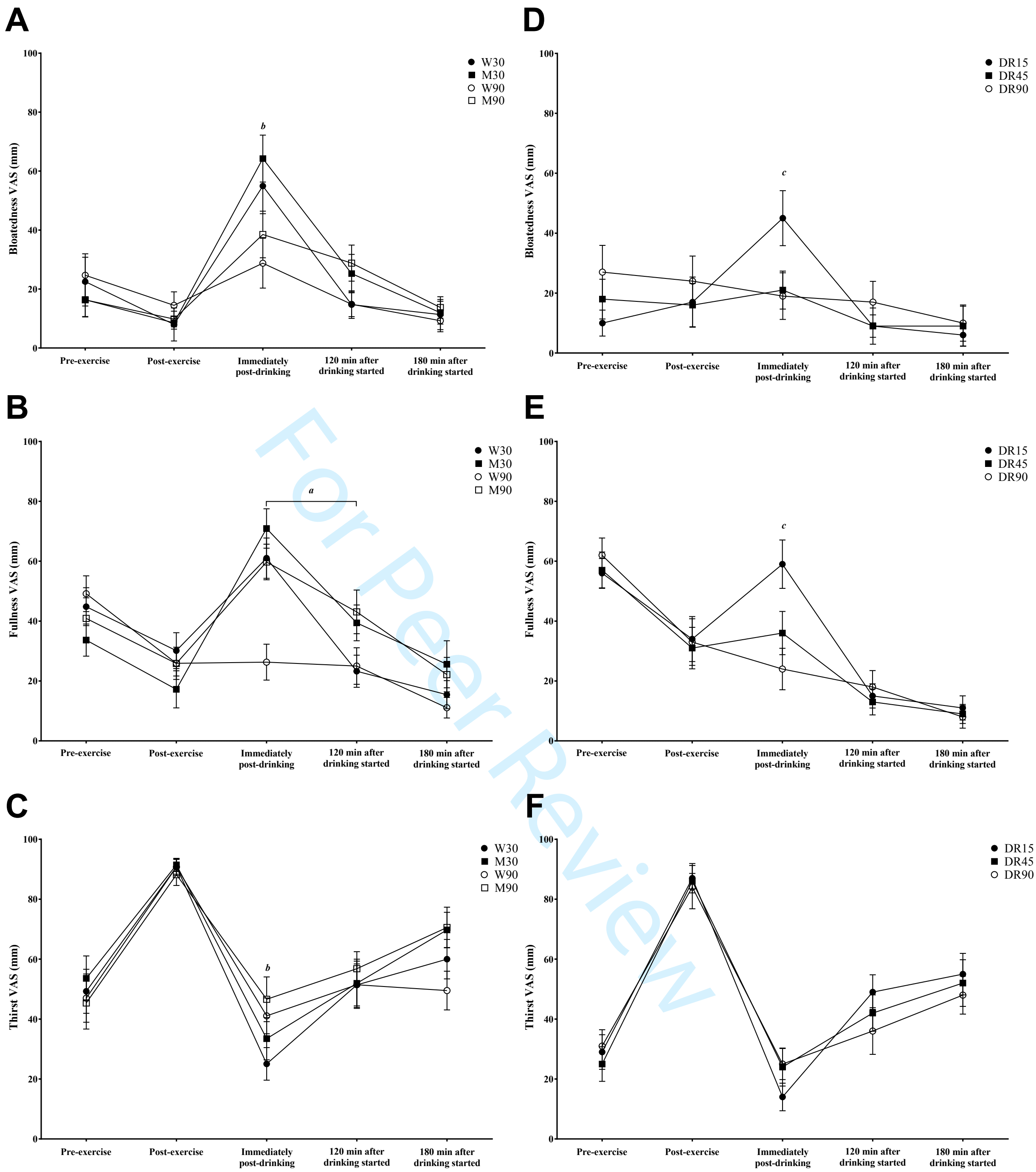

A
Bloatedness VAS (mm)
W30
M30
W90
M90
b
B
Fullness VAS (mm)
a
C
Thirst VAS (mm)
D
DR15
DR45
DR90
c
E
F
Pre-exercise
Post-exercise
Immediately post-drinking
120 min after drinking started
180 min after drinking started

Table 1. Pre-trial conditions and impact of exercise-induced dehydration

Part A	**W30**	**W90**	**M30**	**M90**	***p*-value**
Pre-Ex U_{SG}	1.015±0.006	1.015±0.007	1.013±0.005	1.014±0.005	0.35
Pre-Ex P_{OSM} (mOsm·kg^{-1})	290±4	292±5	290±6	289±5	0.67
Pre-Ex BM (kg)	77.10±9.67	77.27±9.78	76.77±9.73	76.57±9.52	0.28
Ex Duration (min)	70±14	70±13	70±13	70±12	0.86
BM Loss (kg)	1.46±0.28	1.42±0.30	1.43±0.32	1.46±0.29	0.79
BM Loss (%)	1.9±0.3	1.9±0.4	1.9±0.4	1.9±0.3	0.82
Part B	**DR15**	**DR45**	**DR90**		***p*-value**
Pre-Ex U_{OSM}	477±218	474±178	443±185		0.76
Pre-Ex P_{OSM} (mOsm·kg^{-1})	303±5	302±3	302± 5		0.36
Pre-Ex BM (kg)	71.60±9.90	71.54± 10.15	71.31± 10.08		0.39
Ex Duration (min)	79±12	81±13	80± 11		0.62
BM Loss (kg)	1.46±0.35	1.51± 0.33	1.45±0.32		0.30
BM Loss (%)	2.0± 0.4	2.1±0.2	2.0±0.3		0.61

BM: Body mass; Ex: Exercise; P_{OSM}: Plasma osmolality; U_{SG}: Urine specific gravity; U_{OSM}: Urine osmolality. Values are Mean±SD.

Table 2. Test-retest trial data (Part B: pooled from DR15 and DR45)

	Initial Trial	**Repeat Trial**	***p-value***
Pre-Trial Conditions			
Pre-Ex U_{OSM}	483±197	479±197	0.30
Pre-Ex P_{OSM} (mOsm·kg^{-1})	307±5	307±7	0.81
Pre-Ex BM (kg)	72.56±11.10	72.38±10.94	0.30
Ex Duration (min)	80.0±13.5	80.8±13.1	0.34
BM Loss (kg)	1.43±0.32	1.39±0.39	0.62
Fluid Retention Data			
Cumulative urine output (g)	792± 280	704±175	0.07
U_{OSM} 180 min after drinking started (mOsm·kg^{-1})	297±75	281±127	0.69
P_{OSM} 180 min after drinking started (mOsm·kg^{-1})	303±4	302±4	0.38
Fluid retention (%)	52.8±7.0	55.0±7.5	0.21

Values are mean±SD.

Response to peer review comments
Manuscript ID IJSNEM. 2019-0176 entitled
"*Effect of drinking rate on the retention of water or milk following exercise-induced dehydration."*

We thank the editor and reviewers for the detailed review, and believe the edits strengthen our manuscript. Please note that as an *Original Research* manuscript, we have addressed reviewer concerns (and made appropriate manuscript amendments) while being respectful of the word limit specified by the journal.

Reviewer: 1

Recommendation: Minor Revision

Comments:
This paper titled 'Effect of drinking rate on the retention of water or milk following exercise-induced dehydration' includes data from 2 separate studies undertaken in Scotland and Australia. The studies are well-conceived and answer relevant research questions with a clear link between the two studies that justifies their inclusion in the same paper. Given that the studies employ methods that have been often used in previous studies in this area, they are generally sound. Overall, the work highlights that varying the duration in which a drink is ingested from 15-90 min post-exercise has little (small effect on drink retention between 15 and 90 min) effect on the amount of drink retained, but that milk was better retained than water.
The authors thank reviewer #1 for the generally complimentary response to the investigation.

Major Points
Whilst there is relatively few issues with the studies presented and the authors have generally done a good job, there are some caveats that should be covered in the discussion in more detail, in my opinion. Firstly, the duration of follow up was relatively short (i.e. total of 3 h from the start of drinking). This is shorter than the vast majority of previous work in the area and may influence the results in a number of ways. If urine volume was not back down to near basal values, then there may be uncaptured fluid losses in response to the differences in drinking strategy that have not been accounted for. In reality, this is unlikely to represent a large amount of fluid, but should be mentioned as it is particularly pertinent when you consider it in the context of the difference drinking durations as in the shorter trials (e.g. 15 min drinking) there will be a longer time from the end of drinking for the drink to be excreted (i.e. 2 h 45 min in this case) compared to the slower drinking trials. This is particularly pertinent since the only other study I'm aware of in this area (published as an abstract; Archer DT, Shirreffs SM. Effect of fluid ingestion rate on post-exercise rehydration in human subjects. Proc Nutr Soc 60: 200A, 2001) followed for 4 h after the start of drinking and saw no difference between drinking over 30 min and 90 min at 3 h, but a significant difference at 4 h. the difference was, however, small. The other difference between the study of Archer and Shirreffs and this one was that they provided a drink volume equal to 150% of the subjects' sweat loss during exercise. Maybe this also accounts for the difference in observation?
The authors thank the reviewer for these detailed comments and suggestions. In response to these comments (and the concerns of Reviewer #2), we have added a limitations paragraph to the Discussion. The paragraph reads as follows:

"*Several methodological limitations require acknowledgement. Firstly, this study did not employ a direct measure of gastric emptying. Hence, while greater fluid retention was achieved during Milk trials, the distribution of the retained fluid (e.g. within the GI tract (as potentially indicated by higher "fullness" ratings) as opposed to vascular space) and therefore physiological relevance of this fluid retention remains unknown. In addition, the recovery period for this study (3 h from the start of drinking) was shorter than previous work in this area (typically ≥4 h), which may have resulted in small volumes of uncaptured fluid losses in response to the differences in drinking strategy. The decision to shorten the duration of the observation was based on a number of factors; (1) the relatively small volumes of urine seen beyond 90 min following the cessation of drinking in our previous study (Desbrow et al 2014), (2) the smaller volume of* *fluid being ingested (100% v's 150% fluid replacement), (3) the practical relevance of 4 h observation, given that many individuals are likely to eat/drink within this period of time and (4) a previous study (Maughan et al 2016) demonstrated the pattern of response in cumulative urine output and calculated hydration index to ingested drinks was observed to be similar at 2 h post-drinking and 4 h post-drinking."*

We were aware of the Archer et al study, but have avoided direct reference to it in the manuscript, as the information was available in abstract form (as mentioned) only and involved a small participant pool (n=6).

The discussion lacked a little in depth and I certainly would liked to have seen more on the points above and also the practical relevance of the finding, particularly given that in the real world food would also be consumed alongside the drinks, which might change the effects reported. Also, from a practical perspective, drinking more quickly might offer an advantage given that other fluids might then be ingested sooner after completing the 'prescribed rehydration'. Could the methods be shortened to allow more space for discussion of these points?
The authors thank the reviewer for this comment. We agree that the practical aspects of rehydration warrant consideration. Indeed, we have attempted to understand the interaction between food and fluid in our recent studies (1-3). However, for this investigation, food was removed to avoid further influences on gastric emptying rate. We acknowledge the suggestion of reducing the methods section, however, the current level of information has been significantly reduced while ensuring the reader is provided with adequate detail to understand the study design, experimental protocol and standardisation procedures employed for scientific rigor and enhanced confidence in the reported data. Thus, for the methods section, we have opted to retain the current level of detail.

As mentioned, a potential practical benefit of rapid drinking has been added to the Discussion. This section reads as follows:

"In a practical sense post-exercise – as an alternative to "In the sporting post-exercise context", athletes typically consume fluids ad libitum and the beverage choice, drinking rate and total volume consumed are determined by many factors, including prior exercise (intensity/duration/type), environmental conditions, thirst, palatability, gastrointestinal tolerance, drink availability, exercise commitments and other, unrelated dietary goals (Minehan et al., 2002; Passe et al., 2000). The rapid consumption of large volumes of milk or water during the immediate post-exercise period may be poorly tolerated by some individuals. However, the range of subjective responses to our most rapid drinking rates highlights individual differences in tolerance. For those who drink beverages rapidly in the immediate post-exercise period, the rates examined in the present study do not appear to compromise fluid retention when a fixed volume is provided and may facilitate the consumption of other fluids after completing a "prescribed" volume of a beverage. Conversely, it is not known whether rapid beverage ingestion compromises subsequent voluntary fluid consumption in ad libitum drinking scenarios due to an action on thirst response mediated via the gut-brain axis (Zimmerman et al., 2019)."

Minor Points
Line-by line comments:

5: Evans et al. (J Appl Physiol 122: 945–951, 2017) provides a more up to date overview of post-exercise rehydration that might be relevant here.
The Evans et al reference has now been included.

14: Interestingly, the study of Shirrreffs et al (2007) and Watson et al. (2008) are the only studies in the literature (or information from (historic nutrition composition tables/ nutrition information etc.) to report such high levels of sodium in milk. Typically (historic nutrition composition tables/ nutrition information etc.), milk is considered to have a sodium concentration in the region of 20 mmol/L, which generally seems to be ineffective at promoting better rehydration.

The authors agree that the sodium content of milk is interesting in that some varieties appear to differ (regionally / seasonally) in amounts that may influence fluid retention. In fact, our original study design was to have milk trials completed at both study sites with the sodium content of the different milks being an important consideration. However, results from the initial trials lead to a change in experimental treatments for Part B. That said, it is also important to recognise that a combination of nutrients within milk may play a role in either delaying gastric emptying and/or attenuating changes in plasma osmolality.

17: The references here are of less relevance given the exercise/dehydration scenario discussed. Perhaps Clayton et al. (Int J Sport Nutr Exerc Metab 24: 79–89, 2014) or even Evans et al. (Br J Nutr 106: 1732-1739) given they deal with dehydration and/or exercise and repeated drink ingestion.

The Clayton et al. reference has been added to provide further support to this section.

56: Great that females were included, but can you add reference here that supports the lack of any menstrual cycle effects on the measured outcomes?

Unfortunately, there remains a limited amount of research in this area. However, a recent review concluded *"most research indicates that the differences caused by hormonal fluctuations in women do not appear to affect performance, fluid regulation, or health risks during exercise"* (4). Our recent study (5) confirmed that in eumenorrheic females (n=10) there was no difference in fluid balance outcomes post-exercise heat stress when assessed in late follicular and mid-luteal menstrual cycle phases. In the current study we confirmed that all female participants (n=3) had regular menstrual cycles as part of the pre-screening questionnaire and none were taking any oral contraceptive medication.

73-74: Is this correct? Given the effects of strenuous exercise on fluid balance, might a longer standardisation period have been appropriate? By this, they could have done 3 h cycling in the heat 4-7pm if arriving for an 8am trial. Further, it is not mentioned whether any activity done on the day before trials was standardised? As per the food/beverage diary? If so, please can you provide a bit more detail here?

The pre-trial procedures are consistent with those previously reported by our laboratory. The participants in our trial were recreationally active and not trained athletes. As such, we were interested in standardising the pre-trial conditions without being unnecessarily restrictive. All participants reported nil strenuous exercise was performed 12 h prior to the commencement of the experiment.

To clarify, the following sentence has been added to the Results. This section reads as follows:

"All participants reported compliance with the standardisation procedures in the 24 h prior to arriving at the laboratory. In Part A, two participants…."

90: What was the volume of fluid ingested here (i.e. fruit juice).

The authors thank the reviewer for highlighting this omission. The paragraph has been edited and reads as follows:

"*Following this initial blood collection, participants were provided with a standardised breakfast in a quantity relative to BM (20 kJ·kg-1 and 1 g CHO·kg-1) that consisted of raisin toast, strawberry jam and fruit juice (200 mL), …"*

106-110: Was the volume of milk adjusted for the volume of water present since 1000 mL milk does not provide 1000 mL water due to the presence of other nutrients?
The authors thank the reviewer for this important comment. The volume of milk was not adjusted for the water present. This was due to our focus being on the applied context of drinking common beverages at different rates on fluid retention. If the volume of milk was increased to account for its water content (i.e. maintaining water intake rates), the participants would have been required to ingest larger (~10%) volumes of milk at a similar rate, creating inconsistencies across key parameters such as GI ratings.

137-141: What was the anticoagulant added to the blood to yield plasma? Was this consistent between sites?
The authors thank the reviewer for highlighting this omission. An indication of the specific anticoagulant has been added to the Method. This was consistent between sites. The section reads as follows:

"*All samples were collected into EDTA pre-treated vacutainers and centrifuged at room temperature for 10 min at ~1350×g.*"

155: Presumably this pair-wise comparison was a t-test, which was adjusted for multiple comparisons using the Bonferroni adjustment?
Correct. This information is included within the Methods "statistical analysis" section.

169-170: Any ideas on why this trial order effect occurred? After the first trial, did you standardise the exercise duration? Or did you always proceed until the same degree of body mass loss. If the latter, then it seems particularly peculiar.
The authors thank the reviewer for this comment. As mentioned, our participants were recreationally active, therefore our trial order effect is likely due to participant's significant adaptation to the exercise protocol. The protocol was set based on the initial trial and then largely replicated. However, there were a small number of occasions when a participant reached fatigue (following slightly longer exercise duration) yet had less fluid loss than on a previous trial.

Reviewer: 2

Recommendation: Minor Revision

Comments:
Many thanks for the opportunity to read this manuscript. Please see my comments below.
The authors thank reviewer #2 for the positive comment and excellent suggestions.

Introduction:

Line-by line comments:

7: what is meant by "delaying euhydration"? Surely for the diuresis to occur, participants would be hyperhydrated and then return to a hypohydrated state?
The authors thank the reviewer for identifying this source of ambiguity. To clarify, the Introduction has been edited. This section reads as follows:

"However, rapidly consuming large volumes of hypotonic fluid has the potential to reduce plasma osmolality (POSM), resulting in increased urinary output (i.e. "fluid induced diuresis"), potentially delaying a return to euhydration *(Mitchell et al., 1994; Robertson, 1974)."*

10 (onwards): I understand the focus on milk as a rehydration solution given the nature of the study in question however I believe some further discussion is warranted on the efficacy of these solutions. In particular, studies that have demonstrated milk is an effective rehydration solution rely on calculations of net fluid balance/cumulative urine output. In turn, this assumes that all of a solution has been emptied from the stomach and absorbed from the intestine. Studies looking at high carbohydrate solutions (Clayton et al. & Evans et al.) have suggested that nutrient dense solutions result in slow gastric emptying rates that effect the calculation of net fluid balance. Consequently, "greater fluid retention" may simply be the result of the calculations being used rather than any physiological relevance.
The authors thank the reviewer for this comment. In order to address the concerns of both reviewers we have added a section to the Discussion which incorporates the limitation of the past (and current) milk-based rehydration research. This section reads as follows:

"Several methodological limitations require acknowledgement. Firstly, this study did not employ a direct measure of gastric emptying. Hence, while greater fluid retention was achieved during Milk trials, the distribution of the retained fluid (e.g. within the GI tract (as potentially indicated by higher "fullness" ratings) as opposed to vascular space) and therefore physiological relevance of this fluid retention remains unknown."

Methods:

Given it was only measured on one occasion, perhaps VO2max should be referred to as VO2peak
$VO2_{max}$ has been replaced with $VO2_{peak}$ throughout the manuscript.

I really like the assessment of inter-site variation - more studies should do this!
The authors thank the reviewer for the positive response to this assessment.

Why was a 3 hour recovery period employed? Given that some of these trials involve 90 minute drinking protocols, monitoring recovery for a further 90 minutes seems on the short side. Particularly as it involves ingesting milk and it is likely that this will have reduced gastric emptying etc.
Further details regarding concerns over the 3 h recovery period have been incorporated into the Discussion. This section reads as follows:

"*Several methodological limitations require acknowledgement. Firstly, this study did not employ a direct measure of gastric emptying. Hence, while greater fluid retention was achieved during Milk trials, the distribution of the retained fluid (e.g. within the GI tract (as potentially indicated by higher "fullness" ratings) as opposed to vascular space) and therefore physiological relevance of this fluid retention remains unknown. In addition, the recovery period for this study (3 h from the start of drinking) was shorter than previous work in this area (typically ≥4 h), which may have resulted in small volumes of uncaptured fluid losses in response to the differences in drinking strategy. The decision to shorten the duration of the observation was based on a number of factors; (1) the relatively small volumes of urine seen beyond 90 min following the cessation of drinking in our previous study (Desbrow et al 2014), (2) the smaller volume of fluid being ingested (100% v's 150% fluid replacement), (3) the practical relevance of 4 h observation, given that many individuals are likely to eat/drink within this period of time and (4) a previous study (Maughan et al 2016) demonstrated the pattern of response in cumulative urine output and calculated hydration index to ingested drinks was observed to be similar at 2 h post-drinking and 4 h post-drinking.*"

Why are some data reported as mean (SD) and others as mean (SEM)? I would suggest choosing one (preferably mean and SD).
The authors would generally agree with this comment. However, in this instance, only the subjective data (presented as a six figure panel) has used SEM (for error bars). On attempting to provide SD error bars the figure becomes difficult to format with consistency. Thus, for this figure only, we have opted to retain SEM.

Discussion:

As above, I think more attention could be given to some mechanistic detail. In particular, while you haven't measured gastric emptying or stomach volumes, you do have some proxy markers of this i.e. bloatedness/fullness. Given that there are some differences in these markers (particularly when milk was ingested) this might indicate some differences in availability of ingested fluid (though, interestingly, POsm doesn't appear to be different at the end of 3h). While not a major issue, I think it is worth discussing in more detail.

The authors thank the reviewer for this comment. An additional comment has been added within the new limitations section of the Discussion to accommodate this point. This section reads as follows:

"*Several methodological limitations require acknowledgement. Firstly, this study did not employ a direct measure of gastric emptying. Hence, while greater fluid retention was achieved during Milk trials, the distribution of the retained fluid (e.g. within the GI tract (as potentially indicated by higher "fullness" ratings) as opposed to vascular space) and therefore physiological relevance of this fluid retention remains unknown. In addition, the recovery period for this study (3 h from the start of drinking) was shorter than previous work in this area (typically ≥4 h), which may have resulted in small volumes of uncaptured fluid losses in response to the differences in drinking strategy. The decision to shorten the duration of the observation was based on a number of factors; (1) the relatively small volumes of urine seen beyond 90 min following the cessation of drinking in our previous study (Desbrow et al 2014), (2) the smaller volume of fluid being ingested (100% v's 150% fluid replacement), (3) the practical relevance of 4 h observation, given that many individuals are likely to eat/drink within this period of time and (4) a previous study (Maughan et*

al 2016) demonstrated the pattern of response in cumulative urine output and calculated hydration index to ingested drinks was observed to be similar at 2 h post-drinking and 4 h post-drinking."

References:

1. McCartney, D., et al., The effect of different post-exercise beverages with food on ad libitum fluid recovery, nutrient provision, and subsequent athletic performance. Physiol Behav, 2019. 201: p. 22-30.

2. McCartney, D., et al., Fluid, Energy and Nutrient Recovery via Ad Libitum Intake of Different Commercial Beverages and Food in Female Athletes. Appl Physiol Nutr Metab, 2018.

3. Campagnolo, N., et al., Fluid, energy and nutrient recovery via ad libitum intake of different fluids and food. Physiol Behav, 2017. 171: p. 228-235.

4. Rossi, K. A. (2017). Nutritional aspects of the female athlete. *Clinics in sports medicine*, *36*(4), 627-653.

5. Rodriguez-Giustiniani, P., & Galloway, S. D. (2019). Influence of Peak Menstrual Cycle Hormonal Changes on Restoration of Fluid Balance After Induced Dehydration. *International Journal of Sport Nutrition and Exercise Metabolism*, *1*(aop), 1-22.