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Effect of Drinking Rate on the Retention of Water or Milk Following Exercise-Induced Dehydration

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1 Introduction

2 Individuals typically do not consume enough fluid during exercise to counteract sweat losses, producing a 3 post-exercise state of body water deficit (i.e. dehydration) (Garth & Burke, 2013). As a result, individuals 4 are encouraged to drink fluid during recovery to reinstate total body water balance prior to recommencing 5 physical activity (Evans et al., 2017; Sawka et al., 2007). However, rapidly consuming large volumes of 6 hypotonic fluid has the potential to reduce plasma osmolality (POSM), resulting in increased urinary 7 output (i.e. "fluid induced diuresis"), potentially delaying a return to euhydration (Mitchell et al., 1994; 8 Robertson, 1974). Hence, there is considerable scientific interest in understanding factors that enhance 9 fluid retention and assist with rehydration after exercise.

10 When consumed without food and matched for volume, nutrient dense beverages (e.g. milk and milk-11 based beverages) appear to promote greater fluid retention compared to water and carbohydrate-12 electrolyte solutions (Desbrow et al., 2014; Seery & Jakeman, 2016; Shirreffs et al., 2007; Watson et al., 13 2008). The effectiveness of milk as a rehydration solution has been attributed to a number of its 14 constituents (i.e. sodium (Merson et al., 2008; Shirreffs & Maughan, 1998), carbohydrate (Osterberg et 15 al., 2009), and protein (Hobson & James, 2015; James et al., 2014; James et al., 2012)), which are 16 believed to delay gastric emptying and/or attenuate changes in POSM, reducing the degree of fluid induced 17 diuresis (Calbet & MacLean, 1997; Clayton et al., 2014; Murray et al., 1999; Vist & Maughan, 1995).

18 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 19 20 different rates, which is likely to influence nutrient delivery and consequently, fluid retention. To date, 21 only two studies have investigated the influence of drinking rate on fluid recovery (Jones et al., 2010; 22 Kovacs et al., 2002). The initial investigation failed to detect differences in fluid retention when a 23 carbohydrate-electrolyte beverage was consumed over 3 h (79±6%) compared to 5 h (82±5%) following 24 exercise-induced dehydration (3.0% body mass (BM) loss). In contrast, Jones et al., (2010) reported 25 significantly greater retention when water was consumed over a 4 h (75±12%) compared to a 1 h

26 (55±18%) drinking period following an exercise-induced 2.0% BM loss. The explanation for the 27 equivocal findings may relate to the subtle differences in the drinking rates and/or the use of beverages 28 with different nutrient profiles (and hence osmolalities). Furthermore, when consumed ad libitum, 29 individuals typically ingest the largest volume of fluid within the first 30 min following exercise (Baguley 30 et al., 2016). To date, the effect of drinking rate on the retention of fluid from beverages with contrasting 31 nutrient profiles has not been systematically examined. In addition, no previous investigation has 32 compared a conservative drink pattern to a rapid ingestion rate (e.g. large volumes consumed in ~30 min), 33 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.

38 Methods

39 Overview of study designs

40 This investigation was intended to systematically explore the effect of drinking rate on subsequent fluid 41 recovery. The investigation was conducted in two parts, with the results from Part A used to inform the 42 design of Part B. Part A explored the impact of drinking rates of different beverages (milk and water) on 43 fluid retention. In Part B, further exploration of different drinking rates was performed. In addition, the 44 trials were conducted in separate laboratories (Part A - Australia, Part B - Scotland). All participants were 45 fully informed of the nature and possible risks of the investigations before providing written informed 46 consent. The investigation was approved by the Griffith University and University of Stirling's Human 47 Ethics Committees and the procedures were conducted in accordance with the principles outlined by the 48 declaration of Helsinki.

49 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.

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 ± 7.6 mL·kg⁻¹·h⁻¹) completed four experimental trials.

59 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.

67 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

recrease (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.

78 Experimental procedures

79 Participants arrived at the laboratory between 05:30 and 08:00 h and verbally acknowledged compliance 80 to the pre-experimental conditions. A urine sample was taken for determination of hydration status (Part 81 A: urine specific gravity (U_{SG}) (Palette Digital Refractometer, ATAGO, USA) and Part B: urine 82 osmolality (U_{OSM}) (Löser Osmometer, Camlab, UK). If participants recorded a $U_{SG} \ge 1.024$ (Sommerfield 83 et al., 2016) or U_{OSM} of >700 mOsm kg⁻¹ (Sawka et al., 2007) they were considered hypohydrated. In Part 84 A, hypohydrated participants were required to consume 600 mL of plain water over 5 min, before 85 providing a second urine sample 30-60 min later. If this urine sample achieved the thresholds for 86 euhydration the participants continued with the trial (this practice was then replicated on all subsequent 87 trials). If the threshold value was not reached within the 60 min period the trial was rescheduled. 88 Participants then rested in a seated position for 5 min prior to venepuncture of a forearm vein. Following 89 this initial blood collection, participants were provided with a standardised breakfast in a quantity relative 90 to BM (20 kJ·kg-1 and 1 g CHO·kg-1) that consisted of raisin toast, strawberry jam and fruit juice (200 91 before completing a questionnaire on GI subjective symptoms, voiding their bladder and obtaining a 92 baseline nude BM measurement (Part A: A&D Company Ltd, Tokyo, Japan, to nearest 20 g; Part B: 93 Marsden, Rotherham, United Kingdom, to nearest 10 g).

94 Exercise-induced dehydration

After completing a brief standardised warm up, participants began cycling in a warm environment (Part
A: 25.2±0.8 °C and 84±11% RH, Part B: 26.4±0.7°C 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

100 achieved. Following exercise, dehydrated participants rested in a seated position for 15 min prior to 101 having a cool shower. Afterwards, participants dried themselves thoroughly, before a cannula was 102 inserted into a forearm vein and a blood sample obtained. Participants then emptied their bladder and 103 provided a urine sample before a final nude BM measure was recorded to determine total fluid loss (30 104 min post-exercise).

105 Post-exercise fluid replacement

106 In Part A, water or low fat cow's milk (Maleny Dairies, Queensland, Australia; 210 kJ Energy, 5.3 g 107 CHO, 4.0 g Protein, 1.4 g Fat, 48 mg Na^{+,1}00 mL⁻¹) were ingested in a quantity equal to 100% of the 108 volume of sweat lost during exercise. The fluid volume was ingested in six equal aliquots spread evenly 109 over either a 30 or 90 min period, resulting in the beverage treatments: Water 30 min (W30), Water 90 110 min (W90), Milk 30 min (M30), and Milk 90 min (M90). Participants were instructed to consume each 111 aliquot at an even pace over 5 or 15 min according to the relevant drinking rate. In Part B, water in a 112 quantity equal to 100% of the volume of sweat lost during exercise was ingested. The volume was 113 provided in three aliquots spaced evenly over either a 15, 45 or 90 min drinking period, resulting in the 114 following beverage treatments: Water 15 min (DR15); Water 45 min (DR45); and Water 90 min (DR90). 115 To assess within individual variation, participants in part B repeated either the DR15 or DR45 trial. To 116 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.

121 Body mass and fluid retention

- 122 BM change (estimate of fluid loss) was calculated by subtracting the post-exercise BM (post-void) from
- 123 the pre-exercise BM. Net BM change was calculated by subtracting the 3 h BM measurement from the

124 pre-exercise BM. Percent fluid retention at the conclusion of the observation period was calculated by the

125 following equation:

Fluid Retained (%) = 100 x (Total beverage ingested (g) – Total urine output (g))

Total beverage ingested (g)

126

127 Urine and blood collection, storage and analysis

128 Additional urine sampling was performed at pre-exercise, post-exercise (immediately pre-drinking), 129 immediately post-drinking and then at 120 min and 180 min after the start of drinking. At each of these 130 urine collection points, participants completely voided their bladder into an empty container for 131 subsequent measures of urine volume. Total urine loss was calculated from the accumulated urine output 132 in the period from the commencement of drinking until the end of the observation period. A sample of 133 urine was retained for determination of urine osmolality. Blood sampling was performed at pre-exercise, 134 post-exercise (immediately pre-drinking), immediately post-drinking and then at 120 min and 180 min 135 after the start of drinking for the determination of POSM. Participants remained seated prior to a 5 mL 136 blood sample being drawn from an antecubital vein. All samples were collected into EDTA pre-treated 137 vacutainers and centrifuged at room temperature for 10 min at ~1350×g. Plasma was analysed in 138 duplicate on a calibrated freezing-point depression osmometer (Part A: Osmomat 030, Germany and Part 139 B: Löser osmometer, Camlab, UK). Cannulas were kept patent by flushing sterile saline (2 mL of 0.9% 140 NaCl; Becton Dickson, NJ, USA) on completion of each sample (with an equivalent volume of blood 141 initially discarded before collection of subsequent samples).

142 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).

146 Statistical analyses

147 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 148 149 (p>0.05) and Mauchly's test (p>0.05), respectively. Where assumptions of sphericity in repeated-150 measures analyses were violated, the Greenhouse-Geisser statistic was applied. One-way repeated-151 measures analysis of variance (ANOVA) were performed to verify that pre-trial conditions and exercise-152 induced fluid loss did not differ across trials. For Part A, a three-factor (i.e. Beverage x Rate x Time) 153 repeated-measures ANOVA was used to compare main outcomes; two-factor (i.e. Beverage x Rate) 154 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 155 156 present. For Part B, two-factor (i.e. Rate x Time) repeated-measures ANOVA were used to compare 157 outcomes between the different beverage ingestion rates. Paired t-tests or Wilcoxon tests were used where 158 appropriate to conduct post-hoc comparisons on significant interaction effects. An adjusted-alpha (i.e. 159 p=0.05 divided by the number of tests performed) was used to account for multiple comparisons. The 160 test-retest reliability was calculated as a coefficient of variation (CV%) using the traditional method and 161 any difference in responses between sites was assessed using an unpaired t-test. Statistical significance 162 was accepted at p < 0.05. All data are reported as Mean±SD, unless stated as Mean±SEM.

163 **Results**

164 Standardisation procedures

165 [All participants reported compliance with the standardisation procedures in the 24 h prior to arriving at 166 the laboratory.] In Part A, two participants were administered water (600 mL) due to a pre-exercise USG 167 \geq 1.024 on Trial 1; this practice was repeated on all subsequent trials to ensure consistency. The remaining 168 participants had a U_{SG}<1.024 at the commencement of each trial. Exercise duration and pre-exercise 169 values for BM, U_{SG} and P_{OSM} were similar across all treatments, and did not differ significantly by trial 170 order (p>0.05). Exercise-induced BM loss differed significantly (p<0.01) by trial order (Trial 1:

171 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,

- 172 counterbalancing ensured that mass loss was similar across treatment conditions (Table 1).
- 173 In Part B, exercise duration and pre-exercise values for BM, U_{OSM} , P_{OSM} , and exercise induced BM loss
- 174 were similar across all treatments (Table 1); and did not differ significantly by trial order (p>0.05).

175 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%).

188 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.

196 In Part B, all experimental trials concluded with participants in a state of negative net fluid balance

197 (DR15: -0.75±0.23 L; DR45: -0.75±0.37 L; DR90: -0.60±0.18 L; Figure 3B). No differences were
198 observed between trials.

199 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·kg⁻¹, p<0.001), but this effect was not evident by 180 min (Water: 290±2 mOsm·kg⁻¹;
Milk: 293±4 mOsm·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·kg⁻¹; DR45: 302±3 mOsm·kg⁻¹; DR90: 303±5 mOsm·kg⁻¹, p>0.05).

207 Subjective measures

208 In Part A, analysis for bloatedness, fullness, and thirst ratings identified a significant effect of time on 209 each variable (p < 0.01). A significant effect of beverage was also observed for fullness (p = 0.022). For 210 bloatedness and fullness there were significant time x beverage interaction effects (bloatedness: p=0.014; 211 fullness p < 0.01). Post hoc comparisons revealed that the 30 min drinking protocol increased feelings of 212 bloatedness (p < 0.01) and decreased feelings of thirst (p < 0.01) immediately after drinking compared to 213 the 90 min protocol. The consumption of milk increased feelings of fullness immediately after drinking 214 (p < 0.01) and at 120 min (p < 0.01), compared to the consumption of water. No other significant 215 differences were observed.

- 216 In Part B, perceived bloatedness and fullness were significantly higher immediately after drinking on the
- 217 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 timepoint (Figure 4).

220 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).

225 Discussion

226 This two-part study explored the effect of drinking rate on fluid retention of different beverages following 227 exercise-induced dehydration. In keeping with our hypothesis, Part A observed that drinking milk resulted 228 in greater fluid retention than water during a 3 h recovery period. This effect was not influenced by 229 drinking rate (i.e. 30 vs. 90 min). Consequently, Part B assessed retention of water consumed over 230 alternative drinking rates (i.e. 15 vs. 45 vs. 90 min), as well as the day-to-day variation in post-exercise 231 fluid retention. Part B, indicated that the 15 min drinking protocol led to a significant reduction in fluid 232 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 233 234 post-exercise fluid recovery is small and that the nutrient composition of a beverage has a more 235 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·h⁻¹ vs. 0.40 L·h⁻¹. Kovacs et al., (2002) had participants ingest a
carbohydrate-electrolyte sports drink at a maximum rate of 1.32 L·h⁻¹ in the first hour, with an average
rate over 3 hours of 0.77 L·h⁻¹ and compared this to fluid retention with a slow drinking rate of 0.53 L·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.

248 The current findings suggest that the nutrient profile of different beverages have a greater impact on fluid 249 retention than ingestion rate. Indeed, when consumed exclusively and matched for volume, milk 250 beverages promote greater fluid retention than water at rest (Maughan et al., 2016) and during the post-251 exercise period (Seery & Jakeman, 2016; Shirreffs et al., 2007; Watson et al., 2008). These effects may be 252 mediated by the composition of milk (whey/casein protein), electrolyte content and insulin response to 253 carbohydrate/protein delivery. In addition, the electrolyte content of milk (Shirreffs et al., 2007) and 254 insulin mediated impacts on renal water transport (Magaldi et al., 1994) both have the potential to 255 enhance fluid retention.

256 In a practical sense post-exercise, athletes typically consume fluids ad libitum and the beverage choice, 257 drinking rate and total volume consumed are determined by many factors, including prior exercise 258 (intensity, duration and type), environmental conditions, thirst, palatability, gastrointestinal tolerance, 259 drink availability, exercise commitments and other, unrelated dietary goals (Minehan et al., 2002; Passe et 260 al., 2000). The rapid consumption of large volumes of milk or water during the immediate post-exercise 261 period may be poorly tolerated by some individuals. However, the range of subjective responses to our 262 most rapid drinking rates highlights individual differences in tolerance. For those who drink beverages 263 rapidly in the immediate post-exercise period, the rates examined in the present study do not appear to 264 compromise fluid retention when a fixed volume is provided and may facilitate the consumption of other 265 fluids after completing a "prescribed" volume of a beverage. Conversely, it is not known whether rapid 266 beverage ingestion compromises subsequent voluntary fluid consumption in ad libitum drinking scenarios 267 due to an action on thirst response mediated via the gut-brain axis (Zimmerman et al., 2019).

268 Several methodological limitations require acknowledgement. Firstly, this study did not employ a direct 269 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" 270 271 ratings) as opposed to vascular space) and therefore physiological relevance of this fluid retention remains 272 unknown. In addition, the recovery period for this study (3 h from the start of drinking) was shorter than 273 previous work in this area (typically ≥ 4 h), which may have resulted in small volumes of uncaptured fluid 274 losses in response to the differences in drinking strategy. The decision to shorten the duration of the 275 observation was based on a number of factors; (1) the relatively small volumes of urine seen beyond 90 276 min following the cessation of drinking in our previous study (Desbrow et al 2014), (2) the smaller 277 volume of fluid being ingested (100% vs. 150% fluid replacement), (3) the practical relevance of 4 h 278 observation, given that many individuals are likely to eat/drink within this period of time and (4) our 279 previous study (Maughan et al., 2016) demonstrated the pattern of response in cumulative urine output 280 and calculated hydration index to ingested drinks was observed to be similar at 2 h post-drinking and 4 h post-drinking. 281

282 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 postexercise fluid retention.

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

- 293 Giustiniani conducted the research and analysed the samples. B. Desbrow, S. D. R. Galloway, C. Irwin,
- 294 N. Rodriguez-Sanchez, D. McCartney and L. Sayer performed the statistical analysis. B. Desbrow, S. D.
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- 296 Sayer wrote the paper. All the authors approved the final version of the paper.
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389 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

396 significantly different to water. Values are Mean±SD.

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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

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.

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

Part A	W30	W90	M30	M90	<i>p</i> -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		<i>p</i> -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. 410 411

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

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Figure 4

Figure 1







Time after participants started drinking









Part A	W30	W90	M30	M90	<i>p</i> -value
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Table 1. Pre-trial conditions and impact of exercise-induced dehydration

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

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Values are mean±SD.