

Accepted Manuscript

Fluid, energy and nutrient recovery via ad libitum intake of different fluids and food

Nadia Campagnolo, Elizaveta Iudakhina, Christopher Irwin, Matthew Schubert, Gregory R. Cox, Michael Leveritt, Ben Desbrow



PII: S0031-9384(16)30320-1
DOI: doi: [10.1016/j.physbeh.2017.01.009](https://doi.org/10.1016/j.physbeh.2017.01.009)
Reference: PHB 11628
To appear in: *Physiology & Behavior*
Received date: 24 May 2016
Revised date: 11 November 2016
Accepted date: 6 January 2017

Please cite this article as: Nadia Campagnolo, Elizaveta Iudakhina, Christopher Irwin, Matthew Schubert, Gregory R. Cox, Michael Leveritt, Ben Desbrow , Fluid, energy and nutrient recovery via ad libitum intake of different fluids and food. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Phb(2017), doi: [10.1016/j.physbeh.2017.01.009](https://doi.org/10.1016/j.physbeh.2017.01.009)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

TITLE

Fluid, energy and nutrient recovery via *ad libitum* intake of different fluids and food.

AUTHORS

Nadia Campagnolo^{1*}, Elizaveta Iudakhina^{1*}, Christopher Irwin¹, Matthew Schubert², Gregory R. Cox³, Michael Leveritt⁴, Ben Desbrow¹

¹Menzies Health Institute Queensland, School of Allied Health Sciences, Griffith University, Gold Coast, Queensland, Australia.

²Dept. of Kinesiology, Auburn University at Montgomery, Alabama, USA.

³Sports Nutrition, Australian Institute of Sport, Gold Coast, Queensland, Australia.

⁴School of Human Movement and Nutrition Sciences, University of Queensland, Brisbane, Queensland, Australia.

CORRESPONDING AUTHOR

Ben Desbrow, PhD

Menzies Health Institute Queensland, School of Allied Health Sciences, Griffith Health Building (G40), 2.83, Griffith University, Gold Coast, Southport, Queensland, 4222, Australia.

E-mail: b.desbrow@griffith.edu.au

Ph: +61 (07) 56789110

Fax: +61 (07) 56780199

* Please note that we are seeking support for the manuscript to indicate this was a co-first author paper. The research aim was divided in two parts – 1. hydration assessment and 2. nutrient intake from different beverage treatments. The two student researchers, (NC and EI), were assigned lead responsibility for one aspect of the study, with the results combined for this manuscript.

ABSTRACT

Introduction: This study compared the effects of ad libitum consumption of different beverages and foods on fluid retention and nutrient intake following exercise. **Methods:** Ten endurance trained males (mean±SD; Age = 25.3±4.9 years, VO₂max = 63.0±7.2 ml·kg·min⁻¹) performed four trials employing a counterbalanced, crossover design. Following 60 mins of exercise (matched for energy expenditure and fluid loss) participants consumed either water (W1 and W2), a sports drink (Powerade® (P)) or a milk-based liquid meal supplement (Sustagen Sport® (SS)) over a four hour recovery period. Additionally, participants had access to snack foods on two occasions within the first two hours of recovery on all trials. All beverages and food were consumed ad libitum. Total nutrient intake, urine volume, USG, body weight as well as subjective measures of gastrointestinal tolerance and thirst were obtained hourly. Plasma osmolality was measured pre, post, one and four hours after exercise. **Results:** Total fluid volume ingested from food and beverages in W1 (2.28±0.42 L) and P (2.82±0.80 L) trials were significantly greater than SS (1.94±0.54 L). Total urine output was not different between trials (W1= 644±202 mL, W2 = 602±352 mL, P = 879±751 mL, SS = 466±129 mL). No significant differences in net body weight change was observed between trials (W1= 0.01±0.28 kg, W2 = 0.08±0.30 kg, P = -0.02±0.24 kg, SS = -0.05±0.24 kg). Total energy intake was higher on P (10179±1484kJ) and SS (10577±2210 kJ) compared to both water trials (W1 = 7826±888 kJ, W2 = 7578±1112 kJ). **Conclusion:** With the co-ingestion of food, fluid restoration following exercise is tightly regulated and not influenced by the choice of either water, a carbohydrate-electrolyte (sports drink) or a milk-based beverage.

Keywords: dehydration, recovery, energy balance, training.

INTRODUCTION

Nutrition recovery is vital to facilitate improved exercise capacity and performance, whilst also promoting optimal training adaptation (6). Appropriate fluid and nutrient intake following aerobic exercise can alter molecular responses to training, modify muscle-damage repair processes, cause changes to the restoration of glycogen as well as restore water and electrolyte balance (12). Evidence-based guidelines highlight the considerations required to address post-exercise nutritional issues (1, 2). These recommendations often focus on individual aspects of recovery such as fluid balance restoration (1), muscle protein resynthesis (2) or substrate replenishment (2). However, in practical terms, these issues require concurrent consideration.

Athletes often finish exercise in fluid deficit (1), which has resulted in considerable interest in understanding factors influencing fluid recovery (19, 31, 33). Studies investigating fluid retention following exercise typically employ prescribed drinking protocols to explore the impact of a single ingredient (e.g. carbohydrate, sodium) or volume manipulations on fluid balance (13-15, 19, 20, 22-24, 28, 32, 34). In contrast, recent evidence suggests that consuming beverages containing a variety of nutrients, such as milk and milk-based supplements, may promote greater fluid retention (9, 30, 35). The consumption of beverages with diverse nutrient profiles are likely to influence many aspects of recovery in addition to fluid retention.

To date, very few studies investigating fluid retention have employed protocols consistent with an applied environment where athletes drink voluntarily (*ad libitum*) and are also encouraged to consume food. Studies using prescribed drink volumes do not consider factors such as thirst, palatability, and gastrointestinal comfort; which are likely to influence the volume of beverage consumed in practice (10). Investigations employing *ad libitum* consumption of beverages accompanying exercise indicate that an individual's consumption of different beverages can be highly variable (21) even with similar

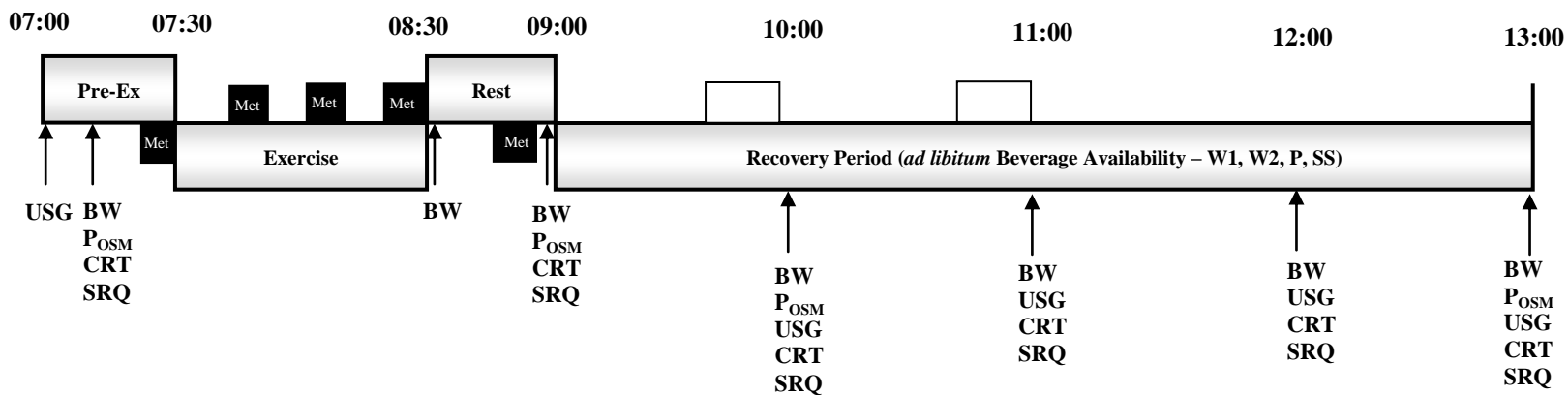
levels of exercise induced fluid loss (4). Furthermore, in the hours following exercise athletes often consume food, in addition to fluid, which is likely to influence both fluid consumption and retention. Only two studies have investigated the impact of food on fluid balance (18, 25). Collectively, these studies indicate that consuming food may enhance fluid retention. However, only limited foods items have been examined (rice/beef meal (18), beef jerky (25)) and on both occasions fluid intakes were prescribed. To date, no study has investigated the impact of *ad libitum* food and beverage consumption on fluid retention and nutrient intake during post-exercise recovery.

Therefore, the aim of this study was to compare the effects of *ad libitum* consumption of different beverages in combination with snack foods on the recovery of exercise-induced fluid loss and energy expenditure. It was hypothesised that when consumed with food, beverages with different nutrient profiles would facilitate different volumes of consumption, yet result in similar fluid retention.

METHODS

Participants. Ten endurance trained (cyclists and/or triathletes) men (mean±SD; age=25.3±4.9 years; height=177.5±6.1 cm; body weight=70.3±5.8 kg; $VO_{2max}=63.0\pm7.2$ ml·kg·min⁻¹) who trained and/or competed ≥ 10 h·week⁻¹ were recruited to participate. Participant numbers were established using power calculation software (G*Power Version 3.1.9.2, University Kiel Germany, 2014) and experience with likely attrition. With an effect size of 0.5, power (1- β) of 0.80 and $\alpha=0.01$, projections suggest 10 participants were required to detect a 0.5kg difference in net fluid balance. This value was established as the least significant difference between group means based on previous rehydration investigations which have also incorporated cross-over study designs (4, 9). The investigation was approved by the XXXX University Human Research Ethics Committee (protocol: XXXX/HREC) (removed for blind review), and all participants provided written, informed consent before enrolment.

Study design. A timeline of the experimental trial is displayed in Figure 1. Each participant attended the laboratory on five separate occasions; one preliminary and four repeated measures experimental trials separated by at least 5 days using a counterbalanced (incomplete Latin square) design. Each trial involved exercise-induced fluid loss prior to the *ad libitum* provision of one of three different trial beverages (W1 – Water, W2 – Water 2, P – Powerade[®], SS – Sustagen Sport[®]) and food. To blind participants from the true nature of the investigation, participants were informed the purpose was to examine the influence of different beverages on post-exercise cognitive function (sham questions and a short cognitive function task were administered throughout the trials). Participants were given access to food for 15 min at the end of the first and second hour of the recovery period prior to undertaking the cognitive function test. Participants were not advised that food intake was being monitored until the completion of the study.

Figure 1. Schematic of experimental trial protocol.

Pre-Ex, Pre-Exercise; BW, Nude body weight; USG, Urine specific gravity; CRT, Choice Reaction Time cognitive task (for blinding); SRQ, Subjective Ratings Questionnaire; P_{OSM}, blood collection for subsequent Posm analysis; Met, Metabolic gas measurement; FOOD, *ad libitum* access to snack foods for 15 minutes; W1, Water 1; W2, Water 2; P, Powerade; SS, Sustagen Sport.

Screening and preliminary testing. Participants were required to be aged between 18 and 40 years; without history of cardiovascular, metabolic or kidney diseases; not taking any medications known to influence lipid or carbohydrate (CHO) metabolism; not known to have food allergies or intolerances. All participants completed preliminary health screening prior to undertaking a graded exercise test on an electronically braked cycle ergometer (Lode Excalibur Sport; Lode BV, Groningen, Netherlands). The protocol began at 100 W, and increased in 50 W increments every 2.5 min until volitional exhaustion with participant's respiratory gases sampled continuously by breathing into a calibrated gas analysis system (Medgraphic Ultima, MGC Diagnostics and Medisoft, USA). Method for determining maximum O₂ consumption (VO_{2max}) and Peak Sustainable Power Output (PPO) have previously been described (Desbrow, Minahan, & Leveritt, 2007) with these values being used to establish initial exercise intensities for subsequent trials.

Pre-trial standardisation. Participants were instructed to abstain from alcohol for 24 h and to avoid caffeine-containing products and moderate-strenuous exercise for 12 h prior to experimental trials. For 24 h prior to the first trial, participants completed a diary recording all food and drink consumed which also included a pre-packaged evening meal for each trial. The meal (Lasagne (Lean Cuisine[®]), frozen garlic bread (Coles[®] Smartbrand) and 600 ml Gatorade (Pepsico[®])) was designed to provide $\sim 60 \text{ kJ}\cdot\text{kg}^{-1}$ body weight (BW) of energy. Participants were required to abstain from food for at least 10 hours prior to arrival at the laboratory for the experimental trial. Participants were also instructed to drink 1000 mL of plain water prior to retiring to bed to assist with hydration. Participants were required to repeat this dietary intake prior to all subsequent trials.

Resting, pre-exercise period. Participants arrived to the laboratory $\sim 0700\text{h}$ with compliance to pre-experimental conditions being verbally acknowledged. An initial urine sample was collected to determine urine specific gravity (USG; Palette Digital Refractometer, ATAGO, USA). The participants were able to start exercising after a USG reading < 1.020 was obtained. Participants who recorded a USG reading ≥ 1.020 were provided with a bolus of plain water (500-1000 mL) to consume within ~ 5 min, followed by a 30 min rest period before a subsequent USG measure was taken. If USG remained ≥ 1.020 the trial was rescheduled. Once a USG reading < 1.020 was recorded, participants rested in a supine position for 15 min prior to respiratory gases (Medgraphic Ultima, MGC Diagnostic Corporation, USA) being collected continuously for 10 min to estimate resting metabolic rate, and to allow body fluid compartments to stabilise (29). Immediately following respiratory measures, a blood sample was collected for analysis of plasma osmolality, and subsequently completed a cognitive function test and subjective feelings questionnaire (Adaptive Visual Analogue Scale (AVAS) (17)). Participants then voided their

bladder completely and a baseline nude BW was measured (HW-PW200; A&D Company Ltd, Tokyo, Japan).

Exercise dehydration period. Fluid loss was induced by exercise on a cycle ergometer (Lode Excalibur Sport; Lode BV, Groningen, Netherlands) in a stable laboratory environment (23°C; 70% RH). During trial one, a warm-up was completed at 100 W for 5 min after which the workload was increased to 65% PPO. Exercise intensity remained stable for 20 min, thereafter participants who expressed likely volitional exhaustion prior to achieving the required BW loss (~1.8%) had workloads reduced by ~5% at 10 min time stages to an intensity that could be tolerated. The minimum allowable workload was 50% PPO. Heart rate and rating of perceived exertion (RPE) (Borg, 1973) were collected every 10 min throughout exercise. During exercise respiratory gases (VO_2 and VCO_2) were collected continuously between ~12-20 min, ~32-40 min and ~52-60 min. Following 60 min of cycling, a nude BW was measured to determine fluid loss. If a BW loss <1.8% from baseline was recorded, participants were required to continue exercise in 10 min intervals. Once a BW loss \geq 1.8% was achieved, exercise ceased. The duration and intensity was recorded and was used as the exercise protocol for all subsequent trials.

Following exercise participants rested in a supine position for 30 min to enable subsequent fluid losses from sweat and to stabilise body fluid compartments. After resting for 15 min, respiratory gases were collected continuously for 10 min. Immediately thereafter, a second blood sample was collected, and participants completed a cognitive function test and responded to the subjective feelings questionnaire before having a cool shower. Following the shower, participants dried themselves thoroughly, and a final nude BW measure was recorded prior to commencing the nutrition recovery period.

Nutrition recovery period. Participants completed a 4 h nutrition recovery period in an observation room adjacent to the exercise laboratory. During this time participants were able to read and watch TV. Participants were given immediate access to one of three trial beverages, in excess of expected consumption. The beverages included either water (repeated trial), a carbohydrate-electrolyte sports drink (Powerade[®], Coca Cola Ltd, Mountain Blast flavor) or a milk-based liquid meal supplement (Sustagen Sport[®], Nestle, Chocolate flavor) (Table 1). All beverages were stored in a personal fridge (4°C) in opaque jugs with participants instructed to “*self-serve*” into cups and to “*drink as much as they like*”. All beverage vessels (i.e. jugs and cups) contained no volume increments to prevent behavioral influences on fluid ingestion.

Table 1. Beverage characteristics.

Beverage	Energy kJ·100 mL ⁻¹	Protein g·100 mL ⁻¹	Fat g·100 mL ⁻¹	CHO g·100 mL ⁻¹	Na⁺ mg·100 mL ⁻¹
Water	0.0	0.0	0.0	0.0	0.0
Sustagen	417.0	6.5	0.2	17.6	67.0
Powerade	129.0	0.0	0.0	7.3	28.0

Participants were provided access to a variety of snack foods on two occasions (for 15 min at the end of the first and second hour of the recovery period). Participants entered a private room containing food and a computer (set up for the cognitive function task) and were informed they could bring their beverage; however, they were asked to refrain from taking in any external items (e.g. mobile phones). This approach was designed to prevent any distractions or interaction with the investigators or other participants and to reinforce the importance of the cognitive task. Food items included sports bars, fresh fruit, breads and condiments (see supplemental materials) with participants being informed to “*eat as much as they like*” and that “*more of the same food would be provided in 1 h but that no food could be removed from the private room due to safety regulations*”. Participants were also informed that no food would be provided in hours three and

four of recovery. In the final 2 min, participants completed the cognitive test and then returned to the main observation room to complete the subjective feelings questionnaire. Participants then provided urine (volume, USG) and blood (plasma osmolality) measures and a nude BW, prior to continuing *ad libitum* beverage consumption. The procedure for urine and BW collection was repeated each hour for the remaining 3h of the recovery period. Blood samples were collected at the end of hours 1 and 4 (Figure 1).

Data Collection

Food and fluid intake measures: Fluid volume and energy and nutrients consumed were calculated by weighing foods and beverages before and after each hour to the nearest 1g. All food weights for each participant were measured by one researcher (EI or NC). Food weights were then entered into a spreadsheet (Excel[®], Microsoft Office 2013) developed using manufacturers' values for packaged foods and Foodworks[®] (Version 6.0, 2014, Xyris Software, Australia) for fresh food items. Duplicate calculations of total macronutrient (protein, carbohydrate, fat), fluid and sodium consumption from food and beverage were verified by the same investigators. Results are described as "Beverage Volume" = total volume of the drink consumed and "Fluid Intake" = the total fluid component of both the beverages and food consumed.

Energy expenditure and compensation: Gas exchange data obtained via indirect calorimetry was averaged in 30 s segments over the final ~8 min of each collection period. Estimated energy expenditure (eEE) was quantified in the following increments: pre-exercise rest period (0-20 min), exercise period 1 (30-50 min), exercise period 2 (50-70 min) and exercise period 3 (70-90 min), post-exercise rest (95-110 min), and total trial (0-110 min). Rates and total substrate oxidation were calculated using the equations of Frayn (11) and assuming negligible protein

oxidation (27). Energy equivalents of $16.75 \text{ kJ}\cdot\text{g}^{-1}$ of carbohydrates and $37.68 \text{ kJ}\cdot\text{g}^{-1}$ of fat were utilised to calculate energy expenditure from substrate oxidation (27).

Blood sampling: Participants rested quietly for ~10 min in a supine position prior to a 5 mL blood sample being drawn from an antecubital vein. Blood samples (4 in total) were drawn pre-exercise (~20 min), post-exercise (~85 min) and during the nutrition recovery period at the end of hours one and four. All samples were collected into BD vacutainers[®] and immediately centrifuged at 10°C for 10 min at ~3000 g. Plasma samples were analysed in duplicate on an Osmomat 030 osmometer (calibrated to $300\text{m Osm}\cdot\text{kg}^{-1}$ using manufacturer's instructions) using the freezing point depression method to determine plasma osmolality.

Urine sampling: At the end of each hour of nutrition recovery, participants completely voided their bladder into a new empty 2 L container (to avoid contamination) for subsequent measures of hourly and total urine volumes. Participants were permitted to urinate throughout the observation period, and on each occasion, the void was collected and added to the hourly urine output. 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 (i.e., 4 h total). A sample of urine each hour was utilised to determine urine specific gravity (USG; Palette Digital Refractometer, ATAGO, USA) as an additional indicator of hydration status.

Total fluid retention: A value for total fluid retention was calculated by the following formula:

$$\text{Fluid retention} = \left[\frac{(\text{total volume of fluid (beverage \& food) consumed} - \text{total urine output})}{\text{total volume of fluid consumed}} \right] \times 100$$

Body weight measures: Nude body weight measures were obtained pre and post exercise and at the end of each hour of nutrition recovery (HW-PW200; A&D Company Ltd, Tokyo, Japan). Net body weight was calculated by subtracting the body weight (post voiding) from the initial body

weight, with faecal losses being excluded via immediate pre–post body weight measurements. When used across an acute time period, it is proposed that body weight changes take into account urinary, sweat and other insensible losses and provides an indication of hydration status (3). Given the *ad libitum* consumption of food, body weight changes can only be considered an approximate measure for fluid restoration.

Subjective ratings questionnaires: Adaptive Visual Analog Scales (AVAS) were used to assess subjective ratings of hunger, thirst, fullness and bloatedness. To distract participants from the primary outcomes of the study, participants also provided ratings of alertness, concentration, muscle soreness and energy levels. All measures were conducted on a 100 mm visual analogue scale, with 0 mm representing ‘not at all’ and 100 mm representing ‘extremely’ using a computerised modifiable software program (AVAS, Marsh-Richard, Hatzis, Mathias, Venditti and Dougherty (17). Subjective feelings were assessed at baseline (0 min), post-exercise (85 min), and at the end of each hour of nutrition recovery.

Data analysis: All statistical analyses were completed using SPSS Statistics for Windows, Version 21.0 (IBM Corp. 2012, Armonk, N.Y., USA). Comparisons between trials for baseline measures (BW, USG, and plasma osmolality), exercise-induced fluid losses, and for eEE, TEI, eREI and total macronutrient and sodium intake were conducted using one-way repeated measures analysis of variance (ANOVA). Post hoc analysis (Bonferroni) was conducted where significant main effects were present. Effect sizes were reported as partial eta squared (η_p^2). Differences between caloric containing beverages were assessed using paired samples t-tests. Two-way repeated-measures ANOVA (trial x time) were used to examine changes in body weight and subjective ratings throughout trials. Post hoc analysis (Bonferroni) was performed on all significant F ratios. Coefficient of variation (CV) for the repeated water trials was determined

using typical methods $((\text{standard deviation}/\text{mean}) * 100)$. Significant differences were accepted as $p < 0.05$. All data are reported as $\text{means} \pm \text{SD}$ unless otherwise specified.

RESULTS

Standardisation procedures. All 10 participants accepted into the study after initial screening and $\text{VO}_{2\text{max}}$ testing (age, 25.3 ± 4.9 years; height, 177.5 ± 6.1 cm; body weight, 70.34 ± 5.75 kg; $\text{VO}_{2\text{max}}$, 63.0 ± 7.2 ml·kg·min⁻¹) successfully completed all 4 experimental trials. On arrival at the laboratory, all participants except one stated compliance with the pre-trial dietary and exercise controls. One participant reported drinking 250 mL of apple juice at approximately 0430h on trial 1. This pre-trial variance was repeated on each subsequent trial to ensure consistency. A number of participants were provided a bolus of plain water (500-1000 mL) pre-trial due to pre-exercise USG values >1.020 . When water was provided on trial 1 ($n=1$, 1000 mL), this was repeated on all subsequent trials; otherwise a one-off bolus (500 mL) was provided as required for trials 2-4 ($n=4$ of 36 trials). Prior to commencing exercise all participants produced a urine sample that registered a USG <1.020 . No significant differences were observed in pre-exercise measures of BW ($W1=70.33\pm 5.49$ kg, $W2=70.48\pm 5.49$ kg, $P=70.04\pm 5.19$ kg, $SS=70.59\pm 5.32$ kg), USG ($W1=1.011\pm 0.01$, $W2=1.013\pm 0.01$, $P=1.016\pm 0.02$ and $SS=1.010\pm 0.01$, $p=0.441$), or P_{OSM} ($W1=304\pm 10$ mOsm·kg⁻¹, $W2=307\pm 12$ mOsm·kg⁻¹, $P=314\pm 19$ mOsm·kg⁻¹, $SS=298\pm 17$ mOsm·kg⁻¹, $p=0.18$) between trials. The total exercise time for each participant was 60 minutes in all trials. The exercise protocol induced a similar reduction in body weight in each trial (see Table 2; $p=0.830$).

Exercise measurements. All participants achieved the target fluid loss following 60 min of exercise. No differences in average RPE, $F(3,27)=1.385$; $p=0.269$ were observed between trials. There was a significant main effect of trial for average HR, $F(3,12)=6.273$; $p=0.008$, however, post hoc analysis failed to reveal any further significant differences between trials ($p>0.05$). Average HR data indicated that participants performed exercise at an intensity corresponding to

~80% HR_{max} in each of the trials. Mean body weight loss and estimated energy expenditure for each trial are detailed in Table 2. Examination of trial order revealed no main effect for energy expenditure, $F(3,21)=0.397$; $p=0.757$ or fluid loss, $F(3,27)=0.336$; $p=0.799$, indicating that trial results were not influenced by any exercise adaptation. No differences in body weight loss, $F(3,27)=0.288$; $p=0.834$ or energy expenditure, $F(3,21)=2.328$; $p=0.104$ were observed between experimental trials.

Table 2. Participant mean body weight loss and estimated energy expenditure from exercise (n=10).

Variable	W1	W2	P	SS	p value
eEE (kJ)	3936±545	4237±468	4363±691	4213±348	0.104
BW loss (%)	2.43±0.59	2.53±0.57	2.47±0.70	2.50±0.68	0.830

BW, body weight; eEE, estimated Energy Expenditure; W1, Water 1; W2, Water 2; P, Powerade; SS, Sustagen Sport. Means±SD.

Beverage intake. Analysis of trial order revealed no main effect for mean beverage volume consumed $F(3,27)=0.054$; $p=0.983$. Mean beverage volumes consumed for each trial during the recovery period were 2121±422 mL, 2076±608 mL, 2651±813 mL, and 1776±544 mL for W1, W2, P and SS, respectively. There were no statistical differences between the two water trials ($p=0.77$, $CV=12.6\%$). A significant difference was observed for the mean beverage volume consumed between experimental trials, $F(3, 27)=7.260$; $p=0.008$; $\eta_p^2=0.45$. Post hoc analysis revealed that participants drank significantly less SS compared with W1 ($p=0.002$) and P ($p=0.028$). There were no significant differences between any other beverages ($p>0.05$).

Total energy intake from food and beverages. The total mean energy intake (fluid and food) was 7826±888 kJ, 7578±1112 kJ, 10179±1484 kJ, and 10577±2210 kJ for W1, W2, P and SS, respectively. Mean energy intake was similar across both water trials ($p=0.55$, $CV=9.7\%$). A significant main effect was observed for total mean energy consumed between trials, $F(3,$

27)=14.635; $p<0.001$; $\eta_p^2=0.62$, with significantly more energy consumed on P and SS trials compared to both water trials (p 's<0.05). Comparison of individual data revealed all participants consumed more energy in the P trial and 9 of the 10 participants consumed more energy in the SS trial compared to both water trials. No difference was observed between P and SS for mean energy intake ($p=1.00$). Comparisons between the P and SS trials indicate some degree of individual variability, with a number of individuals consuming the same ($n=3$), less ($n=3$) or more ($n=4$) energy on the SS trial compared to the P trial.

Food weight and nutrient intake during recovery. Food weight and nutrient intake from food and beverages are displayed in Table 3. Participants consumed less food by weight in the SS trial compared to all other trials $F(3, 27) = 10.864$; $p<0.001$; $\eta_p^2=0.55$. Macronutrient consumption (beverage, food and total intake) was not significantly different between the water trials ($p>0.05$). Significant effects were observed between SS and all other trials with a higher total intake of protein, $F(3, 27) = 38.589$; $p<0.001$; $\eta_p^2=0.81$, and a lower total intake of fat, $F(3, 27) = 22.609$; $p<0.001$; $\eta_p^2=0.72$. CHO intakes for SS and P were not different from each other, however, both were significantly different to W1 and W2 trials, $F(3, 27) = 53.720$; $p<0.001$; $\eta_p^2=0.86$. Sodium intake was significantly higher during the P trial than all other trials $F(1.47, 13.23) = 7.166$; $p=0.012$; $\eta_p^2=0.44$.

Table 3. Total nutrient intake during 4 h recovery period (n=10).

<i>Total</i>	<i>W1</i>	<i>W2</i>	<i>P</i>	<i>SS</i>
<i>El, kJ</i>	7826±888 ^a	7578 ±1112 ^b	10179±1484	10577±2210
<i>Protein, g</i>	67±16	63±16	56±15	139±33 ^c
<i>CHO, g</i>	197±52 ^a	195±70 ^b	382±55	419±101
<i>Fat, g</i>	80±21	75±23	64±20	31±12 ^c
<i>Sodium, mg</i>	1541±403	1419±291	2030±428 ^d	1796±356
<i>Beverage</i>				
<i>El, kJ</i>	0	0	3396±934	7406±2270 ^c
<i>Protein, g</i>	0	0	0	115±35 ^c
<i>CHO, g</i>	0	0	196±58	313±96 ^c
<i>Fat, g</i>	0	0	0	4±1 ^c
<i>Sodium, mg</i>	0	0	753±221	1190±365 ^c
<i>Food</i>				
<i>El, kJ</i>	7826±888	7578±1112	6783±1834	3171±1063 ^c
<i>Protein, g</i>	67±16	63±16	56±15	24±12 ^c
<i>CHO, g</i>	197±52	195±70	186±69	107±54 ^c
<i>Fat, g</i>	80±21	75±23	64±20	28±13 ^c
<i>Sodium, mg</i>	1541±403	1419±291	1277±515	606±343 ^c
<i>Food weight, g</i>	520±98	526±114	523±148	343±143 ^c

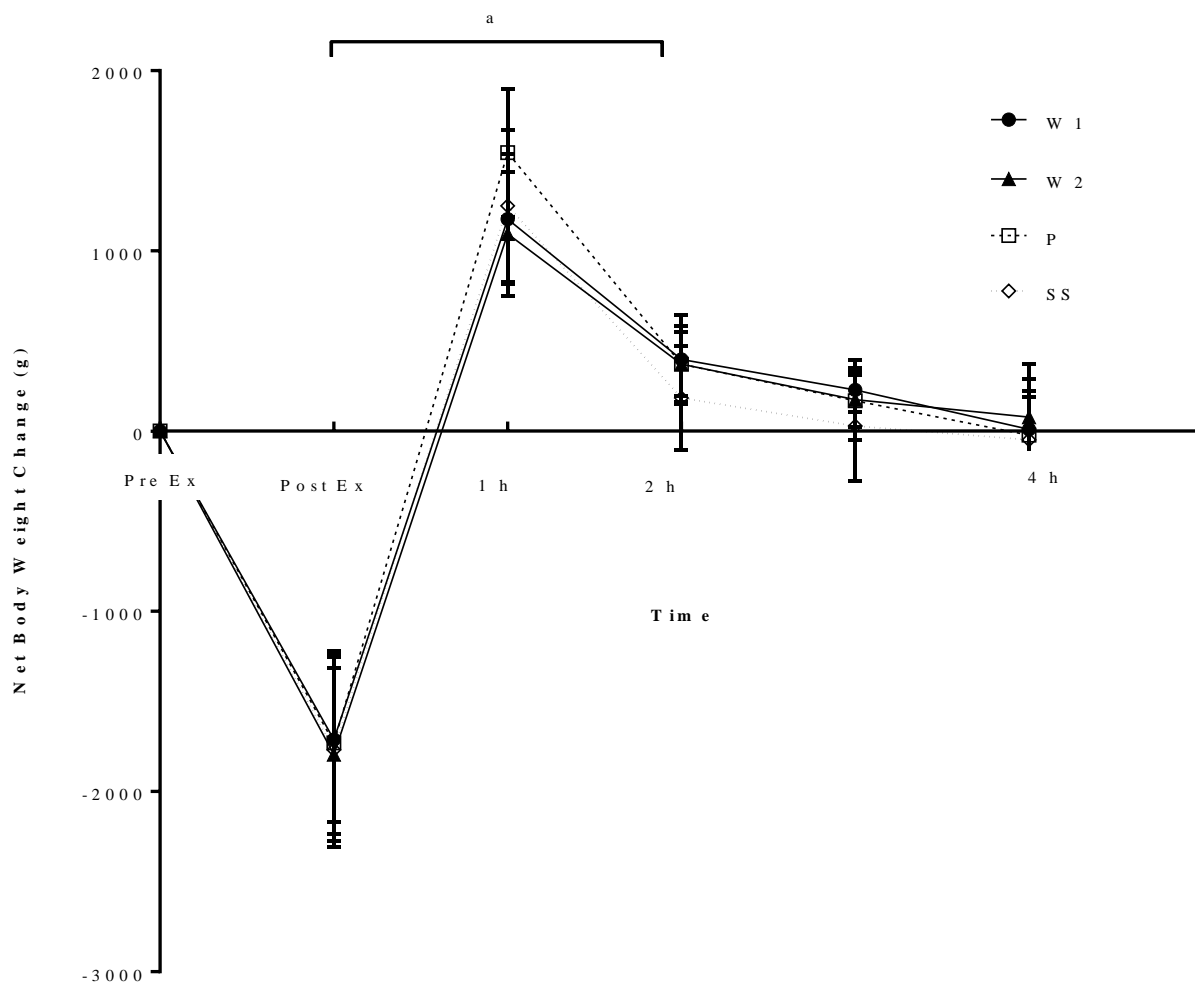
El, Energy intake, W1, Water 1; W2, Water 2; P, Powerade; SS, Sustagen Sport. ^aSignificant difference between W1 and P and SS. ^bSignificant difference between W2 and P and SS. ^cSignificant difference between SS and all other trials. ^dSignificant difference between P and all other trials. Means±SD.

Urine loss and total fluid intake and retention. At the end of recovery, participant's total urine outputs were W1=644±202 mL, W2=602±352 mL, P=879±752 mL and SS=466±129 mL. There were no main effects for total urine volume collected over the trial, $F(1.276, 11.484)=2.127$; $p=0.171$. Participant's total fluid intakes (food and beverage) were W1=2277±417 mL, W2=2213±619 mL, P=2819±800 mL and SS=1939±536 mL with $\leq 3\%$ of total fluid provided by fluid in food. A significant difference was observed for the total fluid consumed between experimental trials, $F(1.694, 15.257)=7.215$; $p=0.008$; $\eta^2=0.45$. Post hoc analysis revealed that participants consumed significantly more fluid on the W1 and P trials compared to SS (all $p<0.05$). There were no significant differences between any other trial ($p>0.05$). Total fluid retention values for each trial were W1=72±8 %, W2=73±11 %, P=72±17 % and SS=74±10 % and were not significantly different from one another, $F(1.455, 13.093)=0.086$; $p=0.861$. At the

end of recovery, participant's final USG and P_{OSM} were $W1=1.012\pm0.01$, $W2=1.012\pm0.01$, $P=1.015\pm0.01$ and $SS=1.020\pm0.00$ and $W1=307\pm14$ mOsm \cdot kg $^{-1}$, $W2=314\pm9$ mOsm \cdot kg $^{-1}$, $P=310\pm6$ mOsm \cdot kg $^{-1}$ and $SS=310\pm21$ mOsm \cdot kg $^{-1}$, respectively. No main effect of trial was observed for USG, $F(3, 27)=0.787$, $p=0.512$ or P_{OSM} values, $F(3, 24)=0.629$, $p=0.603$.

Net body weight change. Mean net BW change for each trial is displayed in Figure 2. All participants entered the recovery period in a state of negative net BW relative to pre-exercise values ($W1=-1.71\pm0.46$ kg, $W2=-1.80\pm0.48$ kg, $P=-1.73\pm0.51$ kg, $SS=-1.77\pm0.54$ kg; $p=0.70$). A significant main effect of trial on net BW change was observed during the recovery period, $F(2.005, 18.049)=5.812$, $p=0.011$; however, post hoc analysis revealed no significant differences between trials ($p>0.05$). There was a significant main effect of time on net BW change during the recovery period, $F(1.857, 16.710)=147.868$, $p<0.001$. Post hoc analysis revealed net body weight increased significantly during the first hour of recovery compared to the post-exercise values ($p<0.05$) for all trials. Net BW change decreased significantly in the second hour of recovery for all trials ($p<0.05$). During the last 2 hours of recovery, net body weight change remained the same in all trials. No trial by time interaction was observed, $F(0.294, 0.147)=1.993$, $p=0.108$.

Figure 2. Net body weight change throughout experimental trials (includes exercise-induced fluid loss and subsequent 4 h recovery period with *ad libitum* consumption of one of four different beverages + food (n=10)).



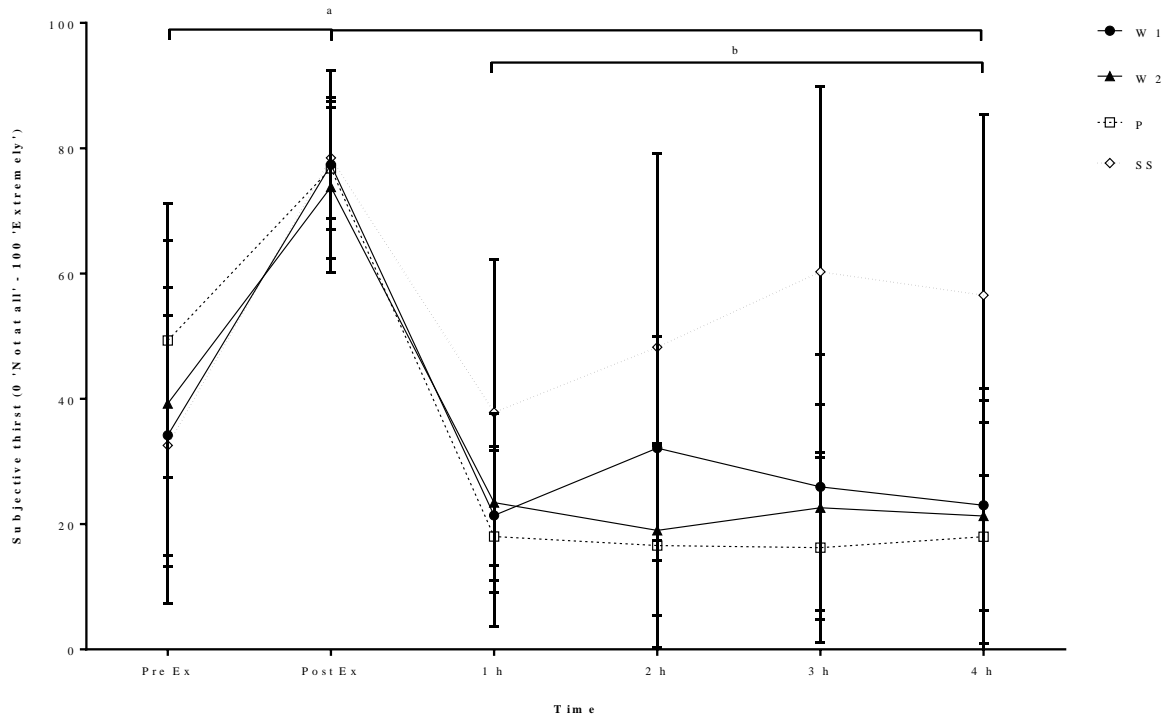
^aSignificant difference in net body weight change compared to previous hour. Pre Ex, Pre-exercise. Post Ex, post-exercise. Means \pm SD.

Subjective feelings. There were no significant trial effects for fullness $F(3, 27) = 2.095$; $p=0.124$, and hunger $F(3, 27) = 0.848$; $p=0.480$. However, there were main effects of time for fullness $F(5, 45) = 42.300$; $p<0.001$, and hunger $F(5, 45) = 38.140$; $p<0.001$. The ratings of perceived thirst and bloatedness are presented in Figure 3 (a and b, respectively). For perceived thirst, there was a significant main effect of trial, $F(3, 27) = 9.408$; $p < 0.001$; time, $F(5, 45) = 30.532$; $p<0.001$ and trial by time interaction, $F(15, 135) = 6.368$; $p<0.001$. Post hoc analysis identified a significant increase in thirst from pre to post exercise and a significant decrease

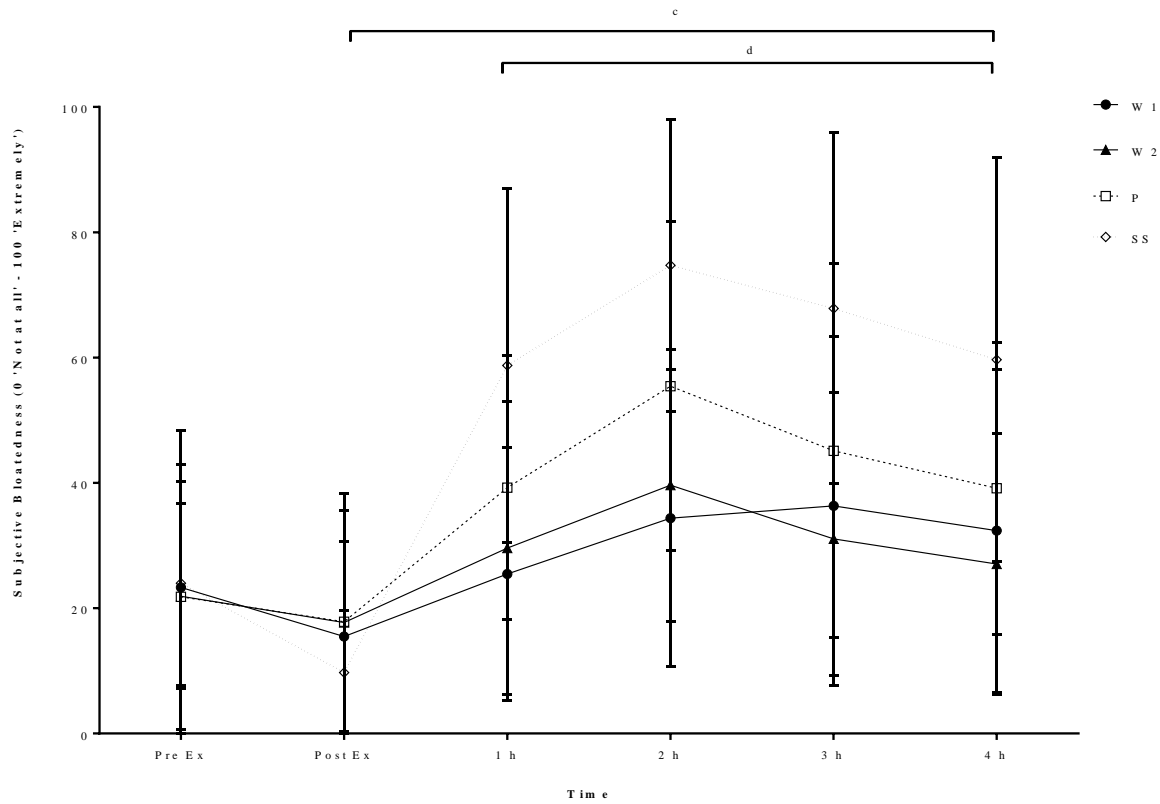
between post-exercise and all subsequent recovery time points ($p < 0.05$). Post hoc analysis also revealed significantly lower thirst rating for P compared to SS from hour 1 through to hour 4 of recovery (all p s < 0.05). No other trial effects were observed at any other time points ($p > 0.05$). There was a significant main effect on bloating for trial, $F(3, 27) = 9.107$; $p < 0.001$; time, $F(5, 45) = 14.573$; $p < 0.001$, and trial by time interaction, $F(15, 135) = 3.804$; $p < 0.001$. Post hoc analysis revealed a significant increase in perceived bloating during SS trials compared to the two water trials. This effect was evident from hour 1 through to hour 4 of recovery (all p s < 0.05). No other trial effects were observed for bloating. Post hoc analysis also revealed a significant increase in subjective ratings of bloating between post exercise and all subsequent time points (all p s < 0.05). No other time effects were observed.

Figure 3. Participant ($n=10$) subjective ratings of thirst (A) and bloatedness (B).

A.



B.



^aSignificant increase in thirst from pre to post exercise and a significant decrease between post-exercise and all subsequent recovery time points ($p < 0.05$). ^bSignificantly lower thirst rating for P compared to SS from hour 1 through to hour 4 of recovery (all $ps < 0.05$). ^cSignificant increase in subjective ratings of bloating between post exercise and all subsequent time points (all $ps < 0.05$). ^dSignificant increase in perceived bloating during SS trials compared to the two water trials (all $ps < 0.05$). No other trial effects were observed for bloating. No other significant effects were observed. Means \pm SD.

DISCUSSION

The purpose of this study was to evaluate the effect of *ad libitum* consumption of different beverages (caloric and non-caloric) and snack foods on fluid retention and total energy and nutrient intake following exercise. The primary finding from this study indicates that when drinking voluntarily and with access to food, individuals achieve similar levels of fluid retention irrespective of beverage type. Furthermore, the consumption of a caloric beverage resulted in greater total energy consumption and differences in nutrient intakes compared to the consumption of water with food. It is imperative when making post exercise nutrition recommendations to consider beverage selection and the availability of food.

The findings from this study demonstrate that the consumption of food following exercise plays an important role in mediating fluid retention when different beverages are consumed. The fluid provided from all beverages and food consumed was equally retained despite different consumption volumes and resulted in participant's body weights returning to near pre-exercise levels (Nb. the food contribution to body weight change was small (~0.34-0.53 kg) in comparison to body weight shifts recorded). Importantly, the beverages selected for this investigation were chosen on the basis of existing evidence suggesting contrasting fluid retention capacity when consumed alone (9, 13, 14). The present results improve our understanding of the interaction between fluid and nutrient recovery following exercise in that the investigation employed *ad libitum* consumption of different beverages and provided access to a variety of foods typically available following exercise. Our results suggest that active individuals unknowingly self-select foods that compensate for the beverage, altering nutrient intake and ultimately fluid retention. This suggests that with consumption of food, fluid restoration is tightly regulated and not influenced by the choice of either water, a carbohydrate-electrolyte (sports drink) or a milk-based beverage.

Previous research has demonstrated that consuming food with beverages post exercise enhances fluid retention compared to carbohydrate-electrolyte sports drinks (18, 25). While the present study had no beverage only trial for direct comparisons, results from previous experiments suggest the consumption of food with fluid can enhance fluid retention (18, 25). The consumption of food with fluid may result in higher nutrient and energy intakes and greater gastric distention, which together, are likely to delay gastric emptying (16). To date, only limited food items have been examined (rice/beef meal (18), beef jerky (25)) and on both occasions food and fluid intakes were prescribed. In practice, individuals may be limited in choice to convenient and easily packaged foods (such as recovery bars, snack foods (e.g. chips) or sandwiches) with their selection and consumption based on personal preferences, attitudes and beliefs. This study provided participants with access to a range of food items that could be consumed voluntarily. The results indicated that individual's food selections differed considerably based on the accompanying beverage. For instance, while total sodium intake was consistent across trials, when consuming beverages with low concentrations of sodium (W1, W2 and P) participants consumed substantially more sodium from food. Likewise, total protein intake was significantly higher in the SS trial, however, protein intake from food was substantially lower on this trial suggesting some form of compensation. These findings prompted further analysis of the food intake data which subsequently did not reveal changes to sodium or protein intake relative to energy consumed (Sodium ~19 mg/100kJ, $p=0.641$, Protein ~14% of total energy, $p=0.238$). Thus, it appears the differences in total nutrient intakes from food were likely a reflection of changes to total food consumption, in contrast to the purposeful selection of specific food items by participant's seeking to moderate the intake of nutrients that were available (or absent) within a trial beverage.

The results of the current investigation provide novel insights that may influence fluid intake advice provided to athletes following exercise. While the effects of consuming different beverages (e.g. protein containing sports drinks or milk) following exercise on subsequent dietary intake have been investigated (5, 7, 26), these studies all prescribed the volume of fluid. In this setting, the prescription of ~500mL of milk or other protein containing beverages does not appear to substantially influence post-exercise energy consumption (5, 7, 26). However, the results of the current study suggest that when presented with the option to self-regulate beverage intake, participant's response to different beverages substantially influences overall dietary intake. After training and/or competition many athletes have concurrent nutrient considerations (e.g. substrate restoration, skeletal muscle adaptation and repair and fluid recovery) and dietary goals (e.g. increasing lean body weight or ensuring an adequate calcium intake). The current results would indicate that in circumstances where weight gain or maximising nutrient intake are desired, the consumption of caloric beverages (P and SS) with food may facilitate simultaneous rehydration, refuelling and positive energy balance. Alternatively, in situations where weight loss or maintenance goals are the priority, water consumption with food should be encouraged to facilitate rehydration without excessive caloric intake.

The method employed in this study allowed participants to voluntarily consume a selected beverage. Studies using *ad libitum* consumption consider factors such as thirst, palatability, and gastrointestinal comfort; which are likely to influence the volume of beverage consumed in practice. The current results indicate that when water is consumed following exercise, less variability is observed in volume consumed compared with previous reports involving repeated trials with sports drinks and milk-based dietary supplements (4). When beverages contribute to nutrient intake beyond fluid alone, there is potential for this to influence

consumption patterns and alter the outcome of recovery. For example, the subjective ratings of bloatedness and thirst on SS were in contrast, with participants being thirsty yet unwilling to consume the beverage in quantities consistent with the other beverage treatments. Clearly, an individual's response to fluid and food following exercise is complex and is likely to be influenced by the interaction of many factors beyond those of this investigation (e.g. nutrition knowledge, beliefs, food preferences, food costs, exercise intensity, gender, social environment, time of day etc.). The influence of psychological, social and physiological factors on eating behavior in a laboratory setting are important considerations when interpreting the current data (8). Further research employing protocols that reflect ecologically valid post-exercise conditions and practices of athletes are warranted.

This study demonstrates that beverages with significantly different nutrient profiles were equally effective at promoting fluid retention, and restoring exercise-induced fluid losses when consumed with snack foods *ad libitum*. The choice of a post-exercise recovery beverage should consider hydration properties in the context of an individual's post-exercise energy requirements and overall dietary goals.

Acknowledgements

The generous help of Dr Surendran Sabapathy, Dr Glenn Stewart, Ms Caroline Young and the research participants is gratefully acknowledged. This study was funded by internal institutional support. No author conflicts of interest exist in relation to the results of this study.

References

1. American College of Sports M, Sawka MN, Burke LM et al. American College of Sports Medicine position stand. Exercise and fluid replacement. *Med Sci Sports Exerc.* 2007;39(2):377-90.
2. American College of Sports Medicine. Joint Position Statement: nutrition and athletic performance. American College of Sports Medicine, American Dietetic Association, and Dietitians of Canada. *Med Sci Sports Exerc.* 2000;32(12):2130.
3. Armstrong LE. Hydration assessment techniques. *Nutr Rev.* 2005;63(6 Pt 2):S40-54.
4. Baguley B, Zilujko J, Leveritt MD, Desbrow B, Irwin C. The Effect of Ad Libitum Consumption of a Milk-Based Liquid Meal Supplement vs a Traditional Sports Drink on Fluid Balance After Exercise. *Int J Sport Nutr Exerc Metab.* 2015.
5. Brown MA, Green BP, James LJ, Stevenson EJ, Rumbold PL. The Effect of a Dairy-Based Recovery Beverage on Post-Exercise Appetite and Energy Intake in Active Females. *Nutrients.* 2016;8(6).
6. Burke L, Deakin V. *Clinical Sports Nutrition, 4th Edition.* McGraw-Hill Education; 2009.
7. Clayton DJ, Stensel DJ, Watson P, James LJ. The effect of post-exercise drink macronutrient content on appetite and energy intake. *Appetite.* 2014;82:173-9.
8. de Castro JM. Eating behavior: lessons from the real world of humans. *Nutrition.* 2000;16(10):800-13.
9. Desbrow B, Jansen S, Barrett A, Leveritt MD, Irwin C. Comparing the rehydration potential of different milk-based drinks to a carbohydrate-electrolyte beverage. *Appl Physiol Nutr Metab.* 2014;39(12):1366-72.
10. Evans GH, Shirreffs SM, Maughan RJ. Postexercise rehydration in man: the effects of carbohydrate content and osmolality of drinks ingested ad libitum. *Appl Physiol Nutr Metab.* 2009;34(4):785-93.
11. Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol Respir Environ Exerc Physiol.* 1983;55(2):628-34.
12. Hawley JA, Tipton KD, Millard-Stafford ML. Promoting training adaptations through nutritional interventions. *J Sports Sci.* 2006;24(7):709-21.
13. James LJ, Clayton D, Evans GH. Effect of milk protein addition to a carbohydrate-electrolyte rehydration solution ingested after exercise in the heat. *Br J Nutr.* 2011;105(3):393-9.
14. James LJ, Evans GH, Madin J et al. Effect of varying the concentrations of carbohydrate and milk protein in rehydration solutions ingested after exercise in the heat. *Br J Nutr.* 2013;110(7):1285-91.
15. James LJ, Gingell R, Evans GH. Whey protein addition to a carbohydrate-electrolyte rehydration solution ingested after exercise in the heat. *J Athl Train.* 2012;47(1):61-6.
16. Kwiatek MA, Menne D, Steingoetter A et al. Effect of meal volume and calorie load on postprandial gastric function and emptying: studies under physiological conditions by combined fiber-optic pressure measurement and MRI. *Am J Physiol Gastrointest Liver Physiol.* 2009;297(5):G894-901.
17. Marsh-Richard DM, Hatzis ES, Mathias CW, Venditti N, Dougherty DM. Adaptive Visual Analog Scales (AVAS): a modifiable software program for the creation,

- administration, and scoring of visual analog scales. *Behav Res Methods*. 2009;41(1):99-106.
18. Maughan RJ, Leiper JB, Shirreffs SM. Restoration of fluid balance after exercise-induced dehydration: effects of food and fluid intake. *Eur J Appl Physiol Occup Physiol*. 1996;73(3-4):317-25.
 19. Maughan RJ, Leiper JB, Shirreffs SM. Factors influencing the restoration of fluid and electrolyte balance after exercise in the heat. *Br J Sports Med*. 1997;31(3):175-82.
 20. Merson SJ, Maughan RJ, Shirreffs SM. Rehydration with drinks differing in sodium concentration and recovery from moderate exercise-induced hypohydration in man. *Eur J Appl Physiol*. 2008;103(5):585-94.
 21. Minehan MR, Riley MD, Burke LM. Effect of flavor and awareness of kilojoule content of drinks on preference and fluid balance in team sports. *Int J Sport Nutr Exerc Metab*. 2002;12(1):81-92.
 22. Mitchell JB, Phillips MD, Mercer SP, Baylies HL, Pizza FX. Postexercise rehydration: effect of Na(+) and volume on restoration of fluid spaces and cardiovascular function. *J Appl Physiol (1985)*. 2000;89(4):1302-9.
 23. Nose H, Mack GW, Shi XR, Nadel ER. Role of osmolality and plasma volume during rehydration in humans. *J Appl Physiol (1985)*. 1988;65(1):325-31.
 24. Osterberg KL, Pallardy SE, Johnson RJ, Horswill CA. Carbohydrate exerts a mild influence on fluid retention following exercise-induced dehydration. *J Appl Physiol (1985)*. 2010;108(2):245-50.
 25. Pryor JL, Johnson EC, Del Favero J, Monteleone A, Armstrong LE, Rodriguez NR. Hydration Status and sodium balance of Endurance Runners Consuming Post-exercise Supplements of Varying Nutrient Content. *Int J Sport Nutr Exerc Metab*. 2015.
 26. Rumbold P, Shaw E, James L, Stevenson E. Milk consumption following exercise reduces subsequent energy intake in female recreational exercisers. *Nutrients*. 2015;7(1):293-305.
 27. Schubert MM, Hall S, Leveritt M, Grant G, Sabapathy S, Desbrow B. Caffeine consumption around an exercise bout: effects on energy expenditure, energy intake, and exercise enjoyment. *J Appl Physiol (1985)*. 2014;117(7):745-54.
 28. Seifert J, Harmon J, DeClercq P. Protein added to a sports drink improves fluid retention. *Int J Sport Nutr Exerc Metab*. 2006;16(4):420-9.
 29. Shirreffs SM. Markers of hydration status. *Eur J Clin Nutr*. 2003;57 Suppl 2:S6-9.
 30. Shirreffs SM, Aragon-Vargas LF, Keil M, Love TD, Phillips S. Rehydration after exercise in the heat: a comparison of 4 commonly used drinks. *Int J Sport Nutr Exerc Metab*. 2007;17(3):244-58.
 31. Shirreffs SM, Armstrong LE, Chevront SN. Fluid and electrolyte needs for preparation and recovery from training and competition. *J Sports Sci*. 2004;22(1):57-63.
 32. Shirreffs SM, Maughan RJ. Volume repletion after exercise-induced volume depletion in humans: replacement of water and sodium losses. *Am J Physiol*. 1998;274(5 Pt 2):F868-75.
 33. Shirreffs SM, Maughan RJ. Rehydration and recovery of fluid balance after exercise. *Exerc Sport Sci Rev*. 2000;28(1):27-32.
 34. Shirreffs SM, Taylor AJ, Leiper JB, Maughan RJ. Post-exercise rehydration in man: effects of volume consumed and drink sodium content. *Med Sci Sports Exerc*. 1996;28(10):1260-71.

35. Watson P, Love TD, Maughan RJ, Shirreffs SM. 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. *Eur J Appl Physiol.* 2008;104(4):633-42.

Highlights

- When food is co-ingested, fluid restoration is tightly regulated and not influenced by the choice of either water, a carbohydrate-electrolyte (sports drink) or a milk-based beverage. This is distinctively different from prescribed beverage volume studies that have indicated a beverage's nutrient composition has a substantial influence on fluid recovery.
- Active individuals self-select foods, altering nutrient intake and ultimately fluid retention.
- Acute energy intake is poorly regulated with the selection of calorie containing beverages (sports drink or milk-based drink) leading to higher energy and altered nutrient intakes. We anticipate our results will initiate significant debate and new inquiry into the utility of beverages specifically formulated for post-exercise recovery when consumed voluntarily by active individuals who also have access to food.