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- 1 Mouth rinsing with a sweet solution increases energy expenditure and decreases
- 2 appetite during 60 minutes of self-regulated walking exercise.

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- 4 Kevin Deighton¹, Lauren Duckworth¹, Jamie Matu¹, Matthew Suter¹, Charlotte Fletcher¹,
- 5 Samuel Stead¹, Shaho Ali¹, Neil Gunby¹, Keelie Korsness¹

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7 ¹Institute for Sport, Physical Activity & Leisure, Leeds Beckett University, UK

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- 9 Correspondence
- 10 Dr Kevin Deighton
- 11 Institute for Sport, Physical Activity & Leisure,
- 12 Leeds Beckett University,
- 13 Leeds
- 14 LS6 3QS
- 15 United Kingdom
- 16 Phone: +44 (0)113 81 23582
- 17 E-mail: K.Deighton@leedsbeckett.ac.uk

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Abstract

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Carbohydrate mouth rinsing can improve endurance exercise performance and is most ergogenic when exercise is completed in the fasted state. This strategy may also be beneficial to increase exercise capacity and the energy deficit achieved during moderate intensity exercise relevant to weight control when performed after an overnight fast. Eighteen healthy men (mean(SD); age 23(4) years, body mass index 23.1(2.4)kg.m⁻²) completed a familiarisation trial and three experimental trials. After an overnight fast, participants performed 60-minutes of treadmill walking at a speed that equated to a rating of perceived exertion of 13 ("fairly hard"). Participants manually adjusted the treadmill speed to maintain this exertion. Mouth rinses for the experimental trials contained either a 6.4% maltodextrin solution with sweetener (CHO), a taste-matched placebo (PLA) or water (WAT). Appetite ratings were collected using visual analogue scales and exercise energy expenditure and substrate oxidation were calculated from online gas analysis. Increased walking distance during CHO and PLA induced greater energy expenditure compared with WAT (mean difference (90% CI); 79(60)kJ; P=0.035; d=0.24 and 90(63)kJ; P=0.024; d=0.27, respectively). Appetite area under the curve was lower in CHO and PLA than WAT (8(6)mm; P=0.042; d=0.43 and 6(8)mm; P=0.201; d=0.32, respectively). Carbohydrate oxidation was higher in CHO than PLA and WAT (7.3(6.7)g; P=0.078; d=0.47)and 10.1(6.5)g; P=0.015; d=0.81, respectively). This study provides novel evidence that mouth rinsing with a sweetened solution may promote a greater energy deficit during moderate exertion walking exercise by increasing energy expenditure and decreasing appetite. A placebo effect may have contributed to these benefits.

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- Key words: appetite regulation, energy balance, metabolism, substrate oxidation, cephalic
- 43 phase response, oral nutrient sensing

Introduction

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Carbohydrate mouth rinsing has become established as a successful strategy to improve endurance performance when exercise is completed after an overnight fast or in a postabsorptive state (> 4 h) (Ataide-Silva et al. 2016; Carter et al. 2004; Chambers et al. 2009; Fraga et al. 2015; Gam et al. 2013; Rollo et al. 2008; Sinclair et al. 2014). However, the ergogenic effect of carbohydrate mouth rinsing is reduced by pre-exercise feeding (Ataide-Silva et al. 2016; Lane et al. 2013) and it remains debated whether there are any beneficial effects when exercise is performed in the postprandial state (Ataide-Silva et al. 2016; Beelen et al. 2009; Lane et al. 2013). Although nutritional recommendations for sports performance advocate the consumption of a high carbohydrate meal within the four hours prior to commencing exercise (ACSM 2016), low and moderate intensity exercise for weight control is often performed after an overnight fast in order to enhance fat oxidation (Deighton et al. 2012). The influence of carbohydrate mouth rinsing on low and moderate intensity exercise is currently unknown but the ergogenic effects observed during high intensity exercise appear to be due to an increased self-selected exercise intensity without a corresponding increase in exertion (Carter et al. 2004; Chambers et al. 2009; Gam et al. 2013; Rollo et al. 2008; Sinclair et al. 2014). This has also been associated with increased feelings of pleasure during exercise (Rollo et al. 2008) and evidence suggests that these ergogenic effects are mediated by an increased activation of the brain centres involved in reward, motivation and motor control (Chambers et al. 2009). It seems plausible that these mechanisms may also improve performance during low and moderate intensity exercise, which would increase energy expenditure and the energy deficit associated with exercise. Based on this rationale, recent review articles have encouraged investigations into the effects of carbohydrate mouth rinsing on exercise protocols that are relevant for weight control (Burke & Maughan 2014; Rollo & Williams 2011).

In addition to benefitting exercise performance, exposure of the oral cavity to nutrients has also been demonstrated to have an anorectic effect. In this regard, masticating and expectorating food from the mouth during modified sham feeding has been shown to reduce appetite perceptions (Heath et al. 2004; Smeets & Westerterp-Plantenga 2006). Such reductions in appetite without the ingestion of nutrients represents another potential benefit of carbohydrate mouth rinsing for weight control but the effect of rinsing a caloric solution rather than masticating solid foods is currently unknown.

Thus, the purpose of this study was to investigate the influence of carbohydrate mouth rinsing on self-selected exercise intensity, energy expenditure, substrate oxidation and appetite perceptions during treadmill walking exercise at a fixed moderate exertion. This represents the first investigation into the effects of carbohydrate mouth rinsing on moderate exertion exercise performance and appetite regulation. We hypothesised that mouth rinsing with carbohydrate would improve walking performance and decrease appetite perceptions.

Methods

Participants

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the Ethics Advisory Committee at Leeds Beckett University. Eighteen recreationally active male participants were recruited for the study and written informed consent was obtained from all participants. Participants were non-smokers, not taking medication, weight stable for at least six months before the study and were not dieting. The physical characteristics of participants (mean (SD) were as follows: age 23 (4) years, body mass 72.2 (2.4) kg, body mass index (BMI) 23.1 (2.4) kg.m⁻²). This population was selected based on evidence that young, healthy men are most sensitive to nutrient manipulation (Appleton et al. 2011; Davy et al. 2007), thereby maximising the likelihood of observing an effect in this proof of concept investigation.

Experimental design

Each participant performed one familiarisation trial and three experimental trials separated by approximately one week. All trials were identical and the experimental trials were completed using a double-blind, counterbalanced Latin Square design. The mouth rinse solutions for the experimental trials consisted of a 6.4% maltodextrin solution sweetened with saccharin (CHO), water sweetened with saccharin (PLA), or plain water (WAT). The addition of saccharin to the CHO and PLA solutions ensured that they were matched for taste. To investigate the possibility of a placebo effect with carbohydrate mouth rinsing, participants were told that both flavoured solutions contained carbohydrate and that the purpose of the study was to compare this with rinsing a water solution (Hulston & Jeukendrup 2009).

Physical activity and dietary standardisation

Participants completed a food diary during the 24-h before the first experimental trial and replicated this before each subsequent trial. Alcohol, caffeine and strenuous physical activity were not permitted during this period. Participants arrived at the laboratory between 0700 h and 0800 h after an overnight fast of at least 10 h and exerted themselves minimally when travelling to the laboratory using motorised transport when possible. Verbal confirmation of dietary and exercise standardisation was obtained at the beginning of each trial.

Experimental trials

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During each trial, participants completed 60-min of walking exercise on a motorised treadmill using a 1% gradient (Model ELG 70, Woodway, Germany). Participants were asked to manually increase their speed using the buttons available on the arm of the treadmill until they reached a speed corresponding to a rating of perceived exertion (RPE) of 13 indicating "fairly hard" (Borg 1973). Participants were required to achieve this speed within 2-min and the 60min walk commenced from this point. Throughout the trial, participants were permitted to alter the speed of the treadmill in order to maintain an RPE of 13. The prescription of exercise at a self-regulated RPE of 13 is supported by previous research that has demonstrated this to produce positive affective responses as well as improvements in fitness and cardiovascular health markers (Parfitt et al. 2012). Participants were able to view the elapsed time during the walk but were blinded from the speed and distance. The only interaction between researchers and participants was during the provision of mouth rinses and collection of subjective scales. No other encouragement or distraction was permitted and the participants did not receive any feedback about the distance covered until the completion of the study. Heart rate was measured at 15-min intervals during the exercise (Polar FT1, Finland). The temperature and relative humidity of the laboratory were maintained at 20 (1) °C and 39 (9) %, respectively.

Expired gas analysis

Expired gas was collected continuously throughout the walk using an online gas analysis system (Metalyzer 3B, Cortex, Germany) and averaged over the 60-min period. During rinsing, the facemask was unclipped and gas analysis data for the subsequent 60-s period was excluded. Energy expenditure and substrate oxidation rates were calculated from VO₂ and VCO₂ values using the equations of Frayn (1983).

Mouth rinse protocol

Participants were required to rinse their mouth with one of the experimental solutions (CHO, PLA or WAT) at -10, -5, 0, and every 7.5 min thereafter throughout the 60-min walk. In accordance with previous research, 25 mL of solution was rinsed around the oral cavity for 10 seconds before being expectorated into a pre-weighed cup (Sinclair et al. 2014).

During each trial, appetite perceptions (hunger, satisfaction, fullness and prospective food

Subjective scales

consumption) were assessed at -10, 0, 30 and 60 min using 100 mm visual analogue scales (Flint et al. 2000). A composite appetite rating was calculated as the mean value of the four appetite perceptions after inverting the values for satisfaction and fullness (Stubbs et al. 2000). Feelings of thirst were assessed at -10, 0, and every 15 min during exercise using an adapted Borg scale (Ispoglou et al. 2015).

Affective responses to exercise were assessed at -10, 0 and every 15 min during exercise using the Feeling Scale to assess pleasure-displeasure (Hardy & Rejeski 1989) and the Felt Arousal Scale to assess perceived activation (Svebak & Murgatroyd 1985). All subjective scales were completed immediately before the administration of mouth rinses. Participants were reminded to maintain an RPE of 13 after the completion of each mouth rinse.

Statistical analysis

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Data were analysed using IBM SPSS statistics version 22 for Windows. Time-averaged area under the curve (AUC) values were calculated for all subjective scales using the trapezoidal method. One-way repeated measures ANOVA was used to assess trial based differences in total distance walked, energy expenditure, substrate oxidation and mean heart rate during exercise, as well as time-averaged AUC values for appetite, thirst and affective responses. Area under the curve values were used for statistical analysis rather than individual timepoints in accordance with the statistical guidance provided by Matthews and colleagues (1990) and the subject-specific guidance provided by Blundell et al. (2010). Where significant main effects of trial were observed, post-hoc analysis was performed using unadjusted Student's paired t-tests. Pearson's product-moment correlation coefficient was used to examine the relationship between BMI and the response to carbohydrate and placebo mouth rinsing compared with water rinsing for the primary outcome variables (distance walked, energy expenditure, substrate oxidation, appetite perceptions). Null-hypothesis significance testing was performed with an alpha value of 5 % in accordance with current convention. In addition to null-hypothesis significance testing, magnitude-based inferences were calculated to examine whether the observed differences were meaningful. This approach has been supported within biomedical and exercise sciences, in addition to other fields such as computer studies (van Schaik & Weston 2016), due to reduced inferential error rates compared with nullhypothesis significance testing (Hopkins & Batterham 2016). This approach also promotes direct interpretation of the magnitude of changes and whether these are meaningful (Buchheit 2016). Subsequently, this approach was utilised and prioritised for the appropriate variables. Using the spreadsheet by Hopkins (2007), the P value was converted into 90% confidence intervals (CI) for inferences about the true value of the effect statistic (Hopkins 2007). An effect was deemed unclear when the upper and lower confidence limits represented meaningful

increases and decreases, respectively. All other effects were deemed clear, and the probabilities that the true effect was a substantial increase, a trivial change, and a substantial decrease were calculated via the sampling t-distribution of the effect in relation to the smallest worthwhile change. The smallest worthwhile change in walking distance was set at 1% to allow comparisons with previous research that has used this threshold to investigate changes in selfregulated running exercise with carbohydrate mouth rinsing (Rollo et al. 2011). An 8 mm difference in composite appetite time-averaged AUC was set as the smallest worthwhile change in appetite perceptions (Blundell et al. 2010). Meaningful changes in the remaining variables are not well-established, which limited the analysis of beneficial, trivial or negative effect magnitudes to walking distance and appetite perceptions only. All results in the text are presented as mean (SD) or 90% confidence intervals where appropriate. Graphical representations of results are presented as mean (SEM) to avoid distortion of the graphs. Effect sizes are presented as Cohen's d and interpreted as ≤ 0.2 trivial, > 0.2 small, > 0.6 moderate, > 1.2 large, > 2 very large and > 4 extremely large (Hopkins 2004). Individual responses are presented within figures to allow further examination of the findings. The inclusion of null-hypothesis significance testing and magnitude-based inferences within the results was intended to support the interpretation of the findings via the most commonly

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employed methods within the subject area.

Results

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Distance walked

One-way ANOVA revealed no significant differences in the total distance walked during the CHO, PLA and WAT trials (P = 0.204; Figure 1). The mean distance walked during the three trials was 5814 m; therefore a threshold value of 58 m was selected as a 1 % meaningful difference in walking distance for magnitude-based inferences. The mean difference in distance covered between the CHO and WAT trials was 163 m (90% CI 3–323 m; 2.9%; P = 0.095; d = 0.21). The mean difference in distance covered between the PLA and WAT trials was 134 m (90% CI 7–261 m; 2.3%; P = 0.084; d = 0.17). The mean difference in distance covered between the CHO and PLA trials was 29 m (90% CI -170-230 m; 0.5%; P = 0.806; d = 0.03). The chance that the true value of the effect has a beneficial, trivial or negative influence on walking distance is 86.5%, 12.1%, and 1.4% for the CHO versus WAT trial; and 84.4%, 14.7%, and 0.9% for the PLA versus WAT trial. The effect between CHO and PLA was unclear due to the upper and lower confidence limits representing meaningful increases and decreases, respectively.

Energy expenditure and substrate oxidation

- One-way ANOVA revealed a significant difference between trials for energy expenditure
- during walking exercise (P = 0.049). Post-hoc analysis demonstrated lower energy expenditure
- 218 in WAT compared with CHO (P = 0.035; d = 0.24) and PLA (P = 0.024; d = 0.27), respectively
- 219 (Figure 2).
- 220 One-way ANOVA revealed a significant difference in carbohydrate oxidation between trials
- (P = 0.040). Post-hoc analysis demonstrated significantly higher carbohydrate oxidation during
- 222 the CHO trial compared with WAT (P = 0.015; d = 0.81) and a trend towards higher
- carbohydrate oxidation during CHO than PLA (P = 0.078; d = 0.47) (Figure 3a). Contrastingly,

- there was no difference between trials for fat oxidation (P = 0.126; Figure 3b). There was no
- significant difference between trials for heart rate during the exercise bout (P = 0.572).

Appetite and affective responses

- There were no baseline differences between trials for composite appetite scores (CHO: 63 (22);
- 228 PLA: 64 (18); WAT: 67 (17); P = 0.653), pleasure-displeasure (CHO: 2 (1); PLA: 2 (1); WAT:
- 229 2 (2); P = 0.719) or perceived activation (CHO: 3 (1); PLA: 3 (1); WAT: 3 (2); P = 0.432).
- Baseline thirst perceptions tended to be different between trials (P = 0.091), indicating higher
- 231 thirst in WAT and PLA than CHO (CHO: 9 (2); PLA: 10 (3); WAT: 9 (2)).
- One-way ANOVA revealed no significant differences in composite appetite time-averaged
- AUC scores between trials (P = 0.101; Figure 4). The mean difference in composite appetite
- time-averaged AUC between the CHO and WAT trials was 8 mm (90% CI 2 15 mm; P =
- 235 0.042; d = 0.43). The mean difference in composite appetite time-averaged AUC between the
- 236 PLA and WAT trials was 6 mm (90% CI -2 15 mm; P = 0.201; d = 0.32). The mean difference
- in composite appetite time-averaged AUC between the CHO and PLA trials was 2 mm (90%)
- CI -3 7 mm; P = 0.524; d = 0.08). Based on a threshold value of 8 mm as a meaningful
- 239 difference in appetite, the probability that the true value of the effect has a beneficial, trivial or
- 240 negative influence is 53.5%, 46.5%, and 0% for the CHO versus WAT trial; 37.1%, 62.5%,
- and 0.4% for the PLA versus WAT trial; and 2.9%, 96.9% and 0.2% for CHO versus PLA,
- respectively. Time-averaged area under the curve for thirst perception tended to be different
- between trials (P = 0.055), indicating higher thirst in WAT than CHO and PLA (WAT: 13 (3),
- 244 CHO: 12 (3), PLA: 12 (3)).
- 245 There were no differences between trials for time-averaged AUC ratings of pleasure-
- 246 displeasure (CHO: 2 (1); PLA: 2 (1); WAT: 2 (2); P = 0.297) or perceived activation (CHO: 4
- 247 (1); PLA: 4 (2); WAT: 4 (1); P = 0.123).

Blinding efficacy, rinse ingestion and correlations

Five out of the 18 participants were able to correctly distinguish between the CHO and PLA solutions (i.e. less than what would be predicted by pure chance), indicating successful blinding. The mean difference in the volume of rinse and expectorate was 0.5, 0.4 and 0.3 mL for each rinse in the WAT, PLA and CHO trials respectively. This resulted in a mean total consumption of 5 mL, 4 mL and 3 mL across the ten rinses for WAT, PLA and CHO, respectively. Body mass index was not significantly correlated with distance walked, energy expenditure, substrate oxidation or appetite perceptions in response to carbohydrate or placebo mouth rinsing compared with rinsing the water solution (all r < 0.5; P > 0.05).

Discussion

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This is the first experiment to investigate the effects of carbohydrate mouth rinsing on selfselected exercise intensity and physiological responses during moderate exertion exercise. The findings demonstrate that mouth rinsing with a sweet solution (either carbohydrate or sweetened placebo) increased self-selected walking speed, which induced a small but significant increase in energy expenditure during the exercise bout. Mouth rinsing with a sweet solution also suppressed appetite during exercise compared with a water solution but increases in carbohydrate oxidation occurred only with carbohydrate mouth rinsing. This study provides novel evidence that the stimulation of carbohydrate and sweet receptors in the oral cavity may have physiological effects beyond those previously reported. In the present study, mouth rinsing with carbohydrate increased the distance walked during 60min of treadmill exercise at a fixed RPE of 13 in comparison with rinsing a water solution. This is the first study to reveal a beneficial effect of carbohydrate mouth rinsing for moderate intensity exercise and the mean improvement of 2.9 % is comparable with previously reported improvements in endurance time trial performance of 1.7 - 3.1 % (Carter et al. 2004; Chambers et al. 2009; Rollo et al. 2008). However, the performance improvements in the present study were only observed in comparison with a water solution and similar benefits occurred with a taste-matched placebo. This contrasts with the previously reported benefits of carbohydrate mouth rinsing, which have been observed in relation to a placebo solution (Ataide-Silva et al. 2016; Carter et al. 2004; Chambers et al. 2009; Gam et al. 2013; Rollo et al. 2008; Sinclair et al. 2014) and suggests that a placebo effect may be responsible for the ergogenic benefits observed in the current study. The reasons for this effect are unclear as the participants were not made aware of any hypothesised benefits of carbohydrate mouth rinsing on walking performance. However, although performance improvements were not attributed to the presence of carbohydrate, mouth rinsing with a sweet solution successfully increased selfselected exercise intensity and walking distance. It seems plausible that the recreationally active participants may have been aware that carbohydrate ingestion improves exercise performance but this was not communicated from the research team. The novelty of the current experiment also demonstrates that the effects of mouth rinsing on the measured variables was unknown for both the researchers and participants. The factors contributing to a potential placebo effect require further investigation, in addition to substantiation of this intervention as a potential nutritional strategy to increase exercise intensity during moderate exertion exercise relevant for weight control. The inclusion of sweetened and unsweetened carbohydrate and placebo solutions in future experiments may be beneficial to elucidate any separate effects of sweetness and carbohydrate content on the variables assessed within the present experiment. The increase in walking distance during the carbohydrate and placebo trials resulted in a corresponding increase in energy expenditure compared with the water trial. Although these effects are small, the observed mean increases of 79 kJ and 90 kJ during exercise in CHO and PLA are greater than that previously reported with carbohydrate mouth rinsing during 30-min of running exercise (Rollo et al. 2008) and similar to the 76 kJ increase in eight-hour energy expenditure during a sit-to-stand intervention in the workplace (Thorp et al. 2016). The practical relevance of such small increases in energy expenditure are unclear but previous physical activity interventions have reported the potential for a cumulative effect that may induce meaningful deficits (Thorp et al. 2016). In this regard, mouth rinsing with a sweetened solution during the completion of five 60-min bouts of walking exercise per week would hypothetically yield an additional weekly energy expenditure of ~425 kJ (~100 kcal). It remains unknown whether the effects observed during the present study would continue with repeated exposures to mouth rinsing during multiple exercise bouts and this requires further investigation. These findings also suggest that increases in walking distance and energy expenditure may not be associated with differences in heart rate between trials. In this regard,

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the present study supports previous evidence that the ergogenic effect of carbohydrate mouth rinsing is not associated with any differences in heart rate during the exercise bout (Lane et al. 2013; Rollo et al. 2008; Sinclair et al. 2014). This accords with the fixed effort of the exercise bout but may also represent low sensitivity to detect differences in heart rate during exercise. Despite similar improvements in self-selected walking speed with carbohydrate and placebo mouth rinsing, changes in substrate oxidation occurred only when rinsing with the carbohydrate solution. To the authors' knowledge, this is the first study to demonstrate this effect and the paucity of research investigating substrate oxidation in response to oral nutrient stimulation (both at rest and during exercise) makes it difficult to draw comparisons with previous literature. However, it remains plausible to speculate that changes in substrate oxidation may be related to the activation of brain centres that has been observed in response to carbohydrate mouth rinsing (Chambers et al. 2009). This includes activation of the insula cortex, which has been shown to increase sympathetic activity (Oppenheimer et al. 1992) and may subsequently increase carbohydrate utilisation (Brooks & Mercier 1994). The investigation of low to moderate intensity exercise in the present study may also have increased the likelihood to detect changes in substrate use associated with this pathway as sympathetic activation during exercise is expected to have been lower than previous studies that have measured substrate oxidation in response to more strenuous protocols (Ataide-Silva et al. 2016; Ispoglou et al. 2015). A mean total of 3 mL of rinse solution (equating to < 0.2 g of carbohydrate) was ingested during the carbohydrate trial, which is insufficient to have mediated the change in substrate oxidation. The concept that changes in substrate oxidation may occur without the ingestion of nutrients is also supported in rodent studies as increases in carbohydrate oxidation have been observed in response to the anticipation of a high carbohydrate meal (McGregor & Lee 1998). This suggests that increases in energy expenditure in response to carbohydrate mouth rinsing may be solely due to increased carbohydrate oxidation without any changes in fat oxidation. Although the

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potential mechanisms remain speculative, the current findings support further investigation into the effects of oral nutrient stimulation on substrate oxidation. Such investigations may help to increase our understanding of the potential physiological effects of carbohydrate mouth rinsing, as well as the relationship between oral nutrient sensing and cephalic phase responses in determining physiological changes with feeding.

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Oral nutrient sensing has also been demonstrated to increase satiety during modified sham feeding whereby a meal of mixed nutrient composition is masticated and expectorated without ingestion (Heath et al. 2004; Smeets & Westerterp-Plantenga 2006). The present study represents the first investigation into the effects of mouth rinsing a liquid solution on appetite perceptions and demonstrates that mouth rinsing with a sweet solution decreases appetite during 60-min of walking exercise. Although some authors have suggested that oral rinsing of a liquid solution is insufficient to stimulate a cephalic phase response (Teff et al. 1995), others have demonstrated cephalic phase insulin release after rinsing the oral cavity with either a sucrose or artificially sweetened solution for 45-seconds (Just et al. 2008). Such changes in circulating insulin concentrations and the appetite suppression observed during modified sham feeding are thought to be primarily mediated via vagal stimulation from oral nutrient sensing (Zafra et al. 2006). The findings of the present study extend evidence of a previously observed cephalic phase insulin response to suggest that mouth rinsing with a sweet solution can also reduce appetite perceptions. Although it was beyond the scope of the present study, it would be beneficial for future experiments to incorporate measurements of circulating insulin and gastrointestinal hormone concentrations as potential mediators of the observed reductions in appetite when mouth rinsing with a sweetened solution. It remains unclear whether this effect would be observed with mouth rinsing in isolation or whether a combination of mouth rinsing and exercise is required. In this regard, it seems plausible that exercise may have enhanced the anorectic effect of mouth rinsing in the present study as previous research has demonstrated

significant appetite suppression during 60-min of moderate intensity walking exercise compared with a resting control (Farah et al. 2012). Thirst perceptions were also lower during the carbohydrate and placebo trials in comparison with the water trial but it seems unlikely that this would have influenced appetite perceptions based on current evidence (Corney et al. 2015; McKiernan et al. 2008).

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Although the findings of the present study have provided novel insights into the effects of carbohydrate mouth rinsing on moderate exertion exercise performance and appetite regulation, this study also contains some notable limitations. Firstly, the use of a manually operated treadmill may have reduced the opportunity for participants to change their speed spontaneously based on how they feel and required conscious decision making. Subsequently the use of an automated treadmill as used by Rollo et al. (2008) may have allowed for more sensitive detection of ergogenic effects and revealed greater changes in walking distance and energy expenditure. Secondly, although this is the first study to investigate a placebo effect with carbohydrate mouth rinsing, a no-rinse control condition was not provided. In this regard, evidence suggests that a no-rinse condition can result in better time trial performance than mouth rinsing with a placebo solution as a result of potential breathing interference and distraction during mouth rinsing (Gam et al. 2013). However, these effects are likely to be of smaller consequence in the present study due to the lower demands of moderate exertion walking exercise in comparison with time trial performance. Thirdly, although meaningful changes in appetite perceptions occurred when mouth rinsing with a sweetened solution, ad libitum energy intake also needs to be assessed to understand whether these changes transpire into reduced energy intake and an enhanced energy deficit from the intervention. Appetite perceptions should also be assessed during the post-exercise period as this is the period when energy compensation may occur and therefore this period is the most relevant for weight management. However, the reduction in appetite during and immediately after the exercise

bout in the present study demonstrates a proof of concept that can be developed with future experiments. Finally, the population sample for this study was comprised of young healthy males; therefore the findings may not generalise to overweight and obese populations where weight management strategies have the most clinical relevance. Although further research is required in different populations, understanding appetite regulation and energy balance in normal weight participants remains important as the prevention of weight gain has been highlighted as a major public health priority (Lawlor & Chaturvedi 2006).

In conclusion, this study has demonstrated that mouth rinsing with a sweet solution can increase self-selected exercise intensity and energy expenditure, in addition to reducing appetite, during 60-min of treadmill walking at a fixed moderate exertion. Mouth rinsing with a carbohydrate solution also increased carbohydrate oxidation during the exercise bout in comparison with placebo and water rinses. These findings provide novel insights into the effects of mouth rinsing on physiological variables during an exercise mode relevant to weight control. Future investigations into the energy intake response to mouth rinsing with a sweetened solution is required to understand whether the observed anorectic effects translate into reductions in energy intake. Understanding the maintenance of the observed effects during multiple exercise bouts is also required but these initial findings demonstrate the potential for mouth rinsing to increase the energy deficit associated with exercise through increased energy expenditure and reduced appetite perceptions.

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509 **Figure 1.** Distance walked in the carbohydrate (CHO), placebo (PLA) and water (WAT) trials expressed as mean (SEM) (a), and the difference in walking distance between trials as 510 individual responses (circles represent values for individual participants) (b). N = 18. 511 512 **Figure 2.** Energy expenditure in the carbohydrate (CHO), placebo (PLA) and water (WAT) 513 trials expressed as mean (SEM) (a), and the difference in energy expenditure between trials 514 as individual responses (circles represent values for individual participants) (b). N = 18. 515 *Significantly different from WAT, P < 0.05. 516 517 Figure 3. Carbohydrate (a) and fat oxidation (b) in the carbohydrate (CHO), placebo (PLA) 518 and water (WAT) trials. Values are mean (SEM). N = 18. *Significantly different from WAT, 519 P < 0.05; *Trend towards being significantly different from PLA, P < 0.10. 520 521 **Figure 4.** Composite appetite scores in the carbohydrate (CHO) (●), placebo (PLA) (○) and 522 water (WAT) (▼) trials expressed as mean (SEM) (a), and the difference in composite 523 appetite time-averaged AUC between trials as individual responses (circles represent values 524 for individual participants) (b). N = 18. 525









