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Original Research

Liking of Sweetness and Amount of Accidental Swallowing are Associated with the Ergogenic Effect of Mouth Rinses on Walking Energy Expenditure in Recreationally Active Men

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Abstract

International Journal of Exercise Science 19(2): 1-22, 2026. https://doi.org/10.70252/IJES2026202 This study aimed to evaluate the effect of mouth rinsing with solutions consisting of binary and reciprocal combinations of sucrose and sucralose on walking energy expenditure. Ten recreationally active men participated in a randomized, double-blind where feasible, within-subject study with five conditions, in which they rinsed with four sucrose-sucralose solutions (sweetness ratios: 1:1, 1:6, 6:1, 6:6) and a control solution during a moderate-intensity 60-minute walking. We measured energy expenditure, heart rate, respiratory exchange ratio and perceived effort. We also assessed ratings of internal state, sweet-liking phenotypes of the participants, and the perception of the solutions. Perceived sweetness in the solution with 1:1 sweetness was significantly lower than the others (except control), and the 1:6 and 6:1 solutions were equi-sweet. Mouth rinse solution did not significantly affect walking energy expenditure and internal state perception ($p > 0.05$). We unexpectedly observed an interaction between sweet liker phenotype and the amount of swallowed solution ($p = 0.021$), but this did not affect energy expenditure. However, the amount of swallowed solution was associated positively with rate of perceived effort ($p = 0.008$), negatively with walking energy expenditure ($p = 0.034$). We conclude that mouth rinsing with different proportions of sucrose-sucralose and total sweetness did not improve walking energy expenditure. We unexpectedly observed that participants that like sweet solutions in general may swallow more of the mouth rinses, and in turn how much they swallow of the mouth rinse is related to perceived effort and physiological variables.

Keywords: exercise metabolism, sucrose, sucralose, perception

Introduction

The presence of a carbohydrate in the mouth even if it is not swallowed may improve performance. For example, Carter et al showed that in nine endurance cyclist performance time during a 1-h time-trial improved by 2.9% for a 6.4% maltodextrin mouthrinse compared to a placebo rinse

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with water.[1] Other studies using the same mouth rinsing protocol showed improved self-selected running speed and distance covered in a 30 min period[2] and greater distance covered running in a 1 hr period.[3] On the other hand various studies showed no performance improvement with a similar protocol. For example, a mouth rinse with 6.4% maltodextrin did not improve multiple sprint performance.[4] Despite inconsistent findings, there is meta-analytic evidence for a performance improvement of 0.15 standard mean difference for a carbohydrate rinse relative to a placebo rinse.[5] This is a small effect size that is meaningful in the context of competitive elite athletic performance, where improvements of around 1% are regarded as worthwhile.[6] If the presence of a carbohydrate improves performance, does a higher concentration of carbohydrate provide additional improvement? In other words, is there a dose-response effect? Various studies showed that there is no linear dose-response relationship.[7,8] When grouping studies in the range of 6-6.5% maltodextrin vs a range of 8-18% maltodextrin, no meta-analytic evidence was observed for an improved benefit from increased concentrations,[5] which suggests that the ergogenic effect of carbohydrate mouth rinses is an all-or-nothing phenomenon.

Various performance-enhancing studies have solved the confounding factor of sweetness with carbohydrate content by comparing the effect of a sweet carbohydrate solution to an equally sweet non-caloric solution. Non-nutritive sugars (sucralose, saccharin, aspartame) generally are used to prepare placebo solutions in those studies. Sucralose is usually the preferred non-nutritive sweetener since, unlike many other non-nutritive sweeteners, it does not have a pronounced off-taste or a lingering bitter after-taste. This makes sucralose the ideal taste-matched control stimulus for sucrose. There are various studies that examined whether equi-sweet carbohydrate solutions lead to greater exercise performance than non-nutritive sweet solutions, but there is (to our awareness) no existing meta-analytic assessment of performance enhancement of sweet-matched carbohydrate solutions relative to control. Deighton et al used mouth rinses with a 6.4% maltodextrin solution sweetened with saccharin, saccharin alone or pure water.[9] Both the carbohydrate and sweet-matched control solution had more energy expenditure than water during 60-min walking exercise, though it must be noted that discrimination of the solutions was not directly assessed. Similarly, Green et al showed that a sweet carbohydrate and sweet non-nutritive mouth rinse both showed a similar improvement in performance relative to a water control.[10] In contrast to these two studies, others have not observed consistent improved performance with non-nutritive sweetened mouth rinses. Hawkins et al assessed the ergogenic effect of nutritive (sucrose) and non-nutritive (sucralose) sweeteners separately.[11] They observed that mouth rinses with sucrose showed greater exercise performance than control (water). Although the sucralose solution (1:1 ratio of sweetness intensity with sucrose) did not improve the performance compared to control, there was a trend for a difference between the sucrose and the higher concentration of sucralose solution (100:1 ratio of sweetness intensity with sucrose). Kulaksız et al did not show any positive effect using both carbohydrate and sweetness (Aspartame and Acesulfame-K) on the time it takes to complete the exercise trial and power out compared to placebo which contains sweeteners.[7] These studies show that there is conflicting evidence on the role of sweetness perception on the ergogenic effect of mouth rinses. We note that also in these studies that there is rarely a manipulation check of matched sweetness, nor a rigorous protocol for excluding perceptual differences between the active and control solutions, especially at the level of the individual participant. Assuming there are no perceptual differences between mouth rinses that confound the difference in performance between sweetened caloric and non-caloric solution in these studies, could a neural mechanism of greater activation of reward pathways by caloric sweeteners relative to non-caloric sweeteners explain the performance enhancement? There is no meta-analytic evidence for a difference in neural responses to caloric and non-caloric sweeteners,[12] but we note that one study found greater connectivity between brain regions for sucralose[13] and that Turner et al[14] observed greater activations in the primary taste cortex and the limbic system in response to a sweet mouth rinse with maltodextrin compared to a taste matched control solution. In summary, in studies of mouth rinses that are intentionally sweet, performance is not consistently enhanced, and the perceptual indistinguishability is not always supported by unbiased data. When we combine the possibility of experienced differences in sweetness from maltodextrin rinses with the observation that only a few studies are in support of ergogenic effects in sweetness-matched solutions, it is clear that more research is needed on the interactions between sweet perception and performance enhancement in mouth rinses.

Here we investigate the effect of mouth rinsing with solutions of similar and different total sweetness, formed by binary and reciprocal combinations of sucrose and sucralose on 60-minute treadmill walking energy expenditure. Our first hypothesis was that a mouth rinse with more carbohydrates would produce a greater ergogenic effect than an equi-sweet solution with less carbohydrates. Our second hypothesis was that the solutions with higher total sweetness would increase walking energy expenditure relative to lower sweetness solutions.

In addition, we also prepared solutions with equal amounts of sucrose and sucralose, one that contains the lowest dose and one that contains the highest dose of both sweeteners. The comparison of the mostly-sucrose and mostly-sucralose solutions to the solution with the mixture of high doses of both solutions then allows us to investigate whether there are supra- or sub-additive ergogenic effects of mixtures of sweeteners. We used standard psychophysical methods to carefully match sweetness perception in a pilot study and analyzed perception in the main experiment to check that the sweetness manipulations were successful and to evaluate supra- or sub-additive effects on perception. We also measured sweet liking phenotypes, to account for individual differences.[18] This study may provide insights into how the combined action of sucrose and sucralose and sweetness perception affects energy expenditure during walking.

Methods

Stimuli

To determine mouth rinse concentrations, we first calculated the amount of sucralose (Metro Chef) matching sucrose (Torku) sweetness of 1%, 3% and 6% w/v according to the formula used by Dubois et al[19] as 0.00186, 0.00465 and 0.00985 gr, respectively. Then in pilot tests with 8 researchers in the lab (data not presented, independent sample), who each tasted (sip-and-spit) three replicates of nine different stimuli (10 ml each, presented in a random sequence with water rinsesn in between) and rated intensity, sweetness and bitterness of the taste of each sample using visual analogue scales (VAS). This procedure confirmed perceptual differences between the 1% and 6% solutions, and we proceeded with a 2x2 factorial design of 1% and %6 of sucrose and sucralose concentrations.

Participants

Twelve recreationally active males were initially enrolled; however, two were excluded due to injuries sustained during their regular exercise routines. Therefore, the study was completed with the remaining ten healthy, recreationally active men (n = 10; age: 21 ± 2.05 years [19–26], height: 176.90 ± 3.54 cm, physical activity level: 5006 ± 3205.7 MET-minutes/week). The inclusion criteria were that the participants are healthy, non-smokers and have an exercise routine of at least 1-hour of aerobic exercise a day for at least 4 days per week. The exclusion criteria were having cardiovascular or metabolic diseases, active infection, musculoskeletal or respiratory problems, using any drug that affects metabolism, or being on a diet. This is a within-subject design with many sessions in a short period of time. Since hormonal cycles in female human participants may introduce systematic influences on walking exercise[20], including female participants would introduce logistical complications with scheduling all test days within a month. For this reasons we recruited only men to the study. Each participant performed all the trials in the same times of the day not to affect the circadian rhythm. All participants came to the laboratory following at least 4-5 hours of fasting to prevent any changes in energy expenditure caused by upper and lower gastrointestinal symptoms and to ensure stable glycemic responses and without consuming tea, coffee, or cigarettes.[21-23] They were not allowed to consume water during the walking protocol. All measurements were conducted at Mersin University, Gait Analysis and Exercise Physiology and Gourmet Brain Laboratory. A sample size of at least 10 participants is required to demonstrate a statistically significant mean difference of 1.54 (effect size) units in heart rate measurements, with 80% power and a 5% Type I error rate.[24] Meta-analytic effect size estimates of energy expenditure were not available at the time of the design of this study. We post-hoc conducted a power analysis during the preparation of this manuscript, with an estimated effect size of 0.15 based on a recently published meta-analysis[5]. We observed that a sample size of 350 participants would be required, which is far beyond feasible for a parallel-arms study with 5 different mouthrinses. We note that studies on the ergogenic effect of mouthrinses commonly use 8-15 participants[5]. The reader should be aware that the current study is likely underpowered. This study was approved by the local ethics committee (25/08/2021, 570), the study was conducted in agreement with the Declaration of Helsinki and all participants gave written informed consent. Participants were informed about the benefits and risks of the study before signing the informed consent form. This research was carried out fully in accordance with the ethical standards of the International Journal of Exercise Science.[25]

Protocol

This study employed a randomized, double-blind where feasible, within-subject crossover design with five conditions and at least four days of washout. Randomization of solutions was created in Excel. A researcher outside the research team was responsible for the order of solutions and assignment to the participants. All participants completed six sessions, which included a familiarization session in addition to the five days with experimental sessions. All experimental sessions were separated by at least 4-5 days to minimize carry-over effects. Five different stimuli, including one control condition, were used in the main study. The concentrations of the mouth rinse solutions were decided according to the results of a pilot study (described above under "stimuli"). Four different solutions with different sucrose-sucralose ratios (1% sucrose+0.00186% sucralose (equivalent to ~1% sucrose sweetness))-1:1 sweetness, (1% sucrose+0.00985% sucralose (equivalent to ~6% sucrose sweetness)-1:6 sweetness, (6% sucrose+0.00186% sucralose)-6:1 sweetness, (6% sucrose+0.00985% sucralose solutions)-6:6 sweetness and a control (demineralized water)-0:0 sweetness solution were prepared.

The goal of preliminary session is to familiarize the participant with the whole protocol on each of the test days. The participant was asked to record their physical activity and dietary intake during the 24-hour period before the first experimental session in order not to create a confounding effect and to ensure standardization in all sessions. One day before the test sessions, the participants were asked to replicate these patterns of physical activity and food intake. Physical and dietary standardization were confirmed verbally at the beginning of each session. The participants were also asked to avoid consuming alcohol and any vigorous physical activity the day before the experimental sessions. Physical activity level of participants was evaluated with the International Physical Activity Questionnaire. To evaluate the sweet-liking phenotype, the participant rated a 1M sucrose solution on a bipolar -50-50 VAS [26] labeled with "not like at all" on the left anchor, "neutral" on the middle anchor, "very liked" on the right anchor.[18]

Standing height was measured to the nearest 0.1 cm with participants standing upright, looking straight ahead, and with the head positioned in the Frankfort horizontal plane. Body mass (to the nearest 0.1 kg) and body composition were assessed using bioelectrical impedance analysis (TANITA BC-601 MA, Tanita Corporation, Tokyo, JAPAN) while participants wore light clothing before participating in each test day. After entering personal data into the device, participants stood barefoot on the foot electrodes to ensure proper contact. During the impedance measurement, participants held their arms straight and slightly abducted from the torso to avoid contact with the body. Each solution was administered to the participant during a moderate-intensity 60-minute walking exercise on 5 different days in a randomized manner (Figure 1). The Physical Activity Readiness Questionnaire for Everyone was applied to assess the health of participants before starting the exercise.

Resting energy expenditure (REE) (kcal/day) was measured with the participant in a supine position on a hospital bed for 15 minutes in each test session with a face mask (Hans Rudolph, USA) covering the mouth and nose to prevent any air leakage. The Borg scale (6-20) is introduced to the participants after REE measurement to evaluate their ratings of perceived exertion (RPE). Before starting the walking protocol, the participant did warm-up exercises. Heart rate was measured during walking by wearing a chest strap (SmartLab). In each session, participants completed exactly 60 minutes of moderate-intensity treadmill walking. The exercise intensity was adjusted to 64-76% of each participant's maximum heart rate which American College of Sports Medicine recommended.[27] Maximum heart rate was calculated using the formula: 220-years. The walking protocol was started when a participant reached 64-76% of their maximum heart rate with increasing the treadmill's (Viasys Health Care, USA) speed and incline. Each participant's treadmill speed was different in both interindividual and between session because the treadmill speed and incline were altered to keep the heart rate between 64-76% of each participant's maximum heart rate. Mouth rinsing with a solution was performed before and every 7.5 min period of the 60-minute walking (12.5% of the 60-minute walking), resulting in a total of 8 periods. After completing each 25% (every 15 min) of the 60-minute walking, the Borg scale was presented to the participant. During the 60-minute walking exercise, participants' RPE values were recorded at 15, 30, 45, and 60 minutes. Physiological measurements were assessed across 8 phases, while RPE was evaluated across 4 phases, that is, at 4 time points. The participant was informed of the time only during the exercise, but no further interventions such as encouragement or distraction were allowed, and no information was given about the covered distance and speed until the end of the exercise. During the 60-minute walking (kcal/day) and REE (kcal/day) measurement, inspired and exhaled gasses were analyzed breath-by-breath using Quark RMR which is an open-circuit calorimeter and OMNIA Cardiopulmonary Diagnostic Software (Cosmed, Rome, Italy). All measurements were performed under laboratory conditions set at 20-24□C and around 50% humidity. Before each test, the flow sensor of the calorimeter was calibrated with a 3 L syringe according to the manufacturer's recommendations, while the gas analyzer was calibrated with a standard gas mixture (5% CO2, 16% O2, Balance N2).

During the rinsing times, the clips of the face mask were opened and it was lifted up just enough for the participant to take the whole solution into his mouth to rinse. The participant rinsed his mouth with one of the 5 different solutions just before starting the walking period and every 7.5 minutes of walking. The participant expectorated the solution into the same container after rinsing for 10 seconds.[21] Before the walking exercise in each session the containers with the mouth rinse solution were weighed. The participant spits the 25 ml solution into the same container after rinsing for 10 seconds.[21] The containers were weighed again after the session. All tests were administered to the participant by the same researcher.

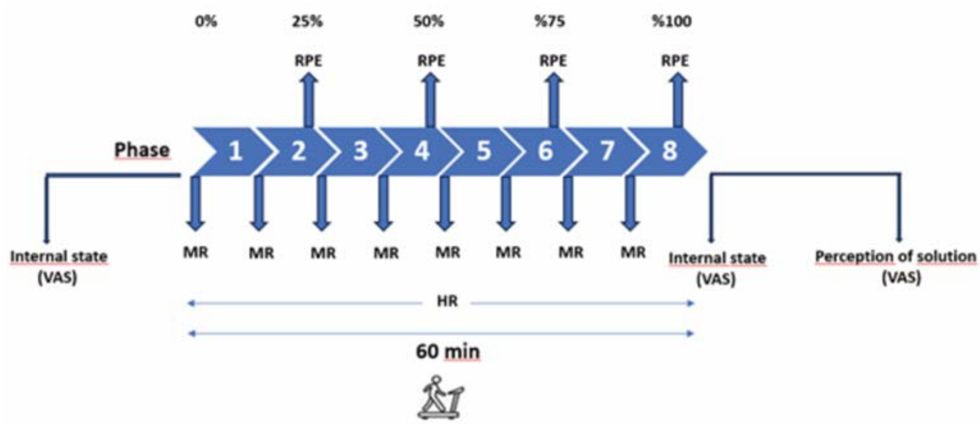

Figure 1. 60 min treadmill walking protocol consisting of 7.5 min phases. The participant rinsed the solution in their mouth before starting the walking and after every 7.5 min of walking. Ratings of perceived exertion (RPE) was assessed using the Borg scale after every 15 min of walking protocol. Visual analogue scale (VAS) was used to assess internal state (hunger, fullness, wanting, thirst) pre and post exercise. Perception of the mouth rinse solution (sweetness, intensity, liking and bitterness) was assessed using VAS at the end of the test. MR: mouth rinsing, HR: heart rate.

In every session 8 different plastic containers were prepared and each container contained 25 ml of each solution, at room temperature. Before each session, all containers filled with rinsing solution were weighed. After the walking exercise, these containers were weighed again and it was evaluated how much of the solution the participant swallowed (a decrease in weight) or how much the participant salivated (an increase in weight).

Internal state (hunger, fullness, thirst, and motivation to consume the drink) and taste perception of the solutions (intensity, sweetness, bitterness and liking) were assessed using the VAS.[26] Hunger, fullness, thirst and motivation to consume (wanting) were rated using a 0-100 mm unipolar VAS with two anchors, labelled with "not at all [...]" on the left anchor and "extremely [...]" on the right anchor at the beginning and end of each walking trial. Intensity, sweetness, bitterness, and liking of solutions were also rated on a 0-100 VAS with two anchors, labelled with "not at all [...]" on the left anchor and "extremely [...]" on the right anchor after completing each walking trial (Figure 1).

Statistical Analyses

Data were analyzed using the JASP (0.16.3) statistics program. We specified a repeated measures ANOVA with two within-subject factors to examine the effect of "solution" (0:0, 1:1, 1:6, 6:1, 6:6) and "phase" (1-8) on each of the measures we use (walking energy expenditure, walking energy expenditure/kg, net energy expenditure, heart rate, respiratory exchange ratio (RER), fat oxidation, carbohydrate oxidation). To analyze RPE, we specified a repeated measures ANOVA with the factors "solution" (as above) and "phase" (2, 4, 6, 8). To examine the effect of solution on each of the perception measures (sweetness, intensity, bitterness and liking), we specified a repeated measures ANOVA with one within-subject factor "solution" (as above). We specified a repeated measures ANOVA with two within-subject factors "solution" (as above) and "time" (pre vs post exercise) for each of the internal state measures (fullness, hunger, wanting and thirst). Sphericity was evaluated with Mauchly's test followed by the Greenhouse-Geisser adjustment where required. If a main effect or interaction effect was observed, post-hoc comparisons were done using paired sample t-test and Holm's correction for multiple comparisons. All data are represented using mean ± standard deviation with alpha set at 0.05. To assess whether sweet liking status, a possibly important between-participants source of variation, exerted an important influence on the observations reported, sweet liker status was also included as a covariate for each of the repeated measures ANOVAs in the following paragraphs, and we assessed interaction with the independent factors for any of the dependent variables. In follow-up analyses related to the finding of an effect of the covariate of "amount of mouth rinse swallowed", we conducted correlational analyses between "amount of mouth rinse swallowed" and the dependent variables related to walking exercise. We averaged across phases for each of the variables, which resulted in one data point per participant per mouthrinse solution, for a total of 50 data points. We conducted correlational analyses to look for a monotonic relationship, and report Spearman's rho if the assumption of normality is violated. Walking energy expenditure, walking energy expenditure/ kg, and net energy expenditure are defined as the primary outcomes. All other outcomes are considered exploratory and should be interpreted with caution.

Results

While the physical activity level of two participants was moderate, eight participants had high intensity physical activity level. Weight, body mass index, fat free mass and fat percentage were measured in every session and a repeated measures ANOVA showed that there was no difference between sessions in terms of these parameters (Supplementary Material, Table 2).

Perception of Solutions

The participant evaluated their perception of the solutions in terms of sweetness, intensity, bitterness and liking using VAS after rinsing their mouth with each of the solutions (0:0, 1:1, 1:6,6:1,6:6) (n=10). As can be seen in Figure 2A, 1:1 was less sweet than the other solutions, 6:6 was more sweet than the others, and 1:6 and 6:1 were approximately equal in sweetness, as intended. A repeated measures ANOVA with factor "solution" with 6 levels showed that solution had a significant effect on sweetness ratings ($F(2.27,20.432) = 18.612$, $p < 0.001$, $\eta 2 = 0.674$, Greenhouse-Geisser [ε] = 0.568). Post-hoc t-tests showed that the 0:0 rinse was less sweet than 1:1 (p=0.018), 1:6 (p=0.001), 6:1 (p=0.001), 6:6 (p=0.001). 1:1 was less sweet than 1:6 (p=0.021), 6:1 (p=0.014), 6:6 (p<0.001). 1:6 and 6:1, as intended, did not differ in sweetness. Unexpectedly, while 6:6 was rated on average as numerically higher in sweetness, statistically 6:6 was not more sweet than 1:6 or 6:1, which is indicative of perceptual sub-additivity.

Intensity ratings (Figure 2B) showed a similar pattern of differences across solutions. A significant effect of solution was also observed on intensity ($F(4,36) = 14.730$, $p < 0.001$, $\eta 2 = 0.621$). Post-hoc t-tests showed that 0:0 was less intense than 1:1 (p=0.003), 1:6 (p=0.001), 6:1 (p=0.001), 6:6 (p=0.001)). 1:1 was less intense than 6:6 (p=0.032). There was no statistical difference between 1:6, 6:1, and 6:6.

There was no effect of solution on bitterness ($F(1.872,16.847)=0.872$, $p=0.430$, $\eta 2=0.088$, Greenhouse-Geisser [ε] = 0.468), which indicates that there was no substantial bitterness perception from the non-nutritive sweetener sucralose.

There was a significant difference between the solutions in liking ($F(4,36) = 5.818$, $p = 0.001$, $\eta^2 = 0.393$) (Figure 2C). Post-hoc t-test showed that 0:0 was less liked than 1:6 (p=0.010), 6:1 (p=0.006), 6:6 (p=0.001). There was no statistical difference between 1:1, 1:6, 6:1, and 6:6.

We conclude that, as intended, 1:6 and 6:1 are equi-sweet. We also note that, although 6:6 was numerically greater in sweetness, there was no significant difference in perception between this solution and the two solutions 1:6 and 6:1. This means that there is perceptual sub-additivity of the solutions. We also note that the lack of perceptual differences between the solutions 1:6, 6:1, and 6:6 means that any subsequent differences in walking energy expenditure cannot be attributed to perceptual differences.

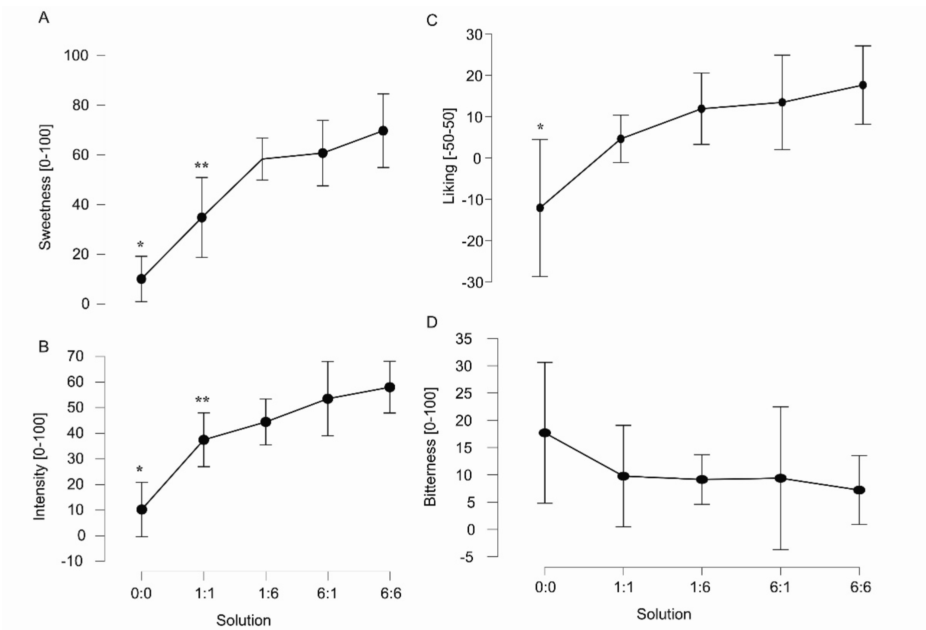

Figure 2. Perception of the mouth rinse solutions (n=10). Graphs show average ratings across participants ± 95 % confidence interval for each solution (on the x-axis). The different perception that were rated on VAS scales are in separate graphs, labeled on the y-axis (* post-hoc corrected p < 0.05 difference between 0:0 and 1:1, 1:6, 6:1, 6:6, **post-hoc corrected p < 0.05 difference between 1:1 and 6:6 in intensity, and 1:6, 6:1, 6:6 in sweetness).

Sweet liker status

To control for effects of individual differences in psychohedonic curves of sweet solutions, we assessed participants' sweet liker phenotypes. In the preliminary session, each participant rinsed with a 1 M sucrose solution and rated sweet taste liking using a VAS, which is a validated proxy of phenotyping sweetness liking across a range of concentrations (n=10).[18] A score of -15 or lower means a sweet disliker phenotype, +15 or higher scores means a sweet liker phenotype. Between -15 and +15 liking scores of VAS refers to inverted-U phenotype. One participant was a sweet disliker, meaning generally their liking of sweet taste goes down as the concentration of the sweet solution goes up. Three participants can be characterized as displaying an "inverted U-pattern", meaning that generally their liking of sweet will initially increase with increasing sweetness, reach an optimum, and then their liking will decrease with continued increasing sweetness. Six participants are sweet likers, meaning that generally their liking of sweet will increase with increasing sweetness. To assess whether sweet liking status exerted an important influence on the observations reported, sweet liker status was also included as a covariate for each of the repeated measures ANOVAs in the following paragraphs, and we found that sweet liking phenotype did not have a significant interaction with the independent factors for any of the dependent variables, with the exception for the dependent variable "amount of mouth rinse that was swallowed" (Supplementary Material, Table 1 and Mouth Rinse.)

Mouth Rinse: amounts swallowed and expectorated

The amounts of swallowed and expectorated solution were evaluated by measuring the weight of the solutions before and after the mouth rinses (n=10). Note that the expectorated solution may be variable due to increased saliva during rinsing. While there is no significant difference between the solutions in terms of swallowed solution ($F(1.822,16.401)=2.706$, $p=0.100$, $\eta2 = 0.231$, Greenhouse-Geisser [ε] = 0.456, Figure 3A), there is a significant difference between the expectorated solutions ($F(4,36)=17.020$, $p<0.001$, $\eta2 = 0.654$, Figure 3C). A greater amount of 6:1 and 6:6 was expectorated compared to 0:0, 1:1, 1:6. A larger volume being expectorated may reflect a greater production of saliva. This means that solutions with 6% sweetness from carbohydrates may induce different physiological responses. We also observed a significant interaction between solution and sweet liker status on the amount of solution that was swallowed ($F(4,32)=3.375$, $p=0.021$, $\eta2=0.032$), but note that including this covariate does not change the effect of solution on amount of swallowed solution ($F(4,32)=2.321$, $p=0.078$, $\eta2 = 0.022$). The sweet liker type groups are too small and uneven to carry out a meaningful statistical analysis with subgroups. But as can be seen in Figure 3B, when the amount of swallowed solution is broken down by sweet liker type, there is a tendency for differences between sweet liker type for solutions 1:6 and 6:6, such that sweet likers swallow more of these solutions.

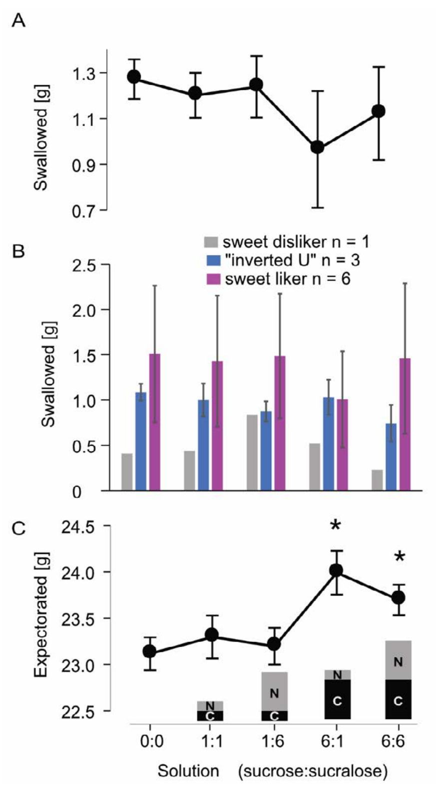

Figure 3. Amount of solution that was swallowed (A, B) and expectorated (C) (n=10). Graphs show average amount in gram across participants ± 95 % confidence interval for each solution (on the x-axis). In panel B this is broken down by sweet liker status (gray: sweet disliker, blue: "inverted U pattern", magenta: sweet liker). Other details as in Figure 2. (P<0.05). (* post-hoc corrected p < 0.05 difference between 6:1 and 6:6 solutions relative to the solutions 0:0 and 1:1, 1:6).

Walking Energy Expenditure

To test how the various rinses affected walking energy expenditure (kcal/day), we specified 5x8 repeated measures ANOVA with factors solution (0:0-6:6) and phase (1-8) (n=10). We observed no main effect of stimulus ($F(4,36)= 0.946$, $p=0.449$, $\eta^2=0.095$) and no interaction between mouth rinse solutions and phase on walking energy expenditure ($F(28,252)=0.883$, $p=0.640$, $\eta^2= 0.018$). We observed a significant main effect of phase ($F(1.344,12.095)= 6.275$, $p=0.021$, $\eta^2=0.108$, Greenhouse-Geisser [ε] = 0.192). We observed a decrease in walking energy expenditure towards the end of the exercise (phase 1 and 7 ($p=0.018$), phase 1 and 8 ($p<0.001$), phase 2 and 7 ($p=0.006$), phase 2 and 8 ($p<0.001$), phase 3 and 7 ($p=0.022$), phase 3 and 8 ($p=0.001$), phase 4 and 8 ($p=0.005$)) (Figure 4A).

We performed additional analyses with transformations of the dependent variable, to account for interindividual differences between participants in body weight and REE. When we normalized the walking energy expenditure for body weight (kcal/kg/day), we observed no main effect of stimulus ($F(4,36)= 0.820$, $p=0.521$, $\eta^2=0.083$). There was a significant main effect of phase ($F(1.387,12.482)= 6.409$, $p=0.019$, $\eta^2=0.120$, Greenhouse-Geisser [ε] = 0.198) and we observed a decrease in walking energy expenditure/kg towards the end of the exercise (phase 1 and 7 ($p=0.018$), phase 1 and 8 ($p<0.001$), phase 2 and 7 ($p=0.005$), phase 2 and 8 ($p<0.001$), phase 3 and 7 ($p=0.018$), phase 3 and 8 ($p<0.001$), phase 4 and 8 ($p=0.004$), phase 5 and 8 ($p=0.048$)). There was no interaction between mouth rinse solutions and phase ($F(28,252)=0.902$, $p=0.612$, $\eta^2= 0.020$) (Figure 4B).

We calculated net walking energy expenditure (kcal/day) by subtracting REE from gross walking energy expenditure. We observed no main effect of stimulus ($F(4,36)=0.631$, $p=0.643$, $\eta^2= 0.066$). There was a significant main effect of phase ($F(1.344,12.095)= 6.275$, $p=0.021$, $\eta^2=0.102$, Greenhouse-Geisser [ε] = 0.192); we observed a decrease in net energy expenditure towards the end of the exercise (phase 1 and 7 ($p=0.018$), phase 1 and 8 ($p<0.001$), phase 2 and 7 ($p=0.006$), phase 2 and 8 ($p<0.001$), phase 3 and 7 ($p=0.022$), phase 3 and 8 ($p=0.001$), phase 4 and 8 ($p=0.005$)). There was no interaction between mouth rinse solutions and phase on net walking energy expenditure ($F(28,252)=0.883$, $p=0.640$, $\eta^2= 0.017$) (Figure 4C).

In summary, there was a decrease across phases in all walking energy expenditure parameters throughout exercise, while mouth rinsing with different solutions has no differential effect.

Heart Rate, Respiratory Exchange Ratio and Substrate Oxidation

We observed no main effect of stimulus ($F(1.347,12.124)=0.398$, $p=0.601$, $\eta 2=0.032$, Greenhouse-Geisser [ε] = 0.337) and no interaction between solution and phase on heart rate ($F(28,252)=0.843$, $p=0.697$, $\eta 2=0.016$), which indicates that there were no consistent differences in intensity of the exercise associated with each solution. We observed a main effect of phase on heart rate ($F(7,63)=2.242$, $p=0.042$, $\eta 2=0.012$). Post-hoc t-tests showed that heart rate in phase 8 was higher than in phase 1 ($p=0.024$). These results show that, as intended, the exercise intensity was similar across test days, and that with the exception of phase 8, it was also similar across phases (Supplementary Material, Figure 1).

To examine whether the mouth rinse solutions affected RER and carbohydrate and fat oxidation (using the Frayn equation) we calculated to monitor how the substrates used in energy metabolism changed during the 60-min walking.[28] A 5x8 repeated measures ANOVA with factors solution (0:0-6:6) and phase (1-8) showed that there was no main effect of solution ($F(4,36)=0.635$, $p=0.641$, $\eta^2= 0.028$) on RER but there was a main effect of phase ($F(2.298,20.683)=72.398$, $p<0.001$, $\eta^2= 0.369$, Greenhouse-Geisser [ε] = 0.328) on RER. There was a decrease in RER towards the end of the 60 min walking. We observed no interaction between mouth rinse solutions and phase on RER ($F(28,252)=1.077$, $p=0.367$, $\eta^2= 0.017$). The average RER values were below 1.1 throughout all trials, indicative of submaximal effort and aerobic metabolism, as intended.

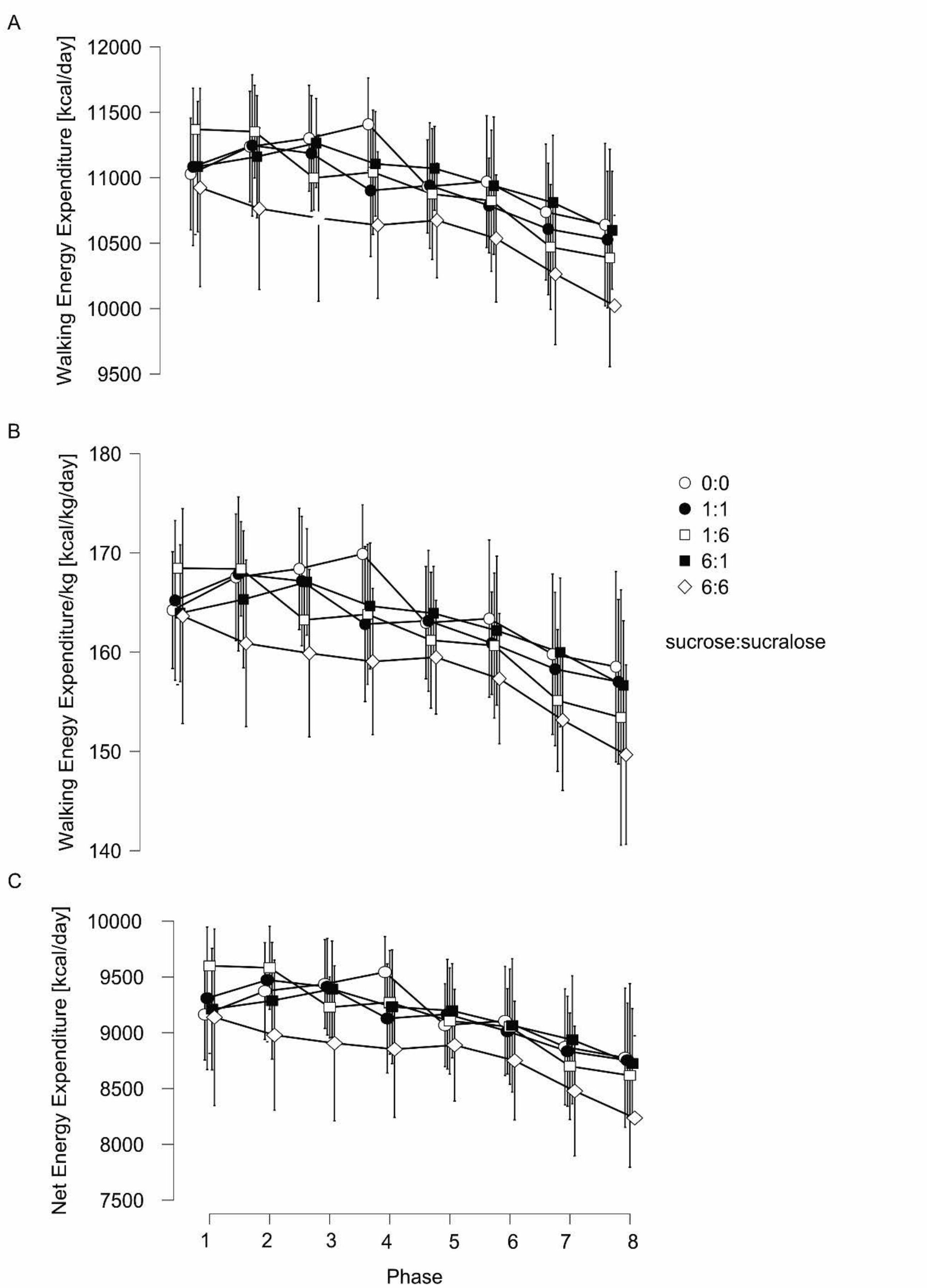

Figure 4. Average walking energy expenditure (kcal/day) values (n=10) obtained from rinsing each of the solutions during the phases of exercise (P<0.05). Each solution is depicted with different symbols, open circles - 0:0 (demineralized water), black circles - 1:1 (1% sucrose+0.00186% sucralose (equivalent to ~1% sucrose sweetness)), open square - 1:6 (1% sucrose+0.00985% sucralose (equivalent to ~6% sucrose sweetness)), black square - 6:1 (6% sucrose+0.00186% sucralose), open diamond - 6:6 (6% sucrose+0.00985% sucralose).

For carbohydrate oxidation, we observed no main effect of solution ($F(4,36)=0.562$, $p=0.692$, $\eta^2= 0.023$), there was a significant main effect of phase ($F(1.668, 15.012)=46.780$, $p<0.001$, $\eta^2= 0.384$, Greenhouse-Geisser [ε] = 0.238), but no interaction between mouth rinse solutions and phase on carbohydrate oxidation ($F(28,252)=1.131$, $p=0.302$, $\eta^2= 0.017$). For fat oxidation we observed a similar pattern: no main effect of solution ($F(4,36)=0.757$, $p=0.560$, $\eta^2= 0.038$), there was a significant main effect of phase ($F(2.663,23.970)=65.973$, $p<0.001$, $\eta^2=0.304$, Greenhouse-Geisser [ε] = 0.380) but no interaction between mouth rinse solutions and phase on fat oxidation ($F(28,252)=1.073$, $p=0.372$, $\eta^2= 0.017$) (See Supplementary Material, Figure 2). As expected these results show that while carbohydrate oxidation was dominant at the beginning of the exercise, fat oxidation was dominant at the end of the exercise. We also observed that substrate utilization was not different for the different solutions.

Ratings of Perceived Exertion

To examine whether the solutions affected perceived exertion during walking, we evaluated the RPE at the end of every 25% of 60-min walking (n=10). Repeated measures of ANOVA showed there is no main effect of mouth rinse solution ($F(4,36)=1.427$, $p=0.245$, $\eta^2=0.070$) and no main effect of phase ($F(1.228,11.055)=1.292$, $p=0.291$, $\eta^2=0.030$, Greenhouse-Geisser [ε] = 0.409) on RPE. We also observed no interaction between mouth rinse and phase on RPE ($F(12,108)=0.779$, $p=0.671$, $\eta^2=0.020$) (Figure 5). These results show that the solutions did not have any effect on RPE values during the walking exercise.

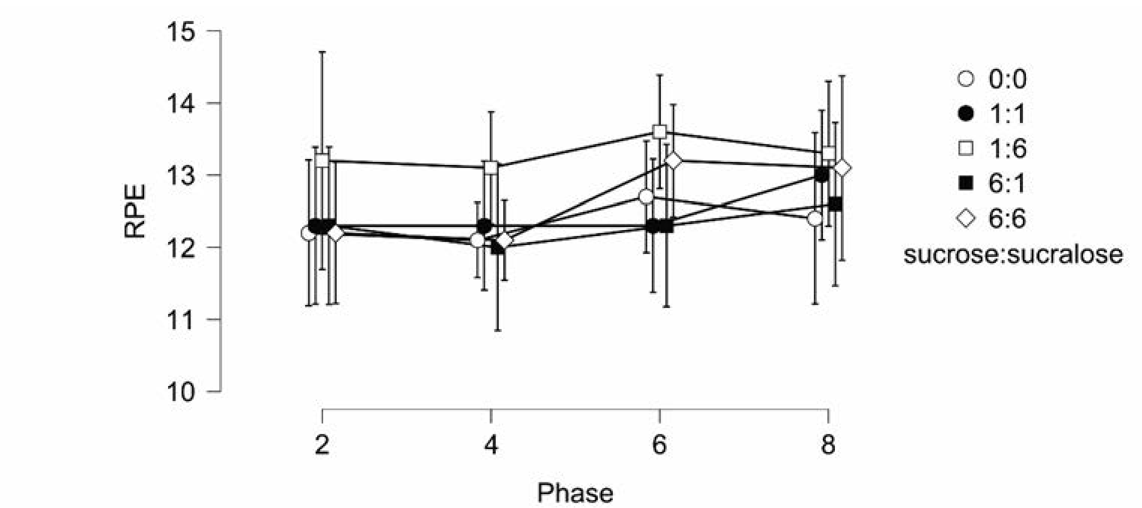

Figure 5. Average RPE values (n=10) obtained from rinsing each of the solutions during the phases of exercise (P<0.05). Details as in Figure 4. RPE, ratings of perceived exertion.

Internal State Ratings

To examine whether the mouth rinse solutions affected fullness, hunger, wanting and thirst before and after walking exercise we conducted a 2x5 repeated measures ANOVA with factors time (pre vs post exercise) and solution (n=10). This analysis showed no main effect of solution ($F(4,36)=0.353$, $p=0.840$, $\eta2=0.015$), no main effect of time ($F(1,9)=0.211$, $p=0.657$, $\eta2=0.010$) and no interaction between solution and time ($F(4,36)=1.887$, $p=0.134$, $\eta2=0.034$) (before and after exercise) in fullness. For hunger, there was no significant main effect of solution ($F(2.130,19.170)=1.300$, $p=0.297$, $\eta2=0.042$, Greenhouse-Geisser [ε] = 0.533), no main effect of time ($F(1,9)=2.116$, $p=0.180$, $\eta2=0.071$) and no interaction between solution and time ($F(4,36)=1.109$, $p=0.367$, $\eta2=0.032$). There was no significant main effect of solution (F(4,36)=1.630, p=0.188, η2=0.063), no main effect of time (F(1,9)=1.148, p=0.312, η2=0.036) and no interaction between solution and time (F(4,36)=0.301, p=0.875, η2=0.009) on wanting to consume more of the solutions. While there was no significant main effect of solution (F(4,36)=0.448, p=0.773, η2=0.007) and no interaction between solution and time (F(1.965,17.687)=2.263, p=0.134, η2=0.046, Greenhouse-Geisser [ε] = 0.491), there was a significant main effect of time on thirst (F(1,9)=32.148, p<0.001, η2=0.480), such that thirst increased from pre- to post exercise (Figure 6). These results show that all solutions had similar internal state ratings regardless of mouth rinse solution or time within session, with the exception of thirst. Thirst ratings likely were increased at the end of the exercise because of a need to rehydrate.

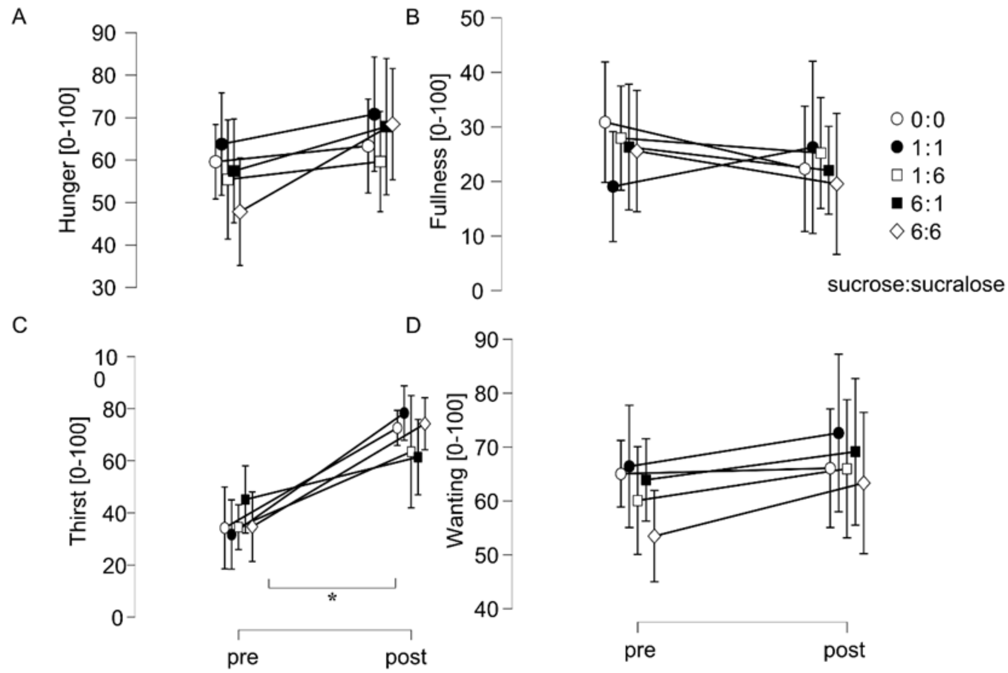

Figure 6. Internal state ratings of each solution pre and post exercise (n=10). Graphs show average ratings across participants with respect to each time ± 95 % confidence interval (on the x-axis). The different perceptions that were rated on VAS scales (A: hunger, B: fullness, C: thirst, D: wanting) are in separate graphs, labeled on the y-axis (P < 0.05). Details as in Figure 4.

Correlations with amount of swallowed mouth rinse solution

Since we unexpectedly observed a significant effect of sweet liking status on the amount of solution swallowed and we observed a coefficient of variation of 0.534 for amount of swallowed solution across all solutions and participants, we explored whether amount of swallowed solution is associated with walking energy expenditure, RER, substrate oxidation, and RPE. We averaged across phases for each of the variables, which resulted in one data point per participant per mouthrinse solution, for a total of 50 data points (n=10). All correlations showed significant bivariate non-normality (Shapiro-Wilk p<.05), so we calculated Spearman's rho. We observed that the amount of swallowed solution correlates negatively with walking energy expenditure (□= -0.301, 95% CI [-0.535, -0.025], p= 0.034), walking energy expenditure/kg (□= -0.366, 95% CI [-0.585, -0.098], p= 0.009), net walking energy expenditure (□= -0.345, 95% CI [-0.569, -0.074], p= 0.014), fat oxidation (□= -0.366, 95% CI [-0.585, -0.097], p= 0.009) and positively with RPE (□= 0.371, 95% CI [0.104, 0.589], p= 0.008). The strength of these correlations can be described as moderate.[29] The amount of swallowed solution was not correlated with RER (□= 0.052, 95% CI [-0.23, 0.326], p= 0.721) or carbohydrate oxidation (□= -0.177, 95% CI [-0.434, 0.107], p= 0.220) (Figure 7). These results show that there is variation in the amount of solution swallowed and that this may affect walking energy expenditure negatively.

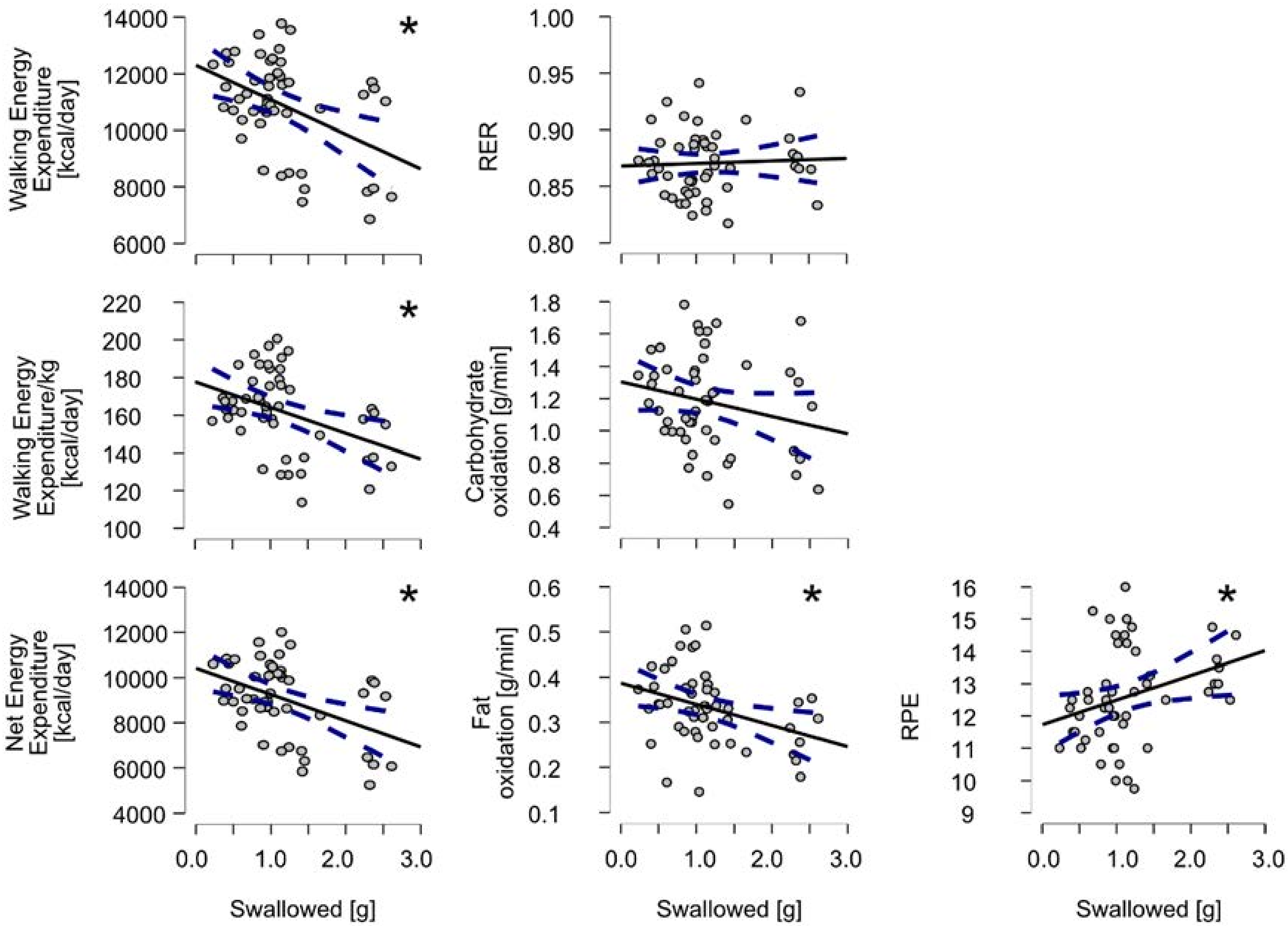

Figure 7. Spearman correlations (n=10) between the amount of swallowed solution and walking energy expenditure, walking energy expenditure/kg, net energy expenditure, RER, carbohydrate oxidation, fat oxidation and RPE. Circles indicate each solution, 5 per participant for a total of 50 data points. The solid line indicates the best fitting linear regression, the dashed blue lines indicate the 95% confidence interval. Asterisk indicates P < 0.05.

Discussion

No significant differences were observed between the two medium-intensity solutions containing reciprocal binary mixtures of sucrose and sucralose in walking energy expenditure parameters. Mouth rinsing with higher total sweetness also did not significantly affect walking energy expenditure. Similarly, neither the composition of sweeteners nor the total sweetness level exerted any significant effect on RER, perceived effort, substrate oxidation, fullness, hunger, or wanting. Previous studies showed that mouth rinsing with relatively non-sweet carbohydrates compared to non-sweet controls improves exercise performance. Mouth rinsing with sweet carbohydrates compared to equi-sweet controls has led to mixed results on performance, with some studies showing enhancement and others showing no effect. While the design of such studies hinges on using similarly non-sweet or sweet controls, mouth rinsing studies generally do not measure sweetness perception and do not take important interindividual differences in perception (such as preferred sweetness) into account. Moreover, the observation by Hawkins et al[11] that a sucralose solution may reduce completion time of exercise over a water control suggests that sweet taste itself - independent of the presence of carbohydrates - plays a role in the ergogenic effect of carbohydrate mouth rinses. If sweetness itself plays an independent role in the ergogenic effect of mouth rinses, then systematic manipulations of sweetness and carbohydrate content should lead to differences in exercise performance.

Regarding the perception of the solutions, we successfully manipulated sweetness intensity differences between the control and 1:1 solution and the solutions containing 6% sweetness from either sucrose or sucralose or both. We also successfully created equi-sweet solutions with 7% total sweetness from reciprocal combinations of sucrose and sucralose. However, the solution that we intended to be strongest in sweetness, the 6:6 solution, received statistically similar sweet ratings as the 1:6 and 6:1 solutions. This may reflect ceiling effects of sweetness concentration, which may be the result of suppression of the different sweeteners on eachother. Perceived sweetness of a mixture of sweeteners can be equal to the sum of the sweetness of the individual sweeteners (additivity), greater (synergy), or less (suppression) than the sum of their individual constituents' intensities. While mixtures at low concentrations generally show synergistic effects for high intensity sweeteners, mixtures at high concentrations may show suppression.[30] Suppression at high concentration may explain why the 6:6 solution was not perceived as greater in sweetness relative to the 1:6 and 6:1 solutions. These results showed that we mostly achieved our perceptual manipulation goals, with the exception of the highest concentration.

When we tested the first hypothesis, that the composition of sweeteners will affect walking exercise, we observed no significant differences between the two medium intensity solutions with reciprocal binary mixtures of sucrose and sucralose in neither the walking energy expenditure parameters, perceived effort, nor substrate oxidation. This may reflect that using a greater proportion of nutritive vs non-nutritive sweeteners does not affect the ergogenic effect of mouth rinses. However, it is also possible that there are other explanations for a lack of effect. The first explanation is that there were too few participants and that the study is underpowered to observe any differences. However, our sample size is within the lower range of previous mouth rinsing study.[9] Additionally, our sample size is large enough to observe robust effects of time (phase) on various physiological measures, such as walking energy expenditure and substrate utilization measures. This indicates that if there are differences from sweeteners mixture compositions, they are relatively small effects. It also means that if they exist, future studies will need larger sample sizes to detect them.

Our second hypothesis, that mouth rinsing with higher total sweetness would increase walking energy expenditure, was not supported by our observations. Our mouth rinse solutions of total sweetness rates were intended as low (2% total, 1:1 sucrose-sucalose ratio), medium (7% total, 1:6 and 6:1 sucrose-sucalose ratio), and high (12% total, 6:6 sucrose-sucalose ratio), but these concentration manipulations did not have a dose-response effect on 60-minute walking exercise. In the first dose-response study conducted by 6% and 12% carbohydrate electrolyte solution mouth rinses delivered during 90-minute running exercise improved performance for both solutions relative to a placebo with non-nutritive sweetener, but no difference was observed between the two carbohydrate concentrations in distance covered.[8] Ispoglou et al[24] also showed that there is no increase in cycling performance for each mouth rinsing solution (placebo, 4%, 6% and 8% carbohydrate concentration). Similar to our results, there was no difference between placebo and other carbohydrate solutions.[24] Moreover, there is no meta-analytic evidence for dose effects in mouth rinses.[5] The lack of dose-response effects from sweetness on walking energy expenditure in the current study is in accordance with the existing literature.

As outlined above, we did not observe effects of changing the relative contribution of nutritive sweeteners, nor of sweetness differences. Unexpectedly, we also did not replicate the ergogenic effect of mouth rinses in general, since we observed that none of the solutions improved walking energy expenditure relative to the control solution. According to the literature, mouth rinsing with carbohydrate solutions has varying effects on performance and these differences may be due to methodological differences. In a meta-analysis, carbohydrate mouth rinse can improve cycling performance by approximately 1.69%,[31] a small effect that shows variation. Most of those studies use high-intensity exercise. In the current study, the effect of solutions during moderate-intensity exercise was evaluated. The effect of solutions with sucrose and sucralose may be more pronounced in high-intensity exercises. If exercise intensity is a systematic moderator of the effect of mouth rinses on energy expenditure, then that may explain that our choice for moderate intensity exercise resulted in no change in walking energy expenditure. We are aware of two studies manipulating exercise intensity. Bastos-Silva et al[32] compared moderate (reported exertion similar to our values) and high intensity cycling showed a greater performance enhancement for the moderate intensity exercise. On the other hand, Karayigit et al[33] observed improved performance for a high intensity exercise, but not for a lower intensity version, but it should be noted that unlike our study and Bastos-Silva et al,[32] they used resistance exercise. More studies on the exercise intensity and type (aerobic vs resistance) as moderator of the ergogenic effect of mouth rinses are warranted.

Unexpectedly we observed an influence of sweet liking phenotype on the amount of the mouth rinse that participants swallowed. We also unexpectedly observed a relation between the amount of swallowed mouth rinse and walking exercise. We underline that these sources of interindividual differences need to be replicated in much larger studies, as a sample size of 10 participants is very small to assess their influence. However, we want to tentatively suggest that our observations indicate that sweetness overall may not matter much, but that individual differences in sweetness may indirectly influence the ergogenic effect of mouth rinses. This is because liking of sweetness may influence how much participants swallow of the solutions and the amount of mouth rinse that is swallowed in turn influences walking exercise negatively. We will first discuss the influence of liking of sweetness and then the influence of the amount of mouth rinse swallowed in separate paragraphs below.

Humans show marked variation in their liking of sweetness. In the current study, the majority of participants (n=6) were sweet likers, three participants showed the inverted U phenotype and one participant was a sweet disliker. We observed an influence of this phenotype on how much of the mouth rinses the participant swallowed. While participants are instructed to not swallow anything and expectorate all of the mouthrinse, there is always a small amount swallowed and there is variation in how much that is. This variation is presumed to be negligent and unsystematic in other studies of the ergogenic effect of mouth rinses. Our findings indicate that sweet liking may be a systematic contributor to variation in swallowed amounts of mouth rinses. At face value, the direction of the effect -a greater amount swallowed for sweet likers- also makes sense; a participant that dislikes sweet solutions may be more diligent at expectorating the solution, and participants that enjoy the taste of the solutions may be less diligent and swallow more as a result. Our observation is also in agreement with the study by Ispoglou et al,[24] who showed that participants swallowed more of the 6% and 8% than they did of 0% and 4% mouthrinses (consisting of a mixture of sucrose and glucose with electrolytes added). This behavior may be an involuntary behavioral driver of systematic or unsystematic varianceand requires further investigation with a larger sample size.

We unexpectedly observed an influence of the amount of swallowed solution on walking energy expenditure. Other studies on the ergogenic effect of mouth rinses generally assume that the amount of swallowed solution is zero or negligent and not systematic. This argument is supported by a lack of changes in blood glucose.[34] However, as we proposed in the previous paragraph, the assumption that this variance is non-systematic may not hold. In the current study we observed that variation in the amount swallowed may be systematic in relation to individual differences in sweet liking and others have shown that there may be systematic differences in relation to mouth rinse concentration.[24] If the amount of swallowed mouth rinse is systematically influenced by other factors, then it should also be considered as a source of variation for exercise performance. We do not know of any studies that directly investigated this. Because we observed an effect of participants' sweetliking phenotype on the amount of swallowed solution, we decided to directly assess the relationship between the amount of swallowed solution and walking energy expenditure. We observed that if more of the mouth rinse is swallowed, there is also lower walking energy expenditure and higher perceived exerted effort. At face value, the direction of this effect may be counterintuitive, after all, the proposed mechanism of the ergogenic effect is that it mimics the effect of carbohydrate consumption on the brain, thus counteracting tiredness and/or augmenting motivation and motor output. So if there is more carbohydrate swallowed then wouldn't one expect this to positively affect exercise performance? We propose that the answer to this may depend on the types of physiological processes triggered by a small amount of swallowed mouth rinse and on the intensity of the exercise. The spreading activation hypothesis proposed by Chambers et al.[35] suggests that taste perception in the mouth activates neural reward pathways in the brain, which in turn stimulates the sympathetic nervous system and may enhance exercise performance. However, this process may not be limited to sympathetic activation alone. For example, sucrose solutions stimulate salivation.[36] In the current study we observed a greater expectorated amount of the solutions with the higher concentrations mouth rinses, which is consistent with greater induced salivation. Small amounts of swallowed mouth rinse may trigger cephalic phase responses that activate the parasympathetic nervous system, potentially increasing blood flow to the digestive system and reducing energy expenditure during moderate-intensity exercise. The increased salivation observed with higher-concentration solutions further supports this mechanism. To test these speculations, future studies should directly manipulate small amounts of swallowed mouth rinse and investigate their effects on exercise performance across low, moderate, and high intensity exercise.

We did not intend for thirst to increase, but this was expected as participants could not drink water. Thirst increased similarly across all mouth rinses, so it is unlikely to be a confounding factor, though excessive thirst might obscure ergogenic effects. Previous studies show mixed results: Ispoglou et al.[24] found increased thirst only in carbohydrate (sucrose and glucose) trials, Deighton et al.[9] observed lower thirst with maltodextrin and placebo, and Přibyslavská et al.[37] found no effect of maltodextrin rinses. Overall, the relationship between mouth rinses, thirst, and exercise performance remains unclear and warrants further investigation.

We observed that mouth rinsing with different relative contributions from nutritive and non-nutritive sweeteners did not make a difference for walking energy expenditure. We also did not observe a dose-response effect, nor did carbohydrate mouth rinses result in any increase in walking energy expenditure compared to the control solution. This study has several limitations. First, the sample size was small and likely underpowered to observe a small effect size. Larger cohorts are needed to accurately characterize interindividual difference. Since only male participants were included in the current study, it is unclear whether mouth rinsing with nutritive and non-nutritive sweet solutions would produce similar ergogenic effects in women, which represents a limitation of this study. Another limitation is that the study was only partially blinded, as sweetness and intensity differed across the solutions. Although participants could perceive differences between the solutions, they were not informed about the specific ingredients or the study hypothesis. Therefore, it is unlikely that awareness of the manipulated variable systematically influenced the results. We also note that, because only the walking energy expenditure measurements were defined as the primary outcomes, all other outcomes are considered exploratory should be confirmed in studies with larger cohorts, and should be interpreted with caution.

Using only water as the control solution made it distinguishable from the other solutions due to the presence of sweet taste. This resulted in participants not being fully blinded to all conditions. Future studies are needed that to understand how ergogenic effects of mouth rinsing with nutritive and non-nutritive varies with gender or sex. Designing study groups consisting of male and female individuals in future studies will contribute to this area. Unexpected, we observed that individual differences in sweet liking influenced the amount of mouth rinse that was swallowed and that this in turn leads to a decreased walking energy expenditure. This indicates the need for a greater experimental control over individual differences and unintentional swallowing in mouth rinsing studies, as well as the need for studies directly investigating such relationships.

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References

1. Carter JM, Jeukendrup AE, Jones DA. The effect of carbohydrate mouth rinse on 1-h cycle time trial performance. *Med Sci Sports Exerc*. 2004;36(12):2107-2111. https://doi:10. 1249/01. MSS. 0000147585. 65709. 6F

2. Rollo I, Williams C, Gant N, Nute M. The influence of carbohydrate mouth rinse on self-selected speeds during a 30-min treadmill run. *Int J Sport Nutr Exerc Metab*. 2008;18(6):585-600. https://doi:10. 1123/ijsnem. 18. 6. 585

3. Rollo I, Cole M, Miller R, Williams C. Influence of mouth rinsing a carbohydrate solution on 1-h running performance. *Med Sci Sports Exerc*. 2010;42(4):798-804. https://doi:10. 1249/MSS. 0b013e3181bac6e4

4. Dorling JL, Earnest CP. Effect of carbohydrate mouth rinsing on multiple sprint performance. *J Int Soc Sports Nutr*. 2013;10(1):1-8. https://doi:10. 1186/1550-2783-10-41

5. Hartley C, Carr A, Bowe SJ, Bredie WLP, Keast RSJ. Maltodextrin-Based Carbohydrate Oral Rinsing and Exercise Performance: Systematic Review and Meta-Analysis. *Sports Med*. 2022;52(8):1833-1862. https://doi:10. 1007/s40279-022-01658-3

6. Hopkins W. Competitive Performance of Elite Track-and-Field Athletes. *Sports Sci.* 2005;9:17-20.

7. Kulaksız TN, Koşar ŞN, Bulut S, et al. Mouth rinsing with maltodextrin solutions fails to improve time trial endurance cycling performance in recreational athletes. *Nutrients*. 2016;8(5):2-15. https://doi:10. 3390/nu8050269

8. Wright BF, Davison G. Carbohydrate Mouth Rinse Improves 1. 5 h Run Performance: Is There A Dose-Effect? *Int J Exerc Sci.* 2013;6(4):328-340. http://www. ncbi. nlm. nih. gov/ pubmed/27990218%0A

9. Deighton K, Duckworth L, Matu J, et al. Mouth rinsing with a sweet solution increases energy expenditure and decreases appetite during 60 min of self-regulated walking exercise. *Appl Physiol Nutr Metab.* 2016;41(12):1255-1261. https://doi:10. 1139/apnm-2016-0344

10. Green MS, Kimmel CS, Martin TD, Mouser JG, Brune MP. Effect of Carbohydrate Mouth Rinse on Resistance Exercise Performance. *J Strength Cond Res.* 2020;36(7):1916–1921.

11. Hawkins KH, Krishnan S, Ringos L, Garcia V, Cooper JA. Running Performance with Nutritive and Non-Nutritive Sweetened Mouth Rinses. *Int J Sports Physiol Perform.* 2017;12(8):1105-1110. https://doi:10. 1123/ijspp. 2016-0577. Running

12. Yeung AWK, Wong NSM. How Does Our Brain Process Sugars and Non-Nutritive Sweeteners Differently : A Systematic Imaging Studies. *Nutrients.* 2020;12(10):3010.

13. Frank GKW, Oberndorfer TA, Simmons AN, et al. Sucrose activates human taste pathways differently from artificial sweetener. *Neuroimage.* 2008;39(4):1559-1569. https://doi:10. 1016/j. neuroimage. 2007. 10. 061

14. Turner CE, Byblow WD, Stinear CM, Gant N. Carbohydrate in the mouth enhances activation of brain circuitry involved in motor performance and sensory perception. *Appetite.* 2014;80:212-219. https://doi:10. 1016/j. appet. 2014. 05. 020

15. Jagim AR, Harty PS, Barakat AR, et al. Prevalence and Amounts of Common Ingredients Found in Energy Drinks and Shots. *Nutrients*. 2022;14(2). https://doi:10. 3390/nu14020314

16. Birch GG. Towards an improved understanding of sweetener synergy. *Trends Food Sci.* 1996;7(12):403-407. https://doi:10. 1016/S0924-2244(96)10041-8

17. Reyes MM, Gravina SA, Hayes JE. Evaluation of sweetener synergy in humans by isobole analyses. *Chem Senses.* 2019;44(8):571-582. doi:10. 1093/chemse/bjz056

18. Iatridi V, Hayes JE, Yeomans MR. Quantifying sweet taste liker phenotypes: Time for some consistency in the classification criteria. *Nutrients*. 2019;11(1):1-24. https://doi:10. 3390/ nu11010129

19. DuBois GE, Walters DE, Schiffman SS, et al. Concentration—response relationships of sweeteners. In: Walters DE, Orthoefer FT, DuBois GE, editors. *Sweeteners: discovery, molecular design, and chemoreception.* Washington (DC): American Chemical Society Publications; 1991. p. 261–76.

20. Janse De Jonge XAK. Effects of the Menstrual Cycle on Exercise Performance. *Sports Med.* 2003;33(11):833-851. https://doi:10. 2165/00007256-200333110-00004

21. Sinclair J, Bottoms L, Flynn C, et al. The effect of different durations of carbohydrate mouth rinse on cycling performance. *Eur J Sport Sci.* 2014;14(3):259-264. https://doi:10. 1080/17461391. 2013. 785599

22. Reynolds AN, Venn BJ. The Timing of Activity after Eating Affects the Glycaemic Response of Healthy Adults: A Randomised Controlled Trial. *Nutrients*. 2018;10(11):1743. https://doi:10. 3390/nu10111743

23. Ribichini E, Scalese G, Cesarini A, et al. Exercise-Induced Gastrointestinal Symptoms in Endurance Sports: A Review of Pathophysiology, Symptoms, and Nutritional Management. *Dietetics*. 2023;2(3):289-307. https://doi:10. 3390/dietetics2030021

24. Ispoglou T, O□Kelly D, Angelopoulou A, Bargh M, O□Hara JP, Duckworth LC. Mouth Rinsing With Carbohydrate Solutions at the Postprandial State Fail to Improve Performance During Simulated Cycling Time Trials. *J Strength Cond Res*. 2015;29(8):2316-2325. https://doi:10. 1519/JSC. 0000000000000882

25. Navalta JW, Stone WJ. Ethical Issues Relating to Scientific Discovery in Exercise Science. *Int J Exerc Sci*. 2019;12(1):1-8. https://doi:10. 70252/eycd6235

26. Klimek L, Bergmann KC, Biedermann T, et al. Visual analogue scales (VAS) - Measuring instruments for the documentation of symptoms and therapy monitoring in case of allergic rhinitis in everyday health care. *Allergo J Int.* 2017;26(1):36-47. https://doi:10. 1007/s40629-016-0006-7

27. American College of Sports Medicine. *Acsm's Guidelines for Exercise Testing and Prescription (8th Ed.)*. Wolters Kluwer Health; 2010. 230 p.

28. Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol.* 2016;121(6):628-634.

29. Dancey CP, Reidy J. Statistics Without Maths for Psychology: Using SPSS for Windows. Prentice Hall; 2004. 644 p.

30. Keast RSJ, Breslin PAS. An overview of binary taste-taste interactions. *Food Qual Prefer.* 2003;14(2):111-124. https://doi:10. 1016/S0950-3293(02)00110-6

31. Brietzke C, Franco-Alvarenga PE, Coelho-Júnior HJ, Silveira R, Asano RY, Pires FO. Effects of Carbohydrate Mouth Rinse on Cycling Time Trial Performance: A Systematic Review and Meta-Analysis. *Sports Med.* 2019;49(1):57-66. https://doi:10. 1007/s40279-018-1029-7

32. Bastos-Silva VJ, Melo A de A, Lima-Silva AE, Moura FA, Bertuzzi R, de Araujo GG. Carbohydrate mouth rinse maintains muscle electromyographic activity and increases time to exhaustion during moderate but not high-intensity cycling exercise. *Nutrients*. 2016;8(3). https://doi:10. 3390/nu8030049

33. Karayigit R, Eser MC, Gur F, Sari C, Cepicka L, Gabrys T. Carbohydrate Mouth Rinse Increases High but Not Low Intensity Repetitions to Failure in Resistance-Trained Males. *Nutrients*. 2022;14(4):1-10. https://doi:10. 3390/nu14040875

34. Clarke ND, Thomas JR, Kagka M, Ramsbottom R, Delextrat A. No Dose-Response Effect of Carbohydrate Mouth Rinse Concentration on 5-km Running Performance in Recreational Athletes. *J Strength Cond Res*. 2017;31(3):715-720. https://doi:10. 1519/JSC. 0000000000001531

35. Chambers ES, Bridge MW, Jones DA. Carbohydrate sensing in the human mouth: Effects on exercise performance and brain activity. *J Physiol.* 2009;587(8):1779-1794. https://doi:10. 1113/jphysiol. 2008. 164285

36. Froehlich DA, Pangborn RM, Whitaker JR. The effect of oral stimulation on human parotid salivary flow rate and alpha-amylase secretion. *Physiol Behav.* 1987;41(3):209-217. https://doi:10. 1016/0031-9384(87)90355-6

37. Přibyslavská V, Scudamore EM, Johnson SL, et al. Influence of carbohydrate mouth rinsing on running and jumping performance during early morning soccer scrimmaging. *Eur J Sport Sci.* 2016;16(4):441-447. https://doi:10. 1080/17461391. 2015. 1020345

Supplementary material

When the sweet taste liking status was included as a covariate in the repeated measures ANOVAs, the solution and sweet liking status interaction (solution x sweet liking status) was not significant for intensity, sweetness, bitterness, liking, fullness, hunger, wanting, thirst, expectorated volume, WEE, WEE/kg, WEEnet, HR, RPE, CHO and fat oxidation. There was no interaction between phase and sweet liking status in WEE, WEE/kg, WEEnet, HR, RPE, CHO and fat oxidation. For swallowed volume there was a significant interaction between solution and sweet liking status.

Table 1. Repeated measures ANOVA results with sweet liking status included as a covariate.

DV	Interaction effect of solution x sweet liking status	Interaction effect of phase x sweet liking status
Intensity	F(4,32)=0.661, p =0.623	NA
Sweetness	F(4,32)=1.799, p =0.153	NA
Bitterness	F(4,32)=0.337*, p =0.696*	NA
Liking	F(4,32)=1.431, p =0.246	NA
Fullness	F(4,32)=0.712, p =0.590	NA
Hunger	F(4,32)=2.128*, p =0.138*	NA
Wanting	F(4,32)=0.174, p =0.950	NA
Thirst	F(4,32)=1.039, p =0.403	NA
Expectorated (g)	F(4,32)=1.215, p =0.324	NA
Swallowed (g)	**F(4,32)=3.375, p =0.021**	NA
WEE (kcal/day)	F(4,32)=0.027, p = .990	F(7,56)=0.904, p=.393*
WEE/kg (kcal/day)	F(4,32)=0.116, p = .976	F(7,56)=0.698, p=.466*
WEEnet (kcal/day)	F(4,32)=0.021, p = .999	F(7,56)=0.904, p=.393*
HR (pbm)	F(4,32)=0.547, p = .524*	F(7,56)=0.276, p=.961
RPE	F(4,32)=0.981, p = .432	F(3,24)=0.243, p=.675*
CHO oxidation (g/min)	F(4,32)=0.661, p = .624	F(7,56)=0.752, p=.464*
Fat oxidation (g/min)	F(4,32)=0.933, p = .457	F(7,56)=0.217, p=.856*

*Sphericity violated, Greenhouse-Geisser correction used
Bold font indicates a significant effect

Table 2. Anthropometric characteristics of participants. Repeated measures ANOVA showed that there was no significant difference across the sessions.

n=10	day 1	day 2	day 3	day 4	day 5	p-value
Weight, kg	67.20±7.49	67.14±7.24	67.29±7.03	67.40±6.98	67.05±7.34	0.473
BMI, kg/m²	21.48±2.17	21.45±2.10	21.48±2.00	21.52±1.99	21.41±2.12	0.502
fat free mass, kg	60.62±7.09	60.35±6.75	60.42±6.74	60.58±6.59	60.53±7.00	0.872
fat percentage,%	9.79±2.99	10.20±2.63	10.22±2.61	10.11±2.14	9.730±2.32	0.629

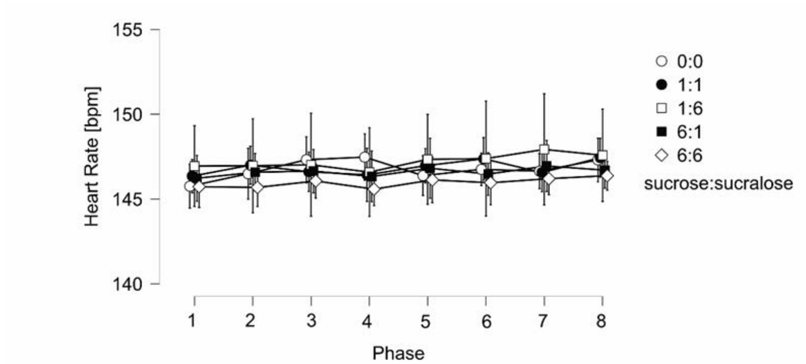

Figure 1. Average heart rate values obtained (n=10) from rinsing each of the solutions during the phases of exercise (P<0.05). Each solution is depicted with different symbols, open circles - 0:0 (demineralized water), black circles - 1:1 (1% sucrose+0.00186% sucralose (equivalent to ~1% sucrose sweetness)), open square - 1:6 (1% sucrose+0.00985% sucralose (equivalent to ~6% sucrose sweetness)), black square - 6:1 (6% sucrose+0.00186% sucralose), open diamond - 6:6 (6% sucrose+0.00985% sucralose). Graph shows average heart rate across phases ± 95 % confidence interval (on the x-axis).

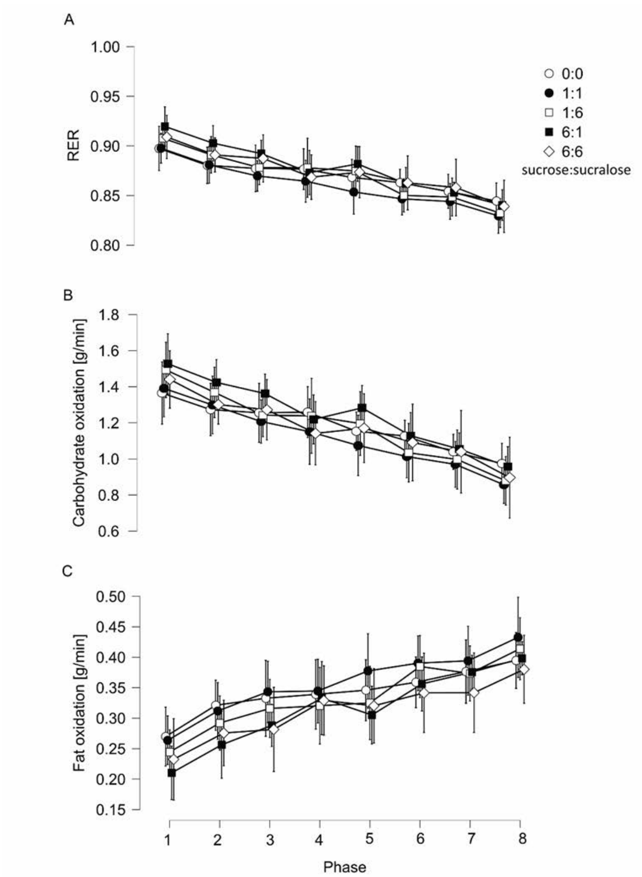

Figure 2. Average RER, carbohydrate and fat oxidation values (n=10) obtained from rinsing each of the solutions during the phases of exercise (P<0.05). Details as in Figure 1. RER, respiratory exchange ratio.