Carbohydrate Mouth Rinse and Hydration Strategies on Cycling Performance in 30 Km Time Trial: A Randomized, Crossover, Controlled Trial

Amanda M.J. Ferreira¹, Luiz F. Farias-Junior¹, Thaynan A. A. Mota¹, Hassan M. Elsangedy¹, Aline Marcadenti^{2,3,4}, Telma M.A.M. Lemos¹, Alexandre H. Okano¹ and Ana P.T. Fayh¹ ^[]

São Paulo/SP, Brazil; ³ IC/FUC, Graduate Program in Health Sciences: Cardiology, Institute of Cardiology/University Foundation of Cardiology of Rio Grande do Sul, Porto Alegre/RS, Brazil; ⁴ UFCSPA, Graduate Program in Nutrition Sciences, Federal University of Health Sciences of Porto Alegre, Porto Alegre/RS, Brazil.

Abstract

The aim of this study was to investigate whether carbohydrate mouth rinse (CMR) improves physical performance of cyclists during a 30-km time trial test and its influence on water balance compared to other strategies of fluid intake. Eleven recreationally trained male cyclists completed a 30 km time trial cycle ergometer under three experimental interventions: (a) CMR, (b) drinking to replace all weight loss (DWL), and (c) drinking "ad libitum" (DAL). Time to complete the 30 km time trial, heart rate, average power, velocity, weight loss, urine color, urine density and pH were evaluated. Statistical analysis was performed using repeated measures analysis of variance (RM-ANOVA) and generalized estimating equations (GEE) with Bonferroni adjustment (p < 0.05). Time to complete the 30 km time trial was similar among CMR 54.5 ± 2.9 min, DWL 53.6 ± 3.9 min and DAL 54.5 ± 2.5 min (p = 0.13). CMR (1.7 \pm 0.4%) elicited similar water loss compared to the DAL ($1.4 \pm 0.6\%$) intervention, but it was higher than the DWL intervention $(0.6 \pm 0.6\%)$ (p < 0.01). CMR did not improve the performance of recreationally trained cyclists in a 30 km time trial test compared to other fluid intake strategies. Furthermore, CMR causes higher water loss compared to DWL intervention.

Key words: Mouth rinsing; endurance performance; exercise; metabolism; nutrition.

Introduction

Cycling time trials are characterized by shorter duration, high intensity and high pedaling cadence (Lucía et al., 1999; Lucía et al., 2001). In cycling competitions, the intensity is higher than 70% of maximum oxygen consumption (VO_{2máx}) and it can reach 90% among elite cyclists (Fernández-García et al., 2000; Lucía et al., 1999; Neumayr et al., 2002). Energy expenditure and cardiopulmonary high demand during cycling competitions challenges water loss control, which has an important role in thermoregulation control (Jeukendrup, 2011; Maughan and Meyer, 2013), and also in depletion of muscle glycogen stores during exercise (Garber et al., 2011; Lepers et al., 2002). Hence, excessive bodily fluid loss (> 2% of body mass), in the form of dehydration, impairs an athlete's physical performance, especially in a hot and humid environment (Maughan and Meyer, 2013; Maughan, 2012; Sawka et al., 2007).

The sports federations advise athletes to drink enough fluid to replace sweat losses during exercise or consume the maximal amount that can be tolerated (Sawka et al., 2007; Thomas et al., 2016). In addition, electrolyte replacement should be considered when exercise leads to high sweat rates, exceeding 900ml/h (Hernandez and Nahas, 2009; Sawka et al., 2007). On the other hand, researchers argue that free-choice fluid intake ("ad libitum") is sufficient to maintain homeostasis (Berkulo et al., 2015; Noakes, 2007; Wall et al., 2015). They believe that the central nervous system is able to indicate the correct fluid volume to be ingested according to afferent information, ensuring control of plasma levels and body temperature (Daries et al., 2000; Goulet, 2011; Machado-Moreira et al., 2006; Noakes et al., 2005; Noakes, 2007). These reports have increased adoption of the "ad libitum" strategy, especially in time trials in which high intensity raises the chance of athletes complaining about gastrointestinal discomfort or the risk of hyponatremia due to excessive water intake (Noakes et al., 2005; Noakes, 2007).

In addition to the recommendations described previously, a new strategy known as carbohydrate mouth rinse (CMR) is being studied in short bouts of exercise (≤ 60 minutes). In this strategy, individuals do not ingest liquids during exercise, but only rinse their mouth with a carbohydrate solution for 5-10 seconds, after which it is expectorated (Thomas et al., 2016). The hypothesis is that carbohydrates would be detected by taste receptors, activating important brain regions related to motivation and motor control, and also improving performance without any ingestion of carbohydrate (Bortolotti et al., 2011; Carter et al., 2004; Jeukendrup, 2014). Carter et al. (2004) were the first to test this hypothesis and to demonstrate performance benefits, though subjects were tested after fasting for 4 hours, which may not be reproducible in practical situations. In addition, CMR was compared to rinsing with a placebo solution containing only water in their composition, which could directly influence the results, because subjects could have perceived the nature of the tested solution. Considering that individuals do not ingest liquids during exercise, its impact on hydration and performance of athletes, along with its effectiveness compared to strategies traditionally recommended are still unknown.

Therefore, the necessity to learn how different nutrition strategies would influence performance of athletes in short high-intensity exercises (< 60 minutes), the present study aimed to investigate whether CMR improve physical performance of cyclists during a 30 km time trial test and

Methods

Participants

Eleven male trained cyclists were recruited to participate in the study. They had a training routine of at least 150 km for 5 hours a week and were heat acclimated, because they lived and trained at an average annual temperature of 28° C and average relative humidity of 83%. Exclusion criteria included smoking, obesity (BMI \ge 30 kg/m²), previous diagnosis of chronic diseases and musculoskeletal injuries within the last 6 months that could interfere with training routine. Participants were recruited via digital media and personal contact, in the Department of Physical Education of the Federal University of Rio Grande do Norte and in cycling teams of the city from April to December 2015. This study was approved by the University Human Research Ethics Committee (CAAE: 31747714.7.0000. 5568). All participants were informed about the procedures and signed the written informed consent. The number of participants included in the present study was calculated based on the study of Carter et al., 2004), considering a test power of 90% and significance level of 5%. The protocol for this trial and supporting CONSORT checklist are available as supporting information.

Study design

Each subject attended the laboratory on five different occasions. In the first visit, subjects performed a body composition assessment (anthropometric technique) and evaluation of performance in a maximal exercise test. The second visit was a familiarization session on the 30 km cycle ergometer time trial, with specific instructions to experimental trials and without ingestion of fluids during exercise. Visits 3-5 consisted of controlled trials, in which participants completed time trial test at self-selected intensity and in a fed state. They were under random influence of the following interventions: CMR = carbohydrate mouth rinse; DWL = drinking to replace all weight loss; DAL = drinking "ad libitum". The order of interventions was randomized by drawing lots for each participant with a minimum of four days between tests. Participants were aware of the intervention selected only at the beginning of test, but they were blinded to the composition of solutions that were offered in cups of translucent coloration.

Anthropometric assessment

Weight and height from all subjects were determined by a portable digital scale coupled to a stadiometer with accuracy of 0.1 kg and 1.0 cm (Welmy[®], W 110 H, Brazil). Body Mass Index was calculated and nutritional status was classified according to the cut-off points defined by the World Health Organization (WHO, 2000). All measurements of skinfolds (triceps, subscapular, chest, biceps, iliac crest, abdominal, medial thigh and calf) were made on the right side of the body by using a compass with accuracy of 0.1 mm (Cescorf[®], Brazil). Identification of sites and measurements were in accordance with the International Society

for the Advancement of Kinanthropometry (ISAK) standards. Skinfold measurement were used for calculation of body density using the generalized equation proposed by Jackson and Pollock (1978), and later converted to fat percentage, according to formula proposed by Siri (1961).

Maximal exercise test

Subjects performed a maximal exercise test on a cycle ergometer (Velotron, Racermate, Inc., Seattle, WA, USA) to determine the maximal oxygen uptake (VO2max) and heart rate (HRmax). The initial workload was 100W followed by 25W increments per minute until exhaustion. Subjects were instructed to maintain cadence ≥ 80 rpm. The gas exchange breath-by-breath was recorded throughout the maximal exercise test (Quark - CPET, Cosmed, Roma, Italy). VO₂max was considered as the highest mean value in 30s recorded during the test. Heart rate was recorded throughout the test using a Polar Monitoring System (Polar RS800cx, Kempele, Finland). All subjects achieved $\geq 95\%$ of age-predicted maximal HR (220 - age) at the moment of volitional exhaustion. The appropriate seat position and handlebar height were determined and replicated for each subsequent visit.

Experimental trials

Before all trials, participants proceeded urine collection and weight measurement (light clothing and no shoes). They were equipped with a heart rate monitor and instructed to perform a 30 km time trial cycle ergometer (Velotron, Racermate, Inc., Seattle, WA, USA) with self-regulated pace in the shortest time possible, under three different nutritional strategies. The evaluation of physical performance was based on the total time to complete the 30 km time trial in each intervention. Heart rate was recorded throughout all trials (Polar RS800 cx, Kempele, Finland). The software coupled to the cycle ergometer recorded performance variables during trials (distance traveled, average power, average speed and cadence). At the end of the test, subjects were directed to a new urine collection and weight measurement. Assessment of hydration status during experimental trials was carried out using three methods: monitoring of weight change, urine color and urine analysis. Weight changes were evaluated by weight loss percentage, which represents weight variation from the beginning to the end of training session (final weight (kg)-starting weight (kg)/100) (Casa et al., 2000). Urine color was evaluated according to the scale of Armstrong et al. (1994). For avoid bacteria contamination, urine was collected after cleaning the genital area with water (but nor soap) before giving a sample. A midstream urine was used - subjects were instructed to interrupt the flow of urine after a few seconds and then collecting this middle portion of the urine in a specific recipient. Urine samples were analyzed immediately after collection by the same researcher, in a well-lit room by holding the samples against a white background and rating them as whole numbers after comparison against a previously published scale (scale colors ranging from 1 [lightest] to 8 [darkest]). Variables such as pH and density of urine were analyzed using urine test strips in a semi-automatic urine analyzer (Labtest®, Cobas U, 411).

All trials were performed in an acclimatized room

(average temperature of 22.1° C and humidity of 72.5%). Participants were instructed to abstain from alcohol, caffeine, tobacco and exercise, and also to stay properly hydrated for 24 hours prior each visit. It was mandatory to ingest 500 ml of fluid two hours before the tests, according to recommendations from the American College of Sports Medicine (Sawka et al., 2007). All trials were conducted at the same time of the day and subjects were in a fed state (\leq 2-3 hours from the last meal). Participants were also instructed not to change the eating pattern in the days leading up to the tests, particularly the last meal prior testing. Furthermore, they were instructed to record all foods and beverages consumed throughout the day. Based on this food diary, nutritional composition was analyzed using a software (DietWin Profissional, DietWin®, Porto Alegre, Brazil).

Solution for CMR comprised 6.4% of unflavored maltodextrin (Atlhetica®, Brazil), in accordance with recommendations from literature (Jeukendrup, 2014). Subjects received 25 ml of the solution after every 12.5% of the 30 km time trial completed. They were instructed to rinse the fluid around their mouths for about 10 seconds, and then, spit the whole solution in a container indicated by the investigator. The expelled volume of liquid was measured to ensure that ingestion of solution was not significant. According to recommendations from the ACSM for fluid replacement (Sawka et al., 2007; Thomas et al., 2016), weight loss during the familiarization session was considered to determine the fluid volume to be replaced in the DWL session. The calculated volume was evenly distributed during the test in cups of 150ml, so that participants drank all the liquid or the maximum scheduled to be tolerated. In addition, 1 capsule of electrolyte supplement (Repor Salt, Atlhetica[®], Brazil) that corresponds to 321 mg of sodium (14 mEq) was used in cases that weight loss was higher than 2%. In the DAL session, water was offered as much as the subjects wanted (ad libitum) in cups of 150 ml, and the volume ingested was registered.

Statistical analysis

The normality of the data and the homogeneity of variance were tested using Shapiro-Wilk and Levene test, respectively. The results were expressed as mean \pm standard deviation. The variables time to complete trial, heart rate, power, energy consumption and food consumption, regardless of time and intervention, were analyzed by comparison of means using repeated measures ANOVA (RM-ANOVA). The variables measured before and after trial such as weight and urinary markers were evaluated according to intervention and time using the Generalized Estimating Equation (GEE: General Estimation Equation) with Bonferroni adjustment for repeated measures. In order to ensure that the effect of one treatment did not influence or alter the effect of subsequent treatments, the order of participants' presentation and residual effects (carry-over) were balanced. The size of the variance effect was calculated by partial eta-squared (η^2_p). All analysis the level of statistical significance was considered as p < 0.05. Statistical analysis was performed using SPSS® 22.0 software and STATA version 1.2 for Windows.

Results

Eleven male recreationally trained cyclists participated in the study (Age = 30.4 ± 6.2 yr; height = 1.73 ± 0.08 m; weight = 73.6 ± 7.81 kg; body mass index = 24.67 ± 2.22 kg/m²; Body fat percentage = $11.2 \pm 3.4\%$, maximum power = 362.5 ± 48.88 W; maximum oxygen consumption = 55.3 ± 5.6 ml·kg⁻¹·min⁻¹). Table 1 shows dietary intake in the previous 24 hours and last meal before experimental trials. There was no significant difference among experimental interventions in the energy consumption of carbohydrates, proteins and lipids, and the average energy in the previous 24 hours and last meal before exercise.



Figure 1. Maximal Heart Rate (A) and Power (B) response for each percentage 30-km time trial distance in CMR (carbohydrate mouth rinse), DWL (drinking to replace all the weight lost) and DAL (drinking "ad libitum") interventions. The values expressed as Mean \pm SD. * = within effect for CMR interventions related to 25% of distance. # = within effect for DWL interventions related to 25% of distance. RM-ANOVA (F_(1.36, 13.60) = 14.8, p < 0.001, $\eta^2 p$ = 0.597).

The mean intensity of 30 km time trials were not significant different (81.3 \pm 5.8, 82.5 \pm 7.9 and 81.1 \pm 7.4 %HR_{max}, F_(2,20)=0.51, p=0.60, $\eta^2 p$ =0.049) among CMR, DWL and DAL interventions. Regarding 30 km time trial performance, there were not significant differences for average power (198.6 \pm 25.9, 210.7 \pm 41.3 and 196.9 \pm 22.4 W, F_(1.31, 13.14) = 2.94, p = 0.25, $\eta^2 p$ = 0.227) and average velocity (33.1 \pm 1.7, 33.8 \pm 2.5 and 33.0 \pm 1.5 km·h⁻¹, F_(2,20)= 3.07, p = 0.06, $\eta^2 p$ = 0.235) among CMR, DWL and DAL interventions, respectively. The distances were divided at 25, 50, 75 e 100% of 30 km time trial for analysis. Figure 1 shows that heart rate increased throughout 30 km time trial in CMR and DWL, reaching higher values in the last stage, but did no change in DAL intervention (F_{(1.36},

 $_{13.60)}$ = 14.8, p < 0.001, $\eta^2 p$ = 0.597). However, there was no significant difference among interventions (F_(6, 60) = 1.37, p = 0.24, $\eta^2 p$ = 0.121). Additionally, there was no change in power of any intervention throughout time trial (F_(6, 60) = 7.13, p = 0.64, $\eta^2 p$ = 0.067). Time to complete 30 km time trial among the CMR, DWL and DAL interventions were not significantly different (54.5 ± 2.9, 53.6 ± 4.0 and 54.5 ± 2.5 minutes,

 $F_{(2,20)} = 2.25, p = 0.131, \eta^2 p = 0.184).$

All interventions elicited weight loss after completion of the 30 km time trial. However, DWL showed lower value of weight loss percentage (0.6 ± 0.6, 1.7 ± 0.4 and 1.4 ± 0.6 %weight loss, $F_{(2, 20)} = 32.9$, p < 0.001, $\eta^2 p = 0.767$) compared CMR and DAL interventions, respectively. Additionally, the volume of fluid intake throughout 30 km time trial was lower in CMR followed by DAL and DWL interventions (10.4 ± 4.3, 433.2 ± 258.8 and 1081.8 ± 337.1 mL, $F_{(2, 20)} = 50.58$, p < 0.001, $\eta^2 p = 0.835$). Table 2 shows results of hydration status. There was increased urinary concentration in all interventions, but it did not differ significantly. The urine density was not significant different over time of test and among interventions.

Food intake	CMR	DWL	DAL	p-value	
24 hours before exercise					
Energy intake (Kcal)	2453.8 ± 752.7	2280.2 ± 547.9	2965.0 ± 1182.9	.226	
Carbohydrate intake (g)	307.4 ± 109.2	278.3 ± 76.6	359.4 ± 150.9	.223	
Carbohydrate contribution (%)	50.5 ± 8.3	48.9 ± 5.9	48.4 ± 7.0	.766	
Carbohydrate contribution (g/kg)	4.1 ± 1.5	3.8 ± 1.0	4.9 ± 2.0	.337	
Protein intake (g)	144.0 ± 44.8	136.1 ± 33.7	196.6 ± 128.4	.228	
Protein contribution (%)	24.1 ± 6.0	24.6 ± 5.8	25.8 ± 7.6	.268	
Protein contribution (g/kg)	2.0 ± 0.6	1.8 ± 0.5	2.7 ± 1.7	.229	
Lipid intake (g)	72.0 ± 38.1	69.2 ± 27.0	82.3 ± 33.6	.611	
Lipid contribution (%)	25.4 ± 9.1	26.6 ± 5.7	25.8 ± 6.2	.915	
Lipid contribution (g/kg)	1.0 ± 0.5	0.9 ± 0.4	1.1 ± 0.5	0.612	
Meal before exercise					
Energy intake (Kcal)	669.2 ± 137.0	641.6 ± 132.3	621.1 ± 92.9	.199	
Carbohydrate intake (g)	74.62 ± 20.1	70.7 ± 17.9	68.2 ± 18.8	.666	
Carbohydrate contribution (%)	44.8 ± 9.1	44.2 ± 8.6	43.5 ± 7.5	.938	
Carbohydrate contribution (g/kg)	1.0 ± 0.3	1.0 ± 0.2	0.9 ± 0.3	474	
Protein intake (g)	62.9 ± 21.4	61.6 ± 18.7	57.2 ± 16.1	.736	
Protein contribution (%)	37.6 ± 9.2	38.1 ± 6.2	36.7 ± 8.8	.922	
Protein contribution (g/kg)	0.9 ± 0.3	0.8 ± 0.3	0.8 ± 0.2	.474	
Lipid intake (g)	12.2 ± 4.1	11.8 ± 4.0	12.5 ± 5.7	.921	
Lipid contribution (%)	16.4 ± 4.7	16.8 ± 5.6	18.7 ± 9.7	.374	
Lipid contribution (g/kg)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	.922	

CMR = carbohydrate mouth rinse. DWL = drinking to replace all the weight lost. DAL = drinking "ad libitum". p-value for repeated measures analysis of variance (RM-ANOVA)

Table 2. Hydration variables in three different intervention	s.
--	----

	CMR		DWL		DAL				
	Pre	Post	Pre	Post	Pre	Post	p ^a	pb	p ^c
Weight (Mean±SD)	73.0 ± 8.1	71.7 ± 7.9	72.5 ± 7.7	72.1 ± 7.5	72.4 ± 7.5	71.5 ± 7.4	< .001	.065	< .001
Diff. ± SE (IC95%)	-1.3 ± 0.1 (-	1.5 at -1.1)	-0.4 ± 0.1 (-	-0.7 at -0.2)	-1.0 ± 0.1 ((-1.3 at -0.7)			
p^d	< .001		< .001		< .001				
Urine Color (Mean±SD)	3.3 ± 1.3	5.3 ± 1.8	2.6 ± 1.5	3.6 ± 1.6	3.1 ± 1.2	4.5 ± 1.5	< .001	.072	.187
Diff. ± SE (IC95%)	2.0 ± 0.6 (0).9 at 3.1)	1.0 ± 0.1 ((0.8 at 1.3)	1.5 ± 0.3	(0.9 at 2.0)			
p^d	<.001		< .001		< .001				
Urine pH (Mean±SD)	6.5 ± 0.5	6.2 ± 0.2	6.4 ± 0.3	6.5 ± 0.3	6.5 ± 0.6	6.4 ± 0.2	.016	.525	.027
Diff. ± SE (IC95%)	-0.3 ± 0.1 (-	0.6 at -0.1)	0.1 ± 0.1 (-0.1 at 0.2)	$\textbf{-0.1}\pm0.2$	(-0.4 at 0.3)			
p ^d	.003		.289		.759				
Urine density (Mean±SD)	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	.283	.267	.633
Diff. ± SE (IC95%)	-0.1 ± 0.1 (-	0.1 at 0.1)	0.1 ± 0.1 (+	-0.1 at 0.1)	$\textbf{-0.1} \pm 0.001$	(-0.1 at 0.1)	1		
p ^d	.45	58	1.	00	.2	210			

CMR = carbohydrate mouth rinse. DWL = drinking to replace all the weight lost. DAL = drinking "ad libitum". Analyzed data according to GEE. Diff = Difference between times; SE = standard error; ^a time; ^b treatment; ^c interaction time * treatment; ^d within effect with adjust Bonferroni.

Discussion

In the present study, we investigate whether carbohydrate mouth rinse improve physical performance of recreationally trained cyclists compared to different strategies of fluid intake during high intensity exercise lasting less than one hour. To the best of our knowledge, this is the first study investigating whether CMR strategy contributes to a better performance than other traditional strategies of fluid intake, evaluating cyclists in a fed state. Additionally, this study aimed to compare the hydration status among different interventions (CMR, DWL and DAL). Our main results were that CMR intervention did not show superior influence on performance of cyclists in 30 km time trial, and it elicited similar water loss compared to DAL intervention, but higher than DWL intervention.

Carter et al. (2004) observed that the use of CMR strategy showed an improvement of 2.9% on the average time for completion of the test, compared to a placebo solution, in a time trial with intensity > 75% VO_{2máx} and lasting ~1 hour. Others researchers have found similar results using CMR (Bortolotti et al., 2011; Carter et al., 2004; Chambers et al., 2009; Correia-Oliveiraet al., 2013), and this strategy became popular between athletes. Although CMR strategy has been included in the ACSM position stand as an alternative to carbohydrates replacement in short-duration exercises (Thomas et al., 2016), the present study did not observe improvements on performance. It is possible that this result is related to the fed state of participants in our study. This condition (fed state) is more frequent between athletes during competitions, and our findings corroborate other similar studies that did not showed changes on performance (Beelen et al., 2009; Ispoglou et al., 2015; Kulaksız et al., 2016; Kumar et al., 2016). Beelen et al. (2009) did not find differences regarding time for completion of the test, power and heart rate in fed athletes rinsing their mouths with carbohydrate when compared to rinsing with water. Therefore, CMR strategies for subjects in a fed state do not appear to improve performance of cyclists in a test lower than 60 minutes.

Studies with CMR strategy aimed to evaluate their ergogenic effect, and did not assess the individual's hydration status during exercise. In high-performance sports, health issues are not valued as much as test scores. However, when this strategy begins to be used by a large number of athletes to improve their performance, without taking into account the potential risks to their health, researchers should pay attention to other outcomes than physical performance. It is important to emphasize that athletes exercising or competing in hot and humid conditions may have hydration disorders using the CMR strategy, especially if their weight loss was upper than the limits of health (>2%).

In the present study, cyclists underwent a physical test with average duration of 1 hour in an acclimatized room, without reaching dehydration, but with considerable water loss. It is postulated that fluid loss higher than 2% of weight decrease exercise performance (Casa et al., 2000). However, variables of weight loss, urine color and density that usually determine hydration status (Arnaoutis et al., 2012; Casa et al., 2000) did not influence the performance of cyclists under different interventions. It is unknown whether a higher water loss would influence the velocity and time to complete exercise test. It is currently discussed in the literature that hydration status is not the main factor influencing physical performance of athletes in this type of exercise (Berkulo et al., 2015; Cheung et al., 2015; Goulet, 2011; Noakes, 2007; Wall et al., 2013). Additionally, the fact that the athletes were acclimated to the hot and humid environment, due to the tropical climate of the city of this study, contributed to the maintenance of performance, regardless of the intervention.

The most accurate parameter to measure body water balance is plasma osmolality. In some situations, urinary specific gravity also shows to be a good marker (Mckenzie and Mu, 2015), followed by other urinary parameters such as density and pH (Mckenzie and Mu, 2015; Shirreffs, 2003). In practice, athletes and coaches use body mass variation to detect dehydration status, and it was used in the present study. Acute changes in body mass over a short time period can frequently be assumed to be due to body water loss or gain, since over a short time period, no other body component will be lost at such a rate (Shirreffs, 2003). We recognize that the use of these parameters is a limitation of the present study. However, these measures are widely used in other studies (Cheuvront et al., 2010; Shirreffs, 2003) and are accessible to the public for the practical applications. Moreover, they were taken with the methodological accuracy necessary for their correct scientific interpretation.

Goulet (2011) have suggested that exercise intensity and duration have a greater impact than hydration status, since water loss up to 4% of weight did not affect performance of athletes in their study. Some authors have confirmed this hypothesis, studying the effect of dehydration to performance in hot environments. Wall et al. (2015) did not observed changes on performance, physiological and perceptual variables in a cycling time trial, even with a weight loss up to 3%. Cheung et al. (2015) demonstrated that weight loss up to 3%, physiological effects and increased thirst perception did not affect submaximal exercise performance of cyclists in hot temperatures, reinforcing the same hypothesis. Berkulo et al. (2015) did not observe negative effects on performance of athletes when they were hypo-hydrated. Cyclists were exposed to three different experimental situations during a 40 km cycling time trial in hot temperature: hydrated with no ingestion of water, hypo-hydrated with no fluid ingestion and hypo-hydrated with ad-libitum water ingestion. Heart rate, temperature response, RPE and thermal sensation variables did not exhibited significant differences among interventions. These results seem to reinforce the idea that, if weight loss during exercise is less than 4%, fluid intake would not improve physical performance, and athletes would use other nutritional strategies such as CMR or ad libitum.

Some studies have pointed out that fluid replacement guided by thirst may be sufficient for thermoregulatory responses maintenance and ability to perform exercise, even with the small involuntary dehydration that frequently occurs in some sports (Goulet, 2011; Noakes, 2007). Furthermore, this practice can also prevent gastrointestinal complaints caused by fluid accumulation (Noakes, 2007). Ad libitum water intake was sufficient to keep the hydrated state of individuals who exercised for an hour in a hot and humid environment, whereas water intake according to weight loss would lead to excessive consumption of water (Machado-Moreira et al., 2006).

Overall, these studies highlight that DAL should be an alternative for athletes, especially considering the possibility of gastrointestinal discomfort due to excessive fluid intake in moderate duration exercise that require great physical effort. Therefore, athletes should develop individualized hydration strategies that prevent damage to health and performance during exercise. Further studies should be conducted to evaluate the effects of using both CMR and DAL strategies together, considering the potential ergogenic effects reported in the literature, which could improve athletic performance, because competitions might be decided with only a few seconds difference.

Conclusion

In conclusion, CMR did not improve the performance of recreationally trained cyclists in 30 km time trial tests compared to other strategies of fluid intake recommended traditionally. However, the water loss in CMR similar to DAL, but higher than DWL, suggests that physical performance of cyclists is not affected when water loss is lower < 4% weight in short high-intensity exercise (< 60 minutes), when performed in controlled temperature and when individuals were in a fed state.

Acknowledgements

No financial funding was obtained for this study. The authors have no conflicts of interest to declare. All experiments comply with the current laws of the country.

References

- Armstrong, L.E., Maresh, C.M., Castellani, J.W., Bergeron, M.F., Kenefick, R.W., LaGasse, K E. and Riebe, D. (1994) Urinary indices of hydration status. *International Journal of Sport Nutrition* 4(3), 265-279.
- Arnaoutis, G., Kavouras, S. a., Christaki, I. and Sidossis, L.S. (2012) Water ingestion improves performance compared with mouth rinse in dehydrated subjects. *Medicine and Science in Sports and Exercise* 44(1), 175-179.
- Beelen, M., Berghuis, J., Bonaparte, B., Ballak, S.B., Jeukendrup, A.E., and Van Loon, L.J.C. (2009) Carbohydrate mouth rinsing in the fed state: Lack of enhancement of time-trial performance. *International Journal of Sport Nutrition and Exercise Metabolism* 19(4), 400-409.
- Berkulo, M. a R., Bol, S., Levels, K., Lamberts, R.P., Daanen, H. a M. and Noakes, T.D. (2015) Ad-libitum drinking and performance during a 40-km cycling time trial in the heat. *European Journal* of Sport Science February, 1-8.
- Bortolotti, H., Altimari, L. R., Vitor-Costa, M. and Cyrino, E.S. (2011) Enxágue bucal com carboidrato: Recurso ergogênico capaz de otimizar o desempenho físico. Revista Brasileira de Cineantropometria E Desempenho Humano 13(2), 158-161.
- Carter, J.M., Jeukendrup, A.E. and Jones, D.a. (2004) The effect of carbohydrate mouth rinse on 1-h cycle time trial performance. *Medicine and Science in Sports and Exercise* 36(12), 2107-2111.
- Casa, D.J., Armstrong, L.E., Hillman, S.K., Montain, S.J., Reiff, R.V., Rich, B.S.E., Roberts, W.O. and Stone, J.a. (2000) National Athletic Trainers' Association Position Statement: Fluid Replacement for Athletes. *Journal of Athletic Training* 35(2), 212-224.
- Chambers, E.S., Bridge, M.W. and Jones, D.a. (2009) Carbohydrate sensing in the human mouth: effects on exercise performance and brain activity. *The Journal of Physiology*, 587(Pt 8), 1779-1794.
- Cheung, S.S., Mcgarr, G.W., Mallette, M.M., Wallace, P.J., Watson, C.L., Kim, I.M. and Greenway, M.J. (2015) Separate and combined effects of dehydration and thirst sensation on exercise performance in the heat. *Scandinavian Journal of Medicine and Science in Sports* 25(S1), 104-111.
- Cheuvront, S.N., Ely, B.R., Kenefick, R.W. and Sawka, M.N. (2010) Biological variation and diagnostic accuracy of dehydration assessment markers 1–4. *American Journal of Clinical Nutrition* 92, 565-573.
- Correia-Oliveira, C.R., Bertuzzi, R., Kiss, M.A.P.D. and Lima-Silva, A.E. (2013) Strategies of dietary carbohydrate manipulation and their effects on performance in cycling time trials. *Sports Medicine* 43(8), 707-719.
- Daries, H.N., Noakes, T.D. and Dennis, S.C. (2000) Effect of fluid intake volume on 2-h running performances in a 25 degrees C environment. *Medicine and Science in Sports and Exercise* 32(10), 1783-1789.
- Fernández-García, B., Pérez-Landaluce, J., Rodríguez-Alonso, M. and Terrados, N. (2000) Intensity of exercise during road race procycling competition. *Medicine and Science in Sports and*

Exercise 32(7), 1002-1006.

- Garber, C.E., Blissmer, B., Deschenes, M.R., Franklin, B.a., Lamonte, M.J., Lee, I.M., Nieman, D.C. and Swain, D.P. (2011) American College of Sports Medicine position stand.Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: Guidance for prescribing exercise. *Medicine and Science in Sports and Exercise* **43**(7), 1334-1359.
- Goulet, E. D. B. (2011) Effect of exercise-induced dehydration on timetrial exercise performance: a meta-analysis. *British Journal of Sports Medicine* **45(14)**, 1149-1156.
- Hernandez, Arnaldo José; Nahas, R.M. (2009). Modificações dietéticas, reposição hídrica, suplementos alimentares e drogas: suplementos alimentares e drogas : comprovação de ação ergogênica e potenciais riscos para a saúde. *Revista Brasileira de Medicina Do Esporte* 15(3), 3-12. (In Portugal).
- Ispoglou, T., O'Kelly, D., Angelopoulou, A., Bargh, M., O'Hara, J.P. and Duckworth, L.C. (2015) Mouth-Rinsing with Carbohydrate Solutions at the Postprandial State Fail to Improve Performance During Simulated Cycling Time Trials. *Journal of Strength and Conditioning Research / National Strength & Conditioning* Association 29(8), 2316-2325.
- Jackson, A.S. and Pollock, M.L. (1978) Generalized equations for predicting body density of men. *The British Journal of Nutrition* 40(3), 497-504.
- Jeukendrup, A. (2014) A step towards personalized sports nutrition: Carbohydrate intake during exercise. *Sports Medicine* **44(Suppl 1)**, S25-S33.
- Jeukendrup, A.E. (2011) Nutrition for endurance sports: marathon, triathlon, and road cycling. *Journal of Sports Sciences* 29(Suppl 1, August), S91-99.
- Kulaksız, T., Koşar, Ş., Bulut, S., Güzel, Y., Willems, M., Hazir, T., & Turnagöl, H. (2016). Mouth Rinsing with Maltodextrin Solutions Fails to Improve Time Trial Endurance Cycling Performance in Recreational Athletes. *Nutrients* 8(5), 269.
- Kumar, N., Wheaton, L. A., Snow, T. K., & Millard-Stafford, M. (2016). Carbohydrate ingestion but not mouth rinse maintains sustained attention when fasted. *Physiology and Behavior* 153, 33-39.
- Lepers, R., Maffiuletti, N. a, Rochette, L., Brugniaux, J., & Millet, G. Y. (2002). Neuromuscular fatigue during a long-duration cycling exercise. *Journal of Applied Physiology (Bethesda, Md. : 1985)* 92(4), 1487-1493.
- Lucía, A., Hoyos, J., Carvajal, A. and Chicharro, J.L. (1999) Heart Rate Response to Professional Road Cycling: The Tour de France. *International Journal of Sports Medicine* 20(3), 167-172.
- Lucía, A., Hoyos, J. and Chicharro, J.L. (2001) Preferred pedalling cadence in professional cycling. *Medicine and Science in Sports* and Exercise 33(8), 1361-1366.
- Machado-Moreira, C.a., Vimieiro-Gomes, A.C., Silami-Garcia, E. and Rodrigues, L.O.C. (2006) Hidratação durante o exercício: A sede é suficiente? *Revista Brasileira de Medicina Do Esporte* 12(6), 405-409. (In Portugal).
- Maughan, R.J. (2012) Investigating the associations between hydration and exercise performance: Methodology and limitations. *Nutrition Reviews* 70(Suppl 2), 128-131.
- Maughan, R.J. and Meyer, N.L. (2013) Hydration during intense exercise training. Nestle Nutrition Institute Workshop Series 76, 25-37.
- Mckenzie, A.L. and Mu, C.X. (2015) Accuracy of Urine Color to Detect Equal to or Greater Than 2 % Body Mass Loss in Men. *Journal* of Athletic Training 50(12), 1306-1309.
- Neumayr, G., Pfister, R., Mitterbauer, G., Gaenzer, H., Sturm, W., Eibl, G. and Hoertnagl, H. (2002) Exercise intensity of cycle-touring events. *International Journal of Sports Medicine* 23(7), 505-509.
- Noakes, T.D. (2007) Drinking guidelines for exercise: what evidence is there that athletes should drink "as much as tolerable", "to replace the weight lost during exercise" or "ad libitum"? *Journal* of Sports Sciences **25(7)**, 781-796.
- Noakes, T.D., St Clair Gibson, A. and Lambert, E.V. (2005) From catastrophe to complexity: a novel model of integrative central neural regulation of effort and fatigue during exercise in humans: summary and conclusions. *British Journal of Sports Medicine* 39(2), 120-124.
- Sawka, M.N., Burke, L.M., Eichner, E.R., Maughan, R.J., Montain, S.J. and Stachenfeld, N.S. (2007) Exercise and fluid replacement. *Medicine and Science in Sports and Exercise* 39(2), 377-390.
- Shirreffs, S. M. (2003). Markers of hydration status. European Journal of Clinical Nutrition 57(Suppl 2), 6-9.

- Siri, W.E. (1961) Body composition from fluid spaces and density: analysis of methods. In: Techniques for measuring body composition. Eds: E.J. Brozek and A. Henschel. Vol. 9. Washington, DC: National Academy of Sciences.
- Thomas, D., Erdman, K. and Burke, L. (2016) American College of Sports Medicine Joint Position Statement. Nutrition and Athletic Performance. *Medicine & Science in Sports & Exercise* 48(3), 543-568.
- Wall, B.A., Watson, G., Peiffer, J.J., Abbiss, C.R., Siegel, R. and Laursen, P.B. (2015) Current hydration guidelines are erroneous: dehydration does not impair exercise performance in the heat. *British Journal of Sports Medicine* 49(16),1077-1083.
- World Health Organization, W.H.O. (2000) Obesity: preventing and managing the global epidemic. Geneva.

Key points

- Carbohydrate mouth rinse is not a better performance improvement technique in compared to other hydration strategies in cyclists.
- Drinking water ad libitum is an efficient strategy to hydrate, and does not harm sports performance compared to the water recommendations of the American College of Sports Medicine.
- The water loss in carbohydrate mouth rinse is similar to strategy ad libitum.
- Heart rate did not change throughout 30 km time trial in drinking water ad libitum intervention.
- All interventions elicited weight loss after completion of the 30 km time trial.

AUTHOR BIOGRAPHY

Amanda Maria de Jesus FERREIRA Employment Nutritionist, Federal Univ. of Rio Grande do Norte, Brazil. Degree MSc **Research interest** Nutritional strategies for cycling athletes E-mail: amandamjf@hotmail.com Luiz Fernando de FARIAS JUNIOR Employment Prof. of PE, PhD Student, Federal Univ. of Rio Grande do Norte, Brazil. Degree MSc **Research interest** Psychophysiological responses to exercise and high intensity interval training. E-mail: lfariasjunior@gmail.com Thaynan Aline de Araújo MOTA Employment Nutritionist, Federal Univ. of Rio Grande do Norte, Brazil. Degree BSc **Research interest** Nutritional strategies for cycling athletes. E-mail: thaynan_ed@hotmail.com

Hassan Mohamed ELSANGEDY Employment Prof. of PE Depart. in Federal Univ. of Rio Grande do Norte, Brazil. Degree PhD **Research interest** Psychophysiological responses to exercise and elderly E-mail: hassan.elsangedy@gmail.com Aline MARCADENTI **Employment** Prof. of Nutrition Depart. in Federal Univ. of Health Sciences of Porto Alegre, Brazil. Degree PhD **Research interest** Cardiovascular disease and nutrition. E-mail: marcadenti@yahoo.com.br Telma M. de Araújo Moura LEMOS **Employment** Prof. of Clinical and Toxicological Analysis Depart. in Federal Univ. of Rio Grande do Norte, Brazil. Degree PhD **Research interest** Metabolic and biochemical variables in Policistic Ovarian Syndrome E-mail: telmaml@yahoo.com.br **Alexandre Hideki OKANO Employment** Prof. of PE Department in Federal Univ. of Rio Grande do Norte, Brazil Degree PhD **Research interest** Psychophysiological responses to exercise. E-mail: emaildookano@gmail.com Ana Paula Trussardi FAYH Employment Prof. of Nutrition Depart. in Federal Univ. of Rio Grande do Norte, Brazil. Degree PhD **Research interest** Nutritional strategies for athletes and physically active persons, obesity and body composition. E-mail: apfayh@yahoo.com.br

🖂 Ana Paula Trussardi Fayh

Department of Nutrition, Federal University of Rio Grande do Norte, Avenida Senador Salgado Filho, 3000, Campus Universitário - Lagoa Nova – Natal/RN- Brazil