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Section II – Exercise Physiology and Sports Medicine

Effects of the Intake of a Glycerol and Sodium Beverage on the Hydration Balance in a Time Trial in Colombian Endurance Cyclists

by
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This article presents a randomized, double-blind, crossover clinical trial designed to evaluate the effects of the intake of a glycerol and sodium beverage on the hydro-electrolytic balance in Colombian cyclists under both the laboratory and field conditions. This study included twelve Colombian cyclists between 18 and 32 years old, who were assessed by an incremental test using an ergo-spirometer. Each participant was tested for three days. A previous hyperhydration period was performed, in which they consumed three types of beverages: glycerol (GLI), glycerol with sodium (GLINA), and a placebo (PLB), with a one-week washout period between the beverage intake. Subsequently, they performed testing consisting of continuous pedaling for one hour at their first ventilatory threshold under laboratory conditions, followed by a 2.61-km uphill time trial with an average gradient of 10.6% in the field, equipped with a portable ergo-spirometer. During the test, hydration, gastrointestinal symptoms, and thirst perception were evaluated. These were the results per beverage: GLI: sweating: 1472.2 ± 275.8 mL/h, urine volume: 878.3 ± 463.5 mL, sweat sodium concentration: 75.93 ± 26.62 mmol/L; GLINA: sweating: 1422.8 ± 333.8 mL/h, urine volume: 1070.8 ± 522.0 mL, sweat sodium concentration: 66.10 ± 22.12 mmol/L; PLB: sweating: 1585.2 ± 383.2 mL/h, urine volume: 1188.3 ± 596.6 mL, sweat sodium concentration: 75.32 ± 25.88 mmol/L. Findings show that the intake of GLINA beverages has the potential to reduce sweating, urine specific gravity, and sweat sodium losses. These findings suggest that the combination of glycerol with sodium could be beneficial for hydration in endurance sports.

***Keywords**: overhydration; ergogenic aid; bicycling; thermoregulation; exercise-induced dehydration*

Introduction

Dehydration is a critical factor that can significantly compromise performance, thus maintaining fluid and electrolyte homeostasis in the body is a fundamental strategy. This can be achieved through the intake of well-studied beverages designed to compensate for fluid and electrolyte losses caused mainly by sweating (Australian Institute of Nutrition, 2022; Vega-Pérez et al., 2016).

In sports nutrition, glycerol has proved to have a hydrating and hyperhydrating capacity as it is considered to have the potential to expand plasma volume, thus increasing retained volume and aerobic performance, decreasing thirst perception, and delaying dehydration. In addition, it promotes water reabsorption in renal tubules through its effect on osmoreceptors and antidiuretic hormone secretion (Goulet et al., 2006, 2018; Savoie et al., 2015; Van Rosendal et al., 2015).

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Despite the above, some concerns remain. Most studies supporting its use were conducted prior to its prohibition by the World Anti-Doping Agency in 2010. Since its removal from the list of banned substances in 2018 and despite its classification as a Type A evidence supplement (indicating strong scientific evidence and safety of use in sport) by the Australian Institute of Nutrition (AIS), related studies have been scarce. Actually, the existing research has focused on recreationally trained individuals, which has yielded inconsistent results in terms of magnitude of effect, but consistent in terms of hydration and performance variables, suggesting a high ergogenic potential (Goulet et al., 2007, 2018; Savoie et al., 2016; Van Rosendal et al., 2015).

In order to provide new information to clarify the role of glycerol and glycerol with sodium in sports nutrition and sports performance, in aspects related to hyperhydration and prevention of dehydration, this study aimed to evaluate the effects of the intake of a beverage containing glycerol and sodium on the hydro-electrolytic balance and performance under laboratory and field conditions in Colombian endurance cyclists.

Methods

Participants

Twelve male competitive road cyclists participated in this study. Their characteristics were as follows (mean ± SD): age: 21.8 ± 4.3 years; training volume: 13.2 ± 2.2 hours/week; distance covered: 385.0 ± 92.1 km/week; training experience: 5.8 ± 4.0 years; mean maximum oxygen uptake (VO_{2max}): 67.5 ± 0.4 mL/min/kg; body mass: 63.7 ± 5.5 kg; body height: 174.3 ± 4.4 cm; muscle mass (calculated using the Lee's equation): 46.1 ± 2.1%; and fat mass (calculated using the Faulkner's equation): 11.2 ± 1.9% with a sum of 8 skinfolds: 62.8 ± 19.9 mm). Inclusion criteria were: (i) age between 18 and 32 years, (ii) training experience >2 years, (iii) weekly training >10 hours, (iv) participation in competitions >3 times a year, and (v) maximal oxygen uptake >50 mL/kg/min.

Athletes with any of the following conditions were excluded: (i) persistent sports injuries; (ii) <10 hours of training per week; (iii) presence of frequent symptoms of gastrointestinal discomfort (<2 days/week with persistent symptoms >30 min); (iv) serious diseases (cardiovascular, metabolic, bone, renal); (v) health conditions affecting sports performance or athletes with smoking habit or frequent alcohol consumption (350 mL of beer, 150 mL of wine, or 45 mL of hard liquor). The protocol was explained in detail and the participants gave written informed consent to take part in this study. The Bioethics Committee of the Center of Sports Sciences of the Ministry of Sports, Bogotá D.C., Colombia, approved all experimental procedures (approval code: Act No. 13; approval date: 12 October 2022).

Measures

Preliminary visit: Prior to the study, the population was characterized by determining body composition, physiological variables through an ergospirometry test, food intake, gastrointestinal symptoms, and thirst perception. Body composition was determined by an ISAK level 1 accredited anthropometrist using the 4-component mass fractionation kinanthropometric method (bone mass calculated with the Rocha's formula, muscle mass calculated with the Lee's formula, and residual mass and fat mass calculated with the Faulkner's formula). A THG digital scale was used for body mass, a Seca 213 portable stadiometer for body height, a NutriMEdu flexible steel anthropometric tape for perimeters, a SlimGuide adipometer for skinfolds, and a Vitruvian Caliper anthropometer for diameters.

To assess the population food intake, a 24-h recall method was employed for three days (including the day of physiological assessment tests). Athletes provided detailed records of food preparations, portion sizes, quantities, and ingredients consumed. Based on this information, food intake was quantified and averaged using the MenusPlus software, which sources its nutritional data from the Colombian Food Composition Tables (TCAC) editions 2018, 2015, 2005, and 1978, as well as the USDA National Nutrient Database for Standard Reference, Release 28. This analysis allowed for the determination of the athletes' average caloric intake and the macronutrient distribution (carbohydrates, fats, proteins and sodium) of their diets.

Maximum oxygen uptake, ventilatory thresholds (aerobic and anaerobic thresholds and maximal workload), the respiratory exchange ratio (RER), and maximal relative oxygen consumption

were determined by ergospirometry using a MetaLyzer 3B-R3 ergospirometer, including a Lode Excalibur Sport 38k4 workload device (925900), and an incremental start test with 100-W load and 30-W increments every minute at 2600 masl. The heart rate was measured using a Polar H10 heart rate monitor. Thirst was assessed using the Thompson and Campbell 7-point categorical scale (Millard-Stafford et al., 2012).

Gastrointestinal symptoms were assessed using a qualitative self-perception questionnaire specifically developed for this study, based on the items described by Beltrán Cháidez et al. (2020). This instrument allowed for the identification of the presence or absence of symptoms, as well as their type.

Pre-test controls: To avoid dietary- and exercise-related biases, the following measures were implemented (Goulet et al., 2018; Savoie et al., 2015):

- **i.** Food intake on the day of the physiological assessment test was recorded using a 24-h recall;
- **ii.** the type of food and amounts consumed on that day were replicated on the three field test days, recalled the previous day, and monitored using photographic records;
- **iii.** participants were asked not to exercise 12 h prior to testing;
- **iv.** training loads were similar on all test days, with low-intensity sessions two days before each test;
- **v.** participants were asked to drink 500 mL of water before going to bed;
- **vi.** they had breakfast two hours before the tests (1.2 ± 0.6 g/kg body mass of carbohydrates);
- **vii.** dairy products and caffeine were to be avoided; and
- **viii.** no dietary or nutritional supplements were allowed 48 h prior to testing.

Design and Procedures

Experimental protocol: This study was based on a randomised, double-blind, crossover clinical trial. After a preliminary characterization conducted one week prior to testing, cyclists underwent three testing sessions, each involving a different beverage as part of a hyperhydration protocol: glycerol with sodium (GLINA), glycerol without sodium (GLI), and water (PLB); each session was spaced at least one week apart. The tests were conducted at the same time of the day for each athlete. The hyperhydration phase lasted 60 min, followed by a 30-min rest interval. Participants then completed a 60-min aerobic endurance test on a stationary bicycle at the first ventilatory threshold, followed by 15 min of active recovery. Finally, an uphill time trial test was conducted with a portable ergo-spirometer (MetaMax 3B; CORTEX Biophysik GmbH). The climb had a length of 2.61 km, with a positive altitude gain of 281 m and an average gradient of 10.6%. Temperature and relative humidity were measured with an HTC-2 thermohygrometer (Figure 1).

Upon arrival at the test site, participants provided a urine sample for the assessment of urine specific gravity (using a POCKER PAL-10S digital refractometer, Atago) and urine colorimetry (using the Armstrong colorimetry chart). In addition, thirst perception, gastrointestinal symptoms, and body mass (measured with an empty bladder and minimal clothing) were studied. During the hyperhydration protocol, gastrointestinal symptoms and thirst perception were assessed. At the end of the rest interval, body mass and blood glucose levels were measured, as well as gastrointestinal and thirst questionnaires were completed. Sweat collection patches (3M Tegaderm + Pad 5 cm x 7 cm) were also applied to the scapular region and the forearm. After one hour of continuous training, participants were weighed again in minimal clothing. Then they completed questionnaires, provided urine samples, and had their glucose measured. Next, sweat patches were applied and sodium in sweat was measured with the LAQUAtwin Na-11 meter. Subsequently, new patches were applied and the portable cycloergometer was used.

At the end of the tests, the sweat rate (sweat loss/exercise time in mL/min) was calculated with the following formula:

$$\left(\frac{mL}{min}\right): \frac{Body\ mass\ change\ (kg) + Volume\ of\ fluid\ intake\ (mL) - Urine\ volume\ (mL)}{Exercise\ time\ (min)}$$

After the uphill time trial, participants were again weighed with minimal clothing. They then completed questionnaires, provided urine samples, and had sodium in sweat measured. After 24 h, gastrointestinal symptoms and thirst perception were reassessed. If athletes needed to urinate during the protocol, the urine was collected and weighed. To estimate whole-body sodium

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losses, the formulas taken from Baker (2016) were applied (Figure 1).

Hyperhydration protocol and beverages provided: For hydration, GLYON® glycerol was used in two forms: one with sodium and one without sodium, both diluted in water. In addition, a placebo beverage (PLB) consisting only of water with a passion fruit-flavored sweetener supplied by Nutritek was used. The sodium additives consisted of sodium chloride ion, sodium citrate, and pharmaceutical grade distilled water.

Each athlete received a different beverage in each test, with a dose of 1.2 g/kg glycerol (equivalent to 1.5 mL/kg Glyon) diluted in 25 mL/kg of water. For the beverage containing sodium, a concentration of 0.5 g/L sodium was added together with a flavoring agent. The beverages were administered over a 90-min pre-test period. During the first 60 min, participants received boluses equivalent to 20% of the total beverage every 15 min, followed by a 30-min rest interval before beginning the endurance tests. Table 1 presents the GLINA (GLYON®) technical data sheet.

Statistical Analysis

The statistical software PSPP (Perfect Statistical Professional Presented) was used for the analysis of quantitative variables. All quantitative variables were reported as means and standard deviations. Basic descriptive statistics were applied to variables related to socio-demographic description, identification of gastrointestinal symptoms, and thirst perception, based on the nature of the variables. The repeated measures ANOVA was used to determine whether there were significant differences in the means of the measured variables, with each participant acting as his own control. A value of $p < 0.05$ was considered statistically significant.

Results

Temperature and humidity: The average temperature and humidity during the first laboratory test were 17.4 ± 1.8°C ($p = 0.49$) and 66.9± 10.3% ($p = 0.27$), respectively. During the uphill time trial test, they were 18.3 ± 1.5°C ($p = 0.43$) and 63.9 ± 6.3% ($p = 0.14$), respectively.

Participants: Tables 2 and 3 present the baseline characteristics of the participants in terms of performance physiological variables in the pre-test periods and body composition using the 4-component fractionation model.

Food intake: The caloric intake of the participants was characterized by an average daily consumption of 2555 ± 551 kcal over a 3-day period. The macronutrient distribution consisted of 17.1 ± 1.9% protein, 55.2 ± 4.3% carbohydrates, and 27.7 ± 4.9% fats, with an average sodium intake of 4.7 ± 0.8 g/day.

Effect of beverages on hydration balance: Table 4 shows differences between the beverages in terms of variables related to the hydro-electrolyte balance of the cyclists during the tests. No statistically significant differences were found in any of the hydration balance variables evaluated.

Urine density: At the end of the 60-min cycling session (continuous test), urine density was lower in athletes consuming the PLB (1.0063 ± 0.0033 g/mL) and higher in those consuming the GLINA beverage (1.0085 ± 0.0031 g/mL), although both values remained within the hyperhydration range. At the end of the time trial, density was higher athletes consuming the GLI beverage (1.0181 ± 0.0027 g/Ml) and lower in those consuming the GLINA beverage (1.0170 ± 0.0021 g/mL). The beverage associated with the largest increase in density from baseline to the uphill time trial was the PLB, with a change of 0.0009 g/mL, while the smallest change was in the GLINA beverage, with a variation of −0.0022 g/mL.

Urine volume: Athletes consuming the PLB showed the greatest losses in urine volume: 1188.3 ± 596.6 mL. The losses following the GLINA beverage intake were 1070.8 mL and for the GLI beverage intake, they were 878.3 mL.

Sweat rate: The highest sweat rate (greater fluid loss) was observed following the PLB intake, with an average of 1585.2 ± 383.2 mL/h, while the GLINA beverage intake resulted in the lowest sweat rate: 1422.8 ± 333.8 mL/h.

Sodium loss through sweat: At the end of the 60-min exercise period, there was a greater loss of sodium through sweat in athletes who consumed the GLI beverage (1.29 ± 0.53 g), while consumption of the GLINA beverage contributed to a lower sodium loss (1.27 ± 0.59 g) ($p = 0.99$). One athlete showed higher sodium losses compared to the other participants: 2.3 g for the GLI beverage intake, 2.8 g for the GLINA beverage intake, and 1.8 g for the PLB intake. All athletes provided samples for this test. Figure 2 shows the individual

dynamics of sodium loss after 60 min of exercise for each cyclist.

At the end of the time trial, sodium loss by sweat increased for all conditions (GLI, GLINA, and PLB). The highest loss was observed following the GLI beverage intake (1.74 ± 0.62 g) and the lowest following the GLINA beverage intake (1.53 ± 0.52 g). The largest difference between the values obtained at baseline and after the time trial occurred following the GLI beverage intake (0.49 ± 0.26 g), while the PLB condition resulted in increases by 0.39 ± 0.19 g. Not all athletes provided samples of sodium through sweat for this test; 7 samples were collected for the GLI condition and 9 samples for both PLB and GLINA conditions. Figure 3 shows the individual behavior of sodium loss after the time trial.

Thirst perception: At the start of the tests, most athletes reported no thirst or mild thirst with any of the three beverages. After the hyperhydration rest period in all tests, the majority (83.3%) reported no or low levels of thirst, with none reporting more than neutral symptoms. However, at the end of 60 min of exercise, 25.0% of athletes reported being thirsty or very thirsty under the GLI and GLINA condition, while 41.7% of athletes under the PLB condition. At the end of the time trial, 16.7% of participants reported feeling "very, very thirsty" only under the PLB condition. At the end of the time trial, the perception of "thirsty" or "very thirsty" was reported by 83.3% of athletes following the GLI beverage intake, 75% following the GLINA beverage intake, and 66.7% following the PLB intake.

Gastrointestinal symptoms: At the beginning of the tests, no athlete reported gastrointestinal symptoms. At the end of the hyperhydration rest period in all tests, most of them remained symptom-free (66.7% for the GLI condition and 83.3% for PLB and GLINA conditions). The following mild symptoms were reported during this period: under the GLINA condition, 16.7% of the athletes experienced bloating and 16.7% experienced burping; under the GLI conditions, 8.3% of the athletes experienced bloating and 8.3% experienced reflux, and following the PLB consumption, 16.7% of the athletes experienced abdominal distension. After 60 min of the exercise, abdominal bloating was reported by 8.3% of participants under both the GLINA and PLB condition. At the end of the time trial, one case of abdominal pain occurred under the GLINA condition (8.3%) and one case of nausea under the PLB condition (8.3%). No gastrointestinal symptoms were observed 24 h after the tests.

Table 1. GLINA technical data sheet (GLYON®)*.

Calories	**127 calories/L**
Active ingredient	Glycerol 46,2 g/L
Electrolytes	Sodium 600 mg/L
	Chlorine 450 mg/L
	Potassium 0 mg

** Information provided by the supplier*

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Table 2. Behavior of physiological variables for maximum performance and oxygen uptake.

Physiological variable	Mean (± SD)
Load in W on VT1	240.0 ± 41..6
W on VT1 per kg of weight (W/kg)	3.8 ± 0.5
HR at VT1 (beats/minute)	157 ± 10
Load in W on VT2	327.6 ± 25.8
W on VT2 per kg of weight (W/kg)	5.2 ± 0.3
HR at VT2 (beats/minute)	178 ± 6
VO_{2max} (mL/min/kg)	67.5 ± 0.4
Load in W maximum	395.5 ± 27.8
W maximum per kg (W/kg)	6.3 ± 0.4
HR in W maximum (beats/minute)	189 ± 8

W: Watts, VT1: first ventilatory threshold, VT2: second ventilatory threshold, HR: heart rate, VO_2: oxygen volume

Table 3. Body composition of cyclists. Four-component fractionation model.

Component	Mean (± SD)
Body mass (kg)	63.7 ± 5.5
Percentage of muscle mass (Lee's equation)	46.1 ± 2.1
Percentage of bone mass (Rocha's equation)	19.8 ± 1.2
Percentage of adipose mass (Kerr's equation)	23.1 ± 3.8
Percentage fat mass (Faulkner's equation)	11.2 ± 1.9
Percentage of fat mass (Carter's equation)	7.7 ± 1.6
Sum of 8 skinfolds (mm)	62.8 ± 19.9

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Table 4. Variables related to the hydroelectrolyte balance of cyclists.

Hydration variable	Beverage	Mean (± SD)	Statistical significance
Pre-hydration urine density (g/mL)	GLI	1.0175 ± 0.0077	0.80
	PLB	1.0171 ± 0.0089	
	GLINA	1.0192 ± 0.0078	
Final urine density continuous test (g/mL)	GLI	1.0078 ± 0.0034	0.25
	PLB	1.0063 ± 0.0033	
	GLINA	1.0085 ± 0.0031	
Uphill time trial final urine density (g/mL)	GLI	1.0181 ± 0.0027	0.58
	PLB	1.0180 ± 0.0036	
	GLINA	1.0170 ± 0.0021	
Urine volume (mL)	GLI	878.3 ± 463.5	0.36
	PLB	1188.3 ± 596.6	
	GLINA	1070.8 ± 522.0	
Sweat rate (mL/h)	GLI	1472.2 ± 275.8	0.48
	PLB	1585.2 ± 383.2	
	GLINA	1422.8 ± 333.8	
Weight gain during overhydration (kg)	GLI	0.98 ± 0.37	0.56
	PLB	0.80 ± 0.47	
	GLINA	0.80 ± 0.46	
Weight change from the start of hyperhydration to the end of the time trial (kg)	GLI	−0.95 ± 0.39	0.096
	PLB	−1.40 ± 0.67	
	GLINA	−1.09 ± 0.46	
Sodium post the continuous test (g)	GLI	1.29 ± 0.53	0.99
	PLB	1.28 ± 0.45	
	GLINA	1.27 ± 0.59	
Sodium after the uphill time trial (g)	GLI (n = 7)	1.74 ± 0.62	0.71
	PLB (n = 9)	1.72 ± 0.58	
	GLINA (n = 9)	1.53 ± 0.52	
Sodium post the continuous test (mmol/L)	GLI	55.95 ± 22.38	0.99
	PLB	55.46 ± 19.24	
	GLINA	55.16 ± 26.65	
Sodium after the uphill time trial (mmol/L)	GLI (n = 7)	75.93± 26.62	0.66
	PLB (n = 9)	75.32 ± 25.88	
	GLINA (n = 9)	66.10 ± 22.12	

GLI: a glycerol beverage; GLINA: a glycerol with sodium beverage; PLB: a placebo

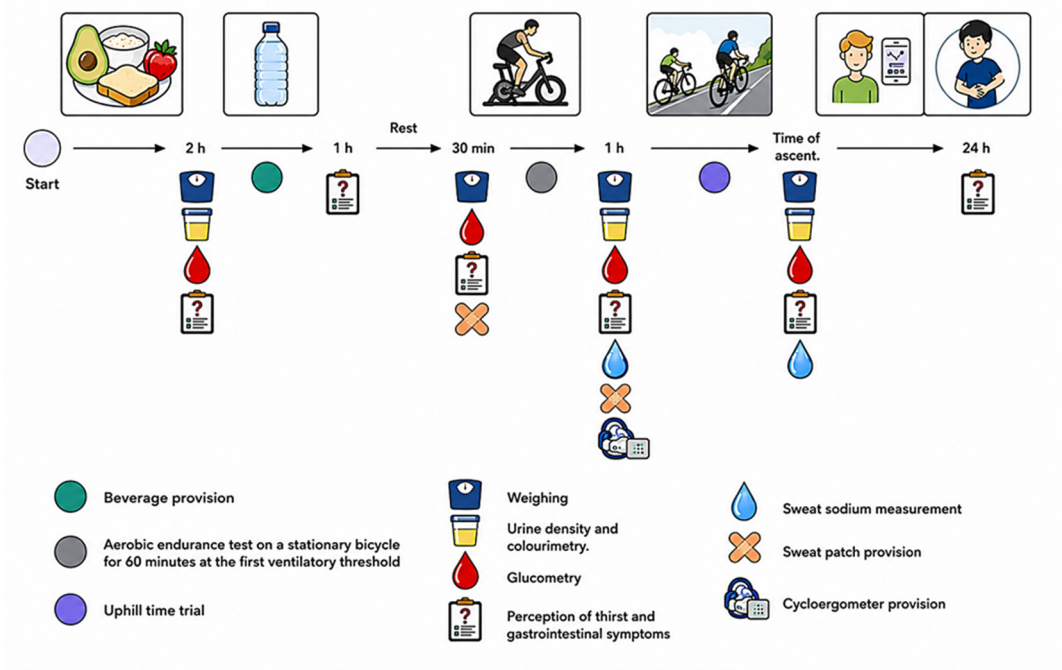


Figure 1. Example test protocol.

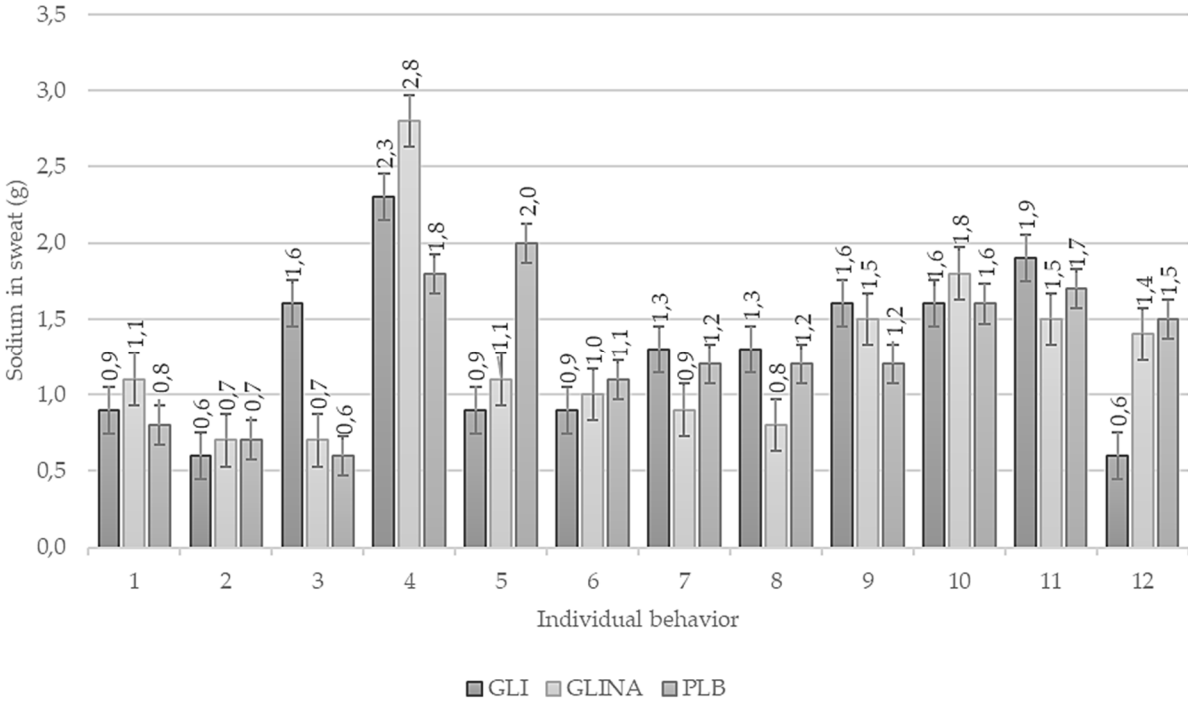


Figure 2. Individual behavior of TC sweat sodium loss (g) after 60 min of continuous cycling.

GLI: participants consuming the glycerol beverage; GLINA: participants consuming the glycerol with sodium beverage; PLB: participants consuming the placebo

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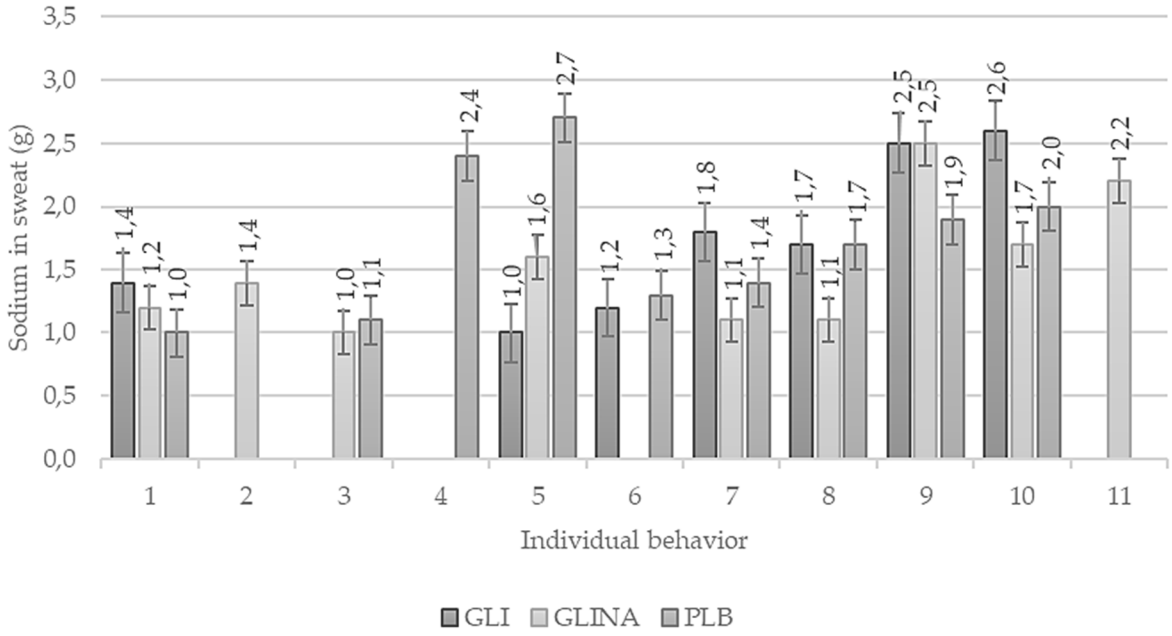


Figure 3. Individual behavior of TC sweat sodium loss (g) after the uphill time trial.
GLI: participants consuming the glycerol beverage; GLINA: participants consuming the glycerol with sodium beverage; PLB: participants consuming the placebo

Discussion

Temperature

The average laboratory temperature in this study was 17.4 ± 1.8°C, with relative humidity of 66.9 ± 10.3%. During the uphill time trial, the average temperature was 18.3 ± 1.5°C with relative humidity of 63.9 ± 6.3%. These conditions were significantly lower than those reported in other studies that also evaluated the effects of glycerol intake. For instance, Savoie et al. (2016) reported average temperatures ranging from 22.0 ± 0.8°C to 23.8 ± 1.0°C and relative humidity ranging from 42.9 ± 3.9% to 46.9 ± 3.0%. Goulet et al. (2018) reported average temperatures from 21.8 ± 1.3°C to 24.3 ± 1.1°C and relative humidity from 51.2 ± 4.2% to 53.2 ± 3.9%. In a previous study, Goulet et al. (2006) reported laboratory temperatures of 25°C and relative humidity from 38% to 42%. The lower temperatures observed in this study, with regard to those in previous research, may have limited the comparability of the results. In temperate climates, such as those in which the tests were performed, heat stress is lower, thus the thermoregulatory capacity of the organism is maintained more efficiently. In contrast, higher temperature and humidity scenarios are more likely to affect thermoregulation, which increases heat production, sweat volume, and perceived exertion (with increases of 0.21 Borg units for each additional 1% loss of body mass) (Bergeron, 2016; Goulet et al., 2006; Nascimento et al., 2020; Skotniczny et al., 2026a; Stellingwerff et al., 2019).

Sweating Rate

Although no significant differences in the sweat rate were found in this study, the highest rate occurred under the PLB condition (1585.2 ±

383.2 mL/h), while the lowest occurred under the GLINA condition (1422.8 ± 333.8 mL/h). These results are consistent with previous research. For instance, a study by Goulet et al. (2006) compared glycerol-induced hyperhydration with the PLB condition in participants who ran at 66% of VO_{2max} for 2 h, followed by an incremental run to exhaustion at 25°C. Their results showed no significant differences in the sweat rate which was slightly lower than ours despite the higher temperature and longer duration of the first protocol (glycerol: 956.92 ± 60.26 mL/h and PLB: 922.67 ± 39.66 mL/h).

In a later study, Goulet et al. (2007) compared hyperhydration and euhydration in a 2-h cycling test at 65% of VO_{2max}, followed by an incremental test to exhaustion. They found a sweat rate in the glycerol group similar to that observed in our study (1304 mL/h), with no significant difference compared to the water euhydration group.

Goulet et al. (2018) suggested that the administration of glycerol before exercise may increase fluid retention and the sweat rate by up to 33% compared to water, which would favor the thermoregulatory process. However, the available evidence, including the results of our study, is neither conclusive nor significant regarding its effects on hydro-electrolyte balance.

In this study, body composition was assessed during the preliminary phase as part of participants' characterization. Given that all three experimental protocols were conducted on the same individuals using a crossover design within a period of less than four weeks, no significant changes in adipose or muscle tissue components were expected between trials. The variations in body mass observed across protocols are attributed primarily to changes in hydration status rather than alterations in tissue mass. Nonetheless, we acknowledge that interindividual differences in body composition (particularly in fat-free mass) may influence the sweat rate (Suarez-Ortegón et al., 2024).

In this study, the potential impact of aerobic capacity (assessed via VO_{2max}) on thermoregulatory capacity and, consequently, on the participants' sweat rate, was also controlled for, as participants served as their own controls. Previous studies have shown that greater aerobic capacity entails higher heat production, which in turn leads to increased cutaneous blood flow and a higher sweat rate (Skotniczny et al., 2026b).

The approach of this study minimized variability associated with individual differences in body composition and aerobic capacity and, therefore, it was not considered a potential source of bias.

Urine Specific Gravity

At the end of the uphill time trial, a higher urine density was observed in the groups consuming GLI (1.0181 ± 0.0027 g/mL) and the PLB (1.0180 ± 0.0036 g/mL), while participants consuming GLINA showed slightly lower urine specific gravity (1.0170 ± 0.0021 g/mL). The assessment of urine specific gravity is important because it provides insight into the hydration balance of an individual. In general, gravity below 1.010 g/mL is associated with a state of hyperhydration, while higher values indicate increased urine concentration and possible dehydration (McCubbin et al., 2020).

Glycerol, which is almost entirely absorbed by the proximal and distal tubules of the kidney, plays a crucial role in fluid retention. In the presence of sodium, as is the case with GLINA, glycerol contributes to increased interstitial osmolarity in the renal medulla, which promotes water reabsorption and fluid retention (Van Rosendal et al., 2012). This synergy between glycerol and sodium could explain the lower urine density observed in the GLINA group, suggesting greater efficiency in water retention and better preservation of the hydro-electrolyte balance.

When comparing our results with previous research, we identified only two studies that assessed urine density after ingestion of a glycerol-based beverage. Both studies used a passive experimental design with physically active participants who were not classified as athletes. The first study, conducted by Savoie et al. (2016), found no significant difference among three beverages (sodium chloride solution, sodium chloride tablets, and glycerol) at the end of the 180-min passive period; urine densities ranged from 1.014 to 1.016 g/mL, with the glycerol group presenting slightly higher urine density values.

In contrast, Goulet et al. (2018) compared the effects of hyperhydration induced by salt, glycerol, and a combination of salt and glycerol. They found significant differences in urine density after 180 min of rest: 1.014 g/mL for the beverage

with salt and 1.018 g/mL for the beverage with glycerol and sodium. In our study, the value obtained for the GLINA group was even lower than that reported by Goulet et al. (2018), despite the pedaling and incremental exercise protocol used. This suggests that hydration balance could be maintained for longer duration under conditions of moderate to intense physical activity.

Sodium Loss through Sweat

Sodium excreted in sweat depends on the rate of sodium reabsorption in the sweat ducts and factors such as the sweat flow rate and Na+/K+-ATPase ion transporter activity; these processes are influenced by multiple variables, thus reference values for sodium in the sweat are highly variable both within and between athletes. The concentration of sodium excreted in sweat can range from 10 to 90 mmol/L, which, in conjunction with a variable sweat rate, can lead to even greater inter-individual differences in sodium losses (American College of Sports Medicine et al., 2007; Baker, 2016; McCubbin et al., 2020; Smith et al., 2021).

When sodium losses were assessed in our cyclists, the average concentration during the 60 min of cycling was 55.52 ± 22.29 mmol/L, increasing to 72.17 ± 24.19 mmol/L at the end of the time trial. These values were within the expected range for endurance athletes, as sodium loss through sweat tends to increase with duration and intensity of exercise. No data are available on sodium concentration in sweat for the beverages analyzed, as most studies have focused on the assessment of sodium in urine.

In prolonged activities such as cycling, cumulative heat stress can be considerably higher, even under favorable environmental conditions, due to the sustained exertion that requires continuous thermoregulation through sweating; and although glycerol contributes to water retention and improves hydration, its effect on sodium losses appears to be more limited during these activities (Goulet et al., 2006; Savoie et al., 2016; Sawka et al., 2007)). In these situations, the constant need to sweat may partially offset the benefits of glycerol, explaining the sodium losses observed in our study. Furthermore, Savoie et al. (2016) noted that glycerol-containing beverages reduced urinary sodium excretion, but did not significantly reduce sweat loss in athletes undergoing intense physical exertion.

Considering the potential impact of food intake on sweat sodium losses, our study replicated the participants' food intake the day prior, and the breakfast on the day of testing, across all three trials for each participant. Therefore, dietary intake was not considered a potential source of bias in this context. However, we acknowledge that sodium intake may influence sodium losses through sweat, as evidenced by the study of Braconnier et al. (2020). Thus, we suggest that future research could incorporate a more in-depth analysis of these dietary factors.

Gastrointestinal Symptoms

Assessing the presence of gastrointestinal (GI) symptoms was critical, as dehydration and high intensity activities can reduce the blood flow to the GI tract, instead favoring the flow to the muscles and skin (Duarte and Giménez, 2015). Costa et al. (2017) mentioned that as exercise intensity and duration increase, the same occurs for the rates of intestinal injury, intestinal permeability, and endotoxemia, leading to impaired gastric emptying, slowed intestinal transit, and malabsorption; these alterations are exacerbated with increased core temperature.

When developing and implementing sports beverages or nutritional ergogenic aids, it is crucial to evaluate the presence of gastrointestinal symptoms and train the nutritional strategy to minimize the occurrence of adverse gastrointestinal symptoms. Furthermore, when experimenting with large volumes of fluids in a short period of time, it is necessary to assess the possible occurrence of gastrointestinal disturbances, since this may induce several side effects that outweigh potential benefits (Duarte and Giménez, 2015; Jardine et al., 2023; Jeukendrup, 2014; Robergs and Griffin, 1998).

In our study, most of the athletes had no adverse gastrointestinal symptoms either at the end of the hyperhydration period or during the time trial period; those who did experience symptoms reported bloating, belching, and reflux.

A systematic review by Jardine et al. (2023) on the effects of hyperhydration found that symptoms such as bloating, nausea, diarrhea, vomiting, and stomach fullness were related to the use of glycerol, although all were classified as mild. Gastrointestinal symptoms, such as abdominal discomfort, nausea, and fullness, can negatively

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impact athletic performance, especially in endurance sports such as cycling.

In contrast, Goulet et al. (2018) reported significant abdominal discomfort with the use of the GLINA beverage, while Savoie et al. (2016) identified statistically significant gastrointestinal discomfort with the use of sodium chloride tablets, but not with glycerol. Van Rosendal et al. (2012) suggested that waiting 30 min after hyperhydration is sufficient for the sensation of stomach fullness to subside.

These findings are relevant as they show that hyperhydration with glycerol and sodium can be well tolerated under controlled conditions, minimizing the likelihood of adverse effects that were reported in other studies. The low incidence of gastrointestinal distress suggests that the hydration strategies used were effective, allowing athletes to maintain optimal hydration without affecting their comfort or performance (Goulet et al., 2018; Savoie et al., 2016).

In this study, a previously validated instrument for athletes was not used, which is acknowledged as a methodological limitation. However, the questionnaire employed allowed for the identification of the presence or absence of symptoms and their type, enabling the assessment of overall tolerance to the beverages evaluated. Future research should consider incorporating validated questionnaires for athletes, as these would allow for the quantification of symptom severity and facilitate more precise associations between glycerol intake and gastrointestinal response.

Conclusions

The ingestion of a sodium-containing glycerol beverage was associated with a decrease in the rate of sweating, lower urine specific gravity at the end of the uphill time trial and a reduction in sodium losses through sweat. These findings, although not statistically significant, suggest that this drink could favor the maintenance of water-electrolyte balance and delay the onset of dehydration. This is relevant for athletes competing in endurance disciplines, where maintaining fluid and electrolyte levels is crucial for optimizing performance and where conditions of poor access to fluids or high thermal stress may occur.

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