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Effects of pre-exercise glycerol supplementation on dehydration, metabolic, kinematic, and thermographic variables in international race walkers

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ABSTRACT

Background: Due to the increase in global temperature, it is necessary to investigate solutions so that athletes competing in hot conditions can perform in optimal conditions avoiding loss of performance and health problems. Therefore, this study aims to evaluate the effect of pre-exercise glycerol supplementation during a rectangular test at ambient temperature mid (28.2ºC) on dehydration variables in international race walkers.

Methods: Eight international male race walkers (age: 28.0 years (4.4); weight: 65.6 kg (6.6); height: 180.0 cm (5.0); fat mass: 6.72% (0.66); muscle mass: 33.3 kg (3.3); VO_{2MAX}: 66.5 ml · kg^{-1}·min^{-1} (1.9)) completed this randomized crossover design clinical trial. Subjects underwent two interventions: they consumed placebo ($n = 8$) and glycerol ($n = 8$) acutely, before a rectangular test where dehydration, RPE, metabolic, kinematic, and thermographic variables were analyzed before, during and after the test.

Results: After the intervention, significant differences were found between groups in body mass in favor of the placebo (Placebo: −2.23 kg vs Glycerol: −2.48 kg; $p = 0.033$). For other variables, no significant differences were found.

Conclusion: Therefore, pre-exercise glycerol supplementation was not able to improve any dehydration, metabolic, kinematic, or thermographic variables during a rectangular test at temperature mid in international race walkers. Possibly, a higher environmental temperature could have generated a higher metabolic and thermoregulatory stress, generating differences between groups like other previous scientific evidence.

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1. Introduction

World athletics competitions (World Championships or Olympic Games) are usually held in summer (July or August) where very high temperatures can be reached, which can

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affect both the performance and the health of the athlete. For example, at the World Athletics Championships in Doha (2019), the competition temperature (T°) was 29.3–32°C with a relative humidity (RH) of 46.3–80.6% in the marathon, 20 km and 50 km race-walker competitions [1].

In addition, at the Tokyo 2021 Olympic Games, moderate-high temperatures were also observed during the marathon, 20 km and 50 km race walk with a temperature of 25.0–31.0°C [2]. At the 2022 World Athletics Race Walking Teams Championships in Muscat (Oman), temperatures of 27.4–29.6°C and RH of 47.3–62.7% were reached in the 20 km race walk (men and women) [3]. Therefore, athletes have to cope with moderate temperatures at international competitions due to the timing and location of the competitions, which are also affected by climate change [4]. Due to the potential impact on the performance and health of athletes, it is an issue that should be addressed as a priority by sports nutritionists, physiologists, and sports scientists.

The practice of sport in hot conditions induces thermoregulatory and other physiological stresses that may induce alterations in endurance exercise capacity [5]. When exercising in heat, there is an increase in blood flow to the skin and in the rate of sweating to allow heat dissipation to the outside of the body. However, these physiological-thermoregulatory adjustments can lead to dehydration, especially during long-term exercise [5]. Progressive dehydration during intense and prolonged whole-body exercise in the heat decreases stroke volume, cardiac output, blood pressure, and blood flow to active skeletal muscle, skin, and brain while simultaneously raising core temperature, heart rate, and total peripheral resistance [6]. An explanation is that the decline in cardiac output with the progression of dehydration and hyperthermia is caused by a decrease in left ventricular filling induced by a reduction in blood volume, largely a dehydration-induced loss of plasma volume [7].

Thermoregulation works through the autonomic nervous system with physiological proportional to elevations in body temperature [8]. Exercise in hot conditions produces cardiovascular changes and affects brain function, increases pulmonary ventilation and alters muscle metabolism, which may contribute to fatigue and affect the ability to maintain power output during aerobic exercise [8].

During high-intensity exercise in heat, the reduction in stroke volume and cardiac output limits arterial oxygen supply to the exercising muscles, and peripheral fatigue will appear as aerobic metabolism becomes inadequate to support ATP resynthesis [9]. In contrast, during moderately intense exercise in heat, the increased heart rate can compensate for the decrease in stroke volume, and muscle oxygen uptake is unaffected, without producing peripheral fatigue [9]. However, hyperthermia-induced alterations in cardiovascular parameters (blood pressure, heart rate, and arterial CO_2 pressure), ventilation, and afferent feedback to the central nervous system from muscle and temperature receptors may influence fatigue [8]. On the other hand, an increase in glycogen utilization [10] and lactate concentration [11] was also observed at the muscle level, with a decrease in intramuscular triglyceride utilization [10] when athletes exercised in hot conditions compared to comfortable temperatures.

These physiological alterations produced during exercise in hot conditions in athletes have motivated different professionals related to high performance to look for solutions. At the level of sports nutrition, one of the most studied supplements has been glycerol for its ability to generate hyperhydration glycerol [12,13]. The great interest in glycerol in hot

conditions is due to its osmotic properties, where hyperhydration with glycerol prior to exercise is the primary objective [13]. In addition, glycerol is a gluconeogenic precursor, and approximately 38% and 79% of the total turnover consists of the conversion of glycerol to glucose in lean and obese individuals, respectively [14]. This attributes an energetic function to glycerol.

The pharmacokinetics of glycerol in blood following glycerol ingestion have been studied. It was found that after a 12-h fast, ingestion of glycerol (85% glycerol solution containing 1.2 g/kg body mass (bm) of glycerol) increased glycerol concentrations by 0.05 mmol/L to a peak of 19.3 ± 3.6 mmol/L in 1.40 ± 0.43 h [15]. It has an estimated distribution half-life (1st$_{/2}$) of 22.6 min. The saturation kinetics of blood glycerol clearance was evident over a 105 min period at serum concentrations between 17 and 19 mmol/L [15]. In addition, a monoexponential decline adjusted to decreasing glycerol concentrations resulted in a 1st$_{/2}$ elimination of 143 min. The total body elimination of glycerol 1st$_{/2}$ was 51.6 ± 10.8 min, and urinary glycerol recovery was 18.9 ± 5.2% of the total. The mean corpuscular volume remained unchanged and there was no evidence of hemolysis [15].

Due to the hyperhydration capacity of glycerol, the effect of glycerol in affecting athletic performance under hot conditions has been studied. Anderson et al. [16] observed that ingestion of 1 g/kg bm of glycerol plus 20 ml/kg bm of water decreased urine volume (−25% in 120 min), heart rate, and rectal temperature (−0.4°C) compared to placebo in endurance athletes trained in ambient conditions of 35°C and 30% RH. However, no differences in muscle glycogenolysis, lactate accumulation, and phosphocreatine degradation were observed when comparing the glycerol group with placebo [16]. However, the glycerol ingestion group performed 5% more than the placebo (252 ± 10 vs. 240 ± 9 kJ; $p \leq 0.05$) in a rectangular test (90 min of steady-state cyclic exercise at 98% of lactate threshold) [16].

Goulet et al. [17] also studied the effect of glycerol supplementation (1.2 g/kg bm of glycerol +26 ml/kg bm) on performance using a rectangular test of 2 h cycling at 65% of maximal oxygen uptake (VO_{2MAX}) interspersed with five intervals of 2 min at 80% VO_{2MAX} at 25–26°C in trained endurance athletes. This author found a post-exercise bm loss of 1.7 ± 0.3% with pre-exercise glycerol intake and 3.3 ± 0.4% with pre-exercise placebo ($p \leq 0.05$). After 2 h of cycling, the pre-exercise hyperhydration protocol significantly increased heart rate and perceived thirst, but rectal temperature, sweat rate, perceived exertion, and perceived heat stress did not differ between conditions [17]. Pre-exercise hyperhydration significantly increased the time to exhaustion and peak power compared to pre-exercise placebo [17].

In contrast to the results of the two previous studies. Marino et al. [18] found no difference after glycerol supplementation (1.2 g/kg bm of glycerol +21 ml/kg bm) compared to placebo in the performance (distance and power) of a rectangular test (longest possible distance in 60 min cycling with 6 × 1 min sprints every 10 min interspersed) in hot conditions (34.5°C and 63.4% RH) in well-trained endurance subjects. This author also found no significant differences between the two groups in rectal T but did find significant differences in heart rate (higher in GLY at high intensity) and sweating (GLY: 1.72 vs PL 1.15 L/h) [18]. One study review found that glycerol supplementation has been shown to improve performance by increasing time to exhaustion (24%) and/or increasing power or work (5%), associated with improvements in thermoregulatory and cardiorespiratory function (↑ plasma volume, sweat rate and ↓ central temperature, perceived

exertion index) [19]. Therefore, based on the studies analyzed on the effects of glycerol intake on performance, there is no clear benefit, as the data are inconsistent.

With the motive of continuing to evaluate the effect of glycerol on performance in hot conditions and in international athletes, since to our knowledge there are no studies on this type of sample. And considering, as we have mentioned before, that international athletics championships are selecting their venues in places with climatic conditions of medium–high temperatures and humidity. This generates a challenge to the professionals who surround the athletes of the highest level, who use all available means so that the performance of these athletes is not affected in this type of events. Therefore, our main objective was to evaluate the effect of glycerol intake in international race walkers under mid-temperature conditions in a rectangular test of varying intensity on dehydration, rate of perceived exertion (RPE), heart rate, metabolic, kinematic, and thermographic variables. This study was conducted before the 2022 World Athletics Race Walking Teams Championships in Muscat (Oman) to test the validity of the use of glycerol in this competition. We hypothesize that pre-exercise glycerol supplementation will improve dehydration and metabolic variables that will improve race walking kinematic and thermographic variables.

2. Material and methods

2.1. Study design

A randomized, crossover experimental design study was conducted. Randomization was performed using software (Randomizer 4.0) to assign codes to the groups generated in this study [20]. Participants were international race walkers who performed a rectangular test under normal temperature (T°): T° mid with placebo intake (PL) (28.1 ± 1.1°C; 31.4 ± 9.9% RH) and T° mid with glycerol (GLY) intake (28.3 ± 0.4°C; 31.5 ± 5.0% RH) 3 weeks before the team World Cup in Muscat (Oman) 2022. All race walkers underwent similar training in both the week before and during the study. In addition, they followed a standardized diet, taking into account bm, the day before and the day of (i.e. breakfast before the tests) by a sports nutritionist (FJMN). Each participant performed the rectangular tests at the same time in the morning (between 9 am and 2 pm) to minimize the effect of the circadian rhythm. Rectangular test visits were separated by 5 days.

2.2. Participants

Eight international race walkers from the Spanish National Race-walking Team participated and completed this study. Importantly, three of these elite athletes won the team gold medal at the 35 km Race Walking World Cup (Oman 2022) and individually finished 2nd, 3rd and 20th overall. The characteristics of the athletes are presented in Table 1, and the final time at the Spanish 35 km Race Walking Championships was ~2 h 34 min. The inclusion criteria were to have 1) a maximum time (2 h 37 min) in the Spanish 35 km Race Walking Championships, 2) qualified for the World Cup (Oman), 3) at least 3 years of experience in international competitions and 3) at least 10 years of experience in race walking. Participants were excluded from the study if they: had suffered a muscle injury at least 3 months prior to the study, had a pathological or

Table 1. General characteristics of the race walkers.

Characteristics	Value
Age (years)	28.0 (4.24)
Body mass (kg)	65.6 (6.60)
Height (cm)	180.0 (5.00)
BF (%)	6.7 (0.66)
MM (kg)	33.3 (3.32)
VO_{2MAX} ($ml \cdot kg^{-1} \cdot min^{-1}$)	66.5 (1.89)

Values are expressed as mean (standard deviation (SD)). BMI = body mass index; BF = body fat (Yuhasz formula); MM = muscle mass; VO2máx = maximum oxygen volume.

metabolic disease, and were taking any supplement or medication that could affect thermoregulation or performance. It is important to bear in mind that the condition of elite race walker is not fulfilled by many individuals, and the n of our sample would be justified in relation to a small cohort of this type of individuals. All participants were informed about the study procedures and signed the informed consent form. The study was conducted in accordance with the Declaration of Helsinki Declaration for Research Involving Human Subjects [21] and was approved by the Ethics Committee of the Catholic University of Murcia (CE102102/29 October 2021). This study was registered in ClinicalTrials.gov with ID: NCT05295836.

2.3. Procedures

The athletes made five visits to the laboratory on different occasions. Visit 1 consisted of a medical examination, a blood draw to determine health status and a familiarization with the rectangular test. At visit 2, the athletes performed an incremental test to establish the exercise zones for the rectangular test. At visits 3 and 4, a rectangular test was performed at room temperature set at 28.1°C with PL intake and at 28.3°C with GLY intake. Bm, urine volume, and urine-specific gravity (USG) were assessed pre and post rectangular test. In addition, lactate was also measured in finger blood. Indirect calorimetry was used to analyze metabolic variables, a power meter (on the foot) was used to assess kinematic variables, and heart rate was recorded during the rectangular test (Figure 1). A warm-up consisting of 8 min of cycling at an intensity of 50 W was performed 15 min before starting the rectangular test. The day before visits 3 and 4, athletes consumed a standardized diet, consisting of 9.0 g/weight carbohydrate, 1.5 g/weight protein, and 0.9 g/weight fat. In addition, participants were instructed to consume a standardized breakfast 2.5 h before the rectangular test, consisting of 1.30 g/weight of carbohydrate, 0.43 g/weight of protein, and 0.57 g/weight of fat.

2.4. Test

2.4.1. Medical exam

The medical examination included a medical history, a resting electrocardiogram, and a medical examination (auscultation, blood pressure, etc.) to confirm that participants were healthy before enrolling in the study.

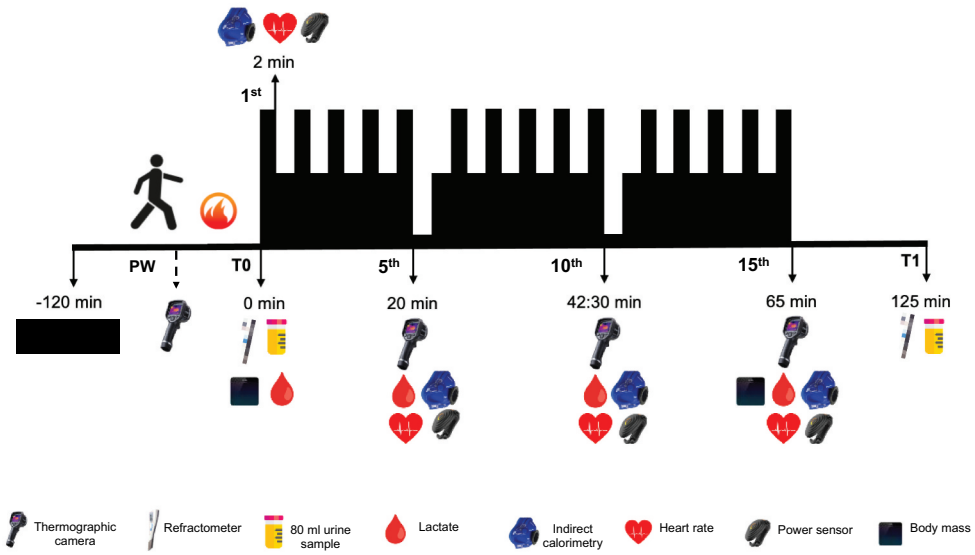

Figure 1. Protocol for visits 3 and 4, for intervention groups PL (28.1°C) and GLY (28.3°C) including a rectangular test and the different variables that were measured. PW = post-warm-up.

2.4.2. Familiarization

During this visit, participants underwent the rectangular test under the same experimental conditions as those used during the experiments, except that they were not required to undergo the hydration protocols beforehand.

2.4.3. Experimental conditions

All athletes underwent the following two interventions: PL, a rectangular test at 28.1°C and intake of 26 ml/kg bm water with flavored (2 h before rectangular test), GLY a rectangular test at 28.3°C and intake of 1 g/kg bm glycerol +26 ml/kg bm water with flavored (2 h before rectangular test). Subjects arrived at the laboratory 2 h before the rectangular test to perform the PL and GLY intake in the laboratory. Throughout the study, subjects were instructed to abstain from strenuous exercise, caffeine, and alcohol for at least 24 h before testing.

2.4.4. Body mass, urine volume, and urine specific gravity (USG)

The bm of the athletes was recorded pre and post rectangular test using a bascule (SECA 780; Vogel & Halke GmbH & Co., Hamburg, Germany). Urine was collected from arrival at the laboratory until the beginning of the rectangular test and 1 h after the end of the rectangular test in 2 L collection bottles. From the urine collected before and after the rectangular test (Σurine), USG was analyzed in an 80 ml aliquot using an Atago PEN-PRO digital refractometer (Atago Co., LTD Tokyo, Japan) in a sterile glass.

$$\Sigma\, urine = (urine\ volume\ pre + urine\ volume\ post)$$

2.4.5. Lactate

Lactate measurements were performed at rest and after 5th, 10th and 15th high intensity runs of the rectangular test using Lactate Pro2 (LT-1730; Arkray, Japan) in capillary blood from the finger.

2.4.6. Incremental test

An incremental maximal test was performed on a treadmill (Technogym Run Excite Med., Cesena, Italy) using a metabolic cart (Metalyzer 3B. Leipzig, Germany) (1029 mBar) to determine VO_{2PEAK} in race walking. The test started with a 5 min of warm up at 5.0 km/h, following by 2 min steps since 9.0 km/h with 1.0 km/h of increments until technical or physiological exhaustion. The data from this test were used to establish the working zones for the rectangular test.

2.4.7. Rectangular test

The rectangular test was performed on a treadmill (Technogym Run Excite Med., Cesena, Italy) (1029 mBar) where the race walkers performed 15 sets of 2 min at high intensity (14.5 km/h = 90–95% of VO_{2PEAK}) and 2.5 min at moderate intensity (11.5 km = 70–75% VO_{2PEAK}) of recovery between sets. After the 5th and 10th sets the race walkers did 1 min of rest followed by 1.5 min at 11.5 km/h and continuing with the 2 min at 14.5 km/h series (Figure 1). Blocks of five series were carried out, where after each block they got off the treadmill quickly to take the temperature with the thermographic camera, lactate, and antioxidants. During this test, the heart rate was recorded after the 1st, 5th, 10th and 15th high-intensity sets. In addition, we also calculated the economy (Ec) during the rectangular test at the same points as the heart rate, using the following formula:

$$Ec = VO2R * velocidad\ min/k$$

Where VO_2/R is the relative oxygen consumption and the speed is expressed in minutes divided by kilometers based on 10.

2.4.8. Thermography protocol

Before the thermographic measurements, all participants were acclimatized to the lab environment (60 m^2) for a period of 15–20 min. The humidity of the room was never higher than 40 ± 0.8%, and the atmospheric pressure was always equal to 1 ATM, which agrees with previous studies [22,23]. No electronic devices were present near the measurement point, and light and heat sources were kept away from the measurement site to avoid thermographic acquisition interference. During this acclimatization period, as suggested by other authors, patients underwent a body temperature check (with a digital thermometer; Berrcom, JXB-178) to rule out changes in basal temperature, and weight, height and BMI were determined to comply with the TISEM protocol [23–25].

The thermograph used was the Flir E75 model (FLIR Systems, Inc., Madrid, Spain), infrared resolution: 320 × 240 pixels, thermal sensitivity <0.04 °C and with fixed thermal recordings from 20 °C to 120 °C. The emissivity was 0.98, as suggested by the manufacturer, and this amount was corroborated in other studies [26,27]. To ensure the machine's correct configuration to the room, it was switched on 1 h before the first recording and placed on a tripod at an inclination of 10–15° and 1 m away from the patient's measurement site.

The race walkers wore sneakers and shorts but no shirt and were positioned a 1 cm artificial turf pad. For all measurements, the regions of interest (ROIs) in the body were defined using conventional anatomical and scientific bibliography [28,29]. A total of four anatomical regions were thermographically measured and analyzed (Figure 1). To guarantee a depth analysis of images, the ROIs were marked on the digitized photo, and the thermographic image was subsequently selected. For the anterior thigh, the proximal ROI began 15 cm below the anterior superior iliac spine and extended to 2 cm above the upper border of the patella (quadriceps region). For the anterior leg, the ROIs started 2 cm below the inferior border of the patella and up to 2 cm above the tibial malleolus (tibia region). The posterior thigh began immediately below the gluteal fold and extended to the upper edge of the Popliteal fossa (hamstring region). In the anterior view of the leg, the ROIs starts 2 cm below the inferior border of the patella up to 2 cm higher than the tibialis malleolus (tibialis region) while in the posterior leg was identified from the inferior border of the Popliteal fossa up to 8 cm above the inferior border of the calcaneus (gastrocnemius region).

2.4.9. Power sensor

Each participant was equipped with a Stryd power meter (Stryd Inc. Boulder CO, USA), which is a 9.1 g carbon fiber reinforced foot-mounted inertial sensor, firmly attached to the shoe and in accordance with the manufacturer's recommendations. The device stores at a sampling rate of 1 Hz the following variables: power (PO), cadence (CD), leg spring stiffness (LSS), from power (FP), from power ratio (FPR), ground contact time (GCT), and vertical oscillation (VO). According to information from the Stryd team, it should not require calibration, accepting a measurement error of 3%. Participants filled in their height and bm prior to use, which is required for PO estimation. As a precautionary measure, the device was fully charged and activated 20 min before the start of the test.

2.4.10. Statistical analysis

IBM Social Sciences software (SPSS, v.21.0, Chicago, IL, USA) was used for statistical analysis. Data are presented as mean ± SD. Homogeneity and normality of the data were checked with the Levene and Shapiro – Wilk tests, respectively. For each ROI variables, a two-way repeated-measures ANOVA with time factor (B vs PW vs 5th vs 10th vs 15th) and group factor (PL vs. GLY) was performed. For indirect calorimetry and power sensor variables, heart rate, and Ec, repeated-measures two-way ANOVA with time factor (1st vs 5th vs 10th vs 15th) and group factor (PL vs. GLY) was applied. For RPE and lactate repeated-measures two-way ANOVA with time factor (T0 vs 5th vs 10th vs 15th) and group factor (PL vs GLY) was applied. Tukey's post hoc analysis was carried out if significance was found in the ANOVA models. For the variables of bm and USG, a two-way ANOVA with time factor (Pre and Post) and group factor (PL vs. GLY) was applied. Tukey's post hoc analysis was carried out if significance was found in the ANOVA models. Independent samples T-test was used for Σurine. Partial eta squared (ηp^2) was also calculated as effect size for time, group and time × group interaction of all variables in the ANOVA analysis. Partial eta square (ηp^2) thresholds were used as follow: <0.01 irrelevant; ≥0.01, small; ≥0.059, moderate; ≥0.138, large [30]. Cohen's d effect sizes (ES) (95% confidence interval) were calculated for comparisons intragroup for bm, urine volume, UGS, and Σurine.

Threshold values for ES statistics were as follows: >0.2 small, >0.5 moderate, >0.8 large [30]. The significance level was set at $p \leq 0.05$.

3. Results

Figure 2 shows the bm, USG, Σurine, and RPE data for the two interventions evaluated (GLY and PL) in a rectangular test in international race walkers. Two-way ANOVA analysis observed a significant time × group interaction in bm (pre-post PL, −2.23 ± 0.60 kg vs pre-post GLY, −2.48 ± 0.73 kg; $p = 0.033$, $\eta p^2 = 0.500$); then, Tukey's post hoc analysis showed a significant pre-post rectangular intragroup decrease ($p = <0.001$) in both interventions. On the other hand, Two-way ANOVA analysis showed no significant time × group interaction in USG (pre-post PL, 0.0064 ± 0.0066 vs pre-post GLY, 0.0030 ± 0.0065; $p = 0.187$, $\eta p^2 = 0.234$) and RPE (PL; T0–0 ± 0, 5th-6.5 ± 1.1, 10th-7.7 ± 1.0, 15th-8.7 ± 1.5 vs GLY; T0–0 ± 0, 5th-6.5 ± 0.5, 10th-8.0 ± 0.5, 15th-8.3 ± 0.9; $p = 0.308$, $\eta p^2 = 0.177$). Regarding Σurine which refers to the sum of urine collected before and after the rectangular test in both groups, the independent sample T-test did not find any significant change (PL; 1,624 ± 439 mL vs GLY; 1,341 ± 367 mL; $p = 0.184$, ES = 0.698).

Figure 3 shows the lactate, relative oxygen uptake (VO_2/R), heart rate, VO_2, CO_2, ratio exchange respiratory (RER), ventilation (V'E), respiratory rate (FR), energy expenditure (EE),

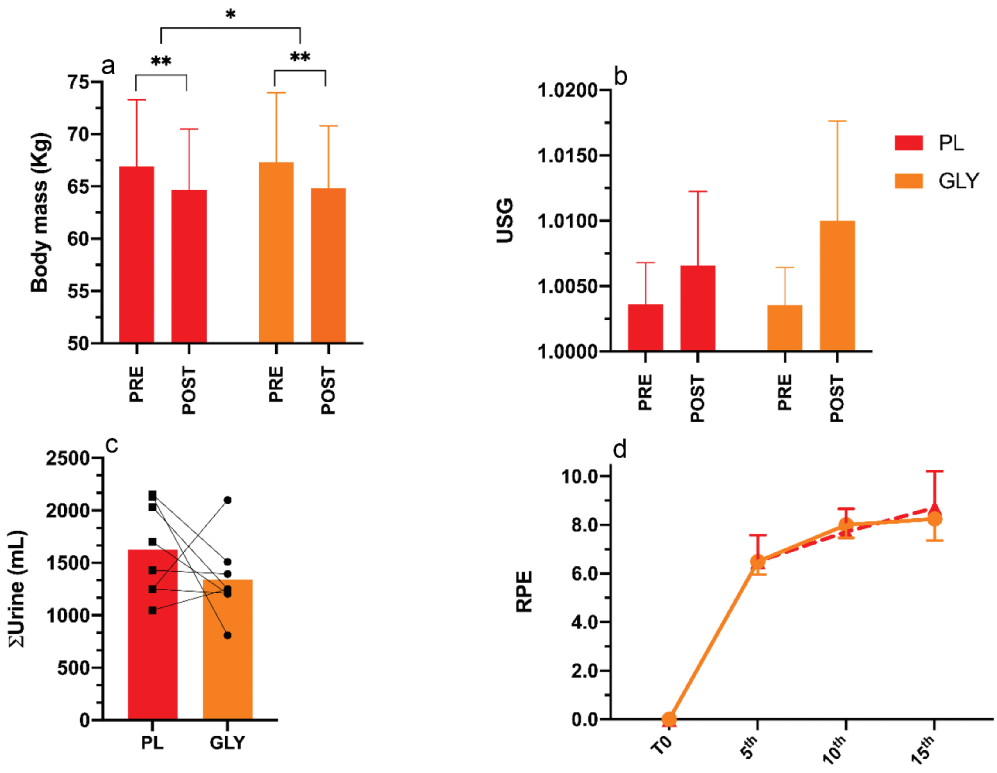

Figure 2. A) pre-post rectangular test changes in bm in international race walker after ingestion of GLY and PL at T° mid. B) pre-post rectangular test changes in USG. C) sum of excreted urine pre-posttest rectangular. D) changes in RPE during the rectangular test. *= p = 0.033; **= p= < 0.001.

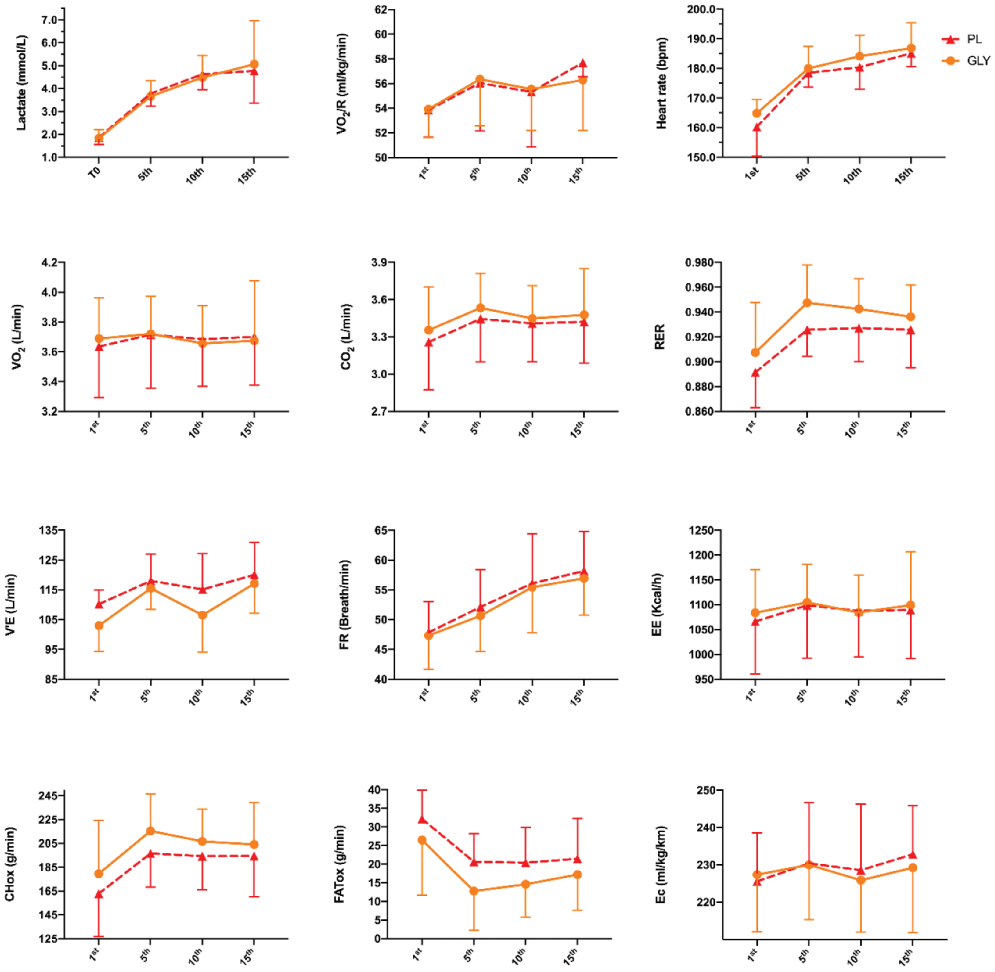

Figure 3. Changes during the rectangular test in metabolic parameters and heart rate after ingestion of GLY and PL at T° mid. CHox = carbohydrate oxidation; CO_2 = production of carbon dioxide; Ec = economy; FATox = fat oxidation; FR = frequency respiratory; FR = frequency respiratory; RER = ratio exchange respiratory; VO_2 = oxygen uptake; VO_2/R = relative oxygen uptake and V'E = ventilation.

fat oxidation (FATox), carbohydrate oxidation (CHox), and economy (Ec) data for the two interventions evaluated (GLY and PL) in a rectangular test in international race walkers. Two-way ANOVA analysis showed no significant time × group interaction in lactate (PL; T0–1.8 ± 0.3, 5th-3.7 ± 0.6, 10th-4.6 ± 0.7, 15th-4.8 ± 1.4 mmol/L vs GLY; T0–1.9 ± 0.4, 5th-3.7 ± 0.7, 10th-4.5 ± 1.0, 15th-5.1 ± 1.9 mmol/L; $p = 0.818$, $\eta p^2 = 0.049$) VO_2/R (PL; 1st-54.8 ± 3.2, 5th-56.5 ± 3.9, 10th-56.2 ± 4.3, 15th-56.2 ± 3.2 L/min vs GLY; 1st-55.4 ± 3.7, 5th-56.2 ± 3.5, 10th-55.3 ± 3.3, 15th-56.3 ± 4.2 L/min; $p = 0.515$, $\eta p^2 = 0.137$) and heart rate (PL; 1st-157 ± 9.9, 5th-177 ± 4.8, 10th-180 ± 7.4, 15th-185 ± 4.5 bpm vs GLY; 1st-163 ± 4.6, 5th-178 ± 7.5, 10th-183 ± 7.1, 15th-186 ± 8.6 bpm; $p = 0.378$, $\eta p^2 = 0.181$).

Moreover, the two-way ANOVA analysis also did not show any significant between time × group interaction in VO_2 (PL; 1st-3.64 ± 0.34, 5th-3.72 ± 0.36, 10th-3.68 ± 0.32, 15th-3.70 ± 0.32 L/min vs GLY; 1st-3.69 ± 0.27, 5th-3.73 ± 0.25, 10th-3.67 ± 0.26, 15th-3.69

± 0.40 L/min;
$p = 0.358$, $\eta p^2 = 0.056$), CO_2 (PL; 1st-3.26 ± 0.39, 5th-3.45 ± 0.35, 10th-3.41 ± 0.26, 15th-3.42 ± 0.33 L/min vs GLY; 1st-3.34 ± 0.35, 5th-3.55 ± 0.28, 10th-3.46 ± 0.26, 15th-3.50 ± 0.37 L/min;
$p = 0.889$, $\eta p^2 = 0.034$), RER (PL; 1st-0.89 ± 0.03, 5th-0.93 ± 0.02, 10th-0.93 ± 0.03, 15th-0.93 ± 0.31 vs GLY; 1st-0.90 ± 0.04, 5th-0.95 ± 0.03, 10th-0.94 ± 0.02, 15th-0.94 ± 0.03; $p = 0.674$,
$\eta p^2 = 0.080$), V'E (PL; 1st-110 ± 4.7, 5th-118 ± 9.0, 10th-115 ± 12.0, 15th-120 ± 10.9 L/min vs GLY; 1st-103 ± 8.6, 5th-117 ± 7.1, 10th-105 ± 12.4, 15th-116 ± 9.9 L/min; $p = 0.128$, $\eta p^2 = 0.265$), FR (PL; 1st-47.9 ± 5.2, 5th-52.1 ± 6.3, 10th-56.1 ± 8.4, 15th-58.2 ± 6.7 breath/min vs GLY; 1st-47.7 ± 5.6, 5th-51.2 ± 6.0, 10th-54.4 ± 7.6, 15th-56.1 ± 6.2 breath/min; $p = 0.910$,
$\eta p^2 = 0.029$), EE (PL; 1st-1,066 ± 106, 5th-1,099 ± 106, 10th-1,088 ± 93, 15th-1,090 ± 98 kcal/h vs GLY; 1st-1,082 ± 87, 5th-1,110 ± 76, 10th-1,089 ± 76, 15th-1,105 ± 107 kcal/h; $p = 0.839$,
$\eta p^2 = 0.045$), CHox (PL; 1st-163 ± 36.0, 5th-197 ± 28.2, 10th-194 ± 28.3, 15th-194 ± 34.3 g/min vs GLY; 1st-176 ± 44.8, 5th-217 ± 31.2, 10th-208 ± 27.0, 15th-207 ± 34.9 g/min; $p = 0.703$,
$\eta p^2 = 0.073$), FATox (PL; 1st-32.1 ± 7.7, 5th-20.7 ± 7.6, 10th-20.4 ± 9.5, 15th-21.5 ± 10.8 g/min vs GLY; 1st-27.7 ± 14.8, 5th-12.5 ± 10.6, 10th-14.7 ± 8.9, 15th-16.4 ± 9.6 g/min; $p = 0.677$,
$\eta p^2 = 0.079$) and Ec (PL; 1st-227 ± 13, 5th-234 ± 16, 10th-233 ± 18, 15th-233 ± 13 ml/kg/km vs GLY; 1st-229 ± 15, 5th-233 ± 15, 10th-229 ± 14, 15th-233 ± 17 ml/kg/km; $p = 0.559$, $\eta p^2 = 0.125$).

Figure 4 shows the form power, cadence, form power ratio, ground contact time, leg spring stiffness, and vertical oscillation data for the two interventions evaluated (GLY and PL) in a rectangular test in international race walkers. Two-way ANOVA analysis showed no significant time × group interaction in form power (PL; 1st-63.3 ± 6.8, 5th-63.8 ± 7.3, 10th-63.8 ± 7.6, 15th-64.5 ± 8.6 W vs GLY; 1st-63.8 ± 6.5, 5th-64.5 ± 6.8, 10th-64.3 ± 7.2, 15th-64.5 ± 6.8 W; $p = 0.138$, $\eta p^2 = 0.300$), cadence (PL; 1st-198 ± 5.6, 5th-196 ± 6.3, 10th-196 ± 6.7, 15th-195 ± 7.1 step/min vs GLY; 1st-198 ± 4.7, 5th-196 ± 6.0, 10th-195 ± 6.0, 15th-196 ± 5.8 step/min; $p = 0.301$, $\eta p^2 = 0.211$), from power ratio (PL; 1st-0.242 ± 0.007, 5th-0.244 ± 0.007, 10th-0.243 ± 0.008, 15th-0.246 ± 0.009 vs GLY; 1st-0.242 ± 0.006, 5th-0.244 ± 0.007, 10th-0.243 ± 0.008, 15th-0.244 ± 0.007; $p = 0.176$, $\eta p^2 = 0.274$), ground contact time (PL; 1st-199 ± 6.0, 5th-200 ± 6.0, 10th-200 ± 6.5, 15th-201 ± 6.4 ms vs GLY; 1st-198 ± 7.3, 5th-199 ± 8.3, 10th-200 ± 7.6, 15th-200 ± 7.2 ms; $p = 0.850$, $\eta p^2 = 0.050$), leg spring stiffness (PL; 1st-11.9 ± 1.4, 5th-11.8 ± 1.3, 10th-11.7 ± 1.4, 15th-11.7 ± 1.5 kN/m vs GLY; 1st-11.2 ± 1.1, 5th-11.1 ± 1.1, 10th-11.0 ± 1.0, 15th-11.0 ± 1.1 kN/m; $p = 0.924$, $\eta p^2 = 0.030$), and vertical oscillation (PL; 1st-5.9 ± 0.35, 5th-6.0 ± 0.4, 10th-6.0 ± 0.4, 15th-6.1 ± 0.5 cm vs GLY; 1st-5.9 ± 0.3, 5th-6.0 ± 0.4, 10th-6.1 ± 0.4, 15th-11.0 ± 0.4 cm; $p = 0.355$, $\eta p^2 = 0.189$).

Figure 5 shows the quadriceps, hamstrings, tibialis, and calf data for the two interventions evaluated (GLY and PL) in a rectangular test in international race walkers. Two-way ANOVA analysis showed no significant time × group interaction in quadriceps (PL; B-33.5 ± 0.8, PW-32.8 ± 0.3 5th-32.9 ± 1.0, 10th-32.6 ± 2.1, 15th-32.2 ± 2.4 °C vs GLY; B-33.5 ± 0.6, PW-33.1 ± 1.1, 5th-32.3 ± 1.0, 10th-32.0 ± 2.0, 15th-32.0 ± 2.4 °C; $p = 0.486$, $\eta p^2 = 0.234$), hamstrings (PL; B-33.2 ± 0.6, PW-32.9 ± 0.6 5th-32.9 ± 1.0, 10th-33.1 ± 1.6, 15th-33.3 ± 1.7 °C vs GLY; B-33.2 ± 0.2, PW-

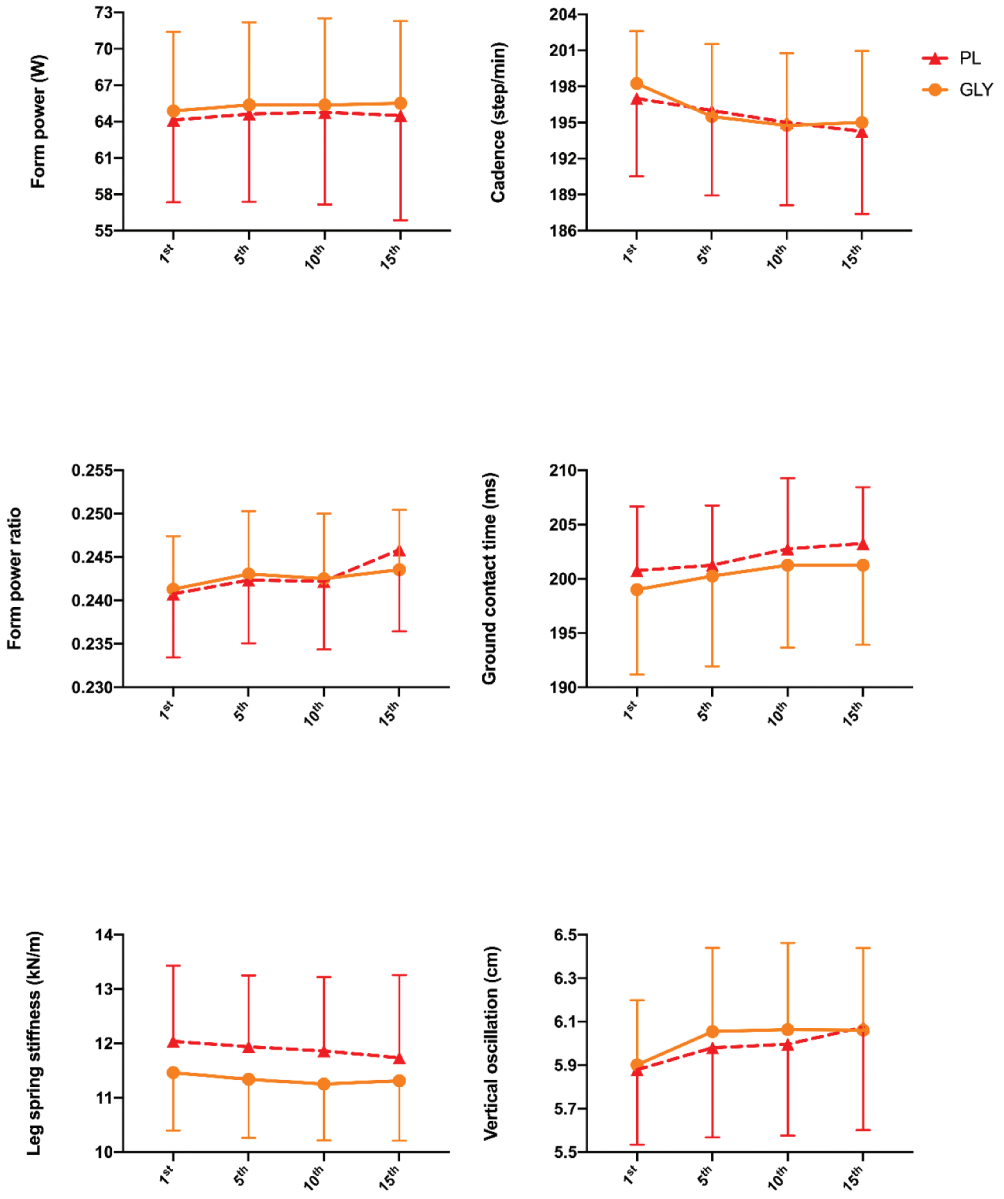

Figure 4. Changes during the rectangular test in kinematic parameters ingestion of GLY and PL at T° mid.

33.2 ± 0.8, 5th-32.8 ± 0.4, 10th-32.5 ± 1.7, 15th-33.1 ± 1.7 °C; $p = 0.497$, $\eta p^2 = 0.230$), tibialis (PL; B-32.9 ± 0.6, PW-33.0 ± 0.4 5th-33.1 ± 0.8, 10th-33.3 ± 1.5, 15th-33.5 ± 1.6 °C vs GLY; B-33.5 ± 0.4, PW-33.1 ± 0.5, 5th-32.9 ± 0.6, 10th-33.2 ± 1.4, 15th-33.5 ± 1.4 °C; $p = 0.246$, $\eta p^2 = 0.343$), and calf (PL; B-32.9 ± 0.4, PW-32.8 ± 0.5 5th-32.8 ± 0.9, 10th-33.1 ± 1.2, 15th-33.5 ± 1.2 °C vs GLY; B-33.2 ± 0.3, PW-33.1 ± 0.6, 5th-32.2 ± 0.5, 10th-32.9 ± 0.9, 15th-33.0 ± 1.5 °C; $p = 0.237$, $\eta p^2 = 0.348$).

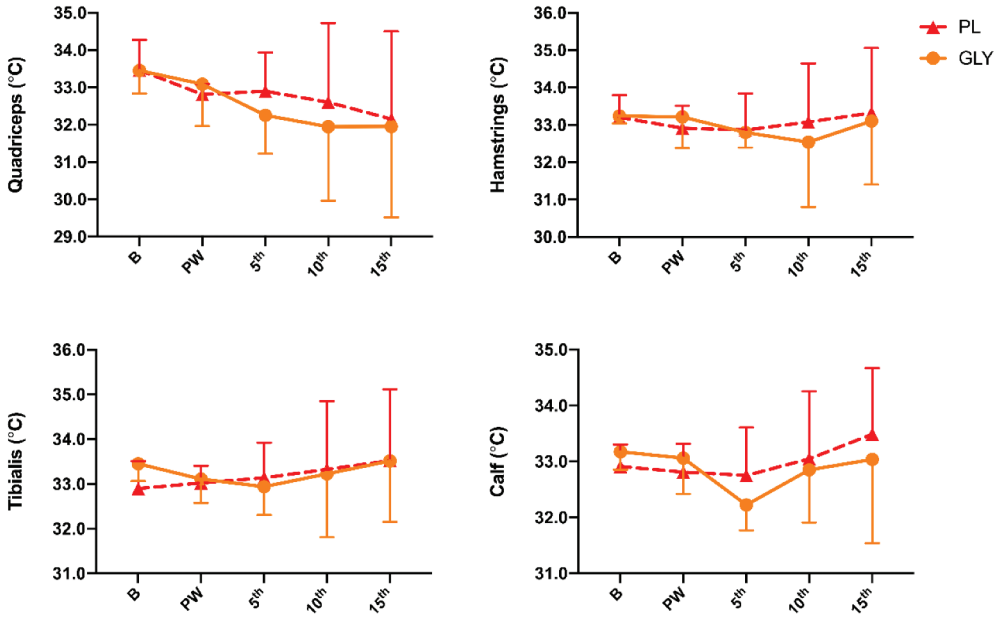

Figure 5. Changes during the rectangular test in the T° of the quadriceps, hamstrings, tibialis and calf after ingestion of GLY and PL at T° mid. B = basal; PW = post warm-up.

4. Discussion

The main aim of the study was to evaluate whether glycerol ingestion was able to improve dehydration, RPE, cardiovascular, metabolic, kinematic, and thermographic variables compared to placebo in environmental conditions of T° mid during a rectangular test. Glycerol supplementation did not show any advantage compared to placebo in a rectangular test in international race walkers under environmental conditions of T° mid in any of the variables studied. Therefore, the results in our study are not in line with our initial hypothesis.

The agency that determines the temperature ranges in which heat stress occurs is the World Meteorological Organization in collaboration with other institutions, and they generate the Universal Thermal Climate Index (UTCI) where they classify the different temperature ranges according to heat stress: "No heat stress" (+9/+26), "Moderate heat stress" (+26/+32), "Strong heat stress" (+32/+38), "Very strong heat stress" (+38/+46) and "Extreme heat stress" (>+46) [31].

In relation to the variables of dehydration and RPE (Figure 2), only in bm we found a time × group interaction, but in favor of the placebo (PL: −2.23 kg vs GLY: −2.48 kg). However, no significant change was found in USG, Σurine, and RPE between groups, but a moderate to large effect size was found. Hitchins et al. [32] evaluated glycerol intake (1 g/kg glycerol in 22 ml/kg bm in carbohydrate-electrolyte drink) and placebo on dehydration and performance parameters at 32°C and HR of 60% during a rectangular test (30 min at functional threshold power and 30 min at maximum sustainable power) in trained cyclists. This author found that glycerol intake resulted in lower urine excretion compared to placebo in the hydration phase (2.5 h pre-exercise), but no significant change (2.8%) in plasma volume between groups [32]. In line with our results, Hitchins et al. [32] also found

no significant change in RPE, but contrary to our results observed no significant change in total sweat loss (GLY: 2.6% bm vs PL: 2.5% bm) expressed as weight loss between both groups during the rectangular test. In our study, although urine excretion (Σurine) in PL was higher (PL: 1.624 mL vs GLY: 1.341 mL) than in GLY, there was no significant difference but a moderate effect size (ES = 0.698); however, there was a significant difference in weight loss (PL: −2.23 kg vs pre-post GLY: −2.48 kg) when comparing the two groups after the rectangular test. There was a difference of about 4°C and a RH of 30% between the environmental conditions used by Hitchins et al. [32] in their study (32°C and RH of 60%) and our study (28°C and RH of 30%), which also included a carbohydrate drink and used a lower amount of GLY.

On the other hand, Marino et al. [18] evaluated glycerol intake (1.2 g/kg glycerol in 21.0 ml/kg bm of water) and placebo on dehydration and performance parameters at 34.5°C and 63.4% RH during a rectangular test (60 min to reach the maximum distance, with 6 sets × 1 min every 10 min) in well-trained cyclists. This author also found that after ingestion of GLY and PL, GLY produced a better urine excretion than PL (GLY: 312 ml vs PL: 430 ml; $p \leq 0.05$) for the duration of the ingestion protocol of both drinks (2.5 h before the rectangular test), obtaining a 24 h urine collection of 1,280 ml for PL and 1,520 ml for GLY, with no significant differences. Furthermore, it also found no significant differences between the two interventions in RPE. In terms of urine excretion, Hitchins et al. [32] and Marino et al. [18] found a beneficial effect on urine excretion in the hydration phase (before the rectangular test) after glycerol intake, but in our case, this was not the case. The difference is that we show the unified pre- and post-rectangular test urine excretion data (Σurine), as this data represents the overall urine excretion during the whole research protocol. The water retention capacity of glycerol is well known [32], which is why it is used in high temperature and RH situations to avoid a loss of performance or to improve it. But there is a problem, and that is that each study uses different environmental temperatures, time of the pre-exercise hydration phase, intake of different amounts of glycerol or together with other molecules, exercise protocol, drink intake during the exercise protocol and sample, so all these factors can lead to different results. In addition, another factor that may bias the data is the hydration status prior to the start of the glycerol [32], intake protocol, but in our case, this was not the situation, as there were no significant differences in urine USG at pre-glycerol intake between the two groups.

Similar to other authors [18,32] who have found no significant differences in RPE after intervention with PL and GLY, we also found no significant differences in this parameter. These authors used similar amounts of glycerol (1–1.2 g/kg bm) and liquid (22–26 ml/kg bm) as in our study. In a 2009 review, eight studies measured RPE during performance after pre-exercise glycerol ingestion, three of them found significant performance improvements and two showed non-significant performance benefits (>5%), although in these studies, RPE was similar between both conditions (GLY vs PL). This may be because glycerol does not cross the blood–brain barrier and is unlikely to exert a direct effect on the central nervous system, and consequently on subjective measures [19]. However, glycerol may improve RPE during exercise by decreasing the extent of dehydration, coupled with cardiovascular and thermoregulatory improvements. Therefore, according to our results, GLY intake does not improve the RPE under T° mid-environmental conditions during a rectangular test in international race walkers.

Regarding the metabolic variables, heart rate, and Ec (Figure 3), we did not find any significant difference between groups. However, we found a moderate effect size (ηp^2) in RER, CHox, FATox, and Ec, and large in VO_2/R, heart rate and V'E. In line with our results, Hitchins et al. [32] also found no significant differences in mean VO_2 in the fixed power phase (78% VO_{2PEAK} for both trials) nor in the variable power phase (GLY: 75% VO_{2PEAK} vs PL: 73% VO_{2PEAK}. Furthermore, no significant changes were detected in lactate during the rectangular test (fixed and variable power phase) between the two groups (GLY: 2.4–6.8 mM vs PL: 2.3–7.0 mM). However, Marino et al. [18] found significant differences during a rectangular test at min 10, 20, 30, 40 and 60 between GLY and PL in heart rate, without significant differences between groups in lactate and blood glucose. These two authors used a glycerol supplementation protocol similar to ours (1–1.2 g/kg bm glycerol and 22–26 ml/kg bm liquid) but with a different exercise protocol than ours.

The effect of glycerol supplementation on metabolic responses is unclear. For example, in fasted rats, glycerol (exogenous) increases gluconeogenesis [33]. This may affect substrate availability and possibly improve performance through increased glucose availability. One human study showed that ingestion of glycerol 45 min before exercise increased blood glucose levels by 14% in the latter stages of an exercise to exhaustion (exercise at 73% of VO_{2MAX}, ~89–108 min, 20–22°C and 40–60% RH), compared to glucose or placebo intake [34]. In addition, blood lactate, alanine, VO_2, CHox, and FATox values during exercise were not different between groups (GLY and PL). However, glycerol intake reduced the magnitude of the increase in exercise plasma FFA compared to PL [34]. These changes are similar to those found in our study, where we found no significant differences in lactate, VO_2, RER, CHox, FATox, and Ec. However, one study found that glycerol at both rest and exercise does not influence the rate of glucose appearance or disappearance, and glucose production is most likely maintained by specific metabolic pathways to balance increases in glycerol [35]. This could be the reason why CHox, and RER did not differ between conditions in the present study. Other possibilities are the method of ingestion and the period (2 h vs. 45 min). Even though theoretically, it sounds good that glycerol can enhance gluconeogenesis and thus performance, there is no clear evidence on this issue. Possibly, one of the factors that makes this impossible is that it has been shown that the human liver, although possessing high levels of glycerol kinase [14,36,37], does not have the gluconeogenic capacity to rapidly convert glycerol to glucose for metabolism during exercise [34,38,39]. Based on the results obtained in our studies and those observed in others, it can be established that glycerol loading modifies metabolic responses during exercise, in the studies where performance increased with glycerol, it is unlikely that muscle metabolism played an important role [32,35]. Based on our results, ingestion of GLY does not provide any advantage in metabolic variables or heart rate in T° mid-environmental conditions during a rectangular test in international race walkers.

In relation to the kinematic variables, in our research we did not find any significant differences between groups (GLY and PL). No studies have evaluated the effects of glycerol intake on kinematic variables. Our hypothesis was that glycerol supplementation at T° mid would improve metabolic variables in international race walkers and avoid negative effects on kinematic variables, but this hypothesis has not been fulfilled. Due to the fact that a large part of the rectangular test takes place in concentrations above 4.0 mmol/L lactate, there is a possibility that increasing the physiological load around the lactate threshold (LT) could affect the variability and fluctuations of kinematic variables. In

this sense, there is evidence that an increase in exercise intensity (incremental test until exhaustion) produces an upward trend in ground contact time and downward leg spring stiffness in middle-distance runners (sub-elite) [40]. In line with our results, the evolution of ground contact time and leg spring stiffness in our study was similar during the rectangular test in both groups. On the other hand, another researcher evaluated how kinematic variables are modified in relation to increasing running speed in an incremental test to exhaustion, and observed that with increasing treadmill speed there was an increase in power relative to weight (W/kg) and a decrease in from power ratio and ground contact time in trained triathletes [41]. These data are different from those observed in our study, as we observed an upward evolution in the from power ratio and ground contact time, this may be due to the differences in biomechanics between runners and race walkers, and to the differences in the exercise protocol tested (incremental test to exhaustion vs ~1 h rectangular test). In addition, recreational runners who reduced ground contact time have been shown to have reduced metabolic demand, probably due to a shorter braking phase, which is metabolically costly [42]. In this regard, previous literature shows that reduced ground contact time is linked to improved running economy [43,44]. Furthermore, a relationship has been seen between decreased vertical oscillation and decreased metabolic demand in a recreational group of runners, but not in an elite group [45]. The relationship found in the recreational group is consistent with the literature, as increased vertical oscillation requires greater support from one's own bm, producing a higher metabolic cost [42,46]. Since some kinematic parameters have a relationship with the metabolic demand quantified by VO_2, it would be interesting to include these variables in future studies where metabolic variables are evaluated, in order to determine the degree of affectation between them.

Finally, in our study, we also evaluated thermographic variables (Figure 5) during the rectangular test in both conditions (GLY and PL). We did not find any significant differences between groups in the different anatomical areas analyzed. However, we did find a large effect size in the quadriceps, hamstrings, tibialis, and calf muscles. To our knowledge, there are no studies that have evaluated the effects of glycerol intake on thermographic variables during a rectangular test. However, Hitchins et al. [32] evaluated core temperature without finding significant changes between groups (GLY: 37.3–38.9°C vs PL: 37.4–39.0°C) during the rectangular test. This author used a glycerol supplementation protocol similar to ours, but with a higher temperature (32°C) and RH (60%). In contrast, other authors have also found a significant reduction in rectal temperature following glycerol ingestion and exercise in dry heat (42°C, 25% RH [47] and 35°C, 30% RH [16]) or hot/humid heat (30°C, 70% RH [48]). Hence, pre-exercise glycerol intake would prevent an increase in core temperature. On the other hand, the effect of environmental temperature on skin temperature changes has been evaluated by thermography, where it has been found that an increase of ~ 11°C (from 17.5°C to 28.2°C) in environmental temperature produces a significant increase in skin temperature in hamstrings, tibialis, and calf muscles during a rectangular test in international race walkers [25]. Therefore, we can see how increased environmental heat stress produces an increase in surface skin temperature, but glycerol ingestion does not have the capacity to improve thermoregulation during exercise at lactic threshold intensity in international race walkers under mid-temperature conditions. It should be mentioned that infrared thermography is a valid tool for the measurement and quantification of the metabolic response of the muscular

system, including in sport [49,50]. Therefore, for future studies, it would be an instrumentation to include to try to find relationships between changes in skin temperature and other performance and metabolic variables, in studies with an intervention with ergogenic aids.

This study has some limitations, and a larger sample would have given more robustness to the results found, especially the results where the ANOVA showed no significance in the time × group interaction but had a large effect size. In addition, it must be taken into account that recruiting athletes at the international level is very difficult and the global cohort of these athletes is smaller than other population cohorts. Also, reproducing indoor weather conditions may be different from the real working in outdoor spaces.

5. Conclusions

The observations made in our study indicate that glycerol intake does not improve any dehydration, heart rate, RPE, metabolic, and thermographic variables in a rectangular test at medium temperature in international race walkers. It is possible that the selected T° and RH (28.2°C and 31.5 RH) were not sufficiently stressful to generate differences in the different variables evaluated. However, it is clear that we must continue to look for solutions for athletes competing in high temperature and humidity conditions, due to the increase in global temperature and the fact that the most important competitions are held in August, which is usually the hottest month of the year.

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References

1. Racinais S, Ihsan M, Taylor L, et al. Hydration and cooling in elite athletes: relationship with performance, body mass loss and body temperatures during the Doha 2019 IAAF World athletics championships. Br J Sports Med. 2021;55(23):1335–1341. doi: 10.1136/bjsports-2020-103613

2. Sugawara M, Manabe Y, Yamasawa F, et al. Athlete medical services at the marathon and race walking events during Tokyo 2020 Olympics. Front Sports Act Living. 2022;4:872475. doi: 10.3389/fspor.2022.872475
3. Galan-Lopez N, Esh CJ, Leal DV, et al. Heat preparation and knowledge at the world athletics race walking team championships muscat 2022. Int J Sports Physiol Perform. 2023;18(8):813–824. doi: 10.1123/ijspp.2022-0446
4. Kakamu T, Wada K, Smith DR, et al. Preventing heat illness in the anticipated hot climate of the Tokyo 2020 summer Olympic games. Environ Health Prev Med. 2017;22(1):68. doi: 10.1186/s12199-017-0675-y
5. Racinais S, Alonso JM, Coutts AJ, et al. Consensus recommendations on training and competing in the heat. Sports Med. 2015;45(7):925–938. doi: 10.1007/s40279-015-0343-6
6. Trangmar SJ, González-Alonso J. Heat, hydration and the human brain, heart and skeletal muscles. Sports Med. 2019;49(Suppl 1):69–85. doi: 10.1007/s40279-018-1033-y
7. Watanabe K, Stöhr EJ, Akiyama K, et al. Dehydration reduces stroke volume and cardiac output during exercise because of impaired cardiac filling and venous return, not left ventricular function. Physiol Rep. 2020;8(11):e14433. doi: 10.14814/phy2.14433
8. Nybo L, Rasmussen P, Sawka MN. Performance in the heat-physiological factors of importance for hyperthermia-induced fatigue. Compr Physiol. 2014;4(2):657–689.
9. Nybo L, Nielsen B. Hyperthermia and central fatigue during prolonged exercise in humans. J Appl Physiol. 2001;91(3):1055–1060. doi: 10.1152/jappl.2001.91.3.1055
10. Fink WJ, Costill DL, Van Handel PJ. Leg muscle metabolism during exercise in the heat and cold. Eur J Appl Physiol Occup Physiol. 1975;34(3):183–190. doi: 10.1007/BF00999931
11. Young AJ, Sawka MN, Levine L, et al. Skeletal muscle metabolism during exercise is influenced by heat acclimation. J Appl Physiol. 1985;59(6):1929–1935. doi: 10.1152/jappl.1985.59.6.1929
12. van Rosendal SP, Coombes JS. Glycerol use in hyperhydration and rehydration: scientific update. Med Sport Sci. 2012;59:104–112.
13. van Rosendal SP, Osborne MA, Fassett RG, et al. Guidelines for glycerol use in hyperhydration and rehydration associated with exercise. Sports Med. 2010;40(2):113–129. doi: 10.2165/11530760-000000000-00000
14. Bortz WM, Paul P, Haff AC, et al. Glycerol turnover and oxidation in man. J Clin Invest. 1972;51(6):1537–1546. doi: 10.1172/JCI106950
15. Robergs RA, Griffin SE. Glycerol. Biochemistry, pharmacokinetics and clinical and practical applications. Sports Med. 1998;26(3):145–167. doi: 10.2165/00007256-199826030-00002
16. Anderson MJ, Cotter JD, Garnham AP, et al. Effect of glycerol-induced hyperhydration on thermoregulation and metabolism during exercise in heat. Int J Sport Nutr Exerc Metab. 2001;11(3):315–333. doi: 10.1123/ijsnem.11.3.315
17. Goulet ED, Rousseau SF, Lamboley CR, et al. Pre-exercise hyperhydration delays dehydration and improves endurance capacity during 2 h of cycling in a temperate climate. J Physiol Anthropol. 2008;27(5):263–271. doi: 10.2114/jpa2.27.263
18. Marino FE, Kay D, Cannon J. Glycerol hyperhydration fails to improve endurance performance and thermoregulation in humans in a warm humid environment. Pflugers Arch Eur J Physiol. 2003;446(4):455–462. doi: 10.1007/s00424-003-1058-3
19. van Rosendal SP, Osborne MA, Fassett RG, et al. Physiological and performance effects of glycerol hyperhydration and rehydration. Nutr Rev. 2009;67(12):690–705. doi: 10.1111/j.1753-4887.2009.00254.x
20. Urbaniak GC, Plous S. *Research randomizer (version 4.0)[computer software]*. 2013.
21. Mundial AM. Editor Declaración de Helsinki de la Asociación médica mundial. Principios éticos para las investigaciones médicas en seres humanos. An Sist Sanit Navar. 2008;24:209–212.
22. Bruning RS, Dahmus JD, Kenney WL, et al. Aspirin and clopidogrel alter core temperature and skin blood flow during heat stress. Med Sci Sports Exerc. 2013;45(4):674–682. doi: 10.1249/MSS.0b013e31827981dc

23. Moreira DG, Costello JT, Brito CJ, et al. Thermographic imaging in sports and exercise medicine: a Delphi study and consensus statement on the measurement of human skin temperature. J Therm Biol. 2017;69:155–162. doi: 10.1016/j.jtherbio.2017.07.006
24. Cabizosu A, Carboni N, Martínez-Almagro Andreo A, et al. Relationship between infrared skin radiation and muscular strength tests in patients affected by emery-dreifuss muscular dystrophy. Med Hypotheses. 2020;138:109592. doi: 10.1016/j.mehy.2020.109592
25. Martínez-Noguera FJ, Cabizosu A, Marín-Pagán C, et al. Body surface profile in ambient and hot temperatures during a rectangular test in race walker champions of the World Cup in Oman 2022. J Therm Biol. 2023;114:103548. doi: 10.1016/j.jtherbio.2023.103548
26. Bernard V, Staffa E, Mornstein V, et al. Infrared camera assessment of skin surface temperature–effect of emissivity. Phys Med. 2013;29(6):583–591. doi: 10.1016/j.ejmp.2012.09.003
27. Tomkinson GR, Olds TS. Secular changes in pediatric aerobic fitness test performance: the global picture. Med Sport Sci. 2007;50:46–66.
28. Marins JB, de Andrade Fernandes A, Moreira DG, et al. Thermographic profile of soccer players' lower limbs. Revista Andaluza de Medicina Del Deporte. 2014;7(1):1–6. doi: 10.1016/S1888-7546(14)70053-X
29. Elsharkawy H, El-Boghdadly K, Barrington M. Quadratus lumborum block: anatomical concepts, mechanisms, and techniques. Anesthesiology. 2019;130(2):322–335. doi: 10.1097/ALN.0000000000002524
30. Cohen J. Statistical power analysis for the behavioral sciences. New York: Academic press; 2013.
31. Blazejczyk K, Bröde P, Fiala D, et al. UTCI - New index for assessment of heat stress in man. Przeglad Geograficzny. 2010;82:49–71.
32. Hitchins S, Martin DT, Burke L, et al. Glycerol hyperhydration improves cycle time trial performance in hot humid conditions. Eur J Appl Physiol Occup Physiol. 1999;80(5):494–501. doi: 10.1007/s004210050623
33. Nikkilae EA, Ojala K. Gluconeogenesis from glycerol in fasting rats. Life Sci. 1962;3(3):243–249. doi: 10.1016/0024-3205(64)90066-9
34. Gleeson M, Maughan RJ, Greenhaff PL. Comparison of the effects of pre-exercise feeding of glucose, glycerol and placebo on endurance and fuel homeostasis in man. Eur J Appl Physiol Occup Physiol. 1986;55(6):645–653. doi: 10.1007/BF00423211
35. Trimmer JK, Casazza GA, Horning MA, et al. Autoregulation of glucose production in men with a glycerol load during rest and exercise. Am J Physiol Endocrinol Metab. 2001;280(4):E657–68. doi: 10.1152/ajpendo.2001.280.4.E657
36. Lin EC. Glycerol utilization and its regulation in mammals. Annu Rev Biochem. 1977;46(1):765–795. doi: 10.1146/annurev.bi.46.070177.004001
37. Terblanche SE, Fell RD, Juhlin-Dannfelt AC, et al. Effects of glycerol feeding before and after exhausting exercise in rats. J Appl Physiol Respir Environ Exerc Physiol. 1981;50(1):94–101. doi: 10.1152/jappl.1981.50.1.94
38. Miller JM, Coyle EF, Sherman WM, et al. Effect of glycerol feeding on endurance and metabolism during prolonged exercise in man. Med Sci Sports Exerc. 1983;15(3):237–242. doi: 10.1249/00005768-198315030-00010
39. Maughan RJ, Gleeson M. Influence of a 36 h fast followed by refeeding with glucose, glycerol or placebo on metabolism and performance during prolonged exercise in man. Eur J Appl Physiol Occup Physiol. 1988;57(5):570–576. doi: 10.1007/BF00418464
40. Hayes PR, Caplan N. Leg stiffness decreases during a run to exhaustion at the speed at VO2max. Eur J Sport Sci. 2014;14(6):556–562. doi: 10.1080/17461391.2013.876102
41. Pardo Albiach J, Mir-Jimenez M, Hueso Moreno V, et al. The relationship between VO(2)max, power management, and increased running speed: towards gait pattern recognition through clustering analysis. Sensors. 2021;21(7):2422. doi: 10.3390/s21072422
42. Moore IS. Is there an economical running technique? A review of modifiable biomechanical factors affecting running economy. Sports Med. 2016;46(6):793–807. doi: 10.1007/s40279-016-0474-4

43. Santos-Concejero J, Granados C, Irazusta J, et al. Differences in ground contact time explain the less efficient running economy in north African runners. Biol Sport. 2013;30(3):181–187. doi: 10.5604/20831862.1059170
44. Nummela A, Keränen T, Mikkelsson LO. Factors related to top running speed and economy. Int J Sports Med. 2007;28(8):655–661. doi: 10.1055/s-2007-964896
45. Aubry RL, Power GA, Burr JF. An assessment of running power as a training metric for elite and recreational runners. J Strength Cond Res. 2018;32(8):2258–2264. doi: 10.1519/JSC.0000000000002650
46. Ackerman J, Seipel J. Effects of independently altering body weight and mass on the energetic cost of a human running model. J Biomech. 2016;49(5):691–697. doi: 10.1016/j.jbiomech.2016.01.016
47. Lyons TP, Riedesel ML, Meuli LE, et al. Effects of glycerol-induced hyperhydration prior to exercise in the heat on sweating and core temperature. Med Sci Sports Exerc. 1990;22(4):477–483. doi: 10.1249/00005768-199008000-00010
48. Easton C, Turner S, Pitsiladis YP. Creatine and glycerol hyperhydration in trained subjects before exercise in the heat. Int J Sport Nutr Exerc Metab. 2007;17(1):70–91. doi: 10.1123/ijsnem.17.1.70
49. de Andrade Fernandes A, dos Santos Amorim PR, Brito CJ, et al. Measuring skin temperature before, during and after exercise: a comparison of thermocouples and infrared thermography. Physiol Meas. 2014;35(2):189. doi: 10.1088/0967-3334/35/2/189
50. Hillen B, Pfirrmann D, Nägele M, et al. Infrared thermography in exercise physiology: the dawning of exercise radiomics. Sports Med. 2020;50(2):263–282. doi: 10.1007/s40279-019-01210-w