

Hyperthermia, but not muscle water deficit, increases glycogen use during intense exercise

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Accepted for publication 30 September 2014

We determined if dehydration alone or in combination with hyperthermia accelerates muscle glycogen use during intense exercise. Seven endurance-trained cyclists ($VO_{2max} = 54.4 \pm 1.05$ mL/kg/min) dehydrated 4.6% of body mass (BM) during exercise in the heat (150 min at 33 ± 1 °C, $25 \pm 2\%$ humidity). During recovery (4 h), subjects remained dehydrated (HYPO trial) or recovered all fluid losses (REH trials). Finally, subjects exercised intensely ($75\% VO_{2max}$) for 40 min in a neutral (25 ± 1 °C; HYPO and REH trials) or in a hot environment (36 ± 1 °C; REH_{HOT}). Before the final exercise bout vastus lateralis glycogen concentration was similar in all three trials (434 ± 57 mmol/kg of dry muscle (dm)) but muscle

water content was lower in the HYPO (357 ± 14 mL/100 g dm) than in REH trials (389 ± 25 and 386 ± 25 mL/100 g dm; $P < 0.05$). After 40 min of intense exercise, intestinal temperature was similar between the HYPO and REH_{HOT} trials (39.2 ± 0.5 and 39.2 ± 0.4 °C, respectively) and glycogen use was similar (172 ± 86 and 185 ± 97 mmol/kg dm, respectively) despite large differences in muscle water content. In contrast, during REH, intestinal temperature (38.5 ± 0.4 °C) and glycogen use (117 ± 52 mmol/kg dm) were significantly lower than during HYPO and REH_{HOT}. Our data suggest that hyperthermia stimulates glycogen use during intense exercise while muscle water deficit has a minor role.

Athletic performance in a hot environment is seldom thought to be affected by energy depletion but rather by hyperthermia or cardiovascular stress (Galloway & Maughan, 1997; Parkin et al., 1999). However, glycogen depletion reduces performance in a thermoneutral environment and could affect performance also when exercising in a hot environment. To our knowledge, the earliest study to focus on the metabolic effects of exercising in a hot environment in humans dates back to 1975 (Fink et al., 1975). In this study, participants exercised in two occasions either in a hot (44 °C) or in a cold (9 °C) environment. They found that after 60 min of exercise, muscle glycogen use was 76% higher in the hot environment. The authors attributed that response to a dehydration-related increase in the activity of glycogen phosphorylase, which was secondary to reduced muscle blood flow and oxygen availability. Years later, Gonzalez-Alonso and coworkers showed that, in fact, prolonged exercise in a 35 °C environment that produced 3.9% of body mass (BM) dehydration reduced blood flow to the contracting muscles (Gonzalez-Alonso et al., 1999). However, leg oxygen consumption was not diminished because of a compensating increase in O_2 extraction. Despite maintaining oxygen supply to the exercising muscles, dehydration increased glycogen use (Gonzalez-Alonso et al., 1999).

In a series of studies, Hargreaves and Febbraio showed elevated muscle glycogen use when exercising (40 min at $70\% VO_{2max}$) in a hot vs thermoneutral environment (40 °C vs 20 °C; Febbraio et al., 1994) or when comparing exercise in a thermoneutral (20 °C) vs a cold environment (3 °C; Febbraio et al., 1996b). The increased glycogen use was accompanied by higher rectal and muscle temperature in the trials with the less favorable conditions to dissipate heat. Apparently, reducing the core temperature elevations during exercise by either exercising in a cooler environment or by rehydrating (Hargreaves et al., 1996; Gonzalez-Alonso et al., 1999) reduces the rates of muscle glycogen use during exercise. These studies were not followed by a performance trial, but it could be thought that preserving muscle glycogen would help to maintain high workloads in the latter stages of a performance trial.

Several factors have been implicated in the increased glycogen use during exercise and heat stress: (a) the increased muscle temperature (Starkie et al., 1999) that habitually occurs along with the increases in core temperature during dehydrating exercise; (b) the increase in circulating adrenaline that accompanies dehydration (Mora-Rodríguez et al., 1996; Febbraio et al., 1998), although the role of circulating epinephrine has been recently argued (Logan-Sprenger et al., 2012, 2013); and

(c) the reduction in O₂ supply with severe dehydration that however does not seem to explain the increased glycogen use during exercise in a hot environment (Gonzalez-Alonso et al., 1999).

Exercise in the heat that induces whole-body dehydration also dehydrates the active muscles (Costill et al., 1976; Neuffer et al., 1991; Hackney et al., 2012). Haussinger and coworkers have found in the liver of rats that water deficit causes reduced glucose transport and increased glycogenolysis (Graf et al., 1988; Lang et al., 1989; Haussinger, 1996). Reduced muscle water in humans, as in the animal model, could stimulate glycogen use. However, to our knowledge, the role of muscle water deficit in the elevated muscle glycogen use observed during dehydrating exercise remains unexplored.

In summary, it is habitually stated that the main cause of fatigue when exercising in the heat is the developing hyperthermia. This notion comes from studies using time to exhaustion protocols as performance trial (Galloway & Maughan, 1997; Parkin et al., 1999). However, athletes do not compete maintaining a fixed workload until exhaustion but rather they vary workload to manage fatigue and complete the event. Then, factors other than hyperthermia could be influencing fatigue during exercise in the heat. Exercise in a hot environment increases glycogen use (Fink et al., 1975; Febbraio et al., 1994, 1996b; Hargreaves et al., 1996). Muscle glycogen content determines fatigue during exercise in a neutral environment (Pernow & Saltin, 1971; Gollnick et al., 1972; Bangsbo et al., 1992; Hargreaves et al., 1995) and could influence performance during exercise in a hot environment. This study is aimed to determine which of the two factors that develop during exercising in the heat (i.e., muscle water deficit and hyperthermia) is the main inductor of the elevated glycogen use. Our hypothesis, based on animal data, was that the rate of muscle glycogen degradation will be increased by the reduction in muscle water content induced by dehydration during exercise in a hot environment.

Methods

Participants

Seven endurance-trained subjects volunteered to participate in the study. Their mean \pm SD for age, height, body mass, percent body fat, and maximal oxygen uptake were 25 ± 10 years, 1.74 ± 0.07 m, 68 ± 7 kg, $14.4 \pm 1.5\%$, and 55 ± 3 mL/kg/min, respectively. All participants were cyclist or triathletes who routinely cycled at least 2 h/day, 4–7 days/week during the last 3 years. Written consent was given by the participants after they were fully informed about the experimental procedures and the possible risks and discomforts associated with the experiment. The study was approved by the University Hospital Research Ethics Committee of Albacete (Spain) in accordance with the latest version of the Declaration of Helsinki.

Preliminary testing and familiarization trial

Before the onset of the experiment, participants underwent a physical examination including rest and exercise ECG (Cosmed

T12, Rome, Italy). They performed an incremental cycling test in an electromagnetically braked cycle ergometer (Cardiostest 100, Seca, Hamburg, Germany) to determine their maximal aerobic capacity (VO_{2max}). After a 5-min warm-up at 100 W, participants began cycling at 125 W with increments of 25 W each minute until volitional fatigue. Gas exchange data were collected using an automated breath by breath system (Quark b2, Cosmed) and averaged every 15 s. VO_{2max} was determined as the highest plateau (two successive maximal readings within 0.15 L/min) reached. Data resulted from this test were used to set the exercise workload of each participants during the experimental trials. Body fat was determined by dual energy X-ray absorptiometry (DXA Hologic Serie Discovery Wi QDR, Bedford, Massachusetts, USA). The following day, a familiarization trial was performed using all the instrumentation of the experimental trial. The preliminary testing and the familiarization trial were scheduled approximately 1 week before the experimental trials and participants refrained from hard physical activity the day prior to testing.

Experimental design

A randomized, crossover, and controlled experimental design was used. On three occasions, separated by at least 1 week to avoid heat acclimation, participants cycled during 150 min (120 min at 65% VO_{2max} and 30 min at 55% VO_{2max}) in a hot environment (33 ± 1 °C; $25 \pm 2\%$ relative humidity, 2.5 m/s air flow) to dehydrate to $4.6 \pm 0.3\%$ BM loss. After dehydration, subjects rested for 4 h while ingesting 250 g of carbohydrate (Powerade® powder, Coca-cola, Atlanta, Georgia, USA) mixed with water to maintain dehydration in one trial (HYPO) and with as much water as needed to replace all body fluid losses in the other two trials (3.1 ± 0.3 L; REH and REH_{HOT} trials). Afterwards, subjects cycled for 40 min intensely (i.e., 75% VO_{2max}) in the three trials. In two trials (i.e., HYPO and REH) pedaling was held in a thermoneutral environment while in a third trial (i.e., REH_{HOT}), subjects exercised in a hot environment to match the core temperature reached during the HYPO trial.

Experimental protocol

The experiment was conducted during fall and winter seasons to avoid heat acclimation caused by high outdoors temperature. Experimental trials started at the same time of day to avoid circadian variation in physiological responses (Krauchi & Wirz-Justice, 1994). The evening before each experimental trial, subjects were required to consume the same meal and to drink 0.5 L of water before they went to bed. Five hours before arriving at the laboratory, participants ingested a telemetric body core temperature pill (Cor-Temp™, HG, Inc., Palmetto, Florida, USA) to measure intestinal temperature during exercise. Participants ate a standardized breakfast (i.e., 3 g/kg of body mass consisting of milkshake, bread, and cacao) and 0.5 L of water 1 h before the trials. Upon arrival at the laboratory, subjects voided and their nude body weight was measured. Afterwards, a urine sample was collected and analyzed for specific gravity (Usg) to confirm euhydration (i.e., < 1.020 ; Sawka et al., 2007; Fernandez-Elias et al., 2014). Then, subjects lay down on a stretcher and were catheterized (20-gauge Teflon®; BD Insyte, Becton Dickinson, Madrid, Spain) in an antecubital vein. Following, baseline blood sample (i.e., 5 mL) was obtained and catheter patency was maintained flushing with 0.5 mL of 0.9% sterile saline each 15 min.

After the first blood sampling, a baseline muscle biopsy was obtained from the midsection of the vastus lateralis. Immediately after, participants dressed up in cleated cycling shoes, attached a heart rate (HR) telemetric band around their chest, and entered in the climatic chamber where they sat quietly on the cycle ergometer for another 15 min while resting HR intestinal temperature (T_{CORE}).

and a pre-exercise upright position blood sample were collected. Then, pedaling started at the prescribed work rate. Upon completion of the dehydrating exercise, participants towed dry and their post-exercise nude body mass was measured again before they voided. Then, participants lay down on a stretcher for an hour to ensure equilibration between body fluids spaces (Mora-Rodriguez et al., 2014). Subsequently a second muscle biopsy was obtained. Participants rested during 3 more hours while they were provided with 250 g of carbohydrate powder (Powerade®) mixed with either 0.4 L of water (HYPO trial) or the same syrup together with as much water as needed to replace all body fluids losses (3.1 ± 0.3 L; REH trials). Then, a third muscle biopsy body weight, blood sample, and urine void were obtained before subjects entered the climatic chamber for the final exercise bout.

Following, subjects sat for 15 min in the cycle ergometer while resting HR and T_{CORE} were recorded. Then, subjects cycled intensely ($75\% \text{VO}_{2\text{max}}$) during 40 min in three different situations: (a) hypohydrated in a thermoneutral environment (HYPO; 25 ± 1 °C, 28% relative humidity and 2.5 m/s air flow); (b) euhydrated in a thermoneutral environment (REH; 25 ± 1 °C, 28% relative humidity and 2.5 m/s air flow); and (c) euhydrated in a hot environment (REH_{HOT}; 36 ± 1 °C, 25% relative humidity and 1.0 m/s air flow). After exercise, subjects' nude body weight was recorded, urine and blood samples were collected, and fourth muscle biopsy was obtained. Subjects were offered food and drinks and instructed about how to take care of the biopsy wounds before being scheduled for the next trial.

Body weight and urine analysis

Body weight was assessed every hour of dehydrating exercise and before and after the final bout of intense exercise using a ± 0.05 sensitive scale (Seca 764, Hamburg, Germany). Urine volume collected before and immediately after exercise was measured using a graduated cylinder (Symax, Proton, Prague, Czech Republic). Urine specimens were analyzed in duplicate for specific gravity (Usg) using a handheld refractometer (Master-Sur/N α , Atago, Japan).

Thermoregulatory measurement

At least 5 h before arriving at the laboratory (Kolka et al., 1997), participants ingested a telemetric body core temperature pill (CorTemp™, HQ, Inc., Palmetto, Florida, USA) to measure intestinal temperature (T_{CORE}) during exercise. Whole-body sweat loss was calculated by subtracting pre- from post-exercise nude body mass, correcting for exhalation of metabolic carbon and respiratory water losses (Mitchell et al., 1972). None of the participants urinated or ingested fluids during exercise and thus no correction was needed.

Cardiovascular measurements

HR was measured using a HR monitor (RS 400, Polar, Kempele, Finland) during exercise. VO_2 and carbon dioxide production (CO_2) were measured using a computerized open-circuit spirometry (Quark b2, Cosmed). Data for these variables were collected during 2 min after 15 min of exercise and at the end of the 40-min intense cycling bout.

Blood sampling and analysis

Blood samples were taken supine before exercise, after 15 min of sitting on the cycle ergometer, and after 0, 15, 60, 120, and 150 min of dehydrating exercise. During the recovery period, blood samples were drawn before exercise, after 15 min of sitting on the cycle ergometer, and after 0, 15, and 40 min during the final

bout of exercise. An aliquot (0.5 mL) of each venous blood sample was immediately analyzed for hemoglobin concentration (ABL-520; Radiometer, Brønshøj, Denmark) and hematocrit (in triplicate) by microcentrifugation (Biocen, Alresa, Madrid, Spain). Relative changes in plasma volume (PV) were calculated using the equations outlined by Dill and Costill (1974). The remaining blood sample (4.5 mL) was allowed to clot into serum tubes (Z Serum Sep Clot Activator Vacuette®, Greiner Bio-One GmbH, Kremsmünster, Austria) and then spun at 2000 g for 10 min in a refrigerated (4°C) centrifuge (MPW-350R, Med. Instruments, Warsaw, Poland). The serum portion was stored at -80 °C for further analyses. Lactate was analyzed using a lactate dehydrogenase reaction (Sigma Aldrich, St. Louis, Missouri, USA) in a multichannel spectrometer plate reader (Versamax, Molecular Devices, Sunnyvale, California, USA).

Biopsies analysis

Muscle samples were obtained by biopsy of the vastus lateralis (Bergström et al., 1967). Two biopsies were obtained from each leg. Incisions were spaced 5 cm advancing from a distal to a proximal location. After biopsy, muscle samples were immediately cleaned of connective tissue, cut in 15 to 20 mg pieces and rapidly weighted on a precision balance with a sensitivity of 0.1 μg (XB220A, Precisa, Dietikon, Switzerland). Muscle samples were snap frozen in liquid N_2 and stored at -80 °C for further analysis. Muscle water content was determined by weight and re-weight muscle samples after thorough freeze-drying in a lyophilizer (Cryodos-50, Telstar, Madrid, Spain). Glycogen content was determined from the measurement of glucose after acid hydrolysis analysis (Passonneau & Lauderdale, 1974). Briefly, muscle samples (~ 20 mg) were homogenized using a glass-on-glass system on ice with deionized water. Then, samples were hydrolyzed in 2N hydrochloric acid and heated for 2 h at 100 °C (Tembloc, JP Selecta, Barcelona, Spain). Finally, samples were neutralized to pH 6.5–7.5 with 1N sodium hydroxide, and glucose concentration was analyzed by colorimetric assay (Enzymatic Glucose Reagent, Thermo Scientific, Waltham, Massachusetts, USA). Data in one subject that underwent 10 resting biopsies within 8 weeks in a euhydrated state (Usg < 1.020 and body weight ± 0.25 kg) confirmed the high reproducibility of this technique in our laboratory in human muscle (i.e., 4% CV).

Statistical analysis

Data are presented as mean \pm SD unless otherwise stated. When Shapiro–Wilk test revealed non-normally data distribution, differences among treatments were analyzed using Friedman's non-parametric test. Normally distributed data were analyzed using one-way analysis of variance (ANOVA) with repeated measures. Data collected repeatedly over time were analyzed using two-way (time \times treatment) repeated measures ANOVA. After a significant F ratio (Greenhouse–Geisser adjustment for sphericity), pair wise differences were identified using Tukey's (honest significant difference) post hoc procedure for normally distributed data, and Mann-Whitney U -test with correction for multiplicity for non-normally distributed data. Cohen's formula for effect size (ES) was used and the results were based on the following criteria: > 0.70 large effect, 0.30 – 0.69 moderate effect, and < 0.30 small effect (Cohen, 1988). Level of significance was set at $P < 0.05$. Data analysis was performed using SPSS software v.18 (IBM, Chicago, Illinois, USA).

Results

Fluid balance and muscle water content

Participants arrived at the laboratory in a similar hydration state as suggested by similar pretrial Usg

Table 1. Plasma volume changes from supine rest (PV), serum lactate concentration, sweat loss, percentage of body mass loss (dehydration %), and urine production during dehydrating exercise (150 min), after 1 h of recovery without rehydration (1 h post), before and after the 40 min final bout of 75% $\dot{V}O_{2max}$ exercise

	Trial	0'	60'	120'	150'	1 h Post	Before final bout	After final bout
PV loss (%)	HYPO	–	-10.9 ± 3.4	-13.7 ± 3.7	-13.1 ± 4.3	-5.2 ± 4.6*	0.3 ± 6.5*	-15.5 ± 5.8*
	REH	–	-11.0 ± 2.5	-13.6 ± 4.6	-12.6 ± 5.6	-5.0 ± 4.9*	3.5 ± 5.8*	-15.9 ± 3.3*
	REH _{HOT}	–	-12.7 ± 3.0	-15.7 ± 3.8	-14.4 ± 4.6	-6.2 ± 6.0*	2.0 ± 4.4*	-14.9 ± 5.4*
Lactate (mmol)	HYPO	1.9 ± 0.8	–	2.9 ± 1.7	1.4 ± 0.9	1.0 ± 0.5	3.1 ± 1.0*	4.3 ± 1.6
	REH	2.1 ± 0.7	–	2.3 ± 0.7	1.9 ± 1.0	1.3 ± 0.5	2.5 ± 0.9*	3.4 ± 1.2
	REH _{HOT}	2.1 ± 0.9	–	2.7 ± 1.3	1.7 ± 0.7	1.4 ± 0.6	2.5 ± 1.2*	5.0 ± 1.3*
Sweat loss (L)	HYPO	–	0.79 ± 0.11	1.26 ± 0.10	0.76 ± 0.16	–	–	0.97 ± 0.29
	REH	–	0.99 ± 0.15	1.14 ± 0.07	0.63 ± 0.10	–	–	0.90 ± 0.07†
	REH _{HOT}	–	0.86 ± 0.16	1.16 ± 0.09	0.68 ± 0.12	–	–	1.24 ± 0.12†
Dehydration (%)	HYPO	–	1.4 ± 0.1	3.5 ± 0.4*	4.7 ± 0.7*	–	4.4 ± 0.3	5.6 ± 0.5*
	REH	–	1.7 ± 0.5	3.6 ± 0.5*	4.6 ± 0.5*	–	1.4 ± 0.7*†	2.7 ± 0.3*†
	REH _{HOT}	–	1.5 ± 0.5	3.5 ± 0.7*	4.6 ± 0.8*	–	1.2 ± 0.7*†	3.0 ± 0.2*†
Urine production (mL)	HYPO	244 ± 207	–	–	116 ± 112	–	186 ± 65	40 ± 63
	REH	344 ± 274	–	–	158 ± 58	–	1027 ± 356†	143 ± 130
	REH _{HOT}	355 ± 356	–	–	80 ± 64	–	912 ± 578†	122 ± 62

Data are presented as mean ± SD.

*Significantly different from prior stage.

†Significantly different from HYPO.

‡Significantly different from REH_{HOT}; ($P < 0.05$).

(1.014 ± 0.008, 1.016 ± 0.006, and 1.014 ± 0.008 for HYPO, REH, and REH_{HOT} trials, respectively) and body mass (69.7 ± 7, 69.6 ± 8, and 69.5 ± 8 kg for HYPO, REH, and REH_{HOT} trials, respectively). Urine production (Table 1) was similar before (313 ± 275 mL) and after dehydrating exercise (118 ± 81 mL). However, before the 40 min final bout of exercise, urine production in the HYPO trial was lower than in the REH trial ($P < 0.05$; ES = 3.98) and the REH_{HOT} trial ($P < 0.05$; ES = 2.25). At the end of the 40 min final bout of exercise, urine production was not significantly different among trials, although it remained lower in the HYPO trial (40 ± 63 mL) than in the REH and REH_{HOT} trials [143 ± 130 (ES = 1.07) and 122 ± 62 (ES = 1.30) mL, respectively]. As expected, there were no differences in percentage of dehydration reached after the initial 150 min of dehydrating exercise (4.7 ± 0.7% in the HYPO trial, 4.6 ± 0.5% in the REH trial, and 4.5 ± 0.7% in the REH_{HOT} trial; Table 1). After the 4 h recovery in the REH and REH_{HOT} trials, subjects were mildly dehydrated (1.5 ± 0.5% and 1.2 ± 0.5%, respectively) while severe dehydration was kept in the HYPO trial (4.4 ± 0.2%; $P < 0.05$; ES = 8.28 REH vs HYPO; ES = 9.14 REH_{HOT} vs HYPO).

Muscle water (mH₂O) was not different before the commencement of any of the three trials, evidencing a similar basal muscle hydration status. After the 150 min of dehydrating exercise, mH₂O decreased similarly in all trials (398 ± 6 to 354 ± 7 mL/100 g of dry muscle (dm); Fig. 2). However, after the rehydration period and before the 40 min final bout of exercise, the mH₂O was significantly lower in the HYPO than in the REH ($P < 0.05$; ES = 1.61) and REH_{HOT} trials ($P < 0.05$; ES = 1.43),

suggesting a different muscle hydration status before the onset of the final exercise bout (Fig. 2).

Blood analysis

PV loss and serum lactate are shown in Table 1. There were no differences between trials in PV changes at any time in the experiment. Also, no differences were found between trials in lactate although it increased with the last bout of exercise only in the REH_{HOT} trial.

Thermoregulatory measurement

At the beginning of dehydrating exercise, subjects' T_{CORE} was not different between trials (36.8 ± 0.2 °C). During the 150 min of dehydrating exercise, T_{CORE} increased significantly over time in all trials ($P < 0.05$) reaching a similar T_{CORE} at the end of exercise (38.6 ± 0.4 °C). Also, there was no difference in sweat loss during dehydrating exercise (Table 1). Right before the start of the final 40-min bout of exercise, T_{CORE} (Fig. 3(a)) was similar in all three trials (37.3 ± 0.3 °C). During exercise, T_{CORE} increased significantly over time but remained lower in the REH trial (38.5 ± 0.4 °C) than in the REH_{HOT} trial (39.2 ± 0.4 °C; $P < 0.05$; ES = 1.58) and HYPO trials (39.2 ± 0.5 °C; $P < 0.05$; ES = 1.37; Fig. 3(a)). In accordance, sweat loss (Table 1) during the 40-min trial was significantly higher in REH_{HOT} than in the HYPO ($P < 0.05$; ES = 1.92) and REH trials ($P < 0.05$; ES = 4.85).

Cardiovascular measurement

There was no difference in HR among trials during the 150 min of dehydrating exercise (147 ± 9, 150 ± 11, and

Table 2. Muscle glycogen concentrations (mmol/kg dm) during the experimental trials at rest (euhydrated), after 150 min of dehydrating exercise (dehydrated 4.7% BM), before and after the final bout of exercise (40 min at 75% $\text{VO}_{2\text{max}}$)

	Euhydrated	Dehydrated (4.6% BM)	Before final bout	After final bout
HYPO	627 ± 74	350 ± 73*	408 ± 51*	223 ± 105*
REH	629 ± 71	357 ± 53*	452 ± 79*	335 ± 56*
REH _{HOT}	636 ± 66	333 ± 77*	442 ± 41*	270 ± 82*

Data are presented as mean ± SD.

*Significantly different from prior stage ($P < 0.05$).

149 ± 13 beats/min for REH, HYPO, and REH_{HOT}, respectively). Before the 40-min final exercise bout, HR was similar in all trials (77 ± 10 beats/min). However, at the end of the 40-min bout, there were significant differences in HR among trials (161 ± 4, 173 ± 5, and 181 ± 5 beats/min for REH, HYPO and REH_{HOT} respectively; $P < 0.05$; Fig. 3(b)).

Muscle glycogen and carbohydrate oxidation

Data concerning muscle glycogen concentration are shown in Table 2. Pre-exercise baseline muscle glycogen concentration was similar among the three trials and decreased similarly after 150 min of dehydrating exercise (631 ± 70 to 347 ± 68 mmol/kg dm; $P < 0.05$). After the recovery period, muscle glycogen was restored similarly in all three trials to a level of 434 ± 57 mmol/kg dm. During the 40-min final bout of exercise, glycogen use and total carbohydrates oxidation were increased during HYPO and REH_{HOT} in comparison with REH (Fig. 4).

Discussion

The aim of this study was to identify if muscle dehydration accelerates glycogen use during a bout of exercise similar to what athletes face in the final stages of long distance competition (i.e., 40 min at 75% $\text{VO}_{2\text{max}}$; Fig. 1). Increased rates of muscle glycogenolysis during exercise would rapidly reduce muscle glycogen stores causing premature fatigue (Pernow & Saltin, 1971; Gollnick et al., 1972; Bangsbo et al., 1992; Hargreaves et al., 1995). However, the reductions in exercise performance in a hot environment are rarely attributed to muscle glycogen depletion but rather to hyperthermia or cardiovascular stress (Galloway & Maughan, 1997; Parkin et al., 1999). These studies use time to exhaustion at a fixed workload as their performance test. However, when performing in a hot environment, athletes do not hold the workload until exhaustion, but reduce the workload to avoid excessive hyperthermia (Cheuvront & Haymes, 2001; Abbiss et al., 2010). In this situation, when athletes do not reach fatigue because of hyperthermia, the rates of glycogen use become a relevant factor

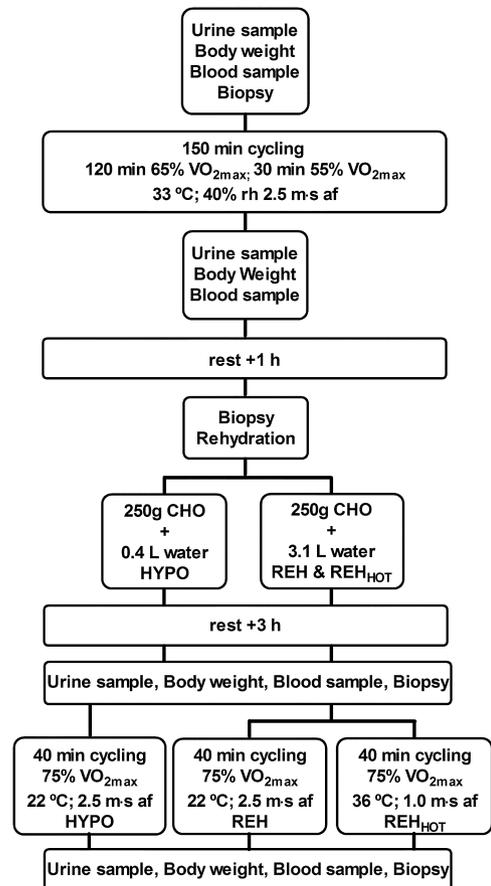


Fig. 1. Experimental design. rh, relative humidity; af, air flow.

determining performance. For this reason, we measured the rates of muscle glycogenolysis when subjects were hyperthermic (REH_{HOT}) or hyperthermic and hypohydrated (HYPO) in comparison with a control trial (REH) where these two factors were not present (Fig. 1).

It is well documented that exercise in the heat (34–44 °C dry bulb) increases the rates of muscle glycogen degradation in comparison with exercise in a cooler environment (Fink et al., 1975; Febbraio et al., 1994). During exercise in the heat, glycogen use is increased when dehydrating in comparison with a trial where rehydration prevents core temperature from increasing (Gonzalez-Alonso et al., 1999). Even during prolonged exercise in a neutral environment (20–22 °C), the rates of glycogen use increase when core temperature rises because of fluid restriction in comparison with a trial where rehydration is allowed (Hargreaves et al., 1996; Logan-Sprenger et al., 2012, 2013). Furthermore, muscle glycogen use decreases when exercising in the cold (i.e., 3 °C; Febbraio et al., 1994) or when cooling the exercising muscles by applying cold water or ice to the skin (Kozlowski et al., 1985; Starkie et al., 1999). Finally, when muscles are heated prior to intense exercise (2 min at 115% of $\text{VO}_{2\text{max}}$; Febbraio et al., 1996a), glycogen degradation rates increase even if core temperature is not elevated. All these evidences suggest that

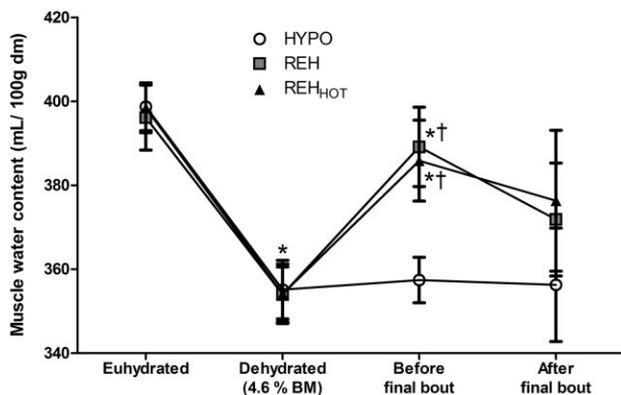


Fig. 2. Changes in muscle water content during the experimental trials. Data are mean \pm SEM. *Significantly different from prior stages. †Significantly different from HYPO ($P < 0.05$).

hyperthermia and particularly elevations in muscle temperature accelerate the rate of muscle glycogen degradation during exercise.

Our study confirms previous findings since glycogen use during the 40 min of intense exercise was $35 \pm 8\%$ higher when subjects remained hypohydrated (i.e., HYPO trial; Fig. 4; $P < 0.05$). Importantly, the difference in glycogen use was not due to different starting muscle glycogen concentrations. Neuffer et al. (1991) already showed that hypohydration did not limit glycogen recovery, so we provided 250 g of carbohydrates in all trials and obtained similar glycogen recovery in all trials. The increased glycogen use during the final bout of exercise was accompanied by the increase core temperature to 39.2 ± 0.5 °C in comparison with when subjects were rehydrated and core temperature only reached 38.5 ± 0.4 °C (i.e., HYPO vs REH trial; Fig. 3(a)). While this association between hyperthermia and glycogen use has been fully described, the novelty of our study is that we addressed if muscle water deficit (Fig. 2) contributes to the observed increase in glycogen degradation.

In the rat liver, tissue dehydration stimulates an array of catabolic responses that include glycogenolysis (Graf et al., 1988; Lang et al., 1989; Haussinger, 1996). However, the confirmation of this catabolic effect of dehydration in the skeletal muscle of humans is, to our knowledge, not available. Based on the animal data, we hypothesize that muscle water deficit by itself could increase glycogen use in our trained subjects. To attain our goal, we include three trials in a crossover repeated measures design testing endurance-trained athletes (Fig. 1). During the first portion of the study, subjects dehydrate by $4.6 \pm 0.3\%$ of BM, which reduced their leg muscle water by $11 \pm 1\%$ (Fig. 2). During recovery (4 h), we either restricted fluid to maintain muscle water deficit (HYPO trial) or allowed full rehydration in another two occasions (REH trials). They finally perform a bout of intense exercise (40 min at $75\% \text{VO}_{2\text{max}}$). In one of the final bouts (i.e., REH_{HOT} trial), subjects were rehydrated,

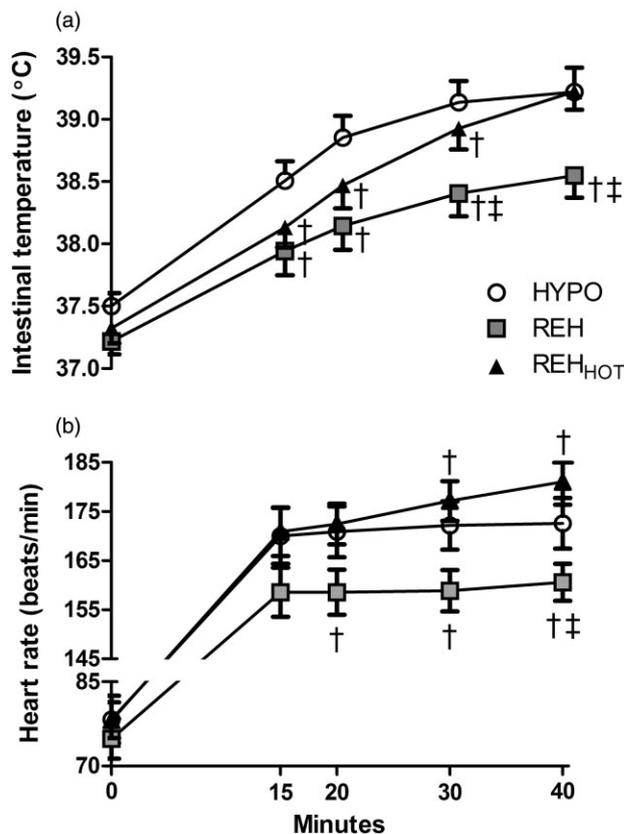


Fig. 3. (a) Intestinal temperature and (b) heart rate during the 40 min of the final exercise bout ($75\% \text{VO}_{2\text{max}}$). Data, for both panels, are mean \pm SEM. †Significantly different from HYPO. ‡Significantly different from REH_{HOT} ($P < 0.05$).

but core temperature was increased to match the levels of the HYPO trial (39.2 ± 0.4 vs 39.2 ± 0.5 °C; Fig. 3(a)) by exercising in an uncompensable (Mora-Rodriguez, 2012) hot environment (36 ± 1 °C) restricting airflow (Mora-Rodriguez et al., 2007). That trial was compared with the HYPO trial to investigate the isolated effects of muscle water deficit on glycogen degradation rates.

We expected increased glycogen use during HYPO above the REH_{HOT} trial. This would have indicated that muscle water deficit stimulates glycogenolysis above the effects of hyperthermia. Contrary to our hypothesis, we found that glycogen use was similarly elevated when muscle water was low (HYPO) or normal (REH_{HOT}; Fig. 2). If we just had compared HYPO with REH trial, we may have concluded that muscle water deficit is an important factor contributing to the increased glycogenolysis with dehydration. However, the lack of difference between HYPO and REH_{HOT} trials reveals that core temperature and likely muscle temperature elevation, is the main factor determining the rate of glycogen use. Furthermore, muscle water deficit seems to have a minor role in glycogen degradation rate during intense aerobic exercise (i.e., $75\% \text{VO}_{2\text{max}}$). Mechanistically, our data suggest that a magnitude of muscle dehydration of $4.6 \pm 0.3\%$ is not a stronger stimulus than hyperthermia

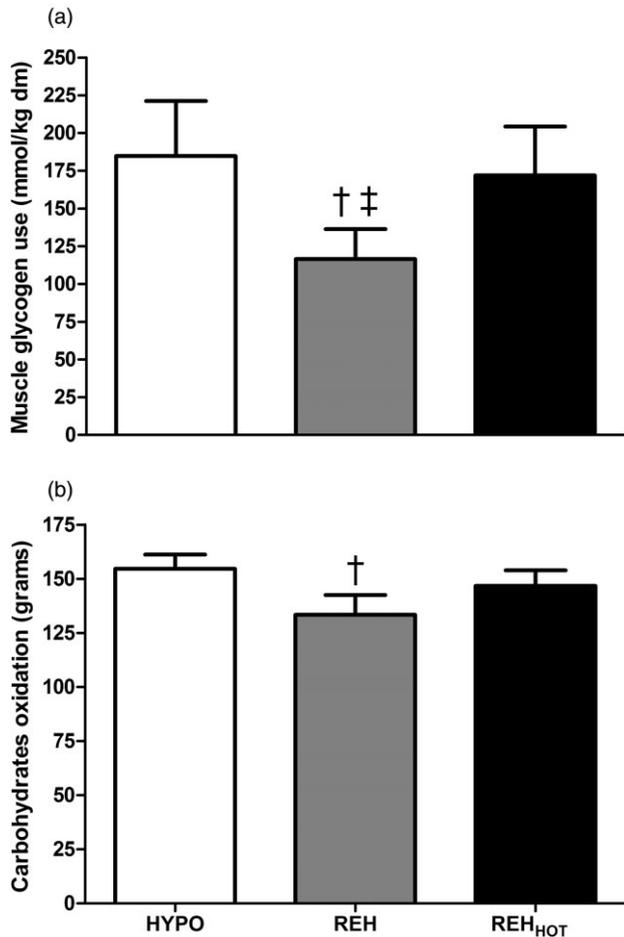


Fig. 4. (a) Muscle glycogen use and (b) total carbohydrate oxidation during the 40 min of the final exercise bout (75% of VO_{2max}). Data, for both panels, are mean \pm SEM. †Significantly different from HYPO ($P < 0.05$). ‡Significantly different from REH_{HOT} ($P < 0.05$).

(39.2 ± 0.15 °C T_{INT}) to increase intramuscular carbohydrate utilization.

Traditionally three factors have been proposed to explain the substrate shift toward higher carbohydrate oxidation and muscle glycogen use when exercising under the effects of heat stress that results in dehydration and hyperthermia (Febbraio, 2001; Logan-Sprenger et al., 2012): (a) a direct temperature effect on key glycolytic enzymes that augment their reaction rates (the Q_{10} effect); (b) increase in sympathetic nerve activity and plasma epinephrine stimulating muscle glycogen phosphorylase activation; and (c) dehydration reducing muscle blood flow and oxygen availability creating an energy unbalance that stimulates glycogen phosphorylase. We tried to add a fourth factor to this list, namely the reductions in muscle water content as a stimulator of glycogenolysis during exercise. However, we did not find basis for its inclusion into that list of factors. Furthermore, our data suggest that the traditional factors were behind our increases in glycogen use. The elevation in muscle temperature was not measured in the present

study. However, the work from Hargreaves et al. (1996), Febbraio (2000), and Gonzalez-Alonso et al. (1999) predicts that muscle temperature would have tracked core temperature and thus been higher during exercise in the HYPO and REH_{HOT} trials.

Plasma epinephrine was not assessed, and thus, we cannot relate its concentration to the effects on muscle glycogen use. However, it is well established that epinephrine secretion is augmented during exercise with heat stress. Studies in humans increasing plasma adrenaline levels via infusion have produced conflicting results. Mora-Rodriguez et al. (2001) found a transient increase in calculated glycogen oxidation when plasma epinephrine was elevated during exercising at 45% VO_{2peak} to the levels observed during exercise at 65% VO_{2max} . However, Chesley et al. (1995) did not find increased glycogen use when infusing epinephrine during exercise at 85% of VO_{2max} . Likely, when exercising at high intensity, glycogen phosphorylase is activated irrespective of adrenaline levels via local factors. Febbraio et al. (1998) conducted a study where epinephrine was infused into individuals exercising in a 20 °C environment to mimic the sympatho-adrenal response observed when exercising at 40 °C. Their results demonstrated that adrenaline increase muscle glycogen utilization in trained men exercising at 70% VO_{2max} . In contrast, Logan-Sprenger et al. (2012) recently reported differences in muscle glycogen use during prolonged exercise that were not accompanied by increases in plasma epinephrine concentrations. Thus, it is controversial if plasma epinephrine elevation is needed to explain the increases in glycogen use during dehydrating exercise.

Finally, we observed a marked blood lactate (Table 1) and HR (Fig. 3) response during our REH_{HOT} trial. This suggests that performing intense exercise in a hot environment results in higher metabolic and cardiovascular stress than when the same workload is undertaken in a thermoneutral environment despite being hypohydrated. Other researchers have suggested that skin temperature (highly influence by ambient temperature) is an important determinant of HR during exercise (Cheuvront et al., 2010). It could be argued that the environmental heat during REH_{HOT} could have increased plasma epinephrine to the levels of HYPO accounting for the similar glycogen use. However, previous studies reveal that plasma epinephrine concentration when dehydrated 1.2% and exercising in the heat is lower than when subjects are 4.6% dehydrated and exercise in cold environment (Gonzalez-Alonso et al., 2000). Nonetheless, we cannot completely discount an indirect effect of increasing skin temperature and sweat rate on accelerating muscle glycogenolysis during exercise in the REH_{HOT} trial.

In summary, contrary to our initial hypothesis, we found that an exercise-induced deficit in muscle water content of $11 \pm 1\%$ has a minor role on the increased glycogen degradation rates observed when individuals are hyperthermic and dehydrated. Conversely, our data

reveal that hyperthermia (i.e., increases in core temperature to 39.2 ± 0.4 °C) during intense aerobic exercise (i.e., 75% $\dot{V}O_{2max}$) is the main cause of the elevated muscle glycogen use even when separated from dehydration. Our findings can be used by sports physicians and nutritionist intending to preserve muscle glycogen in athletes that compete in hot environments.

Perspectives

Our data suggest muscle dehydration is not a stronger stimulus than hyperthermia (39.2 ± 0.4 °C T_{CORE}) to increase intramuscular carbohydrate utilization. However, we are not encouraging athletes to remain dehydrated during prolonged exercise. On the contrary, rehydration is the more ecological mode to delay hyperthermia during exercise in a hot environment. Our data suggest that we should not only worry about maintaining

athletes rehydrated but also preventing them from hyperthermia because of reduced convective and/or evaporative heat loss. Our data sustain that a hyperthermic athlete, although rehydrated, uses muscle glycogen at the same rate than when dehydrated. This could lead them to reductions in athletic performance.

Key words: Muscle glycogen, muscle water content, hydration status, thermoregulation, performance.

Acknowledgements

We thank the help and collaboration of Dr. Jeffrey F. Horowitz and Dr. Rachael K. Nelson, from the Kinesiology Department at the University of Michigan (Ann Arbor). We also acknowledge the dedication of each of the seven athletes that participated in this study. V. E. F.-E. was supported by a predoctoral fellowship from the Junta de Comunidades de Castilla-La Mancha.

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