Rehydration with glycerol: endocrine, cardiovascular, and thermoregulatory responses during exercise in the heat

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IT IS WELL ESTABLISHED THAT intense physical exercise in the heat increases the risk of heat illnesses and decreases exercise performance, whereas significant dehydration can augment these responses (4, 17, 38, 43). Additionally, since the 1940s, scientists have observed that, when people exercise in the heat, they become dehydrated, even if they have free access to water (38). It is generally accepted that dehydration is difficult to prevent during heavy training or competition in the heat (7). In prolonged events and in some individuals with high sweating rate, total body water loss may be as much as 8% of initial body weight (3). This phenomenon has been described as involuntary dehydration (18). On the other hand, rapid fluid and electrolyte restoration after exercise is a slow process that requires at least 3 h and >100% of fluid losses, along with a significant amount of electrolytes (45).

However, ingesting a large volume of fluids, even in dehydrated subjects, rapidly decreases arginine vasopressin (AVP), even before plasma volume or osmolality have been restored, leading to increased urinary output (12). Additionally, thirst sensation decreases in response to plasma volume and osmolality restoration, mouth wetness, and oral-pharyngeal stimulation (33). For these reasons, rehydration is a major part of the recovery process after exercise, especially when individuals must undertake repeated bouts of exercise-heat stress.

In 1987, glycerol, used with water, was first shown to induce hyperhydration more efficiently than water alone, and this effect persisted for 4 h (40). More recently, the effect of glycerol-induced hyperhydration on fluid-regulating hormones was investigated (14). The authors demonstrated a small, nonsignificant increase in plasma vasopressin and no change in circulating aldosterone (Aldo) or atrial natriuretic factor. They reported that there may have been a direct glycerol effect on the kidneys that increased fluid reabsorption and induced a positive fluid balance.

Glycerol is a safe agent that does not approach toxic levels when administered orally in doses of <5 g/kg body wt (47). Glycerol also is an attractive compound for use in studies that involve fluid balance and exercise, because it is not a major energy source during intense exercise (15, 32, 34, 37). Furthermore, glycerol-induced hyperhydration has been suggested to (1) increase overall exercise performance (2, 9, 19) or time to exhaustion (36) in both temperate (36) and hot (2, 9, 19) environments; (2) increase sweat rate (28); (3) decrease heart rate (HR) (36) and rectal temperature (Tre) during moderate-intensity cycling exercise (2); and (4) last for 32–48 h after dosing (23). However, several other studies have shown no performance (20, 29, 31, 48) or thermoregulatory benefit (9, 19, 25, 26, 31, 36) following glycerol-induced hyperhydration.

The purpose of the present investigation was to examine the effect of partial oral rehydration with glycerol on a subsequent exhaustive exercise test in the heat. Surprisingly, although there is a vast amount of information on the role of glycerol as a hyperhydration agent, very limited information is available on its effect in rehydration (44). We hypothesized that glycerol...
would stabilize cardiovascular function, reduce heat stress, and enhance exercise capacity after partial rehydration.

MATERIALS AND METHODS

Subjects

Eight endurance-trained male cyclists agreed to serve as subjects in this study. Their mean (±SE) characteristics were as follows: age, 24 ± 1 (range 19–29) yr; body mass, 70.1 ± 1 (range 66.7–73.6) kg; height, 181 ± 2 (range 174–185) cm; fat-free mass, 56.6 ± 0.4 (range 52.6–61.7) kg; and peak O2 uptake (V˙O2 peak), 61.4 ± 0.8 (range 58.9–65.9) ml·kg⁻¹·min⁻¹. Subjects were selected after their physical activity and medical history questionnaires were reviewed, without regard to race or ethnic origin. These men were heat acclimatized, trained regularly, competed in road cycling or mountain bike races, were nonsmokers, and reported no previous history of endocrine, cardiovascular, renal, or thermoregulatory disorders. All athletes had completed their competitive season at least 2 mo before the study and were on their training season. The study was approved by the Institutional Review Board for Studies Involving the Use of Human Subjects at the University of Connecticut, and all volunteers gave their written, informed consent after attending an informational meeting that addressed the study purpose, methods, and attendant risks and benefits.

Preliminary Testing

Body composition. The hydrostatic weighing technique was used to determine body density. Each subject performed the measurement at least 20 times, on 2 separate days, before the actual data were collected. Before this measurement, residual volume was calculated based on the vital capacity measured with a hand-held spirometer. Calculation of the percentage of body fat from body density was based on the Brozek equation (6).

V˙O2 peak test. V˙O2 peak was determined in a thermocomfortable environment (27°C) using an incremental resistance exercise test on a mechanically braked cycle ergometer (Monark ergomedic 818E, Sweden). The exercise test consisted of continuous cycling at a constant cadence (90–100 rpm), while resistance increased by 0.5 kp every 2 min until volitional exhaustion. Breath-by-breath analysis of the expired gases during the test was performed with an open-circuit respiratory apparatus (model CPXD, MedGraphics Cardiopulmonary Exercise System, St. Paul, MN). Two of the following three criteria were used to verify the attainment of V˙O2 peak: 1) no increase in oxygen uptake (V˙O2 <150 ml/min) with an increase in ergometer resistance, 2) HR >90% of predicted maximal value (i.e., 220 – age), and 3) respiratory exchange ratio >1.1. The average duration of the V˙O2 peak test was 10.01 ± 0.96 min.

Experimental Protocol

Each subject completed three experimental trials, which differed only with regard to rehydration. The rehydration treatments were as follows: no fluid (NF), which served as a control; glycerol followed by water (G), and water alone (W). To ensure double-blind design, a glycerol solution. All other doses in G and W were identical and consisted of aspartame-flavored water to mimic the sweetness of the Penta Manufacturing, Livingston, NJ) that was administered as 1 g glycerol/kg body wt. For W, the first dose consisted of aspartame-flavored water to mimic the sweetness of the glycerol solution. All other doses in G and W were identical and consisted of water. A noncaloric, nonsodium, flavored powder (Kool-Aid) was added to both G and W to provide consistent sweetness, flavor, and color. All fluids were served chilled (10°C). The total fluid ingested was equal to 3% of the euvulated body weight for each subject and was designed to partially rehydrate the subjects. Ten minutes following rehydration, subjects were taken back into the next trial.

Table 1. Indexes of fluid-electrolyte status during dehydration and rehydration

<table>
<thead>
<tr>
<th>Trial</th>
<th>Weight, kg</th>
<th>USG</th>
<th>Osm沛, mosmol/kgH2O</th>
<th>Weight, kg</th>
<th>Dehydration, %</th>
<th>USG</th>
<th>Osm沛, mosmol/kgH2O</th>
<th>Fluid intake, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>No fluid</td>
<td>71.3 ± 1.3</td>
<td>1.005 ± 0.001</td>
<td>279.4 ± 2.4</td>
<td>68.5 ± 1.3</td>
<td>3.91 ± 0.26</td>
<td>1.028 ± 0.02</td>
<td>289.4 ± 2.4</td>
<td>0</td>
</tr>
<tr>
<td>Glycerol</td>
<td>70.8 ± 1.3</td>
<td>1.005 ± 0.001</td>
<td>280.1 ± 1.6</td>
<td>68.0 ± 1.2</td>
<td>3.86 ± 0.17</td>
<td>1.029 ± 0.01</td>
<td>289.3 ± 1.6</td>
<td>2.148 ± 0.30</td>
</tr>
<tr>
<td>Water</td>
<td>71.8 ± 1.1</td>
<td>1.005 ± 0.002</td>
<td>284.0 ± 1.0</td>
<td>68.9 ± 1.1</td>
<td>4.00 ± 0.35</td>
<td>1.029 ± 0.01</td>
<td>291.6 ± 1.0</td>
<td>2.142 ± 0.40</td>
</tr>
</tbody>
</table>

Values are means ± SE. Euhyd, euhydrated baseline; USG, urine specific gravity; Dhy-Base, dehydrated baseline; Osm沛, plasma osmolality; Rhy, rehydration.
environmental chamber (36.7 ± 0.2°C) and sat quietly in the wheelchair for 20 min before another 25-ml blood sample was obtained.

**Exercise test.** Following the blood sample, subjects sat on a mechanically braked cycle ergometer (Monark ergomedic 818E). Seat height was the same for all trials, and toe clips were used. The exercise test (time to exhaustion expressed in minutes) consisted of steady-state cycling (74.0 ± 1.1% VO2 peak) at 80–100 rpm. Subjects continued cycling until one of the following criteria for termination was met: inability to maintain cadence (decrease by >20 rpm), increase in Trec to 39.5°C for >5 min, or signs or symptoms of heat exhaustion. The following ambient conditions were maintained in the climatic chamber during exercise: 36.8–44.4°C, 48.1 ± 1.6% relative humidity, and wind speed by fan of 2.54 m/s. All clocks and stopwatches were removed from subject view.

Trec and skin temperatures (Tsk) were recorded every 4 min, whereas HR was obtained at 2-min intervals during exercise. Arterial blood pressure, forearm skin blood flow (SKBF), VO2, carbon dioxide production (VCO2), respiratory quotient (R), respiratory rate (RR), pulmonary ventilation (Ve), and cardiac output (Q) were measured at 0, 5, 15, 30, 45, and 60 min of exercise. Blood samples (5 ml) were taken at 0, 5, 15, 30, 45, and 60 min of exercise, and a final blood sample (25 ml) was drawn immediately after the end of exercise.

**Thermoregulatory measurements.** Trec was measured by using a rectal thermistor (model 401, Yellow Springs Instruments, Yellow Springs, OH) inserted 10 cm past the external anal sphincter. Tsk were recorded via skin thermistors (model 409, Yellow Springs Instruments) at four sites: chest, triceps, thigh, and calf. Mean-weighted Tsk was calculated according to the equation of Ramanathan [Tsk = 0.2 (Tchest + Ttriceps) + 0.3 (Tthigh + Tcalf)], where T is temperature (39).

Forearm SKBF was measured for 2 min with a laser Doppler flowmeter for microvascular perfusion (model Periflux PFZB, Perimed, Stockholm, Sweden). During the measurement, subjects rested their forearm in a neutral position with 90° flexion of the elbow. Data were expressed as cutaneous vascular conductance (CVC) by dividing SKBF, in volts, over mean arterial pressure (MAP), in 100 mmHg. Whole body sweat rate during exercise was calculated from body weight changes, corrected for estimated respiratory water losses (35), blood sample volume, and urine output.

**Cardiovascular.** HR was measured by a lead I configuration, using a telemetric cardiotachometer (model Vantage XL, Polar Electro). Breath-by-breath analysis of the expired gases was performed with an open-circuit respiratory apparatus (model CPX, MedGraphics Cardiopulmonary Exercise System) to determine VO2, VCO2, R, RR, and Ve. Q was measured via CO2 rebreathing, employing the exponential method (1, 10). VO2 and VCO2 were measured, as described above, for 3 min before each rebreathing procedure. To calculate stroke volume (SV), HR was recorded just before the onset of the rebreathing technique. Arterial systolic (SBP) and diastolic blood pressures (DBP) also were measured with an aneroid sphygmomanometer and stethoscope. MAP was calculated as shown in the following formula: MAP = (SBP − DBP)/3 + DBP.

**Blood collections.** All blood samples were drawn without stasis at the points described above. From the 5-ml blood samples, a 4-ml aliquot was transferred to a test tube containing lithium heparin to determine OsmE, plasma sodium concentration ([Na+]P), plasma potassium concentration, plasma glucose concentration, plasma lactate concentration, total plasma proteins (TPP), and plasma Aldo concentration ([Aldo]P). The remaining aliquot (1 ml) was analyzed immediately for hematocrit (Hct) and hemoglobin (Hb). From the 25-ml blood sample, a 5-ml aliquot was treated as just described. From the remaining sample, a 7-ml aliquot was transferred into an EDTA-treated test tube for plasma AVP ([AVP]P) and glycerol ([Glycer]P) analysis. A separate 5-ml aliquot was transferred into a chilled EDTA-treated test tube that contained 2,000 kallikrein-inhibitor units of aprotinin (Sigma Diagnostics, St. Louis, MO) for plasma atriopeptin ([AP]P) analysis. An 8-ml aliquot was transferred into a chilled EDTA-treated tube for plasma renin activity (PRA) analysis. These samples were centrifuged immediately at 1,800 g and 4°C for 12 min. Plasma samples for hormonal assays were refrigerated at −80°C for later analysis.

**Blood and urine analyses.** USG and TPP were measured by refractometer (model A300CL, Spartan). Hct was determined in triplicate from whole blood by the microcapillary technique, following centrifugation for 4 min at 9,500 g. No corrections were made for trapped plasma or for peripheral venous sampling. Hb was measured in triplicate from whole blood with the cyanmethemoglobin technique (Sigma Diagnostics). Percent changes in plasma volume were calculated with the following formula (11):

\[
\Delta PV = 100 \left( \frac{Hb_B/Hb_A}{[1 - (Hct_B/100)]/[1 - (Hct_A/100)]} - 1 \right)
\]

where ΔPV is percent change of plasma volume, subscript B is before (control), and subscript A is after (experimental). OsmE and OsmI were determined in duplicate by freezing point depression the same day (model 3DII, Advanced Digimatic Osmometer, Norwood, MA). [Na+]P and plasma potassium concentration were determined by ion-sensitive electrodes on fresh plasma samples (model 984-S, AVL Scientific, Roswell, GA). Plasma glucose and lactate concentrations were determined in triplicate with an enzymatic technique (model 2003, Yellow Springs Instruments). [Glycer]P was determined in duplicate with a colorimetric technique (Sigma Diagnostics). [AVP]P was determined by a commercially available radioimmunoassay kit (Nichols Institute, San Juan Capistrano, CA). The with- and between-assay coefficients of variation for [AVP]P at midrange (13 pg/ml) were 1.8 and 5.8%, respectively. The sensitivity of the assay and the average extraction recovery were 1.3 pg/ml and 63.8%, respectively. PRA was evaluated in duplicate by radioimmunochemical determination of plasma angiotensin I, generated during 1-h incubation at pH 6.0 (Incstar, Stillwater, MN). The within- and between-assay coefficients of variation of this assay at midrange (1.2 ng·l−1·s−1) were 4.6 and 4.8%, respectively. The sensitivity of the assay was 0.05 ng·l−1·s−1. [Aldo]P was determined by a radioimmunoassay technique (Peninsula Laboratories, Belmont, CA), after extraction on octadecyl-silica C18 cartridges (Sep-Pak C18, Waters Associates, Milford, MA). The coefficient of variation of the assay was <5%. [Aldo]P was determined with a radioimmunoassay technique (Coat-A-Count, Diagnostic Products, Los Angeles, CA) that had a within-assay coefficient of variation for the mid- (395 pmol/l) and the high-range (1,020 pmol/l) of 3.3 and 1.9%, respectively. The sensitivity of the assay was 44 pmol/l. To reduce interassay variations, each subject’s plasma samples were analyzed within the same assay run. All hormonal analyses were performed in duplicate.

**Statistical Analysis**

Statistical evaluation of the data was accomplished with a two-way analysis of variance with repeated measures (treatment × time). Significant differences between the means were determined by Newman-Keuls post hoc test. Statistical differences were determined at the P < 0.05 level of confidence. All values were reported as means ± SE.

**RESULTS**

Hydration indexes, hydration status, and amount of fluid ingested by the subjects are presented in Table 1. The euhydrated body weight and fluid status of subjects before each trial, the degree of dehydration achieved, pretest USG, and OsmE after dehydration did not differ significantly (P > 0.05). The amount of fluid ingested during G and W was almost identical. During rehydration, urine output for the NF, G, and W were 0, 138 ± 63, and 523 ± 93 ml, respectively. It should be noted that, during G, only two subjects were able to urinate. As a result of the urine output, although the amount of fluid
intake was the same, hydration levels before the beginning of exercise were −4.06, −0.89, and −1.37% for NF, G, and W, respectively. No statistically significant differences were found in urine output or level of hydration between G and W before the beginning of exercise. The dehydration levels at the end of the exercise were greater in NF (−5.5 ± 0.3%) compared with G (−3.3 ± 0.4%) and W (−3.4 ± 0.4%), whereas no differences were found between G and W. Because volunteers exercised more during G, it seems that the rate of dehydration is likely reduced during the G trial. Urine output after exercise for NF (118 ± 20 ml) was significantly lower (P < 0.05) than for G (205 ± 32 ml) and W (336 ± 112 ml), but was not different between G and W.

None of our subjects reported nausea, headache, or gastrointestinal problems during, or the 24 h following, G. One subject was stopped by the investigators during the G exercise test (time: 51 min and 45 s), as his core temperature reached 39.5° for >5 min (see criteria for termination in Experimental Protocol section).

Exercise time to exhaustion during G (32.5 ± 3.8 min) was 19% longer than during W (27.1 ± 3.3 min) and 72% longer than during NF (18.9 ± 2.7 min). Whereas seven out of the eight subjects exercised longer in G than in W, all subjects exercised longer in G and W than in NF. However, no differences were observed among the three trials for VO2, R, Ve, or RR, both at rest and during exercise (Table 2).

Plasma Responses

Glycerol ingestion significantly increased (P < 0.05) [GlycP], by ~100 times (from 0.06 ± 0.01 to 9.94 ± 0.23 mmol/l) above NF (from 0.06 ± 0.01 to 0.08 ± 0.02 mmol/l) and W (from 0.06 ± 0.01 to 0.11 ± 0.04 mmol/l). Glycerol rehydration induced a higher level of OsmP than W at 0, 10, and 15 min of exercise and immediately postexercise (Fig. 1). However, [Na+]P was lower (P < 0.05) during G than W. [Na+]P during exercise was significantly higher for NF (P < 0.05) than both G and W (Fig. 1). TPP also was higher before and during exercise during NF (vs. G and W). Rehydration with glycerol significantly increased plasma volume (vs. W and NF; P < 0.05), and those differences were maintained throughout the exercise test (Fig. 1). Plasma glucose did not change significantly during any of the trials (Table 2), but was higher than the 15-min value during all trials. However, at 15 min of exercise and immediately postexercise, plasma glucose was significantly higher during NF compared with G and W. During NF, plasma lactate was higher than G and W at 15 min of exercise, whereas immediately postexercise this was true only compared with G (Table 2).

Cardiovascular Responses

Q was maintained to a similar level from 5 min (NF: 21.0 ± 0.8, W: 20.6 ± 0.9, and G: 21.4 ± 0.8 l/min) to 15 min of exercise (NF: 20.0 ± 0.7, W: 21.0 ± 0.9, and G: 20.5 ± 0.8 l/min) for all trials. HR responses during exercise (Fig. 2) were not different between the G and W trials (P > 0.05). However, during the first 15 min of NF, HR was significantly higher than both the G and W values. HR at the end of the exercise test did not differ among trials. The SV at 15 min of exercise (Fig. 2) for NF was significantly (P < 0.05) lower than the G and W. During the G, there was a slight trend to maintain a higher SV

Table 2. Selected measurements at Dhy-Base, preexercise, and 5, 15, and 30 min of exercise

<table>
<thead>
<tr>
<th>Variable Title</th>
<th>Dhy-Base</th>
<th>Preexercise</th>
<th>5 min</th>
<th>15 min</th>
<th>End (Post-Ex for Glucose, Lactate, and Glycerol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO2, l/min</td>
<td>No fluid</td>
<td>0.45±0.03</td>
<td>3.17±0.12</td>
<td>3.13±0.12</td>
<td>3.17±0.12</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>0.53±0.06</td>
<td>3.19±0.11</td>
<td>3.25±0.10</td>
<td>3.20±0.11</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>0.47±0.03</td>
<td>3.21±0.12</td>
<td>3.23±0.11</td>
<td>3.20±0.12</td>
</tr>
<tr>
<td>R</td>
<td>No fluid</td>
<td>0.81±0.04</td>
<td>0.97±0.02</td>
<td>0.99±0.02</td>
<td>0.98±0.02</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>0.80±0.05</td>
<td>0.97±0.02</td>
<td>0.97±0.02</td>
<td>0.97±0.02</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>0.81±0.04</td>
<td>0.99±0.02</td>
<td>0.96±0.02</td>
<td>0.98±0.02</td>
</tr>
<tr>
<td>Ve, l/min</td>
<td>No fluid</td>
<td>14.0±1.2</td>
<td>91.4±5.3</td>
<td>108.0±6.5</td>
<td>99.2±5.1</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>15.7±2.5</td>
<td>86.7±4.5</td>
<td>98.4±4.7</td>
<td>95.3±4.1</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>14.8±1.6</td>
<td>93.0±5.5</td>
<td>103.2±6.5</td>
<td>99.1±5.8</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>No fluid</td>
<td>84.0±1.7</td>
<td>89.6±3.1</td>
<td>90.7±3.5</td>
<td>90.5±3.1</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>82.4±2.7</td>
<td>90.0±2.1</td>
<td>88.0±2.8</td>
<td>85.8±2.9</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>85.6±2.0</td>
<td>92.2±3.7</td>
<td>89.1±3.8</td>
<td>87.6±3.8</td>
</tr>
<tr>
<td>RR, breaths/min</td>
<td>No fluid</td>
<td>17±2</td>
<td>42±4</td>
<td>52±4</td>
<td>46±4</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>17±2</td>
<td>37±3</td>
<td>45±4</td>
<td>44±4</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>17±2</td>
<td>40±4</td>
<td>48±4</td>
<td>45±4</td>
</tr>
<tr>
<td>Plasma glucose, mmol/l</td>
<td>No fluid</td>
<td>4.9±0.2</td>
<td>4.8±0.1</td>
<td>4.9±0.2</td>
<td>6.4±0.4</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>4.8±0.1</td>
<td>5.0±0.1</td>
<td>4.9±0.2</td>
<td>5.4±0.4*</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>5.0±0.1</td>
<td>4.7±0.1</td>
<td>4.9±0.1</td>
<td>5.5±0.4*</td>
</tr>
<tr>
<td>Plasma lactate, mmol/l</td>
<td>No fluid</td>
<td>1.0±0.1</td>
<td>1.0±0.0</td>
<td>5.1±1.0</td>
<td>8.8±2.0</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>1.0±0.1</td>
<td>1.3±0.1</td>
<td>4.5±0.9</td>
<td>6.8±1.3*</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>1.0±0.1</td>
<td>1.1±0.1</td>
<td>5.2±1.0</td>
<td>7.3±1.9*</td>
</tr>
</tbody>
</table>

Values are means ± SE. VO2, oxygen uptake; R, respiratory quotient; Ve, pulmonary ventilation; MAP, mean arterial pressure; RR, respiratory rate; End, end of exercise; Post-Ex, postexercise. Significantly different from *no fluid and †preexercise value: P < 0.05.
than for W, but the difference was not statistically significant.

We were unable to collect enough \( \dot{Q} \) data after 15 min, since several subjects stopped exercising before the 30-min mark.

**Thermoregulatory Responses**

\( T_{re} \) (Fig. 3) was significantly elevated \( (P < 0.05) \) during NF compared with G and W at 0, 4, 8, and 12 min of exercise. \( T_{sk} \) (Fig. 3) was significantly higher before the beginning of exercise in NF, but not different during exercise. SkBF and CVC were similar among the three experimental trials immediately before the exercise test (Fig. 4). During exercise, however, both SkBF and CVC were significantly higher \( (P < 0.05) \) during G vs. W and NF. Sweating was higher \( (P < 0.05) \) during G and W (G: 1,426 \( \pm \) 152 ml and W: 1,395 \( \pm \) 128 ml) than NF (798 \( \pm \) 72 ml).

**Hormonal Responses**

\([AVP]_p\) was significantly decreased after rehydration during G and W, but increased \( (P < 0.05) \) in response to exercise for all of the trials. The postexercise level of vasopressin for NF was significantly higher \( (P < 0.05) \) than for G and W (Fig. 5). [AP]_p level was similar before rehydration and did not change during rehydration and exercise (Fig. 5).
PRA (Fig. 6) decreased during rehydration for G and W and increased significantly \((P < 0.05)\) at the end of exercise (vs. preexercise) in all experimental conditions. No differences were found among the different trials. [Aldo] was greater \((P < 0.05)\) after exercise (vs. preexercise) for all trials. At the end of exercise, [Aldo] was lower \((P < 0.05)\) in NF than W and G (Fig. 6).

**DISCUSSION**

This study examined the endocrine, thermoregulatory, and cardiovascular responses to exercise in the heat after oral rehydration with glycerol. We hypothesized that rehydration with glycerol (G trial) would prolong exercise time to exhaustion, elicit greater cardiovascular stability, and lower thermoregulatory strain compared with NF or W trials. Rehydration with glycerol did prolong exercise time to exhaustion in the heat. However, this improved exercise capacity in the heat with glycerol rehydration was not associated with any clear thermoregulatory or cardiovascular advantage compared with rehydration with water only. Although glycerol rehydration resulted in a higher SkBF during exercise in the heat, the environmental conditions prevented any heat loss by radiation or convection. As such, the improved SkBF had no thermoregulatory impact.

Similar improvements in exercise time to exhaustion have been observed in the only other study in which glycerol was used as a rehydration agent (44), as well as in a hyperhydration study (36). Increase in exercise performance has been observed in some studies that utilized glycerol-induced overhydration before exercise (2, 9, 19), whereas others reported no performance improvement (20, 29, 31, 48). Although previous glycerol-induced overhydration studies have reported gastrointestinal discomfort, headaches, or even blurred vision (9, 25, 26), in our study, none of the subjects experienced any of those symptoms during G or the 24 h following the study. The absence of side effects has been also shown by others (27, 36, 40), and it has been speculated that high-concentration glycerol solutions (i.e., 50%) are likely to induce these symptoms.

Although rehydration during G did not induce a statistically significant better fluid balance compared with W, the small difference in hydration (due to smaller urinary output) may have provided physiologically greater body water “availability” during the glycerol trial. Furthermore, since volunteers exercised more during G but sweated similarly with W, we found that the rate of dehydration was reduced. Glycerol, on the other hand, is a solute that increases the tonicity of blood, and, although 2 liters of water were consumed, the 100-fold
increase in plasma glycerol (Table 2) led to maintenance of the dehydration-induced elevation of Osmp. This osmotic effect, along with a slight improvement in hydration level, could induce the increased plasma volume observed during G compared with W. A similar response of plasma volume has been reported in other glycerol studies when plasma volume changes were calculated from Hct and Hb values, like in the present study (21, 29, 37, 44), or with Evans blue dilution technique (21).

Both Montner et al. (36) and Anderson et al. (2) indicated that glycerol-induced hyperhydration maintained HR at a lower level than a placebo. In the present study, although plasma volume for G was always greater than that for W, HR, V̇O₂, blood pressure, and SV were not significantly different (G vs. W), although SV showed a nonsignificant trend to be higher during G (Fig. 2). It should be noted that we were unable to measure SV for all subjects after 15 min of exercise, since few of the volunteers managed to reach the 30-min time point at which the CO₂ rebreathing maneuver was performed for the SV estimation (NF: 1, G: 5, and W: 3 subjects). We speculate that SV would have been higher in the G compared with W and NF at a later exercise stage, when cardiovascular strain was likely greater. Based on the first 15 min of exercise, our data are in agreement with a previous study by Latzka et al. (26), in which glycerol-induced hyperhydration before uncompensable exercise-heat stress offered no cardiovascular advantage compared with water-induced hyperhydration.

It was hypothesized that glycerol-related ergogenicity could enhance thermoregulatory responses, despite the fact that most studies have not reported thermoregulatory benefits (9, 19, 36, 44). In the present study, because the environmental temperature was always higher than the Tsk, sweat evaporation was the only means of heat dissipation. In contrast to the work of Lyon et al. (28), which reported that glycerol-induced hyperhydration increased sweating and decreased exercise core temperature, no difference was found in the sweat rates of W and G.

Glycerol is a gluconeogenic substance that can be metabolized in the liver and provides energy. Studies in experimental animals have shown that rats fed with high doses of glycerol performed better: they were protected from hypoglycemia and exhibited both liver and muscle glycogen sparing (46). This finding was not reported for humans, during 90 min of continuous running (34), cycling to exhaustion for ~90 min (15), or even cycling to exhaustion (80–92 min) following a 36-h fast (32). These data suggest that the human liver may not be able to metabolize glycerol fast enough to provide sufficient energy during intense exercise. Similarly, we found no differences between G and W in plasma glucose, lactate, V̇O₂, and R, indicating that glycerol did not contribute substantially as an energy substrate.
In the present study, glycerol ingestion (G) altered fluid-regulating hormone responses to exercise in the heat (AVP, AP, and Aldo) and PRA in ways that were not observed when water alone was consumed (W). Glycerol increased OsmP; regulating hormone responses to exercise in the heat (AVP, other fluid-regulating hormones. maximal exercise in the heat, with or without glycerol, is not a during exercise.

Cardiovascular or thermoregulatory advantages, although CVC during intensive cycling in the heat, likely by maintaining hydration with glycerol increased exercise time to exhaustion state of hydration does not affect exercise-induced responses of both PRA and Aldo increased significantly as 0.05), whereas Aldo did not change significantly. In response to exercise (AP, H11022 plasma AP increases in response to atrial stretch (16). AP tended to decrease with rehydration and increase in response to exercise (P > 0.05). This may have occurred because plasma AP increases in response to atrial stretch (16). Freund et al. (13) reported an increased [AP]p as a result of maximal exercise in both trained and untrained subjects, whereas Kraemer et al. (24) reported that submaximal exercise (71% maximum VO2) did not alter [AP]p. In the present study, AP remained unchanged during exercise, suggesting that submaximal exercise in the heat, with or without glycerol, is not a potent secretory stimulus. In agreement with this finding, Freund et al. (14) showed that glycerol did not affect ANP and other fluid-regulating hormones.

PRA decreased during both rehydration conditions (P < 0.05), whereas Aldo did not change significantly. In response to exercise, both PRA and Aldo increased significantly as previously reported (8), but no changes were found among the trials. Brandenberger et al. (5) also observed that the initial state of hydration does not affect exercise-induced responses of Aldo and PRA.

In conclusion, this study supported the hypothesis that hydration with glycerol increased exercise time to exhaustion during intensive cycling in the heat, likely by maintaining greater plasma volume. Glycerol did not provide significant cardiovascular or thermoregulatory advantages, although CVC was significantly increased. Furthermore, glycerol did not have a significant effect on fluid-regulating hormones, before or during exercise.

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