Failure to reduce body water loss in cold-water immersion by glycerol ingestion

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Arnall DA, Goforth HW. Failure to reduce body water loss in cold-water immersion by glycerol ingestion. Undersea & Hyperbaric Med 1993; 20(4):309–320.—The efficacy of ingesting an aqueous glycerol solution to enhance body water retention during prolonged cold-water dives was evaluated. Nine Naval Special Warfare divers performed a 3-h dive in 13°C water. Divers were assigned to either a water-treatment group (WT) or a glycerol-treatment (GT) group. WT ingested 30 ml water/kg lean body mass (LBM). GT ingested a solution consisting of 1.2 ml glycerol/kg LBM and 30 ml water/kg LBM. Blood was drawn at prehydration, 90 min after hydration, and 20 min after the 3-h dive for serum glycerol, glucose, free fatty acids, lactate, and electrolyte determinations. Fluid intake and output was recorded and urine analyzed for osmolality, electrolytes, and specific gravity. Serum glycerol values in GT were 200 times greater at posthydration than prehydration and 100 times greater at postdive than at prehydration. Urine output, total body weight loss, and non-urine weight loss during posthydration and dive sampling periods were not significantly different between treatment groups. Hyperhydration with an aqueous glycerol solution of 1.2 ml glycerol/kg LBM seems ineffective in significantly reducing body water loss in divers during prolonged cold-water immersion.

glycerol, hyperhydration, cold water immersion, divers, human subjects, natriuresis, kaliuresis, cold

In humans, water immersion, whether head-out or whole body, represents an environmental stress that elicits unique physiologic responses (1, 2). Immersion has been shown to significantly increase diuresis (1, 3–6). Increased losses of sodium and potassium often accompany increased urine production (1, 7). Exposure to cold air alone significantly increases diuresis and plasma volume loss (8); however, cold-water immersion produces a more powerful stimulus (4, 9). All of these physiologic responses occur when subjects are immersed in cold water (4, 8, 10) or thermoneutral water (1, 11–14).

It is generally accepted that the underlying mechanisms of immersion-induced diuresis involve neural, hormonal, and hemodynamic factors. A primary factor seems to be the redistribution of blood into the thoracic region and a concomitant increase
in right atrial and pulmonary transmural pressure (2). This in turn stimulates atrial receptors and the release of natriuretic and diuretic "factors," e.g., atrial natriuretic factor (ANF) (9, 15, 16).

Prolonged immersion diuresis, with or without supplemental water intake, produces significant plasma volume loss over a range of 8–18%, resulting in whole body dehydration (1, 4–6, 10, 17). Dehydration (18, 19), hypohydration (20, 21), and plasma volume loss negatively affect work capacity (22–24). Prolonged cold exposure (10°–18°C) compounds the problems associated with immersion diuresis by causing loss of muscular power and strength (21, 25, 26), loss of mental faculties (27, 28), and possible death due to severe hypothermia.

An obvious strategy to overcome the large fluid volume loss during water immersion would be to hyperhydrate the diver. However, since the kidneys depend on adequate vascular pressures and volume to filter the blood, an increased vascular volume becomes a stimulus for increased diuresis (1). Therefore, forced hydration with water before and during cold and thermoneutral immersion tests has proven unsuccessful in compensating for diuresis and preventing dehydration (1, 4, 5, 10). Infusion of plasma expanders to investigate changes in oxygen delivery capacity or to study improved temperature regulation in the heat during exercise have been examined (7, 29–32). For example, infusion of albumin is reported to acutely increase plasma oncotic pressure and maintain an elevated plasma volume in the face of a heat-stress challenge (30). However, the use of plasma expanders to expand blood volume or to inhibit diuresis has not been tested on human subjects during cold-water immersion.

Glycerol is an endogenous triose and a metabolic intermediate possessing several biochemical fates resulting in an estimated biological half-life of 30–60 min (33). Glycerol has the advantage of being highly miscible in water and can easily be administered orally or intravenously in large volumes (33). Furthermore, it is readily absorbed by the gastrointestinal tract, reaching peak serum values in 60–90 min, and equilibrates rapidly across most non-neurologic tissues (33). Also, glycerol readily equilibrates with the extracellular space (33, 34). Lyons and associates and Riedesel et al. reported that ingesting a glycerol bolus followed by water enhanced plasma volume retention in subjects exercising in hot (35, 36) or in thermoneutral (37, 38) terrestrial environments. Glycerol and water ingestion is therefore an appealing intervention because of its non-invasive administration and its reported ability to sequester water temporarily in the interstitial spaces (33, 34).

The purpose of this study was to examine the utility of ingesting an aqueous glycerol solution to enhance body water retention during a 3-h, cold-water dive.

METHODS AND MATERIALS

Subjects

A full explanation of risks and benefits and a review of the research procedures were given to each subject before signing a consent form. This study was approved by the Committee for the Protection of Human Subjects, Naval Health Research Center, San Diego, California.
Table 1: Anthropometric Measurements of the Subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>TBM, kg</th>
<th>LBM, kg</th>
<th>Fat, %</th>
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<tbody>
<tr>
<td>Wi</td>
<td>30</td>
<td>170.2</td>
<td>80.3</td>
<td>66.8</td>
<td>17</td>
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<tr>
<td>Ce</td>
<td>28</td>
<td>172.7</td>
<td>74.8</td>
<td>62.7</td>
<td>16</td>
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<tr>
<td>Hn</td>
<td>28</td>
<td>172.7</td>
<td>69.2</td>
<td>58.1</td>
<td>16</td>
</tr>
<tr>
<td>Tt</td>
<td>27</td>
<td>177.8</td>
<td>77.6</td>
<td>67.0</td>
<td>14</td>
</tr>
<tr>
<td>Le</td>
<td>28</td>
<td>175.3</td>
<td>88.0</td>
<td>73.7</td>
<td>16</td>
</tr>
<tr>
<td>We</td>
<td>32</td>
<td>167.6</td>
<td>66.8</td>
<td>55.7</td>
<td>17</td>
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<tr>
<td>Ve</td>
<td>43</td>
<td>177.8</td>
<td>78.6</td>
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<td>Bn</td>
<td>29</td>
<td>180.3</td>
<td>77.1</td>
<td>67.2</td>
<td>13</td>
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<tr>
<td>Lr</td>
<td>31</td>
<td>180.3</td>
<td>98.2</td>
<td>77.6</td>
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<td>x̄</td>
<td>31</td>
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<td>78.9</td>
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<td>±SEM</td>
<td>±1.6</td>
<td>±1.5</td>
<td>±3.2</td>
<td>±2.3</td>
<td>±0.7</td>
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</table>

Nine Naval Special Warfare divers (SEALs) volunteered to participate. The subjects' individual anthropometric data are summarized in Table 1. Body fat was estimated according to the skinfold technique of Durnin and Wormersley (39). Subjects were asked to refrain from using nicotine products and caffeinated beverages for 24 h before the start of the study and throughout the 6-h experimental period (3 h for predive activities and 3 h of immersion).

Data analyses and treatment groups

Three subjects volunteered to perform a repeated-measures design experiment in which they would first undergo the glycerol treatment (GT) and then receive the water treatment (WT). Each treatment trial was separated by 1 day. Six other subjects, who could not participate in a repeated-measures design, were assigned randomly to either WT or GT. Analysis of the data from the repeated-measures trials, using a Wilcoxon signed-rank test, demonstrated no significant differences in blood or urine base-line metabolite values before beginning the WT or GT predive hydration trials. Additionally, a Mann-Whitney U test revealed no significant differences between treatment responses of the repeated-measures and non-repeated-measures subjects. Therefore, the data were pooled to form two treatment groups, the WT group (six observations) and the GT group (six observations). The Mann-Whitney U test was then applied to these data to determine differences between the two treatment groups. Statistical significance was evaluated at the $P < 0.05$ level. Table and figure representations of these data are expressed as means $±$ SEM of six observations per treatment group.

Experimental protocol

The subjects arrived at the dive facility after an overnight fast (12–16 h) and remained seated for 20–30 min before having a base-line blood sample taken. Two to three hours before the dive all were fed a high carbohydrate (CHO) meal, which
contained 686–833 kcal, comprising 140–160 g CHO (78% of total kcal), 12–18 g fat, and 5–8 g protein. This was designed to ensure that all subjects began the dive in a similar nutritional state and were not hungry during the 3-h cold-water dive.

After completing the meal and before commencing the hydration phase of the experiment, subjects voided their bladders. Subjects were then weighed (± 0.1 kg) on an electronic digital scale (West Scales). From this point on, the volume or weight or both of all fluids ingested and urine and feces excreted were measured and recorded.

All subjects were blind to the treatments in which they participated. They ingested individually determined volumes of either the aqueous glycerol solution or a control water solution. Each diver drank a solution containing 30 ml water/kg lean body mass (LBM) representing an average of 2 liters of solution per subject. The control solution, approximating the glycerol solution in flavor and color, was made by adding 2 teaspoons of saccharine and 2 tablespoons of concentrated orange juice to 2 liters of water. Only the orange juice concentrate was added to the glycerol solution. The glycerol treatment solution was individually calculated as 1.2 ml glycerol/kg LBM and averaged 81 ± 4 ml (mean ± SEM) of glycerol per subject in the GT group. This amount of glycerol is considered a safe medical dose (33) and falls within the range used by other researchers (35–38).

Equipment

Divers were outfitted with a layered, passive, thermal protection system comprising a vulcanized rubber dry suit (Viking), a Thinsulate-400 suit (3M Corporation), and polypropylene underwear (Thermax); the overall clo value totaled approximately 1.8. Additionally, divers wore wet-suit hoods and gloves made of 6.35-mm thick neoprene. Divers remained completely submerged in a sitting position in 5 ft of fresh water maintained at 13° ± 0.5°C for 3 h. During the dive, divers used an MK-15 closed-circuit underwater rebreathing apparatus which recirculated the diver’s air, maintaining the partial pressure of oxygen and extracting carbon dioxide. Externally fitted condomlike urinary catheters (Hollister) were worn as part of an overboard urine discharge system that exited the dry suit via an 8-mm diameter tube. For the purpose of collecting urine samples, the exit tube was fitted with a quick-release connector attached to a 1-liter collection bag. This permitted the research team to disconnect the urine sample bag and refit a new collection bag while the diver was submerged.

Blood and urine collection and analysis

Blood was sampled 3 times from the cubital vein. The first collection of blood, the prehydration (PREHYD) sample, was taken 20 min after arriving at the dive facility. This blood sample was used to establish the PREHYD base-line values for metabolites and electrolytes. The second collection of blood, the posthydration/predive sample (POSTHYD), was taken 60 min after completing the hydration protocol. It was used to establish a POSTHYD base line to compare with the postdive values. The third blood sample, the postdive (POSTDIVE) one, was taken after divers exited the water, removed their diving equipment, and had been sitting quietly for 20–30 min. Serum samples were prepared and later analyzed for glucose (sGLU),
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glycerol (sGLY), free fatty acids (sFFA), lactate (sLAC), and the following electrolytes: sodium, potassium, calcium, chloride, creatinine, and phosphate. Hematocrit was determined from heparinized whole blood in triplicate using a microhematocrit centrifuge. Free fatty acids in serum were analyzed using the colorimetric method of Novak (40). Glycerol concentrations were determined in serum and urine samples using the spectrophotometric enzymatic assay of Kreutz (41). Serum lactate concentrations were determined using a lactate analyzer (Yellow Springs Instruments, Inc., model 23L). Calibration of the analyzer against standards of varying lactate concentrations was performed at the beginning of each assay run and regularly after each fifth sample determination. Also, triplicate samples were run randomly on serum samples to check the reliability of the analyzer.

Urine samples were obtained hourly by exchanging the collection bags attached to the diver’s overboard urine discharge tube. Sample volumes were measured and subsamples frozen awaiting analysis for urine glycerol (uGLY). Additionally, another sample set was refrigerated and analyzed for urine concentration of sodium, potassium, calcium, chloride, creatinine, and phosphate, as well as for the determination of osmolality and specific gravity.

RESULTS

The volume of water ingested by the subjects in the GT and WT trials was 2,034 ± 38 ml and 1,918 ± 32 ml (mean ± SEM), respectively, and did not differ significantly between the two treatment groups. The mean volume of glycerol in the GT solutions was 81 ± 4 ml (mean ± SEM). The mean nutritional values for the predive meals consumed by each treatment group did not differ significantly in kilocalories, CHO, fat, or protein.

Serum data

There were no significant differences in mean sGLU levels between the WT or GT group within each sampling time (Fig. 1A). Likewise, within a given treatment (glycerol or water), there were no significant differences in sGLU values across the three sampling times. All subjects maintained normal blood glucose concentrations of 5.24–5.63 mM or 94–101 mg/dl throughout the 3-h experimental dive.

Concentrations of sFFA at POSTDIVE, for both the WT and the GT trials, were significantly higher ($P < 0.02$) than at the PREHYD and POSTHYD times (Fig. 1B). POSTDIVE sFFA concentrations were 63 and 65% higher than PREHYD values in the WT and GT trials, respectively. Additionally, the initial PREHYD sFFA values of subjects in the WT group were significantly higher ($P < 0.001$) than in the GT group.

Concentrations of sLAC for the WT and GT subjects did not differ significantly between groups at any of the three sampling times (Fig 1C). Both WT and GT demonstrated a significantly lower sLAC concentration ($P < 0.02$) at POSTDIVE compared to sLAC concentrations at POSTHYD. Mean sLAC values for all subjects throughout the study were within the normal range of 1.0–2.4 mM for nonexercising subjects.
FIG. 1.—Mean of six observations ± SEM. A, serum glucose; B, serum free fatty acids; * = significant difference ($P < 0.05$) from PREHYD and POSTHYD sampling times within the same treatment group; $\dagger$ = significant difference ($P < 0.05$) from corresponding glycerol value. C, serum lactate; $\ast$ = significant difference ($P < 0.05$) from POSTHYD values within the same treatment. D, Hematocrit percentage; $\ast$ = significant difference ($P < 0.05$) from PREHYD and POSTHYD values in the glycerol subjects.

Hematocrits of GT subjects increased significantly ($P < 0.05$) during the dive (Fig. 1D). Unfortunately, the more critical parameter, plasma volume, could not be calculated (42) because technical difficulties prohibited the determination of hemoglobin concentrations.
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At PREHYD the concentration of sGLY was 0.05 mM for all subjects (Fig 2A). The subjects in WT continued to demonstrate low sGLY values of 0.05 mM throughout the entire study. As expected, the mean sGLY values for GT increased 200-fold above base line at POSTHYD and were still approximately 100-fold above base-line sGLY values at POSTDIVE.

FIG. 2—Means ± SEM. A, serum glycerol; * = significant difference (P < 0.05) from PREHYD values; § = significant difference (P < 0.05) from values of the water trials at the corresponding sampling time. B, urine glycerol; * = significant difference (P < 0.05) from corresponding values in the water trial group. Numbers in parentheses are observations by each sampling time point.
Serum electrolytes (sodium and potassium) did not differ significantly between treatments and across all sampling periods.

### Urine data

Prehydration urine samples collected on arrival at the dive facility had a mean glycerol concentration of 0.05 mM. Glycerol concentrations in urine of sampling times 2–5 (Fig. 2B) were significantly greater ($P < 0.001$) in GT than in WT. For the GT group, the amount of glycerol excreted during the 1.5-h period before the dive represented only 4.1% of the total glycerol ingested, whereas an additional 10.2% was excreted during the 3-h dive.

Glycerol treatment subjects excreted significantly more ($P < 0.02$) sodium than WT control subjects during the POSTHYD period. All other urinary electrolyte data did not differ significantly between treatments or within treatments across all sampling periods. During the dive, both WT and GT groups lost equivalent amounts of urinary sodium (67 and 69 meq, respectively). During the entire 6-h experimental period, divers lost a total of 90–120 meq sodium and 23–40 meq potassium. The sodium and potassium lost during the experiment is well within the recognized normal daily losses for these two electrolytes; therefore, this loss does not represent a significant metabolic disturbance (43).

Urine output, total body weight loss, and non-urine weight loss during POSTHYD and dive sampling periods did not differ significantly between the WT and GT groups (Table 2).

#### Table 2: Water and Body Weight Changes During Glycerol and Water Treatments

<table>
<thead>
<tr>
<th></th>
<th>Fluid in, liters</th>
<th>Fluid Out, Liters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hydration Volume</td>
<td>PH</td>
</tr>
<tr>
<td>Water (WT)</td>
<td>1.98</td>
<td>0.78</td>
</tr>
<tr>
<td>± 0.19</td>
<td>± 0.28</td>
<td>± 0.59</td>
</tr>
<tr>
<td>Glycerol (GT)</td>
<td>2.06</td>
<td>1.03</td>
</tr>
<tr>
<td>± 0.18</td>
<td>± 0.68</td>
<td>± 0.44</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Fluid Intake, kg</th>
<th>Fluid Total, kg</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonurine Weight, kg</td>
<td>Total Weight,</td>
<td></td>
</tr>
<tr>
<td>Water (WT)</td>
<td>1.30</td>
<td>2.12</td>
<td>3.0</td>
</tr>
<tr>
<td>± 0.59</td>
<td>± 0.30</td>
<td>± 0.40</td>
<td>± 1.0</td>
</tr>
<tr>
<td>Glycerol (GT)</td>
<td>1.36</td>
<td>1.81</td>
<td>2.0</td>
</tr>
<tr>
<td>± 0.44</td>
<td>± 0.21</td>
<td>± 0.53</td>
<td>± 1.0</td>
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</tbody>
</table>

*Values are expressed as means of six observations ± SEM.*
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Diver’s thermal status

After 3 h of immersion in 13°C water, all subjects reported feeling cold. The subjects’ skin and rectal temperatures were not monitored during the 3-h dive period. Other studies conducted by this laboratory have documented rectal temperature decrements of no more than 1.0°C in divers wearing similar dry-suit ensembles immersed at similar water temperatures for 3 h.

DISCUSSION

Riedesel et al. (38) reported a total mean water retention of 400 ml of water in subjects at rest for 4 h in a thermoneutral terrestrial environment. These subjects ingested a glycerol bolus of 1.5 g/kg total body mass (TBM) followed by 1.5 liters of water. In the present study, our subjects ingested a glycerol solution containing 1.2 g/kg LBM. When this amount of glycerol is recalculated using TBM instead of LBM, the divers in GT ingested a glycerol dose of 1.0 g/kg TBM. This resulted in a non-significant mean water retention of 310 ml greater in GT than in WT, as indicated by the smaller total weight loss of 0.310 kg in GT (Table 2B). Given the 0.5 g/kg TBM difference in the glycerol dose used in the present study, the relative amount of water retained is quite similar in the two studies. Creatinine clearance was determined to be not significantly different between treatment groups and to be normal (WT = 104 ml/min; GT = 93 ml/min), indicating that all divers had normal renal function. Plasma and urine osmolality were also within normal limits and not significantly different between the two treatment groups.

Claybaugh et al. (3) found that physically trained subjects exhibit less diuresis and natriuresis than untrained subjects during head-out immersion. Their findings suggest that physical fitness may lessen the diuretic response to increased thoracic volume caused by whole-body or head-out immersion. SEALs are physically well-trained, which may have blunted the amount of diuresis that would otherwise have been experienced by less physically fit subjects. Additionally, Rochelle and Horvath (6) found that surfers who were chronically exposed to cold water exhibited a smaller plasma volume loss than control subjects during cold stress. On the other hand, cold acclimation was recently reported not to influence a subject’s diuresis or plasma volume loss (8). Considering these controversial findings, it would be important in follow-up studies to match the degree of physical conditioning and cold acclimation of the subjects to reduce individual variation and show a significant difference in water retention. It may also be necessary to alter the timing and concentration of the glycerol doses to achieve a significant effect during cold-water immersion.

Peak sGLY concentrations are reported to occur within 60–90 min after ingestion (44). In the present study, sGLY increased from 0.05 mM at PREHYD to a peak of 13.4 mM 90 min after ingesting the glycerol solution. At POSTDIVE, sGLY concentrations were still significantly elevated to 4.9 mM in GT. The sGLY concentration peaks and nadirs in this study agree quite closely with the findings of Gleeson et al. (45) and Miller et al. (46). Although sGLY concentrations were significantly elevated during the experiment in GT subjects, there was no significant difference in urine output across all subjects. While glycerol may easily cross over into the interstitial space (33,34), it does not seem to exert sufficient osmotic draw to sequester
significant volumes of fluid under the conditions of cold exposure and immersion pressures. The combined physiologic stressors of cold and immersion experienced by our divers may alter the water retention capability of glycerol reported by Lyons et al. (35–37) and Reidel and associates (38) in their terrestrial studies.

The liver is considered the organ responsible for 75% of glycerol clearance from the blood, with the kidneys removing the remaining glycerol (34). Inasmuch as only 14% of the ingested glycerol was excreted in the urine from POSTHYD through POSTDIVE, it is likely that much of the remaining glycerol was metabolically destined for the liver or peripheral tissues, such as skeletal muscle. Possible biochemical fates for glycerol are that it is a) sequestered into the interstitial space; b) taken up by the liver and used for sustaining blood glucose concentrations (e.g., for CNS functions); c) used by the liver as a gluconeogenic substrate for glucose-6-phosphate production and glycogen synthesis; or d) used for the production of triacylglycerides by the liver.

In summary, consuming a single glycerol solution (2 liters of water containing 1.2 g glycerol/kg LBM) seems ineffective in significantly decreasing body water loss under the combined stress of cold-water and whole-body immersion. It may be that these stressors, individually or in combination, overcome the ability of glycerol to sequester body water into the interstitial space. Even though glycerol has been reported to slow body water loss in terrestrial studies (35–38), it does not seem to have the same effect in cold water immersion. Suggestions for future research might entail an attempt to reduce variation in intersubject immersion diuresis by selecting subjects having similar physical training and cold acclimation backgrounds. Additional studies are also needed to determine the effect of glycerol dose concentration and timing of ingestion on body water dynamics.

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REFERENCES

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