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**Glycerol Hyperhydration Alters Cardiovascular and Renal Function**

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**ABSTRACT**

Six subjects randomly completed four experimental trials consisting of 2 hours of controlled fluid ingestion at rest, followed by 110 min of cycle ergometry exercise at  $44 \pm 11$  %  $\text{VO}_2$ max. Fluid was also ingested during exercise, and the trials differed in either the pre-exercise or during exercise fluid ingestion. The control trial consisted of the pre-exercise ingestion of 26 mL/kg of flavored water, and 5 mL/kg every 20 min of 5% glucose during exercise (WC). For the remaining trials the solutions ingested were 1.2 g/kg glycerol in a total of 26 mL/kg (GH) pre-exercise, and either 5% glucose (GC), 0.5% glycerol in 5% glucose (GCGA), or 1.5% glycerol in 5% glucose (GCGB) during exercise. Compared to WC, GH decreased urine flow ( $4.7 \pm 2.2$  vs  $7.6 \pm 3.7$  mL.min,  $P < 0.001$ ) and free water clearance ( $-1.4 \pm 1.3$  vs  $2.5 \pm 1.3$  mL/min,  $p < 0.001$ ). Consequently, pre-exercise hydration was largest with GH ( $0.9 \pm 0.4$  vs  $0.3 \pm 0.3$  L for the mean of GC, GCGA and GCGB vs WC). Compared to WC, GH also increased serum osmolality ( $283.3 \pm 3.3$  to  $281.0 \pm 2.6$  vs  $284.3 \pm 0.2$  to  $291.0 \pm 2.3$  mOsmol/kg,  $p < 0.01$ ) despite no difference in serum antidiuretic hormone (ADH) ( $2.3 \pm 2.0$  vs  $2.4 \pm 1.0$  pg/mL at 120 min). When comparing WC to GCGA and GCGB, continued glycerol ingestion during exercise increased cardiac stroke volume ( $163.3 \pm 27.9$  vs  $174.4 \pm 22.8$  mL,  $p < 0.01$ ), and decreased heart rate ( $128.2 \pm 19.0$  vs  $122.0 \pm 14.5$  b/min,  $p < 0.01$ ). GH increases body hydration by decreasing renal free water clearance via a non-ADH mediated mechanism. Continued glycerol and water ingestion during exercise increases the cardiovascular benefits of pre-exercise GH, and prolongs the state of hyperhydration.

**Key Words:** *Osmolality, Antidiuretic Hormone, Free Water Clearance, Stroke Volume*

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## **Introduction**

Compared to the ingestion of an equal volume of water, ingestion of glycerol and water providing approximately 1.0 g/kg body weight of glycerol in a total volume of 20 to 26 mL/kg body weight (glycerol hyperhydration, GH) can significantly decrease urine volume and cause a fluid retention of between 300 to 730 mL (1-4). GH has also been shown to improve evaporative cooling during exercise in a hot environment (2), and prolong time to fatigue during endurance exercise (3). The combination of the pre-exercise ingestion of glycerol, followed by added glycerol ingestion during exercise has not been studied. However, Koenigsberg et al. (5) showed that compared to the ingestion of water, continued glycerol and water ingestion can maintain an increased body hydration for up to 40 hours. The increase in body water from GH is known to occur without an increase in plasma ADH (4), and has been theorized to result from a glycerol mediated increase in water reabsorption in the distal tubules and collecting ducts of the kidney (4,6).

Since previous studies (7-10) of fluid infusion to expand the plasma volume found increases in stroke volume and lowered heart rates, GH may also provide benefits to cardiovascular function during exercise. For example, glycerol ingestion has been shown to lower heart rates during exercise in the heat (2), yet contradictory evidence exists for an expansion of plasma volume following GH (1,2,4,11).

Prior research has mainly compared pre-exercise glycerol hyperhydration to an equal volume of saline (1), orange juice (2) or water ingestion (3,4). The generalizability of using water as a control solution can be questioned due to the known superiority of carbohydrate-electrolyte solutions over water for sustaining hydration during exercise (12), and for improving rehydration from dehydration (13).

Based on our summary of prior research, it was our intent to compare pre-exercise GH to water ingestion. As most athletes ingest carbohydrate beverages during exercise, we also wanted to compare pre-exercise GH to GH followed by liquid carbohydrate ingestion during exercise. We hypothesized that the hyperhydration induced by GH would be larger than from water, and better sustained during exercise with additional glycerol ingestion. We also hypothesized the GH and continued glycerol ingestion would retain better hydration and cardiovascular benefits compared to GH and liquid carbohydrate ingestion during exercise. Based on the findings of Freund et al. (4), we further hypothesized that hyperhydration from GH would occur without increases in serum ADH, yet still be associated with a reduction in free water clearance.

## **Materials and Methods**

### ***Subjects and Approval***

Six subjects, four men and two women, volunteered to participate. The female subjects were eumenorrheic and were studied in the follicular phase of their menstrual cycle, as suggested by verbal interview and verified by a serum progesterone concentration <5 ng/mL. The study was approved by the Human Research Review Committees of each agency involved in this research.

### ***Procedures***

Subjects were first familiarized with the exercise equipment, and a preliminary cycle ergometry test to  $\text{VO}_2\text{max}$  was completed to determine relative submaximal workloads for the subsequent exercise trials. For all testing, exercise was performed on a semi-recumbent cycle ergometer, ambient conditions were maintained at 23.5 - 24.5 °C and 25 - 27% humidity, and the altitude was 1600m.

Subjects completed four trials in a randomized, double blind procedure. Trials were performed at

least one week apart, and at the same time of day. Subjects were admitted to a National Institutes of Health supported Clinical Research Center 48 hours prior to each trial. During this period, identical self-selected diets were given to each individual at each admission, and training activities were curtailed. After breakfast on the morning of each trial, subjects were transferred to the exercise testing facility. After arrival, a nude, post-void weight ( $\pm 0.1$  kg) (Pennsylvania 3000 Electronic Scale) for each subject was recorded and baseline urine specimens were obtained. An antecubital vein heparin lock (18 gauge) was placed, and subjects remained in a seated position for 10 min, after which venous blood specimens (7 mL) were obtained.

For the control hydration condition (WC), 26 mL/kg body weight of aspartame sweetened and artificially flavored water was ingested over a 2 hour period before exercise and followed by 5% CHO (5 mL/kg body weight every 20 min) during exercise (Table 1). For the GH trials, subjects ingested 1.2 gm/kg body weight of glycerol in 26 mL/kg of solution over a 2 hour period before exercise, and every 20 min during exercise ingested 5 mL/kg body weight of 5% CHO (GC), 5% CHO in 0.5% glycerol (GCGA), or 5% CHO in 1.5% glycerol (GCGB). The glucose solutions ingested during exercise also had 20 mEq/L of sodium chloride, and all solutions were kept at 4°C. Pre-exercise GH involved the ingestion of a bolus volume of concentrated glycerol (200 g/L), providing 1.0 g/kg body weight during the initial 30 min, as described in detail by Montner et al. (3). Thereafter, water was ingested every 30 min to 120 min, and at 60min the remaining glycerol (0.2 g/kg) was ingested. The total volume of water ingestion was the balance of 26 mL/kg body weight minus the volume of the glycerol solution.

During the 2 hour hydration period, subjects maintained a sitting position. All urine during this period was collected, the volume measured, and samples from baseline and 120 min were used for

analyses of creatinine and osmolality. Following the 2-hour pre-exercise hydration period, the subjects performed 110 min of exercise in a semi-recumbent position on a cycle ergometer (Model 846T, Quinton Instrument Co., Seattle WA) at  $44 \pm 11\%$   $\dot{V}O_{2max}$ . This workload and position was selected to ensure that subjects could complete the exercise duration, and allow for more easily determined and accurate stroke volume measurements using Doppler flowmetry. Venous specimens for blood elastic yield stress (EYS) were taken every 40 min, and at 60 and 120 min of rest for serum osmolality and ADH. Serum glycerol was determined at baseline, at 120 min of rest and after 110 min of exercise. Nude body weight was also measured at the end of hydration (120 min), and after 110 min of exercise each subject towel dried and was again weighed nude.

**Table 1: Constituents of the Different Drinks**

<i>Drink</i>	<i>Glycerol (g/kg)</i>	<i>Glucose (g/100 mL)</i>	<i>Electrolytes (mEq/L- NaCl)</i>
<i>Glycerol Bolus</i>	1.2	0	0
<i>Glucose Solutions During Exercise</i>			
<i>GC</i>	0	5.0	20.0
<i>GCGA</i>	0.5	5.0	20.0
<i>GCGB</i>	1.5	5.0	20.0

### *Analytical Procedures and Calculations*

#### Indirect Calorimetry

The exercise protocol used to determine  $\dot{V}O_{2max}$  consisted of 1 min duration increments at between 20 to 30 Watts/min to ensure a total test duration between 10 to 15 min. Expired gas analysis was completed using a time averaged automated system (Incarepulmobil, Erich Jaeger, Rockford, IL) involving volume measurement with a heated pneumotach. Expired fractions of oxygen and carbon dioxide were determined using oxygen (zirconian cell) and carbon dioxide (infra-red) analyzers. Prior to each test the pneumotach was calibrated with seven volumes of a 1 L<sub>ATPS</sub> calibrated syringe, and analyzers were calibrated to room air and medically certified gasses (100% nitrogen; and

15% O<sub>2</sub>, 5% CO<sub>2</sub>, balance nitrogen).

### Central Cardiovascular Hemodynamics

Stroke volume was determined by pulsed Doppler (PD) flowmetry (Medsonics, Fremont, CA) utilizing a suprasternal approach (14-17). This standard noninvasive method has been described in detail and validated by us in previous studies (14,15) and by others (16,17). In our hands, the 95% confidence levels for precision (percent changes) and accuracy (absolute values) are  $\pm 7\%$  and  $\pm 13\%$  respectively. The measurements were taken with a 2.0 MHz transducer at time 0, 45, and 120 min of the pre-exercise period, and at the following intervals of the exercise: 10, 30, 50, 70, 90 and 110 min. Heart rate was determined from PD waveform intervals. The PD data were subsequently processed by blinded observers to yield data of Doppler angle, aortic diameter and mean blood flow velocity. Pulse volume was calculated using the Doppler equation.

### Blood and Urine Assays

Blood and urine samples were assayed in duplicate for creatinine (calorimetric method, IL Monarch 2000, Lexington, MA, or reflectometric method, Kodak Ektachem 700XR, Rochester, NY), and osmolality (freeze point depression, Nichols Inst., CA). Serum ADH was assayed by radioimmunoassay (Nichols Inst., CA) with a test-retest coefficient of variation  $<5\%$ . Glycerol was assayed by enzymatic spectrophotometry (Boehringer Diagnostics, CA), and EYS was quantified as a marker of blood viscosity (Vilastic 3, Vilastic Scientific, Inc).

### Renal Function

Renal free water clearance was calculated as urine flow - ((urine osmolality x flow) / serum osmolality). Creatinine clearance was calculated as ((urine creatinine x flow) / serum creatinine).

### Statistics

Due to concerns over statistical power, descriptive

statistics were first completed on all variables to determine mean differences, variance and effect sizes. For the variable of stroke volume, determined to be the most difficult to detect significance for during exercise with 6 subjects per group, the mean difference between WC and GCGB at 50 min was 15.2 mL ( $162.8 \pm 26.5$  vs  $178.0 \pm 16.2$  mL), with an effect size of 0.71 and a  $\beta$  error = 0.85 from a one-tailed t-test (Statmate, Graphpad Software, San Diego CA). To improve statistical power, we decided to conduct statistical analyses from specific planned comparisons after collapsing specific trials to compare water vs GH, and glycerol ingestion during exercise to CHO ingestion (Statistica, Statsoft Inc., Tulsa OK).

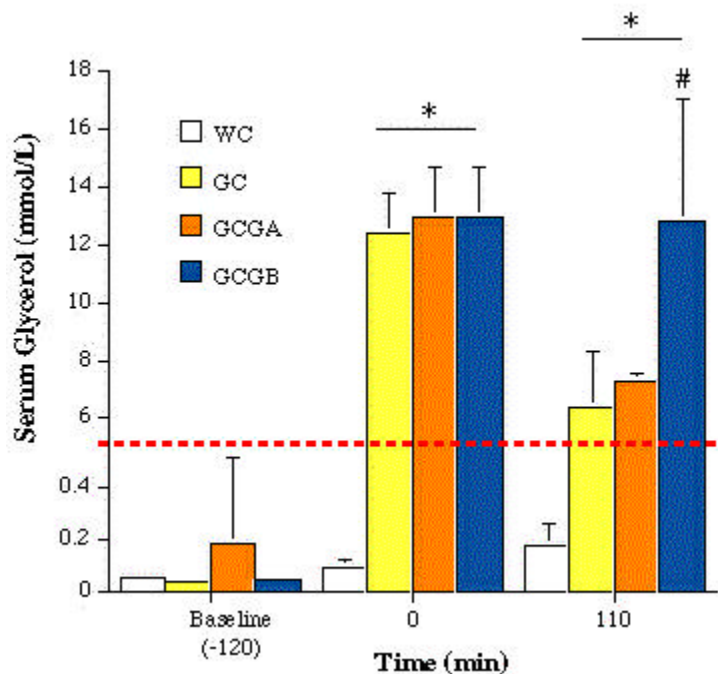
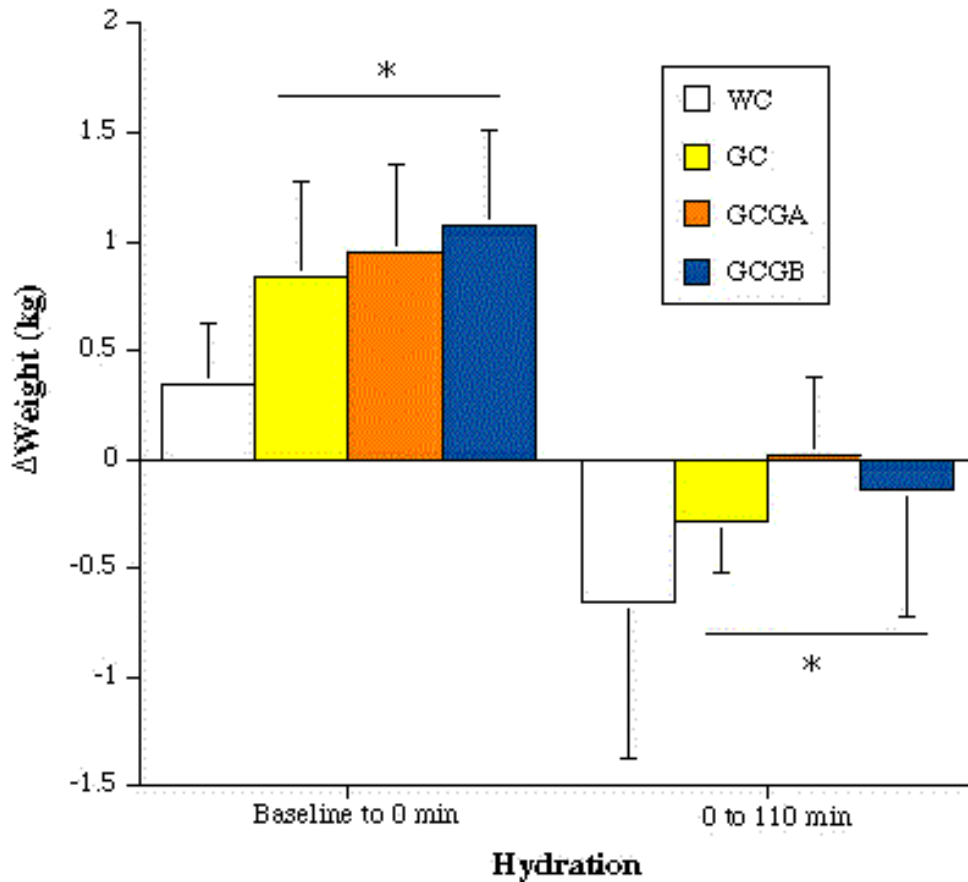


Figure 1: The change in serum glycerol over time and during each trial. \* =  $p < 0.01$  from WC and baseline, # =  $p < 0.01$  from GC and GCGA.

Due to repeat analyses of means and apriori protection against type II errors, a Bonferoni adjustment was used to protect against type I errors, resulting in statistical acceptance at  $p = 0.017$  ( $0.05/3$ ). This approach enabled us to improve

statistical power, as indicated by the comparison of stroke volume data of WC (N=36) compared to the combined data from GCGA and GCGB (N=60) throughout exercise (mean difference = 11.4 mL, effect size = 0.4,  $\beta$  error = 0.3). Data are presented as mean  $\pm$  SD.

analyses, group mean data for pre-exercise GH represent the combined data from GC, GCGA and GCGB trials. Pre-exercise water ingestion did not change serum osmolality ( $283.3 \pm 3.3$  to  $281.0 \pm 2.6$  mOsmol/kg). However, pre-exercise GH significantly increased serum osmolality at 120 min compared to baseline ( $284.3 \pm 0.2$  to  $291.0 \pm 2.3$  mOsmol/kg, for WC vs GH trials,  $p < 0.01$ ), but did



**Figure 2: The change in body weight from baseline to 120 min pre-exercise ( $\Delta$ weight), and from baseline to 110 of exercise during each trial. \* =  $p < 0.01$  for WC vs GC, GCGA and GCGB.**

## Results

### Subjects

Subjects trained regularly with cycling or running and had a mean age, body mass, and maximal oxygen uptake ( $VO_{2max}$ ) of  $27 \pm 3$  yr.,  $68 \pm 6$  kg, and  $55.0 \pm 2.8$  mL  $kg^{-1}$   $min^{-1}$  respectively.

### Pre-exercise Hydration

Due to the need to perform apriori statistical

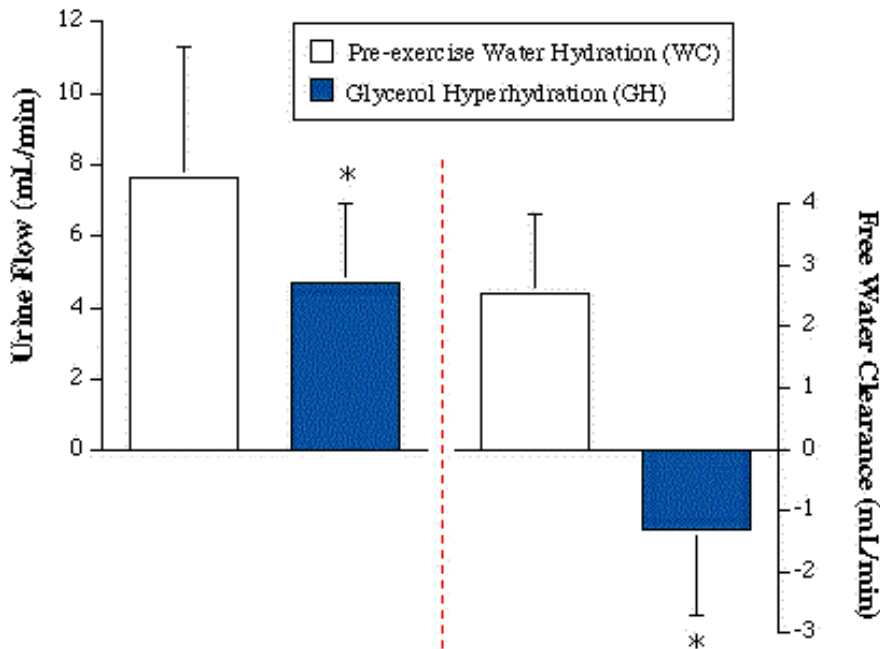
not increase serum ADH ( $2.3 \pm 2.0$  vs  $2.4 \pm 1.0$  pg/mL at 120 min for WC vs GH

trials). Pre-exercise GH significantly increased serum glycerol at 120 min compared to baseline, and compared to WC (Figure 1). Body weight gain at 120 min was significantly larger with GH (Figure 2), resulting in an additional 600 mL of water storage ( $p = 0.0015$ ). These body weight changes coincided with significant reductions in urine flow and free water clearance for WC compared to GH (Figure 3), but no change in creatinine clearance ( $133 \pm 35$  vs  $110 \pm 45$  mL/min for WC and GH trials). EYS results

did not differ between trials, and the mean values during pre-exercise hydration were  $0.12 \pm 0.02$  dyne/cm<sup>2</sup> for both WC and GH.

### Exercise Hydration

During the GH trials, blood glycerol remained elevated above WC during exercise (Figure 1). Continued ingestion of glycerol in the GCGA and GCGB trials did not cause a detectable increase in serum glycerol until after 110 min of exercise, when serum glycerol in the GCGB trial was significantly larger compared to all other trials (Figure 1).



**Figure 3: The decreases in pre-exercise urine flow and free water clearance, averaged over 2 hours, for the WC and GH (GC, GCGA, GCGB) trials. \* =  $p < 0.01$  for WC vs GH.**

Pre-exercise GH and the continued ingestion of glycerol and liquid carbohydrate during exercise (GC, GCGA, GCGB) significantly ( $p < 0.01$ ) improved body weight maintenance (retained hydration at 110 min of exercise compared to baseline) compared to WC (Figure 2). Mean SV

during exercise was significantly larger for the trials involving added glycerol ingestion (GCGA and GCGB) compared to WC and GC, and mean HR was significantly lower in GCGA and GCGB than WC and GC (Figure 4a&b).

### Discussion

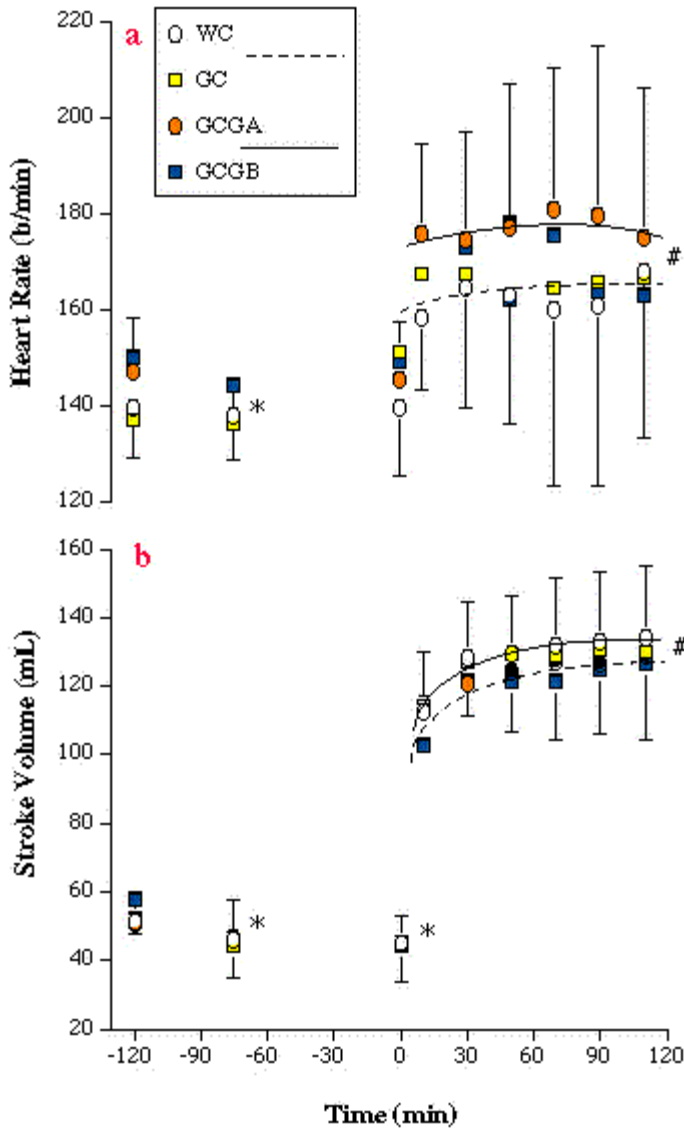
As in the previous studies of Riedesel et al. (1), Lyons et al. (2), Montner et al. (3) and Freund et al. (4), we found that GH, compared with water ingestion, resulted in an increased fluid retention. Furthermore, as with the study of Freund et al. (4) we documented that GH increased osmolality,

reduced free water clearance, but did not increase plasma ADH. Thus, glycerol appears to be an ineffective osmole for ADH, like urea and glucose (16). Murray et al. (11) found that ADH increased more from baseline with glycerol ingestion during exercise (3.2 to 9 pg/mL) compared to water ingestion alone (3.2 to 6 pg/mL). However, Murray et al. (11) did not follow a pre-exercise GH procedure, thereby preventing a glycerol hyperhydration which others have shown requires an approximate 2 hour pre-exercise hydration regimen (1-4). It remains unclear why the protocol of Murray et al. (11) caused an increase in

blood ADH.

We also reported that pre-exercise GH and glycerol ingestion during exercise better preserved body hydration during exercise compared to water and liquid carbohydrate ingestion (Figure 1). This finding supports the research of Lyons et al. (2), who documented that GH provided more fluid for sweat output during exercise in dry heat compared to water/orange juice, without further compromising body water stores. It appears that GH enables the

body to tolerate water loss better by simply providing more fluid rather than by decreasing fluid loss during exercise/heat stress.



The causal mechanism of the body water gain from GH has not been clearly established. However, the mechanism for the hyperhydration afforded by GH has been theorized to result from the increased glycerol reabsorption in the kidney which also induces an increased water reabsorption (4,6,18). Due to the high diffusive properties of glycerol, the

**Figure 4: The changes in a) heart rate (HR) and b) stroke volume (SV) during the pre-exercise and exercise hydration periods of each trial. For HR, \* =  $p < 0.01$  for all trials from baseline (-120 min). # =  $p < 0.01$  for all of exercise for WC and GC vs GCGA and GCGB. For SV, \* =  $p < 0.01$  for all trials from baseline (-120 min). # =  $p < 0.01$  for all of exercise for WC and GC vs GCGA and GCGB.**

sustained elevation in blood glycerol causes glycerol and water to be evenly distributed throughout the body water spaces external to the brain and eyes (18). It is therefore logical to assume that the hydration benefits of GH are an expansion of the intra-cellular, interstitial, and vascular body water compartments (18). Riedesel et al. (1) hypothesized that as glycerol was metabolized or excreted, the increased volume of body water would become available for metabolic use. This remains a viable explanation for the eventual benefits of GH to cardiovascular and thermoregulatory capacities during exercise.

Although we did not quantify plasma volume in this study, an improved retention of plasma volume during exercise (less hemoconcentration) is a possible explanation for our documented increases in cardiac stroke volume. Based on this interpretation, we feel it is important to identify some difficulties in detecting a plasma volume expansion following GH. Despite the theoretical rationale for GH to increase all body water compartments (18), there are contradictory findings of a plasma volume expansion following glycerol ingestion. Gleeson et al. (19) found that 400 mL of a 1 g/kg glycerol solution increased PV by 10% during pre-exercise hydration while placebo and glucose feeding did not. PV changes were calculated from changes in Hb and Hct as described by Dill and Costill (20). With ingestion of similar amounts of glycerol and water during exercise, Murray et al. (11) also found that glycerol maintained PV better than a water placebo or glucose solution. Conversely, Freund et al. (4) reported no differences in blood and plasma volumes following glycerol vs water ingestion, despite the

500mL increase in total body water with glycerol ingestion.

The difficulty in detecting an increase in plasma volume after GH is not surprising, as the largest increase in fluid retention seen with GH has been 730 mL over a 2 hour period (3). This time frame would allow the glycerol and water to more evenly diffuse throughout the total body water. As plasma volume is the smallest of the body water compartments, a 730 mL increase in total body water evenly distributed among body water compartments would cause an approximate 45 mL increase in plasma volume, which would be difficult to detect given the errors of dye dilution, and the relatively small numbers of subjects used in past research (1-4).

Interestingly, the significant findings for an expansion of plasma volume by Gleeson et al. (19) and Murray et al. (11) may be related to the decreased time provided to subjects after glycerol ingestion (45 min and 0 min, respectively). This shorter time frame may have prevented an equilibration of the glycerol and water load throughout the total body water volume, resulting in a greater plasma volume expansion.

No previous published studies have directly measured SV after GH. In the present study, SV was increased with continued ingestion of glycerol during exercise compared to liquid CHO ingestion after water or pre-exercise GH (Figure 4b). These results coincided with reciprocal changes in heart rate (Figure 4a). These functional measurements may be physiologically more significant than the difficulty in documenting a change in plasma volume after GH, as previously explained. The cardiovascular benefit of pre-exercise GH may not be in the absolute increase in plasma volume, but the potential for using added extravascular water stores to better maintain plasma volume during exercise/heat stress.

In a 1982 study of plasma volume expansion by Kanstrup and Ekblom (10), HR was an average of 5 bpm lower than control at submaximal exercise intensities. Furthermore, Fortney et al. (7) infused 533 mL of isotonic albumin and lactated Ringers solution before exercise, and found that HR during exercise was 3-6 bpm lower than control and SV was an average of 13 mL higher than control. Hopper et al. (9) found that during submaximal cycle ergometry, infusion of 403 mL of dextran solution increased SV by 11% (14ml) and CO by 7% (1.32 L/min), and decreased HR from 141 bpm to 138 bpm. The HR and SV findings of our study are in the range found in these studies involving artificial PV expansion.

Another important finding of this study was the added benefit of glycerol ingestion during exercise to pre-exercise GH. Surprisingly, pre-exercise GH did not reveal results during exercise that were significantly different from water ingestion followed by liquid carbohydrate (Figures 2 and 3). These findings differ to the results of Montner et al. (3) and Lyons et al. (2). However, the exercise intensity of Montner et al. (3) was larger (74%  $\text{VO}_2\text{max}$  to exhaustion) than the present study, and the subjects of Lyons et al. (2) were required to exercise in a hot environment (90 min at 60%  $\text{VO}_2\text{max}$  in 42°C). Perhaps the maximal potential for benefits from pre-exercise GH are observed during more severe exercise and/or heat stress.

The benefit of continued glycerol and water ingestion during exercise is supported from data of glycerol removal after pre-exercise GH. Robergs and Griffin (18) have calculated that the rate of glycerol removal from the body after GH approximates 30-50 g/hr. Thus, two hours after GH more than 50% of the glycerol ingested has been removed from the body via urinary excretion and metabolism. Based upon the direct action of glycerol on water reabsorption in the distal tubule,

the continued retention of added body water would require a sustained elevation in blood glycerol. This result was clearly seen in the blood glycerol data of the GCGB trial after 110 min of exercise (Figure 1).

### **Conclusions**

Pre-exercise GH resulted in fluid retention by reducing renal free water clearance, but through a non-ADH mediated mechanism. Compared to pre-exercise water or GH followed by liquid carbohydrate ingestion, pre-exercise GH followed by continued glycerol and water ingestion during exercise increases stroke volume and lowers heart rate during exercise. These additional cardiovascular benefits of GH during exercise occurred with an additional 5 g glycerol/hr in 350 mL/hr. It remains unclear whether larger amounts of glycerol ingestion during exercise would further sustain improved hydration, and whether these responses during recumbent low to moderate intensity cycling could relate to improved exercise performance at higher relative exercise intensities.

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