The effects of glycerol on changes in body fluid compartments in males at rest

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The Effects of Glycerol on Changes in Body Fluid Compartments in Males at Rest

by

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Date
Numerous studies have confirmed the hyperhydrating effect of glycerol ingestion combined with added fluid intake during resting conditions. Extensive monitoring of fluid changes in the various fluid compartments of the body during glycerol hyperhydration has yet to be carried out. In most studies, changes in total body water (TBW) are simply determined by changes in nude body weight or by differences between fluid intake and urine output, while changes in intracellular water (ICW) and extracellular water (ECW) remain inconclusive. In this investigation we utilized multi-frequency bioelectrical impedance analysis (BIA)(Xitron Hydra ECF-ICF Model 4200) to examine glycerol-induced changes in TBW, ECW, and ICW compartments in resting subjects over a 4-hr time period. Two trials were completed, one with glycerol and one with a placebo. The hydration regimen consisted of a glycerol beverage (1.2 g/kg nude body weight (NBW) in a 20% solution of water) or a placebo followed by a bolus of distilled water (total volume = 26 ml/kg NBW) consumed over fifty minutes. The glycerol trial exhibited a significantly greater NBW and fluid retention at 160 and 240 minutes thus verifying its hyperhydrating effect. Fluid retention based on BIA failed to demonstrate hyperhydration with glycerol. BIA measures underestimated the quantity of fluid retained. Despite this, BIA measures did indicate significantly greater ICW stores at 160 and 240 minutes in the glycerol trial. In both trials ECW stores exhibited a significant decrease at 80, 160, and 240 minutes relative to o minutes. The results of this study suggest that the hyperhydrating effect of glycerol may not be accurately depicted by BIA over an acute period of time. Changes in body hydration and altered compartmental volume ratios may be responsible for this effect.
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Lastly, this work is dedicated to all those who have chosen to free themselves from society’s programming and remain true to their own spirit.
# Table of Contents

**Chapter 1: Introduction**  
Introduction 1  
Problem 4  
Subproblem 4  
Research Hypotheses 4  
Significance of Study 9  
Rationale for Study 9  
Limitations 10  
Delimitations 10  
Definition of Terms 11

**Chapter 2: Review of Related Literature**  
13

**Chapter 3: Methodology**  
Descriptive Data 27  
Experimental Procedure 28  
Figure 3.1 30  
Research Design and Statistical Procedures 34  
Table 3.1 35

**Chapter 4: Results**  
Descriptive Data 36  
Table 4.1 36  
Trial Measurements 37  
Figure 4.1 41  
Figure 4.2 41  
Figure 4.3 42  
Figure 4.4 42  
Figure 4.5 43  
Figure 4.6 43  
Figure 4.7 44  
Figure 4.8 44  
Figure 4.9 45  
Figure 4.10 45  
Figure 4.11 46

**Chapter 5: Discussion and Conclusions**  
Trial Measurements 47  
Conclusions 58  
Topics for Further Research 59

**References** 60

**Appendix I: Attachments**  
Institutional Review Board, Informed Consent, Health History Questionnaire, and Data Sheet 65

**Appendix II: Manuscript** 73

**Appendix III: Statistical Results** 118
Chapter One: Introduction

Introduction

Hydration status of the body is a key factor regulating work performance and thermoregulation during exercise in a hot environment (Sawka et al., 1985). If fluid loss, through sweat and respiratory water loss, exceeds fluid intake then the body becomes hypohydrated. Hypohydration leads to a number of detrimental effects: decreased cardiovascular performance (Fortney et al., 1981), increased heart rate and core temperature (Nadel et al., 1980), decreased cutaneous blood flow and sweat rates (Nadel et al., 1980 and Sawka et al., 1983), and decreased work performance (Sawka, 1992). The physiological mechanisms which may be responsible for these effects include plasma hypertonicity, hypovolemia, and intracellular electrolyte imbalances (Sawka, 1992 and Fortney et al., 1984).

Attenuation of the decline in plasma volume normally exhibited during prolonged exercise in the heat helps maintain central blood volume thus allowing for maintenance of cardiac output without an increase in heart rate. Also, maintenance of plasma volume fosters an increased cutaneous blood flow, which allows for a reduced core temperature (Fortney et al., 1988). The two most common methods used to facilitate such an effect on plasma volume are the infusion of albumin with saline and hyperhydration through fluid ingestion. The former method has limited applicability in the athletic setting for two reasons. The first and most obvious reason is that infusions are impossible to perform during most, if not all, athletic events. The second reason is that this technique may be viewed as a controversial performance enhancing aid and would be banned by the sport's
viewed as a controversial performance enhancing aid and would be banned by the sport's governing body. The latter method has met with a limited degree of success depending upon the timing and contents of the fluid ingested. Thermoregulation was enhanced in young soccer players who underwent a weeklong water hyperhydration period (Rico-Sanz et al., 1996). Despite an increase in urine output these players did exhibit a significant increase in total body water (TBW). Drinking large amounts of fluid immediately prior to exercise in a hot environment may also result in an initial increase in TBW and plasma volume, but this is rapidly counteracted by the renal, cardiovascular, endocrine, gastro-intestinal, and central nervous systems. These systems interact to maintain a physiological state of euhydration, thus any increases in TBW through fluid ingestion are only transient.

Researchers have overcome this homeostatic response by having subjects ingest glycerol along with large amounts of fluid. Glycerol is used as a hyperhydrating agent because of its rapid absorption, its even distribution among body fluid compartments, its presence as a natural metabolite, its osmotic action, and its safety when presented in oral doses of 1 g/kg body weight every 6 hours (Lyons et al., 1990). A number of studies have confirmed the hyperhydrating effect of glycerol ingestion along with added fluid intake during resting conditions (Montner et al., 1996; Freund et al., 1995; Lyons et al., 1990; Riedesel et al., 1987). Lyons et al. (1990) demonstrated that hyperhydration with glycerol prior to exercise leads to a decreased core temperature and an increased sweating rate during moderate exercise in the heat. In another study, glycerol-enhanced hyperhydration prolonged cycling endurance time and facilitated a lower submaximal
heart rate, but did not lower core temperature or increase sweat rate (Montner et al., 1996). Other studies involving the ingestion of glycerol have failed to demonstrate any thermoregulatory or cardiovascular advantages over the ingestion of water alone (Murray et al., 1991 and Meyer et al., 1995). The inability to produce hyperhydration in these studies can be attributed to differences in the total amount of fluid ingested, timing of glycerol ingestion, and exercise protocol employed.

Extensive monitoring of fluid changes in the various fluid compartments of the body during glycerol hyperhydration has yet to be carried out. In most studies, changes in TBW are simply determined by changes in nude body weight or by differences between fluid intake and urine output, while changes in intracellular water (ICW) and extracellular water (ECW) are left open to speculation (Inder et al., 1998; Montner et al., 1996; Koenigsberg et al., 1995; Freund et al., 1995; Lyons et al., 1990; Riedesel et al., 1987). Siefert et al. (1995) in a published abstract did, in fact, monitor changes in fluid compartment volumes after glycerol ingestion, but unfortunately the method by which this was performed was not reported. Lyons and Riedesel (1993) examined shifts in fluid compartment volumes in response to glycerol ingestion in rats. TBW was determined by injecting the rats with a radioisotope of water. ECW was determined by injecting the rats with a radioisotope of inulin with tricarbocyanine dye. Intracellular water and interstitial water were determined mathematically. Although they were able to establish values for each fluid compartment, they only did so at one time point other than baseline. No study to date has used multi-frequency bioelectrical impedance analysis (BIA) to monitor
changes in fluid compartment volumes during an extended period of glycerol hyperhydration.

Problem

The purpose of this investigation was to utilize multi-frequency bioelectrical impedance analysis (BIA) to examine changes in total body water (TBW), extracellular (ECW) and intracellular (ICW) water compartments in response to glycerol hyperhydration.

Subproblem

A secondary purpose of this study was to determine if the Xitron-Hydra ECS-ICS (Model 4200, San Diego, CA) BIA system is sensitive enough to detect small changes in TBW, ECW, and ICW as a result of glycerol hyperhydration.

Research Hypotheses

Hypothesis One

There will be differences in changes in nude body weight such that the glycerol trial will show greater gains in body weight when compared to the placebo trial.

Justification

In this study any increase in body weight will be due to fluid retention. The osmotic action of glycerol increases the body’s fluid reservoir. A study by Murray at al. (1991) showed an increased ADH release in response to elevated blood glycerol levels. ADH (anti-diuretic hormone) slows down the formation of urine. Freund et al. (1995) demonstrated a similar trend in ADH concentrations, but also proposed that glycerol...
exerts its effect by lowering the rates of free water clearance in the proximal and distal tubules of the kidney. Without these mechanisms acting in the placebo trial, the fluid that is consumed will be rapidly excreted in the form of urine; therefore, this trial will exhibit lesser gains in body weight.

**Hypothesis Two**

There will be differences in the volume of fluid retained such that the glycerol trial will retain a greater volume of fluid when compared to the placebo trial.

**Justification**

Fluid retention will be calculated by subtracting the volume of urine output from the volume of fluid ingested. Urine production is lowered after glycerol ingestion (Robergs and Griffin, 1998; Montner et al., 1996; Koenigsberg et al., 1995; Lyons et al., 1990; Riedesel et al., 1987); therefore, the volume of fluid retained will be greater in the glycerol trial when compared to the placebo trial.

**Hypothesis Three**

There will be a difference in the volume of urine output such that the placebo trial will produce a greater volume when compared to the glycerol trial.

**Justification**

Urine formation is reduced after glycerol ingestion due to its potential for increasing ADH levels (Murray et al., 1991) and reducing free water clearance (Robergs and Griffin, 1998 and Freund et al., 1995). A reduced urine production will lead to a reduced volume
of urine voided; therefore, the placebo trial will exhibit a greater volume of urine output when compared to the glycerol trial.

**Hypothesis Four**

There will be differences in total body water (TBW) such that the glycerol trial will exhibit greater increases when compared to the placebo trial.

**Justification**

In this study any increase in body weight will be interpreted as an increase in TBW. The osmotic action of glycerol leads to decreased urine formation and subsequent decreased fluid loss (Robergs and Griffin, 1998; Lyons et al., 1990; Montner et al., 1996; Koenigsberg et al., 1995; Riedesel et al., 1987). The homeostatic responses to maintain euhydration in the placebo trial will lead to greater fluid losses, which will promote lower increases in TBW compared to the glycerol trial.

**Hypothesis Five**

There will be differences in extracellular water (ECW) volumes such that they will be higher in the glycerol trial when compared to the placebo trial.

**Justification**

Upon absorption glycerol is evenly distributed throughout the body's fluid compartments (Lin, 1977). As glycerol enters these compartments water will follow due to the osmotic gradient created by glycerol, thereby facilitating a higher ECW volume in the glycerol when compared to the placebo trial (Seifert et al., 1995).
Hypothesis Six

There will be differences in intracellular water (ICW) volumes such that they will be higher in the glycerol trial when compared to the placebo trial.

Justification

Upon absorption glycerol is evenly distributed throughout the body’s fluid compartments (Lin, 1977). As glycerol enters these compartments water will follow due to the osmotic gradient created by glycerol, thereby facilitating a higher ICW volume in the glycerol when compared to the placebo trial (Lyons and Riedesel, 1993).

Hypothesis Seven

There will be no differences in plasma volume changes between the glycerol trial and the placebo trial.

Justification

Although the study by Gleeson et al. (1986) does indicate an increase in plasma volume in response to glycerol hyperhydration, the majority of the existing research does not support this finding (Montner et al., 1996; Koenigsberg et al., 1995; Freund et al., 1995; Seifert et al., 1995; Lyons et al., 1993; Lyons et al., 1990; Riedesel et al., 1987).

Hypothesis Eight

There will be differences in plasma glycerol concentrations such that the values obtained during the glycerol trial will be higher than those obtained during the placebo trial.
**Justification**

Glycerol ingestion at doses between 1.0 and 1.5 g/kg bodyweight can raise blood glycerol concentrations from .05 mmol/L to between 15 and 20 mmol/L (Robergs and Griffin, 1998). During the glycerol trial the subjects will be ingesting glycerol; therefore, it is expected that the plasma glycerol concentrations will be higher during this trial when compared to the placebo trial.

**Hypothesis Nine**

There will be differences in plasma osmolality such that values obtained during the glycerol trial will be higher when compared to the placebo trial.

**Justification**

Ingestion of glycerol leads to increases in plasma glycerol concentrations, which are accompanied by increases in plasma osmolality (Robergs and Griffin, 1998). In a study by Riedesel et al. (1987) 85-95% of the increase in blood osmolality was due to the rise in plasma glycerol concentration. During the glycerol trial the subjects will be ingesting glycerol; therefore, it is expected that during this trial plasma osmolality values will be higher when compared to the placebo trial.

**Hypothesis Ten**

There will be differences in urine osmolality such that values obtained during the glycerol trial will be higher than those obtained during the placebo trial.
**Justification**

Decreased free water clearance may lead to more highly concentrated urine when comparing the glycerol trial to the placebo trial. Also, urine osmolality may increase as urine glycerol concentration increases due to decreased reabsorption in the kidney. Glycerol is excreted in the urine when its concentration in the plasma exceeds 1.6 mmol/L (Robergs and Griffin, 1998).

**SIGNIFICANCE OF THE STUDY**

The use of glycerol as a means to induce hyperhydration and benefit exercise performance in the heat has been investigated since 1987 (Riedesel et al., 1987). The exact physiological mechanisms behind glycerol's effectiveness have yet to be delineated. The results of this study may help researchers standardize the procedures involved in the study of the effects of glycerol hyperhydration on exercise performance.

Cellular hydration state has been proven to be a potent determinant of cellular protein turnover in health and disease (Haussinger et al., 1993). An increase in hydration stimulates anabolism, whereas a decrease in hydration stimulates catabolism. The results of this study may have implications leading to the use of glycerol as an anti-catabolic agent in post-surgical and chronically ill patients.

**RATIONALE FOR THE STUDY**

Only one study examining alterations in fluid compartment volumes during glycerol hyperhydration in man has been performed, but, unfortunately, the instrumentation and
methodology implemented were not reported in the abstract (Siefert et al., 1995). Another study, involving glycerol hyperhydration in rats, utilized radioisotopes of water and inulin and dye dilution techniques to discern shifts in fluid compartment volumes (Lyons and Riedesel., 1993). These techniques may have limited applicability in human research due to financial and ethical reasons. Bioelectrical impedance analysis is a simple, relatively inexpensive, portable and easy way to measure body water. Its use as a valid measurement tool has been verified by numerous studies (Vache et al., 1998; Segal et al., 1991; and van Marken Lichtenbelt et al., 1994). Multi-frequency BIA monitoring of fluid shifts during glycerol hyperhydration is necessary for a more complete understanding of the physiological mechanisms underlying glycerol’s ability to provide thermoregulatory and cardiovascular benefits during exercise in the heat.

LIMITATIONS

i. Non-randomized sample. The sample for the study was not randomly selected; however, the subjects served as their own controls by using a repeated measures design.

ii. Instrumentation. There is inherent error associated with all instrumentation. Error was minimized by using trained testers and calibrated instruments.

DELIMITATIONS

i. Type of subjects. To eliminate possible confounding by hormonal differences which may influence fluid retention only males participated in this study.
ii. Blood sampling. In order to minimize trauma to the subjects, blood samples were taken only four times (every 80 minutes) over the 4-hr time period. More frequent blood sampling may have provided valuable information regarding changes in plasma volume, plasma glycerol, and plasma osmolality during glycerol hyperhydration.

iii. Glycerol and water dosage. To maximize hyperhydration, the glycerol and water dosages were based on recommendations by Robergs and Griffin (1998). The glycerol dosage was 1.2 g/kg body weight in a 20% solution. The total volume of water ingested was 26 ml/kg NBW.

DEFINITION OF TERMS

**Hypohydration:** A condition of decreased body water content (Greenleaf, 1992).

**Hypertonicity:** A condition of increased plasma osmolality (Greenleaf, 1992).

**Hypovolemia:** A condition of decreased blood volume (Sawka, 1992).

**Plasma volume:** The volume of blood comprised of the liquid, non-cellular, components. Plasma constitutes ~55% - 60% of total blood volume. Plasma is 90% water.

**Hyperhydration:** A condition of increased body water content (Greenleaf, 1992).
**TBW**: Total body water is the sum of the volume of water in the body’s intracellular (ICW) and extracellular (ECW) fluid compartments.

**ICW**: Intracellular water is the volume of water found within the body’s cells. It constitutes ~ 66% of TBW (Wilmore and Costill, 1994).

**ECW**: Extracellular water is the volume of water found outside the body’s cells. It is found in the form of plasma, interstitial fluid, lymph fluid, and other body fluids. It constitutes ~ 33% of TBW (Wilmore and Costill, 1994).

**Euhydration**: The normal state of hydration or daily body water content (Greenleaf, 1992).

**Osmolality**: The ratio of solutes (such as Na⁺, K⁺, CL⁻, MG⁺ and glycerol) to fluid. Generally expressed in mOsm / L units.

**ADH**: Anti-diuretic hormone. A hormone, released by the posterior pituitary in response to an increase in plasma osmolality, which increases the permeability of the kidney’s collecting ducts thereby reducing the amount of water excreted in the urine (Wilmore and Costill, 1994).

**BIA**: Bioelectrical impedance analysis is a method of determining body water content and body composition based on the electrical-conductive properties of the body’s tissues.
Chapter Two: Review of Related Literature

Physiological Consequences of Hypohydration

During exercise-heat exposure, a large amount of body water may be lost through sweat output. If a sufficient volume of water is not ingested at a rate equal to the rate of sweat loss then a state of decreased body water, or hypohydration, supervenes. Hypohydration leads to plasma hypertonicity, hypovolemia, and intracellular electrolyte imbalances (Sawka, 1992 and Fortney et al., 1984). All of these mechanisms may contribute to the following adverse effects which hypohydration imposes upon exercise performance: decreased cardiovascular performance (Fortney et al., 1981), increased heart rate and core temperature (Nadel et al., 1980), decreased cutaneous blood flow and sweat rates (Nadel et al., 1980 and Sawka et al., 1983), and decreased work performance (Sawka, 1992).

With the understanding that attenuation of the hypohydration-induced decline in plasma volume facilitates maintenance of cardiac output and increased cutaneous blood flow (Fortney et al., 1981), scientists looked for ways to prevent hypohydration. Maintenance of circulatory and thermoregulatory function during exercise in the heat has been accomplished with the infusion of whole blood or protein-saline solutions (Fortney et al., 1981 and Fortney et al., 1988). Another method which attempted to allay the onset of hypohydration was hyperhydration through increased fluid intake. Increased fluid intake over the period of a week increased body water reserves and enhanced thermoregulatory function in young, heat-acclimated soccer players (Rico-Sanz et al., 1996). Contrary to these findings, hyperhydration through increased fluid intake immediately prior to a
potentially hypohydrating environment has met with limited success due to the rapid response of the homeostatic fluid regulating mechanisms in the body, i.e. the excess fluid is lost to urine formation. Researchers have been able to override these mechanisms by adding glycerol to the pre-exercise hyperhydration regimen (Robergs and Griffin, 1998; Montner et al., 1996; Freund et al., 1995; Lyons et al., 1990; Riedesel et al., 1987).

**Glycerol**

Glycerol is a 3-carbon alcohol occurring as a natural metabolite in the human body. Due to its potent osmotic-dehydrating action, glycerol has experienced widespread use in the clinical setting for the past 40 years in the treatment of intracranial and intraocular hypertension (Robergs and Griffin, 1998; Frank et al., 1981; Tourtellotte, et al., 1972).

The earliest studies concerning exogenous glycerol in the realm of exercise performance focused on its role as an energy substrate during prolonged exercise. Terblanche et al. (1981) found that pre-exercise glycerol feeding in rats significantly slowed the depletion of muscle and liver glycogen, postponed the onset of hypoglycemia, and increased exercise time to exhaustion. During strenuous sub-maximal exercise in man, glycerol has failed to produce similar results. Man cannot utilize glycerol as a gluconeogenic substrate rapidly enough to serve as a significant energy substrate during strenuous exercise (Maughan and Gleeson, 1988; Gleeson et al., 1986; Miller et al., 1983).

Another finding worth mentioning here from the study by Gleeson et al. (1986) was that the ingestion of glycerol (1 g/kg NBW in 400 ml fluid) increased plasma osmolality and
plasma volume (PV) to a greater extent in the pre-exercise period than did the ingestion of glucose or a placebo.

Riedesel et al. (1987) were the first to directly assess glycerol's potential as a hyperhydrating agent. In the first series of experiments the researchers examined the effects of different glycerol doses on the retention of a dilute saline solution. They had subjects ingest 0.5, 1.0, or 1.5 g glycerol/ kg body weight (BW) and drink 21.4 ml/kg of a 0.1% NACL solution within 40 min. The three glycerol solutions were compared to each other and to a control trial in which no glycerol was added to the saline solution. The subjects remained seated throughout the 4-hr experiment. They found that the glycerol doses of 1.0 and 1.5 g/kg BW plus the added fluid promoted a state of hyperhydration which lasted up to 4 hr. Urine formation was significantly reduced during the 2nd and 3rd hour after glycerol ingestion. There were no changes in plasma volume, despite a significant increase in plasma osmolality, which peaked within 60 min.

In the second series of experiments the researchers examined the effects of a 1.0 g glycerol/ kg BW dosage on fluid retention when the added fluid was consumed over a 3.5-hr period rather than acutely over a 40-min period. The experimental protocol was the same as in the first series except for the duration of ingestion of the added fluid. The researchers essentially reproduced the same results as in series one; therefore, it was concluded that glycerol plus added fluid ingestion is an effective method of increasing total body water regardless of whether the added fluid is ingested rapidly or over an extended period of time.
Lyons et al. (1990) investigated the effects of glycerol hyperhydration (GH) on thermoregulatory and cardiac responses during exercise in the heat. Three fluid regimens were employed in this study: one involved ingesting glycerol (1.0 g/kg BW) at time 0 and each hour (0.1 g/kg BW) after 2 hr until 4 hr; the second involved ingesting similar volumes of fluid without the glycerol; and the third involved limited fluid intake. Total volumes of fluid ingested for each regimen were: 28.4 ml/kg, 28.4 ml/kg, and 5.4 ml/kg, respectively. After resting for 2.5 hr in the lab, the subjects ran on a treadmill for 1.5 hr at an intensity equivalent to ~60% VO\textsubscript{2} max at temperature of 42° C (107.6° F) and relative humidity of 25%. It was found that glycerol plus fluid ingestion significantly reduced urine output, decreased core temperature (T\textsubscript{c}), and increased sweat rate during the exercise in the heat when compared to the ingestion of water alone. Plasma osmolality and glycerol concentrations were significantly increased. No changes were found in plasma volume or serum electrolyte concentrations. It was concluded that pre-exercise GH is an effective means of reducing the thermal stress associated with moderate exercise in the heat.

Murray et al. (1991) examined the physiological responses associated with glycerol ingestion during exercise. They had subjects consume either a 10% glycerol solution, a 6% carbohydrate-electrolyte beverage, a 6% carbohydrate-electrolyte beverage plus 4% glycerol, or a water placebo at regular intervals during the first 60 min of cycling exercise at ~52% VO\textsubscript{2} peak in a warm environment (30° C and 45% relative humidity). The total glycerol dose in the 10% solution was 1.2 g/kg BW. Total fluid intake was ~650 ml. Blood samples were taken every fifteen minutes throughout the 90 minutes of exercise.
Based on urine volume and body weight data, it was found that the subjects showed no indication of hyperhydration. It was also found that sweat rate, esophageal temperature, heart rate, RPE, aldosterone levels, and cortisol levels were unaltered by beverage treatment. Glycerol solutions did produce a significant increase in plasma osmolality and significantly attenuated the decrease in plasma volume associated with the placebo beverage. The authors concluded that consumption of 4 and 10% glycerol solutions during exercise confers no substantial thermoregulatory or cardiovascular benefits.

Lyons and Riedesel (1993) investigated the effects of GH on fluid compartments in rats. Either 20 ml water/kg BW or a 5% glycerol (1g/kg BW) solution was intragastrically administered to the rats. Total body water (TBW), extracellular fluid (ECF), and plasma volume (PV) were determined by $^{3}$H$_{2}$O, $^{14}$C-inulin, and dye dilution, respectively, at 2 hr post-ingestion. Urine volume and fluid retention were also measured throughout the experiment. The data indicated that glycerol ingestion significantly enhanced fluid retention (by 50%) and reduced urine output for 3 hr when compared to water ingestion alone. At 2 hr post-ingestion there was a significant increase in TBW, which primarily resulted from intracellular fluid (ICF) expansion. No significant differences in ECF, PV, or interstitial fluid (ICF) were found. The authors concluded that glycerol solutions have a greater effect at increasing TBW and ICF than equal volumes of water.

Seifert et al. (1995) (abstract) examined the effects of glycerol ingestion on fluid compartments during exercise in man. Subjects ingested 1.5 g/kg BW of glycerol or a placebo with 3.3 ml/kg BW of orange juice followed by 21 ml/kg BW of water 2.5 hr
before exercise. The exercise bout consisted of three 30-min cycling intervals at ~75% VO$_2$ max followed by a 600 revolution time trial. The method of measuring fluid compartments was not reported. It was found that glycerol ingestion did significantly increase fluid retention when compared to the placebo. Glycerol significantly reduced core temperature and increased plasma osmolality. The data also indicate that changes in ISF volume were greater at rest as a result of the glycerol treatment. No significant differences between beverage treatments were found for heart rate, PV, and sweat rate. The authors concluded that glycerol attenuated changes in fluid status and in thermoregulatory and circulatory functions. ISF was used to support PV during exercise and ICF was used to replenish ISF and PV during recovery.

Meyer et al. (1995) tested the effects of three hydration beverages on temperature regulation, circulatory function and work performance during prolonged heat exposure. The subjects lived in a climatic chamber simulating desert conditions (25-45° C, 20% relative humidity) for 60 hr. Subjects performed three exercise sessions on a treadmill at 4-hr intervals each day. The three beverage solutions consisted of: a water placebo, a 5% CHO-electrolyte drink, and a 4% CHO-electrolyte drink with 1% glycerol. Subjects drank 250 ml of the test beverage before entering the environmental chamber. Fluids were continuously available to them throughout the study. Although no attempt was made to hyperhydrate the subjects they were encouraged to drink fluid at a rate of ~1 L/h. Glycerol ingestion significantly increased sweat rate during all 3 days. Core temperature and body mass changes and work performance were unaffected by beverage treatment.
The researchers concluded that water supports the overall physiological responses to exercise-heat exposure as well as either of the other two beverages.

Attempting to delineate the physiological mechanisms underlying GH, Freund et al. (1995) examined its associated renal, vascular, and hormonal responses. Subjects completed three trials, two of which required hyperhydration and one in which no fluid was ingested. During the hyperhydration trials, glycerol (1 g/L TBW in 5.0 ml/L TBW of water) or water alone (5.0 ml/L TBW) served as the experimental solution. A bolus of water (32 ml/L TBW) was ingested after each experimental solution resulting in a total fluid consumption of 37 ml/L TBW. Testing lasted 4 hr and did not require any exercise. It was found that glycerol ingestion resulted in greater fluid retention, which was attributed to decreased free water clearance. Hyperhydration had no effect on atrial natriuretic peptide and aldosterone concentrations. Heart rate and plasma volume also remained unaffected by beverage treatment. Although not significant, there was a trend toward increased ADH concentrations during the glycerol trials (P=0.07); therefore, the authors concluded that ADH may be partly responsible for glycerol’s effectiveness. The other mechanism posited was an increase in the medullary concentration gradient in the kidney, which would lead to decreased free water clearance.

The idea of maintaining glycerol-induced hyperhydration for a prolonged period of time was researched by Koenigsberg et al. (1995). In series 1, attempts were made to keep subjects hyperhydrated for 49 hr and in series 2, for 32 hr. In series 1, 3.12 g/kg BW of glycerol mixed with 13.54 g/kg of orange juice and 34.43 ml/kg BW of water were
consumed per 24-hr period. In series 2, glycerol (3.12 g/kg/day) was presented in a 20% solution with artificially flavored water. Both experimental solutions were compared to a water control over the same period of time. Subjects were restricted to sedentary activities throughout the experiment. Subjects were allowed meals and ad libitum fluid intake, but were required to record all fluid intake. Urine volume was significantly reduced as fluid retention was increased in response to glycerol ingestion. Plasma osmolality and volume were unaffected by glycerol ingestion. The authors concluded that it is possible to maintain hyperhydration for extended periods of time, possibly reducing the need to ingest fluid prior to or during dehydrating situations.

The potential for glycerol hyperhydration to benefit exercise was addressed by Montner et al. (1996). Study I was conducted to test the effects of pre-exercise GH without rehydration during exercise on HR, $T_e$ and endurance time. Study II examined the effects of rehydration during exercise following GH. Subjects ingested either a 20% glycerol (1 g/kg BW) solution or a water placebo followed by a bolus of water, amounting to a total ingested volume of 26 ml/kg BW. Exercise was started 1 hr after the 90-min hydration period. Subjects cycled at ~61% $V_O_2$ max until exhaustion. The same fluid and exercise protocol was used in study II except for the ingestion of a 5% dextrose solution every 20 min. The results of both studies indicate that GH lowers HR during submaximal exercise. Plasma volume measures taken after hydration, at the end of exercise and after exercise were unaffected by GH. It was also found that GH did not lower $T_e$ or increase sweat rate as was expected. Endurance time was prolonged in both studies in response to
GH. The authors concluded that pre-exercise GH enhances endurance time and lowers HR, with and without rehydration during exercise.

Latzka et al. (1997) investigated the efficacy of pre-exercise hyperhydration for improving thermoregulatory functioning during compensable exercise-heat stress. The subjects completed five trials under the following conditions: euhydration, glycerol hyperhydration with and without rehydration during exercise, and water hyperhydration both with and without rehydration during exercise. During the hyperhydration trials, subjects drank 3.9 ml/kg lean body mass (LBM) of the experimental solution (glycerol or water). The glycerol dosage in the experimental solution was 1.2 g/kg LBM. A bolus of water (25.2 ml/kg LBM) was consumed after the experimental solution, amounting to a total fluid intake of 29.1 ml/kg LBM over a 30-min period. The subjects attempted 120 min of exercise on a treadmill at ~45% of max VO$_2$ at a temperature of 35° C. No differences in plasma volume, T$_c$, sweating rates, or TBW were observed among the hyperhydration trials. From this data, the authors concluded that hyperhydration, even with the addition of glycerol, provides no thermoregulatory advantage compared with euhydration during compensable exercise-heat stress.

Latzka et al. (1998) also examined the effects of hyperhydration on thermoregulatory and circulatory function during uncompensable exercise-heat stress. This study consisted of three trials: control, glycerol hyperhydration, and water hyperhydration. The fluid consumption and exercise protocol for this study was the same as that of Latzka et al. (1997) except for the fact that subjects had to wear chemical protective clothing and

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exercise until exhaustion. No differences were observed between the hyperhydration treatments for Tc, sweat rate, cardiac output, and HR. It was concluded that GH provides no physiological advantage over water hyperhydration during uncompensable exercise-heat stress. GH does delay hypohydration during uncompensable exercise-heat stress when compared to euhydration.

Inder et al. (1998) examined the effect of both glycerol and desmopressin on hydration and exercise performance in athletes ingesting routine volumes of fluid. Subjects exercised for 1 hr at 70% VO$_{2\text{peak}}$ followed immediately by an incremental increase in workload every 2 min until exhaustion. Glycerol (1 g/kg BW in 500 ml of water) was ingested 4 hr prior to the start of exercise. Desmopressin was taken 30 min prior to the start of exercise. Total water ingestion over the 1-hr exercise and the incremental exercise bout was 900 ml. There was no significant effect of glycerol or desmopressin on exercise performance. Glycerol ingestion did not effect body mass; therefore, glycerol ingestion with modest fluid intake did not result in significant hyperhydration. Urine osmolality was significantly higher after glycerol treatment when compared to the control. The authors concluded that the ingestion of glycerol 4 hr before exercise in conjunction with routine, pre-race fluid intake had no effect on exercise performance or hydration status in triathletes exercising for 1 hr.

Jimenez et al. (1999) assessed plasma volume (PV) changes in response to exercise- and heat-induced dehydration and glycerol-induced hyperhydration. The researchers compared the use of Evans blue dye dilution method with the "Hct-Hb" method for
determining changes in PV. Eight trained men completed four trials: euhydration, passive-heat-induced dehydration, exercise-induced dehydration, and glycerol-induced hyperhydration. The glycerol hydration regimen consisted of the ingestion of 1.1g glycerol /kg body mass diluted in 256 ml water. Then 30, 60, and 90 min later the subjects ingested a mean volume of 456 ml water containing 1.2g NaCl/ L, for a total ingested volume of 21.4 ml/kg body mass. PV measures were obtained before, during, and after each hydration regimen. The researchers found that the Evans blue dye PV measurements were in agreement with the Hct-Hb measurements. The main finding concerning glycerol was that PV was significantly higher at 60 and 120 min during the hydration phase and 30 and 90 min after the hydration phase.

Hitchins et al. (1999) examined the effects of glycerol hyperhydration on exercise performance in hot, humid conditions. In this study, 8 trained cyclists completed two 1-hr cycling time trials. Each trial was preceded by ingestion of a glycerol solution (1g/kg body mass) diluted in a carbohydrate-electrolyte drink or a placebo of equal volume. Total fluid intake was 22 ml/kg body mass. The hydration period commenced 2.5 hr before each trial and was completed within 30 min. The 1-hr trial was divided into a 30-min fixed power-output phase and a 30-min self-paced, variable power-output phase. Glycerol did lead to greater water retention after 2 hr compared to the placebo. The main finding of this study was a 5% improvement in cycling performance during the final 30 min following glycerol hyperhydration. This improvement was associated with an increased cardiac frequency during the first 10 min of the self-paced portion of the performance trial, which the authors attribute to a greater work output at these time-
points. There were no differences in core temperature and sweating rates between trials, suggesting that glycerol did not confer any thermoregulatory advantages.

Montner et al. (1999) investigated the mechanisms responsible for glycerol-induced fluid retention and heart rate reduction during exercise. Subjects ingested a water placebo 26ml/kg BW or a glycerol solution (1.2/kg BW) in 26 ml/kg BW of water over a 2-hr period before exercise. During the placebo trial subjects also ingested a 5% CHO solution every 20 minutes throughout the exercise. During the glycerol trial the exercise fluid regimen consisted of a 5% CHO solution with 0%, .5%, or 1.5% glycerol every 20 min. Subjects completed 4 cycling trials of 120 min duration at ~45% VO2 max. The data indicated that the glycerol solutions significantly increased fluid retention and reduced free water clearance. It was also found that stroke volume (SV) during exercise in the glycerol trials significantly increased from baseline when compared to the placebo trial. ADH values did not differ between the glycerol and placebo treatments. The authors concluded that glycerol facilitates fluid retention through a non-ADH mechanism and this fluid retention facilitates a reduced exercise HR due to an increased SV.

**Bioelectrical Impedance Analysis (BIA)**

BIA is a method of determining body water content and body composition based on the electrical-conductive properties of the body's tissues. It is a fast, portable, easy way to assess the volume of body water compartments. Its validity in measuring body water
compartments has been verified utilizing isotopic dilution techniques (Vache et al., 1998; van Marken Lichtenbelt et al., 1994; Van Loan, 1990). Although the validity of utilizing BIA has been established, some authors have suggested that continued research is needed for the development of prediction equations applicable to athletic, obese, diseased, and any other populations where fluid volumes and distributions differ from those of the normal reference population (Thomas et al., 1998; Bedogni et al., 1996; Van Loan, 1990).

Saunders et al. (1997) examined the effects of varying hydration states on BIA in endurance trained individuals. Fifteen trained subjects participated in all four trials: normal hydration, exercise-induced hypohydration, rehydration, and superhydration. BIA, body weight, and hydrostatic weighing measurements were obtained during each trial. The researchers found that the BIA measures of body composition varied during the periods of altered hydration. Altered hydration was accompanied by altered resistance measurements. The authors concluded that varied hydration levels across measurements may preclude the ability of BIA to accurately assess body composition.

Bedogni et al. (1996) assessed the reliability of BIA in predicting fluid compartment volumes in children afflicted with juvenile rheumatoid arthritis (JRA). The normal ratio of ECW to ICW in these children is altered such that the ECW volume is greater than in the normal population. Isotopic dilution was utilized to assess the validity of using BIA in predicting fluid compartments in thirty-nine JRA patients. The results indicated that BIA underestimated TBW and ECW. The authors concluded that the altered ECW-ICW
ratio in JRA patients invalidates the use of BIA prediction equations for TBW and ECW developed on healthy children.

In summary, the studies regarding the effects of glycerol hyperhydration on thermoregulatory and circulatory function and exercise performance have yet to provide any conclusive results. The disparities in the findings of these studies may be due to methodological flaws regarding glycerol dosage and the timing of exercise and blood sampling post-ingestion. Few studies have directly addressed the issue of changes in fluid compartment volumes in response to glycerol hyperhydration and no study has used bioelectrical impedance analysis to monitor these changes.
Chapter Three: Methodology

Setting
All testing took place in the Human Performance Laboratory (Department of Health and Human Performance) at the University of Montana, McGill Hall Room 121. All blood and urine analyses were performed at this location.

Subjects
The subjects for this study included 13 males who were free from any physical and/or mental condition that may be aggravated by the study. The subjects that volunteered for the study read and completed an Institutional Review Board (IRB) approved consent form and health history questionnaire before any measurements were taken.

Descriptive Data

Height and Weight
Height (cm) was measured using a conventional stadiometer. Nude body weight (NBW)(kg) was measured using a calibrated, digital scale (Befour Inc. Model PS6600T, Cedarburg, WI).

Total Body Water
Total body water, extracellular water (ECW) plus intracellular water (ICW), were determined for all subjects through multi-frequency bioelectrical impedance analysis (BIA) using the Xitron-Hydra ECF-ICF (Model 4200, San Diego, CA) BIA system.
Prior to the analysis the subjects were required to lie in a supine position for 5 minutes in a climate-controlled lab (~19°C, 30% relative humidity).

**Experimental Procedure**

Each subject completed two hydration trials, a placebo trial with water alone and a treatment trial with glycerol supplementation. Each trial was separated by at least 4 days with the order being randomized. All subjects were instructed to refrain from eating or drinking for 12 hours prior to each trial. They were also instructed to refrain from participating in exercise, consuming alcohol, or using tobacco products within 24 hours of testing.

**TBW (ECW + ICW) Measures**

Both trials were performed in the manner described below. After arrival at the laboratory, subjects were instructed to void their bladder. Height and weight were determined as described above. With subjects in a supine position the ankle and wrist electrodes of the BIA instrument were applied in accordance with the instrument’s operating manual. The ankle and wrist areas were cleaned with alcohol and any excessive body hair was removed by shaving. Two current-injection electrodes were placed at the right hand and foot on the dorsal surfaces proximal to the metacarpal-phalangeal and metatarsal-phalangeal joints, respectively. The center of the two voltage-detector electrodes was placed on the mid-line between the prominent ends of the right radius and ulna of the wrist and the mid-line between the medial and lateral malleoli of the right ankle. The injection and detection electrodes were placed at least 5 cm apart.
according to manual specifications (Xitron Technologies Manual, 1997). The same electrode-placement distances were used for both trials. Electrode distances ranged from 5.1 to 7.0 cm and 8.1 to 12.8 cm on the wrist and ankle, respectively.

The subjects were instructed to lie motionless for 10 minutes with arms abducted ~ 15° and legs slightly apart. At minute 5, BIA measurements of TBW, ECW, and ICW were obtained. These measures were repeated prior to the hydration treatment and every 80 minutes thereafter until completion at minute 240. Immediately after TBW measurement and prior to the hydration treatment a 7 mL blood sample was drawn to determine baseline values for Hb, Hct, osmolality, and glycerol concentration. Blood samples were also taken immediately following all BIA measurements throughout each trial. Urine was collected between BIA measurements (as necessary) and the subject was also asked to produce a final sample 5 minutes prior to each BIA measurement. Nude body weight was measured at baseline and after each final urine collection prior to BIA.

Hydration Procedures

Nude body weight (as determined on that day) was used to determine the total volume of fluid (26 mL/kg NBW) and the amount of glycerol (1.2 g/kg NBW) to be ingested during each trial. The glycerol was diluted with water to a 20% solution. The control solution consisted of an equal volume of water used to create the glycerol solution. Subjects ingested the solutions during the first 5 minutes of each trial. The subjects then ingested a bolus of water over the next 45 minutes. Drinking was paced evenly over the
45 minutes by presenting 20% of the total volume to be ingested every 9 minutes. Figure 3.1 depicts the experimental procedure.

**Figure 3.1 Experiment Timeline**

![Experiment Timeline Diagram]

- **= hydration period**
- --- = urine collection (as necessary)
- ↓ = last urine collection, NBW
- \( \nabla \) = TBW (ECW + ICW)
- ♦ = Blood Draw (7 mL)

**Blood Work**

Blood samples (7mL) were taken immediately following all TBW measurements at time 0, 80, 160, and 240. All blood draws were collected in non-additive 7 mL vacutainer tubes. Samples were obtained from alternating anticubital arm veins.

**Plasma Volume**

Plasma volume (PV) changes were calculated from appropriate hematocrit (Hct) and hemoglobin (Hb) values, as determined by Dill and Costill (1974). To determine the plasma volume changes the Hb concentration was determined first. Twenty \( \mu \text{L} \) of whole
blood were transferred into 3 mL of drabkins reagent and then incubated at room temperature for 20 minutes. Using a spectrophotometer (Milton Roy Spectronic 401, Rochester, NY) the sample absorbance was measured at 540 nm. Hb concentration was determined using equation 3.2, which is based on the molecular weight of hemoglobin and the millimolar extinction coefficient of cyanmethemoglobin measured under the standard conditions of a 1 cm light path at 540 nm. Hct was determined using 40 μL microhematocrit tubes filled with whole blood. Each microhematocrit tube was sealed and spun in a microcentrifuge (Jouan A13, Winchester, VA) for 5 minutes. Hct was then expressed as the percentage of packed cell distance to total cell distance. The value obtained was then multiplied by a factor of .96 to account for plasma trapped in the cell mass. Percent changes in plasma volume were calculated based on equation 3.3 (Latzka, 1996). All Hb and Hct samples were analyzed in triplicate.

**Equation 3.2 Hemoglobin Concentration Calculation**

\[
[Hb] \text{ g/dl} = \frac{\text{Abs} \times \text{Sample Dilution}}{44} \times \text{Mr Hb} \times (1000)^{-1} \\
\text{Abs} = \text{Absorbance of sample} \\
\text{Mr} = \text{molecular weight of Hb} = 64,458 \text{ g/mole} \\
44 = \text{millimolar absorptivity of cyanmethemoglobin}
\]

**Equation 3.3 Plasma Volume Change Calculation**

\[
\% \Delta PV = 100 \times \frac{(Hb_{pre})(Hb_{post})^{-1}(1-Hct_{post} \times 10^{-2})(1-Hct_{pre} \times 10^{-2})^{-1} - 100}{1}
\]
Plasma Osmolality

Plasma osmolality was found using freezing point depression (Precision Systems µOsmette Model 5004, Sudbury, MA). The osmometer was calibrated prior to any analyses to ensure accurate results. After blood samples were obtained they were centrifuged (Juoan Mr22i, Winchester, VA) at 3,000 rpm for 15 minutes and the plasma was withdrawn. Fifty µL of the plasma was pipetted into 2mL disposable tubes for analysis. The sample was then placed in the osmometer for measurement. All samples were done in duplicate.

Plasma Glycerol Concentration

Plasma glycerol concentrations were determined using a Triglyceride (GPO-Trinder) assay kit (Sigma Diagnostics, St. Louis, MO) and spectrophotometer. All samples were analyzed in duplicate.

Urine Collection

Urine samples were collected in plastic specimen cups prior to the initial TBW measurement and throughout the 4-hr experimental period. Samples collected in between TBW measurements were pooled together for total volume and osmolality measurements for the respective time points.

Urine Volume and Fluid Retention

Urine volume was determined simply by pooling together all urine samples collected for the respective time points and measuring the volume in a graduated cylinder. The
volume of fluid retained was determined by subtracting the volume of urine output from the volume of fluid ingested. See equation 3.4. Fluid loss from sweat evaporation was considered negligible because the subjects were resting in a thermoneutral environment.

**Equation 3.4 Volume of fluid retained calculation**

\[ V_{\text{ret}}(\text{mL}) = V_{\text{ing}}(\text{mL}) - V_{\text{uri}}(\text{mL}) \]

where

- \( V_{\text{ret}} \) = the volume of fluid retained
- \( V_{\text{ing}} \) = the volume of fluid ingested
- \( V_{\text{uri}} \) = the volume of urine output

**Urine Osmolality**

Urine osmolality was found using freezing point depression (Precision Systems \( \mu \)Osmette Model 5004, Sudbury, MA). The osmometer was calibrated prior to any analyses to ensure accurate results. Fifty \( \mu \)L of the urine were pipetted into 2mL disposable tubes for analysis. The sample was then placed in the osmometer for measurement. All samples were done in duplicate.
Research Design and Statistical Procedures

Descriptive analyses involved the calculation of means and standard deviations. The experimental design involved comparisons being made between the subjects' results from their experimental and placebo trials across all four sampling time-points. A 2-within-factor analysis of variance (ANOVA), with experimental treatment being the first factor and sampling time the second factor, was used to determine if there were significant main and interactive effects. Differences in fluid retention as well as fluid loss resulting from measurement methodologies utilized were assessed using a 3-within factor ANOVA, with experimental treatment being the first factor, sampling time the second factor, and methodology employed the third factor. Differences were considered significant when $p \leq .05$. Multiple comparisons were performed to determine differences when significant main effects or interactions resulted. However, the number of multiple comparisons adhered to the $k - 1$ restriction where $k$ equals the number of cell means for the main effect or interaction of interest. Dependent variable (DV) comparisons are represented in table 3.1.
Table 3.1 Variables being compared between experimental and control trials

<table>
<thead>
<tr>
<th>DV</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>ΔNBW</td>
<td>X</td>
</tr>
<tr>
<td>TBW</td>
<td>X</td>
</tr>
<tr>
<td>ECW</td>
<td>X</td>
</tr>
<tr>
<td>ICW</td>
<td>X</td>
</tr>
<tr>
<td>Hb</td>
<td>X</td>
</tr>
<tr>
<td>Hct</td>
<td>X</td>
</tr>
<tr>
<td>ΔPV</td>
<td>X</td>
</tr>
<tr>
<td>Plasma Osm.</td>
<td>X</td>
</tr>
<tr>
<td>Plasma Glycerol</td>
<td>X</td>
</tr>
<tr>
<td>Urine Volume</td>
<td>X</td>
</tr>
<tr>
<td>Fluid Retained</td>
<td>X</td>
</tr>
<tr>
<td>Urine Osm.</td>
<td>X</td>
</tr>
</tbody>
</table>

Note: "X" denotes at which time points the variables were compared.
Chapter Four: Results

Descriptive Data

Thirteen male subjects, aged 19-39 years, participated in the study. All subjects were free from any prior health condition that would preclude their participation in the study. All subjects filled out an IRB approved consent form. All subjects completed both the experimental and control trials. Two subjects were excluded due to vomiting during the hydration phase. One subject vomited during the glycerol trial in response to the sweet taste of the glycerol solution. The other subject, despite being able to tolerate the fluid volume during the glycerol trial, vomited in response to the fluid load during the placebo trial. Descriptive data of the subjects are provided in Table 4.1.

<table>
<thead>
<tr>
<th></th>
<th>13 males</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>13 males</td>
</tr>
<tr>
<td><strong>Height (m)</strong></td>
<td>1.76 ± 0.09</td>
</tr>
<tr>
<td><strong>Nude Body Weight (kg)</strong></td>
<td>77.0 ± 10.0</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>24.7 ± 6.0</td>
</tr>
<tr>
<td><strong>Total Body Water (L)</strong></td>
<td>46.02 ± 5.85</td>
</tr>
<tr>
<td><strong>ECW (L)</strong></td>
<td>18.79 ± 2.44</td>
</tr>
<tr>
<td><strong>ICW (L)</strong></td>
<td>27.23 ± 3.91</td>
</tr>
<tr>
<td><strong>Total Fluid Intake (L) – placebo</strong></td>
<td>2.0 ± .27</td>
</tr>
<tr>
<td><strong>Total Fluid Intake (L) – glycerol</strong></td>
<td>2.0 ± .26</td>
</tr>
</tbody>
</table>

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Trial Measurements

Nude Body Weight

The trial x time interaction was significant (F = 17.07, p = 0.0001; Figure 4.1). Multiple comparisons revealed that NBW was significantly higher at 160 and 240 minutes in the glycerol versus the placebo trial. In the glycerol trial, NBW was significantly higher at 80, 160, and 240 minutes versus 0 minutes. However, in the placebo trial, NBW was significantly higher only at 80 and 160 minutes versus 0 minutes.

Fluid Retention (fluid intake – urine output)

The trial x time interaction was significant (F = 21.88, p = 0.0001; Figure 4.2). Multiple comparisons revealed that fluid retention was significantly greater at 160 and 240 minutes in the glycerol versus the placebo trial. In both trials the amount of fluid retained at 160 and 240 minutes was significantly lower versus 80 minutes.

Urine Output

The trial x time interaction was significant (F = 15.82, p = 0.0001; Figure 4.3). Multiple comparisons revealed that urine output was significantly greater at 160 minutes in the placebo versus the glycerol trial. Also, in the placebo trial urine output was significantly greater at 160 minutes versus 80 minutes.

Total Body Water (BIA)

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The trial x time interaction was not significant ($F = 1.43, p = 0.248$; Figure 4.4). However, the main effect of time was significant ($F = 11.03, p = 0.0001$). Multiple comparisons revealed that, for both trials, TBW was significantly higher at 80 minutes versus 0 minutes.

**Extracellular Water (BIA)**

The trial x time interaction was not significant ($F = 0.61, p = 0.613$; Figure 4.5). However, the main effect of time was significant ($F = 7.91, p = 0.0004$). Multiple comparisons revealed that, for both trials, ECW was significantly lower at 80, 160, and 240 minutes versus 0 minutes.

**Intracellular Water (BIA)**

The trial x time interaction was significant ($F = 2.90, p = 0.048$; Figure 4.6). Multiple comparisons revealed that ICW was significantly higher at 160 and 240 minutes in the glycerol versus the placebo trial. In the glycerol trial, ICW was significantly higher at 80, 160, and 240 minutes versus 0 minutes. In the placebo trial, ICW was significantly higher at 80 and 160 minutes versus the 0 minutes.

**Fluid Retention (actual vs. BIA)**

The trial x time x method interaction was not significant ($F = 0.50, p = 0.613$). However, the time x method interaction was significant ($F = 3.60, p = 0.043$; Figure 4.9). Multiple
comparisons revealed that fluid retention values calculated from BIA measures were significantly lower than actual fluid retention measures at 80, 160, and 240 minutes.

**Plasma Volume**

The trial x time interaction was not significant (F = 0.93, p = 0.408; Figure 4.8). Similarly, the main effects of trial (F = 0.04, p = 0.836) and time (F = 0.22, p = 0.803) were not significant.

**Plasma Glycerol**

The trial x time interaction was significant (F = 718.52, p = 0.0001; Figure 4.9). Multiple comparisons revealed that plasma glycerol concentrations were significantly higher at 80, 160, and 240 minutes in the glycerol versus the placebo trial. Also, in the glycerol trial plasma glycerol concentrations were significantly higher at 80, 160, and 240 minutes versus 0 minutes.

**Plasma Osmolality**

The trial x time interaction was significant (F = 71.46, p = 0.0001; Figure 4.10). Multiple comparisons revealed that plasma osmolality was significantly greater at 80, 160, and 240 minutes in the glycerol versus the placebo trial. In the glycerol trial, plasma osmolality was significantly greater at 80, 160, and 240 minutes versus 0 minutes. In the placebo
trial, plasma osmolality was significantly lower at 80, 160, and 240 minutes versus 0 minutes.

_Urine Osmolality_

The trial x time interaction was significant ($F = 3.84$, $p = 0.018$; Figure 4.11). Multiple comparisons revealed that urine osmolality was significantly higher at 80, 160, and 240 minutes in the glycerol versus the placebo trial. Also, in both trials, urine osmolality was significantly lower at 80, 160, and 240 minutes versus 0 minutes.
Figure 4.1  Nude body weight changes in response to treatments (*p<0.05, glycerol vs. placebo; †p<0.05, vs. glycerol 0; Δp<0.05 vs. placebo 0)

Figure 4.2  Changes in actual fluid retained (ml) calculated from the change in fluid intake – urine output. (*p<0.05 vs. placebo; †P<0.05 vs. glycerol 80; Δp<0.05 vs. placebo 80)
Figure 4.3 Differences in urine output (ml) in response to treatments (*p<0.05 vs. glycerol; †p<0.05 vs. placebo 80)

Figure 4.4 Differences in TBW (L) in response to treatments as measured with BIA (*p<0.05 vs. 0; main effect of time)
Figure 4.5 Differencs in ECW in response to treatments as measured with BIA (*p<0.05 vs. 0; main effect of time)

Figure 4.6 Differences in ICW in response to treatments as measured with BIA (*p<0.05 vs. placebo; †p<0.05 vs. glycerol 0; Δp<0.05 vs. placebo 0)
Figure 4.7 Differences in fluid retained (mL) as measured from BIA analyses and actual (fluid intake - urine output). (*p<0.05 vs. actual)

Figure 4.8 Changes in plasma volume (%) in response to treatments

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Figure 4.9  Differences in plasma glycerol concentrations in response to treatments (*p<0.05 vs. placebo; †p<0.05 vs. glycerol 0)

Figure 4.10  Differences in plasma osmolality (mOsm) in response to treatments (*p<0.05 vs. placebo; †p<0.05 vs. glycerol 0; ∆p<0.05 vs. placebo 0)
Figure 4.11 Differences in urine osmolality (mOsm) in response to treatments (*p<0.05 vs. placebo; †p<0.05 vs. glycerol; Δp<0.05 vs. placebo)
Chapter Five: Discussion and Conclusions

The use of glycerol as a hyperhydrating agent has been well documented in prior research (Montner et al., 1999; Hitchins et al., 1999; Montner et al., 1996; Seifert et al., 1995; Freund et al., 1995; Lyons and Riedesel, 1993; Lyons et al., 1990; Riedesel et al., 1987). However, the effects of glycerol hyperhydration on fluid compartment distribution remains inconclusive. The majority of prior research has simply used fluid retention and increased total body weight to determine increases in TBW and have subsequently speculated on the changes in ECW and ICW. The present study utilized a multi-frequency BIA system to examine fluid compartment shifts in response to glycerol hyperhydration in resting male subjects. A secondary purpose was to determine if the Xitron Hydra ECF-ICF (Model 4200) BIA system was sensitive enough to accurately detect changes in fluid compartment volumes in response to glycerol hyperhydration.

Trial Measurements

The conclusions that can be drawn from the following data apply only to the sample tested and the protocol implemented.

Nude Body Weight

Nude body weight was significantly higher at 160 and 240 minutes in the glycerol versus the placebo trial. This finding is in agreement with the previous work by Montner et al. (1999) and Montner et al. (1996) but conflicts with the results obtained by Inder et al. (1998), who did not show an increase in nude body weight in response to glycerol.
ingestion. The failure to achieve an increase in body weight in the study by Inder et al. (1998) is most likely due to the fact that the subjects did not consume as large a dose of glycerol (1g/kg vs. 1.2g/kg) and volume of liquid (~9 ml/kg vs. 26 ml/kg) as the aforementioned studies. The greater body weight in our glycerol trial is attributed to the greater fluid retention at 160 and 240 minutes.

**Fluid Retention (fluid intake - urine output)**

Fluid retention was significantly greater at 160 and 240 minutes in the glycerol versus the placebo trial. This finding reflects the difference in body weights obtained between trials at these two time points. Four hours after the hydration regimen started ~41% of fluid ingested was retained in the glycerol trial compared to ~16% retention in the placebo trial. These results coincide with previous studies which have assessed the effects of glycerol hyperhydration during resting conditions (Montner et al., 1999; Montner et al., 1996; Seifert et al., 1995; Freund et al., 1995; Koenigsberg et al., 1995; Lyons and Riedesel, 1993; Lyons et al., 1990; Riedesel et al., 1987).

It is interesting to note, however, that in the studies by Latzka et al. (1997) and Latzka et al. (1998) glycerol hyperhydration produced no significant increases in fluid retention (as indicated by TBW measures derived from deuterium dilution) when compared to water hyperhydration. Their first post-hydration TBW measurement was taken 30 minutes after the hydration regimen was complete. The lack of any significant difference at this time point coincides with the findings of our study. Throughout the exercise heat-stress trials, which commenced at this time, still no differences in TBW were shown. Based on our
findings in resting subjects it would appear that exercise and heat-stress had an equalizing effect on glycerol-induced fluid retention. The reduced diuresis that accompanies exercise and heat exposure is the most likely mechanism responsible (Latzka et al., 1997).

_Urine Output_

Urine output was significantly lower at 160 minutes in the glycerol versus the placebo trial. At 80 and 240 minutes the observed differences were not statistically significant. This is in agreement with previous studies which have assessed the effects of glycerol hyperhydration during resting conditions (Montner et al., 1999; Montner et al., 1996; Seifert et al., 1995; Freund et al., 1995; Koenigsberg et al., 1995; Lyons and Riedesel, 1993; Lyons et al., 1990; Riedesel et al., 1987) and disagreement with others (Inder et al., 1998; Latzka et al., 1998; and Latzka et al., 1997). Obtaining an initial urine volume measure at 30 minutes pre-exercise, which equates to 3.5 post-glycerol ingestion, in conjunction with a considerably lower fluid intake (~9 ml/kg vs. 26 ml/kg) may explain the discrepancy in the study by Inder et al. (1998). The lack of difference in urine output measures in the Latzka et al. (1997) and (1998) studies is most likely a result of the decreased diuresis in response to exercise and heat exposure. When cumulative urine volumes were expressed as a percentage of fluid intake we found that 20, 43, and 21 percent of the fluid ingested was excreted at 80, 160, and 240 minutes, respectively, in the placebo trial. However, in the glycerol trial 22, 20, and 17 percent of the fluid ingested was excreted at 80, 160, and 240 minutes, respectively. The non-significant difference in urine output at 80 minutes is most likely due to the fact that the increase in
serum glycerol concentrations and the concomitant increase in serum osmolality, the two most likely factors responsible for reduced diuresis during glycerol hyperhydration (Robergs and Griffin, 1998), did not peak until this time. The non-significant difference at 240 minutes may be an indirect consequence of the considerable difference in urine output (859 L vs. 401 L, placebo and glycerol, respectively) shown at 160 minutes.

Total Body Water

Multi-frequency BIA of TBW revealed no significant differences between hyperhydration trials. This finding is in agreement with that of Lyons and Riedesel (1993). This support is presented with great caution due to a number of methodological differences. First, in their study the researchers used radio-labeled water as opposed to BIA to assess TBW changes. Second, they used an animal (rat) model, thus limiting the extent to which conclusions can be made to human populations. Third, the concentration of the glycerol solution, the total fluid volume, and the method of administration were different from ours. Last, TBW was only measured at one time point after glycerol administration. Interestingly, when these researchers expressed TBW as a percentage of body weight they did find a significant difference in TBW between the glycerol and control treatments. In our study, converting TBW to a percentage of NBW had no such effect. However, the main effect of time was significant in our study, indicating a significant increase in TBW at 80 minutes versus pre-hydration in both trials. The failure of BIA to indicate any significant differences in TBW between trials is in direct opposition to a host of previous studies (Montner et al., 1999; Montner et al., 1996; Seifert et al., 1995; Freund et al., 1995; Koenigsberg et al., 1995; Lyons and Riedesel,
1993; Lyons et al., 1990; Riedesel et al., 1987) which have assessed changes in TBW via changes in body weight, urine output, and fluid retention. It is also in direct opposition to our findings regarding the same variables. Reasons for this disparity will be discussed later in the section comparing actual fluid retention (fluid intake − urine output) with BIA measures of fluid retention.

Extracellular Water

Multi-frequency BIA of ECW revealed no significant differences between hyperhydration trials. This finding is in agreement with Lyons and Riedesel (1993). Analyzing the decay characteristics of $^{14}$C-inulin, Lyons and Riedesel (1993) showed no differences in the ECW compartment between the glycerol and control trials. Again, this support is presented with considerable reserve due to the methodological differences previously reported. To a certain extent, our findings are in disagreement with those of Seifert et al. (1995). In a published abstract they reported that one component of the ECW compartment, the interstitial fluid volume (ISFV), at rest was greater in response to glycerol versus water hyperhydration. No significant difference in PV changes was observed between treatments. The disparity between these findings and ours may be a result of the measurement techniques involved. In our study we were unable to address any changes in ISFV utilizing BIA. Utilizing BIA to measure ECW causes both ISFV and PV to be summed together; therefore, any volume change in one of these variables may, depending upon the magnitude, be offset by a change in the other if it occurs in the opposite direction. Measuring the individual components of the ECW compartment
through the use of radio-labeled isotopes may serve as a more sensitive technique for assessing any subtle changes.

**Intracellular Water**

ICW was significantly higher at 160 and 240 minutes in the glycerol versus the placebo trial. The findings of Lyons and Riedesel (1993) support these results when they express ICW as a percentage of body weight. Our findings are also in accordance with those reported by Seifert et al. (1995). In both of these studies glycerol induced a state of increased TBW, which to a great extent was due to ICW compartment expansion. The osmotic characteristics and the distribution of glycerol are primarily responsible for this phenomenon. After absorption glycerol is freely distributed throughout body fluid compartments barring the brain, cerebrospinal fluid, and the aqueous humor (Lin, 1977). The distribution of glycerol into the various fluid compartments causes a consequent increase in the osmotic gradient of these compartments, thus favoring the movement of water into these spaces during hyperhydration. ICW volumes at 160 and 240 minutes were significantly greater after glycerol ingestion versus water ingestion thus suggesting the movement of glycerol into these compartments by these time points. The non-significant ($p = 0.10$), yet greater, ICW volume at 80 minutes in the glycerol trial suggests that the movement of glycerol and/or water into this fluid compartment had not yet reached equilibrium.
Fluid retention (actual versus BIA)

When comparing the two different methods of measuring fluid retention we reported that BIA measures of fluid retention at 80, 160, and 240 minutes were significantly lower than actual fluid retention measurements for both trials. Although the trend in fluid retention is the same for both methods, BIA consistently underestimated the volumes involved. As this was the first study to utilize multi-frequency BIA to monitor changes in fluid compartment volumes in response to glycerol hyperhydration, it is difficult to determine the reasons for this discrepancy. The possibility of measurement error concerning the use of the BIA system cannot be entirely ruled out, although great care was taken to minimize confounding due to variables such as electrode placement, degree of limb abduction, and length of time the subject was supine prior to the measurement. Changes in the hydration state of subjects has been shown to adversely affect impedance measurements (Saunders et al., 1997). Also, Bedogni et al. (1996) reported that alterations in normal body water distribution adversely affected BIA measurements of water compartments in juvenile rheumatoid arthritis patients. According to Sharfetter et al. (29) altered ion concentration in response to insufficient water intake and eventual dehydration can affect ECW and ICW predictions by 1-2% and 4-5%, respectively. Increased ion concentrations can lead to overestimation of body water as a result of decreased resistance values. Our subjects were initially tested after a minimal 12-hr food and water fast. Initial serum osmolality measures were 290 and 288 mOsm for the placebo and glycerol trials, respectively. The euhydration criteria (<286 mOsm) set by Latzka et al. (1997) suggest that our subjects were slightly dehydrated. Because plasma electrolyte concentrations were not measured in this study it is difficult to determine whether they
may have compromised BIA measurements. According to Thomas et al. (1998) the increased resistivity due to increased concentrations of physiologically relevant molecules such as glucose has not been studied in great detail; therefore, it is possible that the increased glycerol concentration may also be responsible for the measurement error. Also worth considering as a possible source of measurement error is the fact that whole body impedance measurements derived using the wrist-ankle methodology are disproportionately sensitive to hydration changes in the limbs versus the trunk (Thomas et al., 1998).

**Plasma Volume**

Except for initial changes, plasma volume changes exhibited no significant differences over time or between trials. These results are in agreement with previous findings in resting subjects (Hitchins et al., 1999; Greenleaf et al., 1998; Montner et al., 1996; Freund et al., 1995; Koenigsberg et al., 1995; Seifert et al., 1995; Lyons and Riedesel, 1993; Lyons et al., 1990; Riedesel et al., 1987) but contradict others (Jimenez et al., 1999; Montner et al., 1999; and Gleeson et al., 1986). Riedesel et al. (1987) and Lyons et al. (1990) hypothesized that the extra fluid which is retained during glycerol hyperhydration is selectively moved to the extravascular fluid compartments in response to an increase in osmolality. However, Freund et al. (1995) suggested that this explanation was rather tenuous considering the heightened serum glycerol concentrations and osmolality exhibited during glycerol hyperhydration. Freund et al. (1995) and Montner et al. (1999) suggested that the failure to produce any significant changes in PV during glycerol hyperhydration may have been a result of the time course of PV...
measurements and insensitive Hct and Hb measurements. Gleeson et al. (1986) reported a significantly greater increase in PV at rest 15, 30, and 45 minutes following the ingestion of glycerol. Based on our PV values it would seem that the timing is a relevant factor. The greatest difference between trials (1.44%) in percent increases in PV that we reported occurred at the first sampling point, 80 minutes, which was obtained 75 minutes after glycerol ingestion. Had we sampled for PV immediately post-hydration (50 minutes) or sooner it is possible that we may have discovered a significant difference. Also, assuming that glycerol has reached equilibrium throughout fluid compartments it is possible to calculate the volume difference in fluid retention in the vascular space based on the fact that blood volume represents only 12% of TBW. If the additional 489 mL retained during the glycerol trial were freely distributed throughout TBW, the calculated blood volume would increase only ~59 mL over values obtained during water hyperhydration. This small volume difference may be beyond the sensitivity limits of the measurement method employed. It is difficult to make a valid comparison between our findings with the findings of Murray et al. (1991) because their glycerol ingestion and PV measures were carried out during exercise. The methodological differences of Montner et al. (1999) also make comparisons difficult. Their subjects ingested glycerol prior to and during exercise and they did not directly measure PV changes. Instead, they inferred an increase in PV based on a greater stroke volume obtained during exercise for the glycerol trial.
**Plasma Glycerol**

Plasma glycerol concentrations were significantly higher at 80, 160, and 240 minutes in the glycerol versus placebo trial as was expected. All studies which have measured plasma glycerol levels during hyperhydration in resting subjects support this finding (Montner et al., 1999; Greenleaf et al., 1998; Montner et al., 1996; Koenigsberg et al., 1995; Freund et al., 1995; Lyons et al., 1990; Riedesel et al., 1987). During the glycerol trial, a maximum glycerol concentration of 13.08 mM was reached at 80 minutes, with a gradual decline thereafter to 6.73 mM by the end of the experiment (240 minutes). These values are in accordance with those described in a review article by Robergs and Griffin (1998) for similar dosages and concentrations.

**Plasma Osmolality**

Plasma osmolality measures were significantly greater at 80, 160, and 240 minutes in the glycerol versus the placebo trial. These results are in agreement with previous research in resting subjects (Jimenez et al., 1999; Montner et al., 1999; Greenleaf et al., 1998; Montner et al., 1996; Freund et al., 1995; Seifert et al., 1995; Lyons et al., 1990; Riedesel et al., 1987) but in disagreement with others (Inder et al., 1998 and Koenigsberg et al., 1995). Inder et al. (1998) did not offer an explanation to rectify the disparity between their findings and previous research. Perhaps the ingestion of the glycerol solution along with a meal minimized the expected osmolality differences. Koenigsberg et al. (1995) speculate that their values, which are inconsistent with previous research conducted in their laboratory, may be due to error induced by sample storage or instrument calibration. Our osmolality data seem to contradict our plasma volume data. If the osmotic drive of
glycerol is responsible for increased water retention then one would expect that greater serum osmolality would lead to greater water retention in the vascular space during glycerol hyperhydration. This did not occur in our study. The relative changes in PV may have been beyond the sensitivity limits of the Hct and Hb method employed.

Urine Osmolality

Urine osmolality decreased from pre-hydration values, as would be expected from the large volume of fluid intake. Urine osmolality was significantly higher at 80, 160, and 240 during the glycerol versus the placebo trial. These results concur with previous research in resting subjects (Inder et al., 1998; Freund et al., 1995; Riedesel et al., 1987). The kidneys reabsorb the majority of filtered glycerol at normal physiological serum glycerol levels (0.05 mM). When serum levels exceed 1.6 mM glycerol is excreted in the urine (Tourtellotte et al., 1972). Urinary excretion of glycerol increases in accordance with increasing serum glycerol levels and at serum levels between 15 and 20 mM urinary excretion of glycerol represents the largest component of total body glycerol clearance (Robergs and Griffin, 1998). Although urine glycerol concentrations were not measured in this study, the current serum glycerol data support the contention that the greater urine osmolality values reported during the glycerol trial were mostly due to the presence of glycerol. Also, the excretion of sodium may have accounted for a small portion of the increase in osmolality (Hitchins et al., 1999; Freund et al., 1995; Riedesel et al., 1987).
Conclusions

This study was the first to utilize multi-frequency BIA to assess changes in body fluid compartments in response to glycerol hyperhydration. Based on the data collected and the protocol used it was concluded that glycerol serves as a more potent agent than water when attempting to hyperhydrate subjects at rest over an acute period of time. This conclusion is based on actual fluid retention and urine output measures. However, when utilizing multi-frequency BIA to assess fluid status changes, glycerol exhibits no difference in its ability to hyperhydrate when compared to water. This conclusion is based on the TBW measures obtained from the BIA system. This finding is not necessarily a reflection of glycerol's hyperhydrating capability; rather, it is most likely a reflection of the wrist-ankle methodology employed and/or the BIA system's prediction equations that are used to derive fluid compartment volumes. Modifying the formulae generated from euhydrated subjects for predicting body water compartments may reduce the degree of error involved. Also, the BIA system did not indicate any differences in ECW between trials. Despite the failure to detect any difference in TBW and ECW between trials, the BIA system did indicate a greater ICW at 160 and 240 minutes during the glycerol trial. This change is supported by previous research (Lyons and Riedesel, 1993) that have shown that glycerol has no effect on ECW but leads to a greater increase in ICW. However, in the present study, this conclusion must be interpreted with caution considering the previously mentioned limitation of the BIA system and its current calculation methodologies.
Topics for Further Research

While this study provided valuable insight regarding the hyperhydrating aspect of glycerol and the measurement limitations of the BIA system, it also proposed the need for further research regarding these topics. Utilizing a similar protocol, tracer dilution techniques should be employed to help develop accurate prediction equations for BIA use in populations with altered hydration status and ECW-ICW ratios. Knowing the volume of the interstitial fluid compartment may also elucidate the effects of glycerol on the ECW compartment. Comparing BIA measurements obtained utilizing the segmental methodology versus the wrist-ankle methodology may elucidate the mechanisms responsible for this system’s inability to accurately determine changes in TBW during glycerol hyperhydration. In order to obtain valid baseline measures a criterion to verify euhydration based on plasma osmolality should be adopted. Measuring fluid compartments sooner after hydration and at more frequent time points may provide greater insight into fluid compartment shifts in response to glycerol ingestion. Due to the limited scope of this study certain variables that could be of importance were not examined. Future research could include measurement of urine glycerol, serum and urinary electrolytes, and fluid and mineral regulating hormones such as aldosterone, ANP, and ADH.
References


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Appendix I

Attachments: Institutional Review Board, Informed Consent, and Health History Questionnaire
The University of Montana
INSTITUTIONAL REVIEW BOARD (IRB) 010-99

CHECKLIST

Submit one completed copy of this Checklist, including any required attachments, for each course involving human subjects. The IRB meets monthly to evaluate proposals, and approval is granted for one academic year. See IRB Guidelines and Procedures for details.

Project Director: Dennis P. Kirby Jr.  Dept.: HHP  Phone: 738-1064
Signature: __________________ Date: 12/15/98

Co-Director(s): __________________  Dept.:  Phone:

Project Title: The effects of physical changes in plasma volume, total body water, intracellular water, and extracellular water

Project Description: This project will examine fluid compartment changes in fasting subjects in response to glycerol ingestion

Please provide the dates requested below:

<table>
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<th>Date Submitted to IRB</th>
<th>Project Start Date</th>
<th>Ending Date</th>
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Students Only:
Faculty Supervisor: Be Allen Ruby  Dept.: HHP  Phone: 2117
Signature: __________________
(My signature confirms that I have read the IRB Checklist and attachments and agree that it accurately represents the planned research and that I will supervise this research project.)

IRB Determination:

- [ ] Exempt from Review
- [ ] Approved by Administrative Review
- [X] Full IRB Determination:
- [ ] Approved
- [X] Conditional Approval (see attached memo)
- [ ] Resubmit Proposal (see attached memo)
- [ ] Disapproved (see attached memo)

Signature/IRB Chair: [Signature]  Date: 1-28-98

(over)
IRB REVIEW AND DETERMINATIONS

TO: Project Director: Dennis Kirby
Faculty Supervisor: Dr. Brent Ruby

PROJECT TITLE: "The effects of glycerol on changes in plasma volume, total body water, intracellular water, and extracellular water"

FROM: Carrie Gajdosik, Chairperson of the IRB, 243-5189

DATE: February 1, 1999

The above project was reviewed during the January 28, 1999 meeting and was conditionally approved, subject to meeting the following conditions:

1. Indicate to the PI if subjects will receive repeated sticks for blood drawing. If so, this should be included on the consent form.
2. Indicate to the Board if there will be compensation to the subjects.
3. Submit a copy of the information that will be on the recruiting poster.
4. Indicate if the health survey that will be used is the same as the one in the past used by Dr. Ruby. If not, please submit the current health survey that will be used for this study.
5. The PI has indicated that an oral dose of glycerol at 1.0 g/kg body weight has been well tolerated. Indicate to the IRB if the amount that the PI's are giving (1.2 g/kg) is also well tolerated according to the literature.
6. Indicate to the IRB how the PI's will decrease the discomfort of the individuals during the 4 hours of the study.
7. Submit to the IRB the procedure that will be used for training the individuals who will be drawing the blood. This pertains to those individuals who are not yet trained phlebotomists.
8. Make the following changes in the consent form: a) The language is too technical throughout the entire consent form. Paraphrase it into simple language eliminating the technical terms. Be sure to explain what glycerol is in simple language. Use either the second person or the third person throughout the consent form. Combining the two makes it confusing when reading. b) Explain why this topic is being studied. c) In the paragraph on the consent form labeled as #1 ("orientation meeting..."), add the amount of time it will take to complete the questionnaires. In paragraph two, simplify the words "lie supine" and write out abbreviations. Also, further explain the sequence of events. d) Paragraph 4, transcribe the 240 minutes into hours so it's clearer to the subject. Simplify the many technical terms in this paragraph such as multi-frequency bioelectrical impedance, current ejection, reception electrodes, fluid compartments, etc. e) Paragraph 6, give information regarding the use of the rectal thermometer, including how privacy will be maintained, who will insert it, how it will be kept in place, and information about comfort or discomfort when wearing it. f) Paragraph 7, indicate if there will be multiple sticks for drawing blood. If not, indicate how you will avoid this. Simplify the wording such as venopuncture.
g) Paragraph 9, indicate that the blood sampling will be done by trained phlebotomists or by students in training under the supervision of the trained phlebotomist. h) Under the line where the participant signs, indicate that the person needs to be 18 years or older. 1) Combine or use both sentences that refer to withdrawing from the study. Clarify that the subject does not need to experience discomfort in order to stop the trial or to drop out of the study. It needs to be made clear that the person may withdraw for any reason.

Please submit your revisions to the Chair of the IRB for final approval before you start data collection.

If a consent form is used, a copy of it should be provided to all participants.

The projects director is expected to immediately notify the IRB Chair, if any material changes occur. These changes include:

1. Substantial change(s) in procedure
2. Significant unanticipated problems
3. Adverse reactions of, or effects on, the subjects

IRB approval expires after one year. If data collection continues beyond 1 year from the date of IRB approval, you must submit a "Continuation Report" to the IRB for reconsideration and re-approval. Forms can be obtained from Research Administration.
Informed Consent Form

The Effects of Glycerol on Changes in Body Fluid Compartments in Man at Rest

Principal Investigator: Dennis Kirby

Co-Investigators: Brent Ruby PhD. Brian Sharkey PhD. Delbert Kilgore PhD.

Location: Human Performance Laboratory
McGill Hall #121
The University of Montana
Missoula MT 59812

The purpose of this investigation is to utilize multi-frequency bioelectrical impedance analysis (BIA) to examine changes in plasma volume (PV) and total body water (TBW) in both the extracellular (ECW) and intracellular (ICW) water compartments in response to an oral glycerol load combined with a bolus of distilled water.

Participation in the study will include:

1) Orientation meeting and pre-screening health assessment. This will involve filling out a health history questionnaire so that only apparently healthy individuals will participate.

2) Two separate hydration trials. All subjects will participate in a control and an experimental trial. Both trials will require the subjects to remain in a semi-supine position for approximately 4 h. Trials will be spaced at least 4 days apart. Prior to the trials subjects will be required to abstain from food and liquids for 12 hours. Also, no exercise, alcohol, caffeine, and tobacco products will be allowed 24 hours prior to each trial.

3) Hydration Regimen. The subject will ingest either an experimental solution (1.2 g/kg nude body weight (NBW) of glycerol in a 20% solution or a control solution (a volume of water equal to the glycerol solution) over a period of 5 minutes. The subject will then ingest a bolus of water at equal intervals over the following 45 minutes (for a total volume of 26 ml/kg NBW).

4) Body water monitoring via multi-frequency bioelectrical impedance analysis (BIA). This will involve the placement of current-injection and reception electrodes on the subjects’ wrist and ankle while they are relaxed in a supine position. An undetectable electric current is then passed through the body to determine water content of the fluid compartments. BIA measurements will be taken prior to the hydration regimen and at 80-minute intervals until minute 240.
5) Blood samples. Blood samples will be drawn to establish changes in plasma volume. A 7 ml blood sample will be drawn (via veno-puncture) immediately after each BIA measurement (Time 0, 80, 160, and 240 min).

6) Urine Collection and Nude Body Weight. Subjects will be asked to produce a urine sample immediately prior to all BIA measurements (Time 0, 80, 160, 240 min). Nude body weight will be measured after urine collection and prior to BIA. Urine collection and body weight measures will be obtained in the laboratory bathroom to ensure privacy.

It is expected that some discomfort (such as headache, nausea, or a bloated feeling) may occur as a result of the glycerol and/or water ingestion. If you experience discomfort during either trial you may stop at any time or drop out of the study. Blood sampling can sometimes be associated with risks of bruising (10%), infection (<1%), and clotting problems (<1%). These risks will be minimized by the use of sterile procedures and trained technicians. The principal investigator and other trained staff will conduct all testing and blood sampling. All results and records will be kept confidential and locked in the Human Performance Laboratory under the supervision of the principal investigator.

"In the event that you are injured as a result of this research you should seek appropriate medical treatment. If the injury is caused by the negligence of the University or any of its employees, you may be entitled to reimbursement or compensation pursuant to the Comprehensive State Insurance Plan established by the Department of Administration under the authority of M.C.A., Title 2, Chapter 9. In the event of a claim for such injury, further information may be obtained from the University’s Claims Representative or University Legal Counsel."

I have read the above statements and understand the risks involved with this study. Any questions which may have occurred to me have been answered to my satisfaction. I understand that if I have additional questions I can contact Dennis Kirby at home (728-1064) or Brent Ruby at home (542-2513) or at the Human Performance Laboratory (243-2117) at any time. I understand that participation is strictly voluntary and that I may withdraw from participation at any time without penalty.

Name of Participant

_____________________________

Signature of Participant _____________________________ Date ________

(by signing, the subject certifies that he is 18 years of age)

Signature of Investigator _____________________________ Date ________
PRE-PARTICIPATION HEALTH QUESTIONNAIRE

NAME________________________________________AGE______________
ADDRESS________________________________________________________________________
PHONE H-_________________________________W-_________________________

IN CASE OF EMERGENCY CONTACT:
NAME________________________________________RELATION______________
ADDRESS_______________________________________________________________________
PHONE H-_________________________________W-_________________________

MEDICAL HISTORY/PHYSICAL PROFILE

1. Have you ever experienced any of the following: (check all that apply)
   ____ High Blood Pressure  ____ Diabetes  ____ Chest Pain
   ____ Heart Problems  ____ Stroke  ____ High Cholesterol
   ____ Kidney Problems  ____ Migraine or headache disorders
   ____ Liver Problems

2. Are you currently: (check all that apply)
   ____ a smoker
   ____ using non-prescription drugs (e.g. aspirin)
   If yes, what and how often? _____________________________________________
   ____ engaged in recreational drug use (i.e. marijuana, etc.)

3. Are you currently taking any prescribed medications?_______
   If yes, what? _________________________________________________________

4. Have you ever experienced a head injury?_____________________________
   If yes, are there any lingering effects or symptoms?_____________________

EXERCISE HISTORY

1. Describe the types of exercises that you currently do.

2. How often do you do these exercises?

** Is there anything that may limit your participation in the study that has not been asked?
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Appendix II

Manuscript for submission to the International Journal of Sport Nutrition
December 13, 1999

Priscilla M. Clarkson, PhD.
International Journal of Sport Nutrition
Department of Exercise Science
University of Massachusetts
Amherst, MA 01003

Dear Dr. Clarkson,

We have enclosed the original and three copies of our manuscript entitled, "The Effects of Glycerol on Changes in Fluid Compartments in Males at Rest". We would appreciate it if you would consider this manuscript for submission into the original research section of the International Journal of Sport Nutrition.

Thank you for your time and consideration. We look forward to your comments in the near future.

Sincerely,

Brent C. Ruby, Ph.D.
Director, Human Performance Laboratory
Department of Health and Human Performance
The University of Montana
McGill Hall #121
Missoula, MT 59801
Phone: (406) 243-2117, Fax: (406) 243-6252
Email: ruby@selway.umt.edu

Authors

Brent C. Ruby
Dennis P. Kirby
The Effects of Glycerol on Changes in Body Fluid Compartments in Males at Rest

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Abstract The purpose of this study was to utilize multi-frequency bioelectrical impedance analysis (BIA) to examine glycerol-induced changes in fluid compartments in resting subjects over a 4-h time period. Two 4-h trials were performed including glycerol or water (placebo). The hydration regimen consisted of either a glycerol beverage (1.2 g/kg nude body weight (NBW) in a 20% solution) or water followed by a bolus of distilled water (total volume = 26 ml/kg NBW) consumed over a fifty minute time period. The glycerol trial exhibited a significantly greater (p < .05) NBW and fluid retention at minutes 160 and 240 thus verifying glycerol’s hyperhydrating effect. Fluid retention based on BIA measurements did not significantly demonstrate hyperhydration with glycerol. BIA consistently underestimated the quantity of fluid retained. However, BIA measures did indicate significantly greater (p < .05) intracellular water (ICW) stores at minutes 160 and 240 in the glycerol trial. In both trials extracellular water (ECW) stores exhibited a significant decrease (p < .05) at minutes 80, 160, and 240 relative to pre-hydration. The results of this study suggest that BIA may not accurately depict the hyperhydrating effect of glycerol over an acute period of time. Variations in resistance in response to changes in hydration status, altered fluid distribution, or osmolality may be responsible for this disparity.

Key Words Bioelectrical Impedance Analysis - Hyperhydration - Total Body Water - Plasma Volume - Osmolality
Introduction

Hypohydration, resulting from inadequate fluid intake during exercise in a hot environment, leads to a number of detrimental effects: decreased cardiovascular performance (4), increased heart rate and core temperature (22), decreased cutaneous blood flow and sweat rates (22, 27), and decreased work performance (26). Encouraging athletes to drink large amounts of fluid prior to exercise in a hot environment is of little long-term benefit because the ensuing increase in total body water (TBW) and plasma volume (PV) is rapidly counteracted by the physiological reflexes involving the renal, cardiovascular, endocrine, gastro-intestinal, and central nervous systems.

Researchers have overcome this homeostatic response by having subjects ingest glycerol along with large amounts of fluid. Glycerol is used as a hyperhydrating agent because of its rapid absorption, its even distribution among body fluid compartments, its presence as a natural metabolite, its osmotic action, and its non-toxicity when presented in oral doses of 1 g/kg body weight every 6 hours (17). A number of studies have confirmed the hyperhydrating effect of glycerol ingestion along with added fluid intake during resting conditions (5, 17, 20, 23). Lyons et al. (17) demonstrated that hyperhydration with glycerol prior to exercise leads to a decreased core temperature and an increased sweating rate during moderate exercise in the heat. In another study, glycerol-enhanced hyperhydration prolonged cycling endurance time and facilitated a lower submaximal heart rate, but did not lower core temperature or increase sweat rate (20). Other studies involving the ingestion of glycerol have failed to demonstrate any thermoregulatory or cardiovascular advantages over the ingestion of water alone (18, 21). The inability to
produce hyperhydration in these studies may be attributed to differences in the total amount of fluid ingested, timing of glycerol ingestion, and exercise protocol employed.

Also of importance is the fact that cellular hydration state has been proven to be a potent determinant of cellular protein turnover in health and disease (Haussinger et al., 1993). An increase in cellular hydration stimulates anabolism in the cell, whereas a decrease in hydration stimulates catabolism. Control of this process is particularly important in the management of post-surgical and chronically ill patients. If proven effective at increasing intracellular water stores, glycerol exhibits the potential of serving as an anti-catabolic treatment agent.

Extensive monitoring of fluid changes in the various fluid compartments of the body during glycerol hyperhydration has yet to be conducted. In the majority of prior research, changes in TBW are simply determined by changes in nude body weight or by differences between fluid intake and urine output, while changes in intracellular water (ICW) and extracellular water (ECW) have not been documented (5, 11, 13, 17, 20, 23). Siefert et al. (28) in a published abstract did, in fact, monitor changes in fluid compartment volumes after glycerol ingestion, but unfortunately the method by which this was performed was not reported. Lyons and Riedesel (16) examined shifts in fluid compartment volumes in response to glycerol ingestion in rats. TBW and ECW were determined using isotopic dilution techniques. ICW and interstitial water were determined mathematically. Although they were able to establish values for each fluid compartment, they only did so at one time other than baseline. No study to date has used
multi-frequency bioelectrical impedance analysis (BIA) to monitor changes in fluid compartment volumes during an extended period of glycerol hyperhydration.

The purpose of this investigation was to utilize multi-frequency bioelectrical impedance analysis (BIA) to examine changes in TBW, ECW, and ICW compartments in response to glycerol hyperhydration in resting male subjects over a 4-h time period.

Methods
Fifteen healthy male human subjects volunteered to participate in the study. All subjects gave written informed consent prior to the study, which was approved by the Institutional Review Board of The University of Montana. All testing took place in the Human Performance Laboratory at The University of Montana. Descriptive data of the subjects is shown in Table 1.

Experimental Procedures
Each subject completed two hydration trials, a control trial with water alone (W) and a treatment trial with glycerol supplementation (G). Trial order was randomized and separated by at least 4 days. All subjects were instructed to refrain from eating or drinking for 12 hours prior to each trial and to refrain from participating in exercise, consuming alcohol, or using tobacco products within 24 hours of testing.

Upon arrival at the laboratory, subjects were instructed to void their bladder. Height (cm) was determined using a conventional stadiometer and nude body weight (NBW) in kg.
was measured using a calibrated, digital scale (Befour Inc. Model PS6600T, Cedarburg, WI). TBW, ECW, and ICW were determined for all subjects from multi-frequency bioelectrical impedance analysis (BIA) using the Xitron-Hydra ECF-ICF (Model 4200, San Diego, CA) BIA system. With subjects in a supine position the ankle and wrist electrodes of the BIA system were applied in accordance with the instrument's operating manual. The ankle and wrist areas were cleaned with alcohol and any excessive body hair was removed by shaving. Two current-injection electrodes were placed on the right hand and foot on the dorsal surfaces proximal to the metacarpal-phalangeal and metatarsal-phalangeal joints, respectively. The center of the two voltage-detector electrodes was placed on the mid-line between the prominent ends of the right radius and ulna of the wrist and the mid-line between the medial and lateral malleoli of the right ankle. The injection and detection electrodes were placed at least 5 cm apart (10). The same electrode-placement distance for each subject was used for both trials. Electrode distances ranged from 5.1 to 7.0 cm and 8.1 to 12.8 cm on the wrist and ankle, respectively and was dependent on hand and foot size. All impedance spectral data were fit to the Cole-Cole model using non-linear curve fitting software (2). ECW and ICW volumes were predicted from the modeled impedance data using equations formulated from Hanai mixture theory (8).

The subjects were instructed to lie motionless for 10 minutes with arms abducted ~15° and legs slightly apart. At minute 5, BIA measurements of TBW, ECW, and ICW were obtained. These measures were repeated prior to the hydration treatment and every 80 minutes thereafter until completion at 240 minutes. Immediately after TBW...
measurement and prior to the hydration treatment a 7 mL blood sample was drawn to determine baseline values for Hb, Hct, osmolality, and glycerol concentration. Blood samples were also taken immediately following all BIA measurements throughout each trial. Urine was collected between BIA measurements (as necessary) and the subject was also asked to produce a final sample 5 minutes prior to each BIA measurement. Nude body weight was measured at baseline and after each final urine collection prior to BIA measurements.

Hydration Procedures

Nude body weight (as determined on that day) was used to determine the total volume of fluid (26 mL/kg NBW) and the amount of glycerol (1.2 g/kg NBW) to be ingested during each trial. The glycerol was diluted with water to a 20% solution (~2340 mOsm). The osmolality of the glycerol solution combined with the bolus of water was ~525 mOsm. The placebo consisted of a volume of water equal to the total volume of the glycerol solution. Subjects ingested the glycerol or placebo solution during the first 5 minutes of each trial. The subjects then ingested a bolus of water over the next 45 minutes. Drinking was paced evenly over the 45 minutes by presenting 20% of the total volume to be ingested every 9 minutes throughout the entire hydration period.

Blood and Urine Analyses

Blood samples (7mL) were taken immediately following all BIA measurements (with the subjects in the supine position for 8-10 minutes) at time 0, 80, 160, and 240. All blood draws were collected in non-additive 7mL vacuutainer tubes. Samples were obtained from alternating anticubital arm veins during the 4-hour experimental period. Plasma
volume (PV) changes were calculated from appropriate hematocrit (Hct) and hemoglobin (Hb) values, as determined by Dill and Costill (3). After blood samples were obtained they were centrifuged (Juoan Mr22i, Winchester, VA) at 3,000 rpm for 15 minutes and the plasma was withdrawn. Plasma osmolality was determined using a freezing point depression technique (Precision Systems µOsmette Model 5004, Sudbury, MA). Plasma glycerol concentrations were determined using an enzymatic, spectrophotometric (Milton Roy Spectronic 401, Rochester, NY) technique and a commercially available assay kit (Triglyceride GPO-Trinder, Sigma Diagnostics, St. Louis, MO).

Urine samples were collected in plastic specimen cups prior to the initial BIA measurement and throughout the 4-h experimental period. Samples collected between BIA measurements were collected to determine total urine output volume. Urine osmolality was determined using those samples which immediately preceded each BIA measurement. Urine volume was determined simply by pooling all samples collected for the respective time points and measuring the volume in a graduated cylinder. The volume of fluid retained was determined by subtracting the volume of urine output from the volume of fluid ingested. Sweat and respiratory water loss were considered negligible under resting conditions in a thermoneutral environment (~19°C, 30% relative humidity). Urine osmolality was determined as indicated above using freezing point depression (Precision Systems µOsmette Model 5004, Sudbury, MA).
Calculations from BIA Measurements were obtained as indicated below

**Fluid Retention (mL):**

\[ R_x = (TBW_x - TBW_{pre}) \times 1000 \]

where

- \( R \) is fluid retention (mL); \( TBW \) is total body water (L); \( x \) is time point in minutes (80, 160, 240); \( pre \) is pre-hydration (0 minutes)

**Fluid Loss (mL):**

- \( L_{80} = I - R_{80} \) (Volume of fluid loss from ingestion to 80 minutes post-ingestion)
- \( L_{160} = R_{160} - R_{80} \) (Volume of fluid loss from 80 minutes to 160 minutes)
- \( L_{240} = R_{240} - R_{160} \) (Volume of fluid loss from 160 minutes to 240 minutes)

where

- \( L \) is fluid loss (mL); \( I \) is total volume of fluid ingested (mL)
- \( R \) is fluid retention (mL)

**Statistical Analyses**

The SuperANOVA software package (Abacus Concepts, Berkeley, CA) was used to analyze all data. Descriptive data are expressed as means ± SD. The experimental design used was a repeated measures with comparisons being made between the subjects' results from their control and experimental trials at all four sampling times. A 2-within-factor analysis of variance (ANOVA), with experimental treatment being the first factor and sampling time the second factor, was used to determine if there were significant main
and interactive effects. Differences in fluid retention and fluid loss between measurement methodologies utilized were assessed using a 3-within-factor ANOVA, with experimental treatment being the first factor, sampling time the second factor, and methodology employed the third factor. Differences were considered significant when p ≤ .05. Multiple comparisons were performed to determine differences when significant main effects or interactions resulted. However, the number of multiple comparisons conducted adhered to the $k - 1$ restriction where $k$ equals the number of cell means for the main effect or interaction of interest.

Results

Thirteen subjects completed both the control and experimental trials. Two subjects were excluded from the study after vomiting during the hydration phase. Vomiting in one subject was triggered by the sweet taste of glycerol. Despite tolerating the entire fluid load (~2.7 L) during the experimental trial, the other subject vomited 48 minutes into the hydration phase during the control trial in response to the large amount of fluid ingested. Table 1 shows the descriptive data obtained.

Nude Body Weight

Increases in NBW were greater with glycerol ingestion. The trial x time interaction was significant ($F = 17.07, p = 0.0001$; Figure 1). Multiple comparisons revealed that NBW was significantly higher at 160 and 240 minutes in the glycerol versus the placebo trial. In the glycerol trial, NBW was significantly higher at 80, 160, and 240 minutes versus 0
minutes. However, in the placebo trial, NBW was significantly higher only at 80 and 160 minutes versus 0 minutes.

**Actual Fluid Retention (fluid intake – urine output)**

Fluid retention was greater with glycerol ingestion. The trial x time interaction was significant (F = 21.88, p = 0.0001; Figure 2). Multiple comparisons revealed that fluid retention was significantly greater at 160 and 240 minutes in the glycerol versus the placebo trial. In both trials the amount of fluid retained at 160 and 240 minutes was significantly lower versus 80 minutes.

**Urine Output (actual fluid loss)**

Urine output was lower with glycerol ingestion. The trial x time interaction was significant (F = 15.82, p = 0.0001; Figure 3). Multiple comparisons revealed that urine output was significantly lower at 160 minutes in the glycerol versus the placebo trial. Also, in the placebo trial urine output was significantly greater at 160 minutes versus 80 minutes.

**Total Body Water (BIA measure)**

Changes in TBW were not different between trials. The trial x time interaction was not significant (F = 1.43, p = 0.248; Figure 4). However, the main effect of time was significant (F = 11.03, p = 0.0001). Multiple comparisons revealed that, for both trials, TBW was significantly higher at 80 minutes versus 0 minutes.
Extracellular Water (BIA measure)

Changes in ECW were not different between trials. The trial x time interaction was not significant \((F = 0.61, p = 0.613; \text{Figure 5})\). However, the main effect of time was significant \((F = 7.91, p = 0.0004)\). Multiple comparisons revealed that, for both trials, ECW was significantly lower at 80, 160, and 240 minutes versus 0 minutes.

Intracellular Water (BIA measure)

The increase in ICW was greater with glycerol ingestion. The trial x time interaction was significant \((F = 2.90, p = 0.048; \text{Figure 6})\). Multiple comparisons revealed that ICW was significantly higher at 160 and 240 minutes in the glycerol versus the placebo trial. In the glycerol trial, ICW was significantly higher at 80, 160, and 240 minutes versus 0 minutes. In the placebo trial, ICW was significantly higher at 80 and 160 minutes versus 0 minutes.

Fluid Retention (actual vs. BIA)

Fluid retention as measured by the difference between fluid intake and urine output was higher than fluid retention as calculated by differences in TBW measured from the BIA. The trial x time x method interaction was not significant \((F = 0.50, p = 0.613)\). However, the time x method interaction was significant \((F = 3.60, p = 0.043; \text{Figure 7})\). Multiple comparisons revealed that fluid retention values calculated from BIA measures were significantly lower than actual fluid retention measures at 80, 160, and 240 minutes.
Fluid Loss (actual vs. BIA)

Fluid loss values were similar for both measurement methodologies. The trial x time x method interaction was not significant (F = 0.26, p = 0.772). However, the time x method interaction was significant (F = 22.38, p = 0.0001; Figure 8). Multiple comparisons revealed that fluid loss values calculated from BIA measures were significantly higher than actual fluid loss values at 80 minutes.

Percent Plasma Volume

Changes in plasma volume were not different between trials. The trial x time interaction was not significant (F = 0.93, p = 0.408; Figure 8). The main effect of trial was also not significant (F = 0.04, p = 0.836) or time (F = 0.22, p = 0.803).

Plasma Glycerol

Plasma glycerol concentration was higher with glycerol ingestion. The trial x time interaction was significant (F = 718.52, p = 0.0001; Figure 9). Multiple comparisons revealed that plasma glycerol concentrations were significantly higher at 80, 160, and 240 minutes in the glycerol versus the placebo trial. Also, the plasma glycerol concentrations showed a significant increase from 0 at all time points during the glycerol trial.

Plasma Osmolality

Plasma osmolality was elevated with glycerol ingestion. The trial x time interaction was significant (F = 714.6, p = 0.0001; Figure 10). Multiple comparisons revealed that plasma osmolality was significantly greater at 80, 160, and 240 minutes in the glycerol
versus the placebo trial. In the glycerol trial, plasma osmolality was significantly greater at 80, 160, and 240 minutes versus 0 minutes. In contrast, the plasma osmolality was significantly lower at 80, 160, and 240 minutes versus 0 minutes during the placebo trial.

**Urine Osmolality**

Urine osmolality was higher with glycerol ingestion. The trial x time interaction was significant ($F = 3.84, p = 0.018$; Figure 11). Multiple comparisons revealed that urine osmolality was significantly higher at 80, 160, and 240 minutes in the glycerol versus the placebo trial. Also, in both trials, urine osmolality was significantly lower at 80, 160, and 240 minutes versus 0 minutes.

**Discussion**

The use of glycerol as a hyperhydrating agent has been well documented in prior research (5, 7, 16, 17, 19, 20, 23, 28). However, the effects of glycerol hyperhydration on fluid compartment distribution remain inconclusive. The majority of prior research has simply used fluid retention and increases in total body weight to determine increases in TBW and has therefore speculated on the changes in ECW and ICW. The present study utilized a multi-frequency BIA system to examine fluid compartment shifts in response to glycerol hyperhydration in resting male subjects.

The data indicate that NBW and fluid retention were greater at 160 and 240 minutes in the glycerol versus the placebo trial thus verifying glycerol's hyperhydrating effect. Four hours after the hydration regimen started ~41% of fluid ingested was retained in the
glycerol trial compared to ~16% retention in the placebo trial. These findings are in agreement with previous studies on glycerol hyperhydration during resting conditions (5, 13, 16, 17, 19, 20, 23, 28), but conflict with the results obtained by Inder et al. (11) who did not show significant increases in NBW in response to glycerol ingestion. The failure to demonstrate an increase in body weight in their study was most likely due to the fact that subjects did not consume as large a dose of glycerol (1g/kg vs. 1.2g/kg) and volume of liquid (~9 ml/kg vs. 26 ml/kg) as the aforementioned studies.

In the studies by Latzka et al. (14, 15), glycerol hyperhydration produced no significant increases in fluid retention (as indicated by TBW measures derived from deuterium dilution) when compared to water hyperhydration. Their first post-hydration TBW measurement was taken 30 minutes after the hydration regimen was complete. The lack of any significant difference at this time point coincides with the findings of our study. Throughout the exercise heat-stress trials, which commenced at this time, still no differences in TBW were observed. Based on our findings in resting subjects it would appear that exercise and heat-stress had an equalizing effect on glycerol-induced fluid retention. The reduced diuresis that may accompany exercise and heat exposure is the most likely mechanism responsible (14).

Urine output was significantly lower at 160 minutes in the glycerol versus the placebo trial. At 80 and 240 minutes the observed differences were not statistically significant. This is in agreement with previous studies that have assessed urine output during glycerol hyperhydration under resting conditions (5, 13, 16, 17, 19, 20, 23, 28) and in
disagreement with that of Inder et al. (11). Obtaining an initial urine volume measure at 30 minutes pre-exercise, which equates to 3.5-h post-glycerol ingestion, in conjunction with a considerably lower fluid intake (~9 ml/kg vs. 26 ml/kg) may explain the discrepancy between the results of our study and those of Inder et al. (11). When cumulative urine volumes were expressed as a percentage of fluid intake we observed that 20, 43, and 21 percent of the fluid ingested was excreted at 80, 160, and 240 minutes, respectively, in the placebo trial. However, in the glycerol trial 22, 20, and 17 percent of the fluid ingested was excreted at 80, 160, and 240 minutes, respectively. The non-significant difference in urine output at 80 minutes is most likely due to the fact that the increase in plasma glycerol concentrations and the concomitant increase in plasma osmolality, the two most likely factors responsible for reduced diuresis during glycerol hyperhydration (24), did not peak until this time. The non-significant difference at minute 240 may be an indirect consequence of the considerable difference in urine output (859 ml vs. 401 ml, placebo and glycerol, respectively) shown at 160 minutes.

The most important findings of this study arise when comparing actual measured values of fluid retention and loss with those calculated from BIA measures of TBW. At all time-points, fluid retention values derived from differences in BIA measures of TBW were significantly lower than actual fluid retention measurements (fluid intake – urine output) for both trials. Although the trend in fluid retention was the same for both methods, BIA consistently underestimated the volumes involved. As this was the first study to utilize multi-frequency BIA to monitor changes in fluid compartment volumes in response to glycerol hyperhydration, it is difficult to determine the reasons for this
discrepancy. The possibility of measurement error resulting from the use of the BIA system cannot be entirely ruled out, although great care was taken to minimize the effects of confounding factors such as electrode placement, degree of limb abduction, and length of time the subject was supine prior to the measurement, as indicated in the instrument operation manual. Changes in the hydration states of subjects have been shown to adversely affect impedance measurements (25). Also, Bedogni et al. (11) reported that alterations in normal body water distribution adversely affected BIA measurements of water compartments in juvenile rheumatoid arthritis patients. According to Sharfetter et al. (29) altered ion concentration in response to insufficient water intake and eventual dehydration can affect ECW and ICW predictions by 1-2% and 4-5%, respectively. Increased ion concentrations can lead to overestimation of body water as a result of decreased resistance values. Our subjects were initially tested after a 12-h food and water fast. Initial plasma osmolality measures were 290±2.9 and 288±4.2 mOsm for the placebo and glycerol trials, respectively. The euhydration criteria (<286 mOsm) set by Latzka et al. (14) suggest that our subjects may have been slightly dehydrated. However, the obtained values for our subjects are within the normal range (280-290) and suggest normal hydration levels. Because plasma electrolyte concentrations were not measured in this study it is difficult to determine whether they may have compromised BIA measurements. According to Thomas et al. (30) the increased resistivity due to increased concentrations of physiologically relevant molecules such as glucose has not been studied in great detail; therefore, it is possible that the observed increase in plasma glycerol concentration may also be somewhat responsible for the underestimation of TBW. Also worth consideration as a possible source of measurement error is the fact that whole body
Impedance measurements derived using the wrist-ankle methodology are disproportionately sensitive to hydration changes in the limbs versus the trunk (30).

Although the BIA system inaccurately assessed the absolute volume of fluid retained, it did, in fact, accurately assess the volume of fluid loss at 160 and 240 minutes. The overestimation of fluid loss that occurred at 80 minutes may be a result of an inaccurate baseline (0 minutes) or 80-minute TBW measurement. An overestimation of TBW at baseline and/or an underestimation at 80 minutes would artificially inflate the fluid loss obtained at 80 minutes. Because the fluid loss values derived from BIA at 160 and 240 minutes (which are dependent on the 80-minute value) were not different from actual urine output measures, it is speculated that the baseline TBW measurement may be in error. The hydration state of the subjects as well as the wrist-ankle methodology employed may have influenced the initial TBW measurement for reasons mentioned above.

Plasma volume changes exhibited no significant differences over time or between trials. These results are in agreement with previous findings in resting subjects (5, 7, 9, 13, 16, 17, 20, 23, 28) but contradict others (6, 12, 19). Riedesel et al. (23) and Lyons et al. (17) hypothesized that the extra fluid which is retained during glycerol hyperhydration is selectively moved to the extravascular fluid compartments in response to an increase in osmolality which is a result of the movement of glycerol into these fluid compartments. However, Freund et al. (5) suggested that this explanation was rather tenuous considering the heightened serum glycerol concentrations and osmolality exhibited during glycerol
hyperhydration. Freund et al. (5) and Montner et al. (19) suggested that the failure to produce any significant changes in PV during glycerol hyperhydration may have been a result of the time course of PV measurements and insensitive Hct and Hb measurements. Gleeson et al. (6) reported a significantly greater increase in PV at rest 15, 30, and 45 minutes following the ingestion of glycerol. Based on our PV values it would seem that the timing is a relevant factor. The greatest difference between trials (1.44%) in percentage increases in PV that we reported occurred at the first sampling point, 80 minutes, which was obtained 75 minutes after glycerol ingestion. Had we sampled for PV immediately post-hydration (50 minutes) or sooner it is possible that we may have obtained results similar to Gleeson et al. (6). Also, assuming that glycerol has reached equilibrium throughout fluid compartments it is possible to calculate the volume difference in fluid retention in the vascular space based on the fact that blood volume represents only 12% of TBW. If the additional 489 mL retained during the glycerol trial were freely distributed throughout TBW, the calculated blood volume would increase only ~59 mL over values obtained during water hyperhydration. This small volume difference may be beyond the sensitivity limits of the measurement method employed. It is difficult to make a valid comparison between our findings with the findings of Murray et al. (21) because their glycerol ingestion and PV measures were carried out during exercise. The methodological differences of Montner et al. (19) also make comparisons difficult. In their study, the subjects ingested glycerol prior to and during exercise and they did not directly measure percentage changes in PV. Instead, they inferred an increase in PV based on a greater stroke volume obtained during exercise for the glycerol trial.
Plasma glycerol concentrations were significantly higher at 80, 160, and 240 minutes in the glycerol versus placebo trial as was expected. The glycerol concentration results are in agreement with prior research in resting subjects (5, 7, 13, 17, 19, 20, 23, 28). Plasma osmolality was significantly higher at 80, 160, and 240 minutes in the glycerol versus placebo trial. These findings are in accordance with prior research (5, 7, 12, 17, 19, 20, 23, 28), but in disagreement with others (11, 13). Inder et al. (11) did not offer an explanation to rectify the disparity between their findings and previous research. Perhaps the ingestion of the glycerol solution along with a meal minimized the expected osmolality differences. Koenigsberg et al. (13) speculate that their values, which are inconsistent with previous research conducted in their laboratory, may be due to error induced by sample storage or instrument calibration. Our plasma osmolality data seem to contradict our plasma volume data. If the osmotic drive of glycerol is responsible for increased water retention then one would expect that greater plasma osmolality would lead to greater water retention in the vascular space during glycerol hyperhydration. Statistically speaking, this did not occur in our study. However, there was a trend towards water retention in the vascular space resulting from glycerol ingestion. The relative changes in PV may have been beyond the sensitivity limits of the measurement method employed.

Urine osmolality decreased from pre-hydration values, as would be expected from the large volume of fluid intake. Urine osmolality was significantly higher at 80, 160, and 240 during the glycerol versus the placebo trial. These results concur with previous
research in resting subjects (5, 11, 23). The kidney’s reabsorb the majority of filtered glycerol at normal physiological serum glycerol levels (0.05 mM). When serum levels exceed 1.6 mM glycerol is excreted in the urine (31). Urinary excretion of glycerol increases in accordance with increasing serum glycerol levels and at serum levels between 15 and 20 mM urinary excretion of glycerol represents the largest component of total body glycerol clearance (24). Although urine glycerol concentrations were not measured in this study, the current plasma glycerol data support the contention that the greater urine osmolality values reported during the glycerol trial were mostly due to the presence of glycerol in the urine. Also, the excretion of sodium may have accounted for a small portion of the increase in osmolality (5, 9, 23).

In conclusion, this study was the first to utilize multi-frequency BIA to assess changes in body fluid compartments in response to glycerol hyperhydration. Based on the data collected and the protocol used it was concluded that glycerol serves as a more potent agent than water when attempting to hyperhydrate subjects at rest over an acute time period. This conclusion is based on changes in NBW and actual fluid retention data. However, when utilizing multi-frequency BIA to assess fluid retention, glycerol exhibits no difference in its ability to hyperhydrate when compared to water. This finding is not necessarily a reflection of glycerol’s hyperhydrating capability; rather, it is most likely a reflection of the wrist-ankle methodology employed and/or the BIA system’s prediction equations that are used to derive fluid compartment volumes. Modifying the formulae generated from euhydrated subjects for predicting body water compartments may reduce the degree of error involved. More importantly, except for the initial fluid loss
measurement the BIA system did accurately assess the loss of fluid over the 4-h time period, thus suggesting the validity of using multi-frequency BIA to track changes in fluid compartments over an acute period of time. The BIA system did not indicate any differences in ECW between trials. Despite the failure to detect any difference in TBW and ECW between trials, the BIA system did indicate a greater ICW at 160 and 240 minutes during the glycerol trial. This change is supported by previous research (16) that has shown that glycerol has no effect on ECW but leads to a greater increase in ICW. However, in the present study, this conclusion must be interpreted with caution considering the previously mentioned limitations of the BIA system and its current calculation methodologies, particularly using the wrist-ankle methodology. In the future, segmental BIA measurements should be obtained to minimize the error resulting from altered hydration status of the trunk and limbs. Also, isotopic dilution techniques (H2O and sodium bromide) for the measures of TBW, ICW and ECW should be employed to help develop accurate prediction equations for BIA use in populations that have altered hydration levels and ECW-ICW ratios. The development of hydration-specific equations and/or adjustments would increase the versatility of this measurement technique.
References


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31. Tourtellotte W.W., J.L. Reinglass, and T.A. Newkirk. Cerebral dehydration action of
glycerol: historical aspects with emphasis on the toxicity and intravenous
Figure Captions

Table 1. Descriptive data of subjects (mean ± SD)

Figure 1. Nude body weight changes in response to treatments (*p<0.05 vs. placebo; \(\ddot{p}<0.05\) vs. glycerol 0; \(\Delta p<0.05\) vs. placebo 0)

Figure 2. Changes in actual fluid retained (ml) calculated from the change in fluid intake - urine output. (*p<0.05 vs. placebo; \(\ddot{p}<0.05\) vs. glycerol 80; \(\Delta p<0.05\) vs. placebo 80)

Figure 3. Differences in urine output (ml) in response to treatments (*p<0.05 vs. glycerol; \(\ddot{p}<0.05\) vs. placebo 80)

Figure 4. Differences in TBW (L) as measured with BIA (*p<0.05 vs. 0; main effect of time)

Figure 5. Differences in ECW in response to treatments (*p<0.05 vs. 0; main effect of time)

Figure 6. Differences in ICW in response to treatments (*p<0.05 vs. placebo; \(\ddot{p}<0.05\) vs. glycerol 0; \(\Delta p<0.05\) vs. placebo 0)
Figure 7. Differences in fluid retained (mL) between actual values (fluid intake - urine output) and values calculated from BIA measures of TBW (*p<0.05 vs. actual)

Figure 8. Differences in fluid loss (mL) between actual values (urine output) and values calculated from BIA derived values of fluid retention ("p<0.05 vs. actual)

Figure 9. Changes in plasma volume (%) in response to treatments

Figure 10. Differences in plasma glycerol concentrations in response to treatments
(*p<0.05 vs. placebo; †p<0.05 vs. glycerol)

Figure 11. Differences in plasma osmolality (mOsm) in response to treatments (*p<0.05 vs. placebo; †p<0.05 vs. glycerol; Δp<0.05 vs. placebo)

Figure 12. Differences in urine osmolality (mOsm) in response to treatments
(*p<0.05 vs. placebo; †p<0.05 vs. glycerol; Δp<0.05 vs. placebo)
Table 1.

<table>
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<td>Total Fluid Intake (L) – glycerol</td>
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</tbody>
</table>
Figure 1.

[Graph showing changes in nude body weight (kg) over time (min) for placebo and glycerol groups. Symbols indicate significant differences between conditions.]

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Figure 3.

[Graph showing urine output over time for placebo and glycerol treatments, with error bars indicating variability.]

* * Coucho Placebo
** Glycerol

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Figure 4.

![Graph showing Total Body Water (kg) over Time (min) for placebo and glycerol treatments.](image-url)

- O placebo
- □ glycerol

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Figure 5.
Figure 6.

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Figure 7.
Figure 8.
Figure 9.
Figure 10.

Plasma glycerol (mM) vs. Time (min) for placebo and glycerol treatments.
Figure 11.

Plasma Osmolality (mOsm)

Time (min)

- Placebo
- Glycerol

* p < 0.05 vs baseline
† p < 0.01 vs baseline

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Figure 12.
Appendix III

Statistical Results
### Nude Body Weight

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Dependent: body weight (L)

#### Means Table

Effect: Trial * time
Dependent: body weight (L)

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#### Actual Fluid Retained (fluid intake – urine output)

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Dependent: fluid retained (ml)
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Effect: trial * time
Dependent: fluid retained (ml)

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Urine Output

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Dependent: urine output (ml)

Means Table
Effect: trial * time
Dependent: urine output (ml)

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## Total Body Water

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Dependent: TBW (L)

### Means Table

**Effect: time**  
Dependent: TBW (L)

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**Means Table**  
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Dependent: TBW (L)

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## Extracellular Water

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Dependent: ECW (L)

### Means Table

**Effect: time**

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### Means Table

**Effect: Trial * time**

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### Intracellular Water

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Dependent: ICW (L)

#### Means Table

**Effect: Trial * time**

Dependent: ICW (L)

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Dependent: fluid retained (actual vs. BIA)
## Means Table

**Effect: time * method**  
**Dependent: fluid retained (actual vs. BIA)**

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## Means Table

**Effect: trial * time * method**  
**Dependent: fluid retained (actual vs. BIA)**

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## Plasma Volume

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Dependent: $\Delta$'s in plasma volume (%)

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### Means Table

Effect: trial * time

Dependent: Δ's in plasma volume (%)

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### Plasma Glycerol

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Dependent: plasma glycerol (mM)

### Means Table

Effect: Trial * time

Dependent: plasma glycerol (mM)

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### Plasma Osmolality

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Dependent: Plasma osmolality

#### Means Table

**Effect:** Trial * time

**Dependent:** Plasma osmolality

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### Urine Osmolality

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Dependent: Urine osmolality
Means Table
Effect: Trial * time
Dependent: urine osmolality

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