Comparing Hyperhydration Ability between a Glycerol Solution and a Sodium Solution on Dehydration and Performance in Runners

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The purpose of this study was to examine how inducing hyperhydration with a glycerol solution and a sodium solution prior to exercise influenced performance and hydration status during a moderate set-intensity exercise bout followed by a time-to-exhaustion bout when compared to a control solution containing water only. Six well-trained runners ages 21 to 38 with an average relative VO$_2$ peak of 57 ml/kg/min participated in three trials. Each trial included a 2.5 hour hyperhydration phase where participants ingested a solution containing water, glycerol, or sodium. Following hyperhydration, participants ran for 90 minutes at 65% of their HRR followed by a time to exhaustion bout at 85% of their HRR. There were no significant differences in performance or changes in total body water between solutions. There was a lower relative percentage of total urine output from sodium (p=0.001) and glycerol (p<0.01) when compared to water. There was less weight loss from initial body weight to post-exercise weight when sodium (p=0.02) and glycerol (p=0.029) were consumed compared to water alone. While sodium appears to be as effective as glycerol in inducing hyperhydration, there were no performance differences when participants induced hyperhydration with glycerol, sodium, or water.

INDEX WORDS: Hyperhydration, Endurance, Running, Sodium, Glycerol, Performance
COMPARING HYPERHYDRATION ABILITY BETWEEN A GLYCEROL SOLUTION AND A SODIUM SOLUTION ON DEHYDRATION AND PERFORMANCE IN RUNNERS

by

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DEDICATION

I would like to dedicate this thesis to my parents and Tom, because without their support, I would not have made it as far as I did.
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CHAPTER 1
INTRODUCTION

It has been shown that dehydration greater than 2% of bodyweight can hinder endurance performance and increase cardiovascular strain, especially in hot environments (Goulet, 2012). For many athletes, maintaining euhydration is not always practical during a competition, but without extra fluids, dehydration will eventually set in and likely hinder performance as the competition continues. Since maintaining euhydration and preventing dehydration is important to performance, putting an athlete into a hyperhydrated state before competition has been shown to delay the time to dehydration, which will theoretically improve performance (Anderson, Cotter, Garnham, Casley, & Febbraio 2001). Hyperhydrating prior to exercise with water alone has been shown to be an ineffective method because most of the water will quickly be excreted in the urine (Mehmet & Husrev, 2011). However, when glycerol is added to water, it has been shown to be an effective hyperhydration agent (Anderson et al., 2001; Marino, Kay, & Cannon 2003). This could be due to its ability to reduce diuresis, and therefore increase fluid retention (Robergs & Griffin, 1998).

Glycerol (1,2,3-propanetriol) is a 3-carbon alcohol that occurs naturally in the body and is also the backbone of triglyceride molecules. At rest, glycerol concentration is approximately 0.05 mmol/L, but serum concentrations can rise to approximately 20 mmol/L when ingested in amounts greater than 1.0 g/kg of body weight (BW). Glycerol has a selective permeability, and is evenly distributed between intracellular fluid compartments and extracellular fluid compartments. The osmotic gradient that glycerol
induces as it is being absorbed, by increasing solute concentrations within the fluid compartments compared to outside compartments, is what helps promote hyperhydration (Nelson & Robergs, 2007).

Melin, Jimenez, Koulmann, Allevard, & Gharib (2002) showed that when using a glycerol hyperhydration protocol, an average of 77% of ingested fluid was retained. This study was different from others because it did not use an exercise protocol, only a hyperhydration period. Subjects were either euhydrated or hyperhydrated. Hyperhydration was induced by consuming 1.1 g/kg BW glycerol in ~256mL of fluid in 1 minute followed by consumption of mineral water containing 1.2 g/L of NaCl in 3 separate equal intakes every 20 minutes. This equated to 21.4 mL/kg of BW. Glycerol hyperhydration increased body weight by approximately 2.5 pounds after the 2-hour hyperhydration protocol, which went down to a 1.2 pound gain after 90 additional minutes. The control group lost an average of 0.6 pounds after 2 hours, and after an additional 90 minutes that increased to a 0.9 pound loss, which was significant (Melin et al., 2002).

Many studies have examined the benefits of using glycerol to put athletes into a hyperhydrated state prior to exercise. The recommended amount to induce hyperhydration ranges from 1.0-1.5 g glycerol/kg BW. There has been a general consensus among many studies that glycerol leads to an increase in total body water and a reduction in urine output (Anderson et al., 2001; Marino et al., 2003); however, there have been conflicting reports about whether glycerol hyperhydration will actually improve performance (Marino et al., 2003; Mehmet & Husrev, 2011; Sheadler, Garver, Kirby, & Devor, 2010). These differences could be due to different protocols regarding
the amount of glycerol consumed, temperature of exercising conditions, or the duration of
the exercise trial. Some studies have shown slight performance improvements (Anderson
et al., 2001; Patlar, Yalcin, & Boyali, 2012), while others have shown only a delay in
time to reach dehydration, but not a statistically significant improvement in performance

In 2010, glycerol made the banned list of the World Anti-Doping Agency
(WADA) as a masking agent due to its ability to expand plasma volume. This could make
it harder to detect banned substances due to diluting the plasma (WADA, 2012). While
this may not pose a problem to some athletes, such as recreational marathon runners, it
could pose a problem to athletes that play for a college or professional team. Because of
this, looking at hyperhydration using a sodium solution may be beneficial to athletes who
must follow WADA policies. This could offer the benefits that glycerol hyperhydration
has been shown to provide, but without using a banned substance. In one study, subjects
consumed a sodium solution drink containing 130 mmol/L of aspartame-flavored sodium
solution (7.5g NaCl) in 26 mL fluid/kg of BW 110 minutes prior to exercise, in three
equal doses every 20 minutes for the first hour. Results suggested that a sodium solution
provided fluid retention comparable to the glycerol technique (Gigou, Dion, Asselin,
Berrigan, & Goulet, 2012). This study also suggested that future research would need to
directly compare sodium to glycerol before any recommendations can be established.
Other research has found that drinking fluids with a higher sodium concentration than
what is found in a typical sports drink before exercise can improve physiological status
and exercise capacity in warm conditions (Sims, Rehrer, Bell, & Cotter, 2007a).
It has been well established that glycerol enhances total body water to help maintain hydration. However, the conflict in literature regarding the effects of glycerol hyperhydration on improving performance has yet to be determined. If it can be shown that a sodium solution has a similar hyperhydration effect as a glycerol solution, this could be beneficial to endurance athletes that are banned from using glycerol. It is crucial for athletes to avoid dehydration while performing, so it is important to find strategies they can utilize to help maximize hydration.

Additionally, delayed gastric emptying can be very uncomfortable for some athletes, which is why some endurance runners may not consume adequate fluids while running due to gut discomfort. This would lead to quicker rates of dehydration due to inadequate intake of fluids during exercise, and possibly performance decrement. However, increased volume can help promote gastric emptying at rest (Gropper, Smith, & Groff, 2009). Therefore, if a runner is able to properly induce hyperhydration with enough time prior to an event, it may allow them to stay properly hydrated during the event, while avoiding GI distress that may emerge if smaller volumes of liquid are consumed while running.

**Purpose Statement**

The purpose of this study was to examine how inducing hyperhydration with a glycerol solution and a sodium solution prior to exercise influenced performance and hydration status during a moderate set-intensity exercise bout followed by a time-to-exhaustion bout when compared to a control solution containing water only.
**Hypothesis**

It is hypothesized that the glycerol solution will pose the greatest benefit to increasing total body water and performance compared to the sodium solution and water, but sodium solution will result in greater benefits compared to water.

**Research Questions**

1. Which solution will have the lowest urine output pre-exercise?
   - Hypothesis: Glycerol hyperhydration will have the lowest urine output pre-exercise.

2. Which solution will have the greatest total body water retention pre-exercise?
   - Hypothesis: Glycerol hyperhydration will have the greatest total body water retention.

3. Which solution will allow for the greatest retention of water (TBW) by the end of the run?
   - Hypothesis: Glycerol hyperhydration is expected to have the greatest retention of total body water post-run.

4. During which method was the greatest performance (as measured by time-to-exhaustion performance bout) seen?
   - Hypothesis: The greatest performance is expected to occur with glycerol hyperhydration, if there are no negative side effects. If negative side effects, such as gut discomfort, occur with glycerol, it is believed that sodium hyperhydration will lead to a greater performance compared to water.
5. Which solution had the fewest number of negative side effects (ie: GI distress)?

   o Hypothesis: Sodium will have the fewest number of negative side effects.

**Rationale**

To date, no studies have directly compared glycerol and sodium loading protocols on the effects of hyperhydration in runners. Examining these two solutions under the same conditions using identical protocols would help to determine whether there is any significant difference on performance or hydration status.
CHAPTER 2
LITERATURE REVIEW

Introduction

Hydration plays a crucial role on physiological parameters within the body. It is important to understand how different levels of hydration including, dehydration, euhydration, and hyperhydration, impact performance and physiological strain. While being properly hydrated prior to the start of a competition may be enough for some athletes, it may not be enough for those exercising for more than 1.5 hours, if they wish to have less physiological strain and a chance at maintaining initial performance levels. It has been stated that hyperhydration has no additional benefit over maintaining hydration during an event (Magal, Cohen-sivan, Heled, 2005); however, it isn’t always practical for athletes to maintain hydration during an event. For example, starting athletes may stay on the field through the entire game, and may only get half time as a chance to hydrate. Another example can be found in endurance athletes, such as marathon runners or triathletes. Even if the opportunity is available to consume fluids during the race, they may find it uncomfortable to run with fluids in their stomach, or they may not want to add time by stopping at a water station. This is why it can be important for some athletes to hyperhydrate prior to exercise.

There are still mixed reviews on whether hyperhydration with glycerol can improve performance, but it has been shown to increase total body water and reduce urine output (Anderson et al., 2001; Marino et al., 2003; Nishijima et al., 2007). Studies involving longer duration exercise have a greater chance of supporting the theory of
improved performance. Unfortunately, glycerol was banned in 2010 by WADA, so some athletes are no longer able to consume glycerol. This is when the idea of hyperhydrating with a sodium solution became an area of interest. Although glycerol and sodium have not been directly compared, past research has examined hyperhydration with sodium and indicates the results may be comparable to the effects of glycerol (Gigou et al., 2012).

Hyperhydration has become a popular research topic. A general consensus has still not been formed on hyperhydration recommendations, which is probably due to numerous discrepancies in methodology designs. As more studies are designed, and similar protocols are followed, perhaps some hyperhydration guidelines can be established.

**Measuring Hydration Status**

Many measurements can be used to assess level of hydration, but none can be used alone to completely measure hydration status (Armstrong, 2007). There is some agreement that total body water, along with plasma osmolality, provides the “gold standard” for measuring hydration status; however, this is likely dependent based on the situation, and is more accurate under controlled laboratory conditions. Hydration status can be obtained by using blood measures, such as plasma osmolality and percent plasma volume change. Urine measurements for hydration include urine osmolality, urine specific gravity, urine color and 24-hour urine volume. Other ways to measure hydration include bioelectrical impedance spectroscopy, body mass change, salivary flow rate, and rating of thirst (Armstrong, 2007).
Effects of General Hydration on Performance and Physiological Markers

Performance has been shown to decrease when water loss is greater than 2% of body weight. Similar studies have found that in warm conditions, post-run heart rate and core body temperatures are higher in dehydrated groups versus hydrated groups (Casa, Stearns, Lopez & Ganio, 2010; Lee, Nilo, Lim, Teo & Byrne, 2010; Lopez, Casa, Jensen, DeMartini, Pagotta, Ruiz, Roti, Stearns, Armstrong, & Maresh 2011). Casa et al. (2010) found that there were no performance differences during submaximal trials; however, there were significant increases in performance during the “all-out” 12km run, in the hydrated group (p=.001). Lopez et al. (2011) found that completion time of the run was significantly slower in the dehydrated group. Lee et al. (2010) suggested that based on results, there could be a possible implication that physiological responses may only be significantly influenced by fluid intake in hypohydrated individuals, and not euhydrated individuals, but it is still important for euhydrated individuals to maintain hydration status. These results demonstrate how dehydration increases the physiological strain on the body and support the importance of proper hydration.

In a study performed by Hillman, Vince, Taylor, McNaughton, Mitchell, & Siegler (2011), it was found that when exercise-induced dehydration occurred, there was an increase in oxidized glutathione (GSSG) concentration, which is a marker of oxidative stress. When subjects maintained euhydration, it helped lessen the rate of increase of GSSG. Dehydration and exercise resulted in an increase of cellular and oxidative stress, emphasizing the importance of euhydration to reduce thermal and oxidative stress during extended exercise in heat.
Hyperhydration and Performance with Glycerol

Glycerol turnover and blood glycerol clearance are both influenced by the ingestion of glycerol. During times when higher amounts of glycerol are ingested (1.0-1.5 g/kg BW), glycerol turnover may increase to a rate of 0.7 g/kg/hr (Robergs & Griffin, 1998).

A review article on glycerol consumption by Robergs & Griffin (1998) found that glycerol consumption did not cause many adverse reactions. However, some studies reported GI distress, headache, and dizziness following glycerol consumption (Gleeson, Maughan, & Greenhalff, 1986; Murray, Eddy, Paul, Seifert & Halaby, 1991), though this was not consistent in the literature. Differences in administration protocols could explain this discrepancy. Gleeson et al. (1986) administered 1 g/kg of BW of glycerol to subjects 45 minutes prior to exercise, while Murray et al. administered a 10% glycerol solution during the first 60 minutes of exercise.

In a study by Melin et al. (2002), glycerol hyperhydration was shown to be effective with an average of 77% of the ingested fluid being retained. This study did not use an exercise protocol, just a hyperhydration period, but significant results were observed. Two hours post-hyperhydration period, the experimental group gained 2.5 pounds while the control group lost 0.6 pounds.

Hyperhydration protocols vary between studies. In a study examining endurance trained athletes using cycling in warm conditions, 1g glycerol/kg BW in 20mL/kg BW diluted lemon-orange sweetener was consumed by the experimental group, while the control group had a placebo (Anderson et al., 2001). Subjects began hyperhydration 120 minutes prior to exercise, then cycled for 90 minutes at a workload that corresponded to
98% of their lactate threshold, followed by a 15-minute performance trial. The pre-exercise urine measurements showed the glycerol group had lower urine volume (p<.05) and higher urine osmolality (p<.05) than the control group (Anderson et al., 2001).

Similarly, in a different study, Marino et al. (2003) gave subjects 2.5 hours to consume 1.2 g/kg BW glycerol in unsweetened, concentrated orange juice to induce glycerol hyperhydration, while the control group just had orange juice. Pre-exercise urine output was significantly lower (p<.05) for the glycerol trial versus the placebo trial. In another study by Mehmet & Husrev (2011), 1.2 g/kg of BW of glycerol in 20 mL/kg of BW of fluid was administered 105 minutes before the exercise trial. Plasma osmolality was significantly lower (p<.05) in the glycerol group following supplementation than before supplementation or at the end of exercise, in comparison to the groups with sports drink or water; however, plasma and blood volumes were not different between glycerol and the other two trials. While glycerol wasn’t superior to water or a sports drink in this study, glycerol hyperhydration did prevent dehydration and maintain euhydration in participants (Mehmet & Hursev, 2011). In a study that administered glycerol differently, the glycerol solution (20% of drink weight and equal to 1.2 g/kg of BW) was administered as a bolus and was consumed within 30 minutes. To then induce hyperhydration and raise total fluid consumption after the glycerol bolus administration, subjects had 1 hour and 20 minutes to consume an amount of water that would bring total fluid consumption to 26mL/kg BW. Hyperhydration started 2 hours and 20 minutes prior to the performance run. Although urine outputs were higher in the placebo trial versus the glycerol trial, and glycerol supplementation did result in greater fluid retention and
weight gain than the placebo, neither of these results reached statistical significance (Scheidler et al., 2010).

In contrast to other studies that used only one method of hyperhydration, Nishijima et al. (2007) sought to determine whether a glycerol solution (1.2 g/kg BW) in 25 ml/kg of water or a high-concentration glycerol (1.0 g/kg BW) bolus in 8 ml/kg BW of water followed by additional fluid, was more effective at hyperhydration. Subjects had 60 minutes to consume either 25 mL/kg of BW of water (control) or 1.2 g/kg of BW glycerol mixed in 25 mL/kg of BW of water (glycerol trial). The glycerol bolus trial was administered differently. Subjects had 90 minutes to complete this protocol, starting with 1.0 g/kg BW glycerol in 8 mL/kg BW of water, followed by intervals of additional water in amounts of 4 ml/kg BW, with minute 60 including 0.2 g glycerol /kg of BW in 5 ml/kg of BW of water. While the glycerol bolus protocol had higher increases in bodyweight and lower urine output than the regular glycerol protocol, these results were not significant. The glycerol trial and glycerol bolus trial had significantly higher values of body weight gains (p<.01) and reduced urine output (p<0.01) than the water trial. The glycerol bolus protocol was then administered to determine the effect of glycerol loading on dehydration. Following exercise, the glycerol group had significantly higher body weight (p<.01) than the placebo group (Nishijima et al., 2007).

Despite studies positivity showing that glycerol induces hyperhydration, many do not show an improvement in performance. One study, involving seven well-trained individuals, tested hyperhydration prior to a cycling test where the goal was to cover as much distance as possible in 60 minutes. Subjects had two trials, one with a placebo drink, and one with the glycerol drink. They had 2.5 hours to consume 1.2 g/kg BW of
glycerol in concentrated, unsweetened orange juice mixed with 21 mL/kg BW of water. The placebo drink was the same, except for the glycerol. Results found no significant difference in power output or distance covered between the glycerol group and placebo group (Marino et al., 2003). In a study involving three 90-minute treadmill runs in hot conditions, nine elite long distance runners had the opportunity to consume three different solutions on three separate occasions. They were given either 1.2 g/kg of BW glycerol, a diluted sports drink, or distilled water, with all solutions in 20 mL/kg of BW of water. The glycerol solution didn’t show any advantage over a sports drink or water in improving endurance performance. Hyperhydration occurred 105 minutes prior to the exercise trial and fluid was divided into 3 equal parts, administered every 30 minutes (Mehmet & Hursev, 2011). Another study showed a similar outcome to the study performed by Mehmet. Subjects began consuming a glycerol (1.2 g/kg of BW) or placebo solution 2 hours and 20 minutes prior to exercise. They had 30 minutes to consume the solution, and then were given 1 hour and 20 minutes to bring total water ingestion to 26 mL/kg of BW. Results found that glycerol hyperhydration had no benefit over hyperhydration with only water in improving endurance running performance in a hot and dry climate (Scheidler et al., 2010).

There have been studies using glycerol hyperhydration that have shown actual improvement in performance (Anderson et al., 2001; Nishijima et al., 2007). Anderson et al. (2001) recruited six endurance-trained males to perform a steady state cycle exercise at 98% of their lactate threshold followed by a 15-minute performance trial. Subjects performed two trials, once with a glycerol solution and once with a placebo solution. Glycerol hyperhydration was induced by subjects consuming 1 g glycerol/kg of BW in 20
ml/kg of BW diluted in a low calorie lemon-orange sweetener. The glycerol group, compared to the control group, performed a significantly higher amount of work that equated to a 5% increase in exercise performance (Anderson et al., 2001). In another cycling study, with six well-trained participants, consuming a glycerol bolus followed by consumption of water induced glycerol hyperhydration. Total glycerol consumption amounted to 1.2 g/kg of BW and total water was 25 ml/kg of BW. Subjects had 90 minutes to consume either the glycerol drink or control drink, and then began the 70 minute cycling exercise 90 minutes after the hydration phase ended. The glycerol group increased workload by 9% during a variable power phase of cycling; however, the results were not statistically significant (Nishijima et al., 2007).

Most studies using glycerol hyperhydration use long duration exercise. However, Patlar et al. (2012) performed a very different protocol, using glycerol hyperhydration for exercise bouts under 10 minutes. 40 males participants performed an anaerobic power test (Wingate Anaerobic Power Test) and aerobic capacity test (Astrand Cycle Ergometer Test) after receiving a glycerol or placebo solution to become hyperhydrated. Subjects were divided into four total groups. There were two main groups, exercise and sedentary, then each main group was subdivided into the placebo or glycerol group. Groups assigned to the glycerol group were given 1.2 g/kg of BW of glycerol in 26 ml/kg of BW of water, while the placebo group was given only 26 ml/kg of BW of water. The glycerol-exercise group had significantly higher anaerobic power, aerobic power and improved the time trial performance when compared with the other three groups (Patlar et al., 2012).

Performance differences with glycerol hyperhydration could be due to different protocols and methodologies. Unlike the consistent guideline of using 1-1.5 g/kg BW of
glycerol to induce hyperhydration, no established guidelines have been set for performance protocol variables such as: differences between time-trial protocols or time-to-exhaustion protocols, amount of time needed before exercise to induce hyperhydration, how long duration of exercise must be to see possible improvements in performance, and how climate and temperature impacts glycerol hyperhydration.

**Hyperhydration and Performance with Sodium**

A few studies have shown hyperhydration with sodium to be effective (Gigou et al., 2012; Sims et al., 2007a,b). However, the benefits of sodium hyperhydration on performance were not consistent. Gigou et al. (2012) found no difference in performance, while Sims et al. (2007a) showed sodium hyperhydration to have a positive influence on performance.

Gigou et al. (2012) used six male participants who performed an 18km (11.2 miles) time-trial in a euhydrated state and hyperhydrated state. During the euhydrated trial, subjects arrived properly hydrated and were given no liquid 110 minutes before exercise. In the hyperhydrated trial, subjects drank 26 mL/kg of BW of fluid of a 130 mmol/L (7.5g NaCl) sodium solution over 60 minutes, administered in 3 equal increments of 6.5 mL/kg of BW every 20 minutes to induce hyperhydration, followed by a 50 minute waiting period prior to exercise. Performance was not different between trials; however, the hyperhydrated state had lower cardiovascular and thermoregulatory stress. Based on their results, this study suggested that sodium could be as beneficial as glycerol in preventing fluid loss. Another important finding of this study was that a weight gain of 1 kg (2.2 pounds) did not impact running speed (Gigou et al., 2012).
Sims and colleagues conducted two individual studies (2007a,b) on men and women using sodium hyperhydration in the heat. In both the male and female study performed by Sims et al. (2007a,b), subjects had 1 hour to consume a high or low sodium solution of 10 mL/kg of BW, given in 7 equal amounts every 10 minutes. The high-sodium loading protocol consisted of sodium citrate (7.72 g/L) and sodium chloride (4.5 g/L).

Sims et al. (2007a) found that time-to-exhaustion in female cyclists was longer in the group that consumed the high sodium drink to hyperhydrate versus the low sodium drink. It also demonstrated that a higher sodium concentration drink could improve physiological status and reduce thermoregulatory strain. An important finding of this study in women was that exercise capacity was increased with sodium loading during the high-hormone phase of the menstrual cycle. Increased exercise capacity was indicated by a reduced perceived exertion rate and a greater time to exercise exhaustion during a 70% VO$_2$ max cycling trial (Sims et al., 2007a).

In another study conducted by Sims, van Vilet, Cotter, & Rehrer (2007b), male participants were asked to run at 70% of their VO$_2$ max until exhausted. It was shown that sodium hyperhydration reduces physiological and thermoregulatory strain in warm conditions and increases resting plasma volume (Sims et al., 2007b). Because sodium hyperhydration in athletes is a new topic of research, more studies must be performed before a standardized sodium protocol can be recommended.
Effect of Weight Changes on Running Performance

There is some conflict in research as to whether or not a change in body weight can impact performance. While it has been reported that dehydration resulting in >2% weight loss can hinder performance (Goulet, 2012), it has also been shown that an exercise-induced weight loss can increase running speed in ultra-marathoners (Knechtle, Knechtle, Wirth, Rust, & Rosemann, 2012). Similarly, Zouhal, Groussard, Minter, Vincent, Cretual, Gratas-Delamarche, Delamarche, & Noakes (2011) found that those who had higher levels of body weight loss had the fastest marathon times. Based on these findings, it would seem that a hyperhydration-induced weight gain would negatively influence performance. However, Gigou et al. (2012) found that a sodium hyperhydration induced weight gain of 1 kg does not impact running speed.

Gastric Emptying and Gastrointestinal (GI) Effects of Glycerol and Sodium

Gastric emptying can be affected by numerous factors, including volume, osmolarity, and chemical composition (macronutrient distribution). Higher volume promotes gastric emptying while an increased osmolarity will delay emptying. In addition, sodium, monosaccharaides and an acidic pH can slow down the rate of gastric emptying (Gropper et al., 2009). Slower gastric emptying could negatively influence runners by giving them GI discomfort while running.

Goulet (2009) reported that no study had assessed the rates of gastric emptying and intestinal absorption of glycerol consumption in humans. A majority of study participants do not experience any adverse effects to glycerol consumption. In a cycling study conducted by Marino et al. (2003), it was reported that none of the seven subjects
experienced any feelings of nausea, headache, or stomach fullness. However, it is possible that subjects can have adverse reactions to glycerol consumption. Anderson et al. (2001) reported that two out of six subjects experienced diarrhea 24 hours post-trial. Nishijima et al. (2007) reported that nearly all of the six subjects experienced drowsiness during glycerol fluid intake, but there were no major complaints.

While sodium can slow down the rate of gastric emptying (Gropper et al., 2009), studies involving sodium hyperhydration (Gigou et al., 2012; Sims et al., 2007a,b) have not reported adverse effects or GI distress that could be due to delayed gastric emptying. Gigou et al. (2012) reported that there was no significant difference in feelings of dizziness, nausea, and abdominal bloating between the sodium hyperhydrated group and the normal hydration group. Conversely, in a gastric emptying study performed by Miller, Mack, & Knight (2010), most of the subjects reported GI distress after consuming 7 mL/kg BW of pickle juice, which had a significantly higher sodium concentration than the deionized water used as a control. However, while subjects did report GI distress, they also reported that they were not extremely nauseous throughout the study.

**Physiological Parameters with Hyperhydration**

When examining physiological parameters during hyperhydration, heart rate is most commonly used, with studies reporting varied findings. During glycerol hyperhydration, some studies showed no difference in heart rate between the glycerol hyperhydration group and the placebo (Mehmet & Hursev, 2011; Nishijima et al., 2007; Scheadler et al., 2010). One study showed a lower heart rate in the glycerol group compared to the control group (Anderson et al., 2001). While another study showed that
overall heart rate during the trial was not different between the glycerol group and placebo group at numerous individual time spots during the trial, heart rate was significantly (p<.05) higher in the glycerol group than the placebo group (Marino et al., 2003). Similar to glycerol hyperhydration, sodium hyperhydration has varied results regarding changes in heart rate during exercise. Heart rate was not different between the sodium hyperhydration group and the euhydrated group in a study performed by Gigou et al. (2012). In addition, Sims et al. (2007b) found no heart rate differences between a high sodium group and a low sodium group; conversely, Sims et al. (2007a) showed that the higher sodium group had a lower heart rate in comparison with the low sodium group.

While hyperhydration protocols with either glycerol or sodium have shown some benefit, to date, there is limited research directly comparing glycerol and sodium. Based on results of past research, it has been noted that sodium could possibly have the same benefits as glycerol (Gigou et al., 2012), but it is impossible to conclude until research directly compares the two substances using the same subjects and methodology.

**Temperature and Humidity**

When comparing studies performed in a lab, many used similar temperatures for testing glycerol hyperhydration. Temperatures ranged from 30-35°C (86-95°F) and humidity percentage varied from 25-63.4% humidity (Anderson et al., 2001; Marino et al., 2003; Mehmet & Hursev, 2011; Nishijima et al., 2007; Scheadler et al., 2010). The indoor cycling studies include Anderson et al. (2001), Marino et al. (2003), and Nishijima et al. (2007). Temperatures ranged from 30.3-35°C, and humidity ranged from 30% to 63.4%. The indoor running studies include Mehmet & Hursev (2011) and Scheadler et al.
(2010). Both studies were performed in 30°C; however, the humidity percentage in Mehmet et al. was 25-35%, while Scheadler et al. used 50% humidity.

In the studies using sodium hyperhydration (Gigou et al., 2012; Sims et al., 2007a,b), Gigou et al. (2012) had runners perform on a treadmill in a temperature of 28°C with 20-30% humidity, while Sims et al. (2007a) had participants on a cycling ergometer in a temperature of 32°C and 50% humidity.

While humidity percentages have a much wider range in studies, it is common to use warmer temperatures when testing hyperhydration.

**Experimental Trial Duration**

Typically, studies varied in length during the experimental trials, ranging from 60 minutes to 99 minutes (Anderson et al., 2001; Gigou et al., 2012; Marino et al., 2003; Mehmet & Hursev, 2011; Nishijima et al., 2007; Scheadler et al., 2010), with some participants going longer. During the study by Sims et al. (2007a), numerous participants went over 100 minutes during parts of the study, which examined time-to-exhaustion, while during another study done by Sims et al. (2007b), at least half of the participant times were under 60 minutes. Some studies had set times (Marino et al., 2003; Mehmet et al., 2011; Nishijima et al., 2007), while others were based on set distances (Gigou et al., 2012; Scheadler et al., 2010; Sims et al., 2007a,b), and one combined a set time with a performance trial (Anderson et al., 2001).

Longer endurance experimental trials are common in hyperhydration studies. However, there is still variation between studies regarding exercise being performed for a set time or exercise being performed until subjects reach a certain distance.
Maximal Oxygen Consumption (VO₂ Max) Testing

Maximal oxygen consumption testing is commonly done at the prior to the start of hyperhydration studies. Some studies use VO₂ max (Mehmet & Husrev, 2011; Nishijima et al., 2007; Sims et al., 2007b) while other studies use VO₂ peak (Anderson et al., 2001; Gigou et al. 2012; Marino et al., 2003; Scheadler et al., 2010; Sims et al., 2007a). The variables obtained from a VO₂ test are typically used to help determine running intensity as a percentage of max heart rate or a percentage of workload. While VO₂ max and VO₂ peak are similar, ACSM has established criteria to be considered a true VO₂ max score, including a plateau in oxygen uptake (≤150ml/min or <2ml/kg/min), venous blood lactate levels ≥8 mmol/l, an RER >1.15 and an RPE >17 on the Borg scale (ACSM, 2006). However, Scharhag-Rosenberger et al. (2011) found that in most cases, VO₂ peak could be confirmed as VO₂ max. Trained runners will have significantly higher VO₂ max scores than untrained individuals, but there won’t necessarily be a difference in their maximum heart rate (Zhou, Ernst, & Wang, 2004). The American College of Sports Medicine has established descriptors to accompany the percentile value rankings for VO₂ max results, with scoring in the 90th percentile being classified as well above average and the 70th percentile being classified as above average (ACSM, 2006).
CHAPTER 3

METHODS

The purpose of this study was to examine how inducing hyperhydration with a glycerol solution and a sodium solution prior to exercise influenced performance and hydration status during a moderate set-intensity exercise bout followed by a time-to-exhaustion bout when compared to a control solution containing water only.

Subjects

Nine male runners were recruited; however, only six male runners participated in this study (ages 21 to 38). Two dropped out after the VO$_2$ peak test due to injuries they sustained during their own workouts, and one dropped out after one trial after being unable to keep his heart rate in the proper ranges during the run. The six individuals who completed the study were well-trained (running $\geq$2 years) endurance runners. Using trained runners ensured they had the physical stamina and mental toughness to endure a long run. Participants did not consume creatine within six weeks prior to the start of the study and had no injury or surgery that could have impacted running performance. In addition, participants did not belong to any of the following populations: diabetics, have irritable bowel syndrome (IBS) or inflammatory bowel disease (IBD), PKU, sickle cell anemia, or have disorders of the cardiovascular system, kidney, liver or migraine and headache disorders. This study was approved by Georgia Southern University’s Institutional Review Board. All participants who were eligible to complete the performance trials received a one-liter water bottle. In addition, participants who
completed all three performance trials were given the opportunity to have their body fat assessed in a BOD POD® (COSMED USA, Inc., Chicago, IL).

**Questionnaire**

Prior to completing any physical testing protocols, participants completed two surveys. The first one was a basic demographic survey that gathered birth date, gender, and ethnicity. The second one was a Health Status Questionnaire. This ensured that participants did not belong to any contraindicated populations, or have previous injuries or surgeries that could impact running performance. The survey also addressed the requirement of running for a minimum of two years. In addition, participants were asked if they took any other supplements and if so, what they were taking and how long they had been taking them. Anyone who listed creatine as a current supplement, or had taken it within the last six weeks, was excluded from the study, as it has been shown to increase total body water (Easton, 2007). Before and after completion of each performance trial, participants were given a qualitative survey to see the effects of the different solutions on gastrointestinal (GI) symptoms. The GI survey asked participants if they experienced any bloating, stomach cramps, nausea, and diarrhea and then asked them to rate the symptoms as mild, moderate, or extreme.

**Anthropometric Measurements, Urine Measurements, Physiological Parameters, and Equipment**

Participants were weighed on a calibrated, electronic weighing scale (Siltec, Bradford, MA) and their height was measured using a Detecto scale height rod (Webb
City, MO). The VO\textsubscript{2} peak trial and endurance performance trials were performed on a Trackmaster treadmill (Model number TMX425C, Newton, KS) wearing a Polar Electro Oy heart rate monitor (Professorintie 5, Kempele, Finland). Blood pressure was taken using an electronic blood pressure cuff (Omron, HEM-712CLC). During the VO\textsubscript{2} peak test, participants expired their gases into a mask (Parvo metabolics, Sandy, Utah). Their urine was collected in 32 oz specimen sample cups (Fischer Scientific, Suwanee, GA) and analyzed using Multistix\textsuperscript{®} 10 SG reagent strips for a Clinitek Status + Analyzer (Siemens, Tarrytown, NY). Total body water was estimated using a Bodystat Multiscan 4000 Bioimpedance Analyzer (Bodystat Limited, Isle of Man, Brisish Isles). Participants used the Borg scale to report their rate of perceived exertion (RPE). A heat stress monitor (3M\textsuperscript{TM} QUESTemp, QT-44) was used to monitor temperature and humidity during the trials.

**Supplement Protocols**

Participants randomly received three different liquid solution treatments throughout the three performance trials. When participants performed the control trial with water, they received 1 liter of tap water. During one of the two experimental trials, they received USP grade glycerol (ChemWorld, Atlanta, GA), in which 1.3 g glycerol/kg of bodyweight (BW) was dissolved in 1 L of water. The other experimental trial involved a sodium solution of 7.5 g NaCl (Consolidated Midland Corporation, Brewster, NY) in 26 mL of water per kg of BW. Each solution was masked with the appropriate amount of an aspartame-based raspberry ice flavored sweetener (Crystal Light, Kraft General Foods, Northfield, IL). All solutions were at room temperature.
Exercise Protocols

Preliminary Testing

On the first visit to the lab, prior to participating in any physical activity, participants signed an informed consent after being informed of the study design and any possible risks they could encounter while participating in the study. They then completed a Health Status Questionnaire to determine if they could proceed with further testing. If they qualified to participate in the preliminary test to determine peak oxygen consumption (VO$_2$ peak), height and weight were taken. Based on a protocol used by Peake et al. (2004), the speed for the VO$_2$ peak test began at 6.2 miles per hour (mph) and was increased every two minutes as follows; 7.5, 8.7, 9.9, 10.6, and 11.2 mph. If participants completed two minutes at 11.2 mph, the grade was raised to a 2% incline and increased by 2% every minute. During the VO$_2$ peak test, gases were collected in a mask and measured for percentages of carbon dioxide and oxygen. Participants’ heart rates were measured via a Polar heart rate monitor and recorded at the end of each speed change. The purpose of the VO$_2$ peak test was to make sure the participant was a well-trained runner by reaching a minimal VO$_2$ peak requirement and to determine their maximum heart rate, and therefore determine the running intensity that was performed during the experimental trials. The heart rate achieved at their final speed determined maximum heart rate. Their maximum heart rate was put into the Karvoven formula to determine their heart rate reserve (HRR), which would be used to dictate the heart rate ranges they were required to stay in during the running trials. In order to participate in the experimental trials, the males 20-29 years old had to score above 52 mL/kg/min, and
males between 30-39 years old had to score above 50 mL/kg/min. This put all participants in the 80th percentile or above for maximal oxygen uptake, as established by the American College of Sports Medicine (ACSM) guidelines.

**Experimental Testing**

In order to prevent diet from influencing performance on trial days, participants were asked to eat the same type and amount of food and drinks two days before and the day of each trial. This was monitored by a 48-hour dietary recall before the first trial. Participants were given a copy of that recall before each additional trial, so they knew what they needed to eat two days prior to and the day of a trial. This was to help eliminate changes in performance that could be caused by different macronutrient and sodium intakes. Participants were also asked to avoid adding excess salt to foods and highly processed foods two days before testing. They were also asked to refrain from consumption of alcohol and tobacco. In addition, participants were asked to refrain from strenuous physical activity 48 hours before testing, specifically resistance or endurance training of the lower body, and 24 hours prior to each trial, participants were instructed to do minimal physical activity. The evening before each trial, participants were instructed to drink an extra liter of water to ensure they were euhydrated, and lastly, participants were asked to go to bed around the same time and get a similar number of hours of sleep each night prior to each of the individual trials. The morning of the trial, participants consumed an extra 0.5 liters of water and arrived at the lab after a 30-minute fast. Participants were instructed to eat a heavier meal a few hours prior to the trial, and consume a lighter meal shortly before. This was to help prevent any excess GI
discomfort. The participants were able to pick what time of day to perform the trial, but all trials were performed within 1 hour of the initial trial start time to help prevent any fluctuations in performance that could result from exercising at different times. Each trial had a washout period of at least one week.

During the second, third, and forth visits to the lab, the three performance trials were performed. Throughout the study, participants were blinded to the order in which they consumed the following: the sodium solution (SODS), the glycerol solution (GLYC), and the water-control (WATC) trial. Participants were randomly assigned to group 1, group 2, or group 3, with each group having a specific order of receiving the supplements. Group 1 received the supplements in the following order: WATC, GLYC, and SODS. The order for group 2 was: GLYC, SODS, and WATC. Group 3 order of supplementation was: SODS, WATC, and GLYC.

**Experimental Procedures**

Prior to each experimental trial, participants were asked to void their bladder and urine was collected. At that time, urine was measured for urine specific gravity to ensure participants entered the lab euhydrated. All participants entered the lab properly hydrated with a urine specific gravity of \(\leq 1.015\). Body weight was then taken. The weight of their clothes was measured and then subtracted from their BW to get their BW. Participants were asked to wear the same clothes to each trial. Total body water (TBW) was obtained before participants began the hyperhydration phase. To measure TBW, participants were asked to lie down on their backs in anatomical position. Then, two Bodystat® disposable
electrodes were placed on their right foot (below their phalanges and between the talus and calcaneus bones), and two were placed on their right hand (directly below the phalanges knuckles and between the bony prominences on the wrist). The analysis was started and TBW results were given. They then had their blood pressure and heart rate taken. Participants were then be given a pre-hyperhydration GI distress questionnaire.

After all measurements were taken, the hyperhydration period began (minute 1) and lasted for 2.5 hours (150 minutes). The performance trial began at the end of the hyperhydration phase, at minute 150. At minute 65, 110, and 135, BW was taken and heart rate was recorded. At the same time intervals, participants were given the option to void their bladder and have their urine collected. Also, at minute 110, participants were given an additional 8 ounces of water. TBW and blood pressure were also measured again at minute 135. While participants were in the hyperhydration phase, the symptoms of overheating, such as dizziness, feeling faint, nausea, were explained to them. They were told to stop immediately if they started to feel anything abnormal while running during the trial.

To induce hyperhydration, participants were given one of the following solutions to consume in 60 minutes: GLYC (1.3 g/kg BW of glycerol mixed in with 1 liter of water), SODS (7.5 g NaCl dissolved in 26 mL fluid/kg BW), or the control solution WATC (1 liter). All solutions had Crystal Lite raspberry ice flavor added to it to help mask the flavor of the solution itself. Administration of solutions was divided into 3 equal measurements and given every 20 minutes for the first 60 minutes. Participants had to finish the solution before it was time to receive the next one-third of the solution.
Following the initial 60 minutes of hyperhydration, participants remained seated until their warm up began except to void their bladder and be weighed. At minute 145, participants began a 5-minute warm up at a self-selected walking pace.

At minute 150 (minute 1p), the performance trial began with the treadmill set at a 1% incline and a speed that allowed the participant’s heart rate to be between 60-65% of their HRR. The lab was set at 21°C ± 2°C Celsius. RPE using the Borg scale was measured every 10 minutes, and heart rate was recorded every 2 minutes during the first 90 minutes. The speed increased or decreased to maintain the proper heart rate ranges. This was followed by a bout of exercise at 85% of their HRR until they reached volatile exhaustion. During the exhaustion bout, heart rate and RPE were recorded every 30 seconds.

Throughout the performance trial, participants were allowed a minimal amount of water, with 8oz being the maximal amount of water they could receive. This was to help prevent them from getting a dry mouth sensation, but was still minimal enough to not influence hydration levels. However, during the second and third performance trials, they were only allowed to consume the amount of water that was consumed during the first performance trial. To simulate external motivation that most runners use, participants were allowed to listen to music during the first 90 minutes of the moderate, set-intensity run. However, when the 85% of HRR exercise bout was performed until exhaustion, participants had to turn off the music to prevent any external motivation from influencing their performance. In addition, the exercise time and distance was covered during the time-to-exhaustion phase.
Once the performance trial was completed, the participants voided their bladder and urine was collected. Then, they were weighed and total body water measurements were taken and a post-exercise qualitative GI symptom questionnaire was administered. They were given fluids and asked to remain seated so their heart rate and blood pressure could be taken post-exercise.

**Study Timeline**

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Days 2, 3, and 4</th>
<th>Study Timeline</th>
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</thead>
<tbody>
<tr>
<td>~45 minutes</td>
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<tr>
<td>-Questionnaire</td>
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<tr>
<td>-Survey</td>
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<tr>
<td>-Informed consent</td>
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<tr>
<td>-VO₂ max test</td>
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<tr>
<td><strong>Initial</strong></td>
<td><strong>Hyperhydration Protocol</strong></td>
<td><strong>Exercise Protocol</strong></td>
</tr>
<tr>
<td>~10 min</td>
<td>2.5 hours</td>
<td>90 min + exhaustion</td>
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<tr>
<td>-Void bladder</td>
<td>Fluids given in 1/3 partial amounts</td>
<td>Min 1p-90p</td>
</tr>
<tr>
<td>-Collect urine</td>
<td>Measurements taken &amp; waiting</td>
<td>90p-end</td>
</tr>
<tr>
<td>-Weight</td>
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<td>60-65% VO₂ max HR</td>
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<tr>
<td>-BP</td>
<td></td>
<td>85% VO₂ max HR</td>
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<td>-TBW</td>
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<td>-RHR</td>
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<tr>
<td><strong>Final</strong></td>
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<td>-Void bladder</td>
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<td>-Collect urine</td>
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<td>-RHR</td>
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</table>

Participants reported to the lab four times during this study. Day one of this study consisted of a study explanation to the participants, signing of an informed consent, a demographic survey and health status questionnaire, and a VO₂ peak test. Days 2, 3, and 4, each separated by at least one week, included the hyperhydration experimental trials and lasted approximately 4.5 hours each.
Limitations and Delimitations

One of the most significant limitations of this study was that participants performed all trials on a treadmill, and not all endurance athletes train on treadmills regularly. The testing was also done in a lab, not during an endurance event, so there could have been limited motivation of participants due to lack of surrounding competition. In addition, sample size was small and consisted only of males. Furthermore, the lab is not a climate-controlled chamber, so temperature and humidity could not be controlled, but they were recorded for each trial.

Delimitations included the requirement of the participant to be an experienced runner (≥ two years), and have met the VO₂ peak requirement. In addition, participants were not allowed to participate if they consumed creatine six weeks prior to initial testing or if they had any lower body or back injury or surgery that would influence their running performance. Participants that belonged to the list of at-risk populations to glycerol consumption were not allowed to participate. These populations included diabetics and disorders of the cardiovascular system, kidney, liver, or migraine and headache disorders. Anyone with IBS, IBD, or PKU was also excluded.

Assumptions

It is assumed that participants answered the health questions honestly and that they performed to the best of their abilities on the treadmill test. It was also assumed that participants followed the protocol during the times in which they are outside of the lab, including accurately keeping food and sleep logs and following them prior to each trial. It
was also assumed that the solutions were properly mixed and recorded by individuals working on the study.

**Data Analysis**

The data from this study was analyzed using SPSS version 21. Statistical significance was set at $p<0.05$ and all numbers were reported as mean ± standard deviation. Descriptive statistics include demographics, VO$_2$ peak scores, heart rates, and RPE. Eight one-way ANOVAs were run to determine any potential effects of experimental arrangements on individual dependent measures. This ensured that the order in which the solutions were given did not have an effect on the results of the study. To compare pre/post urine specific gravity within each solution, three two-tailed t-tests were performed. To determine differences between hyperhydration solutions, eight repeated-measures ANOVAs were used to compare the hyperhydration solution (GLYC, SODS, or WATC) on performance as well as hydration status, in terms of percentage urine output pre-exercise, weight change from pre-hydration to post-hydration, change in TBW from pre-hydration to post-hydration, change in TBW from pre-hydration to post-exercise, weight loss from pre-hydration to post-exercise, overall urine output, and overall percentage of excreted urine output. In addition, five repeated-measures ANOVAs were performed to evaluate differences in RPE and heart rate during the 90-minute run and during the time-to-exhaustion phase, as well as the overall percentage of body weight lost from pre-hyperhydration to post-exercise.
Data was normally distributed and there were no significant outliers, with the exception of one variable of one participant during one trial. Based on the number of participants in this study, it would have made a substantial impact on the results to exclude this participant. Each participant was included in all three treatments. Sphericity was verified for all analyses, and if violated, a Huynh-Feldt correction was applied. If significance was found, within-subjects contrasts were used to determine specific significance. To determine outliers, histograms and stem and leaf plots were analyzed.
Participant characteristics, VO\textsubscript{2} peak performance, and heart rates are shown in Table 1. The average age and weight of participants was 29.3 years old 179.5 cm, respectively.

| TABLE 1. Physical characteristics, heart rate and VO\textsubscript{2} peak of participants. |
|-----------------------------------------------|-----------------|-----------------|-----------------|
| Age (yr)                                      | 29.3 ± 7.5 [21-38] |
| Height (cm)                                   | 179.5 ± 7.5 [173-188] |
| Weight (kg)                                   | 77.7 ± 9.7 [70-96] |
| Relative VO\textsubscript{2} Peak (ml/kg/min) | 57.1 ± 3.7 [53.4-59.7] |
| Maximum Heart Rate (bpm)                      | 188.5 ± 6 [182-199] |
| Resting Heart Rate (bpm)                      | 51.2 ± 9 [40-65] |
| Data are presented as mean ± standard deviation [minimum-maximum]. (N=6). |

The average relative VO\textsubscript{2} peak of participants was 57.1 ml/kg/min. Lab temperature and humidity and differences between solutions in heart rate and RPE during the 90-minute steady state (SS) run as well as during the time to exhaustion bout are shown in Table 2.

| TABLE 2. Average lab temperature and humidity, as well as heart rate (HR) and RPE (using the Borg Scale). |
|------------------------------------------------|-------------|-------------|-------------|
| GLYC                                          | SODS        | WATC        |
| Lab Temp (°C)                                 | 21.5 ± 0.6  | 21 ± 1.1    | 21.8 ± 0.8  |
| Lab %Humidity                                 | 40.2 ± 20.2 | 38.5 ± 15.3 | 44 ± 10.1   |
| SS HR (bpm)                                   | 138 ± 8.9\textsuperscript{†} | 139 ± 9.1   | 139 ± 8.8   |
| TTE HR (bpm)                                  | 168 ± 7.5   | 167 ± 8.9   | 168 ± 7.7   |
| SS RPE (Borg)                                 | 12.1 ± 1.4  | 11.9 ± 1.7  | 12.1 ± 1.9  |
| TTE RPE (Borg)                                | 18.5 ± 0.5  | 17.8 ± 1.9  | 18 ± 1.1    |
| Data are presented as mean ± standard deviation. (N=6). \textsuperscript{†}Significantly lower HR (p<0.001) than SODS and WATC. |
Table 3 shows differences between hyperhydration solutions in regards to urine output, change in total body water, weight changes, and performance.

| TABLE 3. Hydration and performance differences between hyperhydration solutions. |
|----------------------------------------|----------------|----------------|
| %UO Pre Exercise | GLYC | SODS | WATC |
| Pre-Exer Wt Ch. (kgs) | 0.27 ± 0.27# | 0.19 ± .49 | -0.34 ± 0.47 |
| TBW (L) Pre Exercise | -0.83 ± 1.58 | 0.25 ± 0.89 | -0.58 ± 0.79 |
| TBW (L) Overall | 0.82 ± 1.09 | 1.28 ± 0.35 | 0.9 ± 1.4 |
| Performance (min) | 18.3 ± 6.38 | 12.67 ± 6.86 | 14 ± 9.06 |
| Weight Loss (kgs) | -1.59 ± 0.38 | -1.51 ± 0.78 | -2.18 ± 0.49‡ |
| %BW Lost | 2.04 ± 0.34 | 1.96 ± 1.03 | 2.88 ± 0.69Δ |
| Overall UO (mL) | 1177 ± 286‡ | 2043 ± 507 | 1800 ± 136 |
| Overall %UO | 1.18 ± 0.29 | 1.02 ± 0.26 | 1.8 ± 0.14† |

-Data are presented as mean ± standard deviation. (N=6). %UO is total urine (mL) excreted versus total fluid consumed (mL). Pre-exercise weight change is the difference in weight from pre-hyperhydration to post-hyperhydration. Total body water (TBW) pre-exercise is the change in liters of body water from pre-hyperhydration to post-hyperhydration (pre-exercise). TBW overall is change in water from pre-hyperhydration to post-exercise. Weight loss is the change in body weight from pre-hyperhydration to post-exercise. %BW lost is (initial weight – final weight) / initial weight x 100.

*Significantly higher than GLYC and SODS. #GLYC significantly different from WATC. ‡WATC significantly higher than GLYC and SODS. ΔWATC significantly higher than GLYC and SODS. ¶GLYC significantly lower than SODS and WATC. †WATC significantly higher than GLYC and SODS.

To ensure study results were not influenced by the order in which solutions were administered, eight one-way ANOVAs were run to determine any potential effects of experimental arrangements on individual dependent measures. No order effect was found for any variable.

For percentage of urine output pre-exercise (Figure 1), the repeated-measures ANOVA revealed a significant difference between groups, F(2, 10)=11.62, p<0.05, η=.70. Simple contrasts indicated that both GLYC (F(1,5)=17.16, p<0.01, η=0.77) and SODS (F(1,5)=24.73, p<0.01, η=0.83) had a lower percentage of urine output than
WATC pre-exercise. There was no significant difference between GLYC and SODS for pre-exercise percentage urine output.

![Pre-Exercise Percentage Urine Output](image)

*FIGURE 1: Water resulted in a significantly higher percentage of urine output pre-exercise than glycerol (p=0.009) and sodium (p=0.004).

When evaluating the change in liters of TBW from pre-hyperhydration to post-hyperhydration (pre-exercise), the repeated-measures ANOVA revealed there was no significant difference between solutions, F(2,10)=3.62, p=0.07, η=0.42.

When evaluating the change in liters of TBW from pre-hyperhydration to post-exercise, the repeated-measures ANOVA revealed there was no significant difference between groups, F(2,10)=0.80, p=0.48, η=0.14.

When analyzing performance, measured by time to exhaustion, between solutions, the repeated-measures ANOVA revealed there was no significant difference between groups, F(2,10)=1.90, p=0.20, η=0.28.

There were no negative GI side effects reported when participants consumed the WATC solution. However, only two participants reported side effects when GLYC was
consumed, while three reported negative side effects during the SODS trial. Side effects were varied, and included mild stomach cramps and bloating to extreme cramps, dizziness, and nausea.

For weight changes from pre-hyperhydration to post-hyperhydration (pre-exercise) (Figure 2), the repeated-measures ANOVA revealed a significant difference between groups, $F(2,10)=5.28, p<0.05, \eta=0.51$. A simple contrast indicated that there was a significant difference in weight change from pre-hyperhydration weight to post-hyperhydration (pre-exercise) weight between WATC and GLYC ($F(1,5)=7.81, p<0.05, \eta=0.61$), with GLYC having an average weight gain and WATC having an average weight loss. However, there were no differences in pre-exercise weight changes between SODS and GLYC ($p=0.68$) or sodium and water ($p=0.06$). WATC had the greatest change in weight; however, weight change for WATC was shown as a weight loss while weight change for GLYC and SODS was shown as weight gain.

*FIGURE 2: When compared to water, weight change with glycerol ($p=0.038$) was significantly different. Sodium was not significantly different from water ($p=0.58$) or glycerol ($p=0.679$) in regards to pre-exercise weight change. Water had the greatest weight change (as a weight loss), while glycerol and sodium weight changes displayed as a gain.*
When analyzing differences in weight loss from pre-hyperhydration to post-exercise (Figure 3), the repeated-measures ANOVA revealed a significant difference between groups, $F(2,10)=4.8$, $p<0.05$, $\eta=.49$. When participants consumed GLYC ($F(1,5)=9.14$, $p<0.05$, $\eta=0.65$) and sodium ($F(1,5)=11.25$, $p=0.02$, $\eta=0.69$), they demonstrated significantly less loss of weight when compared to WATC. There was no significant difference in overall weight loss between SODS and GLYC.

For total urine output (mL), repeated-measures ANOVA revealed a significant difference between groups, $F(2, 10)=8.64$, $p<0.01$, $\eta=0.63$. Simple contrasts indicated that GLYC had significantly lower total urine outputs than WATC ($F(1,5)=21.44$, $p<0.01$, $\eta=0.81$) and SODS ($F(1,5)=9.93$, $p<0.05$, $\eta=0.67$), but there was no difference between WATC and SODS ($p=0.30$).

Since SODS was greater in liquid volume than GLYC or WATC, percentage of urine output in relation to the amount of mL consumed was analyzed. For overall percent
urine output (Figure 4), the repeated-measures ANOVA revealed a significant difference between solution groups, F(2, 10)=16.57, p=.001, η=.77. Simple contrasts indicated that both GLYC (F(1,5)=21.44, p<.01, η=.81) and sodium (F(1,5)=44.33, p=.001, η=.90) had a lower percentage of urine output than WATC. There were no significant differences between GLYC and SODS solutions with regards to percent urine output.

Pre-hyperhydration to post-exercise urine specific gravity was compared within each of the hyperhydration solutions. Two-tailed dependent t-tests showed no significant difference in pre and post urine specific gravity for any solution.

*FIGURE 4: When evaluating overall total percentage urine output, water had a significantly higher percentage of urine output than glycerol (p=0.006) and sodium (p=0.001).
CHAPTER 5

DISCUSSION

The purpose of this study was to examine how inducing hyperhydration with a glycerol solution and a sodium solution prior to exercise influenced performance and hydration status during a moderate set-intensity exercise bout followed by a time-to-exhaustion bout when compared to a control solution containing water only. The primary finding of this study was that sodium and glycerol both had a significantly lower percentage of urine output and less of an overall weight change than water. In addition, there was no significant difference between groups regarding performance.

Hydration is an important component of physical activity and can influence numerous variables. In the current study, it is important to note that while glycerol and water were administered as 1 L of liquid solution, sodium was administered in 26 mL water/kg, which equated to 1.8-2.5 times the amount of liquid that was consumed when compared to water and glycerol, depending on the weight of each participant. When examining true values of urine output, the sodium solution resulted in significantly higher output than the glycerol solution, but showed no difference from the water. Due to the unequal amounts of liquid consumed between solutions, results for urine output were evaluated and reported as percentage of mL consumed.

When looking at percentage of mL consumed, the glycerol and sodium solution had a significantly lower total urine output than water pre- and post-exercise. Similar to the current study, Anderson et al. (2001) and Marino et al. (2003) also found that urine output was lower when hyperhydration was induced by a glycerol solution in comparison
to a placebo. In contrast, Sheadler et al. (2010) found that glycerol hyperhydration did not result in a greater fluid retention (difference between fluid ingested and urine volume) than water alone. However, unlike the current study, which used a percentage of urine output compared to the amount of fluid ingested, Sheadler used the difference, so the results are not directly comparable. Studies using sodium to hyperhydrate (Gigou et al, 2012; Sims et al, 2007a,b) did not analyze the percentage of urine output based on total mL consumed, so it is unclear how the sodium results of the current study compare to others.

In the current study, when evaluating weight changes from pre-hyperhydration to post-hyperhydration (pre-exercise), glycerol and sodium both resulted in weight gain while water resulted in weight loss. Although not significant, glycerol led to a greater weight gain than sodium. This is similar to results found by Nishijima et al. (2007), which found that weight gain pre-exercise was significantly higher with glycerol than with a diluted sports drink.

While Gigou et al. (2012) found that a sodium hyperhydration induced weight gain of 1 kg does not impact running speed, the current study did not evaluate the impact that weight gain may have had on running speed or performance. However, it should be noted that in the current study, hyperhydration did not result in weight increases over 1 kg.

Although changes in TBW were not significantly different between solutions in this study, the sodium solution did produce higher increases in TBW than glycerol or water from pre- to post-exercise. This could have been due to the increased fluid consumed with sodium, and not the sodium itself. However, it still is important to note
that SODS was the only solution to have an increase in pre-exercise TBW. Since hyperhydration is an excess of water in the body (hyperhydration, n.d.), an increase in TBW could theoretically increase the time it takes to reach dehydration, which could be beneficial to endurance athletes. While SODS did not lead to an increase in performance in the current study, further research evaluating different amounts of liquid consumed with sodium could lead to insight as to whether or not it was the sodium or the increased liquid that lead to the increase in TBW. Armstrong (2007) states that some authorities claim the widely accepted idea that total body water, in combination with plasma osmolality, provides the “gold standard” for hydration assessment. While many studies use plasma osmolality, no other hyperhydration studies have analyzed TBW, so results of the current study cannot be compared to others.

When evaluating weight loss from pre-hyperhydration to post-exercise, both glycerol and sodium had a significantly lower weight loss than with water. This is similar to Nishijima et al. (2007), which found that while participants still lost weight over the course of the trial with glycerol and a placebo, the weight lost with glycerol was significantly less than weight lost with a placebo. Scheadler et al. (2010) also found that weight gain with glycerol was higher than with a placebo, but results were not significant. Since it has been shown that dehydration greater than 2% of bodyweight can hinder endurance performance (Goulet, 2012), it is important to note that when examining means in the current study, sodium was the only solution to stay below the 2% BW loss threshold; however, statistically, these results were not significantly different than glycerol. Nonetheless, this could be beneficial to endurance athletes, especially when exercising outside in the heat, as greater rates of dehydration can also increase.
cardiovascular strain (Goulet, 2012). In the current study, even though sodium resulted in the poorest performance of any solution, it is thought that the GI issues were the main cause of this.

The amount of sodium used in the current study was based on research by Gigou et al. (2012), which suggested that 7.5 g of sodium chloride dissolved in 26 mL/kg of BW might produce comparable hyperhydration results to glycerol. In the current study, based on a percentage using total urine output and total liquid consumed, SODS actually had a lower urine output than GLYC. Although not significant, SODS had a greater post-exercise TBW and less weight loss from pre-hyperhydration to post-exercise than both glycerol and water. In addition, the overall percentage of weight lost with sodium was not significantly different from glycerol. For this reason, it appears that sodium’s ability to induce and maintain a hyperhydrated state is at least equal to that of glycerol.

It is important to evaluate any variable that could influence performance. The impact of hyperhydration on performance has yet to be determined. In the current study, performance was determined by amount of time participants could run at 85% of their HRR until exhaustion after finishing a 90-minute moderate intensity bout. Results found no significant difference between groups regarding performance. Sodium had the lowest average performance of all three solutions, and while the average performance times with glycerol were better than sodium and water, none of these results were significant. The researchers believe that sodium likely had the lowest average performance time due to the GI discomfort that accompanied drinking the sodium solution.

Similar to the current study, Marino et al. (2003), Mehmet and Husrev (2011), and Scheadler et al. (2010) did not find that glycerol hyperhydration significantly
improved performance in comparison to a placebo solution. In addition, Gigou et al. (2012) found that sodium hyperhydration did not improve performance better than receiving no fluids prior to exercise. However, while there was no statistical significance in the current study, it is important to note that when hyperhydration was induced with glycerol, participants ran an average of four to six minutes longer than when consuming the other solutions. The practical implications of this could possibly be substantial. In addition, Anderson et al. (2001) found that glycerol hyperhydration improved performance, which was measured by power output on a cycle ergometer. In addition to Anderson using a different modality to test endurance, improvement could also have been because Anderson accounted for individual body weight by administering 1 g/kg of glycerol in 20 ml/kg of liquid while the current study administered the glycerol in 1 L of water. The current study had a more varied weight range than the Anderson study (77.7 ± 9.7 versus 72.0 ± 4.3, respectively). This could have impacted the glycerol results of heavier participants who received the same amount of fluid as lighter participants.

In the current study, it is thought that lab temperature could have impacted performance. While many studies investigating hyperhydration took place in lab temperatures ranging from 28-35° Celsius (Anderson et al. (2001), Marino et al. (2003), Mehment & Hursev (2011), Nishijima et al. (2007), Sheadler et al. (2010), Gigou et al. (2012), Sims et al. (2007a,b)), the temperature used for the current study averaged 21° Celsius and was unable to be changed. The researchers believe that if lab temperatures had been warmer during the current study, higher rates of dehydration and differences in performance would have been observed when using glycerol and sodium to hyperhydrate in comparison to water. Some studies using trained runners in warmer environments did
not show that glycerol or sodium had a positive impact on performance (Mehmet & Hursev, 2011; Scheadler et al., 2010; Gigou et al., 2012). However, Mehmet & Hursev was the only study to have participants run at a pace based on a physiological parameter (65% of their VO$_2$ max), while studies by Scheadler and Gigou were based more on a self-selected pace. This could be one reason that performance differences were not seen. If participants did not push themselves to the fullest extent, the results would not be as meaningful. In this current study, while no solution was shown to have a statistically significantly benefit to performance, glycerol had the highest average time to exhaustion, and it is thought that warmer conditions would have had enough influence to show significant results.

In addition, any uncomfortable side effects from solutions could have impacted running performance. Side effects for glycerol included mild stomach cramps for one participant and slight dizziness and headache for another, both of which subsided prior to running. For sodium, side effects included moderate bloating and extreme stomach cramps for one participant, mild nausea for another, and bloating in the lower abdomen for another participant. Also, because performance in the current study was based on volatile exhaustion, results could have varied based on how internally motivated the participant was feeling that day.

Changes in physiological variables can be influenced by several factors including the intensity and duration of a workout, and nutrient and hydration status. There is contrast in the literature on the effects of hyperhydration on physiological variables. In the current study, average heart rates and RPE during the 90-minute run appeared to be almost identical between glycerol, sodium, and water. RPE, taken every ten minutes
during the 90-minute run, was not significantly different between solutions. However, statistics revealed that heart rate, which was taken every two minutes during the 90-minute run, was significantly lower when participants consumed the glycerol solution versus the sodium and water solution.

Similar to the current study, Anderson et al. (2001) found that heart rate was lower during the glycerol trial when compared to a control. Conversely, Marino et al. (2003) found that heart rate was higher when consuming glycerol versus a placebo, while Mehmet and Hursev (2011) and Scheadler et al. (2010) found that heart rates when using a glycerol hyperhydration solution were not different when compared to other solutions; however, like the current study, they did find that glycerol hyperhydration did not lower RPE. Discrepancies in heart rates could be due to differences in the sensitivity of equipment used throughout studies.

Sims et al. (2007b) found that final RPE was lower in the high-sodium trials versus the low-sodium trials; however, no significant difference was observed in heart rate between the high-sodium solution versus the low-sodium solution. Gigou et al. (2012) found that hyperhydration with a sodium solution prior to an 18km running trial resulted in a significantly lower heart rate compared to beginning the run euhydrated.

However, because there was statistical significance with heart rate and not RPE, the researchers believe there is little practical implication with heart rate significance. The current study adjusted treadmill speed to keep each participant’s heart rate in specified zones, so the differences are likely due to equipment error and it was unexpected to see statistical differences in heart rate. Due to the design of the current study, it is thought that instead of heart rate, a more practical way to evaluate solution
differences would be to see how far participants could run throughout the trial within their given heart rate zones. Since there are no available published studies that have evaluated heart rates when directly comparing sodium, glycerol, and water, it is unknown how this study would directly compare to others.

Gastrointestinal (GI) distress can severely impact a runner’s ability to perform well. For this reason, it is important to evaluate the impact that each solution had on participants. An artificial sweetener was added to all three solutions, and most subjects reported little, if any, difference in taste between the water and glycerol solution. However, all participants reported a negative and/or different taste when they consumed the sodium solution. This is different from Sims et al. (2007b), which mentioned that participants had no comments on the flavor being salty regardless of receiving a high or low sodium beverage. In the current study, participants noticed the increased fluid for the sodium solution. The sodium solution was dissolved and administered in 26 mL/kg of fluid (1820 – 2496 mL, depending on the participant). Whereas, the glycerol and water solutions equaled 1 L of liquid, which was roughly 10.5 – 14.5 mL/kg of fluid, depending on the participant. While all participants in the current study were able to consume the entire solution for all three trials in the allotted time, most had a harder time consuming the sodium solution because of the increased volume. Gigou et al. (2012) also reported that some subjects had a difficult time consuming all of the sodium solution.

While many participants reported increased fullness right after finishing the glycerol hyperhydration phase, only two reported adverse affects to glycerol. One reported dizziness and headache during the hyperhydration phase, which was similar to findings by Nishijima et al. (2007). The other participant reported feeling mildly bloated
during the exercise phase, which was similar to findings by Sheadler et al. (2010), who also had participants report nausea or bloating with glycerol hyperhydration. Anderson et al. (2001) had two participants report diarrhea within 24 hours post-trial; however, no participants in the current study mentioned this.

In regards to sodium, three participants reported adverse reactions. It is likely that more participants experienced adverse reactions because sodium can delay gastric emptying, therefore creating discomfort (Gropper et al., 2009). One reported moderate bloating and extreme stomach cramps within 20 minutes of the hyperhydration phase, which lasted throughout the exercise protocol. Another participant reported feeling bloated in the lower abdominals during the exhaustion phase of running. The final participant to experience an adverse reaction to sodium actually vomited during the hyperhydration phase and had to repeat the trial at a later date. During the retrial, he complained of mild nausea and stomach churning during the hyperhydration phase, but feelings subsided prior to exercise. On the contrary, Gigou et al. (2012) found that subjective feelings of GI distress and dizziness weren’t different between sodium-induced hyperhydration and receiving no fluids. The current study based sodium hyperhydration administration on the study by Gigou and colleagues. However, the exercise protocol used for that study was an 18km time-trial study, which found no differences in time to complete the trial between sodium hyperhydration and receiving no fluids. The differences in GI responses to sodium are likely just due to varied individual tolerances.

The most significant limitation of this study was the small sample size. Due to the large time requirement and the rural area in which this study took place, it was difficult to find people that were available for the entire experiment. Also, with little being known
about female responses to hyperhydration, another limitation of this study was that only males were recruited. Furthermore, while many studies evaluate the benefits of hyperhydration in warm conditions, this study was unable to be conducted in a warm room due to the inability to adjust the thermostat in the lab. It is believed that warm conditions would have had a significant impact on results. Also, the study was performed in a lab on a treadmill, and not during an endurance event, so motivation could have been impacted. The fact that this study was based on participants giving their best until they reached volatile exhaustion is another limitation, as some participants may have had busier weeks or been more stressed depending on the point in the semester, which could have impacted their internal motivation during some trials and not during others. The time of day was also another limitation of the study, as participants chose what time to participate. This lead to discrepancies in times between participants, with some participants running first thing in the morning after only a small breakfast and others in the afternoon after a full breakfast and lunch. In addition, another possible limitation of study is that exact mL of excreted urine could not be compared because there was more liquid consumed in the sodium solution, so the percentage of mL consumed versus mL excreted had to be evaluated. Additionally, while the entire liquid amount during the sodium trial was based on body weight, it was not in the water and glycerol trial. This is another limitation because weight varied between participants, yet they all received only 1 L of liquid. The final limitation of this study was the one outlier. This involved the total body water readings of one participant during one specific trial, which was likely due to machine error, as proper measurement protocols were followed. While the participant’s
TBW was drastically higher than the other participants, the weight change was similar to other participants, indicating that the measured TBW was inaccurate.

Based on findings from the current study, several ideas for further research were developed. First, it is recommended that this study be duplicated in warm conditions to see how each solution would compare to one another. This could be done inside a climate controlled lab or outside in the heat. Also, since 2.5 hours was enough time to induce hyperhydration, it is recommended to try shortening that time frame to see if effects remain the same. This could help establish a more practical time frame when applied to a real-life setting.

In addition, many participants complained about the taste of the sodium solution. For this reason, it would be beneficial to see if swallowing sodium tablets with the water, instead of dissolving them in the water, would have the same effect on water retention, while making it more palatable. Since sodium was shown to reduce urine output in 26 mL/kg of water, it would also be beneficial to see if it would have the same impact using less water, such as 1 L, making it easier and more realistic for people to consume in the allotted time period.

While participants did keep a food log and were told to eat the same thing prior to each trial, it may be beneficial to have all participants on a standardized diet to help eliminate discrepancies in various diets. It is also recommended that future studies have more participants to help obtain more meaningful results.

This study was performed using runners. In the future, another possible option is to replicate this study using cycling to see if power output is influenced by hydration levels and to see if the GI distress has less of an impact when cycling. In addition,
hyperhydration studies using females are very limited. It is recommended that this study be duplicated in females to see if responses are similar.

In conclusion, when evaluating pre- to post-exercise, glycerol and sodium had significantly less weight loss and significantly less percentage of urine output when compared to water, but had no significant differences between each other. Sodium had greater increases in TBW, but results were not significant. All of the previously mentioned factors indicate an increase in fluid retention, which may help reduce the negative side effects of dehydration. Hyperhydration with glycerol or sodium is more effective than with water alone. Sodium-induced hyperhydration appears to be as effective as glycerol-induced hyperhydration in terms of reducing percentage of urine output in relation to total mL consumed, increasing total body water, and minimizing weight lost during a long run. This could be beneficial to athletes who may get tested by WADA, as glycerol is a banned substance and sodium is legal.

There were no significant differences in performance between solutions, even though average performance with glycerol was the highest and average performance with sodium was the lowest. However, hyperhydration with glycerol and sodium was not superior to water in improving performance.
REFERENCES


APPENDIX A

INFORMED CONSENT
COLLEGE OF HEALTH AND HUMAN SCIENCES
DEPARTMENT OF HEALTH AND KINIESIOLOGY

1. **Principal Investigators:**
   Stephanie Marz, Graduate student, Department of Health and Kinesiology, 912-690-0335
   Amy Jo Riggs, RD, Ph.D., Associate Professor, Department of Health and Kinesiology, 912-478-7753

2. **Purpose of the Study:** The purpose of this study is to examine how performance and hydration status are influenced during a moderate set-intensity exercise bout followed by a time-to-exhaustion bout in warm conditions, when using a hyperhydration protocol prior to exercise, with a glycerol solution versus a sodium solution, and comparing it to a control solution containing water only.

3. **Procedures to be followed:** All participants will be asked to complete a basic demographic survey and Health Status Questionnaire prior to any testing. This will ensure that you have not consumed creatine within the last six weeks and that you have not been injured or have had surgery in the past that would have an impact on running performance. If you have any of the following conditions, you will not be able to participate in this study: Diabetes, Cardiovascular, kidney, or liver disease, Irritable Bowel Syndrome, Inflammatory Bowel Disease, migraines or headache disorders, or have sickle cell anemia or PKU. Individuals that qualify for the study will then be asked to perform a treadmill VO\textsubscript{2} max test. Prior to VO\textsubscript{2} max test, subjects’ blood pressure will be taken to ensure it falls within a normal level (≤120/80). In addition, you will be informed of risks associated with performing a VO\textsubscript{2} max test. You will be asked to discontinue running at any point if you start to feel nauseous, lightheaded, dizzy, or experience muscle cramps, or begin to feel like you will no longer be able to run long enough to reach maximal capacity. If the researcher notices signs of exhaustion or heat-related problems, you will be asked to immediately stop. In addition, there will be someone positioned near the back to the treadmill to act as a “catcher” in case you were to lose balance at any point. The PI is certified in both CPR and First Aid to ensure proper protocol will be followed if an emergency situation would happen to occur.
4. Participants will then sign up for their first experimental trial. Prior to trial days, participants will be asked to keep a detailed food record including all food and beverages 48 hours before and the morning of the trial. Whatever is consumed during this time prior to trial one, participants will be asked to eat and drink the same thing for the second and third trials. A copy of the food record will be given back to participants so they can remember what they need to consume for the future trials. In addition, participants will be asked to avoid alcohol and tobacco as well as strenuous exercise 48 hours before each trial day. Participants will be encouraged to get the same amount of sleep the night before each trial. They will also be asked to avoid eating 30 minutes before they arrive to the lab for testing. During all three experimental trials, participants will be weighed and heart rate and blood pressure will be measured. Total body water measurements will also be taken. Then, a 2.5-hour hyperhydration phase will begin where a substance (glycerol or sodium) will be ingested with a large volume of water. Intervals will occur during the hyperhydration phase where weight and heart rate will be taken, as well as collecting any excreted urine in specimen cups. At the end of the hyperhydration phase, participants will begin the exercise protocol. A 90-minute run on the treadmill at 60-65% of VO$_2$ max will begin, with heart rate and rate of perceived exertion (RPE) being monitored. Following the 90-minute run, the time-to-exhaustion bout will begin. This will involve running at 85% of VO$_2$ max until exhaustion. At time of exhaustion, subjects will remove themselves from the treadmill to be weighed and void their bladder. After that, total body water will be taken again and participants will be asked to complete a qualitative GI survey. Fluids will be provided once total body water is taken and the GI survey is completed. In addition, blood pressure and heart rate will be measured.

5. **Discomforts and Risks**: Possible risks are associated with performing a VO$_2$ max test. These include, but are not limited to: lightheadedness, dizziness, fainting, nausea, vomiting, irregular heartbeat, and in rare cases, cardiac arrest. Possible discomfort may arise when participants are asked to void their bladder, collect their urine in a specimen cup, and then bring it directly to the lab. This may be embarrassing for some people, so participants will be given a paper bag or container to put the cup in to help minimize any embarrassment. Participants will be asked to consume a large volume of liquid prior to running, which could lead to some GI distress, such as a feeling of fullness, bloating or stomach cramping. If they feel too uncomfortable, they may chose not to run. Signs and symptoms of exhaustion and heat-related problems will be explained to participants and they will be asked to stop exercising immediately if they experience any of these symptoms. Lastly, subjects will be asked to complete a gastrointestinal (GI) distress survey that will ask if they experienced any bloating, stomach cramps, nausea, and/or diarrhea. This could be embarrassing for some, so researchers will try to make participants feel as comfortable as possible.
6. **Benefits**: Learning about your individual VO\textsubscript{2} max can help determine the intensity of your training program in order to help improve performance. If the outcomes of this study supports that sodium hyperhydration is as beneficial as glycerol hyperhydration, this could allow many athletes the chance to use hyperhydration prior to practices or competition, and therefore hopefully reduce the incidence of dehydration, heat exhaustion, or a decrement in performance. Every participant that completes the study will have the opportunity to have their body composition measured. This measurement will provide valuable information to you for future training. In addition, each performance trial can act as your long run for the week.

7. **Duration/Time**: Total time required from the participant is approximately 14.5 hours. The initial assessment will take approximately 1 hour. Each exercise experimental trial will take roughly 4.5 hours each time.

8. **Statement of Confidentiality**: All scientific and personal data collected on subjects for presentation purposes will be kept confidential and stored in a locked file drawer in Hollis 2121-A. This information will be available only to the principal investigators. Your identity will not be revealed in publications or presentations that result from this study so as to protect your privacy and confidentiality. All data will be reported as means and standard errors. Data will be kept for 3 years and then destroyed. Electronic data will be kept on a password protected, encrypted private hard drive and will be destroyed as soon as the data analysis has been run and the manuscript is complete. The destruction of all data and records will be done at Georgia Southern University.

9. **Right to Ask Questions**: You have the right to ask questions and have those questions answered. If you have questions about this study, please contact Stephanie Marz, graduate student, Department of Health and Kinesiology, 912-690-0335, sm00454@georgiasouthern.edu or Dr. Amy Jo Riggs, Associate Professor, Department of Health and Kinesiology, 912-478-7753, ajriggs@georgiasouthern.edu. For questions concerning your rights as a research participant, contact Georgia Southern University Office of Research Services and Sponsored Programs at 912-478-0843.

10. **Compensation**: You will receive a water bottle on the first visit to the lab. Any participant that completes the study will have the opportunity to have their body composition measured.
11. **Voluntary Participation**: Your participation in this study is entirely voluntary. If you decide to participate, you are free to withdraw your consent and to stop participating at any time without penalty or loss of benefits to which you are otherwise entitled.

12. **Penalty**: If you decide not to participate, you will not be penalized, and you will not lose any benefits or services to which you are otherwise entitled.

13. You must be 18 years of age or older to consent to participate in this research study. If you consent to participate in this research study and to the terms above, please sign your name and indicate the date below.

You will be given a copy of this consent form to keep for your records. This project has been reviewed and approved by the GSU Institutional Review Board under tracking number H14104.

**Title of Project**: Comparing Hyperhydration Ability Between A Glycerol Solution and Sodium Solution on Dehydration and Performance in Runners

**Principal Investigator**: Stephanie Marz, 912-690-0335, sm00454@georigasouthern.edu

Dr. Amy Jo Riggs, 912-478-7753, ajriggs@georgiasouthern.edu

______________________________________  _____________________
Participant Signature     Date

I, the undersigned, verify that the above informed consent procedure has been followed.

______________________________________  _____________________
Investigator Signature     Date
APPENDIX B

PRE-EXERCISE TESTING HEALTH QUESTIONNAIRE

Name _______________________________________________  Date______________
Preferred Method of Contact ______________________________
Person to contact in case of emergency________________________________________
Emergency Contact Phone ___________________________Birthday (mm/dd/yy)_____/_____/_____
Personal Physician ___________________________Physician’s Phone ______________________
Gender_________  Age ______(yrs) Height ______(ft)______(in) Weight______(lbs)
Does the above weight indicate:  a gain____   a loss___   no change____ in the past year?
If a change, how many pounds?___________ (lbs)

A.  JOINT-MUSCLE STATUS (✓ Check areas where you currently have problems)

Joint Areas                                        Muscle Areas
(  ) Wrists                                      (  ) Arms
(  ) Elbows                                      (  ) Shoulders
(  ) Shoulders                                   (  ) Chest
(  ) Upper Spine & Neck                          (  ) Upper Back & Neck
(  ) Lower Spine                                 (  ) Abdominal Regions
(  ) Hips                                        (  ) Lower Back
(  ) Knees                                       (  ) Buttocks
(  ) Ankles                                      (  ) Thighs
(  ) Feet                                        (  ) Lower Leg
(  ) Other__________________________             (  ) Feet
(  ) Other__________________________             (  ) Other__________________________

B.  HEALTH STATUS (✓ Check if you currently have any of the following conditions)

(  ) High Blood Pressure                        (  ) Acute Infection
(  ) Heart Disease or Dysfunction               (  ) Diabetes or Blood Sugar Level
Abnormality                                      (  ) Anemia
(  ) Peripheral Circulatory Disorder            (  ) Hernias
(  ) Lung Disease or Dysfunction               (  ) Thyroid Dysfunction
(  ) Arthritis or Gout                          (  ) Pancreas Dysfunction
(  ) Edema                                       (  ) Liver Dysfunction
(  ) Epilepsy                                    (  ) Kidney Dysfunction
(  ) Multiply Sclerosis                         (  ) Phenylketonuria (PKU)
(  ) High Blood Cholesterol or Triglyceride Levels (  ) Loss of Consciousness
(  ) Allergic reactions to rubbing alcohol      (  ) Sickle Cell Anemia
(  ) Migraine and/or headache disorders

*NOTE: If any of these conditions are checked, then a physician’s health clearance will required.
C. PHYSICAL EXAMINATION HISTORY
Approximate date of your last physical examination______________________________

Physical problems noted at that time________________________________________________________________________

Has a physician ever made any recommendations relative to limiting your level of
physical exertion? _________YES __________NO
If YES, what limitations were recommended?___________________________________________

D. CURRENT MEDICATION USAGE (List the drug name and the condition being managed)

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<tr>
<th>MEDICATION</th>
<th>CONDITION</th>
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E. PHYSICAL PERCEPTIONS (Indicate any unusual sensations or perceptions. ✓Check if you have recently experienced any of the following during or soon after physical activity (PA); or during sedentary periods (SED))

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<td>Chest Pain</td>
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<tr>
<td>( )</td>
<td>( )</td>
<td>Extreme Weakness</td>
<td>( )</td>
</tr>
<tr>
<td>( )</td>
<td>( )</td>
<td>Numbness</td>
<td>( )</td>
</tr>
<tr>
<td>( )</td>
<td>( )</td>
<td>Mental Confusion</td>
<td></td>
</tr>
</tbody>
</table>

F. FAMILY HISTORY (✓Check if any of your blood relatives . . . parents, brothers, sisters, aunts, uncles, and/or grandparents . . . have or had any of the following)

( ) Heart Disease
( ) Heart Attacks or Strokes (prior to age 50)
( ) Elevated Blood Cholesterol or Triglyceride Levels
( ) High Blood Pressure
( ) Diabetes
( ) Sudden Death (other than accidental)

G. EXERCISE STATUS
Do you regularly engage in aerobic forms of exercise (cycling, walking, etc.)? YES   NO

How long have you engaged in this form of exercise? ______ years ______ months
How many hours per week do you spend for this type of exercise? ________ hours
Do you regularly lift weights?  
YES  NO
How long have you engaged in this form of exercise?  ______ years ______ months
How many hours per week do you spend for this type of exercise?  _______ hours

Do you regularly play recreational sports (i.e., basketball, racquetball, etc.)?  YES  NO
How long have you engaged in this form of exercise?  ______ years ______ months
How many hours per week do you spend for this type of exercise?  _______ hours

H. DIET (✓ Check the nutritional supplements you are currently taking or have taken within the past 9 weeks.)

- ( ) Ribose
- ( ) Protein
- ( ) Protein Drinks
- ( ) Creatine Monohydrate
- ( ) Vitamins (multi-vitamins, etc)
- ( ) Calcium
- ( ) Other: _____________________________________________

____________________________________  _____________________
Participant Signature  Date

I, the participant verify that the answers above in this Health History questionnaire are accurate and true.

____________________________________  _____________________
Investigator Signature  Date
APPENDIX C

VO₂ MAX DATA COLLECTION SHEET

SUBJECT ID: ____
DATE: _______
SEX: Male
AGE: _____
WEIGHT: ____ lbs ; _____ kg    WAIST: ____ inches
HEIGHT: ____ inches; _____ cm   HIPS: ____ inches
RESTING HR: ____

<table>
<thead>
<tr>
<th>STAGE</th>
<th>SPEED</th>
<th>Time (min)</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.2 mph</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7.5 mph</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8.7 mph</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9.9</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10.6</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>11.2</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>11.2 + 2%</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>11.2 + 4%</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>11.2 + 6%</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>11.2 + 8%</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>11.2 + 10%</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

VO₂ MAX: ____ (L/min)   ____ (ml/kg/min)
Max Speed: ______    Max Heart Rate: ____ bpm
Total Time: _______

Heart Rate Ranges using the Karvonen Formula:
[(MHR-RHR) x %intensity] + RHR
60%: ____ BPM    65%: ____ BPM    85%: ____ BPM
APPENDIX D

BORG SCALE RATE OF PERCEIVED EXERTION

<table>
<thead>
<tr>
<th>Borg Rating of Perceived Exertion (RPE) Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>9</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>11</td>
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<tr>
<td>12</td>
</tr>
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<td>14</td>
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<tr>
<td>15</td>
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<td>18</td>
</tr>
<tr>
<td>19</td>
</tr>
<tr>
<td>20</td>
</tr>
</tbody>
</table>

Borg RPE Scale
APPENDIX E

FOOD LOG

Record your food the day before the trials as well as the morning of the trial.

<table>
<thead>
<tr>
<th>Time of Day</th>
<th>Quantity of Food</th>
<th>Food Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:30am</td>
<td>1 cup: ¼ c</td>
<td>Rolled oats with skim milk</td>
</tr>
<tr>
<td></td>
<td>1 tbsp.</td>
<td>Honey</td>
</tr>
<tr>
<td></td>
<td>¼ cup</td>
<td>Frozen blueberries</td>
</tr>
<tr>
<td></td>
<td>1 cup</td>
<td>Water</td>
</tr>
<tr>
<td>10:00am</td>
<td>½ cup</td>
<td>Greek vanilla yogurt</td>
</tr>
<tr>
<td>12:15pm</td>
<td>1 sandwich</td>
<td>2 slices of bread, 4 slices deli turkey, handful of spinach, 1 tbsp. mustard, 1 tsp. honey</td>
</tr>
<tr>
<td>12:30-4pm</td>
<td>1.5 L</td>
<td>1 liter of water consumed over 3.5 hours</td>
</tr>
<tr>
<td>6:30</td>
<td>1 salad</td>
<td>1 cup of fresh spinach, dried herbs, 1 small chicken breast, ¼ cup pasta, 1 tangerine, ¼ cup grapes, drizzled in 2 tbsp. Balsamic vinegar</td>
</tr>
<tr>
<td>6:30-9pm</td>
<td>1 L</td>
<td>1 liter of water consumed over 2.5 hours</td>
</tr>
<tr>
<td>8:00am</td>
<td>3 eggs</td>
<td>2 whole eggs 1 egg white omelet with 1 tomato</td>
</tr>
<tr>
<td>8:15am</td>
<td>0.5 L</td>
<td>0.5 liters of water</td>
</tr>
</tbody>
</table>
APPENDIX F

QUALITATIVE GI SURVEY

Subject Code: __________

Date: ________________

Pre- Qualitative GI Survey:
Prior to your arrival (and currently), did you experience any of the following?
   o Bloating
   o Stomach Cramps
   o Nausea
   o Diarrhea

If you experienced any of the above symptoms, which symptoms were they and would you describe them as mild, moderate, or extreme?

Post- Qualitative GI Survey:
During or following the test, did you experience any of the following?
   o Bloating
   o Stomach Cramps
   o Nausea
   o Diarrhea

If you experienced any of the above symptoms, which symptoms were they and would you describe them as mild, moderate, or extreme?