World Applied Sciences Journal 12 (7): 1114-1124, 2011
ISSN 1818-4952

Effects of Glycerol-Induced Hyperhydration on Cardiovascular Functions and Endurance Performance in Athletes During the Course of Treadmill Exercise Performed at High Temperatures

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Abstract: The purpose of this study was to determine the effects of glycerol induced hyperhydration on cardiovascular functions and endurance performance in athletes during the course of 90 minutes treadmill run at 30°C. In a randomized, double-blind cross over experimental design 9 male elite long distance runners were tested three times with 3 days intervals (wash out) following ingestion of 20 ml.kg-1BW of three different mixture of solutions: 1) water with 1.2 gr.kg-^{1} BW glycerol (G) 2) diluted sports drink (SD) and 3) aspartame flavored distilled water (W). Exercise trials were conducted at an exercise intensity of 65% maximal oxygen consumption (VO_2max) for 90 min at 30±1.8°C and 25-35% relative humidity. Blood parameters (Na^+, K^+ and Cl^- concentrations, blood and plasma volumes, plasma osmolality), VO_2, VCO_2, respiratory exchange ratio (RER), heart rate (HR), perceived rate of exertion (RPE), perceived thirst levels (PTL) and abdominal discomfort (AD) were measured pre and post fluid ingestion, at the 30th, 60th and 90th min of exercise trials. Data were analyzed using two-way (treatment x time) analyses of variance (ANOVA). Significance was defined as $p<0.05$. There was no significant difference in blood parameters, VO_2, VCO_2, RER, HR, RPE, PTL or AD among the three trails ($p>0.05$). The findings of this study showed that glycerol supplementation in elite endurance athletes has no advantages effect on cardiovascular functions or improving endurance performance during 90min of treadmill run with an intensity of VO_{2max}% 65 performed at 30°.

Key words: Glycerol · Hyperhydration · Supplementation · Ergogenic aid

INTRODUCTION

Hyperthermia and dehydration are the most common complications of exercise activities performed in warm and humid environments. In these conditions, the first warning signal of the negative effects of dehydration is the deterioration in performance [1]. Dehydration due to the loss of body weight by 2% deteriorates endurance performance [2-5], increases thermal and cardiovascular stress [6] increases perceived rate of exertion [7] and deteriorates metabolic and central nervous system [8]. Accordingly, decrement in physcial performance leaves the athletes vulnerable to heat injuries [9]. American College of Sport Medicine [10] and National Athletic Trainers Association [11] recommends proper fluid replacement before and during exercise activities to prevent or delay the negative physiological effects of dehydration. It has been reported that hyperhydration prior to exercise is beneficial in preventing or delaying the deletioures effects of dehydration due to sweating [3, 12-15].

Water induced hyperhydration is not efficient because excess water is excreted from the body very quickly as urine. Therefore, different strategies of hyperhydration have been developed and used in athletics. In recent years, it has been shown that ingesting a mixture of water and glycerol solution has positive effects on body hydration and thermoregulatory functions during exercise performed at hot humid environments [9]. Glycerol increases fluid retention due to its high reabsorbility in the proximal and distal renal tubulus of nephron [16, 17] accordingly the plasma volume [18-26]. Glycerol hyperhydration decreases cardiovascular strain by increasing stroke volum and

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cardiac output and decreasing heart rate [27]. Moreover, it has been shown that glycerol hyperhydration has an ergogenic effect on endurance performance [9]. Therefore, glycerol supplementation is preferred compared to water as a hyperhydration fluid. On the other hand, controversial findings have been reported in the literature on the effects of gycerol ingestion on heart rate during exercise. According to these studies heart rate decreases [25, 27, 18], remains unchanged [14, 20, 22, 29], or increases [30] in glycerol induced subjects compared to those of controls. Although [27] has reported that glycerol hyperhydration increases stroke volume [29], has reported that cardiac output remained unchanged during exercise. These controversial findings on the effects of gylcerol hyperhydration have been attributed to the differences in methodology of the studies, such as, glycerol dose or concentration, environmental conditions or exercise intensity and duration.

The findings in the literature with regard to the positive effects of glycerol hyperhydration on sports performance are equivocal, as well. Inder *et al*. [31] and Murray *et al*. [28] have reported that glycerol hyperhydration prior to exercise has no advantageous effect on thermoregulatory and physiologic functions. In a recent meta-analysis study by Goulet *et al*. [32] it has been emphasized that the effects of glycerol hyperhydration on sports performance should be studied by using different methodologies and more comprehensive approaches.

Although the effects of glycerol ingestion on sports performance are quite equivocal glycerol has been reported as a masking agent in 2011 WADA Prohibited List [33]. The reasons for that prohibition might be the glycerol potential effect on increasing plasma volume and its damaging effects on renal functions. However, this study had been conducted before WADA's prohibition of glycerol.

The purpose of this study was to determine the effects of glycerol-induced hyperhydration on cardiovascular functions and endurance performance in athletes during submaximal exercise performed for 90 minutes at 30°C.

MATERIALS AND METHODS

Subjects: Nine elite long distance runners (trained at least for 3 years) participated in this study voluntarily. Their mean (±SD) age, body weight, height, percent body fat, lean body mass and VO_{2max} were; 18,7±1,3 years, 58,8±6,6 kg, 170,7±5,2 cm, 9,74±2,58%, 52,8±5,8 kg, 63,94±3,04 ml.kg^{-1}. The aim of the study was explained to the subjects and a letter of informed consent was obtained from each subject. The study protocol has been approved by the Ethics in Medicine, Surgery and Drugs Research Committee of Faculty of Medicine of Hacettepe University (date: 14.10.2005, number: LUT 05/74–7).

Overview of the Experimental Trials: Subjects visited the laboratory four times: one for preliminary testing and three for experimental testing. On the first visit, body weight, height and body composition of the subjects were measured. Then, they underwent an incremental treadmill test to determine the running speed corresponding to the. After 3 days, experimental trails were started. Subjects were tested three times with 3 days intervals following ingestion of three different beverages. For each trial subjects were asked to drink 20ml.kg^{-1} BW fluid and then a submaximal (65% of VO_{2max}) exercise test was administered for 90 minutes at 30±1.8°C and 25-35% relative humidity. Blood parameters, VO_2, VCO_2, respiratory exchange ratio (RER), heart rate (HR), perceived rate of exertion (RPE), perceived thirst levels (PTL) and abdominal discomfort (AD) were measured pre and post fluid ingestion, at the 30th, 60th and 90th min of exercise trials. Additionally, VO_2, VCO_2, RER and HR values measured at every 3 minutes during the exercise test.

Preliminary Test (Determination of VO_{2max}): One week before the first experimental trial, subjects' VO_{2max} were determined by an incremental treadmill test protocol. The purpose of this test was to determine the running speed of each subject corresponding to 65% of VO_{2max} to be used during the 90min exercise test. The test started after 3 min of warm up on the treadmill at a speed

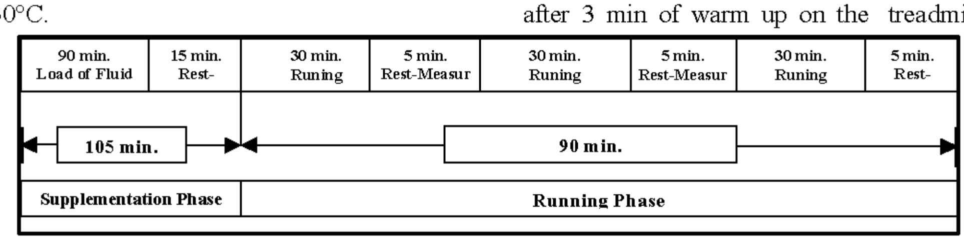

Fig. 1: Protocol design for each experimental trial.

of 6 km.h^{-1} and 0° slope. Throughout the testing protocol, treadmill speed has been increased by 1 km.h^{-1} at every 3 minutes until exhaustion [34]. VO_2, VCO_2 and RER values were measured by CosMed K_4b^2 analyzer. The peak VO_2values measured during the test was accepted as VO_{2max} of the subject. The same test protocol was repeated after 3 days and the highest VO_{2max} value measured during the two tests was recorded as the VO_{2max} of the subject. Then, the running speed of the subject corresponding the 65% of VO_{2max} was calculated from interpolation fitting the VO_2max values on fourth degree curves.

Pre-Exercise Hyperhydration: On the day of each trial, subjects were asked not to have breakfast before arrival to the laboratory. Upon arrival to the laboratory subjects were provided with 500ml water and 500ml fluid nutrient (ENSURE) to standardize the nutritional status and hydration levels of the subjects prior to the trial.

The three experimental beverages were given in a randomized, double-blind and counterbalanced order with subjects serving as their own control. The beverages were glycerol mixed with water (G, 1.2 gr.kg^{-1} BW), diluted sports drink (SD) and distilled water (W) flavored with aspartame. Subjects consumed a total of 20 ml.kg^{-1}BW fluid, 105 minutes before the exercise trial. Beverages were of similar sweetness, color, flavor and temperature (10°C). The total amount of glycerol provided by the G solution was 70.56±3.7ml. During each trial subjects drank a total of 1176±98ml fluid. The total volume of the berages was divided into three equal volumes and subjects were asked to drink each volume every 30 minutes.

Submaximal Exercise Test: Subjects underwent on a submaximal running test on a motorized treadmill (Woodway-PPS Med; USA) at a running speed corresponding the 65% of VO_{2max} for 90 minutes at 30±1.8°C and 25-35% relative humidity. During the 30th and 60th minutes of the exercise test resting periods of 5 minutes were allowed and blood samples were taken and VO_2, VCO_2, RER, HR, RPE, PTL and AD were measured. The same measurements were repeated after 5 minutes following the termination of exercise test. VO_2, VCO_2, RER and HR values measured at every 3 minutes during the exercise test.

Blood Parameters: Blood samples were collected through a catheter inserted into a vein in the antecubital fossa by a certified nurse. Following each blood sampling heparin (2500 I.U. heparin /100ml saline) is flushed into the catheter to prevent blood from clotting in it. A total of 10.5 ml. venous blood was drawn from each subject. 3.5 ml of blood samples were placed into the glass tubes with EDTA, HEPARIN and an empty biochemistry tube. Blood samples placed into the tubes with heparin were centrifuged at 4000 rpm for 40 minutes at room temperature. Blood samples were analyzed to determine blood glucose and lactate levels, blood volume and osmolality and blood electrolytes (Na, K, Cl) at the Biochemistry laboratory of Hacettepe University.

VO_2, VCO_2, RER and HR: VO_2, VCO_2 and RER values were measured with a breath-by-breath portable gas analyzer (CosMed-K_4b^2; ITALY). Heart rates were measured by Polar telemetry (Polar-S610i Heart Rate Monitor, FINLAND).

PRE, PTL and PAP: Rate of perceived exertion (Borg 20-point scale: 6, very, very light; 20, very, very hard), perceived thirst level (11-point scale: 1, none; 11, extreme) and abdominal discomfort (5-point scale: 1, none; 5, extreme) were measured at the 30th, 60th and 90th minutes of the exercise test [35].

Data Analysis: One Way Repeated Measures Analysis of Variance test was used to determine test-retest reliability of VO_2max values obtained by the two incremental treadmill tests. Kolmogorov-Smirnov test was used to verify the normality of distribution. Two-way (treatment x time) analyses of variance (ANOVA) test was used to compare the effects of experimental trials. When a significant difference was found LSD *post-hoc* test was used pair-wise comparisons. Significance was defined as $p<0.05$.

RESULTS

There was no significant difference among the three hyperhydration trials with regard to blood Na^+, K^+ or Cl^- concentrations ($p>0.05$, Na^+ F_{sup}= 3.661, K^+ F_{sup}= 2.339, Cl^- F_{sup}= 0.684) (Table 1). Na concentration in glycerol treatment decreased following supplementation and then decreased at the end of the exercise test ($p<0.05$). It increased at the end of exercise test compared to post supplementation ($p<0.05$) in water treatment. There was no change in Na concentrations with time points in sports drink treatment ($p>0.05$). K concentration in glycerol treatment decreased significantly at the end of exercise test compared to the pre-supplementation value ($p<0.05$).

Table 1: Blood electrolyte concentrations before and after hyperhydration and at the end of exercise test for three experimental trials with ANOVA results

		PreSup	PostSup	90th min		
n= 9		Mean±SD	Mean±SD	Mean±SD	p_{sup}	p_{time}
Blood Na^+ (mEq/l)	G	140.00±1.50	137.00±2.06	139.37±1.81	0.050	0.005
	SD	140.67±1.58	139.71±3.02	140.35±1.92		
	W	137.67±3.77	137.29±2.73	141.00±2.01		
Blood K^+ (mEq/l)	G	4.54±0.57	4.17±0.40	3.96±0.19	0.129	0.930
	SD	4.27±0.25	4.14±0.22	4.16±0.28		
	W	4.29±0.28	4.44±0.13	4.22±0.30		
Blood Cl^- (mEq/l)	G	101.33±2.54	101.66±1.50	105.67±5.63	0.519	0.077
	SD	101.00±1.58	99.78±9.48	102.22±6.92		
	W	100.33±1.73	103.33±5.24	104.56±5.70		

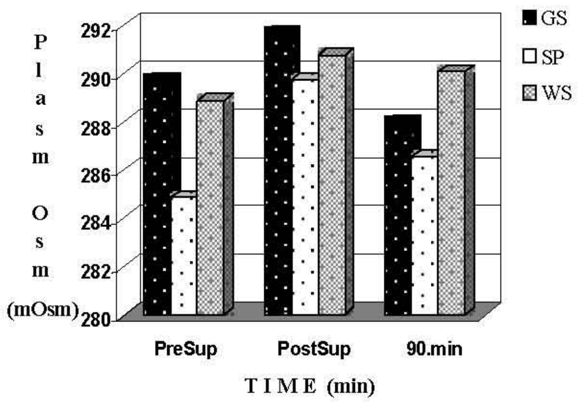

Fig. 2: Plasma osmolality changes over time

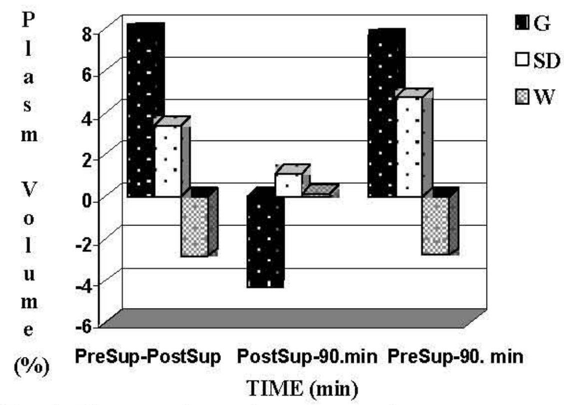

Fig. 3: Plasma volume changes over time.

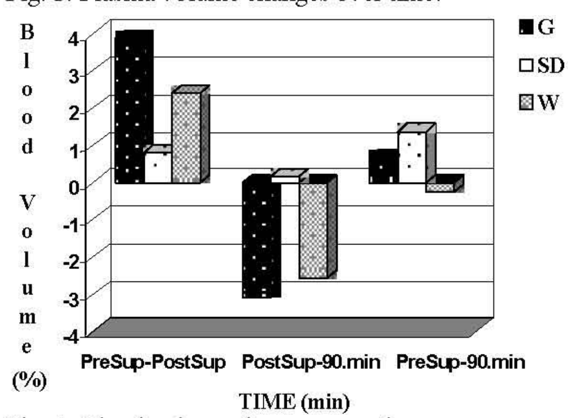

Fig. 4: Blood volume changes over time.

In contrast, Cl concentration increased at the end of the exercise test compared to both pre and post supplementation values in the same treatment ($p<0.05$).

No significant difference was found among the three hyperhydration trials with respect to plasma osmolality (Plasma Osm F_{sup}= 2,863, $p>0.05$, Figure 2). On the other hand, it changed significantly with time points (Plasma Osm F_{time}= 5,079, $p<0.05$) only in G trial. Plasma osmolality values following glycerol supplementation was significantly lower compared to those measured before supplementation and at the end of exercise test ($p<0.05$).

Percent changes in plasma and blood volumes didn't differ among the three trials (F_{sup}= 0.430, F_{sup}= 0,352, respectively, $p>.05$, Figure 3 and Figure 4). On the other hand, significant changes were observed in both parameters with time points (F_{time}=15.254, F_{time}= 10,366, respectively, $p<.05$).

Plasma volumes decreased significantly at the end of exercise test compared to post-supplementation values for the three treatments ($p<0.05$, Figure 3). Blood volumes increased following hyperhydration and then decreased at the end of the exercise. However, it was still higher compared to pre-supplementation.

Percent changes in blood volumes calculated for three time points were significantly different from each other for glycerol treatment. For the water treatment the change occurred from post-supplementation to minute 90 was significantly higher compared to the changes calculated for pre supplementation to post supplementation and pre supplementation to min 90 ($p<0.05$).

With respect to blood glucose and lactate levels there was no significant difference among the trials (F_{sup}= 0.776, F_{sup}= 0.781, respectively, $p>0.05$,

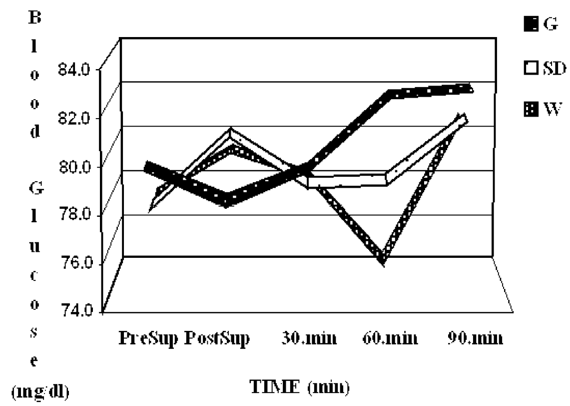

Fig. 5: Blood glucose changes over time.

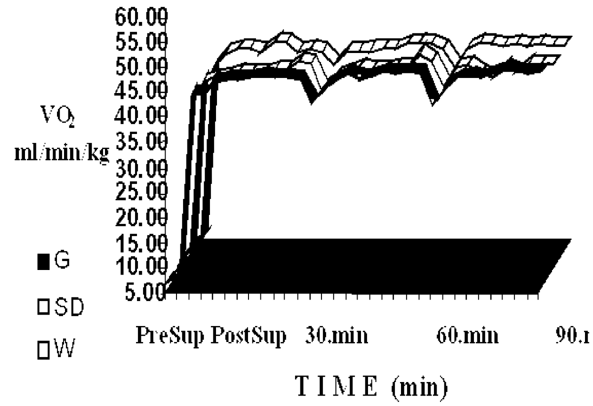

Fig. 7: Changes in VO_2 over time.

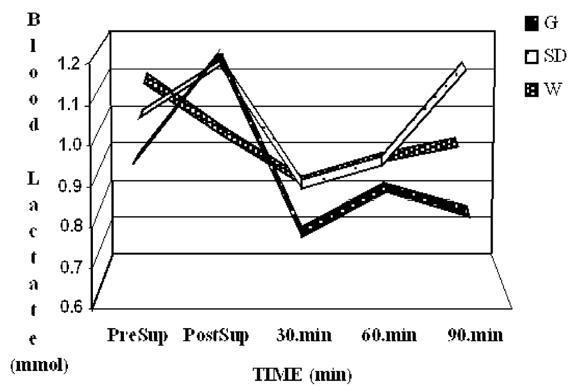

Fig. 6: Blood lactate changes over time.

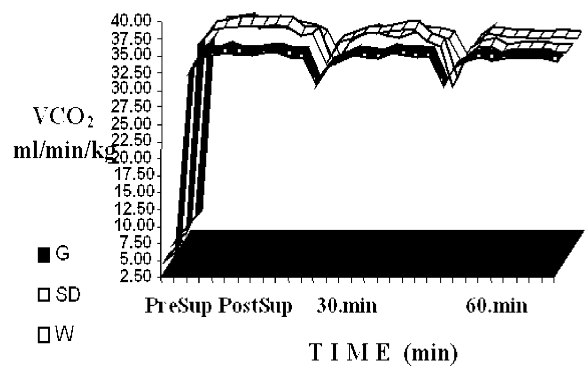

Fig. 8: Changes in VCO_2 over time.

Table 2: RPE, PTL and PAP at the 30th, 60th and 90th minutes of exercise test with ANOVA results

n= 9		30th min Mean±SD	60th min Mean±SD	90th min Mean±SD	p	P_{sup}	p_{time}
Perceived rate of exertion	G	7.22±1.92	7.78±3.15	7.88±3.14	0.183	0.291	0.020
	SD	8.22±3.31	8.67±3.39	9.11±3.95	0.298		
	W	8.22±2.77	8.78±2.95	9.56±3.88	0.038*		
Thirst level	G	1.44±0.73	1.44±1.01	2.89±1.76	0.230	0.534	0.307
	SD	1.78±1.30	2.56±2.83	2.78±2.95	0.148		
	W	2.67±2.06	2.56±1.94	2.44±2.46	0.960		
Abdominal discomfort	G	1.00±0.00	1.00±0.00	1.11±0.33	0.390	0.633	0.633
	SD	1.00±0.00	1.00±0.00	1.00±0.00	-		
	W	1.00±0.00	1.11±0.33	1.00±0.00	0.390		

* = min 30 is different from min 90 ($p<0.05$)

Figure 5 and 6). With regard to time points there was no significant difference in glucose levels (F_{time}= 0.435, $p<0.05$), however, blood lactate levels were differed (F_{time}= 5.479, $p<0.05$) only for the glycerol treatment. Blood lactate level following supplementation was significantly higher compared the values of min 30, 60 and 90 ($p<0.05$). Blood glucose parameters were similar among the trials up to min 30, however, statistically not significant differences were observed among trials from min 30 to min 60 during exercise. Blood glucose parameters were similar among the trials up to min 30, however, statistically not significant differences were observed among trials from min 30 to min 60 during exercise. After the 60th minute glucose values has started to increase and similar levels of blood glucose were observed among trials.

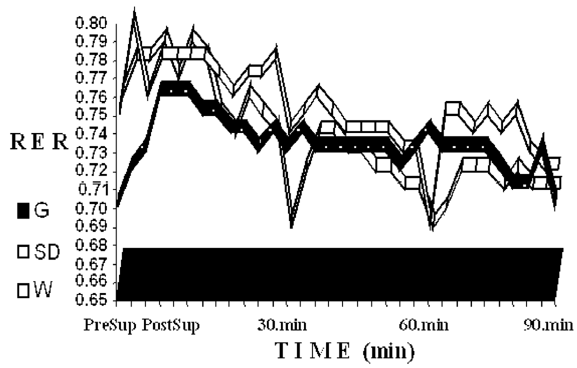

Fig. 9: Changes in RER over time.

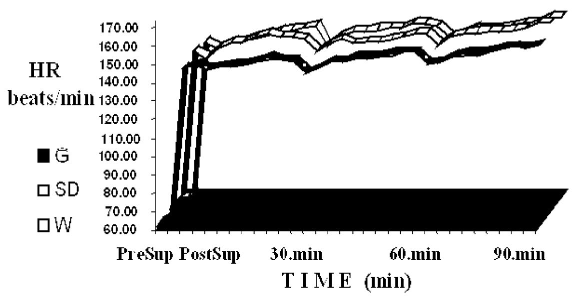

Fig. 10: Changes in HR over time.

There was no significant difference in perceived effort (F_{sup}= 1.336), thirst level (F_{sup}= 0.653) or abdominal discomfort (F_{sup}= 0.471) among the treatments (p>0.05, Table 2). Actually, no subjects complained of any abdominal discomfort or other side effects throughout the experimental trials. With respect to time points, RPE differed significantly (F_{time}= 5.042, p<0.05). RPE at min 90 was significantly higher than that of min 30 for water treatment (p<0.05). On the other hand, TL or AD didn't change with time points (p>0.05, F_{time}= 1.272, F_{time}= 0.471, respectively).

VO_2 values didn't differ among trials (F_{sup}= 0.499, p>0.05), but it did change with time points (F_{time}= 208.357, p<0.05) at min 30 and min 60 of exercise trial. On the other hand, VCO_2 differed significantly both among trials (F_{sup}= 4.854, p<0.05) and among time points (F_{time}= 237.789, p<0.05). Significant differences were found in VCO_2 at min 30 between SD and W, at min 60 between G and W and at min 63 between G and SD and W treatments.

RER and HR values didn't differ among trials (F_{sup}= 0.698 and F_{sup}= 2.257, respectively, p>0.05). They both changed significantly among time points (RER F_{time}= 8.077 and HR F_{time}= 154.327, p<0.05). The changes in RER with time points were not significant in glycerol treatment. However, significant changes were observed in RER values with time points (at min 30 and min 60) in sport drink and water trials. Heart rate values differed with time points (min 30. 60) among all trials.

DISCUSSION AND CONCLUSION

The purpose of this study was to compare the effects of three different fluid supplementations (glycerol, diluted sports drink and water) on cardiovascular and endurance performance during 90 minute treadmill run at high temperature. Findings of the study revealed that glycerol supplementation was not superior to sports drink (4% CHO) or water in improving cardiovascular and endurance performance.

Maintenance of hydration status throughout the experimental trials indicated that all fluid supplementations were given sufficiently to create a hyperhydration status. It is recommended that the amount of glycerol or other supplementation fluids should be consumed in a quantity that will compensate the loss of water through sweating [36]. Plasma and blood volumes and plasma osmolality are the main variables used to determine the hydration status of an athlete. Increase in plasma volume results in improvements in venous return and cardiovascular responses (i.e., decrease in heart rate and increase in cardiac output) [37]. In the present study, plasma and blood volumes increased following fluid supplementations and then decreased at the end of the exercise for all experimental trials. However, the percent changes in plasma and blood volume from pre-supplementation to min 90 showed that they were maintained throughout the trials and dehydration didn't occur (Figures 3 and 4). Nevertheless, maintenance of blood and plasma volumes was not sufficient to observe any improvement in cardiovascular responses. This finding is similar to the findings of O-Brian *et al.* [38] in that they didn't observe any improvement in cardiovascular responses as a result of glycerol hyperhydration. Studies [19, 20, 23, 28, 39, 40] have reported that glycerol supplementation increases plasma volume due to the increase in blood glycerol concentration and this increase in plasma volume was associated to the environmental temperature where exercise performed. Magal *et al.* [23] have reported a 7% increase in plasma volume in response to glycerol hyperhydration. However, they [23] also reported that the increase in plasma volume was not sufficient to prevent hypohydration during exercise.

Pre-supplementation plasma osmolality values in the present study were similar for all treatments (G: 289,78±2,99; SD: 291,67±4,21; W: 288,00±2,18 mosm/kg) (Figure 2). Marino *et al.* [24] have reported pre-supplementation plasma osmolality values for placebo and glycerol treatment groups as 281±2 and 282±1 mOsm/kg, respectively. In that study Marino *et al.* [24]

although the plasma osmolality increased over time, the only significant increase was observed in placebo group between the values of pre and post exercise. Similarly, in a study by Riedesel *et al.* [26] pre-exercise plasma osmolality values were found to be higher in glycerol and glycerol plus excess fluid intake groups compared to other groups. Plasma osmolality values increased during exercise in glycerol and water groups without excess fluid intake, however, no change was observed in other experimental groups [26]. Finding of our study with respect to effect of plasma osmolality is in contrast to the finding of Riedesel *et al.* [26] reporting increased plasma osmolality in response to glycerol supplementation.

Another determining factor of plasma osmolality is plasma Na^+ concentration [1]. Increase in plasma Na^+ and osmolality values were found to be associated with increase in esophageal temperature and this might be the reason for not sweating during exercise at high environmental temperatures [1]. Consuming fluids during exercise may prevent the increase in plasma Na^+ and osmolality values [26]. Glycerol hyperhydration may improve endurance performance by decreasing blood osmolality and electrolyte content [41]. Hitchins *et al.* [419 has suggested that these changes may delay the onset of either muscle or central fatigue.

Although glycerol is not oxidized directly in skeletal muscles [42, 43] it is a substrate for hepatic gluconeogenesis [44-46]. Conversion of glycerol to glucose, particularly in liver [44, 45] takes place so slowly that metabolic side effects of glucose intake are prevented. These features of glycerol have led the scientists to investigate the effects of glycerol on endurance performance in rats. Glycerol significantly decreased the rate of blood and liver glycogen consumption, delayed hypoglycemia and improved fatigue threshold during running [47]. On the other hand, in humans, increase in gluconeogenesis due to glycerol supplementation was not able to improve energy metabolism significantly during prolonged strenuous exercise [48]. In this study [48], the only noticeable effect of increased plasma glycerol was the delay of hypoglycemia by 30 minutes. However, it didn't slow down the rate of decrease in blood glucose level [48]. Similarly, Gleeson *et al.* [49] have reported that blood glucose levels in glycerol supplemented group 45 minutes prior to exercise was up to 14% higher in the later stages of exercise and at exhaustion compared to the placebo or glucose trials. In line with the literature, blood glucose levels in the present study didn't decrease and blood glucose levels didn't differ among trials (Figure 5). However, studies have found results that are contrary to these findings. Anderson *et al.* [18] have found no change in muscle glycogenolysis, lactate accumulation or fosfocreatine degradation in response to glycerol hyperhydration. In line with the findings of Anderson *et al.* [18] we found no change in blood lactate levels among the trials (Figure 6).

Glycerol induced hyperhydration has been suggested to increase metabolic reaction and improve performance. However anderson *et al.* [18] stated that these improvements cannot be attributes to the changes in muscle metabolism.

These findings have led to the studies investigating the effects of glycerol supplementation on carbohydrate and fat utilization during exercise by examining RER values. RER values have been found lower in almost all studies of glycerol supplementation. In our study we found no significant change in RER values in three trials (Figure 9). The lowest and the highest RER values found in the present study were 0.67±0.08 and 0.79±0.07, respectively.

In severe dehydration conditions during prolonged exercise or exogenous heat cardiovascular strain increases so that heart rate increases, stroke volume and cardiac output decrease [50]. Therefore, preventing dehydration during exercise is a great importance for preventing heat injuries and performance decrements. Glycerol has been found to retain body fluid and delay dehydration. However, Wendtland *et al.* [51] reported that although glycerol supplementation increased body fluid retainment it didn't improve cardiovascular or thermoregulatory functions. In contrast anderson *et al.* [18] have found that glycerol hyperhydration attenuated cardiovascular and thermoregulatory strain and so that improved endurance performance.

In studies finding performance improvement in response to glycerol supplementation HR has been found to be lower in glycerol supplemented groups compared to controls in both hot and warm environmental conditions [15, 18, 25, 52, 53]. There several studies reporting no change in heart rate in response to glycerol supplementation at any time points [22, 28, 36, 40, 48, 54, 55, 57]. In a study by Hitchins *et al.* [21] although glycerol treatment significantly increased performance by 5% compared with the placebo group, there was no change in either HR or VO_2 values. On the other hand, Marino *et al.* [56] found surprisingly higher heart rate values in glycerol supplemented group compared to placebo. Our findings regarding heart rate responses are in line with the studies reporting no change among the trials or time points (Figure 10).

Gycerol hyperhydration has been studied as an ergogenic aid in sports performance [58] and it has been shown that glycerol hyperhydration decreases cardiovascular strain [27] and thermoregulatory stress [9] by preventing dehydration [18, 19, 21]. Hitchins *et al.* [21] compared the effects of glycerol and placebo hyperhydration trials during 60-min cycle ergometer exercise split into two 30-min phases: a fixed-workload phase and a variable workload phase at hot and humid environment. Glycerol hyperhydration improved performance by 5% only during exercise with variable workload [21]. Additionally, perceived rate of exertion was found to be similar [21]. Anderson *et al.* [18] have reported%5 increases in endurance performance in glycerol trial. In this study [18], baseline body weight of subjects didn't differ at the end of the exercise; in contrast control subjects' values were lower. Therefore, the enhancement in sport performance in glycerol trial was attributed to decreased thermoregulatory stress and cardiovascular strain due to the prevention of dehydration. Contrary to these findings, others [23, 27, 29-31, 57, 60, 63] have reported that glycerol hyperhydration didn't improve exercise performance of prolonged, low and submaximal intensity. Findings of our study with respect to endurance performance parameters are on line with the findings of later studies. We didn't find any improvement in HR, VO_2 or VCO_2 variables in response to glycerol treatment during 90 minutes of treadmill run. These findings revealed that glycerol hyperhydration didn't improve endurance performance considering the current study protocol.

Inconsistency in findings regarding the effect of glycerol hyperhydration on endurance performance has been attributed to methodological differences of the studies [24, 27, 29, 31, 61, 62]. Leutlemeir *et al.* [61] have previously reported that exercise protocols may affect endurance performance and warned the researchers. Researchers have stated that in studies reporting no improvement in endurance performance exercise intensity (45-60% VO_{2max}) [27, 29, 62] or duration (30-35 min) [24, 31] might not be sufficient to create a hypohydration state. Moreover, several researchers reported that giving not enough time following glycerol supplementation and starting exercise trial may diminish the retention of body fluid [29, 63]. Robergs *et al.* [64] have stated that consumption of glycerol 1.0 $g.kg^{-1}$ BW and 1.4 to 2.0 lt of fluid containing glycerol is an appropriate strategy to create a hyperhydration state and this hyperhydration strategy was superior to water placebo trial in retaining body fluid between 2.5 to 4h following consumption. Very recently, Goulet *et al.* [65] has developed a new method for inducing glycerol hyperhydration to maximize endurance performance.

In conclusion, the findings of this study showed that glycerol hyperhydration was not superior to sports drink or water in improving cardiovascular responses or thermoregulatory system during 90m minutes of exercise performed at 30°C. Moreover, although glycerol hyperhydration prevented dehydration and maintained euhydration state it didn't affected endurance performance variables within the limits of this study protocol.

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