Influence of an Isotonic Sports Drink during Exercise and Recovery on Subsequent Endurance Capacity and Aldosterone Response in the Heat in Well-Trained Endurance Athletes

Mohammad Fayiz AbuMoh’d¹
¹Yarmouk University, Department of Sports/Movement Sciences, Irbid, Jordan

Abstract
This study was designed to investigate the influence of isotonic sports drink ingestion during exercise and recovery on subsequent endurance capacity, aldosterone, and other physiological responses in the heat. Twelve male well-trained endurance athletes (27.55±3.87 yrs) performed three experimental trials in which they ingested a sports drink (750 ml), water only or none. Each trial consisted of 3000 m run, 1 h recovery, and time to exhaustion test. The trials were randomized and separated by seven days. Drinks were administered in a crossover design, with the double-blind provision of sports drink and water. Blood samples were collected before and immediately after 3000 m run, following recovery, and at exhaustion. A repeated-measures ANOVA test revealed no differences in the physiological responses before and after 3000 m run (p>0.05). After recovery, heart rate was significantly lower (p=0.050) in the sports drink trial than with water and control, with no differences were noted in the other physiological responses (p>0.05). At exhaustion, however, the data showed no differences in oral temperature and heart rate, but the differences were shown in aldosterone, blood glucose, and sodium (p<0.05). A post hoc Bonferroni test revealed that aldosterone was lower with the sports drink (602.33±18.68 pmol/L) than with water (688.08±29.03 pmol/L) and control (695.25±49.21 pmol/L). Endurance capacity was significantly greater with sports drink (56.53±2.53 min) than with water (51.16±1.80 min, p=0.001) and the control (50.09±3.00 min, p=0.001), without differences between the water and control trials (p=0.178). In conclusion, the ingestion of isotonic sports drink increases endurance capacity and maintains aldosterone more effectively than with or without water probably due to improved fluid retention.

Keywords: dehydration, fluid deficit, oral temperature, hyperthermia, glycogen

Introduction
Prolonged moderate-intensity exercise can cause the depletion of muscle glycogen stores (Thomas, Morris, & Stevenson, 2009; Rollo & Williams, 2010), which can impair excitation-contraction coupling (Urdampilleta et al., 2015), and subsequently decrease performance. In addition, a fluid deficit during prolonged exercise may also develop (Pruna et al., 2016; Logan-Sprenger, Palmer, & Spriet, 2011), specifically when the exercise is performed in hot (> 30 °C) (Coso, Estevez, Baquero, & Mora-Rodriguez, 2008; Urdampilleta et al., 2015) and humid (> 60%; 30-60% refers to normal relative humidity) environments (Lee, Nio, Ang, Law, & Lim, 2011). Exercising for prolonged durations in the heat without adequate fluid intake can result in dehydration and hyperthermia.
Experimental design

Twelve well-trained endurance male athletes from Jordan were recruited for the present study. The descriptive characteristics of the participants are presented in Table 1.

### Table 1. Subjects demographic data

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean±SD</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>27.55±3.87</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169.75±2.66</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>61.33±3.83</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>21.23±0.45</td>
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<tr>
<td>resting HR (bpm)</td>
<td>51.58±2.78</td>
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<tr>
<td>VO₂_max (ml/kg/min)</td>
<td>60.08±4.96</td>
</tr>
<tr>
<td>Training volume (min/week)</td>
<td>707.50±61.87</td>
</tr>
<tr>
<td>Training experience (years)</td>
<td>10.00±3.47</td>
</tr>
</tbody>
</table>

All athletes were accustomed to training six times a week. Before trials, informed written consent form was provided by all participants. This study was performed in accordance with the ethical standards of the national research committee and with the 1964 Helsinki Declaration. The study was approved by the Local Scientific Committee (No. 02-07/2019 M).

**Experimental procedure**

One week prior to the commencement of the trials, the participants visited the laboratory three times. On the first visit, the participants’ characteristics were measured. After two days (on the second and third visits), each athlete was familiarized with the treadmill (TEC-GYM-EXC-700-UNITY, USA) at different speeds for at least 15 min, which was done to confirm a typical cadence. All athletes were instructed to standardize their dietary ingestion 24 h prior to the start of each experimental trial for not to affect their performance. The athletes were asked to avoid intensive training and physical activity and refrain from breakfast, coffee, and any ergogenic aids 24 h prior to each trial. They were asked to arrive at the location of the trials at 7:00 AM after an overnight fast.

water to counteract dehydration (Čugura et al., 2014), electrolytes, namely sodium, to avoid hyponatraemia and hyperthermia (Urdampilleta et al., 2015), and carbohydrate to increase muscle glycogen content (Lee et al., 2011).

Aldosterone, a steroid hormone secreted by the adrenal cortex, is the most potent mineralocorticoids (Vaidya, Brown, & Williams, 2015). It is responsible for fluid and blood volume homeostasis through sodium and chloride reabsorption via the renal tubules and excretes potassium and hydrogen ions by urine (Catena et al., 2017); therefore, it plays an essential function in the human body during prolonged exercise in the heat. Most studies that have investigated the effect of commercially sports drinks have been focused on cycling endurance capacity (Rollo & Williams, 2010; Lee et al., 2011; Čugura et al., 2014), and sodium and potassium electrolytes (Palmer & Spriet, 2008). However, no research has investigated the aldosterone response to the ingestion of isotonic sports drink.

Consequently, the current study aimed to investigate the influence of isotonic sports drink ingestion during exercise and recovery on subsequent endurance capacity, aldosterone and other physiological (oral temperature, blood glucose, heart rate, and sodium) responses in the heat compared to water ingestion. The hypothesis was that the consumption of a commercially isotonic sports drink would be more effective in improving endurance capacity, probably due to its contents that may maintain plasma volume.

**Methods**

**Participants**

Twelve well-trained endurance male athletes from Jordan were recruited for the present study. The descriptive characteristics of the participants are presented in Table 1.
Athletes wore the same attire and shoes for each trial.

Hydration protocol
All athletes were instructed to intake 500 ml of water 2 h prior to the beginning of a trial and refrain from eating and drinking thereafter, unless a trial procedure was indicated. The ingredients of isotonic sports drink and water are described in Table 2.

<table>
<thead>
<tr>
<th>Table 2. Composition of test drinks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Contents</strong></td>
</tr>
<tr>
<td>Energy (kcal/L)</td>
</tr>
<tr>
<td>Protein (g/L)</td>
</tr>
<tr>
<td>Fat (g/L)</td>
</tr>
<tr>
<td>Sucrose (g/L)</td>
</tr>
<tr>
<td>Glucose (g/L)</td>
</tr>
<tr>
<td>Na⁺ (mmol/L)</td>
</tr>
<tr>
<td>K⁺ (mmol/L)</td>
</tr>
<tr>
<td>pH</td>
</tr>
</tbody>
</table>

Legend: Energy, electrolytes, and macronutrients content were obtained from the label of drink manufacturer.

These drinks were served at 18 °C. Athletes were provided to intake either 150 ml of sports drink or water after 5 min of the beginning of 3000 m run. During the recovery period, athletes were asked to ingest either 150 ml of sports drink or water every 15 min. The total volume of each drink was 750 ml. The drinks were served in indistinguishable bottles, so that the participants did not know which drink they had ingested.

3000-m run
Participants entered the track of a stadium and completed a 10-min warm-up including 4 min stretching. Then, they ran 3000-m around the track in which the environmental temperature was warm in all three trials (25–28 °C) with normal relative humidity of 49–52%. Athletes were instructed to run each round on the track in 1.5 min to avoid reaching a heart rate at 160 bpm for preventing myocardial strain (Tortora & Derrickson, 2010), possible fatigue, and high fluid loss via sweating. The heart rate was monitored using a heart rate-vest monitor (Samsung Electronics, South Korea). The environmental temperature and relative humidity were observed using a thermo-hygrometer (A 1, China). The entire 3000-m run took 11.45–12.00 min.

Recovery period
On completion of the 3000-m run, athletes exited the track and entered a chamber at the laboratory in which the ambient temperature was set at 22 °C. Athletes sat resting in the chamber for one hour, during which they ingested their drinks. Recovery ensures the return of the muscle to its pre-exercise state after performance. It is suggested that the recovery period ensures performance in subsequent endurance capacity.

Endurance capacity test
Following the recovery period, athletes completed the endurance capacity test in all trials at a hot temperature (31–33 °C) and normal relative humidity (44–47%). The endurance capacity (time to exhaustion test) was carried out on the treadmill. After a standardized 5 min warm-up on the treadmill, athletes initially ran at 8 km/h, increased by 1 km/h every five minutes until volitional exhaustion. Athletes received verbal encouragement to continue running as long as possible. Running tolerance was defined as the incapacity to maintain a regular step cadence on the treadmill. At that point, the examiner pressed on the emergency stop button, and time to exhaustion was recorded.

Blood samples collection and analysis
Blood samples were collected from each athlete in the three trials and were withdrawn from the median vein of the athlete’s left arm. Samples were taken at pre-trial, immediately after the 3000-m run, at the end of the recovery period, and immediately after the endurance capacity test. A total of 3 ml of blood was dispensed into a plain tube containing clot activator to measure blood glucose using Integral 400 (Switzerland), and 3 ml was dispensed into an anticoagulant EDTA tube. Oral temperature was measured by Digital thermometer-SDT-10A (Samsung, Korea). The plain blood tube was centrifuged at 3 °C and 3500 rev/min for 10 min to allow for the extraction of serum. Serum was used to measure concentrations of sodium using an AL 480 Beckman electrolyte analyser (CO, Japan) and aldosterone hormone using chemiluminescent immunoassay (Elecsys Roche GmbH, Germany). The reference ranges of variables were as follows: 3.9–6.1 mmol/L for blood glucose, 111–860 pmol/L for serum aldosterone, 135–152 mmol/L for serum sodium. The normal range of body temperature is 36.6–37.4 °C.

Statistical analysis
Descriptive statistics are reported as Mean ± standard deviation (SD). Significance was set at p<0.05 for all analyses. A Shapiro-Wilk test was applied to check for normal distribution. All variables (aldosterone, oral temperature, blood glucose, heart rate, and sodium) at all the time points of the protocol were normally distributed (p>0.05). A repeated-measures analysis of variance (ANOVA) with a Greenhouse-Geisser correction was used to determine possible differences between trials in physiological responses and endurance capacity. When a significant F rate was achieved, post hoc tests using the Bonferroni correction was used for pairwise comparisons using adjusted means. Statistical analysis was conducted using SPSS version 18.0 and Microsoft Excel.

Results
There were no significant differences in the physiological parameters (aldosterone, oral temperature, blood glucose, heart rate, and sodium) measured before the commencement of all experimental trials (Table 3).
No differences existed between trials for any physiological parameters, insuring that the all parameters were normally distributed (p>0.05).

Table 4 illustrates the results of responses of these physiological parameters to isotonic sports drink, water or control at three time points: 1) after 3000 m run, 2) following recovery, and 3) at exhaustion. There were no differences in all physiological parameters after the 3000 m run between trials (p>0.05). After recovery, no differences were noted in the physiological parameters (p>0.05) except for heart rate that was significantly lower in the sports drink trial (p=0.050) than with water and control.

At exhaustion, data showed no differences in oral temperature and heart rate (F=1.321, p=0.061; F=1.354, p=0.303, respectively), but differences were present in aldosterone, blood glucose, and sodium (F=128.361, p=0.001; F=15.854, p=0.031; F=4.866, p=0.014, respectively, Table 4). Post hoc using Bonferroni with adjusted means revealed that aldosterone concentration was lower with sports drink than with water (p=0.001) and control (p=0.001), blood glucose levels were elevated with sports drink than with water (p=0.001) and control (p=0.001), and revealed greater serum sodium with sports drink than with water (p=0.046) and control (p=0.024). However, data reported no differences in aldosterone, blood glucose, and sodium between the water and control trials.

There were significant differences between trials (F=44.649, p=0.001) in endurance capacity (treadmill time to exhaustion). Endurance capacity was greater with the sports drink (56.53±2.53 min) than with water (51.16±1.80 min, p=0.001) and control (50.09±3.00 min, p=0.001), but no differences were observed between the water and control trials (p=0.178) (Figure 1).
Post hoc with a Bonferroni adjusted means was used to determine the differences in the endurance capacity between the three trials. *Sports drink significantly different from the control trial (p=0.001). †Sports drink significantly different from the water trial (p=0.012). No differences existed between the water and control trials (p=0.460). Significance level was set at p<0.05.

**Discussion**

The results of this study revealed that the endurance capacity (treadmill time to exhaustion) was longer with the isotonic sports drink than with water and the control. This result could be explained by the availability of carbohydrates in the sports drink during 3000 m run and recovery, contributing to maintained glycolysis during running, particularly at high speed on the treadmill. It is worth noting that a small elevation in carbohydrates promotes glucose and fluid uptake (Shirreffs, 2009), ensuring sustained energy supply. This result agrees with the study of Lee et al. (2011) who reported that in a hot environment (32 °C and 65% rh), the greater endurance capacity with a sports drink (1.5 L) was about 17.7 min longer than with water and about 13.5 min longer than with placebo in physically active males. However, the recovery period in that study was 5 h after 75 min run, which enhanced glycogenogenesis before beginning an endurance capacity test. Bilzon et al. (2000) showed 16 min longer running with a carbohydrate-electrolyte than with a placebo in healthy males. Pruna et al. (2016) found that time to exhaustion following 1 h run on a treadmill was significantly longer with ingesting 1 L of sports-electrolyte-glutamine drink compared to no hydration trial in 12 male endurance athletes. In the same study, they reported improved reactive ability to multiple visual stimuli in a 60-sec test following sports beverage ingestion compared to no hydration status. However, the trials of that study were performed in a cooler environment (22.9 °C and 44% rh). Thomas and colleagues (2009) reported improved cycle to exhaustion after ingesting chocolate milk (32 ± 11 min) compared to carbohydrate replacement drink (21 ± 8 min) and fluid replacement drink (about 23 min) in trained cyclists. They suggested that beverages containing protein are likely to benefit performance rather than carbohydrate or fluid alone. Stevenson et al. (2009) found improved performance and alertness in male golfers as a result of consumption of an isotonic drink (6.4 g carbohydrate and 16 mg caffeine per 100 ml) before a golf game and 1.6 mg/kg of caffeine plus 0.64 g/kg of carbohydrate during a game. In contrast, Rollo and Williams (2010) showed that ingestion of carbohydrate-electrolyte beverage (489 ml of water containing 6.4% carbohydrate) did not increase total running distance (13.6 min) compared to a placebo (13.5 min) in endurance-trained male runners.

The main finding of the present study is that athletes significantly completed the endurance capacity test with lower aldosterone concentrations in the sports drink than that of the water and control trials. This result might be attributed to sodium replacement by ingesting isotonic sports drink that can promote blood pressure homeostasis. Aldosterone is secreted from adrenal cortex as a result of the renin-angiotensin system (Vaidya et al., 2015). When blood flow is decreased, renin is synthesized from blood prorenin in the renal juxtaglomerular cells, which is, in turn, converted to angiotensin I by angiotensinogen found in the liver (Tortora & Derrickson, 2010; Guyton & Hall, 2006). Angiotensin I is then converted to angiotensin II by the angiotensin-converting enzyme, which stimulates the secretion of aldosterone from the adrenal cortex (Tortora & Derrickson, 2010). Aldosterone stimulates the renal tubules to increase reabsorption of sodium and water (Catena et al., 2017) to increase plasma volume and subsequent blood pressure. Thus, aldosterone was higher in the water and control trials to maintain plasma volume and electrolyte balance. In addition, the correlation between aldosterone and sodium is paralleled. The higher sodium content in the sports drink in comparison to water could explain this. This elevation may maintain a higher plasma volume and extracellular fluid (Below & Coyle, 1995), promoting an equilibrium of osmosis. Of relevance, sodium can improve performance through enhanced fluid retention during exercise (Lee et al., 2011). In line with this, Below and Coyle (1995) showed that the ingestion of 550 mg of sodium elevated its concentration
in bloodstream and enhanced the maintenance of plasma volume during 50-min cycling. Lee et al. (2011) also found higher sodium level at cycling exhaustion in a sports drink trial compared to water or a placebo.

The finding of the present study implies that blood glucose at exhaustion was significantly elevated following sports drink ingestion compared to the water and control trials. This result might be attributed to sufficient glycogen synthesis during recovery; what is more important is that blood glucose levels in the three trials were within the normal ranges, which might be related to the theory that stated blood glucose is elevated or maintained while exercise is performed in the heat (Kay & Marino, 2000; Yaspelkis, Scropo, Wilmore, & Ivy, 1993). Furthermore, elevated blood glucose in the heat is related to the increased levels of catecholamines (Febbraio et al., 1994), which are responsible for glycogenolysis. Thus, blood glucose is unlikely to be the reason for exercise cessation in the present study.

Mean heart rates were not different between trials at all the time points except for following recovery, though the endurance capacity was significantly longer with the sports drink than with the water and control trials. This result could be explained by the role of sports drink in cardiovascular preparation during recovery, facilitating heart muscles to work effectively during the endurance capacity test. For instance, heart rates in the present study were insignificantly lower in the sports drink trial compared to the water and control (Table 4). Lee et al. (2011) suggested that sports drink reduced cardiovascular strain compared to water or placebo.

Data reported that mean oral temperatures were similar across trials at exhaustion. The explanation of this result might be attributed to the achieved greater time to exhaustion in the sports drink. This result agrees with the study of Lee et al. (2011), who found that mean core temperatures were similar across trials at exhaustion (sports drink: 38.7 ± 0.5 °C, placebo: 38.7 ± 0.4 °C, water, 38.6 ± 0.5 °C). However, the range of core temperature in all trials in that study was 38.0–39.7 °C, indicating that several athlete’s temperatures have reached the point of hyperthermia (≥ 39 °C). In the present study, the range of oral temperatures was 37.8–38.7 °C. Nevertheless, the core temperature is higher than oral temperature by about 0.5 °C (Coso et al., 2008), ensuring that athlete’s oral temperatures in the present study reached the point of hyperthermia similarly with those in the study of Lee et al. (2011).

Exercise in the heat poses a challenging effect on the ability of an athlete to control his/her body internal environment because of the high ratio of metabolic heat production and heat gain from the environment (Maughan & Shirreffs, 2004), which may result in the incidence of hyperthermia. A hot and humid environment impairs thermoregulation due to the competition between the skin and active muscles for blood flow (Butts et al., 2016). Furthermore, exercise-induced increases in thermal strain result in alterations in central activation and muscle contractile function (Pointon et al., 2012). Subsequently, the oral temperature in the present study might be the main reason for exercise cessation. A limitation of the present study was that urine specific gravity (USG), urine volume, and atrial natriuretic peptide (ANP) hormone were not measured, so further research to measure those variables is recommended.

According to the composition of the commercially available sports drink consumed in the present study, further studies should prove the significance of its influence. No studies evaluated the aldosterone hormone response to ingesting a commercially sports drink. Based on the current findings, this study concluded that the ingestion of isotonic sports drink attenuates elevation in aldosterone concentrations more effectively than with or without water ingestion. For athletes engaging in multiple endurance runs in a single day, consuming isotonic sports drink during exercise and recovery may improve subsequent endurance capacity.


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\[ \text{Conflict of Interest} \]
The authors declare that there is no conflict of interest.

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\[ \text{References} \]


