The Effects of Sports Drink Osmolality on Fluid Intake and Immunoendocrine Responses to Cycling in Hot Conditions

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Summary We investigated the effects of two carbohydrate-based sports drinks on fluid intake and immunoendocrine responses to cycling. Six well-trained male cyclists completed trials on three separate days that involved cycling at 60% VO2peak for 90 min in hot conditions (28.1±1.5°C and 52.6±3.1% relative humidity). During each trial, the subjects consumed ad libitum (1) an isotonic sports drink (osmolality 317 mOsm/kg), (2) a hypotonic sports drink (osmolality 193 mOsm/kg) or (3) plain water. The cyclists consumed significantly (p<0.05) more of the isotonic drink (1.23±0.35 L) and hypotonic drink (1.44±0.55 L) compared with water (0.73±0.26 L). Compared with water (−0.96±0.26 kg), body mass decreased significantly less after consuming the hypotonic drink (−0.50±0.38 kg) but not the isotonic drink (−0.51±0.41 kg). Blood glucose concentration was significantly higher at the end of the isotonic and hypotonic drink trials compared with the water trial. Neutrophil count and the plasma concentrations of catecholamines, interleukin 6 (IL-6), myeloperoxidase, calprotectin and myoglobin increased significantly during all three trials. IL-6 and calprotectin were significantly lower following the hypotonic drink trial compared with the water trial. In conclusion, hypotonic sports drinks are appealing for athletes to drink during exercise, and may help to offset fluid losses and attenuate some inflammatory responses to exercise.

Key Words heat stress, endurance exercise, hydration, cytokines, inflammation

An extensive amount of research has investigated the effects of sports drinks on immunoendocrine responses to exercise. This research has predominantly focused on the effects of differences in the amount of carbohydrate contained within sports drinks. Carbohydrate attenuates the mobilization of leukocytes into the circulation by reducing the systemic release of stress hormones such as cortisol, catecholamines and growth hormone (1–3). Carbohydrate also reduces the circulating concentrations of cytokines (1, 3–6) and cytokine gene expression in skeletal muscle (5). Most research on the effects of sports drinks on immunoendocrine responses to exercise has used isotonic drinks containing a combination of sucrose+glucose+fructose or glucose+maltodextrin. In contrast, little research has systematically compared the influence of the osmolality based on the carbohydrate composition of sports drinks on immunoendocrine responses to exercise.

The osmolality and carbohydrate composition of sports drinks may influence immunoendocrine responses to exercise for two reasons. First, drink osmolality influences personal preference for fluid consumption during exercise (7) and exercise-induced changes in plasma volume and serum osmolality (8, 9). Personal preference for fluid consumption influences voluntary fluid intake and changes in body temperature during exercise (10), which may in turn affect immunoendocrine responses to exercise (11–15). Second, the type of carbohydrates consumed during exercise influences the rate of endogenous carbohydrate oxidation (16–19), which may in turn affect metabolic demands and immunoendocrine responses to exercise (1, 3–6). The purpose of this study was to compare the effects of two sports drinks of differing osmolality and carbohydrate composition on fluid intake, core temperature and immunoendocrine responses to exercise. We hypothesized that ingesting a hypotonic drink containing a combination of sucrose, fructose and maltodextrin would attenuate immunoendocrine responses to exercise more effectively than consuming an isotonic drink containing a combination of glucose and sucrose.

MATERIALS AND METHODS

Experimental design. This study was a randomized,
placebo-controlled study consisting of three separate experimental trials. During the exercise trials, subjects consumed (1) an isotonic sports drink (osmolality 317 mOsm/kg) containing 4% glucose and 2% sucrose, (2) a hypotonic sports drink (osmolality 193 mOsm/kg) containing 2.4% sucrose, 1.2% fructose and 0.5% maltodextrin or (3) plain water. The containers for each drink were painted black, and the taste of drinks was flavored identically so that the subjects would not recognize which drink they were consuming. The drinks were provided to the subjects ad libitum on request. This study was approved by the Human Research Ethics Committee of the Faculty of Sport Sciences of Waseda University for use of human subjects in accordance with the Declaration of Helsinki. Prior to participation, the subjects provided their informed consent.

Subjects. Six well-trained male cyclists volunteered to take part in this study. They exercised more than 2 h a day five times a week. Their means (±standard deviations: SD) characteristics were as follows: age (19.0±0.6 y), body mass (64.1±8.9 kg), height (1.71±0.1 m) and maximum aerobic power (VO2max: 58±6 mL·kg⁻¹·min⁻¹). At the time of the study, none of the subjects were accustomed to exercising in hot conditions.

Preliminary testing. Peak oxygen consumption (VO2peak) was measured using a maximal graded exercise test with an electromagnetically braked cycle ergometer (Combi RS-232, Combi; Tokyo, Japan). The initial workload was 60 W, and the workload was increased thereafter by 30 W every 3 min starting at 60 W until subjects could not maintain the required pedalling frequency (60 rpm). Heart rate (WEP-7404, Nihon Kohden Corp.; Tokyo, Japan) was monitored throughout the exercise. During the progressive exercise test, the expired gas of subjects was collected, and the rates of oxygen consumption (VO2) and carbon dioxide production (VCO2) were measured and averaged over 30-s intervals using an automated breath-by-breath gas analyzing system (Aeromonitor AE-300S, Minato Medical Science; Tokyo, Japan).

Experimental trials. Following the preliminary testing, the subjects all completed three separate experimental trials. Each experimental trial was performed on separate days at least 1 wk apart. In all three trials, the subjects cycled at 60% VO2peak for 90 min in a climate chamber in hot conditions (28.1±1.5˚C and 31.5% relative humidity). The workload corresponded to 60% VO2peak was determined from the graded exercise test by plotting power output against 60% VO2peak. During each trial, the subjects consumed one of the three drinks described above ad libitum. These drinks were provided for the subjects at 10±2˚C. The hypotonic or isotonic drinks were of the same flavor, and served to the subjects in the same type of bottle. In this manner, the subjects were blinded as to which drink they were consuming.

Fluid consumption and changes in body mass were recorded following exercise. To measure rectal temperature during exercise, the subjects self-inserted a rectal probe 10 cm past the anal sphincter. Heart rate was monitored continuously during exercise.

Blood sampling and analysis. Blood lactate concentration was measured in finger prick samples at the end of exercise. Venous blood samples were collected by venipuncture from an antecubital vein before exercise, after 45 min, at the end of exercise (90 min) and after 30 min of recovery. Blood samples were collected into serum separation tubes or vacutainers containing EDTA. A portion of whole blood was used to measure hemoglobin, hematocrit and full blood cell counts using an automatic blood cell counter (PocH100i, Sysmex; Kobe, Japan). The serum separation tubes were left at clot at room temperature for 30 min, while the vacutainers containing EDTA for plasma separation were immediately centrifuged at 1,000 ×g for 10 min. Serum and plasma was then removed and stored at −80˚C for later analysis. Serum osmolality was measured by an automatic osmometer (OSMOSTAT, Kyoto Daichii Kagaku; Kyoto, Japan). Serum free fatty acid concentration was measured on an automated analyzer (Hitachi model 7170, Hitachi, Ltd.; Tokyo, Japan) using an enzymatic reaction involving acyl-coenzyme A synthetase and acyl-Coenzyme A oxidase (NEFA HR-II, Wako Pure Chemical Industries, Ltd.; Osaka, Japan). Plasma glucose concentration was measured spectrophotometrically on an automated analyzer (Hitachi model 7170) using an enzymatic reaction involving hexokinase (GLU-HK (M), Shinotest; Tokyo, Japan). Plasma was analyzed for lactate using an enzymatic assay (Determliner LA, Kyowa Medics; Tokyo, Japan) and an automated analyzer (JCA-BM12, JIEL; Tokyo, Japan). Commercial enzyme-linked immunosorbent assay (ELISA) kits were used to measure the plasma concentrations of cytokines interleukin 1 receptor antagonist (IL-1ra), IL-6, and monocyte chemoattractant protein 1 (MCP-1) (R&D Systems; Minneapolis, MN), IL-8, IL-10, and IL-12p40 (Becton Dickinson Biosciences; San Diego, CA), myoglobin (Life Diagnostics; West Chester, PA), the neutrophil activation markers myeloperoxidase and calprotectin (HyCult Biotechnologies; Uden, The Netherlands) and catecholamines (Labor Diagnostika Nord: Nordhorn, Germany). ELISA measurements were performed according to the instructions for each ELISA kit using a microplate reader (VERSAmax, Molecular Devices; Sunnyvale, CA). The plasma concentrations of all these variables were adjusted for changes in plasma volume (20).

Statistical analysis. Data were analyzed using either a 3×4 factor analysis of variance for normally distributed (or log-transformed data) or Friedman’s rank test for non-normally distributed data (IL-1ra, IL-8 and IL-10). When significant time effects and time×trial interactions were evident, paired t-tests with Bonferroni correction were used to compare changes within each trial and differences between trials. Data were analyzed using Sigma Stat 3.1 Software (Systat; Point Richmond, CA). Significance was set at p<0.05.
RESULTS

Physiological responses

The subjects consumed a significantly greater volume of both the isotonic drink (1.23±0.35 L; p=0.022) and the hypotonic drink (1.44±0.55 L; p=0.035) compared with water (0.73±0.26 L). Body mass decreased significantly less (p<0.05) after consuming the hypotonic drink (−0.50±0.38 kg) compared with water (−0.96±0.26 kg). The change in body mass after consuming the isotonic drink (−0.51±0.41 kg) also tended to be smaller compared with drinking water (p=0.057). Compared with pre-exercise values, serum osmolality was significantly lower (p<0.05) at the end of the hypotonic drink trial (−7±5%) and the water trial (−11±4%), but not the isotonic drink trial (−5±10%). Changes in serum osmolality were not significantly different between trials. Plasma volume decreased by −7.1±9.3% after the isotonic drink trial, −8.0±5.7% after the hypotonic drink trial, and −11.7±4.5% after the water trial, with no significant differences between the trials. Core temperature at the end of exercise was 38.8±0.3°C after the isotonic drink trial, 38.7±0.3°C after the hypotonic drink trial, and 38.8±0.5°C after the water trial, with no significant differences between the trials. Heart rate at the end of exercise was 172±16 beats/min after the isotonic drink trial, 163±20 beats/min after the hypotonic drink trial, and 169±18 beats/min after the water trial, with no significant differences between the trials. Blood lactate concentration at the end of exercise was 1.4±1.2 mmol/L after the isotonic drink trial, 1.0±0.7 mmol/L after the hypotonic drink trial, and 1.2±0.8 mmol/L after the water trial, with no significant differences between the trials.

Energy substrate responses

The average amount of carbohydrate consumed was 74±21 g in the isotonic drink trial, and 59±23 g in the hypotonic drink trial. Plasma glucose concentration increased significantly (p<0.05) above pre-exercise values throughout the isotonic drink trial (Fig. 1). During the hypotonic drink trial, plasma glucose increased significantly (p<0.05) during the first 45 min of exercise, but then returned to pre-exercise values at the end of exercise and during recovery. Glucose remained significantly higher throughout the isotonic and hypotonic drink trials compared with the water trial (p<0.05).

Serum free fatty acid concentration was significantly elevated (p<0.05) at the end of exercise in all three trials, and remained significantly higher after the water trial compared with the isotonic and hypotonic drink trials (p<0.05). Serum free fatty acid concentration was significantly elevated (p<0.05) at the end of exercise in all three trials, and remained significantly higher after the water trial compared with the isotonic and hypotonic drink trials (p<0.05).

Catecholamine responses

Plasma epinephrine concentration was significantly higher (p<0.05) than pre-exercise values after exercise in the isotonic drink and water trials, but not in the hypotonic drink trial (Fig. 2). Plasma norepinephrine concentration was significantly higher (p<0.05) than pre-exercise values after exercise in all three trials, and remained elevated 30 min post-exercise in the isotonic drink trial.
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Changes in catecholamines were not significantly different between the trials.

**Immune responses**

Neutrophil counts were significantly higher \((p<0.05)\) than pre-exercise values 30 min after exercise in all three trials, but the pattern of changes was not significantly different between the trials (Fig. 3). Plasma IL-6 concentration was also significantly higher \((p<0.05)\) than pre-exercise values at the end of exercise and 30 min post-exercise in all three trials (Fig. 3). IL-6 was significantly lower \((p<0.05)\) at the end of exercise in the hypotonic drink trial compared with both the isotonic drink and water trials. The plasma concentrations of IL-1ra, IL-8, IL-10, MCP-1 and IL-12p40 (Table 1) remained unchanged following exercise in all three trials. Plasma myeloperoxidase concentration was significantly higher \((p<0.05)\) than pre-exercise values at the end of exercise in the water and isotonic drink trials, but not the hypotonic drink trial (Fig. 4). Plasma calprotectin concentration was significantly higher \((p<0.05)\) than pre-exercise values at the end of exercise in all three trials (Fig. 4). Calprotectin was significantly lower 30 min post-exercise in the hypotonic drink trial compared with the water trial. Plasma myoglobin concentration was significantly higher \((p<0.05)\) than pre-exercise values 30 min post-exercise in all three trials, but was not significantly different between the trials (Table 1).

**Table 1.** Plasma cytokine and myoglobin concentrations.

<table>
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<th>PRE</th>
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<td>17±6</td>
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</table>

*Significantly different from pre-exercise values. Data for IL-1ra, IL-8 and IL-10 are geometric mean±95% confidence interval \((n=6)\). PRE, pre-exercise; 45 min, 45 min during the 90-min cycling exercise; END, end of exercise; REC, recovery at 30-min post-exercise.
The aim of this study was to compare the effects of the carbohydrate content and osmolality of sports drinks on immunoendocrine responses to endurance exercise. Voluntary consumption of the hypotonic drink was significantly greater than water consumption, which suggests that the athletes preferred to drink the hypotonic sports drink. Consuming the hypotonic drink attenuated plasma concentrations of IL-6 and calprotectin following exercise compared with consuming water. These findings suggest that consuming hypotonic sports drinks may offset fluid losses and reduce some inflammatory responses following exercise.

On average, fluid losses (as indicated by change in body mass) were around 50% lower in the isotonic and hypotonic drink trials compared with the water trial. This difference most likely reflects the greater volume of fluid consumption in the isotonic and hypotonic drink trials compared with the water trial. The experimental design of the present study was different to other exercise studies examining the effects of the fluid osmolality on fluid balance and physiological responses. Nevertheless, some comparisons are worth noting. Rather than allowing ad libitum fluid consumption, Galloway and Maughan (8) provided subjects with a 2% carbohydrate drink (20 g glucose; 239 mOsm/kg) at a rate of 3.57 mL/kg every 10 min, or a 15% carbohydrate drink (20 g sucrose, 130 g glucose polymer; 324 mOsm/kg) at a rate of 1.79 mL/kg every 10 min. Maughan et al. (9) provided subjects with an isotonic drink (200 mmol/L glucose; 310 mOsm/kg) or a hypotonic drink (90 mmol/L glucose; 240 mOsm/kg) at a rate of 100 mL every 10 min. Similar to the present study, these studies reported no significant differences between the isotonic and hypotonic drink trials in rectal temperature or blood lactate concentrations at the end of exercise (8, 9). In contrast with the present study, both studies observed smaller changes in serum osmolality during exercise after consuming the hypotonic drinks compared with the isotonic drinks (8, 9). Furthermore, Galloway and Maughan (8) found that plasma volume decreased to a smaller extent during exercise when consuming the hypotonic drink compared with the isotonic drink. Some of these differences may relate to the type and total amount of carbohydrate in the drinks, and the total volume of fluid consumed during exercise.

The present study is the first to compare different types of carbohydrate intake on immunoendocrine responses to exercise. Most research on the effects of sports drinks on immunoendocrine responses to exercise has used only one isotonic drink containing a combination of either sucrose + glucose + fructose or glucose + maltodextrin. Our findings indicate that blood glucose concentration during exercise was similar after consuming the hypotonic drink containing 2.4% sucrose, 1.2% fructose and 0.5% maltodextrin compared with the isotonic drink containing 4% glucose and 2% sucrose. However, blood glucose concentration was higher (non-significantly) at the end of exercise in the isotonic drink trial compared with the hypotonic drink trial.

Other research has consistently reported that consuming carbohydrate-based sports drinks during exercise suppresses the rise in circulating neutrophil counts during exercise, most likely by reducing the secretion of stress hormones that regulate neutrophil mobilization (1–3). In the present study, neither carbohydrate drink reduced neutrophil counts or plasma stress hormone concentrations following exercise. This discrepancy may relate to the lower intensity and duration of exercise in the present study compared with other studies, which have typically involved more intense (i.e., ≥70% \(V_{\text{O}2\text{max}}\)) and/or prolonged exercise (i.e., ≥2 h) (1–3). In the present study, plasma glucose concentration at the end of exercise in the isotonic and hypotonic drink trials was similar to or higher than other studies on the effects of carbohydrate supplementation on immunoendocrine responses to exercise (3–5, 21–24). Plasma glucose concentration at the end of exercise in the water trial in the present study was also higher than in some other studies (3, 23, 24).

The effects of carbohydrate intake on exercise-induced change in neutrophil activity are variable. Some studies report that carbohydrate prevents a decline in neutrophil elastase release (3) and oxidative burst activity (25), whereas other studies demonstrate no effect of carbohydrate (2, 22–24, 26). These discrepant findings are likely due to variation in various factors including exercise protocols, the training status of subjects, timing of blood samples and assays of neutrophil function (27, 28). In the present study, consistent with other findings (29), carbohydrate intake did not influence changes in

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**DISCUSSION**

![Diagram of fluid consumption comparison](image)

Fig. 4. Plasma myeloperoxidase and calprotectin concentrations. Data are mean±SD (n=6). See Fig. 1 for explanation of letters and symbols.
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plasma myeloperoxidase concentration. The increase in plasma myeloperoxidase concentration during the isotonic and water trials may reflect the release of IL-6, which have greater potential for neutrophil degranulation (30–32). Indeed, consuming the hypotonic drink did reduce plasma calprotectin concentration in the present study. Calprotectin is released from neutrophils in vitro (33): however, the mechanisms regulating calprotectin release and the biological role of calprotectin during exercise are uncertain at present.

Most (1, 3–5, 21), but not all, studies (6, 28, 34) report that carbohydrate ingestion attenuates plasma IL-6 concentration following exercise. In the present study, plasma IL-6 concentration was lower at the end of exercise in the hypotonic drink trial compared with both the isotonic drink trial and the water trial. The increase in plasma IL-6 concentration following all three trials reflects the release of IL-6 from skeletal muscle to maintain blood glucose concentration during exercise (35, 36). The lower plasma IL-6 concentration following the hypotonic trial was therefore most likely due to lower release of IL-6 from skeletal muscle during exercise (36).

It is difficult to determine whether changes in circulating immune cell counts and markers of cellular activation and inflammation in response to exercise and carbohydrates are clinically important for resistance to allergy and/or infection. There are currently no clinical ‘cut-offs’ available to establish the risk of immunosuppression after exercise. In the case of IL-6, it is unclear whether suppressing the IL-6 response to exercise is beneficial. IL-6 down-regulates the synthesis of TNF-α during exercise (37) whereas it up-regulates production of immunosuppressive cytokines such as IL-1ra and IL-10, neutrophil mobilization and priming (30, 31, 32), which indicates that IL-6 likely plays an important role in immunosuppression and systemic inflammation following heavy exertion.

There were several limitations in this study, including differences in the volume of drink consumed, and therefore the amount of carbohydrate consumed during exercise. Other research has provided sports drink (or placebo drinks) at a fixed rate. We chose not to fix the rate of fluid consumption, because we were interested in evaluating personal preferences for the types of drinks. Despite the difference in the volume of drink consumed during exercise, key variables related to physiological strain such as heart rate and core temperature during exercise were not significantly different between the trials. Differences in the amount of carbohydrate consumed might have influenced blood glucose kinetics during exercise, and therefore some of the immunoendocrine responses to exercise. Voluntary fluid intake during exercise is an important factor to consider. Setting fluid and carbohydrate consumption at a fixed rate in research studies may not necessarily reflect the interindividual variability in fluid and carbohydrate intake that would normally occur in practice.

In conclusion, a hypotonic sports drink containing sucrose, fructose and maltodextrin is favorable for athletes to drink during exercise. Consuming this type of drink may help to reduce fluid losses and attenuate some inflammatory responses to exercise. More research is warranted to investigate (i) the magnitude of exercise-induced immune changes that are associated with increased risk of allergy/infection, and (ii) whether nutritional interventions such as carbohydrate supplementation during exercise are clinically beneficial for the health of athletes.

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Conflict of interest

At the time of writing this article, one of the authors was employed by Asahi Soft Drinks Company which manufactured and supplied the beverages in this study.

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