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The effect of ice-slushy consumption on plasma vasoactive intestinal peptide during prolonged exercise in the heat

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Abstract

The aim of this study was to determine the effect of exercise in the heat on thermoregulatory responses and plasma vasoactive intestinal peptide concentration (VIP) and whether it is modulated by ice-slushy consumption. Ten male participants cycled at 62 % VO₂max for 90 min in 32 °C and 40 % relative humidity. A thermoneutral (37 °C) or ice-slushy (-1 °C) sports drink was given at 3.5 ml·kg⁻¹ body mass every 15 min during exercise. VIP and rectal temperature increased during exercise (mean ± standard deviation: 4.6± 4.4 pmol·L⁻¹, P=0.005; and 1.3 ± 0.4 °C, P<0.001 respectively) and were moderately associated (r=0.35, P=0.008). While rectal temperature and VIP were not different between trials, ice-slushy significantly reduced heat storage (P=0.010) and skin temperature (time*trial interaction p=0.038). It appears that VIP does not provide the signal linking cold beverage ingestion and lower skin temperature in the heat.

Keywords: beverage temperature, endurance exercise, thermoregulation, cold drink
1.1 Introduction

It has been suggested that the temperature of fluids ingested during exercise may influence thermoregulation (Lee and Shirreffs, 2007). The hormone vasoactive intestinal peptide (VIP) is a potent vasodilator (Jenssen et al., 1988) that can alter peripheral blood flow (Said and Mutt, 1970b) and may have a role in circulatory and thermoregulatory adaptations to exercise (Hilsted et al., 1980). The rationale behind the present study was to investigate whether there was a link between changes in plasma VIP and cold beverage ingestion.

It is well documented that endurance exercise in a hot environment increases body core temperature which triggers sweating and an increase in skin blood flow (SBF) which helps to dissipate body heat (Gisolfi and Wenger, 1984). As substantial fluid loss from sweating results in a decrease in plasma volume which may further contribute to the rise in rectal temperature (Tr) (Montain and Coyle, 1992), regular and adequate fluid ingestion is recommended (Sawka et al., 2007). Recently, there has been interest in consumption of cold beverages to enhance thermoregulation and exercise performance in the heat. Cold beverage ingestion has been observed to decrease rectal (Tr) and skin temperature (Tsk) compared to thermoneutral beverages (Armstrong et al., 1985; Lee and Shirreffs, 2007; Lee et al., 2008b; Wimer et al., 1997). The mechanism underpinning the effect of cold beverage ingestion on thermoregulation (Tr, Tsk and heat storage) during prolonged exercise requires further investigation. Specifically, the association between cold beverage consumption and lowered Tsk suggests that a signal may exist between the two factors.

Skin temperature is considered a quasi-index of SBF (Charkoudian, 2003) which increases during exercise in the heat to assist with heat loss via convection, radiation and sweat evaporation (Gisolfi and Wenger, 1984). A reduction in Tsk and/or SBF
coupled with a reduction in heart rate (HR) is consistent with a reduction in cardiovascular strain following cold beverage ingestion (Armstrong et al., 1985; Lee and Shirreffs, 2007; Lee et al., 2008b; Wimer et al., 1997). This reduction in Tsk and skin blood flow may be due to a decrease in the circulating hormone VIP. Previous studies have demonstrated that VIP increases with exercise duration (4-20 pmol.L\(^{-1}\)) (Galbo et al., 1979; Hilsted et al., 1980; Schaffalitzky de Muckadell et al., 1977) and with passive heat exposure (Jenssen et al., 1988). VIP is known to have receptors in both the skin and gastrointestinal (GI) tract and is a potent vasodilator, including of the skin (Bennett et al., 2003; Said and Mutt, 1970a) and is a candidate for the signal between cold beverage ingestion and decreased Tsk. A possible mechanism is that ingestion of the cold beverage inhibits release of VIP from nerve fibres in the gut into the plasma which leads to lower cutaneous vasodilation and reduced skin blood flow and skin temperature. Therefore, the aim of this study was to examine the effect of serial consumption of a cold beverage during exercise on VIP and thermoregulatory responses. We hypothesised that during prolonged exercise in the heat, serial consumption of ice-slushy (ICE) would reduce Tsk and VIP compared to a thermoneutral control beverage (CON).

2.1 Methods

Healthy, male, naturally heat acclimatised, endurance cyclists or triathletes were targeted for recruitment. After completing a medical screening questionnaire, participants gave written informed consent which was obtained according to the Declaration of Helsinki. Ten participants (data given as mean ± standard deviation; age: 30.1±7.0 years; height: 175± 6.5 cm; body mass: 75.1±9.4 kg; estimated body fat...
12.3±2.7%; VO2max: 61.8± 5.6 ml.kg⁻¹.min⁻¹) completed the study which was approved by the university Human Research Ethics Committee.

2.2 Preliminary measures

At the beginning of the first visit, participants were measured for nude body mass (Mettler ID 1, Albstadt, Germany) and stretch stature to the nearest 0.5cm using a stadiometer (Harpenden, United Kingdom). Hydrostatic weighing was used to estimate body composition. Participants wore a nose-clip, expired maximally and were submerged sitting on a chair suspended from a scale (Chatillon, New York). Weight was recorded when participants were motionless under water. Residual volume was estimated (van der Ploeg et al., 2000) by assessing the composition of oxygen and carbon dioxide in rebreathed air (5L pure oxygen). Body density (Goldman and Buskirk, 1961), fat free mass and percent body fat (Siri, 1956) were calculated. Underwater weighing was repeated three times and the result accepted if at least 2 measures were within 1%.

Peak aerobic capacity was measured on a cycle ergometer (Lode Excalibur, Groningen, Netherlands). The test consisted of four sub-maximal steady-state power outputs of five min each (100, 150, 200, 250 W) followed by an incremental increase in power (30 W.min⁻¹) until volitional fatigue. Expired air was collected using a Douglas bag for a minimum of 40 s during each stage and prior to fatigue. Samples were analysed with Servomex Pm1111E and Ir1507 sensors (Servomex, Crowborough, UK) to determine oxygen and carbon dioxide fractions. Gas volume was measured with a dry gas meter (Harvard, UK). Power output and VO₂ during the sub-maximal exercise was used to calculate workload for the following trials using linear regression.

2.3 Experimental design

Participants attended the laboratory for three sessions: a preliminary (described above) and two experimental trials, ingesting either ice slushy -1 °C (ICE) or thermoneutral...
°C (CON) beverages. The experimental trials were performed in a randomised order separated by 7-21 days.

During the experimental trials, participants cycled on the ergometer at steady state for 90 min in a climate chamber at 32 °C, 40 % relative humidity (RH) and wind speed set at 3.6 km·h⁻¹. The selected power output based on the previous peak aerobic capacity test was calculated to elicit 60 % of VO₂peak using self-selected cadence. A commercially available 7.4 % carbohydrate-electrolyte sports drink (Powerade Isotonic, Coca-Cola Amatil, Australia) was consumed every 15 min at 3.5 ml per kg body weight in both trials. The temperature of CON was controlled by a thermostatic water bath (E-5A, Julabo, Germany) and ICE was made using a commercial ‘slush’ machine (Iceotonic, Essential Slush, Australia). Beverage temperature was checked prior to consumption using an electronic thermometer (Thermistor 400 series, Cole Parmer, Illinois, USA).

Beverage composition and carbohydrate consumption was the same for both trials. Heart rate (S410, Polar Electro, Kempele, Finland) was taken every minute during SS. Expired air was collected using a Douglas bag for 1 min at 10, 30, 60 and 90 min. Rectal temperature was recorded via a custom made rectal probe every minute. Skin temperature was recorded every minute using four skin thermistors (DS1921H-F5 ibutton, Maxim, USA) placed on the left side (upper chest, mid humerus, mid calf and mid thigh) and were combined to give an overall temperature: Tsk = 0.3T_c + 0.3T_a + 0.2T_t + 0.2T_l (Ramanathan, 1964). Whole body skin blood flow was calculated from Tr and Tsk measurements using the following equation: Q_sk = 1/C × h/(Tr − Tsk), where Q_sk is skin blood flow, C is specific heat of blood (≈0.87 kcal·°C⁻¹·l⁻¹) and h is work measured by VO₂ (L·min⁻¹) (Rowell, 1986). Body heat storage (HS, W·min⁻²) was estimated as: (0.8·ΔTr +0.2·ΔTsk ) ·cₚ, where cₚ is specific heat of body tissue (Havenith et al., 1995). The specific heat of body tissue was adjusted for percent fat mass (3.49 kJ·°C⁻¹·kg⁻¹; (Aoyagi et al., 1996)).
To control for the effect of diet and hydration status, guidelines to consume a minimum of 6g of carbohydrate per kilogram of body-mass were provided to participants and they were instructed to ingest 30 ml per kilogram of body mass. To improve compliance to dietary control, these guidelines were based on food consumed when participants completed a three day food diary prior to commencement of the study. This recommended diet was consumed in the 24 h prior to each visit and confirmed with a 24 h food diary. Dietary intake was analysed using Australian dietary analysis software (FoodWorks Version 7.0.2921, Xyris Pty Ltd). Participants refrained from strenuous activity and alcohol and replicated caffeine consumption for 24 h before fasting for 6 h (except water consumption) prior to presenting to the lab.

Participants commenced each trial at the same time each day.

2.4 Blood analysis

2.4.1 Osmolality

At rest, a cannula was inserted into the antecubital vein. Prior to and post exercise 4 ml blood was collected, left to clot, centrifuged, the serum removed and osmolality determined by a cryoscopic osmometer (Osmomat 030, Gonotec, Berlin, Germany). Euhydration was considered to be a blood osmolality <290 mOsmol/kg.

2.4.2 VIP

At rest, 30 min, 60 min and post exercise, 6mL blood was collected in EDTA tubes containing Trasylol (3,000 KIU in a 6 ml tube) and the tube placed in an ice bath. Following centrifugation at 4 °C the plasma was removed and stored at -85 °C. Samples were analysed for VIP using a commercial RIA kit (EURIA- VIP, Euro Diagnostica, Malmo, Sweden). Tubes were counted using a Wizard 1470 gamma counter (Perkin Elmer, MA, USA). The sensitivity of the VIP assay was 3 pmol·L⁻¹.

2.5 Statistical Analyses
Statistical analysis was performed using SPSS version 19.0 (SPSS Inc., Chicago, IL) with all outcome variables checked for normality. A two-way repeated-measures ANOVA was used to evaluate differences between and within trials. To correct for violations of the assumption of sphericity with the repeated factor, the Huyn-Feldt correction was applied to the F ratio. Simple contrasts were used to further analyse the time effect where it was significant. Where a significant interaction effect was found pairwise differences were identified using the Bonferroni procedure. Pearson’s correlation coefficient were used to determine the association between VIP, Tr and Tsk. Statistical significance was set at $P<0.05$. Data are reported as mean ± standard deviation.

### 3.1 Results

No significant differences were detected in any of the observed variables (Tr, Tsk or VIP) at baseline and no order effect was found. Participant food diaries confirmed compliance with dietary carbohydrate prescription. Participants consumed $186 \pm 36 \text{kJ}$ of total energy per kilogram of body mass, which equates to roughly $13,950 \text{kJ}$. Carbohydrate consumption was $6.9 \pm 1.2 \text{g}$ per kilogram of body mass. There was no difference in total energy or carbohydrate consumption between trials ($P=0.38$ and $P=0.34$ respectively). Participants arrived hydrated to the laboratory for all trials with no observed difference between trials for body mass or serum osmolality ($76.5 \pm 9.6 \text{kg}; 287.5 \pm 3.3 \text{mOsmol\cdot kg}^{-1}$, $P=0.99$ for both).

While Tr increased over time ($P<0.001$), no time*trial difference was detected ($P=0.75$) (Figure 1a). There was no difference in Tr between trials at the end of exercise (overall mean $38.2 \pm 0.4 ^\circ \text{C}$, $P=0.79$) or in absolute change from rest to 90 min (overall mean $1.3 \pm 0.4 ^\circ \text{C}$, $P=0.88$).

A significant decrease over time in skin temperature was detected (Figure 1b, $P=0.001$) with ICE tending to be lower vs. CON ($P=0.063$) and a time*trial interaction ($P=0.038$).
The decrease in skin temperature from rest to end for ICE ( -1.1 ± 0.5 °C) was significantly different to CON ( -0.6 ± 0.7 °C, P=0.046). At the completion of exercise Tsk was lower with ICE (32.9 ± 0.5 °C) vs CON (33.5 ± 0.8 °C) (P=0.007).

Heat production was similar between trials (overall mean 396 ± 39 W.min⁻², P=0.9). During the exercise period ICE (14.6 ± 7.8 W.min⁻²) significantly reduced heat storage versus CON (22.1 ± 5.8 W.min⁻²; P=0.010).

Heart rate increased over time during exercise (P<0.001) but was not different between trials (P=0.09). Calculated whole body SBF decreased during exercise (P<0.01) with no significant difference between trials detected (P=0.46).

VIP (n=7) increased over time (Figure 2, P=0.005) in all participants with no significant difference between trials (P=0.16). Post hoc analysis showed that VIP at 30, 60 and 90 min was greater than rest (p<0.05). A moderate, positive and significant correlation was found between VIP and Tr (r=0.35, P=0.008) and between VIP and Tsk (r=0.42, P=0.003) (Figure 3).

4.1 Discussion

Cold beverage ingestion during prolonged exercise in the heat has previously been shown to improve thermoregulation via a reduction in Tr and particularly Tsk (Wimer et al., 1997), which suggests a link between the two factors. It was hypothesised that VIP may explain part of this link. This study found that consumption of ice-slushy reduced Tsk and heat storage. While we also observed an increase in VIP during exercise in the heat, regular consumption of ICE did not alter the concentration of circulating VIP. This lack of effect of beverage temperature on VIP, independent of the effect of environmental temperature and/or exercise duration (Galbo et al., 1979; Jenssen et al., 1988; MacLaren et al., 1995) suggests that changes in VIP do not provide the
physiological signal for the thermoregulatory effects of cold beverage consumption during exercise.

The findings of this study are consistent with previous literature where serial cold beverage ingestion during exercise was associated with a reduction in heat storage and Tsk but not Tr (Burdon et al., 2010; Lee et al., 2008a). The reduction in heat storage suggests the formation of a heat sink, which has been hypothesised to absorb some of the metabolic heat produced during exercise (Kay and Marino, 2000; Lee et al., 2008a; Mundel et al., 2006). Thermoreceptors in the GI tract (Villanova et al., 1997) may sense this heat sink and signal a reduction in heat loss via convection and sweat evaporation (evidenced by decreased SBF and Tsk) (Banerjee, 1970). A decreased SBF after consumption of a cold fluid bolus has been observed previously (Wimer et al., 1997) but the small serial volume consumed in this study did not alter SBF or heart rate.

Similarly to previous investigations involving exercise (Galbo et al., 1979; MacLaren et al., 1995) or heat exposure (Jenssen et al., 1988), circulating VIP increased during exercise in the heat. Contrary to our hypothesis, no significant difference in VIP was detected with exposure to ICE versus thermoneutral beverage temperature. Serial cold beverage (ICE) ingestion may not be a strong enough stimulus to overcome the requirement for increased circulating VIP to vasodilate skin for heat loss. This suggests that VIP is not part of the mechanism by which cold beverage consumption improves thermoregulation during prolonged exercise in the heat. No difference in circulating VIP with bolus cold beverage ingestion would suggest that the mechanism by which Tsk is reduced is mediated by other hormones or a neural mechanism. One potential neural mechanism is via stimulation of receptors in the GI tract (Villanova et al., 1997), sending signals to the brain and skin to modify thermoregulation.
In conclusion, the present study has shown that ice slushy ingestion led to a reduced heat storage and a lower skin temperature at the end of exercise. In addition, plasma VIP increased during exercise and was correlated with Tr, but was not affected by ice slushy ingestion. While ice slushy ingestion is a potentially useful method for enhancing athletic performance in the heat, further research is needed to investigate the mechanism by which cold beverage ingestion affects thermoregulation.
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Figure captions:

Figure 1: (a) Rectal temperature; and (b) skin temperature during steady-state exercise with consumption of ICE or CON. * $P<0.05$ ICE vs CON

Figure 2: Plasma vasoactive intestinal peptide (VIP) during steady-state exercise in the heat with CON or ICE ingestion. VIP increased with exercise but there were no differences between trials. * significantly different to rest $p<0.05$

Figure 3: There was a significant correlation between plasma VIP and both $T_r$ and $T_{sk}$
The effect of ice-slushy ingestion on body temperature and plasma VIP was examined.

Skin temperature was lower with ice-slushy consumption.

Plasma VIP was not changed with cold beverage ingestion.

There was a correlation between plasma VIP and rectal temperature.
Rectal temperature (°C) Skin temperature (°C)

Figure
Figure 2
Figure 3

- $r = 0.35$, $P = 0.008$
- $r = 0.42$, $P = 0.003$

- Rectal temperature
- Skin temperature