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Skin application of 4% menthol enhances running performance in hot and humid climate

Running head: Skin menthol and external cooling: similar effect on performance

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Abstract

Aerobic performance is negatively impacted by tropical climate due to impairment of thermoregulatory mechanisms. We tested the hypothesis that a torso application of a 4% menthol solution would have the same effect on a best performance 10-km run as an external use of cold water. Thirteen trained male athletes completed four outdoor 10-km runs ($T=29.0\pm1.3^{\circ}\text{C}$, relative humidity $59.0\pm13.6\%$) wearing a tee-shirt soaked every 2-km either in a cold ($\sim 6^{\circ}\text{C}$) or warm/ambient ($\sim 28^{\circ}\text{C}$) solution, consisting in water or in a 4% menthol solution, (CTL, MENT-Amb, CLD and MENT-CLD). Run performances were improved from 4.8 to 6.1% in CLD ($51.4\pm5.5\text{min}$), MENT-Amb ($52.2\pm5.9\text{min}$) and MENT-CLD ($51.4\pm5.1\text{min}$) conditions (vs. CTL, $55.4\pm8.4\text{min}$, $P<0.05$), without differences between these three conditions, whereas heart rate ($177\pm13\text{bpm}$), body temperature ($38.7\pm0.6^{\circ}\text{C}$) and drink ingestion ($356\pm170\text{g}$) were not modified. Thermal sensation after running was lower in MENT-CLD (vs. CTL, $P<0.01$) and thermal acceptability was higher in CLD and MENT-Amb (vs. CTL, $P<0.05$), but thermal comfort, feeling scale and rate of perceived exertion remained unchanged. The use of menthol on skin enhances aerobic performance in a tropical climate, and no differences in performance were observed between menthol and traditional percooling strategies. However, combining both menthol and traditional percooling brought no further improvements.

INTRODUCTION

Hot and humid climate decreases physical performances, especially in aerobic sports [1]. Although the mechanisms leading to these alterations are not fully understood, it is well established that thermoregulatory system dysfunction is involved into the loss of ability to dissipate excess metabolic heat production [2]. These mechanisms, already impaired in hot environment are further altered in humid climate, which drastically limits evapotranspiration due to water-saturated atmosphere [3]. Hence, running may be the most impacted sport by tropical climate as heat dissipation by circulating air convection around the body, as in cycling, is reduced [4]. To limit its effect in endurance sports and to enhance thermoregulatory processes, several countermeasures have been studied with various outcomes on performance: acclimation, hydration, pacing and cooling strategies [1]. Among the latter, external cooling solutions exist in the form of pads containing low-temperature inserts and may be applied on different body parts such as arms, legs, torso and neck [5]. Used before (pre-) or during (per-) exercise, they allow better performance in hot and humid environment by limiting the raise of body core temperature [6]. For example, performances from sprints to short distance running (until 5 km) are enhanced by wearing ‘ice vests’ during warm-up [7], and the use of a cooled neck-collar augments the limit time to exhaustion at aerobic sub-maximal intensity [8]. Performances are further improved when methods are combined (pre- and per-) [9] and/or used with more “aggressive” cooling temperature, around 0°C [6]. Indeed, the disequilibrium between heat production and heat loss during a prolonged exercise, leading to hyperthermia, has a negative impact on physiological functions and exercise performance: heat-dissipating mechanisms (skin vasodilatation, sweating response) are not enough to maintain an optimal body core temperature. Strategies relying on pre- (before exercise) and percooling (during) then augment heat storage and diminish the thermoregulatory strain. Beyond traditional approaches of body cooling, efficient in laboratory-controlled environment but difficult to use in ecological conditions, alternative strategies have been recently developed to maximize performance in tropical conditions. One of them is menthol, a compound largely used in food and tobacco industries, and popular in Asia for its therapeutic properties [10]. It mostly acts on TRPM8 and TRPA1 thermoreceptors [11] on skin and internal mucous membranes: when activated by menthol on skin, they induce a local cold sensation, prone to modify thermal sensation, especially in hot environment [12], leading to a reduced fatigue perception during maximal and submaximal exercise, and potentially to better performances [13,14]. These preliminary observations should have led to further comparisons between the respective uses of menthol and external proven cooling. However, to our

knowledge, there is no study directly comparing the use of menthol at ambient temperature on skin and traditional cooling strategies, to test if menthol could allow the same level of performance in ecological hot and humid environment, without the equipment burden. In other conditions, in a laboratory-controlled environment (34°C, RH 30%), there was no difference of performance between the use of cold 4% menthol gel on skin and cold pads, both applied around the heat-sensitive neck compared to control condition [15]. Barwood et al. showed no difference in running performance between sprayed 0.05% menthol and water at 20°C [16].

Hence, in the lack of data comparing skin-applied menthol and cold-water cooling, as aerobic performance is potentially reduced by the neurosensitive perception of environmental heat and humidity, we hypothesize that the use of menthol on skin would blunt these perception inhibition mechanisms and lead to enhanced performances, similar to the external use of cold water for body cooling.

SUBJECTS AND METHODS

Subjects.

Thirteen middle to well trained and heat-acclimatized male athletes, with a performance level from 3 to 4 out of 5 [17], participated in this study (age: 21 ± 4 years, height: 176 ± 6 cm, body mass: 70 ± 9 kg, maximal aerobic speed 16.2 ± 1.3 km.h⁻¹). All participants went under prior medical examination to check for any cardiopulmonary disease or previous heat stroke, and were tested for their maximal aerobic speed [18]. The study was approved by the National Ethic Committee (CPP, registration number 2018-A00295-50) and subjects gave their informed consent. Procedures were conformed ethics in sport and exercise science [19].

Experimental design

The protocol was conducted in ecological conditions: participants in this field study ran outside, on a flat paved road, directly exposed to the tropical heat and humidity of French West Indies (WBGT: $29.0 \pm 1.3^\circ\text{C}$, relative humidity 59.0 ± 13.6 %), as any athletes would be confronted in endurance races held in the same environment.

Subjects completed four 10-km run tests as fast as they could on a flat course, 4 to 7 days apart, in a randomized cross-over design including four experimental conditions in which athlete's shirt would be soaked in four different solutions: (1) Control (CTL): water at ambient temperature ($T = 28.7 \pm 2.9^\circ\text{C}$); (2) Cold water (CLD) at low temperature ($T = 6.0 \pm 0.8^\circ\text{C}$); (3) Menthol at ambient temperature (MENT-Amb): 4% menthol solution at ambient temperature ($T = 28.2 \pm 2.3^\circ\text{C}$); and (4) Menthol at low temperature (MENT-CLD): 4%

menthol solution at cold temperature ($T = 6.1 \pm 0.6^{\circ}\text{C}$). The menthol solutions were prepared from an 86% menthol-concentrated menthol solution (Robertet, Grasse, France) which was diluted to obtain a 4% solution. Athletes were asked to restrain from training the day before the tests and avoid caffeine on test day. For every athlete, trials were undertaken at the same time of the day to limit both variations of wet bulb globe temperature (WBGT) throughout the day and circadian variations of core temperature. Finally, athletes were asked to wear the same outfit at all sessions, except the white shirt provided by the experimenter.

Experimental procedure

Heart rate (HR), stride rate and run duration were recorded continuously (sampling frequency 1 sec) using a M400 Polar watch appaired with a H7 strap belt or a OH1 sensor [20,21] (Polar Electro Oy, Kempele, Finland). WBGT was continuously measured during the trials with a HD32.2 device (Delta Ohm, Padoue, Italia), placed immediately next to the run course. Core temperature (T_{co}) was telemetrically measured via ingestible temperature measurement pills (BodyCap, Caen, France), with an embedded memory (sampling frequency 30 sec): athletes were instructed to ingest these pills 6 to 8 h before trials to ensure the pill was out of the stomach, thereby avoiding changes in T_{co} due to fluid consumption. Each trial included a prior 15 to 20 minutes standardized warm-up. After 5 minutes of gear equipment and weighing, they started the 10-km run on the 1-km out-and-back course. At start, and every 2 km, athletes stopped for 30 to 40 seconds, during which: 1) athletes took their shirt off; 2) the experimenter soaked it in a solution corresponding to the tested condition (CTL, CLD, MENT-Amb or MENT-CLD), adding or renewing between 250 and 300 g of solution in tee-shirt fabric; 3) athletes put back their shirt on; 4) athletes could hydrate *ad libitum* with water at ambient temperature ($T = 26.9 \pm 1.6^{\circ}\text{C}$). The 2-km stops were necessary due to the pronounced sweating rate in the hot and humid condition, which would drip the solution off the shirt and blunt its potential effects. Finally, psychological parameters were assessed via oral or written questionnaires immediately before start: feeling scale (FS, from -5 ‘Very bad’ to +5 ‘Very good’) [22], thermal comfort (TC, from -3 ‘Very uncomfortable’ to +3 ‘Very comfortable’) [23], thermal sensation (TS, from -3 ‘Very cold’ to +3 ‘Very hot’) [24], thermal acceptability (TA, from -1 ‘Clearly unacceptable’ to +1 ‘Clearly acceptable’) [25]; and after the race: FS, TC, TS, TA and rate of perceived exertion (RPE, from 6 ‘Very, very light’ to 20 ‘Very, very hard’) [26–28].

Data collection and statistical analyses

Following the guidelines of the National Ethic Committee, the different run conditions were strictly randomized to avoid effects of trial order. Run duration, HR and stride rate data were measured and averaged on the successive 2-km intervals and on the whole run, and were retrieved from Polar platform website. T_{co} values were telemetrically updated and downloaded at the end of each session from monitors. Using calibrated balances (Terraillon, Croissy-sur-Seine, France), athletes were weighed-in, dry, shoes and shirt off, immediately before and after each run and water consumption (g) was measured after each stop: water loss during the run was then calculated by adding the weight variation to the quantity of consumed water, and the percentage of weight loss was extracted using initial body weight. Psychological parameters were compiled before and after each run. Normality of data on each condition was verified by a Kolmogorov-Smirnov test. Thus, repeated measures ANOVAs were conducted with Condition (i.e., CTL, CLD, MENT-Amb, MENT-CLD) as the between-factor and Time (i.e., 2-km intervals) as the within-factor. Posthoc Student t-tests were performed when applicable, to compare mean values between successive intervals ([0-2 km] vs. [2-4 km], [2-4 km] vs. [4-6 km], [4-6 km] vs. [6-8 km] and [6-8 km] vs. [8-10 km]) to assess the kinetics of the run pace throughout the 10-km course, and between conditions. Finally, the effect size was assessed by computing the Hedge's g when applicable. Statistical significance was set at $P < 0.05$.

RESULTS

Results are presented in table 1 and in figures 1 and 2.

-----Insert Figure 1-----

Environmental conditions

WBGT and relative humidity are presented in table 1. There was no difference between conditions.

10 km performance (fig. 1A)

Athletes were faster in CLD (- 6.1 %), MENT-Amb (- 4.8%) and MENT-CLD (- 6.1 %) when compared with control ($P < 0.05$, $g = 0.55$, 0.44 , 0.57 respectively). However, no differences were observed between MENT-Amb, MENT-CLD and CLD (fig. 1A).

2 km intervals

Interval splits (i.e., running performance) increased for all conditions ($P<0.01$, fig. 1A), meaning that athletes were getting slower throughout the run.

There was a condition effect on 2-km splits ($P<0.01$, fig. 1A). No differences were observed between conditions on the first two intervals. From the [4-6 km] interval to the last, split durations were longer in CTL (vs CLD, Ment-Amb and Ment-CLD, $P<0.05$, fig. 1A and 1B).

Interval splits continuously increased from the start in CTL condition ([0-2 km] vs. [2-4 km], $P<0.05$; [2-4 km] vs. [4-6 km], $P<0.001$; [4-6 km] vs. [6-8 km], $P<0.01$) before stabilizing on the two last intervals. In CLD, Ment-Amb and Ment-CLD, after some pace variations (or not), speed was not modified on the last three intervals ([4-6 km], [6-8 km] and [8-10 km]).

Heart Rate

No differences were observed between conditions (fig. 1C and 1D).

-----Insert Figure 2-----

Stride Rate

Stride rate was not modified by condition (fig. 2A and 2B).

Core temperature

T_{co} increased throughout the run ($P<0.001$), for each condition (fig. 2D, $P<0.001$). However, there was no difference between conditions (fig. 2C).

-----Insert Table 1-----

Perceptual measures

At the end of the run, FS, TS, TC and TA were all lower ($P<0.001$ and $g=1.12$, $P<0.05$ and $g=0.40$, $P<0.001$ and $g=0.71$, and $P<0.05$ and $g=0.44$, respectively) compared to the trial start. TS at the end of the run was lower in MENT-CLD vs. CTL, and vs. CLD ($P<0.01$, $g=1.23$ and 1.22 respectively). Similarly, TA was increased in CLD, MENT-Amb (vs. CTL, $P<0.05$, $g=0.74$ and 1.04 respectively) (table 1). Condition had an effect on TA ($P<0.05$, table 1). RPE, FS and TC were not modified by conditions (table 1).

Drink ingestion (table 1)

Fluid ingestion increased throughout the run ($P<0.01$), although there was no difference in drink ingestion between immediate successive intervals. However, some differences could be

observed between intervals [0-2 km] and [4-6 km] (CLD, $P<0.01$; Ment-CLD, $P<0.05$), and between [0-2 km] and [6-8 km] (Ment-CLD, $P<0.001$).

Condition had no effect on drink ingestion.

Weight loss (table 1)

As expected, participants were lighter at the end of the run ($P<0.001$), but conditions had no effect on weight loss nor on weight loss percentage.

DISCUSSION

Our main finding points out that the use of menthol on skin in men enhances aerobic performance in a hot and humid climate, and that there is no difference in performance gain between menthol and traditional percooling strategy; core temperature was not raised by a faster pace, and menthol brought a lower thermal sensation. However, combining both menthol and percooling did not bring any further improvements.

First, overall performance was positively impacted by cold and menthol conditions whereas Barwood reported no difference between menthol and water sprays (at ambient temperature) in run speed on a 5-km run held in hot and humid environment [16]. In a laboratory-controlled study, an only acute applying of an 8% menthol gel on the face increased the time limit in a high intensity exercise by more than 20% in a time-to-exhaustion tests on ergometer [13], demonstrating a positive effect of menthol on performance.

Second, run speed decreased continuously from the start in CTL condition (fig. 1A and 1B), which did not happen in the other conditions. In the study of Barwood et al.[16], there was no difference in performance between conditions and athletes' speed remained steady during the 5-km run, whereas we found a negative effect of time on performance during our longer run. Moreover, HR (fig. 1C and 1D) and core body temperature (fig. 2C and 2D) remained stable between conditions (fig. 1C and 1D), even if athletes were performing better, which was also reported in other works [14,16,26,29,30]. As for the heat-related perception measures, participants felt the same lower TS at the end of the run than reported in other works [13,16,26,27,31] while TC was also decreased [32] or maintained/augmented [13,16,26,27] (table 1). It is confirmed here by a better acceptability of the heat both in cold and menthol conditions. RPE was not modified by menthol, meaning that subjects performed all the tests with the same maximal perceived intensity whereas run duration was better for some conditions: this therefore suggests an existing enhancing-performance effect in these conditions [31]. The underlying physiological mechanisms are still to be fully understood, but

the type of activity (running, cycling) or the environmental conditions (dry or humid heat) might play a role.

In our study, cold water cooling and 4% menthol produced a similar effect on performance: the first strategy is long-known to buffer and help dissipate the excess metabolic-heat [6] whereas the second modifies the perception of the environmental heat and humidity [33]. Our study design also allowed us to study the effects of separate (CLD, Ment-Amb) and combined (Ment-CLD) conditions, but we found no further improvement of the latter compared to CLD or Ment-Amb taken separately, as an additive effect could have been expected. This underlines a limit in the combination of multiple strategies to maintain a thermal homeostasis when exercising in a tropical climate. If the effect of external cooling is well understood, mainly by augmenting the heat capacity, the mechanisms elicited by the internal or external use of menthol remain to be fully understood.

The absence of difference in T_{co} between conditions (fig. 2C) implies that, at a faster pace, the thermoregulatory system would have to dissipate a greater amount of metabolic heat. If we assume that the low temperature solutions ($\sim 6^{\circ}\text{C}$) soaking the tee-shirt in CLD and MENT-CLD conditions were able to absorb a portion of the excess heat, this mechanism was not possible in the MENT-Amb condition, as the solution temperature remained above 28°C . Regarding the non-modified T_{co} in all conditions, this raises interrogations about the functioning of thermoregulatory mechanisms, already impaired by the humid atmosphere, and by the limited cooling by air convection during running [2]. Moreover, this excess of heat could not be evacuated through the process of water ingested / loss of water (sweat), as there was no difference, on one hand, between environment and beverage temperatures (heat capacity), and on the other hand, between weight loss in the different conditions (physically evacuated heat through sweat). If aerobic performance strongly depends on core temperature and by its capacity to dissipate excess heat, an alternative mechanism may play a key role in thermoregulation under these harsh conditions, here potentially triggered by menthol applied on skin. This mechanism could be related to the effect of menthol on peripheral blood circulation. A first study reported a skin vasoconstriction when a 3.5% menthol gel was applied on skin [34], which was only noted only during the first 5 min after application. In more recent studies, a vasodilatation phenomenon was observed from 5 min after application and was maximal between 15 and 45 min; a dose-dependent activation was also recorded, optimal from 4 to 7% concentration [35], associated with a proportional related-cold sensation [36]. These menthol-related mechanisms in peripheral vasodilatation would activate, other than TRPM8 thermoreceptors [11], multiple vasodilator pathways, such as nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF), hence increasing blood flow.

Although these studies were performed at rest in thermoneutral conditions, these recent advances could lead to further studies in order to assess these effects at exercise, in hot/humid environment. This opening could also bring a novel light to the central governor model [37], regulating afferent and efferent mechanisms to prevent the occurrence of bodily harm, for example by adjusting race pace under a high thermal stress. Whereas the cold sensation elicited by the use of menthol would act on the central governor through thermosensitive afferences and therefore “trick” it into adopting a higher exercise intensity [38], locally NO-mediated and EDHF actions would promote a cutaneous vasodilatation to counteract a greater metabolic heat production. This would lead to a higher core temperature, and therefore maintain a system homeostasis as long as possible, or as long as the central command is under the influence of TRPM8 afferences. Regarding core temperature, it’s noticeable that numerous previous studies using menthol reported no increase in T_{co} [14,16,26,29,30]. This could be interpreted as a defense mechanism, peripherally triggered, and would compensate a dysfunction from the central command potentially leading to body harm. Moreover, although experimental conditions were designed to preserve a single-blind protocol, it was possible that the aroma from menthol impregnated in the tee-shirt fabric would reach the respiratory system through the nasal passages. As inhaled menthol provokes a large increase of ventilation at rest and exercise [39], an additional heat loss could be made through the augmented respiratory process [40].

Finally, as menthol promotes a higher exercise intensity through sensory nerve-dependent mechanisms [38], our TS data confirm a significant cooling sensation felt by athlete after the run, as observed in other works [13,16,26,27,33,34], potentially acting on central command. This is also confirmed in our study by a higher TA in MENT-Amb condition (vs. CTL, $P<0.05$, table 1). However, we did not observe changes in TC (table 1), unlike other works [16,26,27,34]. To summarize, when using menthol in tropical climate, athletes ran faster and felt “comfortably fresher” than in CTL condition.

In conclusion, our works show improved 10-km performance in ecological conditions when using a 4% menthol skin application. This enhancement was the same as using cold water or cold menthol. The use of a higher menthol concentration (4%) than those used in most studies underlines a dose effect of menthol, while physiological parameters, such body core temperature and heart rate, did not show further impaired thermal stress compared to control condition. The underlying mechanisms are not fully understood yet, but recent findings on menthol-related cutaneous vasodilatation open new perspectives of research and bring a novel light on the central governor theory. Regarding the potential physiological mechanisms induced by the application of menthol on skin, future studies may focus on the effects of

335 different concentration of menthol on aerobic performance, especially in long-duration
336 running.

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Captions

Figure 1

A and B: overall run duration in CTL, CLD, MENT-Amb and MENT-CLD conditions (upper panel: A, individual values and mean $\pm$ SD) and by 2 km-interval per condition for each condition (lower panel: B, mean $\pm$ SEM).

C and D: average heart rate in CTL, CLD, MENT-Amb and MENT-CLD conditions (upper panel: C, mean $\pm$ SD) and by 2 km-interval per condition for each condition (lower panel: D, mean $\pm$ SEM).

*: $P < 0.05$, CLD/MENT-Amb/MENT-CLD vs. CTL.

Time effects were not reported for readability.

Figure 2

A and B: average stride rate in CTL, CLD, MENT-Amb and MENT-CLD conditions (upper panel: A, mean $\pm$ SD) and by 2 km-interval per condition for each condition (lower panel: B, mean $\pm$ SEM).

C and D: average core temperature in CTL, CLD, MENT-Amb and MENT-CLD conditions (upper panel: C, mean $\pm$ SD) and by 2 km-interval per condition for each condition (lower panel: D, mean $\pm$ SEM).

Time effects were not reported for readability.

Table 1

Mean values ($\pm$ SD) of rate of perceived exertion (RPE, from 6 ‘Very, very light’ to 20 ‘Very, very hard’), feeling scale (FS, from -5 ‘Very bad’ to +5 ‘Very good’), thermal sensation (TS, from -3 ‘Very cold’ to +3 ‘Very hot’), thermal comfort (TC, from -3 ‘Very uncomfortable’ to +3 ‘Very comfortable’), thermal acceptability (TA, from -1 ‘Clearly unacceptable’ to +1 ‘Clearly acceptable’), ingested drink and percentage of weight loss.

* $P < 0.05$, ** $P < 0.01$: vs. CTL

++ $P < 0.01$: vs. CLD

† $P < 0.05$: vs. MENT-AMB

468 **Table 1**

469 Mean values ($\pm$ SD) of environmental condition (WBGT and relative humidity RH) rate of perceived exertion (RPE, from 6 ‘Very, very light’ to 20
 470 ‘Very, very hard’), feeling scale (FS, from -5 ‘Very bad’ to +5 ‘Very good’), thermal sensation (TS, from -3 ‘Very cold’ to +3 ‘Very hot’), thermal comfort
 471 (TC, from -3 ‘Very uncomfortable’ to +3 ‘Very comfortable’), thermal acceptability (TA, from -1 ‘Clearly unacceptable’ to +1 ‘Clearly acceptable’),
 472 ingested drink and percentage of weight loss.

473 *P<0.05, **P<0.01: vs. CTL

474 ++P<0.01: vs. CLD

475 †P<0.05: vs. MENT-AMB

		CTL	CLD	MENT-AMB	MENT-CLD
Environmental conditions	WBGT (°C)	29.1 $\pm$ 1.5	28.9 $\pm$ 1.2	28.8 $\pm$ 1.5	29.1 $\pm$ 1.2
	RH (%)	57.7 $\pm$ 12.9	60.0 $\pm$ 13.4	63.6 $\pm$ 14.5	54.6 $\pm$ 13.3
RPE (n.u.)		15.8 $\pm$ 2.2	14.5 $\pm$ 3.1	14.6 $\pm$ 1.8	15.6 $\pm$ 1.7
FS (n.u.)	Pre	2.0 $\pm$ 2.0	2.3 $\pm$ 2.1	2.5 $\pm$ 2.1	1.9 $\pm$ 2.3
	Post	-0.8 $\pm$ 2.3	0.1 $\pm$ 2.3	0.7 $\pm$ 2.0 *	-0.9 $\pm$ 1.7 †
TS (n.u.)	Pre	1.2 $\pm$ 0.9	0.7 $\pm$ 0.9	1.0 $\pm$ 0.8	1.1 $\pm$ 1.0
	Post	1.2 $\pm$ 1.4	1.1 $\pm$ 0.9	0.0 $\pm$ 1.7	-0.4 $\pm$ 1.2 ** ++
TC (n.u.)	Pre	0.5 $\pm$ 0.7	0.6 $\pm$ 0.6	0.8 $\pm$ 0.7	0.6 $\pm$ 0.8
	Post	-0.5 $\pm$ 1.2	-0.1 $\pm$ 1.2	0.3 $\pm$ 0.9	0.2 $\pm$ 0.6
TA (n.u.)	Pre	0.3 $\pm$ 0.4	0.3 $\pm$ 0.3	0.5 $\pm$ 0.3	0.3 $\pm$ 0.4
	Post	-0.1 $\pm$ 0.5	0.2 $\pm$ 0.4 *	0.4 $\pm$ 0.5 *	0.2 $\pm$ 0.5
Drink ingested (g)		363 $\pm$ 173	367 $\pm$ 162	318 $\pm$ 164	379 $\pm$ 198
% weight loss		-1.3 $\pm$ 0.8	-1.0 $\pm$ 0.6	-1.4 $\pm$ 1.0	-1.2 $\pm$ 0.6

476

Figure 1

Figure 2