Relationship of osmotic inhibition in thermoregulatory responses and sweat sodium concentration in humans

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Received 26 May 2000; accepted in final form 12 October 2000

Takamata, Akira, Tetsuya Yoshida, Naoko Nishida, and Taketoshi Morimoto. Relationship of osmotic inhibition in thermoregulatory responses and sweat sodium concentration in humans. Am J Physiol Regulatory Integrative Comp Physiol 280: R623–R629, 2001.—Heat acclimatization improves thermoregulatory responses to heat stress and decreases sweat sodium concentration ([Na+]sweat). The reduced [Na+]sweat results in a larger increase in plasma osmolality (P osmol) at a given amount of sweat output. The increase in P osmol inhibits thermoregulatory responses to increased body core temperature. Therefore, we hypothesized that the inhibitory effect of plasma hyperosmolality on the thermoregulatory responses to heat stress should be attenuated with the reduction of [Na+]sweat due to heat acclimatization. Eleven subjects (9 male and 2 female) were passively heated by immersing their lower legs into water at 42°C (room temperature 28°C and relative humidity 30%) for 50 min following isotonic or hypertonic saline infusion. We determined the increase in the esophageal temperature (Tes) above 38.5°C during upright exercise (21). In addition to improving thermoregulatory function, heat acclimation expands the blood volume (BV) or plasma volume (PV) (2), increases sweat output at a given thermal drive, and reduces sweat sodium concentration ([Na+]sweat) (6, 15).

Increased BV or PV contributes to the production of a higher cutaneous blood flow and probably plays a role in the increase in sweating rate during heat stress because saline infusion (12), water immersion (10), a supine position (5), or negative pressure breathing (9) all increased maximal cutaneous blood flow during exercise by removing the leveling off of the cutaneous vasodilatory response to increased body core temperature, which usually occurs at an esophageal temperature (Tes) above 38.5°C during upright exercise (21).

Reduced [Na+]sweat results in a larger increase in plasma osmolality (P osmol) at a given sweat output, which is beneficial in minimizing the reduction of PV due to sweating, because a larger increase in P osmol withdraws more water from the intra- to extracellular space (13). In contrast, a larger increase in P osmol inhibits thermoregulation even more. Takamata et al. (19) recently reported that thermoregulatory CVD and sweating were attenuated linearly with the increase in P osmol by increasing the rise in body core temperature required to elicit these responses.

Taken together, we hypothesized that the inhibitory effect of plasma hyperosmolality on thermoregulatory responses to increased body core temperature should be attenuated in heat acclimated individuals and that this attenuation is one of the mechanisms that allows heat-acclimated individuals to maintain higher sweating rate and cutaneous blood flow during progressive dehydration induced by extensive sweating. To examine this hypothesis, we determined the relationship between osmotic inhibition of thermoregulatory responses to increased body core temperature and [Na+]sweat in 11 subjects.

METHODS

The experimental protocol was approved by the Review Board on Human Experiments, Kyoto Prefectural University of Medicine. Nine male and two female subjects gave their written informed consent prior to participating in this study. Their age was 20.6 ± 0.4 yr, body wt 59.7 ± 2.0 kg, and
height 168.3 ± 2.8 cm. Five subjects engaged in regular “Kendo” (Japanese fencing) practice and were supposed to be heat acclimated, whereas other subjects were not active but did not participate in any regular exercise program. To quantify the osmotic inhibition of thermoregulation in each subject, we determined the increase in body core temperature required to elicit sweating (ΔTses threshold for sweating) and CVD (ΔTcap threshold for CVD) twice during passive body heating hyperosmotic (HOSM) and normosmotic (NOSM) conditions. HOSM was induced by hypertonic saline infusion and NOSM by isotonic saline infusion prior to passive body heating. Therefore, the conditions were hyperosmotic hyperosmolaria (HOSM) and normosmotic hyperosmolarity (NOSM).

The experiments were separated for a period of at least 1 wk, and the order of the experiments was randomized. In the female subjects, the experiments were conducted during the follicular phase. All of the experiments were conducted during summer (August to September).

Protocol. Subjects reported to the laboratory at 9 AM. They had refrained from heavy exercise for 24 h and from fatty, spicy, and caffeine for 15 h before arriving at the laboratory. They were instructed to eat a light breakfast and drink at least 200 ml of water. On reporting to the laboratory the subjects voided, drank 400 ml of water, and then were kept in the seated position for 1 h during a control period (ambient temperature 28°C, relative humidity 40%). During this period, subjects were inserted with a 20 G catheter (Insyte, Becton Dickinson Infusion Therapy Systems) for blood sampling and infusion into an antecubital vein. At the end of the control period a blood sample was drawn.

After the control period, the subjects were infused with either isotonic (0.9% NaCl) or hypertonic (3% NaCl) saline through the catheter for 90 min. The infusion rate was 0.2 ml·kg⁻¹·min⁻¹ for isotonic saline infusion and 0.125 ml·kg⁻¹·min⁻¹ for hypertonic saline.

Thirty minutes after the end of the infusion period and preceded by a 10-min preheating control measurement, the subjects immersed their lower legs in water at 42°C (ambient temperature 36°C, relative humidity 40%). Forearm and chest sweat was collected during the second exercise bout with a plastic arm bag and a filter paper disk covered with a Plexiglas capsule, respectively. The arm bag and capsule were set in place after washing the skin with distilled water and wiping with a clean dry towel. The collected filter paper disk was transferred immediately to a plastic screw-capped bottle to prevent evaporation. After the filter paper disk was weighed, 1 ml of distilled water was added, whereafter it was soaked for at least 1 h. Thus the chest [Na⁺]low was measured in a diluted state, whereas forearm [Na⁺]low was measured without dilution (18). We conducted this experiment because the sweat output during passive body heating was too small to collect enough sweat for the measurement of [Na⁺]low.

Measurements. Tses as an index of body core temperature was measured with a copper-constantan thermocouple probe in the polyethylene tubing (PE-90, Clay Adams), placed at a distance one-fourth of the standing height from the external nares. Skin temperature (Tsk) was measured at the forehead, chest, upper arm, forearm, abdomen, thigh, and calf. Mean Tsk was calculated from the body surface area distribution and thermal sensitivity of each skin area (8). Heart rate and blood pressure were measured noninvasively every 1 min (Colin BP-780, Komaki, Japan). The sweating rate on the chest (SRch) was measured by the capsule ventilation method. The capsule (12.56 cm²) was affixed on the chest skin with elastic surgical tape and ventilated with dry air at a flow rate of 2 l/min, and the relative humidity and temperature of the outlet air were measured continuously with a humidity and temperature sensor (Visala HMP233L, Helsinki). Measurement of both inlet and outlet air flow through the capsule with flowmeters demonstrated no air leakage during the experiments. Skin blood flow was measured with a laser-Doppler flowmeter on the forearm skin (Advance ALF21, Tokyo). Tcap,Tsk, relative humidity, and temperature of the ventilated air, and output voltage of laser-Doppler flowmeter were measured every 1 s, and the average of every 30-s period was used for data analyses.

Aliquots of blood sample for measurements of osmolality were centrifuged immediately and separated plasma was stored at −20°C until the measurements were performed. Blood for the assays of plasma arginine vasopressin concentration ([AVP]p) and plasma aldosterone concentration was transferred into an ice-chilled EDTA tube and centrifuged at 4°C, and the separated plasma was stored at −80°C until each assay was performed. The remaining blood was immediately prepared for the hematocrit (Hct) and hemoglobin concentration ([Hb]) measurements.

Hct was determined by the microcapillary centrifugation method and [Hb] by the cyanometahemoglobin method (Sigma Hemoglobin Kit), and plasma protein concentration by refractometry (Atago Refractometer). Posmol was measured by freezing point depression (Fiske one-ten osmometer, Norwood, MA). The undiluted forearm [Na⁺]low and diluted chest [Na⁺]low were measured with a flame photometer (Corning 480 Flamephotometer, Medfield, MA). [AVP]p and plasma aldosterone concentration were determined by radioimmunoassay (AVP RIA Kit and Aldosterone RIA Kit, Mitsubishi Chemical). Intra- and interassay coefficients of variation for 1.17 pg/ml AVP were 5.5 and 7.0%, respectively. The minimal detection limit of the AVP assay was 0.21 pg/ml in this experiment (0.063 pg/tube). Intra- and interassay coefficient of variation for 27.2 ng/dl aldosterone were 6.4 and 8.8%, respectively. All of the samples from a given subject were determined with the same assay kit.

Data analyses and statistics. The percent change in PV was calculated from the change in Hct and [Hb] according to the following equation (3)

$$\Delta PV(\%) = 100 \times \left( \frac{[Hb ]_B}{[Hb ]_A} \right) \times \left( \frac{1 - Hct_B/100}{1 - Hct_A/100} \right) - 100$$

where PV is the percent change in plasma volume from the control, subscript B indicates before (control), and subscript A indicates after (experimental).

SRch was calculated from the air flow through the capsule and the difference in water content between inlet and outlet air. The water content of the air was calculated from the measured relative humidity and temperature. SRch was expressed as mg·min⁻¹·cm⁻². Cutaneous vascular conductance (CVC) was calculated from laser-Doppler flowmeter output voltage and mean arterial pressure and presented as the percent change from the mean of the preheating values.

To quantify the osmotic inhibition of the thermoregulatory responses, we determined the increase in Tses required to eliciting sweating and CVD (ΔTses thresholds for sweating and CVD, respectively) for each subject in each condition. ΔTses was presented as the difference from the mean of the 10-min
preheating values. We employed the $\Delta T_{es}$ thresholds for these responses instead of absolute $T_{es}$ thresholds, because the day-to-day variation of $T_{es}$ was larger than the $\Delta T_{es}$ thresholds in NOSM and because the $\Delta T_{es}$ thresholds were linearly correlated with $P_{osmol}$ in our previous data (19). Osmotic inhibition of thermoregulatory sweating and CVD was quantified as the increase in the $\Delta T_{es}$ threshold per unit rise in $P_{osmol}$ using the following equation:

$$\text{osmotic inhibition} = \frac{[\Delta T_{es} \text{ threshold}]_{HOSM} - [\Delta T_{es} \text{ threshold}]_{NOSM}}{(P_{osmol})_{HOSM} - (P_{osmol})_{NOSM}},$$

where $[\Delta T_{es} \text{ threshold}]_{HOSM}$ is the change in $T_{es}$ threshold determined during passive body heating in HOSM and $[\Delta T_{es} \text{ threshold}]_{NOSM}$ in NOSM. $(P_{osmol})_{HOSM}$ is the $P_{osmol}$ before passive body heating in HOSM and $(P_{osmol})_{NOSM}$, the $P_{osmol}$ before passive body heating in NOSM. We also determined the thermal responsiveness of sweating and CVD (the slope of the relationship between these responses and $\Delta T_{es}$ thresholds) for each subject in each condition.

Data were shown as the means ± SE of 11 subjects. Regression analysis was performed using the standard least-squares method. Paired t-test was performed to examine the difference between NOSM and HOSM. $P < 0.05$ was considered significant.

RESULTS

The mean forearm $[Na^+]_{sweat}$ in 11 subjects was 36.3 ± 6.0 meq/l (range 16.5–87.0 meq/l) and chest $[Na^+]_{sweat}$ was 55.9 ± 9.3 meq/l (range 25.8–124.5 meq/l). The chest $[Na^+]_{sweat}$ was higher than the forearm $[Na^+]_{sweat}$ in all of the subjects, and the chest and forearm $[Na^+]_{sweat}$ were highly correlated (chest $[Na^+]_{sweat} = 1.52 \times$ forearm $[Na^+]_{sweat} - 0.55, r = 0.965$). The $[Na^+]_{sweat}$ was not significantly correlated with plasma aldosterone concentration, ranging from 48 to 115 ng/dl ($r = 0.130$).

Figure 1, top and middle, shows PV and $P_{osmol}$ before infusion, and before and during passive body heating. The increase in PV before passive body heating was 8.8 ± 0.7% following isotonic saline infusion and 13.1 ± 1.6% following hypertonic saline infusion, and PV remained unchanged during passive body heating. $P_{osmol}$ increased by 10.4 ± 0.9 mosmol/kgH$_2$O following hypertonic saline infusion and was unchanged following isotonic saline infusion. $P_{osmol}$ in NOSM remained constant during passive heating, whereas $P_{osmol}$ in HOSM decreased slightly during passive body heating, but this change was relatively small. [AVP]$_p$ did not change throughout the experiment in NOSM, whereas [AVP]$_p$ increased in HOSM following infusion and increased further during passive body heating (Fig. 1, bottom).

Preheating $T_{es}$ was 36.90 ± 0.07°C in HOSM and 36.78 ± 0.07°C in NOSM, demonstrating no significant difference between the two conditions. The increase in $T_{es}$ during 50-min passive body heating was much larger in HOSM (1.03 ± 0.06°C), compared with the increase in $T_{es}$ in NOSM (0.54 ± 0.05°C), even though the subjects received the same heat load (Fig. 2, top). The increase in SR$_{ch}$ was delayed in HOSM compared with NOSM, and the area under the curve of the SR$_{ch}$ response in HOSM was significantly lower than in NOSM (Fig. 2, bottom). The response of CVC during passive body heating was similar to the SR$_{ch}$ response in both conditions (Fig. 2, bottom). Preheating mean $T_{sk}$ was not different between the two conditions (33.29 ± 0.10°C in NOSM and 33.39 ± 0.10°C in HOSM). The $T_{sk}$ increased immediately after the onset of immersion because of increased lower leg temperature in both conditions (33.93 ± 0.10°C in NOSM and 33.98 ± 0.11°C in HOSM), and started to decrease during the passive body heating in NOSM when sweating started, whereas $T_{sk}$ did not decrease significantly during the passive body heating in HOSM. However, the difference of $T_{sk}$ between the two conditions was <1°C.

The mean $\Delta T_{es}$ threshold for sweating of the 11 subjects was 0.17 ± 0.04°C in NOSM and 0.69 ± 0.06°C in HOSM (Fig. 3, top), and the mean $\Delta T_{es}$ threshold for CVD was 0.19 ± 0.04°C in NOSM and 0.63 ± 0.06°C in HOSM (Fig. 3, bottom). The $\Delta T_{es}$ thresholds for these responses in HOSM were significantly higher than in NOSM. A comparison of HOSM and NOSM showed

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Fig. 1. Percent change in plasma volume ($\Delta$PV, top), plasma osmolarity ($P_{osmol}$, middle), and plasma arginine vasopressin concentration ([AVP]$_p$, bottom) before infusion, and also before and during passive body heating following isotonic (NOSM) or hypertonic (HOSM) saline infusion. Values are the means ± SE of 11 subjects. *Significant difference between NOSM and HOSM.
that the responsiveness of SR_{ch} and CVC to increased T_{es} (the slope of these relationships above the respective thresholds) were similar (Fig. 3).

Figure 4, top, shows the relationship between the osmotic elevation of the ΔT_{es} threshold for sweating and forearm [Na^+]_{sweat}, showing a high correlation; i.e., the elevation of ΔT_{es} threshold for sweating per unit rise in P_{osmol} was lower in those subjects with a lower [Na^+]_{sweat}. The osmotic shift in the ΔT_{es} threshold for CVD was also correlated with [Na^+]_{sweat} (Fig. 4, middle), but the correlation coefficient was lower (r = 0.628) compared with the relationship between the osmotic shift in ΔT_{es} threshold for sweating and [Na^+]_{sweat} (r = 0.858). The sensitivity of the osmotic AVP secretion (increase in [AVP]_p per unit rise in P_{osmol}) was not significantly correlated with [Na^+]_{sweat} (Fig. 4, bottom).

**DISCUSSION**

Body fluid status has a strong impact on thermoregulatory function. It has been well demonstrated that both a reduction in PV and an elevation of P_{osmol} inhibit thermoregulatory responses to increased body temperature (1, 7). Furthermore, it has been reported that heat acclimation reduces [Na^+]_{sweat} in addition to improving thermoregulatory function (6, 11, 15). Reduced [Na^+]_{sweat} and increased sweating rate at a given thermal drive induced by heat acclimatization would result in a larger elevation of P_{osmol} at a given amount of sweat output (13). A greater increase in P_{osmol} will withdraw more water from intra- to extracellular space and will minimize the reduction of PV (13), which will be advantageous to maintain body core temperature at a lower temperature during prolonged heat stress (13). In contrast, we have shown that increased P_{osmol} inhibits thermoregulatory responses to increased body temperature (17, 19), and the ΔT_{es} thresholds for CVD and sweating elevated linearly with the increase in P_{osmol}, indicating that the ΔT_{es} thresholds for thermoregulatory responses are osmo-

**Fig. 2.** Changes in esophageal temperature (ΔT_{es}, top), local chest sweating rate (ΔSR_{ch}, middle), and cutaneous vascular conductance (ΔCVC, bottom) before and during passive body heating. Values are the means ± SE of 11 subjects.

**Fig. 3.** The ΔSR_{ch} (top) and ΔCVC (bottom) as a function of ΔT_{es}. Values are the means ± SE of 11 subjects.
Thus we determined the relationship between osmotic inhibition of thermoregulatory responses to increased $T_{es}$ and $[\text{Na}^+]_{\text{sweat}}$. Our hypothesis was that the elevation of the $\Delta T_{es}$ thresholds for thermoregulation per unit rise in $P_{\text{osmol}}$ should be attenuated in the subjects with lower $[\text{Na}^+]_{\text{sweat}}$. We also determined the relationship between osmosensitivity for AVP secretion ($[\text{AVP}]_p$ per unit rise in $P_{\text{osmol}}$) and $[\text{Na}^+]_{\text{sweat}}$ to elucidate whether the attenuated osmosensitivity with a lower $[\text{Na}^+]_{\text{sweat}}$ is specific for thermoregulation or general in the osmoregulatory responses.

In the present study, we confirmed that elevated $P_{\text{osmol}}$ inhibits both sweating and CVD by elevating the $\Delta T_{es}$ thresholds for these responses (Fig. 3). The responsiveness, represented by the slope of the relationship between the responses and $T_{es}$ above the thresholds, for sweating and CVD were similar between NOSM and HOSM. In addition, the increase in $T_{es}$ during passive body heating was highly correlated with the $\Delta T_{es}$ thresholds for sweating and CVD (Fig. 5). Therefore, the shifted $\Delta T_{es}$ thresholds for these responses must be the main factor resulting in the excessive increase in $T_{es}$ during passive body heating in HOSM, and the shift in $\Delta T_{es}$ threshold is likely to accurately represent the osmotic inhibition of the thermoregulatory responses.

The most significant finding of this study was that there exists a highly significant correlation among osmotic shifts in the $\Delta T_{es}$ thresholds for sweating, the $\Delta T_{es}$ threshold for CVD, and $[\text{Na}^+]_{\text{sweat}}$, respectively, i.e., the inhibitory effect of plasma hyperosmolality on
thermoregulatory responses was smaller in those subjects with a lower \([\text{Na}^+]_{\text{sweat}}\). The data obtained in this study suggest that the osmoregulatory inhibition of thermoregulatory responses to increased body temperature would be attenuated in heat-acclimated individuals as \([\text{Na}^+]_{\text{sweat}}\) decreases. In contrast to the significant correlation between the osmotic shift in the \(\Delta T_{es}\) thresholds for thermoregulatory responses (increase in the \(T_{es}\) thresholds per unit rise in \(P_{\text{osmol}}\)) and \([\text{Na}^+]_{\text{sweat}}\), the osmosensitivity for vasopressin secretion (increase in \([\text{AVP}]_p\) per unit rise in \(P_{\text{osmol}}\)) was not correlated with \([\text{Na}^+]_{\text{sweat}}\), suggesting that heat acclimation seems to modify the osmosensitivity for the inhibition of thermoregulation selectively.

Although the osmotic shift in the thresholds for both sweating and CVD correlated with \([\text{Na}^+]_{\text{sweat}}\), the correlation coefficient of the relationship between \(\Delta T_{es}\) threshold for CVD and \([\text{Na}^+]_{\text{sweat}}\) was lower compared with that \(\Delta T_{es}\) threshold for sweating and \([\text{Na}^+]_{\text{sweat}}\). One possible reason for this is that the control of cutaneous blood flow is influenced by more factors than the control of sweating, and cutaneous blood flow is controlled by the vasoconstrictor and vasodilator systems (4). Increased \(P_{\text{osmol}}\) may attenuate the thermoregulatory efferent system, but it may not influence the nonthermal control of these responses. The lower stability of the measurement of CVD by laser Doppler flowmetry might be another factor involved in the lower correlation coefficient of the relationship between \(\Delta T_{es}\) threshold for CVD and \([\text{Na}^+]_{\text{sweat}}\).

In the present study, we determined the relationship between the osmotic shift in the thresholds for thermoregulatory responses to heat stress and \([\text{Na}^+]_{\text{sweat}}\) using the data obtained from 11 subjects (cross-sectional study). It would of course be better to determine the changes in the \(\Delta T_{es}\) thresholds for thermoregulatory responses and \([\text{Na}^+]_{\text{sweat}}\) before and after a heat acclimation program (longitudinal study). We determined the relationship twice (in NOSM and in HOSM), and the experiments were separated by at least 1 wk with the order of experiments randomized. Thus it was impossible to examine the effect of a short-term acclimation program on the \([\text{Na}^+]_{\text{sweat}}\) and osmotic inhibition of the thermoregulation, because acclimation status should be changed between the two experiments (15, 20). It is expected that studies will be performed to examine the effect of long-term acclimation.

The forearm \([\text{Na}^+]_{\text{sweat}}\) in the present study was relatively low (36.3 ± 6.0 meq/l). In a different series of experiments in our laboratory, the mean forearm \([\text{Na}^+]_{\text{sweat}}\) measured with the same experimental procedure in different subjects during winter was 62.7 ± 5.7 meq/l (39.4–83.8 meq/l, \(n = 9\)). In the present study, we performed the experiments at the end of summer, and five subjects participated in regular “Kendo” practice in which they wore heavy protectors in a hot environment. Thus we speculate that the relatively low forearm \([\text{Na}^+]_{\text{sweat}}\) in the present study was not due to measurement error, but rather due to a higher acclimation status of these subjects. In the present study, plasma aldosterone concentration was not correlated with \([\text{Na}^+]_{\text{sweat}}\), thus the increased responsiveness of the sweat gland may be augmented in the subjects with a lower \([\text{Na}^+]_{\text{sweat}}\) (6). We found a regional difference of \([\text{Na}^+]_{\text{sweat}}\) between the forearm and chest. All of the subjects showed higher \([\text{Na}^+]_{\text{sweat}}\) in the chest than in the forearm, and forearm \([\text{Na}^+]_{\text{sweat}}\) and chest \([\text{Na}^+]_{\text{sweat}}\) were strongly correlated (\(r = 0.965\)), suggesting that there is extremely low individual variation in the regional \([\text{Na}^+]_{\text{sweat}}\) difference. The regional difference in \([\text{Na}^+]_{\text{sweat}}\) might be due to the difference in sweat collection methods. However, a strong correlation between osmotic inhibition of thermoregulatory responses and \([\text{Na}^+]_{\text{sweat}}\) was not influenced by the method for sweat collection or collection site.

In summary, we confirmed that increased \(P_{\text{osmol}}\) inhibits both thermoregulatory sweating and CVD by elevating \(\Delta T_{es}\) thresholds for these responses. The osmotic inhibition of thermoregulation, represented by the elevation of the \(\Delta T_{es}\) thresholds per unit rise in \(P_{\text{osmol}}\), and \([\text{Na}^+]_{\text{sweat}}\) were highly correlated, and the inhibitory effect of plasma hyperosmolality was smaller in those subjects with a lower \([\text{Na}^+]_{\text{sweat}}\). The results of this study suggest the possibility that heat acclimation attenuates the osmotic inhibition of thermoregulatory responses in addition to reducing \([\text{Na}^+]_{\text{sweat}}\), which would be beneficial in maintaining thermoregulatory sweating and CVD during prolonged heat stress accompanying a large amount of sweating.

**Perspectives**

It has been reported that the thermoregulatory system interacts with other functional systems, including the body fluid regulatory system (16). Heat acclimation status might be acquired as a result of integrated adaptation of several functional systems. In the present study, we demonstrated that osmoregulatory adaptation, i.e., attenuated osmosensitivity for the inhibition of thermoregulation, may be involved in the acquisition of heat-acclimation status by which heat-acclimated individuals can maintain lower body core temperature by sweating and CVD during prolonged heat stress, even though their \([\text{Na}^+]_{\text{sweat}}\) is lower than unacclimated individuals. Although we demonstrated the strong correlation between the osmotic inhibition of thermoregulation and \([\text{Na}^+]_{\text{sweat}}\), the present study was a cross-sectional study. A longitudinal study that examines the effect of heat acclimation on osmotic inhibition and \([\text{Na}^+]_{\text{sweat}}\) would provide further information on the acquisition mechanism of heat acclimation.

We thank Yoshiko Kawaguchi, Mikako Matoba, and Yoko Fujiwara for technical assistance.

This work was supported in part by the Ministry of Education, Science, Sports and Culture of Japan and the Descente and Ishimoto Memorial Foundation for the Promotion of Sports Science to A. Takamata.

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