Effects of hypohydration on thermoregulation during exercise before and after 5-day aerobic training in a warm environment in young men

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Submitted 12 October 2010; accepted in final form 3 February 2011

Ikegawa S, Kamijo Y, Okazaki K, Masuki S, Okada Y, Nose H. Effects of hypohydration on thermoregulation during exercise before and after 5-day aerobic training in a warm environment in young men. J Appl Physiol 110: 972–980, 2011; doi:10.1152/japplphysiol.01193.2010.—We examined whether enhanced cardiovascular and thermoregulatory responses during exercise after short-term aerobic training in a warm environment were reversed when plasma volume (PV) expansion was reversed by acute isotonic hypohydration. Seven young men performed aerobic training at the 70% peak oxygen consumption rate (V\text{O}_2\text{peak}) at 30°C atmospheric temperature and 50% relative humidity, 30 min/day for 5 days. Before and after training, we performed the thermoregulatory response test while measuring esophageal temperature (T_{es}), forearm skin vascular conductance, sweat rate (SR), and PV during 30 min exercise at the metabolic rate equivalent to pretraining 65% V\text{O}_2\text{peak} in euhydration under the same environment as during training in four trials (euhydration and hypohydration, respectively). Hypohydration targeting 3% body mass was attained by combined treatment with low-salt meals to subjects from ~48 h before the test and administration of a diuretic ~4 h before the test. After training, the T_{es} thresholds for cutaneous vasodilation and sweating decreased by 0.3 and 0.2°C (P = 0.008 and 0.012, respectively) when PV increased by ~10% after PV expansion before and after training was reduced to a similar level, ~10% reduction from that in euhydration before training, the training-induced reduction in the threshold for cutaneous vasodilation increased to a level similar to hypohydration before training (P = 0.093) while that for sweating remained significantly lower than that before training (P = 0.004). Thus the enhanced cutaneous vasodilation response after aerobic training in a warm environment was reversed when PV expansion was reversed while the enhanced SR response remained partially.

skin blood flow; sweat rate; heat acclimation; baroreflexes

PHYSICAL TRAINING-HEAT ACCLIMATION improves thermoregulatory and cardiovascular responses to heat stress during exercise and there are many studies demonstrating the thermoregulatory, and cardiovascular advantages of increased body fluid volume, including plasma volume (PV) (30); however, to our knowledge, only a limited number of studies have attempted to compare the impact of hypohydration and/or hypovolemia on the responses before and after physical training-heat acclimation.

Buskirk et al. (1) assessed the effects of dehydration on heart rate (HR) and rectal temperature during exercise on a treadmill at 25°C atmospheric temperature (T_a) before and after 3-wk aerobic training in a hot environment and suggested that the dehydration-induced increases in HR and rectal temperature responses during exercise were similar before and after training. Lately, Sawka et al. (29) reconfirmed the results by having subjects walk on a treadmill in hot-dry, hot-wet, and comfortable environments when euhydrated or dehydrated after 10-day aerobic exercise training in a hot environment. Although these results suggest that the effects of dehydration on temperature and cardiovascular responses during exercise were similar before and after training, few studies have confirmed these results by measuring thermoregulatory responses (cutaneous vasodilation and sweat rate) to hyperthermia and plasma constituents [PV and plasma osmolality (P_{osmol})] potentially affecting the responses during exercise (12, 20, 23).

We have recently accumulated evidence suggesting that PV expansion after physical training-heat acclimation significantly contributes to enhanced thermoregulatory responses (10, 25, 26). Goto et al. (10) suggested that a mixture of protein and carbohydrate (CHO) supplementation immediately after 30-min aerobic exercise at 70% peak oxygen consumption rate (V\text{O}_2\text{peak}) for five consecutive days at 30°C T_a and 50% relative humidity (RH) in an artificial climate chamber evoked greater increases in plasma albumin content (Alb_{cont}) and plasma oncotic pressure. This caused a fluid shift from the interstitial fluid space and thereby more PV expansion than placebo supplementation and, interestingly, was accompanied by greater increases in cutaneous vasodilation and sweat responses to increased esophageal temperature (T_{es}), and an attenuated increase in T_{es} during exercise. In the study, because the environmental conditions and exercise intensity were controlled similarly and total sweat loss was confirmed to be similar during daily training between the two groups, they postulated that the increased thermoregulatory responses with protein-CHO supplementation were caused mainly by greater PV expansion.

Based on these findings, in the present study, we hypothesized that enhanced thermoregulatory responses after aerobic training using the same protocol as in the previous study (10) were reversed when PV was reversed by acute hypovolemia. To examine this, subjects underwent four trials, euhydration and hypohydration before and after aerobic training, respectively, and compared the impact of acute PV reduction by administration of a diuretic on thermoregulatory responses before and after acclimation. In addition, we measured cardiac stroke volume (SV) during exercise while measuring thermoregulatory responses to examine any effects of altered PV on cardiac filling pressure, which has been suggested to be involved in controlling cutaneous vasodilation and the sweat rate during exercise in a warm environment through baroreflexes (6, 7, 14, 21, 22, 23).
Methods

Subjects

This study was approved by the Review Board on Human Experiments, Shinshu University School of Medicine. Seven young male volunteers gave written informed consent before participating in this study. All subjects were students at our university who were recreationally active in sports/exercise and were nonsmokers with no history of cardiovascular or pulmonary diseases. Their physical activity and physical characteristics were similar to our previous study (10). They were 20.6 ± 2.8 (mean ± SD) [18–25 (range)] years in age, 173.5 ± 8.2 (161–183) cm tall, 64.6 ± 10.0 (48.4–74.0) kg body weight, 50.1 ± 5.8 (43.4–58.8) ml·min⁻¹·kg⁻¹ V̇O₂peak, and 193 ± 4 (180–204) beats/min peak HR.

Trials and Protocol

Subjects underwent four trials (euvhydration and hypohydration before and after 5-day aerobic training). Euvhydrated and hypohydrated trials before training were performed consecutively in that order after a recovery day between the trials, and both trials were completed ≥5 days before training. Euvhydrated and hypohydrated trials after training were performed similarly to before training, starting on the 2nd day after the termination of training. All trials were conducted at the same time of day to avoid any effects of circadian variations.

V̇O₂peak was determined as described below >1 wk before the experiment. For thermoregulatory response tests, food was controlled from breakfast on the day before the test. The meals were designed to meet total caloric requirements per day for subjects performing moderate physical activity. For the evhydrated trials, the content of salt in the meals was reduced from 1% for Japanese in euhydrated trials (16). On the other hand, in hypohydrated trials, it was controlled from lunch on the 2 days before the test. The meals were designed to meet total caloric requirements for choosing 5 days as the training period was that this was suggested to be sufficient to evoke most reductions in HR and Ta during exercise in a hot environment in human subjects (30).

HR was continuously monitored during exercise, and the exercise intensity was readjusted at 5 min after the start of exercise each day so that subjects exercised at a target HR equivalent of 70% V̇O₂peak, determined from the relationship between the oxygen consumption rate (V̇O₂) and HR at the V̇O₂peak measurement before training. The reason for adopting the HR at 5 min as the target HR to be readjusted was that the HR depended on the relative exercise intensity before the body temperature started to increase. The power outputs for training significantly increased from 174 ± 7 W on the 1st day to 192 ± 9 W on the 5th day (P = 0.005). During exercise, the subjects were not allowed to drink any fluids. Subjects ingested 6.4 ml/kg of a protein and CHO mixture (56 kcal, 8.3 g CHO, 5.6 g protein, per 100 ml) containing 3.6 kcal/kg, 0.53 g CHO/kg, and 0.36 g protein/kg within 10 min after each day of exercise to accelerate PV expansion (10, 25, 26). Total sweat loss estimated from body weight loss after exercise was 621 ± 22 g/day on average for the 5-day training period.

Dietary Intake During Training

Subjects were instructed to maintain their dietary habits, except for the supplement, during the study period; however, they were instructed to refrain from any food and fluids, except for the supplement and tap water, for >2 h before and after exercise each day. Furthermore, they were instructed to report the food consumed during the 5-day training period to the laboratory by answering a questionnaire prepared by a dietician. When supplement intake was excluded, total calories from daily dietary intake and protein were 2.295 ± 186 kcal and 81 ± 7 g, respectively.

Measurements

V̇O₂peak. V̇O₂peak was measured with graded exercise using a cycle ergometer in an upright position at Ta of 25.0 ± 0.1°C (mean ± range) and RH of 46 ± 1%. After baseline measurements at rest for 3 min, subjects started pedaling at 60 cycles/min without loading. Exercise intensity was increased by 60 W every 3 min until 180 W and, above this intensity, by 30 W every 2 min until 240 W and then by 15 W every 2 min until exhaustion. VO₂ was determined every 15 s (Aeromonitor AE260; Minato), and HR was recorded every minute (EKG, Life Scope 8; Nihon Kohden). V̇O₂peak was determined by averaging the three largest consecutive values at the end of exercise.

PV. On the day of measurement, subjects were weighed in the nude, on short’s and shoes, and an entered an environmental chamber controlled to a Ta of 30.0 ± 0.1°C (mean ± range) and RH of 50 ± 1%. An 18-gauge Teflon catheter was then placed in the right antecubital vein for blood sampling and dye injections. After subjects rested quietly in a sitting position for >45 min, PV was determined by the Evans blue dye dilution method (11). Briefly, after baseline blood
samples were taken, the dye was injected, blood samples were taken 10, 20, and 30 min after injection, and the absorbance (620 and 740 nm, U-1500; Hitachi) of a 10-min plasma sample was used to calculate PV.

Thermoregulatory response test. Subjects rested quietly in a semi-recumbent position in the contoured chair of the cycle ergometer for 60 min while all measurement devices were applied. After resting in the recumbent position in the contoured chair of the cycle ergometer for 10 min, subjects performed cycling exercise in the semi-recumbent position at 65% of their pretraining V0peak for 30 min without fan cooling. Blood samples were taken at 10 and 5 min before and 5 (Ex5), 10 (Ex10), and 30 (Ex30) min after the start of exercise and used to determine blood properties as described below. HR, arterial blood pressure (BP), Tsk, mean skin temperature (Tsk), chest sweat rate (SR), forearm skin blood flow (FBF), and cardiac output (CO) were measured as described below. After the test, subjects wiped off any sweat and then were weighed again in the nude.

HR and BP. During the thermoregulatory response test, HR was recorded every minute as described above, and systolic (SBP) and diastolic (DBP) BP were measured every minute from the right upper arm at the heart level by inflation of the cuff with sonometric pickup of Korotkoff’s sound (model STBP-780; Colin). Mean BP (MBP) was calculated as DBP + (SBP - DBP)/3.

Tsk and Tsa. Tsk was monitored with a thermocouple in polyethylene tubing (PE-90). The tip of the tube was advanced to a distance of one-fourth of the subject’s standing height from the external nares. Tsa was monitored as Tsa = 0.25Tsk + 0.43Tch + 0.32Tfn (27), where Tsk, Tch, and Tfn were surface temperatures in the right forearm at 10 cm below the cubital line on the radial line, the right chest at 10 cm below the midclavicle, and the right anterior thigh at 15 cm above the patella on the middle line, which were measured with thermocouples, respectively. Tsk and Tsa were recorded every 5 s and presented every minute on average.

FBF and SR. FBF was measured by venous occlusion plethysmography with a mercury-in-Silastic tube strain gauge placed around the upper side of the subject’s left forearm positioned above the heart level, with the hand eliminated from the circulation by inflating the occlusion cuff to supra-arterial pressure (280 mmHg) (34). SR was recorded every 5 s, and FBF was measured two times every minute and presented for the injected amount of ICG and hemoglobin concentration ([Hb], g/dl) in blood samples taken before dye injection. SV was calculated from CO and HR measured at the same time. The reproducibility of this measurement, examined in another group of young subjects during graded cycle ergometer exercise, was confirmed to be 0.4–0.9 l/min of 95% confidence limit over the range of 3.3–22.8 l/min, covering the range in the present study (26).

Blood properties. An aliquot of the blood sample was transferred to a heparin-treated tube and used to determine hematocrit (Hct, %, microcentrifuge) and [Hb] by the sodium lauryl sulfate method (Sigma Chemical) in triplicate. The remaining aliquot of sample was transferred to a heparin-treated tube and centrifuged for 3 min with 40,000 rpm, and the separated plasma was stored at −85°C until the assays were performed. The plasma was used to determine total plasma protein concentration ([TP], g/dl) by the biuret method; Wako Chemical), plasma albumin concentration ([Alb], g/dl) by the bromocresol green method; Wako Chemical), and [Alb] by the sodium lauryl sulfate method.

Data Analyses

Forearm skin vascular conductance. Forearm skin vascular conductance (FVC) was calculated as FBF/MBP as a unit of milliliters per 100 milliliters per minute per 100 mmHg.

FVC and SR responses to increased Tsk. The Tsk threshold for increasing FVC (ΔFVC/ΔTsk) and SR (ΔSR/ΔTsk) and the increase in FVC (ΔFVC/ΔTsk) and SR (ΔSR/ΔTsk) at a given increase in Tsk were determined from Tsk vs. FVC or SR relationships in each subject as described previously (32). Briefly, the Tsk vs. FVC relationship in each subject was fitted with three linear regression lines determined visually. The first was determined from the first sharp increase in Tsk before the rapid increase in FVC, the second from the rapid increase in FVC, and the third from measurements after the second component. The ΔFVC was determined as the Tsk at the cross point of the first and second regression lines. The ΔFVC/ΔTsk was determined on the second component. Similarly, the Tsk values at the THsk and ΔSR/ΔTsk were determined. These determinations were performed by three separate investigators who were familiar with the methods but blinded to the trials of the subjects, and the three values were averaged.

PV during exercise. In the euhydrated trials before and after training, PV during exercise was calculated as the product of PV at the baseline determined by the dye dilution method and the percent change of PV from the baseline estimated from changes in Hct and [Hb] (11). In the hypohydrated trials before and after training, we calculated PV from the PV at the baseline in euhydration and changes in Hct and [Hb] from the baseline, respectively.

Statistics

Values are expressed as means ± SE for seven subjects in each trial except where noted. We tested any significant differences in variables during the thermoregulatory response test between any pair of trials using two-way [2 within (trial) × (time)] ANOVA for repeated measures (Tables 1 and 2). In Table 2, the analyses were performed every minute although the values are presented at rest, Ex5, Ex10, and Ex30 to avoid complicating the table. One-way [1 within (trials)] ANOVA for repeated measures was used to examine any significant differences in thermoregulatory responses to increased Tsk between any pair of trials in Table 3. This model was also used to test any significant differences in body weight loss and total urine volume between any pair of trials. One-way [1 within (time)] ANOVA was performed to determine the effect of training on all variables and for repeated measures was used to examine any significant differences in trend changes in PV, CO, and SV in each trial. The statistical power to detect the effects of training was 0.908 and 0.189 on PV, 0.999 and 0.996 on HR, 0.985 and 0.936 on Tsk, 0.904 and 0.383 on THFVC, and 0.853 and 0.975 on THsk at α = 0.05 in euhydration and hypohydration, respectively. Similarly, the statistical power to detect the effects of hypohydration was 1.000 and 0.999 on PV, 0.968 and 0.977 on HR, 0.656 and 0.547 on Tsk, 0.599 and 0.552 on THFVC, and 0.354 and 0.998 on THsk before and after training, respectively. Subsequent post hoc tests to examine any significant differences in various pairwise comparisons were performed using the Tukey-Kramer test. The null hypothesis was rejected when P < 0.05.
Body weight, kg

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<tr>
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<td>Pre-Ex</td>
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<td>Post-Ex</td>
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<td>64.10 ± 3.75</td>
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PV, ml

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<tr>
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<td>Ex10</td>
<td>2.659 ± 170</td>
<td>2.957 ± 161*</td>
</tr>
<tr>
<td>Ex30</td>
<td>2.569 ± 164</td>
<td>2.846 ± 158*</td>
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[TP]p, g/dl

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</thead>
<tbody>
<tr>
<td>Rest</td>
<td>7.0 ± 0.1</td>
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<tr>
<td>Ex5</td>
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<td>Ex10</td>
<td>7.6 ± 0.1</td>
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<td>Ex30</td>
<td>7.8 ± 0.1</td>
<td>7.7 ± 0.1</td>
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[Alb]p, g/dl

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<tr>
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</tr>
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<td>Ex10</td>
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<tr>
<td>Ex30</td>
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TPcont, g

<table>
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<th>Time</th>
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<tbody>
<tr>
<td>Rest</td>
<td>211 ± 13</td>
<td>232 ± 13*</td>
</tr>
<tr>
<td>Ex5</td>
<td>202 ± 13</td>
<td>220 ± 13*</td>
</tr>
<tr>
<td>Ex10</td>
<td>202 ± 13</td>
<td>222 ± 11*</td>
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<tr>
<td>Ex30</td>
<td>201 ± 13</td>
<td>218 ± 12*</td>
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Albcont, g

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<tr>
<td>Rest</td>
<td>139 ± 9</td>
<td>155 ± 7*</td>
</tr>
<tr>
<td>Ex5</td>
<td>133 ± 9</td>
<td>146 ± 7*</td>
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<td>Ex10</td>
<td>132 ± 9</td>
<td>145 ± 7*</td>
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<tr>
<td>Ex30</td>
<td>130 ± 9</td>
<td>142 ± 7*</td>
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Posmol, mosmol/kgH2O

<table>
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<th>Time</th>
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</thead>
<tbody>
<tr>
<td>Rest</td>
<td>290.3 ± 0.5</td>
<td>293.0 ± 2.4</td>
</tr>
<tr>
<td>Ex5</td>
<td>295.6 ± 1.2</td>
<td>297.9 ± 1.3</td>
</tr>
<tr>
<td>Ex10</td>
<td>297.1 ± 0.9</td>
<td>300.2 ± 1.8</td>
</tr>
<tr>
<td>Ex30</td>
<td>298.6 ± 0.6</td>
<td>298.9 ± 1.0</td>
</tr>
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</table>

Values are the means ± SE for 7 subjects. BL, baseline; Pre-Ex and Post-Ex, before and after exercise, respectively; PV, plasma volume; TPcont, total plasma protein content; Albcont, plasma albumin content; Alb, plasma albumin concentration; TP, total plasma protein content; TP, total plasma protein content; Alb, plasma albumin concentration; Posmol, plasma osmolality.

RESULTS

Table 1 shows body weight and plasma constituents during the thermoregulatory response test. After training, body weight at baseline remained unchanged in euhydration; however, in hypohydration, the body weight just before the thermoregulatory response test decreased by 1.9 ± 0.6 kg (29%, mean ± range) and 2.3 ± 0.3 kg (35%) compared with that at the baseline in euhydration before and after training, respectively (both, P < 0.001), being slightly greater after than before training (P = 0.024) because of greater response to low-salt meals (P = 0.043). After training, PV increased (P = 0.006) with TPcont and Albcont (P = 0.002, and P = 0.007, respectively) in euhydration; however, in hypohydration, the increased PV was reduced to the level in the hypohydration trial before training (P = 0.182). There were no significant differences in Posmol among trials during the test.

Table 2 shows cardiovascular and thermoregulatory responses during the thermoregulatory response test in four trials. In euhydration, the increases in HR and Tes during exercise were attenuated while those in FVC were enhanced after training compared with before training (P = 0.001, 0.003, and 0.013, respectively). In hypohydration, although the increases in HR and Tes were enhanced while those in FVC were attenuated compared with in euhydration before and after training (all, P < 0.03), HR and Tes remained significantly lower than before training (P < 0.002 and P = 0.006, respectively) while FVC did not (P = 0.305). On the other hand, there were no significant differences in Tsk and SR throughout the test among trials (P > 0.1 and P > 0.2, respectively).

Figure 1 shows the relationships between Tsk vs. FVC or SR during the thermoregulatory response test in euhydration and hypohydration before and after training. As shown in Fig. 1, although FVC and SR responses to increased Tes were enhanced while those in FVC were enhanced in euhydration before and after training, respectively, however, in hypohydration, they were both reduced to the responses in the hypohydration trial before training.

Table 3 summarized the statistical analyses on the relationships between Tsk vs. FVC or SR before and after training. As shown in Fig. 1, although FVC and SR responses to increased Tes were enhanced while those in FVC were enhanced in euhydration before and after training, respectively, however, in hypohydration, they were both reduced to the responses in the hypohydration trial before training.
training (both, \( P > 0.4 \)). Moreover, we found that SV decreased at Ex30 from that at Ex10 in euhydration and hypohydration before training \( (P = 0.004 \) and 0.038, respectively) but not after training \( (P = 0.280 \) and 0.161, respectively).

**DISCUSSION**

The major findings in the present study are that the enhanced cutaneous vasodilatory response after 5-day aerobic training in a warm environment was reduced to the response in the hypohydrated trial before training when PV was reduced to a similar level while the enhanced SR response remained partially.

**Hydration State in Hypohydration Before and After Training**

In the present study, hypohydration was attained by the administration of low-salt meals and a diuretic before the thermoregulatory response test in the same protocol before and after training; however, the body weight loss in response to low-salt meals was slightly but significantly greater after than before training (Table 1) while the urine volume response to a diuretic was similar between trials. As a result, PV was reduced to a similar level as before training (Fig. 2). Because extracellular fluid volume was likely expanded after physical training—heat acclimation because of enhanced function of Na and water retention hormones during training \( (2, 4, 24) \), greater total body water loss (body weight loss) might be needed to attain a similar level of heat acclimation.

We found that the lowered \( \text{TH}_{\text{FVC}} \) and \( \text{TH}_{\text{SR}} \) in euhydration after training moved toward higher \( T_{\text{es}} \) in hypohydration and, as a result, there were no significant differences in \( \text{TH}_{\text{FVC}} \) between the hypohydrated trials before and after training.

Table 3. **FVC and SR responses to increased \( T_{\text{es}} \) during thermoregulatory response test**

<table>
<thead>
<tr>
<th>Time</th>
<th>Before</th>
<th>After</th>
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<tbody>
<tr>
<td>Work load, W</td>
<td>143 ± 7</td>
<td>143 ± 7</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>64 ± 3</td>
<td>60 ± 3*</td>
</tr>
<tr>
<td>MBP, mmHg</td>
<td>86 ± 3</td>
<td>83 ± 3</td>
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<tr>
<td>T_{es}, °C</td>
<td>36.74 ± 0.12</td>
<td>36.50 ± 0.06*</td>
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<tr>
<td>FVC, units</td>
<td>33.95 ± 0.18</td>
<td>33.83 ± 0.15</td>
</tr>
<tr>
<td>SR, mg·cm^{-2}·min^{-1}</td>
<td>35.11 ± 0.27</td>
<td>35.20 ± 0.25</td>
</tr>
</tbody>
</table>

Values are the means ± SE for 7 subjects. HR, heart rate; MBP, mean blood pressure; \( T_{es} \), esophageal temperature; \( T_a \), mean skin temperature; FVC, forearm skin vascular conductance; SR, sweat rate. *\( P < 0.05 \) vs. euhydration before training. $\( P < 0.05 \) vs. euhydration after training. †\( P < 0.05 \) vs. hypohydration before training. Other abbreviations are the same as in Table 1.
whereas the difference in THSR remained significant (Fig. 1 and Table 3).

THFVC and THSR during exercise are reportedly altered by Tsk (18), Posmol, and relative exercise intensity (31) in addition to PV (6, 7, 19, 23). Because there were no significant differences in Tsk and Posmol between trials, altered exercise intensity due to training or reduced PV likely changed THFVC and THSR.

Experimentally, in euhydration, HR decreased from 144 to 137 beats/min at Ex5 after training, equivalent to an 5% increase of pretraining VO2peak in euhydration according to the relationship between VO2 and HR, consistent with our previous study (10). On the other hand, in hypohydration, HR increased from 144 to 147 beats/min before training, equivalent to an 3% decrease of pretraining VO2peak in euhydration while it increased from 137 to 143 after training, equivalent to an 5% decrease of posttraining VO2peak in euhydration, therefore returning to pretraining VO2peak in euhydration.

Regarding the relationship between relative exercise intensity and THFVC, Mitono et al. (17) reported that the upward shift of THFVC with increasing relative exercise intensity was abolished when the increase in Posmol with a rise in exercise intensity was recovered by hypotonic saline infusion, suggesting that Posmol was a major factor in increasing THFVC with a rise in relative exercise intensity. More importantly, in the present study, Ichinose et al. (12) recently suggested that the sensitivity of an upward shift of THFVC with increased Posmol by hypertonic saline infusion was attenuated after 10-day aerobic training, and, furthermore, the attenuation was greater in subjects with higher PV expansion with a high significant correlation (n = 9, r = −0.89, P < 0.005). These results suggest that the change in THFVC in the present study was caused by altered sensitivity of the cutaneous vasodilatory response to increased Posmol during exercise, which was caused by altered PV. In other words, the similar THFVC in the hypohydrated trials before and after training might be explained by the similar level of PV.

Also, PV might be a major factor in decreasing THSR after training since it was increased by reduced PV although the possibilities of direct effects of decreased relative exercise intensity or the reduced sensitivity to Posmol were not excluded. Experimentally, Fortney et al. (7) suggested in exercising subjects that THSR at the chest increased by ~0.1°C, although not significantly, when the blood volume was reduced by 6.8 ml/kg, equivalent to the PV loss in the present study, by administration of a diuretic before exercise. Recently, Mack et al. (15) assessed this issue by applying lower-body negative pressure to subjects performing cycle ergometer exercise in a supine position and suggested that the SR response to increased Tes was suppressed with an upward shift of THSR. On the other hand, a few studies have suggested that skin temperature was reduced during lower-body negative pressure to decrease SR (5, 33). Therefore, the effects of altered PV on SR responses remain controversial; however, the results in the present study suggest that the downward shift of THSR after aerobic training was caused at least in part by an increase in PV, although the evidence was not so strong. On the other hand, the significantly lower THSR after training than before training in hypohydration despite the similar level of PV suggests that mechanisms other than PV were involved in the enhanced SR response after training (3, 20).

ΔFVC/ΔTes and ΔSR/ΔTes

Also, there have been many studies suggesting that ΔFVC/ ΔTes was enhanced by actions to increase venous return to the heart: PV expansion (6, 7, 23), head-out water immersion (22), posture change from upright to supine position (14), and continuous negative pressure breathing (21). On the other
hand, the sensitivity was reduced by actions to decrease venous return to the heart, e.g., hypovolemia (6, 7, 15, 19). In the present study, in euhydration, we confirmed that FVC/Tes increased in 6/7 subjects after training. On the other hand, in hypohydration, FVC/Tes after training decreased to the level in the hypohydrated trial before training when PV was reduced to a similar level. Moreover, we found that SR/Tes was not significantly different between any pair of trials. These results suggest that the increased FVC/Tes after training was at least partially caused by PV expansion while SR/Tes was not.

SV

As in Fig. 2, in euhydration, SV increased in 6/7 subjects at Ex30 after training. In hypohydration, SV decreased significantly compared with euhydration before and after training, with no significant difference between the hypohydrated trials before and after training. In addition, we found that SV at Ex30 significantly decreased after Ex10 in euhydration and hypohydration before training.

It has been suggested that the decrease in SV during exercise in a warm environment was caused by increased HR, which was evoked by a rise in body temperature (8) and also by hypovolemia in dehydration (9). When applying the concept to the present results, in euhydration, the higher SV after training might have been caused mainly by increased venous return to the heart because of PV expansion. Moreover, the lack of a gradual decrease in SV during exercise might have been caused by less increase in HR due to less increase in Tes. In contrast, in hypohydration, SV was significantly lower than that in euhydration before and after training, and, in addition, it gradually decreased along with exercise before training. These results suggest that PV was a major factor in the increase or maintenance of SV during exercise after training by enhancing heat dissipation mechanisms and thereby decreasing Tes and HR. In addition, SV might reflect cardiac filling pressure, which might control cutaneous vasodilation through mechano-receptors in the cardiac walls (6, 7, 14, 21, 22, 23).

HR and Tes Before and After Training in Hypohydration

As in Table 2, because we found that HR and Tes in hypohydration were significantly lower than before training despite the similar PV and SV at rest and during exercise, mechanisms other than PV might be involved in their improved responses after training. Indeed, again, we found that THSR in hypohydration was significantly lower after than before train-
ing (Table 3), suggesting that the enhanced SR response after aerobic training partially remained even in hypohydration and likely contributed to the less increases in HR and $T_{es}$ during exercise.

**Limitations**

In the present study, we failed to find any significant increases in $\Delta FVC/\Delta T_{es}$, $\Delta SR/\Delta T_{es}$, and SV in euhydration after training, probably because of interindividual variation of responses to training, different from the results in the previous study (10) in which the same protocol of aerobic training was adopted as in the present study. Also, probably for the same reason, we failed to find any significant reductions in $\Delta FVC/\Delta T_{es}$ by hypohydration before training, different from the results in many previous studies (6, 7, 15, 19). However, we are certain that these results do not weaken the conclusion of this study because we found significant changes in $TH_{FVC}$ and $TH_{Sm}$ among trials, strongly supporting the conclusion.

In the present study, we did not perform any control trials (groups); however, the major purpose was to compare the impact of reduced PV on the thermoregulatory response before and after training; therefore, the hypohydrated trial before training can be regarded as a control trial where a diuretic was given similarly to the trial after training. To avoid any interindividual variation of thermoregulatory responses to the treatments, we used the same subjects, which made it difficult to allocate them for such a long period to additional control trials.

In the present study, greater body weight loss was needed after than before training to attain a similar PV, suggesting that more extracellular fluid volume was lost with low-salt meals after training; however, the greater loss after training was only $\sim 0.4$ liters, $\sim 3\%$ of the total, suggesting that the hydration state can be regarded as almost identical between the hypohydrated trials before and after training. Moreover, in the present study, we did not examine any effects of hyperosmolarity on the thermoregulatory responses, although it usually occurs in thermal dehydration in the field; however, PV expansion after training might attenuate the effects by blunting sensitivity, as we suggested previously (12).

In conclusion, the enhanced cutaneous vasodilation after 5-day aerobic training in a warm environment was reversed when increased PV was reversed in acute hypohydration while the enhanced SR response remained partially.

**ACKNOWLEDGMENTS**

We are grateful to Otsuka Pharmaceutical, Higashisefuri, Kanzaki, Japan, for supplying the supplement used in the present study.

**GRANTS**

This study was supported in part by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

**DISCLOSURES**

No conflicts of interest are declared by the authors.

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