Effect of voluntary hypocapnic hyperventilation on the relationship between core temperature and heat loss responses in exercising humans

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Submitted 14 April 2014; accepted in final form 17 September 2014

Fujii N, Honda Y, Komura K, Tsuji B, Sugihara A, Watanabe K, Kondo N, Nishiyasu T. Effect of voluntary hypocapnic hyperventilation on the relationship between core temperature and heat loss responses in exercising humans. J Appl Physiol 117: 1317–1324, 2014. First published September 25, 2014; doi:10.1152/japplphysiol.00334.2014.—Two thermolytic thermoregulatory responses, cutaneous vasodilation and sweating, begin when core temperature reaches a critical threshold, after which response magnitudes increase linearly with increasing core temperature; thus the slope indicates response sensitivity. We evaluated the influence of hypocapnia induced by voluntary hyperventilation on the core temperature threshold and sensitivity of thermoregulatory responses. Ten healthy males performed 15 min of cycling at 117 W (29.5°C, 50% RH) under three breathing conditions: 1) spontaneous ventilation, 2) voluntary normocapnic hyperventilation, and 3) voluntary hypocapnic hyperventilation. In the hypocapnic hyperventilation trial, end-tidal CO2 pressure was reduced throughout the exercise, whereas it was maintained around the normocapnic level in the other two trials. Cutaneous vascular conductances at the forearm and forehead were evaluated as laser-Doppler signal/mean arterial blood pressure, and the forearm sweat rate was measured using the ventilated capsule method. Esophageal temperature threshold was higher for the increase in cutaneous vascular conductance in the hypocapnic than normocapnic hyperventilation trial at the forearm (36.88 ± 0.36 vs. 36.68 ± 0.34°C, P < 0.05) and forehead (36.89 ± 0.31 vs. 36.75 ± 0.31°C, P < 0.05). The slope relating esophageal temperature to cutaneous vascular conductance was decreased in the hypocapnic than normocapnic hyperventilation trial at the forearm (302 ± 177 vs. 420 ± 178°C baseline/°C, P < 0.05) and forehead (236 ± 164 vs. 358 ± 221°C baseline/°C, P < 0.05). Neither the threshold nor the slope for the forearm sweat rate differed significantly between the hypocapnic or normocapnic hyperventilation trials. These findings indicate that in exercising humans, hypocapnia induced by voluntary hyperventilation does not influence sweating, but it attenuates the cutaneous vasodilatory response by increasing its threshold and reducing its sensitivity.

respiratory alkalosis; temperature regulation; skin; evaporation; nonthermal factor

HYPERTHERMIA PROMOTES TWO THERMOLYTIC THERMOREGULATORY RESPONSES, CUTANEOUS VASODILATION AND SWEATING IN HUMANS. It also causes an increase in ventilation at rest (8, 15, 18, 50, 54) and during exercise (18, 21, 36, 42, 53) in humans as it occurs in panting animals (30, 40). This increase in ventilation in humans, hyperthermia-induced hyperventilation, consistently causes arterial CO2 pressure to decline from its normal level, leading to hypocapnia, which means that hypocapnia and enhanced thermoregulatory responses can occur together during hyperthermia. The potential interaction between hypocapnia and thermoregulatory responses are not completely understood, however.

Albert (1) demonstrated that the sweat rate during hyperthermia at rest is reduced by voluntary hypocapnic hyperventilation but not by voluntary normocapnic hyperventilation. In addition, Robinson and King (41) later reported that during hyperthermia at rest, hand blood flow, an index of glabrous cutaneous blood flow, was lower during voluntary hyperventilation with hypocapnia than hyperventilation with normocapnia. More recently, we found that nonglabrous cutaneous vasodilation observed under hyperthermic resting conditions was less pronounced during voluntary hypocapnic hyperventilation than during voluntary normocapnic hyperventilation (17). Taken together, these findings suggest that hypocapnia attenuates thermoregulatory responses under resting conditions. However, there have been no studies examining the effect of hypocapnia on thermoregulatory responses during exercise. Thermoregulatory responses can be modulated by exercise (23, 47) as well as by exercise-associated nonthermal factors, e.g., central command, muscle mechanoreflex, and muscle metaboreflex (2, 3, 31, 44). It remains to be seen whether hypocapnia continues to attenuate thermoregulatory responses in exercising humans despite the possible influences of exercise and/or nonthermal factors.

The preoptic anterior hypothalamus contains warm-sensitive neurons and is thought to integrate thermal information from the body (7). During hyperthermia, the preoptic anterior hypothalamus sends effector signals to the cutaneous blood vessels and sweat glands, which results in cutaneous vasodilation and sweating, respectively (35). Because the influence of core temperature is dominant relative to skin temperature for thermolytic sweating (33) and cutaneous vasodilation (51), the physiological control of thermoregulatory responses is frequently described by the relationship between core temperature and thermoregulatory responses, i.e., the core temperature threshold for thermoregulatory responses and the sensitivity evaluated as the slope relating core temperature to response amplitude (19, 46). It has been hypothesized that the core temperature threshold is determined by the central drive, while the response sensitivity reflects the responsiveness of peripheral cutaneous vessels and sweat glands to the central drive (34). Importantly, no study has evaluated whether the hypocapnia-induced impairment of thermoregulation is due to alteration of core temperature threshold, sensitivity, or a combination of both. In rats, the activity of warm-sensitive neurons in the preoptic anterior hypothalamus is enhanced by increases in arterial CO2 pressure (49); conversely, hypocapnia may attenuate the activity of these neurons, lowering central drive to...
thermal effector outputs. Alteration of central drive appears to be plausible, given that during exercise hypocapnia profoundly reduces cerebral blood flow (20, 38) and thus O$_2$ supply and H$^+$ washout in the brain, which may ultimately influence the central nervous system, including the preoptic anterior hypothalamus.

Accordingly, we hypothesized that in exercising humans, hypocapnia induced by voluntary hyperventilation attenuates thermoregulatory responses by increasing the core temperature threshold and thus lowering central drive. In this study, we also evaluated cerebral circulation to affirm hypocapnia-induced reduction of cerebral blood flow.

**MATERIALS AND METHODS**

**Ethical approval.** The experimental protocols were approved by the Human Subjects Committee of the University of Tsukuba, and conformed to the provisions of the Declaration of Helsinki. All participants provided written informed consent before participating in the experiment.

**Subjects.** Ten healthy males participated in this study. None of the subjects were cigarette smokers or were taking prescription medications. The sample size of 10 was larger than the minimal sample size for the hypocapnia-induced reduction in forearm cutaneous vascular conductance. The sample size of 10 was larger than the minimal sample size for the hypocapnia-induced reduction in forearm cutaneous vascular conductance.

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head, and was normalized to the baseline values, i.e., normothermic
spontaneous ventilation at rest after warm-up. The sweat rate on
the forearm was measured using the ventilated capsule method. Nitrogen
gas was applied at a rate of 800 ml/min to a capsule attached to the
forearm skin with area of 3.46 cm². The humidity in the nitrogen gas
obtained from the capsule was then measured as an index of sweat rate
using a capacitance hygrometer (HMP 45ASPF, Vaisala, Helsinki,
Finland).

Esophageal and skin temperature data were collected via copper
constantan thermocouples, sampled and recorded on a computer every
1 s via a data logger system (WE7000, Yokogawa, Tokyo, Japan).
Skin temperature was measured at eight sites (forearm, forehead,
chest, upper arm, hand, foot, thigh, and calf), and mean skin tempera-
ture was estimated as an unweighted mean value using the following
seven skin sites: forehead, upper arm, hand, foot, calf, thigh, and
chest.

Heart rate was monitored via a three-lead electrocardiogram, and
beat-to-beat changes in arterial blood pressure were monitored using
finger photoplethysmography (Finometer, Finapres Medical system,
Amsterdam, Netherlands). The right arm was placed on a table to keep
the middle finger at heart level. Mean arterial blood pressure was
calculated as the diastolic arterial blood pressure plus one-third of the
pulse pressure.

Middle cerebral artery mean blood velocity was determined using
transcranial Doppler ultrasound (WAKI1TC, Atys Medical, St. Gen-
isval, France). A 2-MHz Doppler probe was placed over the tem-
poral window and fixed with an adjustable headband. The middle
cerebral artery vascular conductance index was calculated as middle
cerebral artery mean blood velocity divided by mean arterial blood
pressure.

Data analysis. The data acquired during spontaneous ventilation at
rest (Rest) were obtained by averaging measurements made over more
than 5 min. The data acquired during voluntary hyperventilation at
rest (Rest2) were obtained by averaging measurements made over the
last 5 min of the 20-min hyperventilation period. The data acquired
during exercise were evaluated by averaging measurements made over
minutes 0–5 (Ex5), minutes 5–10 (Ex10) and minutes 10–15
(Ex15) of the exercise period. We also evaluated core temperature
threshold and response sensitivity based on the relations of esophageal
temperature with cutaneous vascular conductance and sweat rate, as
described by Cheuvront et al. (9). Briefly, we first evaluated the two
regression lines with the smallest residual sums of squares fit to each
curve. The inflection point, where the two lines crossed, was defined
as core temperature threshold, while the slope of the second regression
line was defined as the sensitivity.

For all cardiorespiratory, temperature, and thermoregulatory vari-
ables, we performed two-way repeated measures analysis of variance
using ventilatory condition (spontaneous ventilation, normocapnic
hyperventilation, and hypocapnic hyperventilation) and time (Rest,
Rest2, Ex5, Ex10, and Ex15) as factors. For core temperature thresh-
old and sensitivity, we performed one-way repeated measures analysis
of variance using ventilatory condition (spontaneous ventilation, nor-
 mocapnic hyperventilation, and hypocapnic hyperventilation) as the
factor. After determining the main effects, pairwise differences were
identified using paired two-tailed t-tests. To maintain the type I error
at 5%, Shaffer’s correction was used when comparing ventilatory
conditions (three comparisons: spontaneous ventilation vs. normocap-
nic hyperventilation, spontaneous ventilation vs. hypocapnic hyper-
ventilation, and normocapnic hyperventilation vs. hypocapnic hyper-
ventilation), while Holm’s correction was used when comparing times
(four comparisons: Rest vs. Rest2, Rest vs. Ex5, Rest vs. Ex10, and
Rest vs. Ex15). All data are reported as means ± standard deviation,
which was used instead of standard error in order to adhere to the
statistical guidelines published by the American Physiological Society
(12). Values of P < 0.05 were considered significant.

RESULTS

Normal ventilation at rest. None of the variables recorded
during normal ventilation at rest differed significantly between
trials (Fig. 1, Fig. 2, and Table 1). This indicates that order
effects on cardiorespiratory, temperature, and thermoregula-
tory variables in the three trials were minimal.

Ventilatory variables. There was an interaction between the
effects of ventilatory condition and time on minute ventilation
(Fig. 1A). An interaction also existed for end-tidal CO₂ pressure
(F(8, 72)=84.4, P < 0.001) and estimated arterial CO₂ pressure
(F(8, 72)=80.1, P < 0.001). During voluntary hyper-
ventilation at rest (Rest 2) and during exercise, end-tidal CO₂
pressure decreased in the hypocapnic hyperventilation trial, but
was maintained around the normocapnic level in the sponta-
neous ventilation and normocapnic hyperventilation trials (Fig.
1B). Hence, we successfully manipulated the level of end-tidal
CO₂. A similar result was obtained for estimated arterial CO₂
pressure (data not shown), and we also found an interaction
with end-tidal O₂ pressure (F(8, 72)=4.93, P < 0.001).
Throughout the protocol, however, end-tidal O₂ pressure
did not increase from the normal ventilation level at rest in any
trial: the end-tidal O₂ pressure during normal ventilation at rest
was 101 ± 6 mmHg in the spontaneous ventilation trial, 102 ±
8 mmHg in the normocapnic hyperventilation trial, and 103 ±
4 mmHg in the hypocapnic hyperventilation trial. This result
indicates that we minimized increases in end-tidal O₂ pressure
usually observed during hyperventilation (10, 17), eliminating
a potential confounding factor.

Fig. 1. Time course of changes in minute ventilation (A) and end-tidal CO₂ pressure (B). Values are means ± standard deviation. Rest, normal ventilation at rest; Rest2, last 5
min of 20 min of voluntary hyperventilation at rest; Ex5-15, corresponding number of
minutes of exercise; *P < 0.05 vs. rest in each trial; †P < 0.05 vs. spontaneous venti-
lilation trial; ‡P < 0.05 vs. normocapnic hyperventilation trial.
Fig. 2. Time course of changes in esophageal temperature (A), forearm cutaneous vascular conductance (B), forehead cutaneous vascular conductance (C), and forearm sweat rate (D). Values are means ± standard deviation. Rest, normal ventilation at rest; Rest2, last 5 min of 20 min of voluntary hyperventilation at rest; Ex5-15, corresponding number of minutes of exercise; *P < 0.05 vs. rest in each trial; †P < 0.05 vs. spontaneous ventilation trial; ‡P < 0.05 vs. normocapnic hyperventilation trial.

Esophageal temperature, cutaneous vascular conductance, and sweat rate. Although there was a main effect of time on esophageal temperature [F(4, 36) = 8.42, P < 0.001] and forehead cutaneous vascular conductance [F(4, 36) = 22.4, P < 0.001], no interactions [both F(8, 72) < 0.74, P > 0.156] on esophageal temperature or forehead cutaneous vascular conductance were seen, indicating that esophageal temperature and forehead cutaneous vascular conductance did not differ between trials at any time point (Fig. 2, A and C). On the

Table 1. Temperature and cardiovascular variables from rest to exercise

<table>
<thead>
<tr>
<th></th>
<th>Normal ventilation at rest</th>
<th>Voluntary hyperventilation at rest</th>
<th>Exercise, 5 min</th>
<th>Exercise, 10 min</th>
<th>Exercise, 15 min</th>
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<tr>
<td>Mean skin temperature, °C</td>
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<tr>
<td>Spontaneous ventilation</td>
<td>33.0 ± 0.6</td>
<td>33.4 ± 0.5*</td>
<td>33.4 ± 0.5*</td>
<td>33.7 ± 0.5*</td>
<td>33.9 ± 0.7*</td>
</tr>
<tr>
<td>Normocapnic hyperventilation</td>
<td>33.3 ± 0.4</td>
<td>33.6 ± 0.3*</td>
<td>33.7 ± 0.3*</td>
<td>33.8 ± 0.3*</td>
<td>33.9 ± 0.5*</td>
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<tr>
<td>Hypocapnic hyperventilation</td>
<td>33.1 ± 0.7</td>
<td>33.5 ± 0.4</td>
<td>33.6 ± 0.3</td>
<td>33.5 ± 0.5‡</td>
<td>33.6 ± 0.6</td>
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<tr>
<td>Forearm skin temperature, °C</td>
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<td>Spontaneous ventilation</td>
<td>32.7 ± 0.8</td>
<td>32.9 ± 0.7</td>
<td>33.0 ± 0.7</td>
<td>33.1 ± 0.8</td>
<td>33.5 ± 0.8</td>
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<tr>
<td>Normocapnic hyperventilation</td>
<td>33.0 ± 0.7</td>
<td>33.1 ± 0.7</td>
<td>33.2 ± 0.8</td>
<td>33.3 ± 1.0</td>
<td>33.5 ± 0.9</td>
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<tr>
<td>Hypocapnic hyperventilation</td>
<td>32.6 ± 0.7</td>
<td>32.9 ± 0.5</td>
<td>33.0 ± 0.6</td>
<td>32.9 ± 0.8†‡</td>
<td>33.2 ± 0.9†‡</td>
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<td>Forehead skin temperature, °C</td>
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<tr>
<td>Spontaneous ventilation</td>
<td>33.2 ± 1.4</td>
<td>33.8 ± 1.1*</td>
<td>34.1 ± 1.0*</td>
<td>34.7 ± 1.3*</td>
<td>34.8 ± 1.4*</td>
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<tr>
<td>Normocapnic hyperventilation</td>
<td>33.4 ± 1.3</td>
<td>34.3 ± 0.5*</td>
<td>34.6 ± 0.5*</td>
<td>34.9 ± 0.8*</td>
<td>35.0 ± 0.9*</td>
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<td>33.4 ± 1.5</td>
<td>34.0 ± 0.9</td>
<td>34.4 ± 0.8*</td>
<td>34.6 ± 1.1*</td>
<td>34.7 ± 1.3*</td>
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<td>Heart rate, bpm</td>
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<td>Spontaneous ventilation</td>
<td>72 ± 12</td>
<td>77 ± 13*</td>
<td>125 ± 16*</td>
<td>139 ± 19*</td>
<td>145 ± 19*</td>
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<tr>
<td>Normocapnic hyperventilation</td>
<td>74 ± 13</td>
<td>77 ± 14*</td>
<td>130 ± 16†‡</td>
<td>143 ± 20*</td>
<td>149 ± 20*</td>
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<tr>
<td>Hypocapnic hyperventilation</td>
<td>73 ± 12</td>
<td>80 ± 14*</td>
<td>123 ± 17†‡</td>
<td>136 ± 19†‡</td>
<td>141 ± 22†‡</td>
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<td>Mean arterial blood pressure, mmHg</td>
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<td>Spontaneous ventilation</td>
<td>78 ± 7</td>
<td>77 ± 7</td>
<td>95 ± 14*</td>
<td>95 ± 11*</td>
<td>95 ± 9*</td>
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<td>Normocapnic hyperventilation</td>
<td>73 ± 7</td>
<td>71 ± 6</td>
<td>84 ± 17</td>
<td>91 ± 13*</td>
<td>92 ± 10*</td>
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<td>75 ± 6</td>
<td>74 ± 5</td>
<td>90 ± 10*</td>
<td>94 ± 9*</td>
<td>93 ± 10*</td>
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<tr>
<td>Middle cerebral artery vascular conductance index, cm/s/mmHg</td>
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<tr>
<td>Spontaneous ventilation</td>
<td>0.85 ± 0.20</td>
<td>0.72 ± 0.19</td>
<td>0.81 ± 0.15</td>
<td>0.81 ± 0.17</td>
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<tr>
<td>Normocapnic hyperventilation</td>
<td>0.88 ± 0.19</td>
<td>0.78 ± 0.17†</td>
<td>0.89 ± 0.33</td>
<td>0.84 ± 0.22</td>
<td>0.84 ± 0.25</td>
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<tr>
<td>Hypocapnic hyperventilation</td>
<td>0.87 ± 0.15</td>
<td>0.55 ± 0.15†‡</td>
<td>0.57 ± 0.10†‡</td>
<td>0.59 ± 0.13†‡</td>
<td>0.57 ± 0.12†‡</td>
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Values are means ± SD. *P < 0.05 vs. normal ventilation at rest in each trial; †P < 0.05 vs. spontaneous ventilation trial; ‡P < 0.05 vs. normocapnic hyperventilation trial.
other hand, an interaction was observed for forearm cutaneous vascular conductance \([F(8, 72)=2.28, P < 0.031]\), which was lower in the hypocapnic hyperventilation trial during \(\text{Ex10}\) than in the spontaneous ventilation or normocapnic hyperventilation trial (Fig. 2B). This is evidence of hypocapnia-induced attenuation of cutaneous vasodilation in exercising humans. We also found an interaction of the forearm sweat rate \([F(8, 72)=3.18, P < 0.004]\), though forearm sweat rate did not differ between trials at any time point (Fig. 2D).

**Core temperature threshold and sensitivity.** We detected a main effect of ventilatory condition on the esophageal temperature threshold for forearm and forehead cutaneous vascular conductance [both \(F(2, 18) > 8.12, P < 0.003\)]. We found that the esophageal temperature thresholds for forearm and forehead cutaneous vascular conductances were higher in the hypocapnic hyperventilation trial than in the spontaneous ventilation or normocapnic hyperventilation trial by 0.14–0.21°C (Fig. 3, A and B). This is consistent with our original hypothesis that hypocapnia lowers core temperature threshold and thus central drive for cutaneous vasodilation. A main effect of ventilatory condition was also observed for the sensitivity of forearm and forehead cutaneous vascular conductance [both \(F(2, 18) > 5.50, P < 0.014\)]. The sensitivities of the forearm and forehead cutaneous vascular conductances in the hypocapnic hyperventilation trial were lower than in the normocapnic hyperventilation trial by 28–34% (Fig. 3, A and B). Thus hypocapnia lowers sensitivity, implying alteration of peripheral mechanisms mediating cutaneous vasodilation. On the other hand, there was no main effect of ventilatory condition on the core temperature threshold \([F(2, 18) = 2.66, P = 0.10]\) or sensitivity \([F(2, 18) = 0.72, P = 0.50]\) for forearm sweat rate, demonstrating there to be no between-trial differences in the core temperature threshold and sensitivity for forearm sweat rate (Fig. 3C).

**Other variables.** An interaction was observed between the effects of ventilatory condition and time on mean skin temperature, heart rate, and middle cerebral artery vascular conductance index [all \(F(8, 72) > 2.27, P < 0.032\), while main effects of ventilatory condition on forearm skin temperature \([F(2, 18) = 4.02, P = 0.036]\) and of time on forearm and forehead skin temperature and mean arterial pressure were detected [all \(F(4, 36) > 4.11, P < 0.008\)]. During voluntary hyperventilation at rest or during exercise, mean skin temperature, forearm skin temperature, heart rate, and middle cerebral artery vascular conductance index were all lower in the hypocapnic hyperventilation trial than in the spontaneous ventilation or normocapnic hyperventilation trial (Table 1). These results show that while hypocapnia causes cerebral vasoconstriction, as expected, it can also lower skin temperatures and heart rate during exercise.

**DISCUSSION**

This is the first report on the impact of hypocapnia induced by voluntary hyperventilation on the core temperature threshold and sensitivity of thermolytic thermoregulatory responses in exercising humans. Our results show that core temperature thresholds for forearm and forehead cutaneous vasodilation are significantly higher, while the sensitivities of forearm and forehead cutaneous vasodilation are significantly lower, during hypocapnic hyperventilation than normocapnic hyperventilation. Neither the core temperature threshold nor the sensitivity of the forearm sweat response differed significantly between any two trials. These results suggest that although hypocapnia induced by voluntary hyperventilation does not affect the sweat response in exercising humans, it attenuates the cutaneous vasodilatory response by both increasing the core temperature threshold and reducing response sensitivity.

**Fig. 3.** Thirty-second averaged plots of cutaneous vascular conductance at the forearm (A) and forehead (B) and sweat rate at the forearm (C) plotted against esophageal temperature. The esophageal temperature thresholds are shown as larger symbols with standard deviations, while regression lines indicate sensitivities. †‡ P < 0.05 vs. spontaneous ventilation trial; †P < 0.05 vs. normocapnic hyperventilation trial.
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Effect of hypocapnia on the relationship between core temperature and heat loss responses. Previous studies showed that hypocapnia induced by voluntary hyperventilation reduces glabrous (41) and nonglabrous (17) cutaneous blood flow during hyperthermia at rest. We showed here that this is also true during exercise, as forearm cutaneous vascular conductance was lower after 10 min of exercise in the hypocapnic trial than in the normocapnic hyperventilation trial (Fig. 2B). Furthermore, we found that the core temperature thresholds for increases in forearm and forehead cutaneous vascular conductance were higher during the hypocapnic hyperventilation trial than during the normocapnic hyperventilation trial by 0.14–0.21°C (Fig. 3, A and B). In addition, the sensitivities of forearm and forehead cutaneous vascular conductance were lower during the hypocapnic hyperventilation trial than the normocapnic hyperventilation trial by 28–34% (Fig. 3, A and B). These results suggest that hypocapnia induced by voluntary hyperventilation attenuates the cutaneous vasodilatory response in exercising humans by affecting both central and peripheral mechanisms.

There are several possible explanations for how hypocapnia may modulate central mechanisms. Firing rates of warm-sensitive neurons in the preoptic anterior hypothalamus are increased by hypercapnia in rats (49). This suggests hypocapnia may directly reduce the activity of warm-sensitive neurons, and thus central drive for cutaneous vasodilation. On the other hand, hypocapnia-induced changes in the activity of warm-sensitive neurons (if any) may be due to secondary decrease in cerebral blood flow during hypocapnia, as reflected by the lower middle cerebral artery vascular conductance index in the hypocapnic than normocapnic hyperventilation trial (Table 1). Along these lines, hypoxia is also known to modulate the activity of warm-sensitive neurons (48). The hypocapnia-induced reduction in cerebral blood flow may mediate the hypoxic effect, reducing the activity of warm-sensitive neurons. Moreover, the earlier observation that increases in CO2 enhance the activity of lingual warm-sensitive receptors (13) suggests hypocapnia may reduce afferent thermal inputs from peripheral thermoreceptors (e.g., skin), thereby contributing to the lower activity of warm-sensitive neurons. In addition, hypocapnia can modulate the activities of peripheral (carotid bodies) and/or central (ventral surface of the medulla oblongata) chemoreceptors, information from which is sent to hypothalamus and may reduce the activity of warm-sensitive neurons.

The hypocapnia-induced peripheral modulation indicated by the lower sensitivity of thermolytic thermoregulatory responses seen in the present study may reflect diminished cutaneous vessel responsiveness to vasodilator substances. The nonglabrous cutaneous vasodilation seen with increases in core temperature is mainly attributable to increased sympathetic cholinergic nerve activity (25). ACh and neuropeptides released from cholinergic nerve terminals during hyperthermia bind to receptors on vessels in the skin, inducing vasodilation (25, 26, 45). Hypocapnia may blunt the responsiveness of those vessels to ACh, as was seen in the pig pulmonary artery (4). Two other mediators involved in cutaneous vasodilation during hyperthermia are nitric oxide and substance P (45, 55). Hypocapnia may lower cutaneous vascular responsiveness to nitric oxide and substance P, as it does in pulmonary artery from pigs and airway smooth muscle from rats (4, 14). In addition, intracellular alkalosis increases vascular smooth muscle contractility through several mechanisms, including increasing levels of intracellular Ca2+ (16). If, in the present study, hypocapnia caused intracellular alkalosis in the skin, increases in vascular smooth muscle contractility may limit the responsiveness of cutaneous vessels to vasodilators during hyperthermia.

In contrast to the cutaneous vasodilatory response, the sweat response was unaffected by hypocapnia (Fig. 3C). Differential effects of nonthermal factors on cutaneous vasodilation and sweating have also been reported previously. For example, in resting heated humans, the baroreflex modulates cutaneous vasodilation but not sweating (6), while central command (43, 44) and the metaboreflex (2, 6, 31) reduce or do not affect cutaneous vasodilation, but consistently increase sweating. The characteristics of central command and the metaboreflex may highlight the reason why hypocapnia attenuates cutaneous vasodilation but not sweating. During exercise, both central command and the metaboreflex are activated and their effect on sweating increases over the exercise period (2, 6, 31), which likely counteracts the hypocapnia-induced reduction in sweating. However, central command and the metaboreflex do not provide an increasing effect on cutaneous vasodilation (2, 6, 31), which allows hypocapnia to reduce cutaneous vasodilation. This notion is indirectly supported by the fact that hypocapnia attenuates the sweat response in subjects resting in the heat (1), when neither central command nor the metaboreflex are activated.

Effect of hyperventilation itself. Similar hypocapnic effects on core temperature threshold and response sensitivity were observed for both forearm and forehead cutaneous vascular conductance (Fig. 3, A and B). However, only forearm cutaneous vascular conductance was significantly lower during exercise in the hypocapnic hyperventilation trial than the other two trials (Fig. 2B). Likewise, forearm but not forehead skin temperature was significantly lower during exercise in the hypocapnic hyperventilation trial than in the other two trials (Table 1). These regional differences are due to the fact that the sensitivity of forehead cutaneous vascular conductance was higher in the normocapnic hyperventilation trial than in the spontaneous ventilation trial by 66% (Fig. 3B), which suggests voluntary hyperventilation itself augments the sensitivity of forehead cutaneous vascular conductance. Enhancement of the effect induced by hyperventilation would counteract the reduction induced by hypocapnia and prevent us from detecting a significant reduction in forehead cutaneous vascular conductance.

Effect of hypocapnia on mean skin temperature. It is noteworthy that mean skin temperature, which can reflect overall cutaneous blood flow, was lower at 10 min of exercise in the hypocapnic hyperventilation trial than in the normocapnic hyperventilation trial (Table 1). This suggests hypocapnia-mediated cutaneous vasoconstriction occurred over most nonglabrous skin. Consistent with that notion, heart rate was significantly lower throughout 15 min of exercise in the hypocapnic than normocapnic hyperventilation trial (Table 1), which may reflect the smaller cardiac output required in the hypocapnic hyperventilation trial due to the reduction in cutaneous blood flow.

Limitations. Because we did not elicit maximal cutaneous vascular conductance, we expressed the data as percentages of baseline at rest rather than as percentages of the maximum response, though the latter is the more standard way to repre-
sent cutaneous blood flow data (32). Because basal cutaneous blood flow is variable (11), use of percentages of baseline at rest has the potential to increase the variability of cutaneous blood flow data, necessitating the use of a larger sample to detect significant effects. Nevertheless, we found a significant effect of hypocapnia on cutaneous vascular conductance, which implies the effect of hypocapnia is strong enough to overpower the potential variability of the data.

The three trials were completed within a single day rather than on separate days to avoid day-to-day variation in laser-Doppler cutaneous vascular responses (11, 32). One may argue that this within-day protocol may have yielded cumulative increases in core temperature (27) and in exercise-related metabolites as the protocol continued. In addition, although no clear evidence exists, exercise-induced modulation of baroreflex control, for example, via muscle sympathetic nerve activity (22, 37), may vary progressively throughout the bouts of exercise. This would ultimately cause an order effect on cardiovascular responses, including arterial blood pressure and cutaneous circulation. To minimize progressive increases in postexercise core temperature, we employed a warm-up before all three trials (see Warm-up in MATERIALS AND METHODS). In addition, the order of the trials was randomized and counterbalanced as much as possible. As a consequence, all resting variables were comparable over the three trials (Figs. 1 and 2, Table 1), and we believe the influence of an order effect was minimal.

Perspectives and significance. Based on the results of the present study, we speculate that if hypocapnia caused by hyperthermia-induced hyperventilation (5, 18, 21, 28, 52) reaches a sufficient level, it may in turn modulate the relationship between core temperature and cutaneous blood flow. For example, baroreceptor unloading induced by lower-body negative pressure induces greater ventilation and hypocapnia during hyperthermia (39), which may partially explain the lower-body negative pressure-induced rightward shift in the mean body temperature threshold, calculated as 0.8 × core temperature + 0.2 × mean skin temperature, for cutaneous vasodilation during hyperthermia reported by Lynn et al. (29). Further studies will be needed to address this possibility.

Conclusion. Consistent with our original hypothesis, we show that in exercising humans, hypocapnia induced by voluntary hyperventilation attenuates the cutaneous vasodilatory response by increasing its core temperature threshold. Different from our original hypothesis, hypocapnia induced by voluntary hyperventilation also reduces the sensitivity of the cutaneous vasodilatory response, but does not influence either the core temperature threshold or the sensitivity of the sweat response.

ACKNOWLEDGMENTS

We sincerely thank all of volunteer subjects participating in the experiment. We greatly appreciate the help of Dr. William Goldman for English editing and critical comments.

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GRANTS

This study was supported by the grants from the Ministry of Education, Culture, Sports, Science and Technology in Japan and the Japan Society for the Promotion of Science. N. Fujii is the recipient of a research fellowship for young scientists from the Japan Society of the Promotion of Science.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: N.F., Y.H., N.K., and T.N. conception and design of research; N.F., K.K., B.T., A.S., and K.W. performed experiments; N.F. and K.K. analyzed data; N.F., Y.H., B.T., and T.N. interpreted results of experiments; N.F. and K.K. prepared figures; N.F. drafted manuscript; N.F. and T.N. edited and revised manuscript; N.F., Y.H., K.K., B.T., A.S., K.W., N.K., and T.N. approved final version of manuscript.

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J Appl Physiol • doi:10.1152/japplphysiol.00334.2014 • www.jappl.org
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