Effect of voluntary hypocapnic hyperventilation on cutaneous circulation in resting heated humans

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Fujii N, Honda Y, Delliaux S, Tsuji B, Watanabe K, Sugihara A, Kondo N, Nishiyasu T. Effect of voluntary hypocapnic hyperventilation on cutaneous circulation in resting heated humans. Am J Physiol Regul Integr Comp Physiol 303: R975–R983, 2012. First published September 12, 2012; doi:10.1152/ajpregu.00169.2012.—Hypocapnia attenuates the sweat rate normally seen in hyperthermic resting subjects, but its effect on the blood flow response in their nonglabrous skin under the same hyperthermic conditions remains unclear. In the present study, we investigated whether hypocapnia induced by voluntary hyperventilation affects the blood flow response to heat stress in the nonglabrous skin of resting humans. Nine healthy male subjects were passively heated using legs-only hot water immersion and a water-perfused suit, which caused esophageal temperature (Tes) to increase by as much as 1.0°C. During normothermia and at +0.6°C Tes or +1.0°C Tes, the subjects performed two voluntary 7-min hyperventilation (minute ventilation = 40 l/min) trials (hypocapnic and eucapnic) in random order. End-tidal CO2 pressure was reduced by 23–25 torr during hypocapnic hyperventilation, but it was maintained at the spontaneous breathing level during eucapnic hyperventilation. Cutaneous blood flow was evaluated as the cutaneous red blood cell flux in the forearm (CBF forearm) or forehead (CBF forehead) and was normalized to the normothermic spontaneous breathing value. Hypocapnic hyperventilation at +0.6°C Tes was associated with significantly reduced CBF forehead, as compared with eucapnic hyperventilation, after 5–7 min of hyperventilation (395 to 429 vs. 487 to 525% baseline, P < 0.05). No significant difference in CBF forehead was seen during hypocapnic hyperventilation compared with eucapnic hyperventilation at +0.6°C Tes or +1.0°C Tes. These results suggest that in resting humans, hypocapnia achieved through voluntary hyperventilation attenuates the increase in cutaneous blood flow elicited by moderate heat stress in the nonglabrous skin of the forearm, but not the forehead.

respiratory alkalosis; skin circulation; hyperthermia; thermoregulation

IT IS WELL ESTABLISHED THAT in both exercising and resting humans, hyperthermia induces sweating and skin vasodilation, two thermoregulatory responses for dissipating heat from the body. Hyperthermia also increases in ventilation in both exercising (3, 15–17, 20, 35, 39, 52, 54, 55) and resting (4, 5, 11, 14, 16, 34) humans. Because this hyperthermia-induced hyperventilation is associated with an increase in alveolar ventilation that occurs above the metabolic demand, CO2 is excessively eliminated from the lung, and arterial CO2 pressure drops from its normal level (hypocapnia) (4, 11, 14–17, 20, 35, 41, 52, 54). In essence, hyperthermia simultaneously evokes thermoregulatory responses and hypocapnia in resting subjects; however, investigation of the effects of hypocapnia on thermoregulatory responses under hyperthermic conditions in humans has been very limited.

Albert (1) demonstrated that the sweat rate in resting subjects in a hot environment (40.5°C) is reduced by voluntary hypocapnic hyperventilation but not by voluntary eucapnic hyperventilation, suggesting hypocapnia attenuates the sweat response to heat stress. Later, Robinson and King (37) reported that the rectal temperature of resting subjects in a hot environment (40.6°C) was higher during 60 min of voluntary hyperventilation with hypocapnia than hyperventilation with eucapnia, which suggests heat dissipation during heat stress is attenuated by hypocapnia and is consistent with the findings of Albert (1). Robinson and King (37) also reported that hand blood flow evaluated using venous occlusion plethysmography was lower during voluntary hypocapnic hyperventilation than during voluntary eucapnic hyperventilation. On the basis of the assumption that hand blood flow is directed mostly to skin, they concluded that hyperventilation-induced hypocapnia attenuates the cutaneous blood flow response during heating (37).

The neural control of skin vasodilation in response to heat stress, as well as the skin vessel architecture, is different in the hand than in nonglabrous skin, such as the forearm or forehead (12, 38), and the effect of hypocapnia on nonglabrous skin during hyperthermia remains unknown. In humans, the vessels of nonglabrous skin are controlled by both noradrenergic and cholinergic sympathetic nerves, with the latter being responsible for 90% of the active vasodilatory response to hyperthermia (30). This active cutaneous vasodilation also involves several of substances in addition to ACh (45), including nitric oxide (NO) (28, 42), histamine (57), prostaglandins (33) and potentially substance P (56). Moreover, the observation that, in animals, hypocapnia attenuates the vasodilatory responses to NO (2), ACh (2), and substance P (10) suggests hypocapnia may attenuate the nonglabrous cutaneous vasodilatory response to heat stress in humans, as it does the sweat response (1). Accordingly, the present study was designed to test the hypothesis that in resting heated humans, hypocapnia induced by voluntary hyperventilation attenuates the increase in blood flow to nonglabrous skin.

METHODS

Subjects. Nine healthy male subjects participated in this study, which was approved by the Human Subjects Committee of the University of Tsukuba and conformed to the provisions of the Dec-
laration of Helsinki. All participants provided written informed consent. None of the subjects were smokers, and none were taking any medication. The subjects weighed 66.8 ± 1.2 (SE) kg and were 1.74 ± 0.01 m in height and 24.3 ± 0.6 years of age. In addition, they exhibited a peak oxygen uptake (V\textsubscript{O2 peak}) of 49.4 ± 3.3 ml·kg\textsuperscript{-1}·min\textsuperscript{-1} during incremental semirecumbent cycling; details of the method for measuring V\textsubscript{O2 peak} were available elsewhere (15, 20–22). Before beginning the present study, the subjects came to the laboratory and performed hypoxic and eucapnic hyperventilation under normothermic conditions to become accustomed to the hyperventilation procedure. As a result of this orientation and the fact that eight of the nine subjects had previously participated in similar passive-heating experiments, we were able to minimize artifacts caused by anxiety and inexperience, which could have influenced the experimental data.

**Experimental procedure.** Subjects were asked to abstain from strenuous exercise, alcohol, and caffeine for 24 h before the experiment. They also refrained from any food or drink for 2 h before the experiment. Five out of nine subjects came to the laboratory in the morning (8:30 AM), while the remaining four subjects came to the laboratory in the afternoon (2:00 PM). After arriving at the laboratory, the subjects initially rested for 30 min sitting in a chair (ambient temperature = 25°C and relative humidity = 50%). During this time, a thermocouple was inserted via the nasal passage to a distance equivalent to one-fourth of the subject’s height to measure esophageal temperature (T\textsubscript{es}). Thereafter, the subject voided urine, and body weight was recorded. The subject then put on a water-perfused suit that covered the upper and lower body with the exception of the head, hands, left arm, feet, and calves. Water circulating through the suit was maintained at a temperature of around 35°C with aid of a heater, and a plastic garment was put over the suit to suppress evaporation. Once the subjects had put on the water-perfused suit, experimenters attached two probes for measuring cutaneous red blood cell flux (i.e., a skin blood flow index) at the forehead and forearm (left side), thermocouples for measuring skin temperature (Tsk) at various skin sites, and cuffs for measuring mean arterial blood pressure (MAP) at middle finger and upper arm (left side). Finally, a mouthpiece and nose clip were attached.

Once all measurements were started, we initially recorded more than 5 min of normothermic resting data, while the subjects breathed spontaneously. The subjects then performed two hyperventilation trials under hypoxic or eucapnic conditions in random order (described in detail below). After completing the first of the two hyperventilation trials, the subjects rested for more than 10 min until their respiratory and circulatory variables had returned to prehyperventilation levels before commencing the second hyperventilation trial. After finishing the two hyperventilation trials, the subject’s legs below the knees were immersed in a hot water bath (42°C), and the temperature of the water circulating through the water-perfused suit was increased to 47–49°C, which caused the subject’s body temperature to rise. In addition, the subjects wore cotton gloves to minimize both dry and evaporative heat loss via their hands. When T\textsubscript{es} reached a level +0.6°C above normothermia, the temperature of the water bath was reduced to 40–41°C, and the temperature of the water circulating through the water-perfused suit was reduced to 40–45°C, and the cotton gloves were taken from the subjects’ hands so that T\textsubscript{es} and mean skin temperature (mT\textsubscript{sk}, see below for calculation of mT\textsubscript{sk}) were maintained within ±0.1°C and ±0.2°C of each other. At +0.6°C T\textsubscript{es}, spontaneous breathing data were acquired for more than 5 min, after which the two voluntary hyperventilation trials were performed as under normothermic conditions. Thereafter, T\textsubscript{es} was further increased to +1.0°C above normothermia, and the hyperventilation trials were repeated. During the heating periods between the hyperventilation trials, the subjects were allowed to consume a commercial sports drink (6.7% carbohydrate, Na\textsuperscript{+} = 21 meq/l, Cl\textsuperscript{-} = 16.5 meq/l, K\textsuperscript{+} = 5 meq/l, osmolality = 323 mosmol/kg H\textsubscript{2}O) (Pocari sweat; Otsuka, Tokyo, Japan) to prevent hypohydration as much as possible. The temperature of the sports drink was set to ~38.0°C to minimize fluctuations in T\textsubscript{es}. Subjects were not allowed to drink while hyperventilating.

**Voluntary hyperventilation.** The level of hyperventilation was set as minute ventilation (V\textsubscript{T}) = 40 l/min, tidal volume (V\textsubscript{T}) = 1.000 ml, and respiratory frequency (f\textsubscript{T}) = 40 breaths/min. The desired f\textsubscript{T} was achieved using auditory cues from a metronome, while the desired V\textsubscript{T} was achieved by providing visual feedback from a computer displaying the V\textsubscript{T} value. During eucapnic hyperventilation, 100% CO\textsubscript{2} was added to the inspired air; the CO\textsubscript{2} flow rate was manually regulated by an experimenter monitoring end-tidal CO\textsubscript{2} pressure (PETCO\textsubscript{2}), which was displayed on the screen of a personal computer. This enabled us to adjust the PETCO\textsubscript{2} to the spontaneous breathing level during the hyperventilation. During hypoxic hyperventilation, the subjects breathed room air without adding CO\textsubscript{2}, and PETCO\textsubscript{2} was allowed to decline.

**Measurements.** T\textsubscript{es} and Tsk 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). T\textsubscript{sk} was measured at eight sites (forearm, forehead, chest, upper back, lower back, abdomen, thigh, and calf), and mT\textsubscript{sk} was estimated as a weighted mean value using the following regional percentages reported previously (50): 22% chest, 21% upper back, 19% lower back, 14% abdomen, 14% thigh, and 11% calf. Temperature data for one subject were lost due to a computer accident after the experiment; consequently, the number of subjects for the temperature data analysis was eight. Heart rate (HR) 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, The Netherlands). The left arm was placed on a table to keep the middle finger at heart level. MAP was calculated as the diastolic arterial blood pressure plus one-third of the pulse pressure. Stroke volume (SV) was calculated from the arterial blood pressure waveform using the model-flow method, incorporating age, sex, height, and weight (53). Cardiac output (CO) was calculated as SV multiplied by HR. Total peripheral resistance (TPR) was calculated as MAP divided by CO.

Cutaneous red blood cell flux, which is not affected by blood flow to underlying skeletal muscle (40), was monitored continuously using a laser-Doppler flowmeter (ALF21, Advance, Tokyo, Japan) and was considered to be an index of cutaneous blood flow (CBF). CBF was measured in the forearm (CBF\textsubscript{forearm}), and forehead (CBF\textsubscript{forehead}) and was normalized to a baseline value (CBF during normothermic spontaneous breathing at rest). Because the laser-Doppler was not calibrated to measure absolute flow, the values obtained indicate the relative changes from those seen at baseline. With respect to the reproducibility of CBF\textsubscript{forearm}, 4 of 9 subjects participated in both the experimental and pilot sessions, where they performed hypoxic hyperventilation at normothermia, +0.6°C T\textsubscript{es} and +1.0°C T\textsubscript{es}. CBF\textsubscript{forearm} at +0.6°C T\textsubscript{es} was greatly reduced at the end of hypoxic hyperventilation relative to the level seen during spontaneous breathing in both the experimental (644 ± 290 to 519 ± 309% baseline) and pilot (653 ± 323 to 494 ± 388% baseline) sessions. On the other hand, the relative reduction in CBF\textsubscript{forearm} induced by hypoxic hyperventilation at +1.0°C T\textsubscript{es} was comparatively small in both the experimental (684 ± 257 to 671 ± 246% baseline) and pilot (716 ± 273 to 644 ± 353% baseline) sessions. Importantly, there were no significant between-session differences in any of the values shown above (P = 0.64 to 0.96), which suggests our data are reproducible.

Cutaneous vascular conductance (CVC, CBF/MAP) at the forearm (CVC\textsubscript{forearm}) and forehead (CVC\textsubscript{forehead}) were also evaluated and normalized in the same manner as CBF.

The subjects breathed through a mouthpiece connected to a two-way valve with their nose occluded. A mass-flow sensor (hot-wire type) and a gas-sampling tube (the sampling speed was below 0.2 l/min) were attached between the mouthpiece and the two-way valve, and the expired volume and gases were analyzed using an electric gas flowmeter (RM300i, Minato Medical Science, Osaka, Japan). The dead space and resistance of the respiratory apparatus and the loss of...
volume due to gas sampling were small enough that we considered their effects on the data to be negligible. The flowmeter was calibrated with the aid of an apportioned calibration instrument able to blow in a fixed volume (2 l) and reference gases of known concentration. Ve, Vt, fR, PETCO2, end-tidal O2 pressure (PETO2), and oxygen uptake (V02) were measured breath to breath.

Data analysis. Temperature and cardiovascular and respiratory variables during spontaneous breathing (Table 1) were analyzed using one-way repeated-measures ANOVA. We set one factor to be core temperature (Tes, normothermia, +0.6°C and +1.0°C). We also employed two-way ANOVA in which the factors were PETCO2 level (hypocapnia and eucapnia) and hyperventilation time (pre, 1 to 7 min of hyperventilation) (Figs. 2A and 3A). After determining the main effects, pairwise differences were identified using paired two-tailed t-tests with Bonferroni’s correction so that the type I error was maintained at 5%. Paired two-tailed t-tests were used to compare all variables except CBFforehead and CBFforehead during the last 1 min of hyperventilation with those seen during spontaneous breathing at each body temperature within a trial. In addition, changes in the all measured values from spontaneous breathing to the last 1 min of hyperventilation at each body temperature were calculated and then compared between the hypocapnic and eucapnic hyperventilation trials using paired two-tailed t-tests (Figs. 2B and 3B). Pearson’s product moment correlation coefficients were used to relate pairs of variables. All values are reported as means ± SE. P values <0.05 were considered significant.

RESULTS

Heating-induced changes in body temperature and respiratory and circulatory variables. Table 1 shows heating-induced changes in body temperature and respiratory and circulatory variables during spontaneous breathing. Significant (P < 0.001) main effects of temperature were found on Tes, mTSk, Tskforearm, VO2, CBFforearm, CBFforehead, HR, SV, CO, and TPR. Significant main effects were also found on fR (P = 0.045), Ve (P = 0.013), PETCO2 (P = 0.014), and MAP (P = 0.002). The measured variables related to body temperature (Tes, mTSk, Tskforearm, and Tskforehead), respiration (Ve, fR, and V02), and circulation (HR, SV, and CO) were all significantly increased by heating, whereas heating led to significant reductions in MAP and TPR.

Respiratory changes during hyperventilation. Figure 1 shows the time courses of the changes in Vt, fR, Ve, PETCO2, and PETO2 during hyperventilation at +0.6°C Tes. Similar changes were also observed at normothermia and +1.0°C Tes. The magnitudes of the increases in Vt (range : 356 to 474 ml), fR (range : 21.9 to 26.2 breaths/min), Ve (range : 29.7 to 33.0 l/min), and PETO2 (range : 39.9 to 44.8 torr) were all similar between the eucapnic and hypocapnic hyperventilation trials at each Tes level. By contrast, the magnitude of the decrease in PETCO2 caused by hyperventilation was significantly greater during hypocapnia than eucapnia (range : −23.1 to −24.8 vs. −0.1 to 1.7 torr).

Cutaneous blood flow changes during hyperventilation. Figure 2 shows the changes in CBFforearm over the course of a 7-min hyperventilation (Fig. 2A) and the delta CBFforearm from spontaneous breathing to the last 1 min of hyperventilation (Fig. 2B). ANOVA performed with the data in Fig. 2A revealed that there was a significant interaction in CBFforearm at +0.6°C Tes (P < 0.001), while a significant main effect of hyperventilation time on CBFforearm was found at normothermia (P = 0.011) and at +1.0°C Tes (P = 0.039). At +0.6°C Tes, CBFforearm values from min 5 to 7 during hypocapnic hyperventilation were significantly lower than during eucapnic hyperventilation (Fig. 2A). Even when we employed CVCforearm instead of CBFforearm, we observed significantly lower CBFforearm values from minutes 6 to 7 during hypocapnic hyperventilation than during eucapnic hyperventilation. The delta CBFforearm at +0.6°C Tes from spontaneous breathing to the last 1 min of hyperventilation was also significantly greater in the hypocapnia trial than the eucapnia trial (Fig. 2B). On the other hand, at normothermia and +1.0°C Tes, the CBFforearm values from minute 1 to 7 during hyperventilation did not significantly differ between the hypocapnia and eucapnia trials (Fig. 2A).

The effects on CBFforehead are shown in Fig. 3, which follows the same format as Fig. 2. ANOVA performed with the data in Fig. 3A showed a significant main effect of PETCO2 level on CBFforehead at normothermia (P = 0.020), while a significant main effect of hyperventilation time on CBFforehead was found at +0.6°C Tes (P < 0.001) and at +1.0°C Tes (P < 0.001). During normothermia, CBFforehead values from minutes 3 and 5 during hypocapnic hyperventilation were higher than during eucapnic hyperventilation (Fig. 3A). By contrast, CBFforehead from minute 1 to 7 during hyperventilation were similar in the hypocapnia and eucapnia trials at +0.6°C and +1.0°C Tes (Fig. 3A). At +1.0°C Tes, eucapnic hyperventilation significantly reduced CBFforehead from baseline at minute 4 of hyperventilation (Fig. 3A).

We compared delta CBF elicited by hypocapnic hyperventilation at +0.6°C Tes in the forehead (Fig. 2B) and forehead (Fig. 3B) and found that the reduction was significantly greater in the forehead than forehead (−182 ± 33 vs. −38 ± 5% baseline, P < 0.05). On the other hand, we observed large

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**Table 1. Temperature and respiratory and circulatory variables during spontaneous breathing under normothermic and hyperthermic conditions**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normothermia</th>
<th>+0.6°C Tes</th>
<th>+1.0°C Tes</th>
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</thead>
<tbody>
<tr>
<td><strong>Temperature</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tes, °C</td>
<td>36.3 ± 0.3</td>
<td>36.9 ± 0.3†</td>
<td>37.3 ± 0.2†</td>
</tr>
<tr>
<td>mTSk, °C</td>
<td>34.2 ± 0.3</td>
<td>36.5 ± 0.2†</td>
<td>36.9 ± 0.4†</td>
</tr>
<tr>
<td>Tskforearm, °C</td>
<td>32.4 ± 0.5</td>
<td>33.0 ± 0.9</td>
<td>34.4 ± 0.6†</td>
</tr>
<tr>
<td>Tskforehead, °C</td>
<td>33.0 ± 0.9</td>
<td>33.6 ± 0.5†</td>
<td>33.6 ± 1.2</td>
</tr>
<tr>
<td><strong>Respiratory</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vt, ml</td>
<td>601 ± 91</td>
<td>602 ± 92</td>
<td>633 ± 93</td>
</tr>
<tr>
<td>fR, breaths/min</td>
<td>14.1 ± 2.2</td>
<td>17.2 ± 2.8*</td>
<td>16.7 ± 3.6</td>
</tr>
<tr>
<td>Ve, l/min</td>
<td>8.3 ± 0.7</td>
<td>10.0 ± 1.4*</td>
<td>10.4 ± 2.1*</td>
</tr>
<tr>
<td>V02, ml</td>
<td>243 ± 29</td>
<td>280 ± 54</td>
<td>333 ± 39†</td>
</tr>
<tr>
<td>PETCO2, torr</td>
<td>44.1 ± 2.9</td>
<td>43.1 ± 1.9</td>
<td>41.5 ± 2.3</td>
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<tr>
<td>PETO2, torr</td>
<td>98 ± 3</td>
<td>101 ± 4</td>
<td>96 ± 12</td>
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<tr>
<td><strong>Circulatory</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CBFforearm, %baseline</td>
<td>100</td>
<td>536 ± 70*</td>
<td>635 ± 77*</td>
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<tr>
<td>CBFforehead, %baseline</td>
<td>100</td>
<td>201 ± 18*</td>
<td>306 ± 36†</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>57 ± 11</td>
<td>74 ± 10*</td>
<td>89 ± 12†</td>
</tr>
<tr>
<td>SV, ml</td>
<td>89 ± 12</td>
<td>102 ± 14*</td>
<td>98 ± 13*</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>5.0 ± 0.9</td>
<td>7.5 ± 1.4*</td>
<td>8.8 ± 1.5†</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>82.6 ± 7.5</td>
<td>75.5 ± 11</td>
<td>71.6 ± 9.2</td>
</tr>
<tr>
<td>TPR, mmHg·l−1·min−1</td>
<td>17.1 ± 3.7</td>
<td>10.3 ± 2.6*</td>
<td>8.3 ± 1.1†</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. Tes, esophageal temperature; mTSk, mean skin temperature; Tskforearm, forearm skin temperature; Tskforehead, forehead skin temperature; Vt, tidal volume; fR, respiratory frequency; Ve, minute ventilation; V02, oxygen uptake; PETCO2, end-tidal P02; PETO2, end-tidal P02; CBFforearm, forearm cutaneous blood flow; CBFforehead, forehead cutaneous blood flow; HR, heart rate; SV, stroke volume; CO, cardiac output; MAP, mean arterial blood pressure; TPR, total peripheral resistance; bpm, beats per minute. *P < 0.05 vs. normothermia; †P < 0.05 vs. +0.6°C Tes.
individual variations (from $-0.9$ to $-291.2\%$ baseline) in the reduction in $\text{CBF}_{\text{forearm}}$ from spontaneous breathing to the last 1 min of hypocapnic hyperventilation at $+0.6^\circ\text{C} \ T_{\text{es}}$. When we performed a correlational analysis of the relationship between the reduction in $\text{CBF}_{\text{forearm}}$ and $\dot{V}_\text{O}_2 \text{peak}$ (range: $29.7$ to $58.7 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), we found there to be a tendency toward a negative correlation ($r = -0.45, P = 0.22$).

Other circulatory changes during hyperventilation. Hyperventilation significantly increased HR, regardless of $\text{PETCO}_2$ and $T_{\text{es}}$ levels, except with eucapnic hyperventilation at $+0.6^\circ\text{C} \ T_{\text{es}}$. The magnitude of the increase in HR was significantly larger under hypocapnic than eucapnic conditions during normothermia ($12.3 \pm 1.8 \text{ vs. } 6.3 \pm 1.3 \text{ beats per minute (bpm)})$, but the magnitudes were similar under the two conditions at $+0.6^\circ\text{C} \ T_{\text{es}}$ and $+1.0^\circ\text{C} \ T_{\text{es}}$ (range: 4.5 to 7.4 bpm). SV was significantly reduced by hyperventilation at $+0.6^\circ\text{C}$ and $+1.0^\circ\text{C} \ T_{\text{es}}$, but not during normothermia, and the magnitudes of the reductions were similar between eucapnia and hypocapnia (range: $-14.5$ to $-2.1 \text{ ml}$). Both eucapnic and hypocapnic hyperventilation significantly increased $\dot{V}_\text{O}_2$ during normothermia, but the magnitude of the increase was significantly greater during hypocapnia than eucapnia ($0.9 \pm 0.1 \text{ vs. } 0.4 \pm 0.1 \text{ l/min}$). MAP was significantly reduced by hypocapnic hyperventilation during normothermia and at $+1.0^\circ\text{C} \ T_{\text{es}}$, and the hyperventilation-induced reduction in MAP was greater under hypocapnic than eucapnic conditions during normothermia ($-7.2 \pm 1.8 \text{ vs. } -0.4 \pm 0.9 \text{ mmHg}$) and at $+1.0^\circ\text{C} \ T_{\text{es}}$ ($-4.0 \pm 1.1 \text{ vs. } -0.3 \pm 0.9 \text{ mmHg}$). Hyperventilation significantly reduced $\text{TPR}$ during normothermia, irrespective of the $\text{PETCO}_2$ level, and the magnitude of the reduction was greater under hypocapnia than eucapnia ($-3.8 \pm 0.5 \text{ vs. } -1.4 \pm 0.6 \text{ mmHg} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$).

DISCUSSION

We examined the impact of hypocapnia achieved through voluntary hyperventilation on nonglabrous forearm and forehead skin circulation in resting heated humans. Our results indicate that hypocapnia significantly reduces cutaneous blood flow in the forearm but not the forehead at $+0.6^\circ\text{C}$ core temperature. This suggests that in resting humans, hypocapnia induced by voluntary hyperventilation attenuates the increased nonglabrous skin blood flow response to moderate heat stress in the forearm but not the forehead.
Effect of hypocapnia on nonglabrous skin circulation. Simmons et al. (46) recently showed that CVC_{forearm} during normothermia and whole body cooling did not differ under conditions of hypocapnic and eucapnic hypoxia, which suggests hypocapnia does not affect the cutaneous vascular response in those situations. A similar result was obtained in the forearm under normothermic, normoxic conditions in the present study. By contrast, CBF_{forehead} was increased by normothermic, hyperventilation (Fig. 2).
Hypocapnic hyperventilation, compared with eucapnic hyperventilation. Although we do not know why hypocapnia increased \( \text{CBF}_{\text{forehead}} \) during normothermia, it is unlikely that this increase was mainly due to withdrawal of sympathetic noradrenergic vasoconstrictor activity, since our experimental normothermic conditions were nearly the same as those in an earlier study in which skin vasoconstrictor nerve activity was minimized (9). In both the present study and the earlier one, local skin temperature was 33–34°C, the lower legs were immersed in 34–35°C water, and the unimmersed portions of the body were covered by suits. Thus, the increase in \( \text{CBF}_{\text{forehead}} \) was likely mainly due to local effects. Given that hypocapnic hyperventilation causes histamine release (31) and that histamine receptor activation contributes to an increase in skin blood flow (57), the increase in \( \text{CBF}_{\text{forehead}} \) at normothermia may have been due to hypocapnia-mediated histamine release.

Hypocapnic hyperventilation reduced \( \text{CBF}_{\text{forearm}} \), more than eucapnic hyperventilation at +0.6°C \( T_{es} \), which supports our original hypothesis that hypocapnia attenuates the increased nonglabrous skin blood flow response to heat stress at rest. Loading or unloading arterial and/or cardiopulmonary baroreceptors can modulate cutaneous vascular tone during hyperthermia (7, 32). Thus, an effect of hypocapnia on these baroreceptors could explain the hypocapnia-induced reduction in \( \text{CBF}_{\text{forearm}} \), seen in the present study. However, our results also indicate that MAP, SV, and CO did not differ between the eucapnic and hypocapnic hyperventilation trials at +0.6°C \( T_{es} \), suggesting the baroreceptors contribute little to the hypocapnia-induced reduction in \( \text{CBF}_{\text{forearm}} \). Other possible contributing factors are 1) attenuation of the vasodilatory response to ACh, NO, and/or substance P, 2) an inhibitory effect on the thermoregulatory center, 3) increased noradrenergic vasoconstrictor nerve activity, and 4) a local CO2 effect on cutaneous vascular smooth muscle.

The nonglabrous cutaneous vasodilation seen with increases in core temperature is mainly the product of sympathetic cholinergic vasodilator nerve activity (30). ACh released from cholinergic nerves enhances the active cutaneous vasodilatory response to heat stress via stimulation of NO synthase (45). That hypocapnia attenuates ACh- and NO-induced pulmonary arterial dilation (2) suggests hypocapnia may reduce nonglabrous cutaneous vasodilation by attenuating the effects of ACh and NO on cutaneous vessels. On the other hand, it has been suggested that cutaneous active vasodilation is related to signaling via the neurokinin-1 receptor, which has high affinity for substance P (56). Given that hypocapnia attenuates substance P-induced relaxation of bronchial smooth muscle (10), the observed hypocapnia-induced reduction in \( \text{CBF}_{\text{forearm}} \) at +0.6°C \( T_{es} \) may reflect the attenuation of the vasodilatory effects of substance P. Hypocapnia could also affect the thermoregulatory center directly and/or through peripheral and/or central chemoreceptors, thereby attenuating active vasodilation and reducing \( \text{CBF}_{\text{forearm}} \). Indeed, the firing rates of warm-sensitive neurons in the preoptic-anterior hypothalamus, which are thought to be responsible for heat dissipation responses during heat stress, were increased by hypocapnia in an earlier animal study (49). Alternatively, it might be that hypocapnia attenuates the activity of warm-sensitive neurons, suppressing the active vasodilator system, resulting in forearm skin vasoconstriction during hyperthermia.

Simmons et al. (47) reported that hypercapnia (+5 torr \( P_{\text{ETCO}_2} \)) increased \( \text{CBF}_{\text{forearm}} \) under normothermic conditions and that this effect was abolished by treatment with bretylium tosylate, which blocks skin noradrenergic vasoconstrictor nerve transmission presynaptically. This suggests hypercapnia affects peripheral and/or central chemoreceptors, leading to nonglabrous skin vasodilation via withdrawal of vasoconstrictor tone; in which case, hypocapnia may increase skin noradrenergic vasoconstrictor tone, leading to the reduction in \( \text{CBF}_{\text{forearm}} \), seen at +0.6°C \( T_{es} \). In addition, subcutaneous injection of 12.5–100% CO2 gas induces increases in skin temperature (8), suggesting that skin vessels dilate in response to elevation of the local CO2 concentration. Conversely, when hypocapnia is induced, vasoconstriction that is independent of sympathetic nerve activity may occur in the skin in response to local CO2 depletion, and this too could have contributed to the reduction in \( \text{CBF}_{\text{forearm}} \) at +0.6°C \( T_{es} \). This local effect, if it occurs, may reflect an effect of increase in extracellular fluid pH on vascular smooth muscle, as is seen in the cerebral circulation (23).

Temperature-dependent hypocapnic effect on nonglabrous skin circulation. Although a hypocapnic effect on forearm skin was evident at +0.6°C \( T_{es} \), no effect of hypocapnia on forearm skin was observed at +1.0°C \( T_{es} \). This may reflect the time course of ACh’s activity. Although ACh appears to contribute to cutaneous active vasodilation early during heating, it does not contribute later during heating (43, 45). Consequently, if hypocapnia-mediated attenuation of ACh-induced vasodilation (2) occurred, as we discussed above, this may have been effective at +0.6°C \( T_{es} \) but not at +1.0°C \( T_{es} \). Another possible explanation reflects the nature of sympathetic vasoconstrictor nerve activity. Skin vasoconstriction induced by the onset of exercise, which is mainly related to increased noradrenergic vasoconstrictor nerve activity (27), was seen during moderate hyperthermia, but little or no vasoconstriction was observed at higher levels of hyperthermia (26). This suggests that sufficient hyperthermia attenuates or even abolishes noradrenergic sympathetic constriction of skin vessels. As a result, hypocapnia-mediated increases in sympathetic vasoconstrictor nerve activity (if any) may have been effective at +0.6°C \( T_{es} \) but not at +1.0°C \( T_{es} \). Finally, the sweat response may contribute to the temperature-dependent difference. Although we did not evaluate the sweat response, it is likely that there was a greater activation of sweat glands during heat stress at +1.0°C \( T_{es} \) than at +0.6°C \( T_{es} \). The greater sweat gland activation could, in turn, lead to greater release of bradykinin, which would increase skin blood flow during heat stress (13), although the absence of a bradykinin effect on skin blood flow has also been reported (29). We speculate that bradykinin-mediated vasodilation was greater at +1.0°C \( T_{es} \), counteracting the hypocapnia-induced reduction in forearm skin blood flow.

Regional differences in the hypocapnic effect on nonglabrous skin circulation. Hypocapnia reduced \( \text{CBF}_{\text{forearm}} \) but not \( \text{CBF}_{\text{forehead}} \) at +0.6°C \( T_{es} \). In addition, the delta \( \text{CBF} \) induced in the forearm by hypocapnic hyperventilation significantly differed from that induced in the forehead (−182 ± 33 vs. −38 ± 5% baseline, \( P < 0.05 \); values are depicted in Figs. 2B and 3B). This suggests there is regional variation in the effects of hypocapnia on skin circulation under hyperthermic conditions, which seems plausible, given that hypocapnia may increase skin noradrenergic vasoconstrictor tone and that fore-
head skin, but not forearm skin, lacks noradrenergic vasoconstrictor nerve control (24, 25). In addition, hypocapnia could increase histamine release, which may increase forehead skin blood flow (see discussion above). Therefore, the hypocapnia-induced reduction in skin blood flow might have been counteracted by a histamine-mediated increase in blood flow in the forehead but not the forearm. Moreover, because the rate of sweating, and thus bradykinin release, tends to be higher in the forehead than in the forearm (6, 48), larger bradykinin-induced increases in forehead skin blood flow (13) may also counteract the hypocapnia-induced reduction in skin blood flow. Finally, since the level of blood flow during hyperthermia before hyperventilation in the forehead was much lower than in the forearm (200 to 300 vs. 550 to 650% baseline), the bioavailability of vasodilators such as ACh, NO, and substance P might have been lower in forehead skin vessels. Consequently, hypocapnia-induced attenuation of vasodilatory responses to ACh, NO, and/or substance P (2, 10) in the forehead might have not been clearly seen.

Individual differences in the hypocapnic effect on nonglabrous skin circulation. The observed reductions in CBF_forehead during hypocapnic hyperventilation at +0.6°C Tes ranged from −0.9 to −291.2% baseline, indicating that there is a great deal of individual variation in the hypocapnic effect. Our observation that VO2_peak, which ranged from 29.7 to 58.7 m·kg−1·min−1, tended to correlate with the reduction in CBF_forehead during hypocapnic hyperventilation at +0.6°C Tes (r = −0.45, P = 0.22) suggests higher aerobic fitness may relate to greater hypocapnia-induced reductions CBF_forehead. Given that aerobic training increases the bioavailability of endothelium-derived NO (18), if hypocapnia attenuated NO-induced arterial dilation (2) of human skin vessels, it seems plausible that aerobically fit subjects would have a greater hypocapnia-induced reduction in skin blood flow. Further study employing more subjects with a wider range of VO2_peak would provide a clearer picture of this relationship. On the other hand, the sensitivity of the central and/or peripheral chemoreceptors and their role in hypercapnic or hypoxic ventilatory response exhibit large individual variations (19, 36). Therefore, we speculate that individual differences in chemoreceptor sensitivity to hypocapnia likely contribute to the observed individual variation in hypocapnic hyperventilation-induced decreases in CBF_forehead.

Hypocapnic effect on arterial blood pressure. Our finding that the effect of hypocapnia on MAP was dependent on body temperature is also noteworthy. During normothermia, MAP was reduced by hypocapnic hyperventilation, compared with eucapnic hyperventilation, which suggests hypocapnia has a suppressive effect on MAP and is consistent with an earlier result (51). A similar effect of hypocapnia was seen at +1.0°C Tes, but not at +0.6°C Tes. Because hypocapnia caused nonglabrous skin vasoconstriction at +0.6°C Tes, this vasoconstriction could have offset the reduction in MAP elicited by hypocapnia at that temperature.

The effect of hyperventilation itself on nonglabrous skin circulation. We found that eucapnic hyperventilation reduced CBF_forehead at +1.0°C Tes, which suggests the hyperventilation itself induced nonglabrous skin vasoconstriction, independent of any hypocapnic effect. That voluntary hyperventilation also reduced SV at +1.0°C Tes suggests the hyperventilation during hyperthermia may change the respiratory pump effect, leading to reductions in CBF_forehead. This effect may be mediated through unloading of cardiopulmonary baroreceptors (7, 32).

Limitations. When breathing spontaneously, VE at +1.0°C Tes was higher than that during normothermia due to hyperthermia-induced hyperventilation (5, 14, 16, 54), which caused the magnitude of the increase in Ve from spontaneous breathing to voluntary hyperventilation to be smaller during hyperthermia than normothermia. However, this difference in the magnitude of increase in Ve is thought to be negligible, given that the increase in Ve at +1.0°C Tes was small (+2.1 l/min) and not sufficient to cause a significant reduction in PETCO2. On the other hand, voluntary hyperventilation was set at 40 l/min Ve, which was accomplished by having VT = 1.000 ml and fx = 40 breaths/min. If a different respiratory pattern and Ve had been used, the results might have been different from those obtained in this study. Furthermore, we did not control PETCO2; consequently, it increased by ~30 torr during hyperventilation. However, it was previously shown that even when breathing 85–100% oxygen, which increased PETCO2 by ~540 torr, or about 18 times more than in the present study, cutaneous vascular conductance was only reduced by about ~15% from the prehyperoxia breathing value (58). Therefore, we believe that the slight hyperoxia induced by hyperventilation had little effect on the nonglabrous skin vessels in the present study. Finally, we estimated CO using the model-flow method. Because this method underestimates CO during passive heating at rest (44), actual CO values could have been lower than those reported here, although this does not limit our interpretation of the effect of hypocapnia on nonglabrous skin vessels during hyperthermia.

Perspectives and Significance

Heat stress evokes cutaneous vasodilation and sweating, both of which are known to be thermoregulatory responses. If these responses are impaired, increases in body temperature are accelerated, which could result in heat-related dysfunction (e.g., syncope). Because it is well known that dehydration attenuates thermoregulatory responses (15), frequent drinking is recommended to prevent dehydration, and thus extreme hyperthermia, in a hot environment. Notably, the present study shows that reducing arterial CO2 pressure, as well as hyperventilation itself, also attenuates one of the thermoregulatory responses: heat stress-induced increases in cutaneous blood flow. Thus hyperthermia-induced hyperventilation accompanying hypocapnia (4, 11, 14–17, 20, 35, 41, 52, 54) may accelerate increases in body temperature. In terms of practical application, we speculate that control of ventilation or rebreathing air in a bag (prevention of hypocapnia) may be a simple means of mitigating increases in body temperature in a hot environment.

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