Effect of continuous negative-pressure breathing on skin blood flow during exercise in a hot environment

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Nagashima, Kei, Hiroshi Nose, Akira Takamata, and Takeshi Morimoto. Effect of continuous negative-pressure breathing on skin blood flow during exercise in a hot environment. J. Appl. Physiol. 84(6): 1845-1851, 1998.—To assess the impact of continuous negative-pressure breathing (CNPB) on the regulation of skin blood flow, we measured forearm blood flow (FBF) by venous-occlusion plethysmography and laser-Doppler flow (LDF) at the anterior chest during exercise in a hot environment (ambient temperature = 30°C, relative humidity = ~30%). Seven male subjects exercised in the upright position at an intensity of 60% peak oxygen consumption rate for 40 min with and without CNPB after 20 min of exercise. The esophageal temperature (Tes) in both conditions increased to 38.1°C by the end of exercise, without any significant differences between the two trials. Mean arterial pressure (MAP) increased by -15 mmHg by 8 min of exercise, without any significant difference between the two trials before CNPB. However, CNPB reduced MAP by -10 mmHg after 24 min of exercise (P < 0.05). The increase in FBF and LDF in the control condition leveled off after 18 min of exercise above a Tes of 37.7°C, whereas in the CNPB trial the increase continued, with a rise in Tes despite the decrease in MAP. These results suggest that CNPB enhances vasodilation of skin above a Tes of ~38°C by stretching intrathoracic baroreceptors such as cardiopulmonary baroreceptors.

active vasodilation; baroreceptors; forearm blood flow; laser-Doppler flow

ONE OF THE LIMITING FACTORS to exercise in a hot environment is the maintenance of cardiac filling pressure to sustain cardiac output at a level high enough for adequate blood flow to contracting muscles. However, retention of blood in the cutaneous vasculature due to elevated core temperature reduces the venous return to the heart, which becomes a limitation to continue exercise (20). One of the feedback mechanisms for maintaining cardiac filling pressure has been reported to be the attenuation of the increase in cutaneous vasodilation when core temperature exceeds ~38°C during upright exercise in a hot environment (4, 8, 17, 22). Because this attenuation is partly prevented by blood expansion (8), the supine position (12), and head-out water immersion (20), baroreflexes are likely to be involved in the regulation of skin blood flow (SkBF).

However, the relative contribution of sinoaortic and/or cardiopulmonary baroreceptors to the regulation of SkBF has not as yet been elucidated. The fall in cardiac filling pressure unloads sinoaortic baroreceptors as well as cardiopulmonary baroreceptors by decreasing cardiac stroke volume. Hence, it is necessary to disting-
inspiratory and expiratory tubes. The pressure in the box was controlled at the desired pressure with a vacuum cleaner. The buffer box was ventilated well enough so as not to accumulate expiratory air at the intensity of ~70% peak \text{V}$\text{O}_2$. The pressure in the box was ~17 and ~13 cmH$_2$O in the inspiratory and expiratory phase, respectively, during exercise.

**Measurements.** Heart rate (HR) was recorded every 2 min from the trace of an electrocardiogram (Life Scope 6, Nihon Kohden, Tokyo, Japan). Systolic and diastolic arterial pressures were automatically measured every 2 min, from the left upper arm placed at heart level, by inflation of a cuff with a sonometric pickup of Korotkoff's sound (model STBP-780, Colin, Komaki, Japan). The method reduced person-to-person variation of measurement, and the values were verified by indicator of Korotkoff's sound or earphone each time. Mean arterial pressure (MAP) was calculated as (SAP + 2·DAP)/3, where SAP and DAP are systolic and diastolic arterial pressures, respectively.

Esophageal temperature (T$_{es}$) was monitored every 5 s from a thermocouple-equipped catheter swallowed to the level of the left atrium (5). Subjects were instructed to avoid swallowing saliva during measurement. Skin temperature was also measured every 5 s with thermocouples attached to seven skin sites. The T$_{es}$ and skin temperature data were averaged for every minute. Mean skin temperature (T$_{sk}$) was calculated according to the equation reported by Nadel et al. (18).

FBF was measured every 2 min except 0 and 20 min of exercise by venous occlusion plethysmography by using a Whitney mercury-in-Silastic strain gauge placed around the right forearm. The venous occlusion cuff was inflated to 40 mmHg, whereas the hand was eliminated from the circulation with a wrist cuff inflated to 270 mmHg. Forearm vascular conductance (FVC) was calculated as FBF/MAP (in ml·min$^{-1}$·100 ml$^{-1}$·100 mmHg$^{-1}$, expressed here as units). LDF (ALF21, Advance, Tokyo, Japan) was monitored at the left anterior chest (at 5 cm below and on the midline of clavicle level) and averaged for every minute. Cutaneous vascular conductance (CVC) was calculated as LDF/MAP (in units).

**Blood analysis.** Two milliliters of blood in the catheter were discarded, and another 5-ml blood sample was taken and transferred to a sodium-heparin-treated tube. Hematocrit (Hct; microcentrifuge), Hb (cyanomethohemoglobin method; Colin, Komaki, Japan). The method reduced person-to-person variation of measurement, and the values were verified by indicator of Korotkoff's sound or earphone each time. Mean arterial pressure (MAP) was calculated as (SAP + 2·DAP)/3, where SAP and DAP are systolic and diastolic arterial pressures, respectively.

Electrolyte concentrations in plasma were expressed in millimoles per liter. Concentrations of sodium ([Na$^+$]) and potassium ([K$^+$]) were determined immediately after the experiment. The remaining blood was centrifuged at 4°C, and plasma was used to determine Na$^+$ ([Na$^+$]), K$^+$ concentrations ([K$^+$]), by flame photometry (Flame photometer 480, Corning, Medfield, MA), lactate concentration ([La$^-$]), with an enzyme electrode (model 27, Yellow Springs Instruments, Yellow Springs, OH), and osmolality (Osmol$_p$) by freezing-point depression (one-ten osmometer, Fiske, MA). Another 10 ml of blood were collected in a chilled tube (sodium EDTA 1.5 mg/ml) for determination of plasma atrial natriuretic peptide (ANP), epinephrine (Epi), and norepinephrine (NE) levels. The samples were stored at ~80°C for ~2 mo until the assays that were performed within 2 mo after experiments. ANP was measured with a radioimmunoassay kit (nonextraction method, Sionogi, Tokyo, Japan). The intra-assay coefficients of variation for ANP measurement were 7.70% at low range (33.9 pg/ml), 4.78% at middle range (113 pg/ml), and 4.20% at high range (410 pg/ml). Epi and NE were measured by high-performance liquid chromatography (model HLC-725CA, Tosoh, Tokyo, Japan). The respective intra-assay coefficients of variation for Epi and NE measurements were 1.33 and 2.44% at 275 and 280 pg/ml, respectively. The lowest detectable levels in the assays were 10 pg/ml for ANP and 5 pg/ml for Epi and NE. Percent change in plasma volume (%PV) was calculated from changes in Hct and Hb after corrections for trapped plasma (0.96) and cell F-ratios (0.91) (10). Electrolyte concentrations in plasma were expressed in millimoles per liter after the correction for plasma solid concentrations. Plasma solids were determined with the use of a regression equation of [Pro$_i$] (refractometry) and plasma solid concentrations (dry-weight method) (23).

**Statistics.** Two-way analysis of variance for repeated measures was used to determine significant changes in the variables between the two trials. The T-method was used for subsequent post hoc analyses to identify significant differences in the various pairwise comparisons (27). All values are presented as means ± SE, and the null hypothesis was rejected at P < 0.05. Regression analysis was performed with the standard least-squares method.

**RESULTS**

Figure 1 shows HR, MAP, and pulse pressure (PP) in the C and N trials. HR in both trials rose to 119 ± 5 and 123 ± 6 beats/min by 6 min of exercise and then continued to rise gradually by the end of exercise.
and 138 ± 5 beats/min, respectively) without significant differences between the two trials. MAP in both trials reached the maximum level by 8 min of exercise (99 ± 6 and 101 ± 3 mmHg in the C and N trials, respectively). After the application of CNPB, MAP in the N trial was lower (P < 0.05) than that in the C trial, except at 20, 22, 26, and 34 min of exercise. MAP in the N trial decreased significantly (P < 0.05) from the maximum value during CNPB (89 ± 3 mmHg at the minimum). PP rose in parallel with MAP and remained constant after 10 min of exercise, without any significant differences between the two trials.

$T_{es}$ rose to 37.6 ± 0.1°C in both trials within the first 10 min of exercise and reached 38.1 ± 0.1°C at the end of the experiment (Fig. 2). $T_{sk}$ rose gradually from 34.8 ± 0.2°C at rest to 35.4 ± 0.2 and 35.2 ± 0.2°C by the end of exercise in the C and N trials, respectively. There were no significant differences in $T_{es}$ and $T_{sk}$ between the two trials. Skin temperature at the anterior chest was significantly higher (P < 0.05) than that at the forearm (35.2 ± 0.2 and 34.9 ± 0.3°C at rest and 35.8 ± 0.3 and 35.6 ± 0.2°C at the end of exercise in the C and N trials, respectively). Forearm skin temperature was 34.2 ± 0.2 and 34.0 ± 0.3°C at rest and 34.5 ± 0.3 and 34.1 ± 0.4°C at the end of exercise in the C and N trials, respectively.

FBF and FVC in both trials increased with the rise in $T_{es}$ (Fig. 3). FBF reached 12.8 ± 1.3 and 14.0 ± 1.2 ml·min⁻¹·100 ml⁻¹ at 18 min of exercise in the C and N trials, respectively. FVC showed a similar pattern with FBF and was 14.1 ± 1.4 units in the C trial and 15.0 ± 1.5 units in the N trial at 18 min of exercise. Then, FBF and FVC in the C trial remained at that level until the end of exercise. On the other hand, FBF and FVC in the N trial increased significantly (P < 0.05) from the 18-min values, increasing by another 3.3 ml·min⁻¹·100 ml⁻¹ and 3.9 units by the end of exercise, respectively. Significant (P < 0.05) differences between the two trials existed during 24–38 min of exercise in FBF and 22–38 min of exercise in FVC.

Figure 4 shows the changes in LDF and CVC at the anterior chest skin, expressed as percent change from the resting values (Fig. 4, A and C). LDF and CVC increased within the first 15 min of exercise in both trials and then leveled off after 17 min of exercise (446 ± 76 and 453 ± 82% in LDF and 418 ± 79 and 413 ± 78% in CVC in the C and N trials, respectively). However, the plateau level varied among experiments even in the same subject. Thus, to evaluate the effect of CNPB, percent change of LDF and CVC from the averaged 17- to 19-min intervals of exercise is shown in Fig. 4, B and D. The percent change in LDF for the N trial increased significantly (P < 0.05) after 23 min of exercise from the 17- to 19-min level and was significantly (P < 0.05) higher than that of the C trial. The
levels at the end of exercise were 92 ± 6 and 108 ± 5% in the C and N trials, respectively. Similarly, percent change in CVC for the N trial increased significantly (P < 0.05) from the 17- to 19-min levels after 22 min of exercise, whereas in the C trial it decreased significantly (P < 0.05) at 24, 32, 36, and 38 min of exercise. The percent change in CVC was 88 ± 6% in the C trial and 112 ± 7% in the N trial at the end of exercise. Significant (P < 0.05) differences were observed between the C and N trials after 20 min of exercise. Figure 5A shows the relationship between T_{es} and FVC. T_{es} thresholds for the initial rise in FVC were both 37.6°C, and the T_{es} for the onset of attenuation was 37.7°C in the two trials. The thresholds were determined by visual inspection. However, FVC in the N trial increased again after application of CNPB and then leveled off above 38.0°C. The slopes of the regression equations of T_{es} vs. FVC were 5.8 and 4.8 units/°C (r = 0.83 and 0.81, P < 0.05) during the initial rise in FVC and 38.2 and 47.0 units/°C (r = 0.99 and 0.99, P < 0.05) during the second phase in the C and N trials, respectively. The slope in the C trial was attenuated to 6.1 units/°C (r = 0.78, P < 0.05) above 37.7°C, whereas in the N trial it was significantly (P < 0.05) increased to 22.9 units/°C (r = 0.94, P < 0.05) above 37.8°C.

The relationship between T_{es} and the percent change in CVC from rest (Fig. 5B) was nearly identical to that between T_{es} and FVC. Percent changes in CVC for the C trial tended to decrease (P < 0.05) above a T_{es} of 37.7°C, whereas that from the 17- to 19-min level (Fig. 5C) increased (P < 0.05) during CNPB and then leveled off above 37.9°C (Fig. 5C). The slopes of the regression equations in Fig. 5C were −29.9%/°C in the C trial (r = −0.83, P < 0.05), and 105.9%/°C during CNPB in the N trial before the percent changes in CVC reached a plateau (r = 0.94, P < 0.05). Hct and Hb concentration and [Pro]p during exercise increased significantly (P < 0.05) from those at rest in both trials (Table 1). Hb concentration at 40 min of exercise was significantly (P < 0.05) higher than that at 20 min in the two trials. However, no significant differences were observed between the values at 20 and 40 min in Hct concentration and [Pro]p, for either trial. %PV decreased (P < 0.05) rapidly within the first 20 min of exercise and then decreased slowly until the end of exercise in both trials. [Na^+]p, [K^+]p, Osmolp, and [La^-]p increased significantly (P < 0.05) during exercise, but there were no significant differences in these values between the C and N trials.

The plasma levels of ANP at rest were too low to be detected in both trials. ANP in the C trial increased significantly (P < 0.05) and then leveled off after 20 min of exercise.
of exercise, whereas ANP in the N trial continued to increase after 20 min of exercise, becoming significantly \((P < 0.05)\) different from that in the C trial at 40 min of exercise. Plasma levels of Epi as well as NE in both trials increased throughout exercise without any significant differences between the C and N trials.

**DISCUSSION**

In the present study, we assessed the effects of CNPB on SkBF during prolonged exercise in a hot environment. The major finding was that the skin vascular conductance increased above a \(T_{es}^\circ\) of 37.7°C during CNPB despite the reduction in MAP.

CNPB mobilized a larger number of inspiratory muscles, e.g., intercostal muscles, to maintain ventilation volume against increased negativity of intrathoracic pressure. Although we did not measure \(V_{O_2}\) rate during the trials, the similar changes in HR as well as plasma concentrations of metabolites, e.g., electrolyte concentrations and \([La^{-}]_p\), indicate that the workload during exercise was similar in the C and N trials.

CNPB has been reported to increase the transmural pressure of the atria, thereby increasing the stretch of cardiopulmonary baroreceptors \((3, 21, 28)\). Although we did not measure the transmural pressure, the significant increase in ANP for the N trial at 40 min of exercise (Table 1) suggests a distension of the atria. The primary stimulus for the secretion of ANP is stretch of the atrium \((2)\), and the effective filling pressure is the major modulator of ANP in humans. Although HR and catecholamines also modulate the ANP secretion \((2, 24)\), there were no significant differences in these parameters (Fig. 1, Table 1) between the C and N trials in this study.

The increases in FVC and CVC with a rise in \(T_{es}^\circ\) were reduced above a \(T_{es}^\circ\) of 37.7°C in the C trial (Figs. 2 and 5A), whereas in the N trial these continued to increase without any significant difference in \(T_{es}^\circ\) and \(T_{sk}^\circ\) between the two trials (Fig. 5B). The involvement of cardiopulmonary baroreceptors has been suggested as the primary afferent pathway of baroreflexes in the regulation of SkBF. Johnson et al. \((13)\) reported that an increase in FVC due to a rise in \(T_{es}^\circ\) was attenuated in parallel with the fall in right atrial pressure without a decrease in aortic pressure by application of lower body negative pressure (LBNP) to \(-20\) mmHg in resting subjects. Recently, Mack et al. \((14)\) obtained similar results in exercising subjects. These results suggest the importance of cardiopulmonary baroreceptors in attenuating SkBF above a \(T_{es}^\circ\) of \(-38°C\) during exercise in a hot environment. However, the effects of sinoaortic baroreflexes have not been completely excluded, because cutaneous vasodilation might be suppressed by even small decreases in MAP and/or PP. Moreover, LBNP might stimulate unknown pressure receptors in the pelvic, abdominal, and lower extremity regions \((16)\). In the present study, CNPB caused a significant decrease in MAP without changes in PP (Fig. 1).

The decrease in intrathoracic pressure would also stretch aortic baroreceptors, acting against the influence of the reduced MAP on carotid baroreceptors. In the present study, the difference in MAP between the two trials at 20-40 min of exercise was 5 mmHg. If we assume that the negativity of intrathoracic pressure was the same level as the airway pressure of 11 mmHg, the transmural pressure of the aortic arch in the N trial would be 6 mmHg higher than that in the C trial by subtracting the 5-mmHg decrease in MAP from the 11-mmHg decrease in intrathoracic pressure. The competitive regulation between carotid and aortic baroreceptors is not clear. However, it has been reported that the operating range and the sensitivity of baroreflex in dogs are similar in carotid and aortic baroreceptors \((11)\), or the sensitivity is much less in aortic baroreceptors \((1)\). Furthermore, Guz et al. \((9)\) suggested that aortic baroreceptors had minimal blood pressure effects in human. Therefore, we suppose that the reduction of MAP was not caused by the stretch of aortic baroreceptors overcoming the effect of unloading of carotid baroreceptors but by cardiopulmonary baroreceptors.

Recently, Crandall et al. \((6)\) examined the relative importance of cardiopulmonary and carotid barorecep-

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**Table 1. Blood properties in C and N trials**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C Trial</th>
<th>N Trial</th>
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<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>20 min</td>
</tr>
<tr>
<td>Hct, %</td>
<td>(43.0 \pm 1.1)</td>
<td>(44.6 \pm 1.0)†</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>(14.5 \pm 0.4)</td>
<td>(15.2 \pm 0.3)†</td>
</tr>
<tr>
<td>PPC, g/dl</td>
<td>(6.9 \pm 0.1)</td>
<td>(7.3 \pm 0.1)†</td>
</tr>
<tr>
<td>%PV</td>
<td>0</td>
<td>(-6.8 \pm 0.71)†</td>
</tr>
<tr>
<td>([Na^+]_p), mmol/kgH₂O</td>
<td>(150.2 \pm 0.6)</td>
<td>(154.2 \pm 0.2)†</td>
</tr>
<tr>
<td>([K^+]_p), mmol/kgH₂O</td>
<td>(5.10 \pm 0.10)</td>
<td>(5.88 \pm 0.12)†</td>
</tr>
<tr>
<td>([La^{-}]_p), mmol/kgH₂O</td>
<td>(1.69 \pm 0.18)</td>
<td>(3.58 \pm 0.62)†</td>
</tr>
<tr>
<td>Osmolp, mosmol/kgH₂O</td>
<td>(287 \pm 1)</td>
<td>(295 \pm 1)†</td>
</tr>
<tr>
<td>([ANP]_p), pg/ml</td>
<td>(45 \pm 13)</td>
<td>(109 \pm 19)†</td>
</tr>
<tr>
<td>([Epi]_p), pg/ml</td>
<td>(160 \pm 16)</td>
<td>(443 \pm 45)†</td>
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Values are means ± SE; \(n = 7\) subjects. C trial, exercise at 60% peak \(O_2\) consumption (\(V_{O_2}\)) with ambient pressure breathing; N trial, exercise at 60% peak \(V_{O_2}\) with continuous negative pressure breathing after 20 min of exercise; Hct, hematocrit; PPC, protein concentration; %PV, percent change in plasma volume; \([Na^+]_p\), plasma \(Na^+\) concentration; \([K^+]_p\), plasma \(K^+\) concentration, \([La^{-}]_p\), plasma \(La^-\) concentration; Osmolp, plasma osmolality; \([ANP]_p\), plasma atrial natriuretic peptide concentration; \([Epi]_p\), plasma epinephrine concentration; \([NE]_p\), plasma norepinephrine concentration. \([ANP]_p\) in C and N trials at rest was below least detectable level (10 pg/ml).

*Significant differences between C and N trials, \(P < 0.05\). †Significantly different compared with value at 0 min, \(P < 0.05\).*
tors in regulating cutaneous blood flow in resting hyperthermic humans. They measured changes in CVC with LBNP or external pressure applied over the carotid sinus area. They reported that CVC remained unchanged with the lower levels (−5 and −10 mmHg) of LBNP, whereas CVC decreased with the higher level (−30 mmHg). At LBNP of −30 mmHg, MAP was reduced with an increase in HR, suggesting that carotid or aortic baroreceptors were also unloaded. However, CVC was not changed by applying external pulsatile pressure of 45 mmHg over the carotid sinus area. From these results, they concluded that neither a low level of selective cardiopulmonary baroreceptors unloading nor selective carotid baroreceptors unloading results in reduction in CVC. Moreover, they speculated that the reduction of CVC with LBNP of −30 mmHg was caused by unloading of aortic baroreceptors. Nose et al. (25) showed the relationship between FVC and right atrial pressure (RAP) during the same exercise protocol as this study. They reported that RAP decreased in parallel with the increase in FVC until pulmonary arterial blood temperature increased to 38°C, and then FVC reached a plateau when RAP decreased by 2 mmHg. Because CVC increased in a similar manner to FVC in the present study, the leveling off of CVC above a T<sub>es</sub> of 38°C would not have occurred before RAP was decreased to some extent. The data are consistent with the results by Crandall et al. (6) that CVC was unchanged at lower levels of LBNP. Extracardiac baroreceptors have greater reflex influence on HR than do carotid baroreceptors in humans (15). However, we did not find any significant decrease in HR during CVPB while CVC increased. It might be difficult to compare our study with the results reported by Crandall et al. because orthostatic responses in the cardiovascular system during exercise are much different from those at rest; arterial blood pressure and cardiac filling pressure increase with the increase in sympathetic nerve activity, and baroreflex control changes during exercise (26). These results suggest that the increases in FVC and CVC by CNPB in this study were mainly caused by the stretch of cardiopulmonary baroreceptors, although the effect of aortic baroreceptors could not be completely excluded.

Bjurstedt et al. (3) assessed cardiovascular responses by CVPB in exercising subjects and reported that cardiac output increased by 12% during −15 cmH<sub>2</sub>O of CVPB at an exercise intensity of 50% maximal V<sub>O2</sub>, whereas MAP remained unchanged. On the contrary, MAP significantly decreased by −10% during CVPB in the present study. If we assume that cardiac output remained unchanged or increased as it did in a study in normothermic humans (3), systemic vascular conductance must have increased 10% or more despite the reduction of MAP. We found that FVC and CVC during CVPB increased by −22% and −12% from the pre-CNPB levels, respectively, as shown in Figs. 3 and 4. The data show that the enhanced increase in vascular conductance of skin was partly involved in the reduction of MAP.

Another factor potentially responsible for the decrease in MAP during CNPB is the significant increase in ANP. Faber et al. (7) reported that α<sub>1</sub>-adrenergic vasoconstrictive effects on dilated arterioles in the cremaster muscle were suppressed by ANP, which suggests that the greater ANP in the N trial might be involved in dilating the peripheral vasculature, thus decreasing MAP.

There were some differences in FVC and CVC between the trials (Fig. 5, A and B). FVC in the C trial remained stable after T<sub>es</sub> exceeded 37.7°C; however, %CVC of the average value at 17–19 min of exercise in the C trial tended to decrease with the rise in T<sub>es</sub>. In addition, FVC increased by −22% after the application of CNPB, whereas the increase in %CVC was only −12%. The discrepancy may be explained by the regional variation of responses in cutaneous vasculature to increased body temperature and/or the effect of vasodilation of forearm muscle vasculature.

Despite the increase in SkBF during CNPB, there was no difference in T<sub>es</sub> between the C and N trials. Heat dissipation by increased SkBF would have been minimal because the temperature gradient between skin surface and environment was small in the present study.

In summary, CNPB enhanced vasodilation of skin and decreased arterial pressure during exercise in a hot environment probably by the stretch of cardiopulmonary baroreceptors, although the effects of aortic baroreceptors were not completely excluded. Furthermore, the plasma ANP increased by CNPB may be involved in the reduction in arterial pressure by decreasing total peripheral resistance.

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