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Effect of hypocapnia on the sensitivity of hyperthermic hyperventilation and the cerebrovascular response in resting heated humans

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Running Head: ‘‘Ventilatory and Cerebrovascular Responses to hyperthermia’’

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ABSTRACT

Elevating core temperature at rest causes increases in minute ventilation ($\dot{V}_E$), which leads to reductions in both arterial CO_2 partial pressure (hypocapnia) and cerebral blood flow. We tested the hypothesis that in resting heated humans this hypocapnia diminishes the ventilatory sensitivity to rising core temperature but does not explain a large portion of the decrease in cerebral blood flow. Fourteen healthy men were passively heated using hot-water immersion (41°C) combined with a water-perfused suit, which caused esophageal temperature (T_{es}) to reach 39°C. During heating in two separate trials, end-tidal CO_2 partial pressure decreased from the level before heating (39.4±2.0 mmHg) to the end of heating (30.5±6.3 mmHg) (P=0.005) in the Control trial. This decrease was prevented by breathing CO_2-enriched air throughout the heating such that end-tidal CO_2 partial pressure did not differ between the beginning (39.8±1.5 mmHg) and end (40.9±2.7 mmHg) of heating (P=1.00). The sensitivity of $\dot{V}_E$ to rising T_{es} (i.e., slope of the T_{es}-$\dot{V}_E$ relation) did not differ between the Control and CO_2-breathing trials (37.1±43.1 vs. 16.5±11.1 $l \cdot min^{-1} \cdot °C^{-1}$, P=0.31). In both trials, middle cerebral artery blood velocity (MCAV) decreased early during heating (all P<0.01), despite the absence of hyperventilation-induced hypocapnia. CO_2-breathing increased MCAV relative to Control at the end of heating (P=0.005) and explained 36.6% of the heat-induced reduction in MCAV. These results indicate that during passive heating at rest, ventilatory sensitivity to rising core temperature is not suppressed by hypocapnia, and that most of the decrease in cerebral blood flow occurs independently of hypocapnia.

(249 words)

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NEW & NOTEWORTHY

Hyperthermia causes hyperventilation and concomitant hypocapnia and cerebral hypoperfusion. The last may underlie central fatigue. We are the first to demonstrate that hyperthermia-induced hyperventilation is not suppressed by the resultant hypocapnia, and that hypocapnia explains only 36% of cerebral hypoperfusion elicited by hyperthermia. These new findings advance our understanding of the mechanisms controlling ventilation and cerebral blood flow during heat stress, which may be useful for developing interventions aimed at preventing central fatigue during hyperthermia.

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Key words: Hyperpnea; Hyperthermia; Hypocapnia; Cerebral blood flow

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INTRODUCTION

A rise in core temperature reportedly leads to increased minute ventilation ($\dot{V}_E$) (hyperthermic hyperventilation) at rest (10, 17, 20, 40) and during exercise (18, 33, 45, 48). Given that oxygen uptake remains largely unchanged from normothermic levels during hyperthermia in passively heated resting subjects (10, 17, 40), the influence of metabolic factors on hyperthermic hyperventilation appears to be trivial. Consequently, this hyperventilation results in excessive elimination of CO_2 from the body, decreasing arterial CO_2 partial pressure (P_aCO_2) (hypocapnia) (20, 33, 40). This hypocapnia likely contributes to the cerebral hypoperfusion observed during hyperthermia (9, 16, 31, 33). By limiting heat exchange in the brain, cerebral hypoperfusion would eventually lead to elevation in brain temperature (34), which may ultimately underlie central fatigue during hyperthermia (39). Therefore, preventing the cerebral hypoperfusion associated with hyperthermic hyperventilation may be an effective strategy for minimizing central fatigue during exercise in the heat. Before implementing this strategy, however, it will be important to better understand the characteristics and underlying mechanisms of hyperthermic hyperventilation, most of which are currently unclear.

When $\dot{V}_E$ is expressed as a function of esophageal temperature (T_{es}; an index of core temperature) during passive heating, $\dot{V}_E$ remains nearly unchanged until T_{es} surpasses ~37.8–38.5°C (10, 17, 44). Above this threshold temperature, $\dot{V}_E$ increases linearly with rising T_{es} such that $\dot{V}_E$ increases by 20–32 l·min^{-1} per 1°C rise in T_{es} (10, 17, 44). The slope relating T_{es} to $\dot{V}_E$ would reflect ventilatory sensitivity to rising core temperature (5, 17, 25), as it reflects the thermosensitivity of sweating and cutaneous vasodilation in those relations (7). The hyperthermic hyperventilation-induced hypocapnia potentially suppresses ventilatory sensitivity, given that ventilatory responses are highly sensitive to P_aCO_2 (14). Along these

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lines, we previously found that during prolonged moderate exercise in the heat, continuous restoration of P_aCO_2 to eucapnia increased ventilatory sensitivity (24). However, it remains to be seen whether this is also true during passive heating at rest, as the characteristics of hyperthermic hyperventilation differ depending on whether one is resting or exercising (17, 44). Previous reports using passive heating at rest showed that acute restoration of end-tidal CO_2 partial pressure (P_{ETCO2}, an index of P_aCO_2) to normothermic levels did not change $\dot{V}_E$ at moderate hyperthermia (core temperature of ~37.6–38.0°C) (16), but it did increase $\dot{V}_E$ at severe hyperthermia (38.8°C) (31). The latter result lends support to the possibility that continuous maintenance of P_aCO_2 at eucapnia augments ventilatory sensitivity at rest. It should be emphasized that to precisely evaluate the influence of hypocapnia on the ventilatory sensitivity to rising core temperature, hypocapnia should be prevented throughout the entire period of heating, as we did previously (24). Acute restoration of eucapnia after a period of hypocapnia during heating does not enable the relationships among hypocapnia, ventilation and core temperature to be precisely characterized.

As mentioned, hyperventilation-induced hypocapnia contributes to the cerebral hypoperfusion during hyperthermia. It is still debatable, however, whether this hypocapnia entirely explains the cerebral hypoperfusion during hyperthermia at rest. When P_{ETCO2} is acutely restored to normothermic levels through CO_2 inhalation during hyperthermia, the decrease in middle cerebral artery blood velocity (MCA*V*; an index of anterior cerebral blood flow) is partially (9, 16) or largely (3, 31) reversed. But it is well recognized that restoration of P_{ETCO2} to eucapnia after sustained hypocapnia causes an overshoot in MCA*V* beyond the original baseline value (37). Thus restoration of P_{ETCO2} after sustained hypocapnia during heating could lead to overestimation of the contribution of hypocapnia to the reduction in MCA*V*. To avoid that issue, it is necessary to clamp P_{ETCO2} at a eucapnic level throughout the

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passive heating, as was previously done during exercise in the heat (24).

Accordingly, in the present study we examined the effect of passive heating with or without clamping P_{ETCO2} at the eucapnic level on 1) the ventilatory sensitivity to rising core temperature and 2) the cerebral blood flow response. We hypothesized that hypocapnia developed secondary to hyperthermic hyperventilation diminishes ventilatory sensitivity and accounts for only a fraction of the reduction in cerebral blood flow.

METHODS

Participants

Fourteen healthy males [age 24 ± 3 years, height 173 ± 4 cm, body mass 67 ± 8 kg] volunteered to participate in this study. The participants were nonsmokers, and none were taking prescription medication. Written informed consent was obtained from all participants. The procedure used in this study was approved by the Human Subjects Committee of the University of Tsukuba and conformed to the provisions of the Declaration of Helsinki.

Experimental Design

Participants performed two trials wherein they breathed room air (Control trial) or CO_2-enriched air (CO_2 trial) that prevented a reduction in P_{ETCO2} during passive heating. The two experimental trials were conducted in a counterbalanced manner and were separated by at least 7 days. Participants were asked to abstain from strenuous exercise, alcohol and caffeine for 24 h before the experiment. The participants drank 500 ml of water on the night before the experiment and then consumed a light meal and 500 ml of water 2 h prior to the experiment.

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Procedure

Each participant arrived at the laboratory at 9:00 a.m. and rested in an environmental chamber regulated to an ambient temperature of 25°C and a relative humidity of 50%. During this time, a thermocouple for measuring T_{es} was inserted through the nasal passage to a distance equivalent to one-fourth of the participant's height. The location of the probe in the esophagus was estimated to be posterior to the lower border of the left atrium (47). The participant then voided his bladder, body mass was recorded, and an ultrasound Doppler probe for measuring MCA*V* was attached to the left side of his head. Thereafter, the participant was instrumented with a heart rate (HR) monitor, thermocouples for measuring skin temperatures and a cuff and electrodes for measuring arterial blood pressure. Once instrumented, the participant put on a water-perfused garment that covered his upper body with the exception of the head, left forearm and hands, which were sheathed in cotton gloves. On the left ventral forearm, a capsule for measuring sweat rate was attached using collodion and a probe for measuring skin blood flow was attached near the capsule. The participant then sat in a chair in a bath (water temperature = 35°C) immersed up to his iliac crest and rested in a sitting posture while the water from the bath was circulated through the water-perfused garment. After putting on a breathing mask, a sensor for measuring inspired and expired gases and a three-way valve customized to add CO_2 to the inspired air were connected to the mask. Baseline measurements were then collected for 5 min while the subjects remained sitting. The temperature of the water in the bath, and thus the garment covering the participant's upper body, was then increased to 41°C and held constant for the remainder of the experiment. The water temperature was controlled using a heater and monitored using a thermocouple placed in the bath.

In the Control trial, participants breathed room air throughout the experiment. In the CO_2 trial, they breathed CO_2-enriched air (a mixture of room air and 100% CO_2), beginning at

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the point when P_{ETCO2} fell below baseline due to hyperthermic hyperventilation. We monitored P_{ETCO2} on a real-time basis and manually added 100% CO_2 to the inhaled air. The fraction of inspired CO_2 was <3.5%. The flow rate of the added CO_2 differed depending on the subject's breathing pattern as well as the levels of $\dot{V}_E$ and the metabolic rate. In a preliminary experiment, 4 participants voluntarily hyperventilated such that $\dot{V}_E$ increased to ~20, 30 and 40 l·min^{-1} (breathing frequency (f_B) of ~20, 25 and 30 breaths·min^{-1}, respectively) with CO_2 supplementation to prevent hypocapnia under normothermic conditions. This experiment enabled us to roughly estimate how much CO_2 we needed to add to the inspired air to prevent hypocapnia during passive heating during the CO_2 trial. When T_{es} reached 39.0°C or the subject could no longer tolerate the heating, the experiment was terminated.

Measurements

Skin temperatures and T_{es} were measured using copper-constantan thermocouples, recorded on a computer (ThinkPad A21p, IBM, Japan) at 1-s intervals via a data logger system (WE7000, Yokogawa, Japan), and averaged over 30-s intervals. Mean skin temperature ($\bar{T}_{sk}$) was calculated from seven skin temperatures weighted to the following regional proportions: 7% forehead, 14% forearm, 5% hand, 7% foot, 13% lower leg, 19% thigh and 35% chest (23). HR was recorded every 5 s using a heart rate monitor (Vantage NV, Polar, Finland) and averaged over 30-s intervals. Blood pressure was measured from the upper right arm every 1 min using an automated sphygmomanometer (STBP-780, Nippon Colin, Japan). Mean arterial pressure (MAP) was calculated as the diastolic pressure plus one-third of the pulse pressure. Inspired and expired gases were analyzed using a metabolic cart (AE310s, Minato Medical Science, Japan), and $\dot{V}_E$, tidal volume (V_T) adjusted to BTPS, f_B, oxygen uptake ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$), P_{ETCO2} and the respiratory exchange ratio were recorded

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breath-by-breath. The flow sensor was calibrated using a calibration syringe able to blow a fixed volume (2 liters) of air. The O_2 and CO_2 sensors were calibrated with room air and reference gas of known concentration (O_2 15.0%, CO_2 5.0%, N_2 balance). To determine MCA*V*, we used the transcranial Doppler ultrasound technique (WAKI 1-TC, Atys Medical, France). A 2-MHz Doppler probe was secured with a customized headband to the left temporal region, and the signal was collected at a depth of 45-60 mm. Before the two experiments, the position and probe angle that provided the optimal Doppler signal were determined and photographed. The pictures enabled us to obtain similar MCA*V* values at baseline in the two trials. Cerebral vascular conductance (CVC) was calculated as MCA*V* divided by MAP. Sweat rate was measured using the ventilated-capsule method. Dry nitrogen was supplied to the capsule (3.46 cm^2) at a rate of 0.8 $l \cdot min^{-1}$. The humidity of the nitrogen gas flowing out of the capsule was measured using a capacitance hygrometer (HMP 45ASPF, Vaisala, Finland). Cutaneous blood flow was measured using a laser-Doppler flowmeter (ALF21, Advance, Japan). Cutaneous vascular conductance was calculated as the cutaneous blood flow divided by the MAP and was normalized to the baseline value. Breathing difficulty and thermal sensation were measured every 5 min on a scale of 10 (e.g., difficulty of breathing 1: very easy, 3: moderate, 5: hard, 7: very hard; thermal sensation 5: neutral, 7: warm, 9: very hot).

Data Analysis

To evaluate the relationship between core temperature and ventilatory responses ($\dot{V}_E$, V_T, f_B, and P_{ETCO2}), the ventilatory variables were plotted as functions of T_{es}, and linear regression analyses were conducted using 30-s averaged data. For these analyses, we used a computer algorithm to determine the thresholds for the increase or decrease in the respective ventilatory variables, as evaluated from the inflection point where two calculated regression lines crossed

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(17, 44). We chose the two best-fit regression lines based on the smallest residual sums of squares. The slopes of the second regression lines relating $\dot{V}_E$ V_T, f_B and P_{ETCO2} to T_{es} were considered as sensitivities. The T_{es} strongly correlated with $\dot{V}_E$ (r = 0.74 ± 0.13), V_T (r = 0.53 ± 0.24), f_B (r = 0.66 ± 0.2) and P_{ETCO2} (r = –0.77 ± 0.20). Furthermore, we previously confirmed high day-to-day reproducibility for the T_{es} threshold (r = 0.83, P < 0.05) and the ventilatory sensitivity (r = 0.97, P < 0.05) of hyperthermia-induced hyperventilation (17).

Statistical Analysis

Two-factor repeated-measures ANOVA was used to analyze the time-dependent data using trial (Control and CO_2) and heating duration (pre, 5, 10, 15, 20, 25 min and end of heating) as factors. Two-factor repeated-measures ANOVA was also employed to analyze the core temperature-dependent data using trial (Control and CO_2) and T_{es} level (36.8, 37.2, 37.6, 38.0, 38.4 and 38.8°C) as factors. When a main effect was detected, *post hoc* multiple comparisons were performed to identify pairwise differences using paired *t*-tests with the Bonferroni correction. Paired *t*-tests were also used for between-trial comparisons of the thresholds and sensitivities of ventilatory parameters to rising T_{es}. When the test of normality (Shapiro-Wilk test) failed, the Wilcoxon signed-rank test was used to assess differences between trials. All data are reported as means ± SD. Values of P < 0.05 were considered significant. All statistical analyses were performed using a commercially available statistics package (version 21.0, SPSS Inc., USA).

RESULTS

Heating duration and body weight loss

Heating duration did not differ between the trials (Control vs. CO_2, 32.9 ± 6.4 vs. 33.8 ±

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8.2 min, $P = 0.58$). Body mass losses over the course of the experiment were nearly identical between the Control and CO_2 trials (1.5 ± 0.5 vs. 1.4 ± 0.5%, $P = 0.20$).

Time-dependent changes

Body temperature and thermoregulatory responses

Time-dependent changes in body temperature and thermoregulatory variables are shown in Table 1 and Fig. 1A. There were main effects of trial on T_{es} ($P < 0.01$) and of heating duration on T_{es} and $\bar{T}_{sk}$ (both $P < 0.01$). Over the course of the hot water immersion, T_{es} gradually increased, and the temperatures at the end of heating were similar in the Control and CO_2 trials (38.9 ± 0.3 vs. 39.0 ± 0.3°C, $P = 0.12$). Although T_{es} at 5–25 min was higher in the CO_2 than Control trial, the difference was minimal (<0.19°C, Fig. 1A). The rate of increase in T_{es} did not differ between the Control and CO_2 trials (4.5 ± 0.6 vs. 4.5 ± 0.9°C·h^{-1}, $P = 0.81$). Likewise, increases in $\bar{T}_{sk}$ were equivalent during heating in the two trials. Because we were unable to measure sweat rate and cutaneous blood flow on the forearms of three subjects due to technical difficulties, the data analyzed were from the remaining 11 subjects. Forearm sweat rate and cutaneous vascular conductance did not differ between trials ($P = 0.52$ and 0.68 for a main effect of trial, respectively), and thermal sensation increased similarly in the two trials ($P = 0.29$ for a main effect of trial).

Cardiovascular and cerebrovascular responses

Similar changes in HR and MAP were observed in the two trials ($P = 0.25$ and 0.99 for a main effect of trial, respectively, Table 1). There was a significant interaction between trial and heating duration affecting MCA*V* and CVC (both $P < 0.05$). In both trials, MCA*V* was decreased at and after 10 min of heating in comparison to the pre-heating value. In addition, MCA*V* was higher in the CO_2 than Control trial at the end of heating ($P = 0.005$, Fig. 1D), and

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257 explains 36.6% of the total decrease in MCA*V* occurring in the Control trial. The changes in
258 CVC were similar to those observed with MCA*V*, and CVC was higher in the CO_2 than
259 Control trial at the end of heating (P = 0.014, Table 1).

260 ***Ventilatory responses***

261 Time-dependent changes in respiratory variables are shown in Table 2 and Fig. 1B and C.
262 A significant main effect of heating duration was detected on all the respiratory variables
263 evaluated (all $P < 0.01$). Similar increases in $\dot{V}_E$ were observed in the two trials (P = 0.52 for
264 a main effect of trial). From pre-heating to 15 min of heating, $\dot{V}_E$ increased slightly, and then
265 increased as heating continued (at the end of heating, 23.8 ± 8.3 and 22.9 ± 7.3 $l \cdot min^{-1}$ in the
266 Control and CO_2 trial, respectively, Fig. 1B). Both V_T and f_B did not differ between trials (P =
267 0.31 and 0.37 for a main effect of trial, respectively). At the end of heating in the Control trial,
268 P_{ETCO2} was significantly lower than before heating. As designed, P_{ETCO2} in the CO_2 trial
269 remained unchanged over the period of heating, and was higher than in the Control trial after
270 15 min of heating (Fig. 1C).

271

272 ***T_{es}-dependent changes in ventilatory and cerebrovascular responses***

273 Figure 2 shows $\dot{V}_E$ plotted against T_{es}. The thresholds and sensitivities of the
274 relationships between ventilatory variables and T_{es} are presented in Tables 3 and 4. Twelve of
275 the 14 subjects showed T_{es} thresholds for increases in $\dot{V}_E$ in both trials. One subject showed
276 no threshold in either trial, and another subject exhibited no threshold in the CO_2 trial only.
277 There was no significant between-trial difference in the threshold (P = 0.15). We also observed
278 T_{es} thresholds for increases in V_T in 8 subjects and for increases in f_B in 11 subjects in both
279 trials, and for decreases in P_{ETCO2} in 13 subjects in the Control trial. There were no significant
280 between-trial differences in the thresholds for V_T or f_B (P = 0.06 and 0.56, respectively).

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Individual data for the sensitivities of ventilatory responses to rising T_{es} are shown in Table 4. In 4 of 12 subjects, the sensitivity of $\dot{V}_E$ to rising T_{es} was higher in the CO_2 than the Control trial. The sensitivities of V_T and f_B to rising T_{es} were higher in the CO_2 than the Control trial in 3 of 8 subjects and in 3 out of 11 subjects, respectively. There were no between-trial differences in the sensitivities of $\dot{V}_E$, V_T and f_B to rising T_{es} (P = 0.31, 0.11 and 0.86, respectively). Although there was a two-fold between-trial difference in the mean value of the sensitivity of $\dot{V}_E$, this difference largely disappeared when 2 outliers were excluded (subjects #4 and #9), resulting in similar mean values in the two trials (Control vs. CO_2 trial: 19.6 ± 14.8 vs. 17.6 ± 11.9 $l \cdot min^{-1} \cdot °C^{-1}$, respectively).

Ventilatory and cerebrovascular responses were compared between trials at various T_{es} (Figs. 3 and 4). There were main effects of T_{es} on $\dot{V}_E$, V_T, P_{ETCO2} and MCAV (all $P < 0.01$). The increase in $\dot{V}_E$ and the respiratory pattern, as evaluated based on V_T and f_B, were similar in the Control and CO_2 trials (P = 0.28, 0.74 and 0.18 for a main effect of trial, respectively, Fig. 3). In the Control trial, P_{ETCO2} was lower at T_{es} = 38.8°C than 36.8°C, but it remained unchanged in the CO_2 trial. A main effect of trial on P_{ETCO2} was detected. At T_{es} = 37.6–38.8°C, P_{ETCO2} was higher in the CO_2 than Control trial (all $P < 0.01$, Fig. 4A). There was no main effect of trial on MCAV (P = 0.30). In both trials, MCAV was lower at T_{es} = 37.2–38.8°C than 36.8°C (Fig. 4B). In the CO_2 trial, restoration of the P_{ETCO2} restored 37.6%, 39.8% and 39.3% of the heating-induced decrease in MCAV in the Control trial at T_{es} = 38.0, 38.4 and 38.8°C, respectively.

DISCUSSION

Our main findings are that during passive heating at rest, 1) the sensitivity of $\dot{V}_E$ to rising T_{es} did not differ between the Control and CO_2 trials; 2) the decrease in MCAV occurred

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early during heating, when there was no clear hyperventilation or resultant hypocapnia; 3) at the end of heating (T_{es} = 39.0), MCA*V* was significantly higher in the CO_2 than Control trial, but hypocapnia explained only 36.6% of the observed reduction in MCA*V*. These results suggest that during passive heating at rest, ventilatory sensitivity to rising core temperature is not suppressed by the resultant hypocapnia, and that hyperthermia decreases cerebral blood flow largely independently of the concomitant hypocapnia.

Effect of hypocapnia on ventilatory sensitivity to rising core temperature

Contrary to our hypothesis, the sensitivity of $\dot{V}_E$ to rising T_{es} was not greater in the CO_2 trial than the Control trial (Fig. 2 and Table 4). Only 4 of 12 subjects showed a higher sensitivity in the CO_2 trial; the others showed somewhat lower sensitivity in the CO_2 than Control trial. Similarly, $\dot{V}_E$ at similar T_{es} levels did not differ between the trials (Fig. 3A). These results suggest that for subjects in a hyperthermic resting state, hypocapnia does not suppress ventilatory sensitivity to rising core temperature. By contrast, we previously reported that, during prolonged moderate-intensity exercise in the heat, restoration of P_aCO_2 to the eucapnic level led to a doubling of ventilatory sensitivity as compared to the sensitivity when $PaCO_2$ was decreased secondary to hyperthermic hyperventilation (19.8 vs. 8.9 $l \cdot min^{-1} \cdot °C^{-1}$) (24). We therefore suggest that hypocapnia diminishes ventilatory sensitivity during exercise but not at rest. Furthermore, the fact that hypocapnia attenuates hyperthermic hyperventilation only during exercise likely contributes to the approximately three-times smaller ventilatory sensitivity to rising core temperature observed during exercise in the heat as compared to resting (17, 44).

The lack of change in ventilatory sensitivity upon P_aCO_2 restoration may be due to the location of the P_aCO_2 threshold for increases in $\dot{V}_E$. The threshold exists at a normal P_aCO_2

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329 level at rest (4, 14), and $\dot{V}_E$ remains unchanged when P_aCO_2 decreases below the threshold.
330 This P_aCO_2 threshold is also reportedly unaffected by a 1.5°C elevation in core temperature at
331 rest (4, 6). Because hyperthermic hyperventilation during passive heating decreases P_aCO_2
332 below normal level, it is plausible that these changes in P_aCO_2 are below the threshold for
333 increases in $\dot{V}_E$ and so did not affect hyperthermic hyperventilation at rest. On the other hand,
334 although the P_aCO_2 threshold for increases in $\dot{V}_E$ is reportedly similar during rest and
335 exercise (11, 13), as mentioned, we previously found that $\dot{V}_E$ increased with restoration of
336 P_aCO_2 to the eucapnic level during exercise in the heat (24). This implies that exercise in
337 combination *with hyperthermia* shifts the P_aCO_2 threshold to lower P_aCO_2 levels than that at
338 rest.

339 We previously observed that acute restoration of P_{ETCO2} to eucapnia does not influence
340 $\dot{V}_E$ at T_{es} = 37.6 or 38.0°C (16). However, similar restoration increased $\dot{V}_E$ at a higher T_{es}
341 (i.e., 38.7 °C) (3). In addition, restoration of normocapnia reportedly decreased $\dot{V}_E$ at a rectal
342 temperature of 39.1°C (39), though the level of hyperthermia should be regarded with caution,
343 as rectal temperature responds more slowly than T_{es} (42). The reason for these disparate results
344 remains unclear. Because we detected no effect of hypocapnia on $\dot{V}_E$, irrespective of core
345 temperature in the present study (Fig. 4A), different core temperatures would not explain the
346 lack of consistency between studies. Other differences such as posture (upright (16, 39) versus
347 supine (3)) might be involved, but that remains to be determined.

348

349 ***Effect of hypocapnia on the cerebrovascular response to rising core temperature***

350 With rising T_{es} in the present study, MCA*V* in the Control trial decreased to levels below
351 the pre-heating baseline, which is in line with previous studies (9, 16, 29, 31). Early during the
352 heating, MCA*V* decreased compared to baseline in both trials, despite hyperthermic

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353 hyperventilation, and thus resultant hypocapnia, being minimal (Fig. 1C and D). This implies
354 that at rest hyperthermia can decrease cerebral blood flow independently of hypocapnia. When
355 the decrease in P_{ETCO2} was prevented in the CO_2 trial, MCA*V* was higher than in the Control
356 trial during the latter part of heating (Fig. 1C); however, the restoration accounts for only
357 36.6% of total decrease in MCA*V* (Fig. 1D). Hence, although hypocapnia appears to contribute
358 to the decrease in cerebral blood flow during passive heating at rest, a larger portion of the
359 response is explained by one or more hypocapnia-independent factors. Furthermore, MCA*V*
360 was similarly restored by 40% across the range of T_{es} = 38.0–38.8°C (Fig. 4B), indicating that
361 the relative contribution of hypocapnia to the change in cerebral blood flow during passive
362 heating is similar within this temperature range.

363 Previous studies reported that restoration of P_{ETCO2} through acute CO_2 inhalation during
364 passive heating increased MCA*V* by ~28% and ~38% at core temperatures of 37.6°C and
365 38.0°C, respectively (16) and increased MCA*V* by ~50% at a core temperature of 38.5°C (9).
366 By contrast, the restoration of P_{ETCO2} reversed reductions in MCA*V* and posterior cerebral
367 artery blood flow velocity by ~67% and ~84%, respectively, at core temperature of 38.8°C (31),
368 and it increased MCA*V* by ~75% at a core temperature of 38.7°C (3). It is however noteworthy
369 that the all the results shown above could be influenced by prolonged exposure of hypocapnia,
370 during which MCA*V* gradually increased despite the sustained hypocapnic state as a result of
371 reductions in brain extracellular bicarbonate and recovery of extracellular pH (8).
372 Consequently, restoration of P_aCO_2 to a eucapnic level after prolonged hypocapnic exposure
373 produces an overshoot in MCA*V* above baseline values (27, 37). We suggest that the different
374 magnitudes of this adaptation, in addition to the postural difference as speculated by Bain *et al*.
375 (2), may underlie the discrepancy among the earlier studies.

376 The mechanism underlying hypocapnia-independent decrease in MCA*V* during heat

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377 stress is unclear. Because there was no decrease in MAP throughout the heating in the present
378 study, the possibility of decreased cerebral perfusion pressure is excluded. On the other hand,
379 blood distribution to the internal carotid artery, from which the MCA branches, reportedly
380 decreases, possibly due to an increase in blood distribution to the external carotid artery, which
381 supplies blood to the cutaneous vessels of the head for thermoregulation (35). Furthermore,
382 blood flow in the internal carotid artery gradually decreases with rising T_{es} (35). Thus,
383 modification of the blood distribution in the brain during passive heating may explain the
384 hypocapnia-independent reduction in MCA*V*. In addition, cerebral vessels are innervated by
385 nerve fibers that originate from peripheral (extrinsic) nerve ganglia and intrinsic nerves (22). In
386 humans, stellate ganglion blockade increases cerebral blood flow (26, 46), suggesting
387 regulation of cerebral blood flow is associated with sympathetic nerve activity. Given that
388 hyperthermia increases skin and muscle sympathetic nerve activities (28), cerebral
389 vasoconstriction during hyperthermia may be due in part to sympathetic vasoconstriction, as
390 previously speculated by Brothers *et al*. (9).
391
392 ***Effect of hypocapnia on other variables***
393 In our earlier study, which employed the same heating protocol (19), we showed that
394 greater hyperthermic hyperventilation correlates with lower MAP. This implies that
395 hyperthermic hyperventilation may lower MAP during passive heating at rest. In the present
396 study, however, MAP did not differ between the Control and CO_2 trials (Table 1), indicating
397 that hypocapnia does not influence MAP during passive heating at rest. Hence hyperventilation
398 itself might decrease MAP during passive heating at rest. This notion is indirectly supported by
399 the earlier finding that lung inflation decreases MAP (36).

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400 There were no between-trial differences in forearm sweat rate or cutaneous vascular
401 conductance (Table 1), which suggests hypocapnia does not influence sweating or cutaneous
402 blood flow during passive heating at rest. This is in contrast to the previous observations made
403 by Albert (1), Robinson and King (38) and Fujii *et al*. (15). The discrepancy may reflect
404 differences in core temperature. For example, Fujii *et al*. (15) reported that hypocapnia reduced
405 forearm cutaneous blood flow when T_{es} increased by 0.6°C but not 1.0°C. Since hyperthermic
406 hyperventilation and resultant hypocapnia during passive heating occurred with increases in T_{es}
407 of ~1.0–1.5°C, as shown in that study, it is plausible that the greater elevation in core
408 temperature masked the hypocapnia-induced suppression of cutaneous blood flow. Whether the
409 same explanation applies to the sweat response remains to be investigated.
410
411 ***Limitations***
412 Coverdale *et al*. (12) recently reported that MCA diameter measured using magnetic
413 resonance imaging decreased by 4% when P_{ETCO2} decreased from 36 to 23 mmHg, which is in
414 contrast to previous reports that MCA diameter is unaffected by decreases in P_{ETCO2} to ~25
415 mmHg (21, 41). It may thus be possible that the degree to which MCA*V* was reduced in the
416 Control trial underestimated the true degree to which MCA blood flow was reduced, as blood
417 flow was actually decreased farther than was reflected by the change in velocity.
418
419 ***Perspectives and significance***
420 Reduced cerebral blood flow during hyperthermia reportedly leads to increased brain
421 temperature (34) and central fatigue (39) without modulating cerebral metabolism (32, 43).
422 The present study demonstrated that hypocapnia induced through hyperventilation does not
423 modulate ventilatory responses to rising core temperature, but does contribute to the decrease

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424 in cerebral blood flow seen during passive heating at rest. Based on this observation, we
425 speculate that restoration of normocapnia by inhaling CO_2 gas is not sufficient to fully restore
426 cerebral perfusion during passive heating at rest, and a hypercapnic state (i.e., increase in
427 arterial CO_2 pressure above normocapnic level) or other countermeasures, such as whole-body
428 skin cooling (30), may be required to prevent cerebral hypoperfusion. Furthermore, we found
429 that inhaling CO_2 gas to prevent hypocapnia during passive hyperthermia does not induce
430 additional hyperventilation or dyspnea, as evaluated based on difficulty of breathing. This is in
431 stark contrast to what was observed during exercise in the heat, wherein preventing hypocapnia
432 by inhaling CO_2 gas increased ventilation (24). Therefore, although inhaling CO_2 gas can
433 reverse cerebral hypoperfusion, irrespective of whether the subjects are resting or exercising,
434 this maneuver can distress the respiratory system during exercise. This is important
435 information to know when employing CO_2 gas inhalation as a strategy to mitigate cerebral
436 hypoperfusion during heat stress.
437
438 ***Conclusion***
439 In summary, our results suggest that the ventilatory sensitivity to rising core temperature
440 is not suppressed by hypocapnia, and that hyperthermia decrease cerebral blood flow largely
441 independently of the hypocapnia during passive heating at rest.

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579 **FIGURE LEGEND**

580 **Figure 1.** Time-dependent changes in esophageal temperature (A), minute ventilation (B),
581 end-tidal CO_2 pressure (P_{ETCO2}; C) and middle cerebral artery blood velocity (MCA*V*; D)
582 during passive heating in the Control and CO_2 trials. *P < 0.05, Control vs. CO_2; †P < 0.05, vs.
583 pre-heating (Pre) in the Control trial; ‡P < 0.05, vs. Pre in the CO_2 trial. Symbols are means ±
584 SD (n = 14).

585

586 **Figure 2.** Esophageal temperature-dependent changes in minute ventilation during passive
587 heating in the Control and CO_2 trials. Symbols show 30-s averaged data. Arrows (dashed:
588 Control; solid: CO_2) show the averaged esophageal temperature threshold.

589

590 **Figure 3.** Esophageal temperature-dependent changes in minute ventilation (A), tidal volume
591 (B) and breathing frequency (C) during passive heating in the Control and CO_2 trials. †P <
592 0.05, vs. 36.8°C in the Control trial; ‡P < 0.05, vs. 36.8°C in the CO_2 trial. Symbols are means
593 ± SD (n = 14). The numbers adjacent to the symbols in the Control trial indicate the number of
594 subjects remaining at the corresponding temperature.

595

596 **Figure 4.** Esophageal temperature-dependent changes in P_{ETCO2} (A) and MCA*V* (B) during
597 passive heating in the Control and CO_2 trials. *P < 0.05, Control vs. CO_2; †P < 0.05, vs.
598 36.8°C in the Control trial; ‡P < 0.05, vs. 36.8°C in the CO_2 trial. Symbols are means ± SD (n
599 = 14). The numbers adjacent to the symbols in the Control trial indicate the number of subjects
600 remaining at the corresponding temperature.

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Table 1. Time-dependent changes in body temperature and thermoregulatory and circulatory variables during passive heating

	pre	5min	10 min	15 min	20 min	25 min	end of heating
Mean skin temperature, °C							
Control trial	34.7 ± 0.6	35.7 ± 0.8†	37.5 ± 0.7†	37.9 ± 0.7†	38.1 ± 0.7†	38.3 ± 0.7†	38.4 ± 0.9†
CO_2 trial	34.8 ± 0.4	35.8 ± 0.6‡	37.6 ± 0.7‡	38.0 ± 0.7‡	38.2 ± 0.6‡	38.4 ± 0.6‡	38.5 ± 0.5‡
Forearm sweat rate, $mg \cdot cm^{-2} \cdot min^{-1}$ (n = 11)							
Control trial	0.0 ± 0.0	0.1 ± 0.1	0.4 ± 0.3†	0.8 ± 0.4†	1.0 ± 0.4†	1.1 ± 0.4†	1.1 ± 0.4†
CO_2 trial	0.0 ± 0.0	0.1 ± 0.1	0.5 ± 0.2‡	0.9 ± 0.4‡	1.0 ± 0.4‡	1.1 ± 0.4‡	1.1 ± 0.4‡
Forearm cutaneous vascular conductance, %baseline (n = 11)							
Control trial	100 ± 0	124 ± 37	295 ± 141†	410 ± 253†	434 ± 291	442 ± 298	444 ± 298
CO_2 trial	100 ± 0	131 ± 50	375 ± 251	456 ± 309	470 ± 301‡	467 ± 283‡	482 ± 282‡
Thermal sensation (n = 12)							
Control trial	–	6.3 ± 2.0	7.3 ± 2.1	7.9 ± 1.6†	8.2 ± 1.2†	8.8 ± 1.0†	9.2 ± 1.1†
CO_2 trial	–	7.0 ± 1.0	7.8 ± 0.9‡	8.1 ± 0.7‡	8.4 ± 0.8‡	9.0 ± 0.4‡	9.3 ± 0.7‡
Heart rate, $beats \cdot min^{-1}$							
Control trial	66 ± 10	71 ± 12†	89 ± 14†	101 ± 14†	109 ± 15†	118 ± 18†	128 ± 17†
CO_2 trial	66 ± 9	73 ± 9‡	93 ± 11‡	105 ± 13‡	114 ± 15‡	121 ± 16‡	130 ± 15‡
Mean arterial pressure, mmHg							
Control trial	85 ± 7	87 ± 6	87 ± 5	88 ± 6	88 ± 6	90 ± 6	90 ± 6
CO_2 trial	83 ± 7	87 ± 7	86 ± 8‡	88 ± 8	90 ± 8‡	91 ± 7‡	91 ± 7‡
Cerebral vascular conductance, $cm \cdot s^{-1} \cdot mmHg^{-1}$							
Control trial	0.76 ± 0.11	0.74 ± 0.12	0.68 ± 0.12†	0.63 ± 0.11†	0.62 ± 0.12†	0.58 ± 0.14†	0.53 ± 0.14†
CO_2 trial	0.78 ± 0.11	0.74 ± 0.10‡	0.69 ± 0.11‡	0.66 ± 0.09‡	0.63 ± 0.09‡	0.61 ± 0.08‡	0.59 ± 0.09*‡

Values are means ± SD. n = 14 except for sweat rate, cutaneous vascular conductance and thermal sensation. *P < 0.05 vs. Control trial; †P < 0.05 vs. pre-heating (pre) in the Control trial; ‡P < 0.05 vs. pre in the CO_2 trial.

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607 **Table 2.** Time-dependent changes in respiratory variables during passive heating

	pre	5min	10 min	15 min	20 min	25 min	end of heating
Tida volume, ml							
Control trial	630 ± 114	633 ± 98	704 ± 126†	778 ± 155†	875 ± 261	936 ± 261†	1050 ± 274†
CO_2 trial	658 ± 103	644 ± 70	670 ± 77	773 ± 130	947 ± 268‡	996 ± 263‡	1080 ± 217‡
Breathing frequency, breaths·min^{-1}							
Control trial	17 ± 3	19 ± 3†	19 ± 4	17 ± 4	17 ± 4	20 ± 5	23 ± 6†
CO_2 trial	17 ± 4	19 ± 3‡	20 ± 4‡	18 ± 7	18 ± 6	19 ± 6	23 ± 8
Oxygen uptake, ml·min^{-1}							
Control trial	252 ± 41	270 ± 35	290 ± 29†	315 ± 36†	338 ± 34†	361 ± 39†	399 ± 53†
CO_2 trial	265 ± 29	279 ± 28‡	291 ± 35‡	324 ± 40‡	347 ± 37‡	361 ± 33‡	372 ± 28‡
Carbon dioxide output, ml·min^{-1}							
Control trial	212 ± 39	235 ± 32	272 ± 31†	299 ± 45†	327 ± 55†	371 ± 80†	453 ± 93†
CO_2 trial	220 ± 25	243 ± 26‡	267 ± 33‡	293 ± 35‡	320 ± 49‡	322 ± 44*‡	340 ± 49*‡
Respiratory exchange ratio, units							
Control trial	0.84 ± 0.03	0.87 ± 0.04†	0.94 ± 0.04†	0.95 ± 0.06†	0.96 ± 0.11†	1.02 ± 0.18	1.12 ± 0.22
CO_2 trial	0.83 ± 0.05	0.87 ± 0.04‡	0.92 ± 0.04‡	0.90 ± 0.06‡	0.92 ± 0.08‡	0.88 ± 0.07*‡	0.91 ± 0.10*‡
Difficulty of breathing							
Control trial	–	1.6 ± 1.1	2.6 ± 1.6	3.5 ± 2.2†	4.3 ± 2.2†	5.2 ± 2.5†	6.2 ± 2.2†
CO_2 trial	–	1.9 ± 1.0	2.7 ± 1.4‡	3.8 ± 1.7‡	4.6 ± 2.5‡	5.6 ± 2.6‡	6.7 ± 2.2‡

608

609 Values are means ± SD. n = 14. *P < 0.05 vs. Control trial; †P < 0.05 vs. pre-heating (pre) in

610 the Control trial; ‡P < 0.05 vs. pre in the CO_2 trial.

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611 **Table 3.** Core temperature thresholds of the indicated ventilatory parameters to rising
612 esophageal temperature

	Control	CO_2
Threshold, °C		
Minute ventilation	38.4 ± 0.4 (38.1–38.6)	38.1 ± 0.6 (37.7–38.5)
Tidal volume	38.4 ± 0.5 (37.9–38.8)	37.7 ± 0.9 (36.9–38.4)
Respiratory frequency	38.1 ± 0.6 (37.7–38.5)	38.3 ± 0.5 (37.9–38.6)
End-tidal CO_2 pressure	38.2 ± 0.5 (37.9–38.5)	—

613

614 Values are means ± SD (95% confidence interval). For data analysis, we did not include a
615 subject who did not exhibit a clear core temperature threshold to the relevant ventilatory
616 variable. As a result, the number of subjects was 12, 8, 11 and 13 for minute ventilation, tidal
617 volume, respiratory frequency and end-tidal CO_2 pressure, respectively.

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618 **Table 4.** Individual sensitivities of the indicated ventilatory parameters to increasing
619 esophageal temperature

	$\dot{V}_E$, l·min^{-1}·°C^{-1}		V_T, ml·°C^{-1}		f_B, breaths·min^{-1}·°C^{-1}		P_{ETCO2}, Torr·°C^{-1}	
Subject	Control	CO_2	Control	CO_2	Control	CO_2	Control	CO_2
1	7.9	13.5	–	–	5.2	21.4	-1.1	–
2	45.2	42.0	988	293	35.1	33.9	-39.5	–
3	19.3	12.3	897	483	7.8	5.9	-16.7	–
4	119.1	12.9	56	285	26.5	8.7	-70.4	–
5	8.8	17.1	147	342	10.1	7.7	-12.3	–
6	6.7	29.2	95	270	3.5	66.2	-5.1	–
7	20.5	27.9	–	–	37.1	8.2	-16.6	–
8	30.1	10.2	727	133	11.3	21.4	-21.0	–
9	130.2	8.5	–	–	27.8	4.2	-31.1	–
10	7.4	5.7	–	–	9.1	1.4	-5.2	–
11	8.1	4.5	719	424	–	–	-2.2	–
12	42.1	13.6	1329	448	27.7	4.3	-53.7	–
Mean	37.1 ± 43.1	16.5 ± 11.1	620 ± 471	335 ± 115	18.3 ± 12.6	16.7 ± 19.2	-21.9 ± 21.3	–
P value	0.31		0.11		0.86		–	

620

621 Values are means ± SD. $\dot{V}_E$, minute ventilation; V_T, tidal volume; f_B, breathing frequency;
622 P_{ETCO2}, end-tidal CO_2 partial pressure.

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Figure. 1

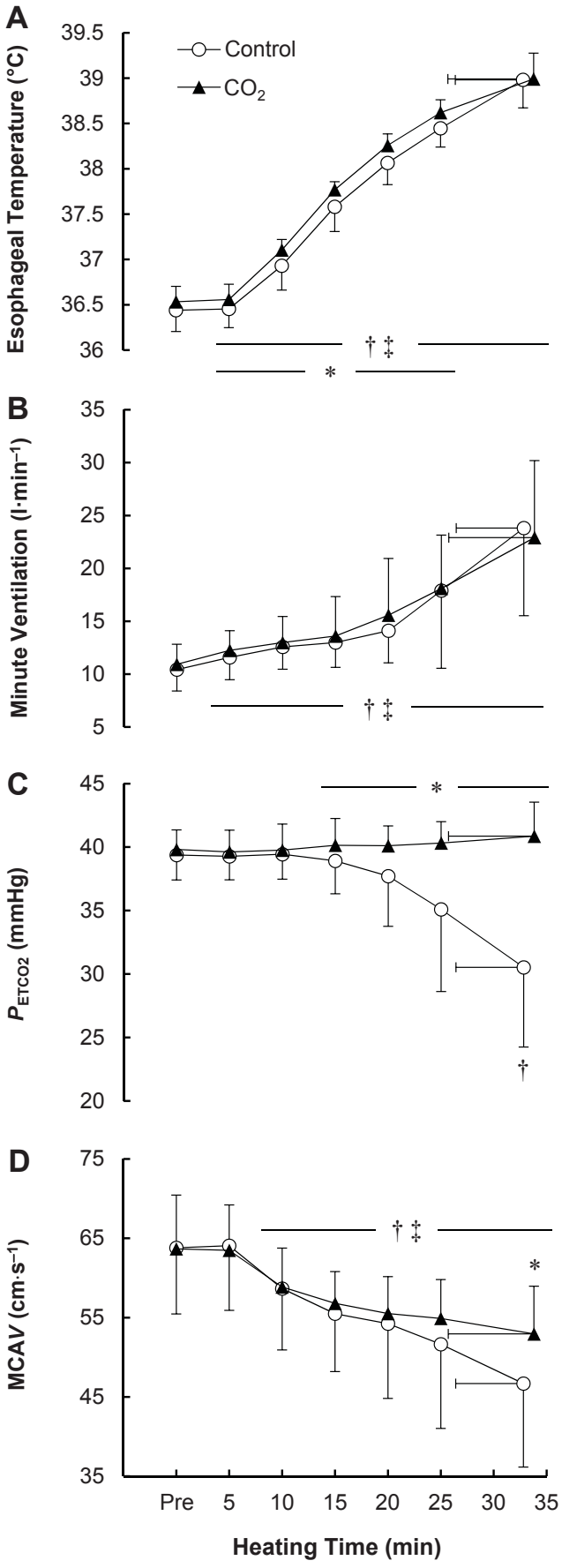

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Figure. 2

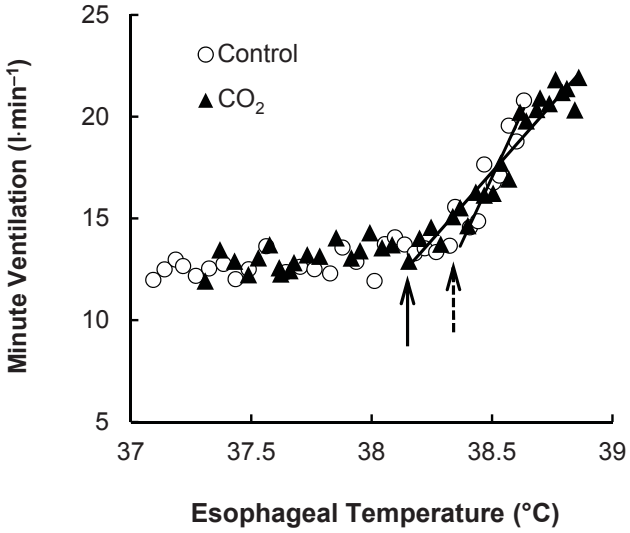

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Figure. 3

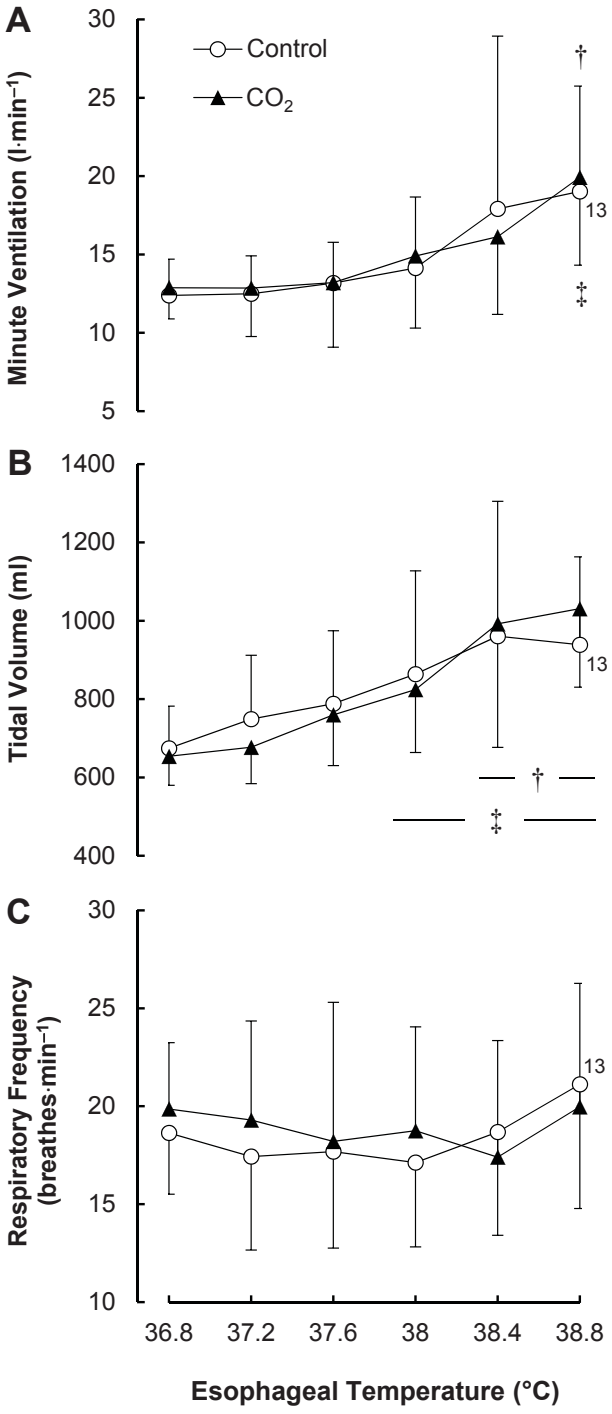

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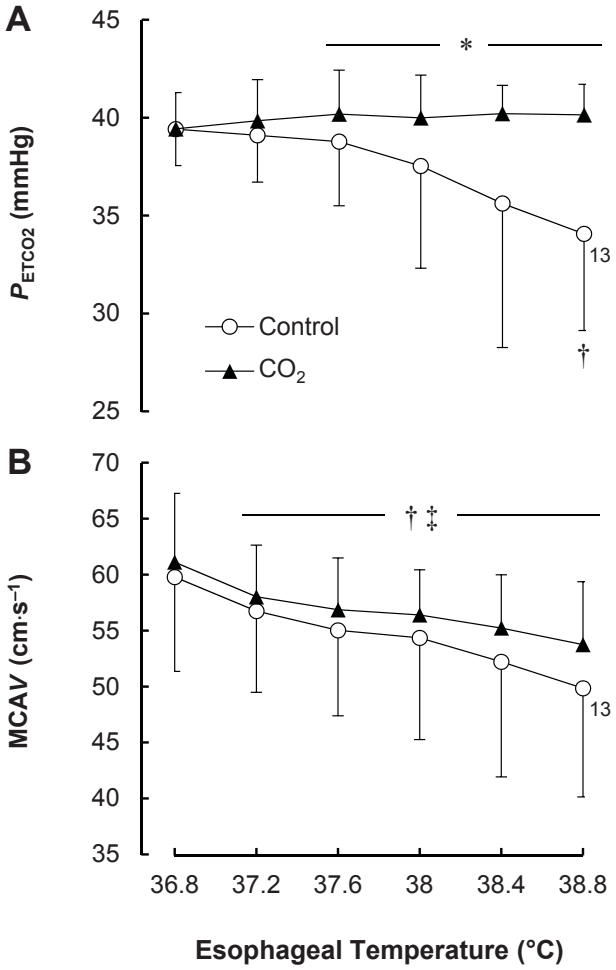
Figure. 4



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