Influence of indomethacin on ventilatory and cerebrovascular responsiveness to CO₂ and breathing stability: the influence of Pco₂ gradients

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¹Department of Physiology, Otago School of Medical Science, University of Otago, Dunedin, New Zealand; ²Department of Medicine, University of Sydney, Sydney, New South Wales, Australia; ³School of Physical Education, University of Otago, Dunedin, New Zealand; ⁴Department of Human Kinetics, Faculty of Health and Social Development, University of British Columbia Okanagan, Kelowna, Canada

Submitted 4 November 2009; accepted in final form 22 December 2009

Fan JL, Burgess KR, Thomas KN, Peebles KC, Lucas SJ, Lucas RAI, Cotter JD, Ainslie PN. Influence of indomethacin on ventilatory and cerebrovascular responsiveness to CO₂ and breathing stability: the influence of Pco₂ gradients. Am J Physiol Regul Integr Comp Physiol 298: R1648–R1658, 2010. First published December 30, 2009; doi:10.1152/ajpregu.00721.2009.—Indomethacin (INDO), a reversible cyclooxygenase inhibitor, is a useful tool for assessing the role of cerebrovascular reactivity on ventilatory control. Despite this, the effect of INDO on breathing stability during wakefulness has yet to be examined. Although the effect of reductions in cerebrovascular CO₂ reactivity on ventilatory CO₂ sensitivity is likely dependent upon the method used, no studies have compared the effect of INDO on steady-state and modified rebreathing estimates of ventilatory CO₂ sensitivity. The latter method includes the influence of Pco₂ gradients and cerebral perfusion, whereas the former does not. We examined the hypothesis that INDO-induced reduction in cerebrovascular CO₂ reactivity would: 1) cause unstable breathing in conscious humans and 2) increase ventilatory CO₂ sensitivity during the steady-state method but not during rebreathing methods. We measured arterial blood gases, ventilation (Ve), and middle cerebral artery velocity (MCAv) before and 90 min following INDO ingestion (100 mg) or placebo in 12 healthy participants. There were no changes in resting arterial blood gases or Ve following either intervention. INDO increased the magnitude of Ve variability (index of breathing stability) during spontaneous air breathing (+4.3 ± 5.2 Δl/min, P ≤ 0.01) and reduced MCAv (−25 ± 19%, P < 0.01) and MCAv-CO₂ reactivity during steady-state (−47 ± 27%, P < 0.01) and rebreathing (−32 ± 25%, P < 0.01). The Ve-CO₂ sensitivity during the steady-state method was increased with INDO (+0.5 ± 0.1 min⁻¹·mmHg⁻¹, P < 0.01), while no changes were observed during rebreathing (P > 0.05). These data indicate that the net effect of INDO on ventilatory control is an enhanced ventilatory loop gain resulting in increased breathing instability. Our findings also highlight important methodological and physiological considerations when assessing the effect of INDO on ventilatory CO₂ sensitivity, whereby the effect of INDO-induced reduction of cerebrovascular CO₂ reactivity on ventilatory CO₂ sensitivity is unmasked with the rebreathing method.

cerebral blood flow; rebreathing

Cerebral blood flow (CBF) and its distribution are highly sensitive to changes in the partial pressure of arterial carbon dioxide (PaCO₂). This local control process, termed cerebrovascular CO₂ reactivity, provides a vital homeostatic function that helps to regulate central pH and, therefore, affects the most important input to respiratory drive (central chemoreceptor stimulus). Indeed, elevations in CBF with hypercapnia increase the washout of CO₂ from the brain, thereby attenuating the rise in central PCO₂. Conversely, decreases in CBF with hypocapnia reduce the CO₂ removal from the brain, thus attenuating the fall in brain tissue PCO₂. Accordingly, the control of CBF plays an important role in stabilizing breathing during perturbations in PaCO₂. Indeed, reductions in cerebrovascular CO₂ reactivity have been implicated in the development of an unstable breathing pattern in patients with congestive heart failure (61) and obstructive sleep apnea (7, 49). Consistent with these reports, observational studies have reported an enhancement in the ventilatory sensitivity to CO₂ and O₂, as well as increased breathing instability in patients with congestive heart failure (21, 32, 52).

Indomethacin (INDO) is a potent reversible cyclooxygenase inhibitor that blocks prostaglandin production in the cerebrovasculature, thereby decreasing CBF and attenuating the cerebrovascular CO₂ reactivity (6, 17, 36, 53) without concomitant changes in metabolic rate (26, 35) or plasma catecholamines (25, 54, 58). This unique feature makes INDO an ideal tool for investigating the role of cerebrovascular CO₂ reactivity in the control of breathing in humans. Previous studies have shown that a reduction in basal CBF and cerebrovascular CO₂ reactivity associated with INDO ingestion (100 mg) elevates resting ventilatory drive (lower plant gain) and ventilatory CO₂ sensitivity (increase controller gain), respectively, presumably due to a blunted H⁺ washout from the brain, thereby causing an increased central chemoreceptor activation (60, 62). Importantly, if the effect of an enhanced controller gain (which serves to destabilize breathing) was greater than the effect of a reduced plant gain (which improves breathing stability), then the net effect of INDO-induced reduction in CBF and cerebrovascular CO₂ reactivity would be reflected in an enhanced ventilatory loop gain, resulting in an unstable breathing pattern. Despite this, the effect of INDO and associated reduction in basal CBF and cerebrovascular CO₂ reactivity on steady-state room air breathing stability has not yet been examined during wakefulness.

An important consideration when assessing the effect of blunted cerebrovascular CO₂ reactivity on ventilatory CO₂ sensitivity is the method used to assess the ventilatory responsiveness. Previous human studies have found INDO-induced reduction in cerebrovascular CO₂ reactivity enhances ventilatory CO₂ sensitivity during the steady-state method (60, 62). Meanwhile, Ivancev et al. (29) reported no changes in ventilatory CO₂ sensitivity with INDO during the rebreathing method in elite breath-hold divers (29). However, because...
breath-hold divers are known to have a blunted ventilatory responsiveness to hypercapnia (24), the effect of INDO on the rebreathing estimate of ventilatory CO2 sensitivity in individuals with normal ventilatory control remains unclear. Since normal PCO2 gradient across the body’s compartment (i.e., end-tidal, arterial, venous, etc.) is essentially abolished during rebreathing (4), the lack of change in ventilatory responsiveness to partial pressure of end-tidal carbon dioxide PETCO2, as reported by Ivancev et al. (29) may be due to a methodological issue when assessing the changes in ventilatory CO2 sensitivity following INDO. For example, during the rebreathing method, the modulatory influence of a blunted cerebrovascular CO2 reactivity on ventilatory CO2 sensitivity would be unmasked due to the absence of a PCO2 gradient. However, to date, no studies have directly compared the ventilatory responsiveness to hypercapnia with (steady state) and without (rebreathing) the influence of the PCO2 gradient following INDO.

The purpose of this study was to examine the effect of INDO on breathing stability during steady-state room air breathing and to compare the effect of INDO on the ventilatory CO2 sensitivity during the steady-state and modified rebreathing method. We examined two hypotheses. First, the effect of enhanced controller gain associated with the INDO-induced reduction in cerebrovascular CO2 reactivity would outweigh the effect of reduced plant gain associated with a reduced CBF, resulting in unstable breathing. Second, the INDO-induced reduction in cerebrovascular CO2 reactivity would cause an increase in the ventilatory CO2 sensitivity during the steady-state method, but not during rebreathing.

METHODS

Participants

Twelve adults (8 male and 4 female) with a mean age of 30 ± 10 yr (mean ± SD), and body mass index of 23 ± 2 kg/m2 participated in this study. Participants were nonsmokers, had no previous history of cardiovascular, cerebrovascular, or respiratory diseases, and were not taking any medications. All participants were informed regarding the purposes and procedures of this study, and informed written consent was given by each participant prior to participation. The study was approved by the Lower South Regional Ethics Committee of the University of Otago, Dunedin, New Zealand, and conformed to the standards set by the Declaration of Helsinki.

Experimental Design

The participants were required to visit the laboratory on three occasions. After a full familiarization with the experimental procedures outlined below (first visit), participants underwent two experimental trials in randomized order (INDO and placebo). Both the INDO (100 mg) and placebo (sugar) trials were administered in identically appearing pill tablets that were ingested with 20 ml of antacid (Maalox). Before each experimental session, participants were informed to abstain from exercise and alcohol for 24 h, caffeine for 12 h, and a heavy meal for the prior 4 h.

With the exception of the arterial blood gas sampling, which was conducted following a 10-min supine rest, all experiments were performed with participants semirecumbent and in controlled temperature conditions (at 22°C). Following 10–15 min of quiet rest, each experimental testing session comprised 1) an arterial blood gas sample, 2) instrumentation, 3) 5-min resting baseline, 4) steady-state and modified rebreathing (detailed below), 5) INDO/placebo administration, 6) 90-min rest, and 7) repeat testing of sessions 1–4. The order of the steady-state and modified rebreathing was randomized, and full recovery (5-min) was permitted between each trial to restore end-tidal gases to baseline resting values.

Steady-state method. The participants breathed through a leak-free respiratory mask (model 8980; Hans-Rudolph, Kansas City, MO) attached to a one-way nonbreathing valve (model 2700; Hans-Rudolph). The inspiratory line contained a Y-valve that allowed switching from room air to a 200-liter Douglas bag containing 7% CO2-93% O2. The steady-state test began with 2-min of baseline room air breathing before participants were switched onto the Douglas bag for 4 min.

Modified rebreathing. The participants wore a nose clip and breathed through a mouthpiece connected to one side of a Y-valve, which allowed switching from room air to a 6-liter rebreathing bag filled with 7% CO2-93% O2. The modified rebreathing test began with a 2-min baseline of breathing room air followed by 5 min of voluntary hyperventilation. For this, participants were instructed and given verbal feedback to increase ventilation (Ve) via depth and frequency to lower and then maintain PETCO2 at 22 ± 2 mmHg. Participants were then switched to the rebreathing bag following an expiration. They were then instructed to take three deep breaths to ensure rapid equalization of PCO2 between the rebreathing bag and the alveolar, arterial, and mixed-venous compartments (39). The rebreathing tests were terminated when: 1) the participant’s PETCO2 reached 60 mmHg or 2) the participant’s partial pressure of end-tidal O2 (PETO2) could no longer be maintained above 160 mmHg or 3) the participant’s Ve exceeded 100 l/min or 4) the participant reached the end of their tolerance.

Measurements

Respiratory variables. Ve and its components of tidal volume and breathing frequency (f) were measured using a heated pneumotachograph (model HR800; Hans-Rudolph) and were expressed in units adjusted to body temperature pressure saturated. The fractional changes in inspired and expired O2 and CO2 were used to calculate PETO2, and PETCO2, by using fast-responding gas analyzers (model CD-3A; AEI Technologies, Pittsburgh, PA; and models ML206 and ML240, ADInstruments, Colorado Springs, CO). The pneumotachograph was calibrated using a 3-liter syringe (model 2700; Hans-Rudolph), and the gas analyzers were calibrated using known concentrations of O2 and CO2 prior to each testing session.

Cerebrovascular variables. Middle cerebral artery velocity (MCAv, an index of CBF) was measured in the right middle cerebral artery using a 2-MHz pulsed Doppler ultrasound system (DWL Doppler, Sterling, VA). The Doppler ultrasound probe was positioned over the right temporal window and was held in place with an adjustable plastic headband. The signals were obtained using search techniques described elsewhere (1). Heart rate (HR) was determined using a three-lead ECG while beat-to-beat mean arterial blood pressure (MAP) was monitored using finger photoplethysmography (Finometer; TPD Biomedical Instrumentation, Amsterdam, The Netherlands). Mean arterial blood pressure measurements by auscultation were also made periodically to check and validate the automated recordings. Cerebrovascular conductance index (CVCi) was subsequently estimated by dividing mean MCAv by MAP within each breath cycle to reveal intrinsic vascular responses to CO2 (11).

Arterial blood gases. Arterial blood gas samples from the radial artery were obtained at rest using a 25-gauge needle in a preheparinized syringe. Following standardized calibration, all blood samples were analyzed using an arterial blood gas analyzing system (NPT 7 series, Radiometer, Copenhagen, Denmark) for pH, partial pressure of arterial O2 (PaO2) and CO2 (PaCO2), bicarbonate concentration ([HCO3−]), and arterial O2 saturation (SaO2).

With the exception of the arterial blood gas variables, all data were acquired at 200 Hz using an analog-to-digital converter (PowerLab; ADInstruments) with commercially available software (Chart version
Ventilatory stability analysis. To establish whether unstable breathing patterns were present, the variability in the $V_{\text{E}}$ trace was examined during spontaneous room air breathing before and after treatments (Fig. 1). First, $V_{\text{E}}$ during the 5-min resting baseline was visually analyzed and classified as stable or unstable based on the absence or presence of Cheyne-Stokes respiration, as characterized by distinct waxing and waning of tidal volume and $f$ (10). If the trace was considered unstable, the difference between the peak and trough of any oscillations in $V_{\text{E}}$ during the final 2 min of the 5-min baseline period was measured (Fig. 1). The average of this $V_{\text{E}}$ difference ($V_{\text{E}}$ variability) was subsequently used as an index of ventilatory stability. In addition, the number of oscillations during this 2-min period was also noted. The presence of Cheyne-Stokes respiration was confirmed by an experimenter blinded to the experimental condition.

Steady-state ventilatory and cerebrovascular reactivity to $CO_2$. Steady-state $V_{\text{E}}$, MCAv, CVCi, MAP, and HR responsiveness to $CO_2$ was estimated from the slope of the mean value of each dependant variable in the final minute of baseline and steady-state hyperoxic hypercapnic breathing. Steady-state hypocapnic cerebrovascular reactivity was estimated from the slope of the mean MCAv in the final minute of baseline and voluntary hyperventilation prior to the rebreathing. It should be acknowledged that the steady-state determination of ventilatory $CO_2$ sensitivity is restricted to the number of data points used in the analysis (39, 41). However, the steady-state $V_{\text{E}}$-$CO_2$ sensitivities (control and INDO) observed in the present study (Fig. 2) was comparable to those reported by Xie et al. (62) who used four steady-state data points (baseline, 2%, 4%, and 6% $CO_2$), thereby supporting the use of two data points in estimating steady-state ventilatory $CO_2$ sensitivity in the present study.

Modified rebreathing estimates of ventilatory and cerebrovascular reactivity to $CO_2$. The modified rebreathing data were accumulated on a breath-by-breath basis and were analyzed using a specially designed program (Full Fit Rebreathing program, version 3.1; by James Duffin, Department of Physiology and Anesthesia, University of Toronto, Toronto, Canada). In brief, breaths from the initial three-breath equilibration, as well as sighs, swallows, and breaths, incorrectly detected by the acquisition software, were excluded from further analysis. Next, the breath-by-breath $PETCO_2$ values were plotted against time and fitted with a least-squares regression line to minimize interbreath variability (16, 39). Subsequently, $V_{\text{E}}$, MCAv, CVCi, MAP, and HR were plotted against the predicted $PETCO_2$ obtained by the regression analysis.

The $V_{\text{E}}$ plot was fitted with a model made up of the sum of two segments separated by one breakpoint (16). The first segment was taken to be resting $V_{\text{E}}$. Thereafter, $V_{\text{E}}$ increased in conjunction with the predicted $PETCO_2$. Since hyperoxia ($PaO_2 \geq 150$ mmHg) is known to silence the peripheral chemoreceptors (13, 20, 38), the observed breakpoint was taken as the ventilatory recruitment threshold of the central chemoreflex, while the second segment was assumed to be the ventilatory $CO_2$ sensitivity attributed to the central chemoreflex alone.

Linear regression was also applied to the MCAv, CVCi, MAP, and HR changes during the modified rebreathing. Unlike the $V_{\text{E}}$ response, there were no differential breakpoints observed in the MCAv, CVCi, MAP, and HR in the majority (8 out of 12) of the participants; thus, a single line was fitted. In all of the above analyses, modeling was based on the sum of least squares for nonlinear regression using LabVIEW software (Levenberg-Marquardt algorithm, LabVIEW 7.1: National Instruments).

Statistical Analysis

Differences between the steady-state method and the modified rebreathing estimates of $V_{\text{E}}$, MCAv, CVCi, MAP, and HR responsiveness to $CO_2$ during control and INDO conditions were assessed using repeated-measures ANOVA with an $\alpha$-level of $P < 0.05$ (SPSS version 17.0; SPSS, Chicago, IL). Post hoc analysis (paired $t$-test) of significant ANOVAs (either method or drug effects) were performed (Bonferroni’s test) to isolate the effect of method and INDO on the dependent measures within participants. Paired $t$-tests were carried out to examine the effect of placebo ingestion, changes in resting baseline variables, $V_{\text{E}}$, MCAv, CVCi, MAP, and HR responsiveness to $CO_2$. To assess the relationship between $V_{\text{E}}$ variability, cerebrovascular $CO_2$ reactivity, and ventilatory $CO_2$ sensitivity following INDO, correlational analyses (Pearson) were carried out between both the absolute values as well as relative changes in $V_{\text{E}}$ variability, MCAv-$CO_2$ reactivity, and $V_{\text{E}}$-$CO_2$ sensitivity during both the modified rebreathing and the steady-state method ($\alpha$-level of $P < 0.05$). Data are reported as means ± SD.

RESULTS

Effect of INDO on Baseline Resting Variables

INDO ingestion reduced resting MCAv and CVCi by 25 ± 19% and 31 ± 22%, respectively, from control values ($P < 0.01$), while no changes were observed with placebo ($P > 0.05$).
Neither placebo nor INDO altered any of the resting respiratory variables or the arterial blood gases (P > 0.05; Table 1). Both INDO and placebo intervention lowered resting HR by 8 ± 6 beats/min and 5 ± 4 beats/min, respectively (P < 0.01), while MAP remained unchanged (P > 0.05; Table 1).

**Effects of INDO on Breathing Stability**

INDO increased the magnitude of the V̇E variability by 4.3 ± 5.2 %/min (P < 0.05) and the incidence of V̇E oscillation by 1.1 ± 1.6 events/min (P < 0.05; Table 2). No change in breathing stability was observed following the placebo intervention (P > 0.05; Table 2).

**Effect of INDO on Cerebrovascular and Ventilatory Response to CO₂**

The coefficient of variation for the MCAv-CO₂ reactivity between repeat trials and within subject were 10 ± 9% during steady-state and 29 ± 33% during modified rebreathing. Meanwhile, the coefficient of variation for the V̇E-CO₂ sensitivity during the steady-state method, thereby supporting the idea that INDO alters breathing control by altering the chemical environment of the central chemoreceptor without affecting the central chemosensitivity per se. MCAv, middle cerebral artery velocity; CVCi, cerebrovascular conductance index.
2.1 ± 0.7 cm·s$^{-1}$·mmHg$^{-1}$; $P < 0.01$; Fig. 2). INDO ingestion lowered the absolute MCAv-CO$_2$ reactivity by 47 ± 27% (2.1 ± 0.7 vs. 1.4 ± 0.4 cm·s$^{-1}$·mmHg$^{-1}$; $P < 0.01$) during steady-state and by 32 ± 25% (2.9 ± 0.9 vs. 1.7 ± 0.9 cm·s$^{-1}$·mmHg$^{-1}$; $P < 0.01$) during modified rebreathing (Fig. 2). INDO augmented the $V\dot{E}$-CO$_2$ sensitivity by 29 ± 34% (4.2 ± 1.3 vs. 3.1 ± 1.5% mmHg; $P < 0.05$; Fig. 2), while the modified rebreathing estimates remained unchanged ($P > 0.05$; Fig. 3).

As a consequence, the difference between the steady-state and the modified rebreathing estimates of MCAv-CO$_2$ reactivity was abolished following INDO ingestion ($P > 0.05$; Fig. 2). Similarly, INDO reduced the normalized CVCi-CO$_2$ reactivity by 111 ± 97% (3.2 ± 1.3 vs. 1.7 ± 1.2% mmHg; $P < 0.01$) during steady-state, while no changes were observed with modified rebreathing ($P > 0.05$; Fig. 2). INDO also reduced the absolute hypocapnic MCAv-CO$_2$ reactivity by 69 ± 29% (1.2 ± 0.3 vs. 0.4 ± 0.3 cm·s$^{-1}$·mmHg$^{-1}$; $P < 0.01$; Fig. 3) and the normalized hypocapnic MCAv-CO$_2$ reactivity by 61 ± 26% (1.7 ± 0.3 vs. 0.7 ± 0.5% mmHg; $P < 0.01$). No changes were observed in the hypercapnic MCAv-CO$_2$ reactivity with placebo during either modified rebreathing or the steady-state method ($P > 0.05$). In contrast, following the placebo, there was an enhancement in the absolute MCAv-CO$_2$ reactivity to hypocapnia by 16 ± 24% (2.0 ± 0.5 vs. 2.3 ± 0.6 cm·s$^{-1}$·mmHg$^{-1}$; $P < 0.05$).

Prior to the interventions, the steady-state estimate of the $V\dot{E}$-CO$_2$ sensitivity was lower compared with the rebreathing method (1.9 ± 1.4 vs. 3.4 ± 1.8 l·min$^{-1}$·mmHg$^{-1}$, respectively; $P < 0.01$; Fig. 2). INDO augmented the $V\dot{E}$-CO$_2$ sensitivity by 0.5 ± 0.5 l·min$^{-1}$·mmHg$^{-1}$ (1.9 ± 1.4 vs. 2.3 ± 1.6 l·min$^{-1}$·mmHg$^{-1}$; $P < 0.01$; Fig. 2) during the steady-state method, while no change was observed during the modified rebreathing method ($P > 0.05$). Nevertheless, the steady-state estimate of $V\dot{E}$-CO$_2$ sensitivity remained lower (2.3 ± 1.6 vs. 3.9 ± 2.4 l·min$^{-1}$·mmHg$^{-1}$; $P < 0.01$; Fig. 2) compared with modified rebreathing values. INDO did not alter basal $V\dot{E}$ (9.0 ± 6.4 vs. 7.7 ± 5.1 l/min; $P > 0.05$), ventilatory recruitment threshold during the modified rebreathing (41 ± 5 vs. 43 ± 4 mmHg; $P > 0.05$), or the x-intercept during the steady-state method (28 ± 8 vs. 26 ± 10 mmHg; $P > 0.05$; Table 3). Placebo did not alter any ventilatory variables during either the steady-state or modified rebreathing method ($P > 0.05$).

### Cardiovascular CO$_2$ Reactivity

No changes were observed in the MAP-CO$_2$ reactivity or the HR-CO$_2$ reactivity following either INDO or placebo intervention ($P > 0.05$).

Prior to INDO administration, there were significant correlations between the MCAv-CO$_2$ reactivity and $V\dot{E}$-CO$_2$ sensitivity during both steady-state ($R^2 = 0.8; P < 0.01$) and modified rebreathing ($R^2 = 0.8; P < 0.01$; Fig. 4). No correlations were observed between the $V\dot{E}$ variance and MCAv-CO$_2$ reactivity and $V\dot{E}$-CO$_2$ sensitivity during steady-state and modified rebreathing ($P > 0.05$; Fig. 4). Following INDO, the correlation between the MCAv-CO$_2$ reactivity and $V\dot{E}$-CO$_2$ sensitivity during modified rebreathing was abolished ($P > 0.05$; Fig. 4A), while the correlation during the steady-state method remained $R^2 = 0.4; P < 0.05$. The INDO-induced

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**Table 1. Resting cerebrovascular, respiratory, and arterial blood gas variables before and after placebo and indomethacin (INDO) ingestion**

<table>
<thead>
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<th>Placebo</th>
<th>INDO</th>
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<td></td>
<td>Control</td>
<td>Control</td>
<td>INDO</td>
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<tr>
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<tr>
<td>MCAv, cm/s</td>
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<td>70 ± 10</td>
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<td>CVCI, cm·s$^{-1}$·mmHg$^{-1}$</td>
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<td>0.90 ± 0.26</td>
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<td>Respiratory</td>
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<tr>
<td>$V\dot{E}$, l/min</td>
<td>13.6 ± 1.7</td>
<td>14.2 ± 2.1</td>
<td>12.7 ± 3.5</td>
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<td>$f$, breaths/min</td>
<td>14 ± 5</td>
<td>16 ± 4</td>
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<td>VT, liters</td>
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<td>0.9 ± 0.4</td>
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<td>PET$_{CO_2}$, mmHg</td>
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<td>PET$_{O_2}$, mmHg</td>
<td>109 ± 9</td>
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<td>MAP, mmHg</td>
<td>81 ± 13</td>
<td>79 ± 10</td>
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<td>92 ± 13</td>
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<td>Heart rate, beats/min</td>
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<td>64 ± 9*</td>
<td>67 ± 10</td>
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<td>Arterial blood gases</td>
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<td>pH</td>
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<td>PA$_{CO_2}$, mmHg</td>
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<td>41 ± 4</td>
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<tr>
<td>PA$_{O_2}$, mmHg</td>
<td>104 ± 13</td>
<td>98 ± 8</td>
<td>106 ± 11</td>
<td>103 ± 6</td>
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<td>S$_{aO_2}$, %</td>
<td>98.3 ± 0.5</td>
<td>98.1 ± 0.4</td>
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<td>97.6 ± 2.7</td>
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<tr>
<td>[HCO$_3$], mmol/l</td>
<td>27.6 ± 3.1</td>
<td>26.7 ± 2.3</td>
<td>27.3 ± 3.2</td>
<td>27.1 ± 2.0</td>
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</table>

Values are means ± SD. MCAv, middle cerebral artery velocity; CVCI, cerebrovascular conductance index; $V\dot{E}$, ventilation; VT, tidal volume; PET$_{CO_2}$, partial pressure of end-tidal carbon dioxide; PET$_{O_2}$, partial pressure of end-tidal O$_2$; MAP, mean arterial pressure; PA$_{CO_2}$, partial pressure of arterial carbon dioxide; PA$_{O_2}$, partial pressure of arterial O$_2$; S$_{aO_2}$, arterial O$_2$ saturation; HCO$_3$-, bicarbonate concentration. *Different from placebo control ($P < 0.01$).

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**Table 2. Effect of placebo and INDO on $V\dot{E}$ variability in resting, semirecumbent participants**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Placebo</th>
<th>INDO</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Control</td>
<td>INDO</td>
<td>INDO</td>
</tr>
<tr>
<td>$V\dot{E}$ variability, Δl/min</td>
<td>3.0 ± 3.3</td>
<td>3.2 ± 3.6†</td>
<td>2.3 ± 3.7</td>
<td>7.0 ± 5.7*</td>
</tr>
<tr>
<td>Incidence of $V\dot{E}$ oscillation, event/min</td>
<td>0.9 ± 0.8</td>
<td>0.7 ± 1.0†</td>
<td>0.7 ± 1.0</td>
<td>1.8 ± 1.5*</td>
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Values are means ± SD. *Different from INDO control ($P < 0.05$); †different from INDO control ($P < 0.01$).

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*Downloaded from http://ajpregu.physiology.org/ by 102.220.33.6 on April 28, 2017*
reductions in the steady-state estimate of MCAv-CO₂ reactivity correlated with the increase in Vₑ variability with INDO ($R^2 = 0.5$; $P < 0.05$; Fig. 4). No significant correlations were observed between the INDO-induced changes in MCAv-CO₂ reactivity and Vₑ-CO₂ sensitivity during either steady-state or modified rebreathing method ($P > 0.05$).

**DISCUSSION**

The new findings from the present study are that INDO ingestion: 1) caused breathing instability to develop in healthy conscious humans, 2) increased ventilatory CO₂ sensitivity when assessed with the steady-state method but not when assessed with the modified rebreathing method, and 3) reduced the cerebrovascular responsiveness to steady-state hypocapnia. These findings confirm our hypotheses that INDO-induced reductions in cerebrovascular CO₂ reactivity: 1) enhances ventilatory loop gain and destabilizes normal breathing patterns and 2) selectively increases ventilatory CO₂ sensitivity during the steady-state method, but not during rebreathing. Collectively, data from the present study support the role of a blunted cerebrovascular CO₂ reactivity in the development of breathing instability during wakefulness. Moreover, our findings highlight important methodological and physiological considerations when assessing the effect of INDO on ventilatory CO₂ sensitivity, whereby the effect of INDO-induced reduction of cerebrovascular CO₂ reactivity on ventilatory CO₂ sensitivity is unmasked with the rebreathing method.

**Methodological Considerations**

**Assessment of breathing instability.** In the present study, we attempted to assess the net effect of INDO on breathing stability at rest. We did this by quantifying the occurrence and magnitude of any Cheyne-Stokes respiration before and following INDO ingestion in conscious resting humans during room air breathing. Since the control of respiration in the brainstem is modulated by cortical influence, the change in breathing instability with INDO observed in the present study (Table 2) may be confounded by factors such as sleep state, drugs, arousal, and emotion (12, 14, 18, 19, 27). However, since no change in Vₑ variability was observed with placebo (Table 2), it seems plausible that the increase in breathing instability in the present study was due to the effect of INDO on ventilatory loop gain.

**Assessment of CBF.** In this study, transcranial Doppler ultrasound was used to measure the MCAv as an index of global CBF responsiveness to CO₂. While numerous studies support the validity of MCAv as an index of regional CBF (5, 40, 45, 50, 56, 57), it is acknowledged that local changes in CBF may differ. For example, if reductions in MCAv are markedly larger than those in the posterior circulation supplying the medulla, then the effect of a blunted cerebrovascular CO₂ reactivity becomes overestimated and, therefore, may not account for the observed increase in the Vₑ response to CO₂ following INDO. However, previous studies using magnetic resonance imaging have demonstrated that INDO causes a similar percentage change in global CBF and MCAv, as well as

![Table 3. Steady-state and modified rebreathing ventilatory test parameters](image)
a comparable reduction in cerebrovascular CO₂ reactivity between brain regions (6, 53, 62). More importantly, INDO has been shown to cause a uniform drop in CBF in the majority of brain regions, including the medulla (26, 47). Therefore, the INDO-induced reduction in resting MCAv and MCAv-CO₂ reactivity observed in the present study should reflect both global and medullary changes in CBF and its responsiveness to CO₂.

**Menstrual cycle.** In the present study, we were unable to control for the menstrual cycle in the four female participants. However, it should be noted 1) that there is very little evidence in the current literature to suggest that cerebrovascular CO₂ reactivity is altered with the menstrual cycle, 2) that the participants acted as their own control, and 3) that the data from the female participants were well within the group mean and SD. Collectively, it seems unlikely that the findings in the present study may be confounded by the influence of the menstrual cycle.

**Effect of INDO on peripheral chemoreceptor activation.** An important consideration when assessing the effect of INDO on ventilatory CO₂ sensitivity is its effects of peripheral chemoreceptor activation. In animal studies, it has been demonstrated that INDO enhances carotid body chemosensitivity to hypoxia and hypercapnia, while no change was observed under normoxic, eucapnic conditions (22, 23). In contrast, carotid denervation studies have demonstrated that the effect of prostaglandin inhibition on Vₑ is unlikely to be mediated by the carotid bodies (31, 37). In humans, Xie et al. (62) reported no difference in the Vₑ response to CO₂ during hyperoxia and normoxia following INDO ingestion. Likewise, unpublished data from our laboratory indicates that there are no changes in ventilatory responsiveness to isocapnic hypoxia following INDO. Therefore, it is unlikely that the observed increase in breathing instability observed in the present study could be attributed to the effect of INDO on the peripheral chemoreflex alone.

**Baseline Ventilatory and Cerebrovascular Responsiveness to CO₂: Comparison with Previous Studies**

Under normal and steady-state hypercapnic conditions, there is a PCO₂ gradient across the compartments in the body (i.e., end-tidal, arterial, brain tissue, jugular venous) that results in a smaller rise in arterial or brain tissue PCO₂ for a given rise in PₑPCO₂. Under such conditions, increases in CBF during hy-
percapnia would lead to a greater central CO2 washout at higher CO2 tensions (39), thereby narrowing the difference between end-tidal and central CO2 (44). In contrast, since the PCO2 gradient is essentially abolished during rebreathing (4), the modulatory effect of increasing CBF on central CO2 is attenuated (3, 39). Accordingly, the steady-state estimates of ventilatory CO2 sensitivity would be less than the rebreathing estimates. In support of this, numerous studies have reported a higher ventilatory CO2 sensitivity with modified rebreathing (4, 30, 42, 55) compared with steady-state estimates. Likewise, we found that the steady-state estimate of Ve-CO2 sensitivity was lower under the control condition compared with modified rebreathing (Fig. 2). While not universal (48), data from the present and previous studies indicate that the ventilatory responsiveness to CO2 obtained with the rebreathing method represents a measurement of Ve-CO2 sensitivity that is independent of the modulatory influence of CBF-induced changes in PCO2 gradient.

In contrast to ventilatory CO2 sensitivity, Pandit and colleagues (41–43) have consistently reported lower estimates of cerebrovascular CO2 reactivity with rebreathing compared with the steady-state method. Similarly, we found the modified rebreathing estimate of cerebrovascular CO2 reactivity was lower compared with the steady-state estimates of the control condition (Fig. 2). Pandit et al. (41) suggested that prior hyperventilation may cause a persistent effect of the cerebrovascular. However, an earlier study by the same group reported a higher steady-state MCAv-CO2 slope compared with Read’s original rebreathing estimates (which includes no prior hyperventilation) (43). Furthermore, reanalysis of data by Pandit and colleagues (41, 43) reveals no difference in the MCAv-CO2 slope obtained using the modified rebreathing and the Read’s original rebreathing method. Therefore, it seems unlikely that prior hyperventilation could account for the lower estimate of cerebrovascular CO2 reaction during modified rebreathing. The reason behind the lower MCAv-CO2 reactivity with the modified rebreathing remains unclear and warrants further investigation.

Effect of INDO on MCAv and MCAv Response to Hypercapnia

Numerous studies using transcranial Doppler ultrasound (29, 36, 62), MRI (6, 53), the 133Xe method (33), and the N2O washout method (17, 59) have reported that INDO reduces basal CBF by 25–35% and lowers cerebrovascular CO2 reactivity by 50–60%. Consistent with these reports, data from the present study demonstrate that INDO ingestion reduced basal MCAv by 25% and blunted MCAv-CO2 reactivity by 47% during the steady-state method and by 26% during modified rebreathing (Fig. 2). Since no changes were observed in the MAP-CO2 reactivity, we speculate that the effect on INDO is restricted specifically to the cerebrovasculature, without any additional effect on the peripheral vascular tone.

Effect of INDO on Resting Ve

In contrast to findings by Xie et al. (62), and consistent with a lack of change in Paco2, we did not observe any alteration in resting Ve following INDO (Table 1), which indicates a preserved background ventilatory drive. Likewise, Markus et al. (36) also reported no changes in Ve with INDO (100 mg), as indicated by a lack of change in PetCO2. It is noteworthy that Xie et al. (62) measured resting Ve during supine rest, while the participants in Markus et al. (36) and the present study were semirecumbent. Therefore, the difference in posture, and therefore Ve-perfusion matching, may explain the discrepancy between these divergent findings.

Effect of INDO on Ventilatory CO2 Sensitivity

The Ve response to CO2 is mediated by the central and peripheral chemoreflex (28). In the present study, for the first time, we compared the effect of INDO on the Ve response to hyperoxic hypercapnia during modified rebreathing and the steady-state method. Since hyperoxia (PaO2 ≥ 150 mmHg) is known to silence the peripheral chemoreceptor activity (13, 20, 38), we attributed the change in ventilatory CO2 sensitivity to alterations in the central chemoreflex alone. Previously, Xie and colleagues (60, 62) found, using the steady-state method, that INDO-induced reduction in cerebrovascular CO2 reactivity caused an enhanced ventilatory sensitivity to hypercapnia, as well as the ventilatory withdrawal to transient hypocapnia. In contrast, Ivancev et al. (29) reported, using the rebreathing method, INDO-induced reduction in cerebrovascular CO2 reactivity failed to alter ventilatory CO2 sensitivity. In the present study, we found INDO administration selectively increased the Ve-CO2 sensitivity during the steady-state method without altering the modified rebreathing estimates (Fig. 2). Moreover, the magnitude of INDO-induced increase in ventilatory CO2 sensitivity was comparable to those reported by Xie et al. (62). The differential effect of INDO on Ve-CO2 sensitivity steady-state and modified rebreathing method may be due to a methodological issue. For example, during hypercapnia, a blunted cerebrovascular CO2 reactivity associated with INDO would cause a greater rise in central CO2 for a given rise in inspired CO2, due to an impaired CO2 washout, thereby widening the Pco2 gradient between the end-tidal and central compartments. Since the normal Pco2 gradient is abolished during rebreathing (4), such increases in Pco2 gradient with INDO would not alter the rebreathing estimate of ventilatory CO2 sensitivity. In support of this, Xie and colleagues (60, 62) reported that the INDO-induced increase in Ve-PetCO2 sensitivity was abolished when the changes in Ve were plotted against estimated PyvCO2, instead of PetCO2. The authors proposed that the reduction in cerebrovascular CO2 reactivity with INDO increased the ventilatory CO2 sensitivity by modifying the chemical environment of the central chemoreceptor without changing the central chemosensitivity per se. Collectively, these findings highlight an important methodological consideration when assessing ventilatory CO2 sensitivity under conditions of altered cerebrovascular CO2 reactivity whereby an INDO-induced increase in the end-tidal to central Pco2 gradient would enhance the ventilatory CO2 sensitivity obtained during the steady-state method, but not during modified rebreathing.

Effect of INDO on Breathing Stability

A novel finding in the present study was the development of breathing instability in conscious humans following INDO (Table 2). We attribute this breathing instability to an increase in ventilatory CO2 sensitivity associated with INDO-induced reduction in cerebrovascular CO2 reactivity (Fig. 2). For ex-
ample, an individual with an enhanced ventilatory CO₂ sensitivity will elicit a higher Ve response for a given rise in Paco₂, thereby increasing the risk of ventilatory overshoot and subsequent hypopcapnia. Conversely, if the Ve withdrawal to decreasing PETCO₂ is enhanced, then the CO₂ reserve (i.e., the difference between eupneic PETCO₂ and apneic threshold) would be lowered, thereby increasing the susceptibility for central apnea (60). Collectively, an enhanced ventilatory CO₂ sensitivity (controller gain) can lead to an enhanced ventilatory loop gain, resulting in breathing instability and increases in an individual’s risk of periodic breathing. In support of this, Chapman et al. (8) demonstrated, using a setup that augmented control system gain, that an enhanced ventilatory responsiveness to CO₂ caused an increase in breathing instability during sleep. Likewise, Xie et al. (60) recently found, using a positive pressure support ventilator that INDO increased controller gain below eupneic PETCO₂, presumably due to a blunted cerebrovascular reactivity to hypocapnia (voluntary hyperventilation) in healthy, conscious participants (Fig. 3). Importantly, such reduction in MCAv-CO₂ reactivity below eupneic PETCO₂ would enhance the ventilatory withdrawal to hypocapnia, thereby reducing the CO₂ reserve and account, in part, for the increased breathing instability following INDO.

An important consideration when examining the effect of INDO on breathing stability is its effect on plant gain (background ventilatory drive). Previous human (62) and goat (9) studies have shown that a reduction in basal CBF leads to an accumulation of CO₂ and H⁺ in the brain, causing an increase in resting Ve and associated reduction in plant gain. Such reduction in plant gain serves to improve breathing stability by increasing the CO₂ reserve (15). In the present study, we did not observe a change in resting Ve following INDO (Table 1), which indicates a preserved plant gain (62). Importantly, this lack of change in ventilatory drive may partially account for the observed increased breathing instability in the present study (Table 2), whereby the destabilizing effect of an increased controller gain associated with INDO is not counteracted by the stabilizing effect of a reduced plant gain (60). Taken together, data from the present study demonstrate, for the first time, that the net effect of a reduced basal CBF and a blunted cerebrovascular CO₂ reactivity associated with INDO ingestion is an enhanced ventilatory loop gain, resulting in breathing instability in conscious humans.

In summary, our findings highlight the role of cerebrovascular function on breathing stability during pathological conditions and raise important methodological considerations when assessing the effect of INDO on ventilatory CO₂ sensitivity.

Perspectives and Significance

It should be acknowledged that the development of breathing instability or apnea associated with transient ventilatory overshoot is primarily dependent on the detection of hypopcapnia by the peripheral chemoreceptors (34, 51, 63). Indeed, peripheral chemoreceptor inhibition with 100% O₂ inhalation significantly blunts periodic breathing in congestive heart failure patients during wakefulness (46). Nevertheless, Reichmuth et al. (49) recently found impaired cerebrovascular reactivity to hypercapnia in patients with obstructive sleep apnea, compared with an age-matched control group. Importantly, they demonstrated that the impairment of the cerebrovascular function, which correlated with the severity of the obstructive apneic events in these patients, was improved with continuous positive airway pressure treatment. In the present study, we found a relationship between the steady-state estimate of MCAv-CO₂ reactivity and breathing instability following INDO ingestion (Fig. 4). Consistent with these findings, we have recently reported that INDO-induced reductions in MCAv-CO₂ reactivity exacerbates the occurrence of both obstructive (7) and central sleep apnea at high altitude (2). Regardless of the mechanism, findings from the present study and those reported recently (2, 7, 49, 60) highlight the role of blunted cerebrovascular CO₂ reactivity in the pathogenesis of breathing instability in humans during wakefulness and sleep.

ACKNOWLEDGEMENTS

The authors thank Prof. J. Duffin who kindly provided technical assistance. Special thanks go to our participants for giving up their time for this study. We also extend our thanks to ADInstruments for the use of their laboratory equipment.

GRANTS

This study was supported by the Otago Medical Research Foundation, Sport and Physical Activity Research Council New Zealand, and The Peninsula Health Care.

DISCLOSURES

No conflicts of interest are declared by the authors.

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