

# Carbon dioxide-mediated vasomotion of extra-cranial cerebral arteries in humans: a role for prostaglandins?

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## Key points

- Cerebral blood flow increases during hypercapnia and decreases during hypocapnia; it is unknown if vasomotion of the internal carotid artery is implicated in these responses.
- Indomethacin, a non-selective cyclooxygenase inhibitor (used to inhibit prostaglandin synthesis), has a unique ability to blunt cerebrovascular carbon dioxide reactivity, while other cyclooxygenase inhibitors have no effect.
- We show significant dilatation and constriction of the internal carotid artery during hypercapnia and hypocapnia, respectively.
- Indomethacin, but not ketorolac or naproxen, reduced the dilatatory response of the internal carotid artery to hypercapnia
- The differential effect of indomethacin compared to ketorolac and naproxen suggests that indomethacin inhibits vasomotion of the internal carotid artery independent of prostaglandin synthesis inhibition.

**Abstract** Extra-cranial cerebral blood vessels are implicated in the regulation of cerebral blood flow during changes in arterial CO<sub>2</sub>; however, the mechanisms governing CO<sub>2</sub>-mediated vasomotion of these vessels in humans remain unclear. We determined if cyclooxygenase inhibition with indomethacin (INDO) reduces the vasomotor response of the internal carotid artery (ICA) to changes in end-tidal CO<sub>2</sub> ( $P_{ETCO_2}$ ). Using a randomized single-blinded placebo-controlled study, participants ( $n = 10$ ) were tested on two occasions, before and 90 min following oral INDO (1.2 mg kg<sup>-1</sup>) or placebo. Concurrent measurements of beat-by-beat velocity, diameter and blood flow of the ICA were made at rest and during steady-state stages (4 min) of iso-oxic hypercapnia (+3, +6, +9 mmHg  $P_{ETCO_2}$ ) and hypocapnia (-3, -6, -9 mmHg  $P_{ETCO_2}$ ). To examine if INDO affects ICA vasomotion independent of cyclooxygenase inhibition, two participant subsets (each  $n = 5$ ) were tested before and following oral ketorolac (post 45 min, 0.25 mg kg<sup>-1</sup>) or naproxen (post 90 min, 4.2 mg kg<sup>-1</sup>). During pre-drug testing in the INDO trial, the ICA dilated during hypercapnia at +6 mmHg ( $4.72 \pm 0.45$  vs.  $4.95 \pm 0.51$  mm;  $P < 0.001$ ) and +9 mmHg ( $4.72 \pm 0.45$  mm vs.  $5.12 \pm 0.47$  mm;  $P < 0.001$ ), and constricted during hypocapnia at -6 mmHg ( $4.95 \pm 0.33$  vs.  $4.88 \pm 0.27$  mm;  $P < 0.05$ ) and -9 mmHg ( $4.95 \pm 0.33$  vs.  $4.82 \pm 0.27$  mm;  $P < 0.001$ ). Following INDO, vasomotor responsiveness of the ICA to hypercapnia was reduced by  $67 \pm 28\%$  ( $0.045 \pm 0.015$  vs.  $0.015 \pm 0.012$  mm mmHg  $P_{ETCO_2}^{-1}$ ). There was no effect of the drug in the ketorolac and naproxen trials. We conclude that: (1) INDO markedly reduces the vasomotor response of the ICA to changes in  $P_{ETCO_2}$ ; and (2) INDO may be reducing CO<sub>2</sub>-mediated vasomotion via a mechanism(s) independent of cyclooxygenase inhibition.

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**Abbreviations** CBF, cerebral blood flow; COX, cyclooxygenase; HR, heart rate; INDO, indomethacin; ICA, internal carotid artery;  $Q_{ICA}$ , internal carotid artery blood flow; ICAv, internal carotid artery blood velocity; MAP, mean arterial pressure; MCA, middle cerebral artery; MCAv, middle cerebral artery blood velocity;  $P_{aCO_2}$ , partial pressure of arterial carbon dioxide;  $P_{ETCO_2}$ , partial pressure of end-tidal carbon dioxide;  $P_{ETO_2}$ , partial pressure of end-tidal oxygen; PG, prostaglandin; TCD, transcranial Doppler ultrasound.

## Introduction

The cerebral vasculature is highly sensitive to alterations in the partial pressure of arterial  $CO_2$  ( $P_{aCO_2}$ ). Elevations in  $P_{aCO_2}$  (hypercapnia) cause a reduction in cerebrovascular resistance and a consequent increase in cerebral blood flow (CBF), while reductions in  $P_{aCO_2}$  (hypocapnia) cause an increase in cerebrovascular resistance and a decrease in CBF (Kety & Schmidt, 1948) – the magnitude of this response is characterized as cerebrovascular  $CO_2$  reactivity. Optimal cerebrovascular  $CO_2$  reactivity acts to attenuate fluctuations in central pH and maintain homeostatic function (Ainslie & Duffin, 2009). Typically it is thought that alterations in  $P_{aCO_2}$  exclusively induce vasomotion (changes in blood vessel diameter) of small pial vessels, with no vasomotion occurring within the larger cerebral arteries (Wolff & Lennox, 1930; Serrador *et al.* 2000). This assumption has persisted despite human (Giller *et al.* 1993) and animal studies (Heistad *et al.* 1978) providing evidence to the contrary. Recent insight from high-resolution magnetic resonance imaging has further revealed that vasomotion of the middle cerebral artery (MCA) occurs in response to both hyper- and hypocapnia (Verbree *et al.* 2014; Coverdale *et al.* 2014). Additionally, using vascular ultrasound combined with automated edge detection analysis software, a tendency for vasomotion of large extra-cranial cerebral arteries across a wide range of  $P_{aCO_2}$  [e.g. internal carotid artery (ICA)] has been reported (Willie *et al.* 2012); however, these latter findings have not been consistently demonstrated when caliper-based manual diameter analysis methods are used (Sato *et al.* 2012; Coverdale *et al.* 2015).

The potential mechanism(s) whereby changes in  $P_{aCO_2}$  result in vasomotion of large extra-cranial cerebral arteries include adenosine (Phillis & DeLong, 1987), nitric oxide (Parfenova *et al.* 1994; Smith *et al.* 1997) and prostaglandins (PGs; Wennmalm *et al.* 1981; Bruhn *et al.* 2001; St Lawrence *et al.* 2002). The last-named mechanism has been ostensibly demonstrated in that administration of indomethacin (INDO), a non-selective cyclooxygenase (COX) inhibitor, reduces basal CBF by ~20–30% and cerebrovascular  $CO_2$  reactivity by ~50–60% (Eriksson *et al.* 1983; Xie *et al.* 2005; Fan *et al.* 2010; Hoiland *et al.* 2015). As illustrated in Fig. 1, this PG-mediated

vasodilatation occurs through up-regulation of cAMP and subsequent phosphorylation (i.e. deactivation) of myosin light chain kinase (Adelstein & Conti, 1978). While animal studies report PG-mediated hypercapnic vasodilatation at the level of the small pial vessels and arterioles (Pickard *et al.* 1980; Busija & Heistad, 1983; Leffler *et al.* 1991), some data collected in post-mortem humans indicate that PG-mediated vasomotion may also take place within the larger intra-cranial cerebral arteries (i.e. MCA; Davis *et al.* 2004). As optimal cerebrovascular  $CO_2$  reactivity is important in stabilizing central pH, and predictive of health outcome (Portegies *et al.* 2014), it is imperative to understand the underlying mechanisms that regulate this response. Although COX inhibition, via INDO, reduces CBF and blunts cerebrovascular  $CO_2$  reactivity (Xie *et al.* 2006; Fan *et al.* 2010; Hoiland *et al.* 2015), it is unknown if this is due in part to a reduction in vasomotion of the larger extra-cranial cerebral arteries (e.g. ICA). Lastly, *in vivo* (Eriksson *et al.* 1983; Markus *et al.* 1994) and *in vitro* (Kantor & Hampton, 1978; Goueli & Ahmed, 1980) evidence indicates that INDO exerts its vaso-motor actions independent of COX inhibition. In humans, administration of COX inhibitors other than INDO (e.g. aspirin and naproxen) do not affect cerebrovascular  $CO_2$  reactivity (Eriksson *et al.* 1983; Markus *et al.* 1994). Using *in vitro* preparations, INDO directly inhibits cAMP (a primary regulator of vascular tone) activity (Kantor & Hampton, 1978; Goueli & Ahmed, 1980).

Therefore, using a single blinded placebo-controlled and randomized design, the purposes of this study were to: (1) resolve the conflicting data surrounding hypercapnic dilatation and hypocapnic vasoconstriction of the ICA, and (2) determine if PGs are implicated in  $CO_2$ -mediated large cerebral artery vasomotion. We hypothesized that: (1) the ICA would dilate in response to hypercapnia and constrict in response to hypocapnia, and (2) COX inhibition with INDO would markedly attenuate the dilatatory response to hypercapnia and the constrictive response to hypocapnia in the ICA. Since INDO has demonstrated a unique ability to inhibit  $CO_2$ -mediated cerebrovascular responses when compared to other COX inhibitors (Eriksson *et al.* 1983; Markus *et al.* 1994), in follow-up experiments, we sought to assess the effects of ketorolac and naproxen – both potent non-selective COX

inhibitors – on CO<sub>2</sub>-mediated vasomotion of the ICA to determine if there is a unique influence of INDO under the conditions of our experimental approach. In this respect, we reasoned that neither ketorolac nor naproxen would affect the vasomotor response of the ICA to hypercapnia and hypocapnia.

## Methods

### Ethical approval

This study was approved by the University of British Columbia Clinical Research Ethics Board and conformed to the *Declaration of Helsinki*. Prior to participation in the study all participants completed written informed consent.

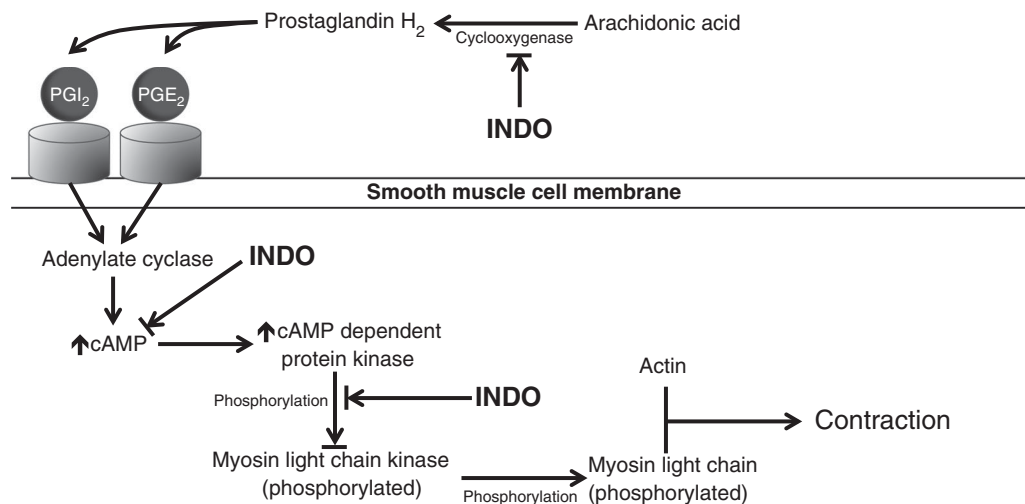
### Participants

Fifteen healthy young volunteers were recruited to participate in this study. The main study, (INDO investigations) included ten participants (one female) with a mean age of  $23 \pm 7$  years, and body mass index of  $22 \pm 2$  kg m<sup>-2</sup>. The female participant was tested on days 1 and 3 of her self-reported follicular phase, thus minimizing any potential sex difference in the response to COX inhibition and CO<sub>2</sub> perturbations (Peltonen *et al.* 2015). In the follow-up experiments, two sub-groups of five participants (all male) were examined for the ketorolac ( $30 \pm 7$  years; body mass index

of  $24 \pm 2$  kg m<sup>-2</sup>) and naproxen ( $24 \pm 3$  years; body mass index of  $22 \pm 1$  kg m<sup>-2</sup>) trials. During familiarization, participants were screened to ensure that reliable ICA ultrasound images and MCA signals could be attained. Participants were familiarized with the remaining experimental equipment and procedures during this session. All participants were free of cardiovascular, respiratory, cerebrovascular, gastro-intestinal and liver disease, were non-diabetic, and were not taking any prescription drugs (other than oral contraceptives;  $n = 1$ ) at their time of participation, as determined by a screening questionnaire.

### Experimental measures

**Cardiorespiratory measures.** All cardiorespiratory variables were sampled continuously throughout the protocol at 1000 Hz via an analog-to-digital converter (Powerlab, 16/30; ADInstruments, Colorado Springs, CO, USA). Heart rate (HR) was measured by a three-lead electrocardiogram (ECG; ADI bioamp ML132), and beat-to-beat blood pressure by finger photoplethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, Netherlands). The Finometer reconstructed brachial waveform was used for the calculation of mean arterial pressure (MAP) after values were back calibrated to the average of three automated brachial blood pressure measurements made over 5 min (Tango+; SunTech, Morrisville, NC, USA). The



**Figure 1. Putative effects of INDO on cerebral smooth muscle cell function**

Vasodilator prostaglandins (prostaglandin I<sub>2</sub> and E<sub>2</sub>; PGI<sub>2</sub> and PGE<sub>2</sub>) produced downstream of arachidonic acid bind prostaglandin receptors, which activate (→) cAMP, leading to up-regulation of cAMP-dependent protein kinase and subsequent inhibition (→|) of myosin light chain kinase (Adelstein & Conti, 1978). By inhibiting this response, downstream phosphorylation of myosin light chain and its consequent contribution to contraction does not occur, resulting in smooth muscle cell relaxation, and/or vasodilatation (Kerrick & Hoar, 1981). INDO probably exerts its affect(s), in addition to COX inhibition, on post-receptor-mediated increases in cAMP (Parfenova *et al.* 1995) and/or by inhibiting cAMP-dependent protein kinase activity (Kantor & Hampton, 1978; Goueli & Ahmed, 1980).

partial pressure of both end-tidal CO<sub>2</sub> ( $P_{\text{ETCO}_2}$ ) and end-tidal O<sub>2</sub> ( $P_{\text{ETO}_2}$ ) were sampled at the mouth and recorded by a calibrated gas analyser (model ML206, ADInstruments), while respiratory flow was measured by a pneumotachograph (model HR 800L, HansRudolph, Shawnee, KS, USA) connected to a bacteriological filter. All data were interfaced with LabChart (Version 7), and analysed offline. Average values for the last minute of each stage were recorded (see *Experimental protocol*).

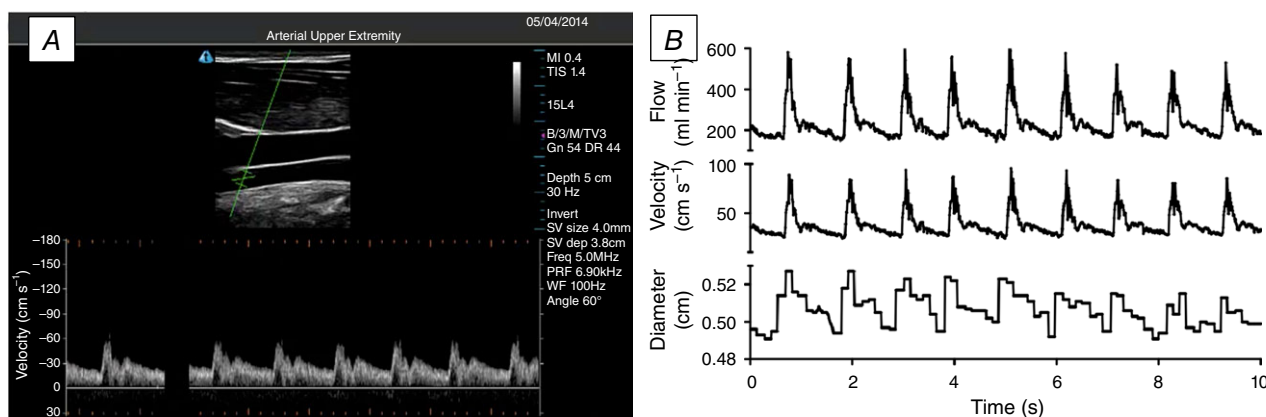
**Dynamic end-tidal forcing.**  $P_{\text{ETO}_2}$  and  $P_{\text{ETCO}_2}$  were controlled by a portable dynamic end-tidal forcing system. This system uses independent gas solenoid valves for O<sub>2</sub>, CO<sub>2</sub> and N<sub>2</sub> and controls the volume of each gas delivered into the inspiratory reservoir through a mixing and humidification chamber.  $P_{\text{ETO}_2}$ ,  $P_{\text{ETCO}_2}$ , both inspiratory and expiratory tidal volume, breathing frequency and minute ventilation were determined for each breath in real time using custom software (Labview 13.0, National Instruments, Austin, TX, USA). Using feedback information regarding  $P_{\text{ETO}_2}$ ,  $P_{\text{ETCO}_2}$ , and inspiratory and expiratory tidal volume, the dynamic end-tidal forcing system adjusts the inspirate on a breath-by-breath basis to control end-tidal gases at a desired target value. Feed-forward control of the inspirate is based on estimates of baseline metabolic O<sub>2</sub> consumption and CO<sub>2</sub> production and employs the alveolar gas equation to determine the required volumes of O<sub>2</sub> and CO<sub>2</sub>. Feedback control is accomplished using a proportional and integral error reduction control system. End-tidal steady-state for each stage (see *Experimental protocol*) was determined once values were within 1 mmHg of the desired target point for at least three consecutive breaths. Our end-tidal forcing system effectively controls end-tidal gases through wide ranges of  $P_{\text{ETCO}_2}$  and  $P_{\text{ETO}_2}$

independent of ventilation at low altitude (Querido *et al.* 2013; Tymko *et al.* 2015, 2016), high altitude (Tymko *et al.* 2016), with hyperthermic interventions (Bain *et al.* 2013) and during exercise (our unpublished observations).

**Cerebrovascular measures.** Blood velocity through the right MCA (MCAv) was measured using a 2 MHz transcranial Doppler ultrasound (TCD; Spencer Technologies, Seattle, WA, USA). The TCD probe was attached to a specialized headpiece (model M600 bilateral head frame, Spencer Technologies), and then secured in place. The MCA was insonated through the middle trans-temporal window, using previously described location and standardization techniques (Willie *et al.* 2011).

Blood velocity and vessel diameter of the ICA were measured using a 10 MHz multi-frequency linear array vascular ultrasound (Terason T3200, Teratech, Burlington, MA, USA). Specifically, B-mode imaging was used to measure arterial diameter, while pulse-wave mode was used to simultaneously measure peak blood velocity (Fig. 2A). Measures of ICA flow ( $Q_{\text{ICA}}$ ) were made ipsilateral to the MCA. The ICA diameter and velocity were measured at least 1.5 cm distal to the common carotid bifurcation to eliminate recordings of turbulent and retrograde flow. Great care was taken to ensure that the insonation angle (60 deg) was unchanged throughout each test. Furthermore, for all experimental sessions, upon acquisition of the first ultrasound image there was no alteration of B-mode gain to avoid any artificial changes in arterial wall brightness/thickness.

All of the ICA recordings were screen captured and stored as video files for offline analysis. This analysis involved concurrent determination of arterial diameter and peak blood velocity at 30 Hz, using customized edge



**Figure 2. Duplex ultrasound image of the ICA and analysis output**

A, a typical image during the recording of ICA flow, diameter and velocity. The velocity sample gate (cross-hairs in the B-mode image) are >1.5 cm distal to the carotid bulb, which would appear just to the right of the visible section if the image were extended. The resultant velocity waveform is free of any retrograde flow and aliasing. B-mode depth is set to 5 cm for this recording. B, data output from a typical ICA trace, showing beat-by-beat flow, diameter and velocity at 30 Hz.



detection and wall tracking software designed to mitigate observer bias (Fig. 2B) (Woodman *et al.* 2001). Based on  $n = 12$  young healthy subjects, unpublished findings from our laboratory show that the variability in analysis is much greater (approximately 25–30%) when using manual compared to automated approaches. Moreover, the intra-observer variability is similarly high when using manual compared to automated approaches. No fewer than 12 consecutive cardiac cycles were used to determine  $Q_{ICA}$ . Volumetric blood flow was calculated using the following formula:

$$Q_{ICA} = \frac{\text{Peak envelope velocity}}{2} \cdot [\pi(0.5 \cdot \text{Diameter})^2]$$

Our within- and between-day coefficients of variation for the assessment of ICA diameter are 1.5 and 4.4%, respectively. Volumetric blood flow (i.e. ICA) and velocity (i.e. MCA) values were calculated within the final minute of each 4 min steady state stage. To help account for MAP in our analysis of the CBF responses, cerebrovascular conductance (CVC) was subsequently calculated as  $Q_{ICA}/MAP$ . As cerebrovascular reactivity to hypercapnia is approximately double that of hypocapnic reactivity, cerebrovascular responses were calculated separately for the hypercapnic and hypocapnic reactivity tests (Willie *et al.* 2012). All response slopes (e.g. mm mmHg  $P_{ETCO_2}^{-1}$ ) were calculated using linear regression (Willie *et al.* 2012; Skow *et al.* 2013; Tymko *et al.* 2016). The Pearson  $r$  correlation for all the diameter responses was  $\geq 0.85$ , indicating linearity of the diameter responses.

### Experimental protocol

On the day of experimental sessions participants arrived in the laboratory at the same time of day having refrained from alcohol, exercise and caffeine for the previous 24 h. Participants were instructed to lie supine for at least 15 min prior to beginning the study protocol and were simultaneously instrumented with the experimental equipment.

**Study 1.** To investigate the role of non-selective COX inhibition via oral INDO we used a single blinded, randomized and counterbalanced placebo-controlled trial requiring two laboratory visits. On each day, following 5 min of baseline measurements (HR, MAP, MCAv,  $Q_{ICA}$ ,  $P_{ETCO_2}$  and  $P_{ETO_2}$ ) while breathing room air, the participants performed two iso-oxic CO<sub>2</sub> reactivity tests (a hypercapnic test followed by a hypocapnic test). The tests were separated by  $\geq 10$  min and a return of MAP, HR and MCAv to baseline values was confirmed prior to proceeding with the hypocapnic test. Thereafter, they were orally administered 1.2 mg kg<sup>-1</sup> of INDO or placebo (sugar pill matched for weight and capsule size), and repeated the baseline measures and the iso-oxic CO<sub>2</sub> tests 90 min later

(Xie *et al.* 2006). This dose of INDO used has been previously shown to effectively inhibit COX activity (Eriksson *et al.* 1983). Test days were separated by  $10 \pm 9$  days. The iso-oxic CO<sub>2</sub> reactivity tests are as follows:

**Test 1: Steady state iso-oxic elevations of  $P_{ETCO_2}$ .** Four minutes of room air breathing were completed to determine baseline  $P_{ETCO_2}$  and  $P_{ETO_2}$  values. Following room air breathing, dynamic end-tidal forcing was utilized to maintain  $P_{ETCO_2}$  and  $P_{ETO_2}$  at baseline (resting) values on an individual basis for 4 min (Tymko *et al.* 2015, 2016). Upon completion of this baseline stage,  $P_{ETO_2}$  remained unchanged while  $P_{ETCO_2}$  was sequentially elevated to +3, +6 and +9 mmHg  $P_{ETCO_2}$  above baseline, with each stage lasting 4 min after reaching steady-state  $P_{ETCO_2}$ . Upon completion of the iso-oxic CO<sub>2</sub> reactivity protocols, participants were removed from the breathing apparatus, and recovered breathing room air.

**Test 2: Steady state iso-oxic reductions in  $P_{ETCO_2}$ .** Four minutes of room air breathing was completed to determine baseline  $P_{ETCO_2}$  and  $P_{ETO_2}$  values. Following room air breathing, dynamic end-tidal forcing was utilized to maintain  $P_{ETCO_2}$  and  $P_{ETO_2}$  at baseline (resting) values on an individual basis for 4 min (Tymko *et al.* 2015, 2016). Immediately after baseline, participants were instructed to actively hyperventilate to sequentially lower their  $P_{ETCO_2}$  to -3, -6 and -9 mmHg below baseline values while  $P_{ETO_2}$  remained unchanged, with each  $P_{ETCO_2}$  step lasting 4 min (after  $P_{ETCO_2}$  had stabilized). Once participants had reached an adequate level of hyperventilation to achieve the desired  $P_{ETCO_2}$ , participants were instructed to maintain constant ventilation for the remainder of the stages (i.e. for -3, -6 and -9 mmHg stages). Inspiratory  $P_{ETCO_2}$  was then altered to achieve the desired  $P_{ETCO_2}$  changes required for each stage.

**Study 2.** To investigate the role of non-selective COX inhibition using orally administered ketorolac (Toradol®), participants attended the lab on one occasion. Following 5 min of baseline measurements (HR, MAP, MCAv,  $Q_{ICA}$ ,  $P_{ETCO_2}$  and  $P_{ETO_2}$ ) while breathing room air, the participants performed the identical two CO<sub>2</sub> reactivity tests as in Study 1 (a hypercapnic test followed by a hypocapnic test). These tests were repeated 45 min later following orally administered ketorolac (0.25 mg kg<sup>-1</sup>). Previous studies have confirmed the effectiveness of COX inhibition by this dose of ketorolac at 45 min (peak plasma concentration) with an associated half-life of ~5–6 h (Jung *et al.* 1989; Jallad *et al.* 1990).

**Study 3.** To investigate the role of non-selective COX inhibition via orally administered naproxen (Aleve®), participants attended the lab on one occasion. Following 5 min of baseline measurements (HR, MAP, MCAv,  $Q_{ICA}$ ,  $P_{ETCO_2}$  and  $P_{ETO_2}$ ) while breathing room air, the participants performed the identical two CO<sub>2</sub> reactivity

tests as in Study 1 (a hypercapnic test followed by a hypocapnic test). These tests were repeated 90 min later following orally administered naproxen ( $4.2 \text{ mg kg}^{-1}$ ). Previous studies have confirmed the effectiveness of COX inhibition by this dose of naproxen (Eriksson *et al.* 1983).

### Statistical analysis

All cardiovascular, cerebrovascular and respiratory variables were analysed within trial (i.e. INDO, placebo, ketorolac and naproxen) using two-way repeated measures ANOVAs. When significant F-ratios were detected, *post-hoc* comparisons were made using Tukey's honest significant difference (HSD) test. To further assess the effect of our interventions on cerebral reactivity (i.e. flow and vasomotor reactivity) specific to study 1, one-way repeated measures ANOVAs were used to compare pre-INDO *vs.* post-INDO *vs.* pre-placebo *vs.* post-placebo reactivity slopes. *Post-hoc* comparisons were made using Tukey's HSD test. Normality of the data was confirmed using the Shapiro–Wilks test. Reactivity slopes (i.e.  $\text{mm mmHg } P_{\text{ETCO}_2}^{-1}$ ) were calculated using linear regression. The effects of ketorolac and naproxen on reactivity slopes were assessed using two-tailed paired *t*-tests (pre- *vs.* post-drug). Pearson *r* correlations were used to assess the relationship between MAP and vasomotor reactivity. All data are expressed as means  $\pm$  standard deviation with *a priori* statistical significance set at  $P < 0.05$ .

## Results

### Study 1: INDO and placebo trials

**Resting CBF and cardiorespiratory variables.** Resting  $Q_{\text{ICA}}$  was reduced by  $40 \pm 12\%$  following INDO ( $257.3 \pm 57.2$  *vs.*  $151.9 \pm 36.6 \text{ ml min}^{-1}$ ;  $P < 0.01$ ), while placebo treatment had no effect ( $257.8 \pm 62.4$  *vs.*  $252.3 \pm 62.4 \text{ ml min}^{-1}$ ;  $P = 0.55$ ). Similarly, INDO reduced resting MCAv by  $36 \pm 11\%$  ( $65.5 \pm 8.6$  *vs.*  $42.0 \pm 8.8 \text{ cm s}^{-1}$ ;  $P < 0.01$ ), while placebo had no effect ( $62.4 \pm 12.4$  *vs.*  $60.7 \pm 14.3 \text{ cm s}^{-1}$ ;  $P = 0.43$ ). Pre-INDO, resting  $Q_{\text{ICA}}$  and MCAv were not different from the pre-placebo and placebo trials. Resting ventilation was unaffected by INDO; however, compared to the placebo, INDO caused a modest increase in MAP ( $77.0 \pm 5.6$  *vs.*  $83.8 \pm 8.7 \text{ mmHg}$ ;  $P = 0.03$ ) and decreased HR ( $57.4 \pm 11.0$  *vs.*  $49.9 \pm 8.6 \text{ beats min}^{-1}$ ;  $P < 0.01$ ).

**INDO CO<sub>2</sub> trials.** Cardiovascular, cerebrovascular and respiratory variables at all stages of the INDO trials are presented in Table 1. Both HR and MAP were elevated at +6 and +9 mmHg  $P_{\text{ETCO}_2}$  pre- and post-INDO in the hypercapnic trial, while MAP was unaltered and HR was reduced at –6 and –9 mmHg  $P_{\text{ETCO}_2}$  during

hypocapnia pre- and post-INDO. There was a main effect of INDO on MAP and HR during hypercapnia, with MAP being higher post-INDO and HR lower. In hypocapnia, HR was lower post-INDO. There was a distinct individual variability in the MAP response (MAP increase ranged from 0 to 15 mmHg above baseline) to hypercapnia, but there was no relationship between MAP reactivity and vasomotor reactivity pre- ( $r^2 = 0.05$ ;  $P = 0.53$ ) or post- ( $r^2 = 0.05$ ;  $P = 0.53$ ) INDO. The change in MAP reactivity and vasomotor reactivity from pre- to post-INDO for the hypercapnic tests were also not related ( $r^2 = 0.02$ ;  $P = 0.73$ ).

Hypercapnia increased MCAv at all stages during the pre-INDO trial, but only at +6 and +9 mmHg  $P_{\text{ETCO}_2}$  during the post-INDO trial. Similarly, MCAv was reduced at all stages during hypocapnia pre-INDO, but only at –6 and –9 mmHg  $P_{\text{ETCO}_2}$  following INDO. Prior to INDO,  $Q_{\text{ICA}}$  was elevated at all stages of hypercapnia; however, this elevation was only apparent at +9 mmHg  $P_{\text{ETCO}_2}$  post-INDO. During hypocapnia,  $Q_{\text{ICA}}$  was reduced at all stages pre-INDO, but only at –6 and –9 mmHg  $P_{\text{ETCO}_2}$  following INDO. Pre- and post-INDO ICA diameter was elevated at +6 and +9 mmHg  $P_{\text{ETCO}_2}$  (Fig. 3A), while during hypocapnia, ICA diameter was reduced at every stage pre- and post-INDO (Fig. 4A). Pre-INDO, CVC was elevated at all stages of hypercapnia, but only at +9 mmHg  $P_{\text{ETCO}_2}$  post-INDO. During hypocapnia, CVC was reduced at all stages pre-INDO, but did not change during the post-INDO trial. There was a main effect of INDO on MCAv,  $Q_{\text{ICA}}$ , ICA diameter and CVC in hypo- and hypercapnia, all of which were lower post-INDO.

**Placebo CO<sub>2</sub> trials.** Cardiovascular, cerebrovascular and respiratory variables at all stages of the placebo trials are presented in Table 2. During hypercapnia, MAP was elevated at all stages while HR was elevated at +6 and +9 mmHg  $P_{\text{ETCO}_2}$ . There was no effect of hypocapnia on MAP or HR.

Hypercapnia increased MCAv and  $Q_{\text{ICA}}$  at all stages pre- and post-placebo, while hypocapnia decreased MCAv and  $Q_{\text{ICA}}$  at all stages pre- and post-placebo. Pre- and post-placebo ICA diameter was elevated at all stages of hypercapnia (Fig. 3B), and decreased during the –6 and –9 mmHg  $P_{\text{ETCO}_2}$  hypocapnic stages (Fig. 4B). Hypercapnia increased CVC at all stages while hypocapnia decreased CVC at all stages.

**Cerebrovascular CO<sub>2</sub> reactivity.** The slope responses of  $Q_{\text{ICA}}$ , MCAv, MAP and ventilation to changes in  $P_{\text{ETCO}_2}$  during the INDO and placebo trials are presented in Table 3. These variables were compared across all conditions (pre-INDO *vs.* post-INDO *vs.* pre-placebo *vs.* post-placebo). Absolute hypercapnic  $Q_{\text{ICA}}$  and MCAv reactivity were reduced by  $69 \pm 20$  and  $59 \pm 28\%$  following INDO ( $P < 0.01$  for both), respectively, and as expected

**Table 1. Cerebrovascular and cardiorespiratory variables before and following INDO during hyper- and hypocapnia**

	Hypercapnia				Hypocapnia				
	Baseline	+3 mmHg	+6 mmHg	+9 mmHg	Baseline	-3 mmHg	-6 mmHg	-9 mmHg	
<i>Q</i> <sub>ICA</sub> (ml min <sup>-1</sup> )	Pre	<b>256.2 ± 62.9</b>	<b>299.6 ± 75.2*</b>	<b>345.1 ± 94.9*</b>	<b>429.5 ± 116.3*</b>	259.9 ± 70.3	224.1 ± 45.5*	207.9 ± 42.7*	184.7 ± 39.6*
	Post	<b>163.1 ± 40.2†</b>	<b>172.0 ± 40.2†</b>	<b>187.4 ± 40.6†</b>	<b>210.0 ± 48.0†*</b>	174.2 ± 40.1†	153.1 ± 37.2†	153.8 ± 34.0†	151.4 ± 32.5†
ICAV (cm s <sup>-1</sup> )	Pre	44.5 ± 4.4	49.9 ± 5.8*	54.6 ± 5.7*	63.2 ± 7.1*	41.0 ± 7.9	36.7 ± 5.4*	34.4 ± 4.6*	31.3 ± 4.5*
	Post	31.4 ± 5.0†	32.8 ± 5.6†	34.9 ± 5.4†	38.8 ± 6.5†	33.1 ± 5.5†	29.8 ± 5.8†	29.9 ± 4.6†	29.5 ± 4.2*
Diameter (mm)	Pre	4.91 ± 0.53	4.99 ± 0.50	5.12 ± 0.53*	5.31 ± 0.54*	5.15 ± 0.45	5.07 ± 0.39*	5.04 ± 0.35*	4.97 ± 0.35*
	Post	4.66 ± 0.52†	4.70 ± 0.51†	4.76 ± 0.53†	4.78 ± 0.51†	4.70 ± 0.45	4.67 ± 0.48*	4.65 ± 0.47*	4.65 ± 0.51*
MCAv (cm s <sup>-1</sup> )	Pre	65.8 ± 8.6	74.1 ± 10.4*	82.7 ± 11.9*	94.7 ± 13.5*	63.7 ± 9.6	57.8 ± 9.0*	53.2 ± 8.4*	50.2 ± 7.5*
	Post	41.3 ± 8.0†	44.2 ± 8.9†	47.9 ± 11.6†	53.1 ± 15.3†	46.0 ± 10.7†	43.8 ± 9.2†	43.5 ± 9.1†	42.3 ± 7.6†
MAP (mmHg)	Pre	79.1 ± 7.2	80.8 ± 7.5	84.6 ± 7.2*	87.2 ± 8.7*	81.4 ± 9.5	81.6 ± 7.7	84.4 ± 9.2	87.5 ± 7.4
	Post	85.6 ± 9.8	87.1 ± 11.6	90.0 ± 9.8*	92.9 ± 8.7*	87.5 ± 7.4†	86.8 ± 9.0	87.0 ± 10.3	86.8 ± 11.2
CVC (ml min <sup>-1</sup> mmHg <sup>-1</sup> )	Pre	3.26 ± 0.88	3.55 ± 0.77*	3.85 ± 0.88*	4.97 ± 1.48*	3.17 ± 0.68	2.74 ± 0.48*	2.46 ± 0.40*	2.18 ± 0.39*
	Post	1.90 ± 0.44†	2.01 ± 0.58†	2.09 ± 0.47†	2.27 ± 0.55†	1.99 ± 0.47†	1.76 ± 0.41†	1.77 ± 0.40†	1.72 ± 0.36†
HR (beats min <sup>-1</sup> )	Pre	58.6 ± 11.5	62.8 ± 11.1	65.1 ± 10.6*	71.1 ± 10.9*	56.6 ± 11.0	59.7 ± 8.8	61.4 ± 8.8*	58.4 ± 9.4
	Post	52.2 ± 8.3	53.0 ± 10.1	56.7 ± 9.3*	62.0 ± 10.1*	52.7 ± 10.2	55.2 ± 9.6	56.9 ± 10.5*	56.0 ± 10.2
<i>V</i> <sub>E</sub> (l min <sup>-1</sup> )	Pre	14.3 ± 3.5	20.4 ± 4.2*	28.5 ± 6.2*	39.1 ± 8.2*	14.3 ± 3.2	24.9 ± 9.1*	26.2 ± 7.0*	26.9 ± 7.5*
	Post	16.5 ± 4.8	22.3 ± 5.3*	32.0 ± 8.5*	43.8 ± 10.7*	18.3 ± 5.4	25.9 ± 3.8*	30.3 ± 6.0*	29.7 ± 6.3*
<i>P</i> <sub>ETCO<sub>2</sub></sub> (mmHg)	Pre	40.7 ± 1.9	43.9 ± 1.8*	46.6 ± 1.8*	49.8 ± 1.7*	40.8 ± 1.7	37.9 ± 1.5*	34.8 ± 1.6*	32.1 ± 1.3*
	Post	40.2 ± 1.3	43.0 ± 1.2*	46.0 ± 1.1*	49.0 ± 1.1*	40.1 ± 1.1	37.1 ± 1.2*	34.1 ± 1.2*	30.8 ± 1.4*
<i>P</i> <sub>ETO<sub>2</sub></sub> (mmHg)	Pre	96.3 ± 3.0	94.9 ± 1.9	95.1 ± 2.4	95.0 ± 2.2	95.7 ± 2.7	95.2 ± 3.4	96.2 ± 3.8	95.1 ± 3.1
	Post	95.9 ± 4.5	95.9 ± 3.9	95.2 ± 3.5	95.5 ± 3.6	96.6 ± 4.5	95.5 ± 3.7	95.5 ± 4.1	96.2 ± 3.4

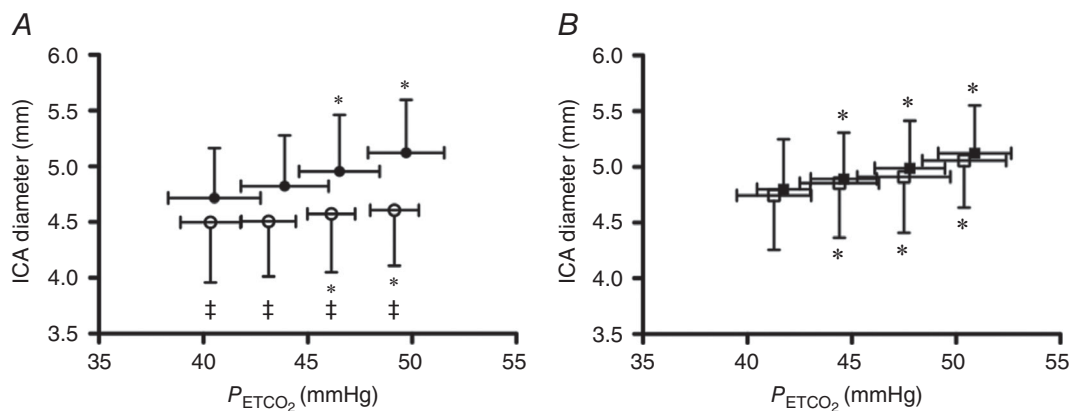
Pre or Post in bold type indicates main effect of the intervention, with the bolded trial significantly greater. \*Significant difference from baseline. †Significant interaction between pre and post. *V*<sub>E</sub>, expiratory tidal volume.

were unaltered by placebo treatment ( $Q_{ICA}$   $P = 0.22$ ;  $MCAv$   $P = 0.69$ ) (Fig. 5). For both  $Q_{ICA}$  and  $MCAv$ , the pre-INDO, pre-placebo and placebo trials did not differ in their respective absolute hypercapnic reactivity. Following INDO, relative hypercapnic  $Q_{ICA}$  and  $MCAv$  reactivity were reduced by  $50 \pm 33$  and  $38 \pm 36\%$ , respectively, but were unaltered by placebo treatment ( $Q_{ICA}$   $P = 0.32$ ;  $MCAv$   $P = 0.31$ ). For both  $Q_{ICA}$  and  $MCAv$ , the pre-INDO, pre-placebo and placebo trials did not differ in their respective relative hypercapnic reactivities.

Although unchanged in the placebo trial ( $Q_{ICA}$   $P = 0.15$ ;  $MCAv$   $P = 0.63$ ), absolute hypocapnic  $Q_{ICA}$  and  $MCAv$  reactivity were significantly reduced by  $72 \pm 25$  and  $79 \pm 25\%$  (both  $P < 0.01$ ), respectively, following INDO (Fig. 6). For both  $Q_{ICA}$  and  $MCAv$ , the

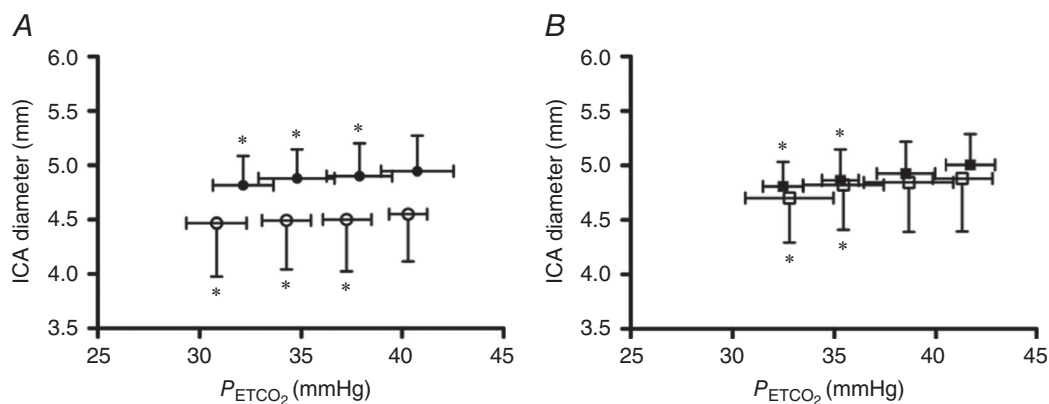
pre-INDO, pre-placebo and placebo trials did not differ in their respective absolute hypocapnic reactivities. Relative hypocapnic  $Q_{ICA}$  and  $MCAv$  reactivity were reduced by  $60 \pm 32\%$  ( $P < 0.01$ ) and  $76 \pm 32\%$  ( $P < 0.01$ ), respectively, following INDO, but were unaltered following the placebo treatment ( $Q_{ICA}$   $P = 0.23$ ;  $MCAv$   $P = 0.48$ ). For both  $Q_{ICA}$  and  $MCAv$ , the pre-INDO, pre-placebo and placebo trials did not differ in their respective relative hypocapnic reactivities.

Hypercapnic reactivity was greater than that during hypocapnia for  $Q_{ICA}$  and  $MCAv$  during all trials (Table 3). During hypercapnia, relative  $Q_{ICA}$  reactivity was greater than that of both  $ICAv$  ( $7.3 \pm 2.0$  vs.  $3.5 \pm 2.2\%$   $\text{mmHg } P_{ETCO_2}^{-1}$ ;  $P < 0.01$ ) and  $MCAv$  ( $7.3 \pm 2.0$  vs.  $4.8 \pm 0.9\%$   $\text{mmHg } P_{ETCO_2}^{-1}$ ;  $P < 0.01$ ) reactivity, which



**Figure 3. The vasomotor response to hypercapnia**

A, the vasomotor response to hypercapnia pre- (●) and post- (○) INDO. B, the vasomotor response to hypercapnia pre- (■) and post- (□) placebo. There was a main effect of INDO, but not placebo, on ICA diameter, which was lower following INDO. Data were analysed using a two-way repeated measures ANOVA and *post-hoc* comparisons were made using Tukey's HSD. \*Different from baseline,  $P < 0.05$ ; ‡between-trial difference in diameter between baseline and intervention,  $P < 0.05$ .



**Figure 4. The vasomotor response to hypocapnia**

A, the vasomotor response to hypocapnia pre- (●) and post- (○) INDO. B, the vasomotor response to hypocapnia pre- (■) and post- (□) placebo. There was a main effect of INDO, but not placebo, on ICA diameter, which was lower following INDO. Data were analysed using a two-way repeated measures ANOVA and *post-hoc* comparisons were made using Tukey's HSD. \*Different from baseline,  $P < 0.05$ .



**Table 2. Cerebrovascular and cardiorespiratory variables before and following Placebo during hyper and hypocapnia**

	Hypercapnia			Hypocapnia					
	Baseline	+3 mmHg	+6 mmHg	+9 mmHg	Baseline	-3 mmHg	-6 mmHg	-9 mmHg	
<b>Q<sub>ICA</sub></b> (ml min <sup>-1</sup> )	Pre	258.9 ± 49.1	310.8 ± 69.1*	357.2 ± 73.5*	413.1 ± 79.2*	261.6 ± 49.4	237.6 ± 51.8*	209.9 ± 49.2*	190.8 ± 39.4*
	Post	263.3 ± 51.9	298.4 ± 56.7*	346.0 ± 70.4*	408.3 ± 67.8*	261.9 ± 58.4	237.1 ± 46.2*	225.2 ± 46.4*	200.2 ± 40.3*
<b>ICAV</b> (cm s <sup>-1</sup> )	Pre	44.0 ± 5.2	49.0 ± 4.9*	56.1 ± 6.9*	61.9 ± 7.7*	40.8 ± 5.6	37.7 ± 4.6*	34.2 ± 4.2*	31.8 ± 4.2*
	Post	45.9 ± 7.6	49.5 ± 6.9*	55.7 ± 7.7*	62.9 ± 7.4*	42.2 ± 4.9	38.9 ± 4.7*	37.6 ± 4.6*	34.9 ± 3.7*
<b>Diameter</b> (mm)	Pre	4.80 ± 0.45	4.89 ± 0.42*	4.99 ± 0.43*	5.12 ± 0.43*	5.01 ± 0.28	4.93 ± 0.29	4.87 ± 0.28*	4.81 ± 0.22*
	Post	4.75 ± 0.49	4.85 ± 0.49*	4.91 ± 0.50*	5.06 ± 0.42*	4.88 ± 0.49	4.84 ± 0.45	4.82 ± 0.41*	4.70 ± 0.41*
<b>MCAV</b> (cm s <sup>-1</sup> )	Pre	63.9 ± 12.8	69.7 ± 13.6*	78.7 ± 15.8*	89.1 ± 15.5*	61.7 ± 12.6	55.1 ± 12.0*	50.0 ± 11.2*	47.5 ± 10.0*
	Post	60.8 ± 14.3	68.3 ± 15.4*	76.6 ± 16.2*	87.3 ± 16.5*	61.2 ± 12.9	55.0 ± 11.8*	50.7 ± 11.2*	48.0 ± 9.1*
<b>MAP</b> (mmHg)	Pre	81.0 ± 6.8	82.7 ± 7.1*	84.0 ± 7.3*	89.0 ± 8.7*	81.1 ± 7.0	81.5 ± 7.9	82.5 ± 8.6	83.8 ± 8.9
	Post	78.8 ± 6.1	82.3 ± 9.3*	86.4 ± 10.0*	89.2 ± 10.9*	82.8 ± 9.6	81.8 ± 6.8	81.1 ± 8.8	82.5 ± 7.7
<b>CVC</b> (ml min <sup>-1</sup> mmHg <sup>-1</sup> )	Pre	3.21 ± 0.61	3.78 ± 0.88*	4.29 ± 1.01*	4.67 ± 0.96*	3.21 ± 0.46	2.91 ± 0.54*	2.55 ± 0.55*	2.28 ± 0.42*
	Post	3.37 ± 0.72	3.66 ± 0.79*	4.05 ± 0.95*	4.63 ± 0.89*	3.17 ± 0.66	2.91 ± 0.58*	2.80 ± 0.62†	2.44 ± 0.51*
<b>HR</b> (beats min <sup>-1</sup> )	Pre	58.1 ± 9.0	59.7 ± 8.4	64.1 ± 6.9*	68.1 ± 5.9*	55.5 ± 8.1	52.5 ± 19.9	57.1 ± 7.3	57.2 ± 7.8
	Post	94.5 ± 3.2	94.0 ± 2.9	94.0 ± 3.0*	93.8 ± 2.9*	57.4 ± 10.7	60.3 ± 11.4	62.8 ± 13.7	63.1 ± 11.5
<b>V<sub>E</sub></b> (l min <sup>-1</sup> )	Pre	14.3 ± 3.3	20.4 ± 4.8*	31.1 ± 8.0*	41.0 ± 11.1*	16.0 ± 5.3	25.3 ± 8.6*	24.9 ± 7.8*	25.0 ± 7.9*
	Post	15.7 ± 4.1	21.9 ± 5.7*	32.2 ± 8.1*	41.7 ± 11.4*	17.5 ± 5.7	23.5 ± 7.1*	25.0 ± 5.9*	27.4 ± 7.2*
<b>P<sub>ETCO<sub>2</sub></sub></b> (mmHg)	Pre	41.7 ± 1.3	44.6 ± 1.6*	47.8 ± 1.7*	50.9 ± 1.7*	41.7 ± 1.2	38.5 ± 1.4*	35.3 ± 0.9*	32.5 ± 1.0*
	Post	41.3 ± 1.8	44.4 ± 1.9*	47.5 ± 2.2*	50.4 ± 2.0*	41.3 ± 1.5	38.7 ± 2.2*	35.4 ± 2.0*	32.8 ± 2.2*
<b>P<sub>ETO<sub>2</sub></sub></b> (mmHg)	Pre	94.1 ± 6.3	95.0 ± 6.5	95.0 ± 6.6	94.7 ± 6.2	94.4 ± 5.4	94.0 ± 5.1	92.9 ± 5.5	93.8 ± 4.7
	Post	94.5 ± 3.2	94.0 ± 2.9	94.0 ± 3.0	93.8 ± 2.9	94.0 ± 2.9	94.6 ± 3.2	94.3 ± 3.3	93.9 ± 3.0

Pre or Post in bold type indicates main effect of the intervention, with the bolded trial significantly greater. \*Significant difference from baseline. †Significant interaction between pre and post. V<sub>E</sub>, expiratory tidal volume.

Table 3. Cerebrovascular and blood pressure responses to CO<sub>2</sub> before and following INDO or placebo

	Units	Absolute reactivity				Relative reactivity				
		Pre-INDO	INDO	Pre-Placebo	Placebo	Pre-INDO	INDO	Pre-Placebo	Placebo	
		Units	Units	Units	Units	Units	Units	Units	Units	
<b>Hypercapnia</b>										
Q <sub>ICA</sub>	(ml min <sup>-1</sup> mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	18.9 ± 7.3 <sup>†</sup>	5.3 ± 3.0 <sup>†*</sup>	17.1 ± 5.3 <sup>†</sup>	15.9 ± 3.3 <sup>†</sup>	(% mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	7.3 ± 2.0 <sup>†</sup>	3.5 ± 2.2 <sup>†*</sup>	6.7 ± 1.9 <sup>†</sup>	6.2 ± 1.2 <sup>†</sup>
MCAV	(cm s <sup>-1</sup> mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	3.2 ± 0.7 <sup>†</sup>	1.3 ± 1.0 <sup>†*</sup>	2.8 ± 0.7 <sup>†</sup>	2.9 ± 0.7 <sup>†</sup>	(% mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	4.8 ± 0.9 <sup>†</sup>	3.0 ± 1.9 <sup>†*</sup>	4.5 ± 1.3 <sup>†</sup>	5.0 ± 1.8 <sup>†</sup>
MAP	(mmHg mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	0.9 ± 0.6	0.8 ± 0.5	0.9 ± 0.4	1.2 ± 0.6	(% mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	1.2 ± 0.8	1.0 ± 0.7	1.0 ± 0.4	1.5 ± 0.4
V <sub>E</sub>	(l min <sup>-1</sup> mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	2.7 ± 0.8	3.1 ± 1.0	3.0 ± 1.2	2.9 ± 1.3	(% · mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	19.8 ± 6.1	19.9 ± 8.1	22.1 ± 10.6	19.7 ± 9.7
<b>Hypocapnia</b>										
Q <sub>ICA</sub>	(ml min <sup>-1</sup> mmHg P <sub>ETCO<sub>2</sub></sub> )	8.2 ± 4.2	2.2 ± 2.0*	7.9 ± 2.2	6.8 ± 2.2	(% mmHg P <sub>ETCO<sub>2</sub></sub> )	3.0 ± 1.0	1.2 ± 0.9*	3.0 ± 0.6	2.6 ± 0.4
MCAV	(cm s <sup>-1</sup> mmHg P <sub>ETCO<sub>2</sub></sub> )	1.6 ± 0.4	0.4 ± 0.5*	1.6 ± 0.4	1.5 ± 0.5	(% mmHg P <sub>ETCO<sub>2</sub></sub> )	2.4 ± 0.5	0.7 ± 0.8*	2.5 ± 0.4	2.4 ± 0.5
MAP	(mmHg mmHg P <sub>ETCO<sub>2</sub></sub> )	-0.4 ± 0.5	-0.08 ± 0.6	-0.3 ± 0.6	-0.04 ± 0.8	(% mmHg P <sub>ETCO<sub>2</sub></sub> )	-0.5 ± 0.7	-0.07 ± 0.7	-0.4 ± 0.8	-0.002 ± 0.9

\*Significantly lower than pre-INDO,  $P < 0.05$ ; <sup>†</sup>significant difference between hypercapnic and hypocapnic reactivities within the experimental trial,  $P < 0.05$ . V<sub>E</sub>, expiratory tidal volume.

could be explained by progressive dilatation of the ICA (see *Vasomotor response to CO<sub>2</sub>*). There was no difference in hypocapnic Q<sub>ICA</sub>, ICAv and MCAv reactivity, probably due to a modest vasomotor reactivity to hypocapnia.

**Vasomotor response of the ICA to CO<sub>2</sub>.** The slope of the vasomotor response to hypercapnia was reduced by  $67 \pm 28\%$  following INDO ( $0.045 \pm 0.015$  vs.  $0.015 \pm 0.012$  mm mmHg P<sub>ETCO<sub>2</sub></sub><sup>-1</sup>;  $P < 0.01$ ) but was unaffected by placebo ( $0.036 \pm 0.006$  vs.  $0.033 \pm 0.006$  mm mmHg P<sub>ETCO<sub>2</sub></sub><sup>-1</sup>;  $P = 0.25$ ). No change in the slope of the response to hypocapnia occurred following INDO ( $0.019 \pm 0.015$  vs.  $0.006 \pm 0.008$  mm mmHg P<sub>ETCO<sub>2</sub></sub><sup>-1</sup>;  $P = 0.08$ ). Vasomotor reactivity was greater during hypercapnia as compared to hypocapnia ( $0.045 \pm 0.015$  vs.  $0.019 \pm 0.015$  mm mmHg P<sub>ETCO<sub>2</sub></sub><sup>-1</sup>;  $P < 0.01$ ).

## Study 2: ketorolac trials

**Resting CBF and cardiorespiratory variables.** Administration of ketorolac had no effect on resting ventilation ( $11.3 \pm 1.5$  vs.  $11.8 \pm 1.9$  l min<sup>-1</sup>;  $P = 0.65$ ), MAP ( $79.2 \pm 8.6$  vs.  $80.0 \pm 5.9$  mmHg;  $P = 0.79$ ), HR ( $55.1 \pm 12.5$  vs.  $53.9 \pm 14.6$  beats min<sup>-1</sup>;  $P = 0.54$ ), Q<sub>ICA</sub> ( $282.8 \pm 66.7$  vs.  $295.6 \pm 80.3$  ml min<sup>-1</sup>;  $P = 0.38$ ) or MCAv ( $56.5 \pm 13.3$  vs.  $58.3 \pm 8.1$  cm s<sup>-1</sup>;  $P = 0.54$ ).

**Ketorolac CO<sub>2</sub> trials.** Cardiovascular, cerebrovascular and respiratory variables at all stages of the placebo trials are presented in Table 4. During hypercapnia, MAP and HR were elevated at +6 and +9 mmHg P<sub>ETCO<sub>2</sub></sub> pre- and post-ketorolac. There was no effect of hypocapnia on MAP while HR was elevated at all stages of hypocapnia pre- and post-ketorolac.

Hypercapnia increased MCAv at all stages pre- and post-ketorolac, while hypocapnia decreased MCAv at all stages pre- and post-ketorolac. Pre- and post-ketorolac Q<sub>ICA</sub> was elevated at +6 and +9 mmHg P<sub>ETCO<sub>2</sub></sub> and reduced at -6 and -9 mmHg P<sub>ETCO<sub>2</sub></sub> during hypercapnia and hypocapnia, respectively. ICA diameter was elevated at +9 mmHg P<sub>ETCO<sub>2</sub></sub> during hypercapnia and reduced at -9 mmHg P<sub>ETCO<sub>2</sub></sub> during hypocapnia. During hypercapnia, CVC was increased at +6 and +9 mmHg P<sub>ETCO<sub>2</sub></sub>, and was decreased during hypocapnia (at -6 and -9 mmHg P<sub>ETCO<sub>2</sub></sub>)

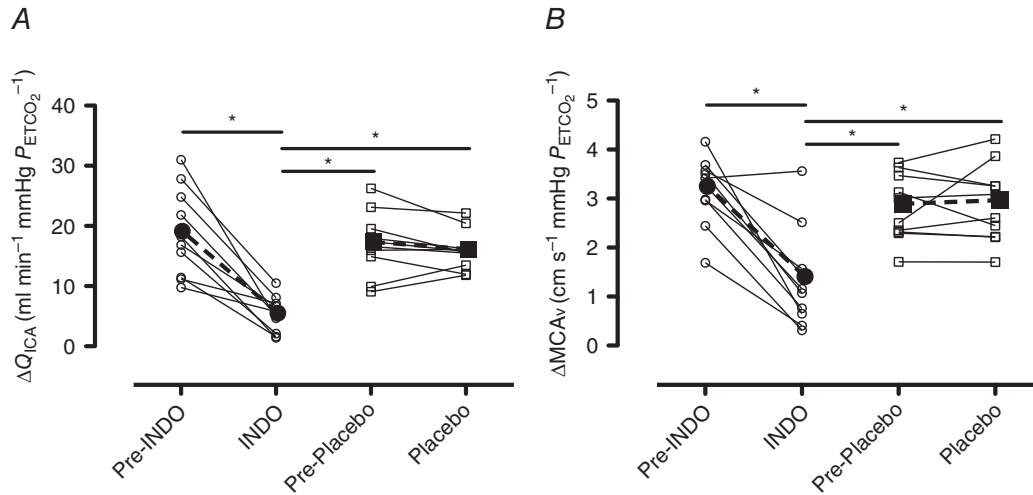
Following ketorolac there was no change in absolute Q<sub>ICA</sub> or MCAv reactivity during both hypercapnia and hypocapnia (Table 5). Relative reactivity for Q<sub>ICA</sub> and MCAv did not differ before and after ketorolac either. The vasomotor response of the ICA to both hypercapnia ( $0.026 \pm 0.015$  vs.  $0.026 \pm 0.022$  mm mmHg P<sub>ETCO<sub>2</sub></sub><sup>-1</sup>;  $P = 0.99$ ) and hypocapnia ( $0.020 \pm 0.024$  vs.  $0.017 \pm 0.014$  mm mmHg P<sub>ETCO<sub>2</sub></sub><sup>-1</sup>;  $P = 0.73$ ) was unchanged following ketorolac.

**Study 3: naproxen trials**

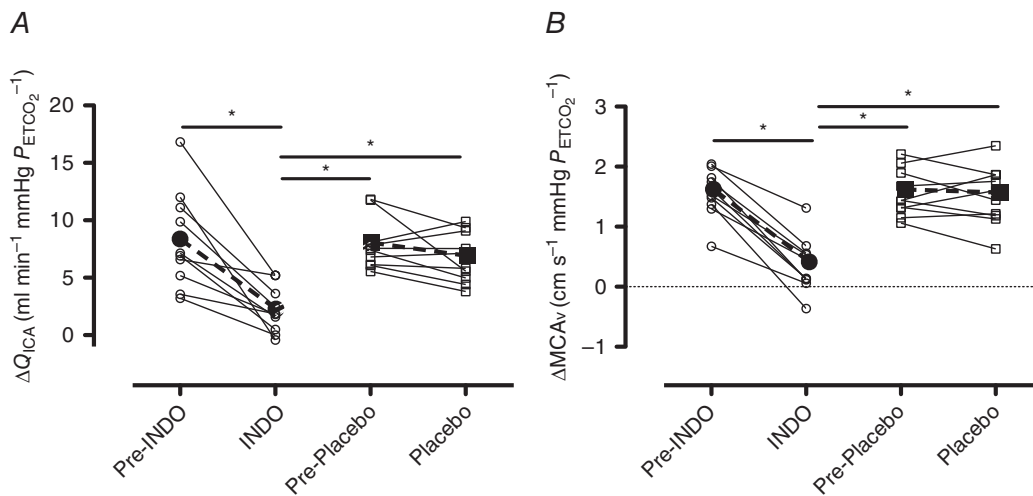
**Resting cerebral blood flow and cardiorespiratory variables.** Administration of naproxen caused a slight increase in resting ventilation ( $10.3 \pm 1.6$  vs.  $12.0 \pm 1.8$  l min<sup>-1</sup>;  $P = 0.04$ ), while MAP ( $77.1 \pm 4.0$  vs.  $74.7 \pm 4.4$  mmHg;  $P = 0.52$ ), HR ( $56.1 \pm 7.5$  vs.  $56.1 \pm 8.4$  beats min<sup>-1</sup>;  $P = 0.99$ ),  $Q_{ICA}$  ( $265.6 \pm 43.5$  vs.

$251.1 \pm 35.5$  ml min<sup>-1</sup>;  $P = 0.38$ ) and MCAv ( $56.5 \pm 9.0$  vs.  $54.0 \pm 8.5$  cm s<sup>-1</sup>;  $P = 0.54$ ) were unchanged.

**Naproxen CO<sub>2</sub> trials.** Cardiovascular, cerebrovascular and respiratory variables at all stages of the placebo trials are presented in Table 6. During hypercapnia, MAP was elevated at +9 mmHg and HR was elevated at +6 and



**Figure 5. Volumetric flow and velocity cerebrovascular reactivity to hypercapnia**  
 A, hypercapnic  $Q_{ICA}$  reactivity prior to and following INDO and placebo treatments. B, hypercapnic MCAv reactivity prior to and following INDO and placebo treatments. Continuous lines and open circles (○) denote individual data, while dashed bold lines and filled circles (●) denote mean data from the INDO trial. Continuous lines and open squares (□) denote individual data, while dashed bold lines and filled squares (■) denote mean data from the placebo trial. Differences between trials were assessed using a one-way repeated measures ANOVA and *post-hoc* tests were made using Tukey's HSD. \*Significant differences between trials,  $P < 0.05$ .



**Figure 6. Volumetric flow and velocity cerebrovascular reactivity to hypocapnia**  
 A, hypocapnic  $Q_{ICA}$  reactivity prior to and following INDO and placebo treatments. B, hypocapnic MCAv reactivity prior to and following INDO and placebo treatments. Continuous lines and open circles (○) denote individual data, while dashed bold lines and filled circles (●) denote mean data from the INDO trial. Continuous lines and open squares (□) denote individual data, while dashed bold lines and filled squares (■) denote mean data from the placebo trial. Differences between trials were assessed using a one-way repeated measures ANOVA and *post-hoc* tests were made using Tukey's HSD. \*Significant differences between trials,  $P < 0.05$ .

Table 4. Cerebrovascular and cardiorespiratory variable before and following Ketorolac during hyper and hypocapnia

		Hypercapnia				Hypocapnia			
		Baseline	+3 mmHg	+6 mmHg	+9 mmHg	Baseline	-3 mmHg	-6 mmHg	-9 mmHg
$Q_{ICA}$ (ml min <sup>-1</sup> )	Pre	299.9 ± 76.3	346.1 ± 96.9	364.9 ± 103.3*	449.3 ± 133.9*	284.9 ± 66.7	261.1 ± 68.2	228.5 ± 62.0*	215.7 ± 54.7*
	Post	303.2 ± 76.6	348.1 ± 95.2	399.3 ± 114.1*	452.9 ± 136.9*	282.5 ± 83.6	254.4 ± 58.7	236.9 ± 64.8*	223.2 ± 55.4*
ICAV (cm s <sup>-1</sup> )	Pre	47.1 ± 6.6	53.3 ± 7.7*	55.8 ± 10.2*	64.1 ± 11.9*	43.2 ± 5.8	40.8 ± 5.5	36.4 ± 5.7*	34.8 ± 2.9*
	Post	46.7 ± 6.9	53.4 ± 8.9*	58.6 ± 10.6*	63.9 ± 11.8*	42.1 ± 8.2	39.0 ± 3.8	36.9 ± 5.7*	35.2 ± 4.6*
Diameter (mm)	Pre	5.15 ± 0.50	5.18 ± 0.55	5.21 ± 0.51	5.38 ± 0.57*	5.25 ± 0.48	5.14 ± 0.50	5.11 ± 0.53	5.06 ± 0.52*
	Post	5.18 ± 0.42	5.20 ± 0.42	5.31 ± 0.47	5.41 ± 0.52*	5.26 ± 0.42	5.21 ± 0.42	5.14 ± 0.41	5.11 ± 0.37*
MCA (cm s <sup>-1</sup> )	Pre	57.0 ± 9.9	63.2 ± 9.7*	70.6 ± 12.3*	79.3 ± 14.2*	54.6 ± 9.4	49.5 ± 7.3*	46.5 ± 6.1*	43.6 ± 5.0*
	Post	59.0 ± 8.8	64.1 ± 9.1*	75.51 ± 10.0*	83.0 ± 11.8*	58.0 ± 8.2	49.3 ± 10.2*	44.5 ± 10.7*	42.7 ± 10.3*
MAP (mmHg)	Pre	80.9 ± 8.5	82.4 ± 8.9	85.1 ± 10.5*	90.4 ± 11.3*	81.4 ± 8.2	80.9 ± 7.5	83.1 ± 7.2	84.9 ± 6.9
	Post	81.3 ± 9.4	83.1 ± 10.1	87.7 ± 11.8*	88.3 ± 11.5*	84.0 ± 10.2	83.3 ± 9.8	84.9 ± 8.9	85.6 ± 9.0
CVC (ml min <sup>-1</sup> mmHg <sup>-1</sup> )	Pre	3.71 ± 0.92	4.20 ± 1.16	4.28 ± 1.12*	4.91 ± 1.15*	3.49 ± 0.67	3.23 ± 0.81	2.74 ± 0.64*	2.53 ± 0.57*
	Post	3.71 ± 0.71	4.18 ± 1.00	4.53 ± 1.07*	5.10 ± 1.35*	3.36 ± 0.94	3.05 ± 0.61	2.76 ± 0.58*	2.60 ± 0.56*
HR (beats min <sup>-1</sup> )	Pre	56.1 ± 13.4	59.0 ± 11.5	59.2 ± 13.1*	62.7 ± 11.8*	52.9 ± 14.6	57.6 ± 13.2*	56.0 ± 14.9*	54.8 ± 14.6*
	Post	53.8 ± 13.8	56.7 ± 13.3	60.0 ± 11.8*	62.6 ± 11.4*	53.7 ± 14.8	57.5 ± 14.3*	57.0 ± 14.3*	57.6 ± 14.0*
$V_E$ (l min <sup>-1</sup> )	Pre	12.5 ± 2.8	17.6 ± 5.1	25.1 ± 9.1	37.7 ± 17.3*	13.3 ± 3.9	30.0 ± 7.9*	30.3 ± 9.0	32.8 ± 9.8*
	Post	14.3 ± 3.2	18.1 ± 7.4	28.1 ± 13.2	40.0 ± 18.6*	14.9 ± 2.8	42.6 ± 29.1*	30.5 ± 6.4	31.02 ± 8.7*
$P_{ETCO_2}$ (mmHg)	Pre	40.57 ± 3.1	43.3 ± 3.0*	46.0 ± 3.0*	49.1 ± 2.9*	40.1 ± 3.2	37.0 ± 3.2*	34.0 ± 3.3*	31.1 ± 3.2*
	Post	39.8 ± 3.5†	42.4 ± 3.4*	46.2 ± 3.1*	49.1 ± 3.5*	39.8 ± 3.4	36.9 ± 3.4*	33.7 ± 3.5*	30.8 ± 3.3*
$P_{ETO_2}$ (mmHg)	Pre	97.5 ± 6.8	98.2 ± 6.6	97.8 ± 6.5	97.8 ± 6.5	97.6 ± 6.0	97.9 ± 6.7	97.9 ± 6.8	97.6 ± 7.0
	Post	96.9 ± 6.1	96.3 ± 5.1	96.6 ± 4.3	96.9 ± 4.9	96.9 ± 4.9	96.5 ± 4.9	96.9 ± 5.1	96.5 ± 4.9

There was no main effect of ketorolac on any variable. †Significant difference from baseline. \*Significant interaction between pre and post.  $V_E$ , expiratory tidal volume.

**Table 5. Cerebrovascular and blood pressure responses to CO<sub>2</sub> before and following ketorolac**

	Units	Absolute reactivity		Units	Relative reactivity	
		Pre-ketorolac	Ketorolac		Pre-ketorolac	Ketorolac
<b>Hypercapnia</b>						
<b>Q<sub>ICA</sub></b>	(ml min <sup>-1</sup> mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	16.6 ± 8.6 <sup>†</sup>	16.2 ± 8.5 <sup>†</sup>	(% mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	5.5 ± 2.5	5.2 ± 2.0 <sup>†</sup>
<b>MCAv</b>	(cm s <sup>-1</sup> mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	2.6 ± 0.6 <sup>†</sup>	2.7 ± 0.6 <sup>†</sup>	(% mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	4.6 ± 0.7 <sup>†</sup>	4.6 ± 1.2 <sup>†</sup>
<b>MAP</b>	(mmHg mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	1.1 ± 0.7 <sup>†</sup>	0.8 ± 0.5 <sup>†</sup>	(% mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	1.4 ± 0.9 <sup>†</sup>	1.0 ± 0.5 <sup>†</sup>
<b>VE</b>	(l min <sup>-1</sup> mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	3.0 ± 2.0	2.8 ± 1.7	(% mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	23.7 ± 13.9	19.0 ± 9.6
<b>Hypocapnia</b>						
<b>Q<sub>ICA</sub></b>	(ml min <sup>-1</sup> mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	8.0 ± 2.1	6.5 ± 4.0	(% mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	2.8 ± 0.7	2.2 ± 0.9
<b>MCAv</b>	(cm s <sup>-1</sup> mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	1.2 ± 0.6	1.7 ± 0.7	(% mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	2.1 ± 0.8	2.9 ± 1.4
<b>MAP</b>	(mmHg mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	-0.4 ± 0.3	-0.2 ± 0.4	(% mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	-0.5 ± 0.3	-0.3 ± 0.5

<sup>†</sup>Significant difference between hypercapnic and hypocapnic reactivities within an experimental trial,  $P < 0.05$ . V<sub>E</sub>, expiratory tidal volume.

+9 mmHg P<sub>ETCO<sub>2</sub></sub>. There was no effect of hypocapnia on MAP while HR was elevated at -9 mmHg of the pre-naproxen trial.

Hypercapnia increased MCAv at all stages pre- and post-naproxen, while hypocapnia decreased MCAv at all stages pre- and post-Naproxen. Pre- and post-Naproxen Q<sub>ICA</sub> was elevated at all stages of hypercapnia and reduced at -6 and -9 mmHg P<sub>ETCO<sub>2</sub></sub> during hypocapnia. ICA diameter was elevated at all stages of hypercapnia but was not significantly reduced at any stage of hypocapnia. During hypercapnia CVC was increased at all stages and was reduced during hypocapnia at -6 and -9 mmHg P<sub>ETCO<sub>2</sub></sub>.

Following naproxen there was no change in absolute Q<sub>ICA</sub> or MCAv reactivity during both hypercapnia and hypocapnia (Table 7). Relative reactivity for Q<sub>ICA</sub> and MCAv also did not differ before and after naproxen. The vasomotor response of the ICA to both hypercapnia (0.030 ± 0.010 vs. 0.032 ± 0.005 mm mmHg P<sub>ETCO<sub>2</sub></sub><sup>-1</sup>;  $P = 0.81$ ) and hypocapnia (0.018 ± 0.026 vs. 0.011 ± 0.025 mm mmHg P<sub>ETCO<sub>2</sub></sub><sup>-1</sup>;  $P = 0.06$ ) was unchanged following naproxen.

## Discussion

The primary novel findings of this study were: (1) the ICA dilates in response to hypercapnia and modestly constricts in response to hypocapnia; (2) vasomotion of the ICA in response to hypercapnia is markedly blunted (-67%) following INDO administration; and (3) INDO, but not ketorolac or naproxen, inhibits cerebrovascular responses to CO<sub>2</sub>, indicating a permissive (i.e. non-COX inhibition-mediated) effect(s) of INDO.

### Cerebrovascular responses to CO<sub>2</sub>

Our volumetric blood flow data have revealed similar hypercapnic reactivity values to that first collected using

the (Kety & Schmidt, 1948) technique of ~7–8% mmHg P<sub>ETCO<sub>2</sub></sub><sup>-1</sup>; such values are consistent with recent studies (Willie *et al.* 2012; Hoiland *et al.* 2015; Tymko *et al.* 2016). This volumetric reactivity, during hypercapnia, greatly exceeds that recorded using TCD measures of MCAv in the current and previous studies (e.g. Ide *et al.* 2003, 2007; Willie *et al.* 2012; Regan *et al.* 2014; Brothers *et al.* 2014; Hoiland *et al.* 2015). As relative MCAv and ICAv reactivity to hypercapnia did not differ, this indicates that vasomotion is responsible for the difference between volumetric flow and velocity indices of cerebrovascular CO<sub>2</sub> reactivity. Consistent with this finding are recent reports that the MCA dilates in response to hypercapnia (Verbree *et al.* 2014; Coverdale *et al.* 2014, 2015) leading to consequent underestimation of cerebrovascular CO<sub>2</sub> reactivity with velocity measures (Ainslie & Hoiland, 2014).

The vasomotor response of the ICA to hypercapnia was markedly greater than that during hypocapnia; thus, there was no appreciable difference in volumetric and velocity indices of reactivity in the hypocapnic range. Upon close examination of the vasomotor profile of the ICA during changes in P<sub>ETCO<sub>2</sub></sub>, it is quite similar to the expected vasomotor profile of the MCA diameter (see fig. 1 in Ainslie & Hoiland, 2014), wherein small changes in P<sub>ETCO<sub>2</sub></sub> do not elicit measurable changes in diameter (Verbree *et al.* 2014; Coverdale *et al.* 2014). It seems that a larger hypocapnic stimulus (e.g. ~9 mmHg below baseline) is required to elicit constriction than the necessary hypercapnic stimulus for dilatation, probably a function of the overall lower cerebrovascular reactivity during hypocapnia. However, using our experimental approach (see *Comparison between studies* below for an explanation) it appears that the ICA may be more sensitive to changes in CO<sub>2</sub> in the hypercapnic range as dilatation at +3 mmHg P<sub>ETCO<sub>2</sub></sub> was observed in both the pre- and post-placebo trials, while the MCA does not appear to dilate (as measured using 3-T magnetic resonance imaging)



Table 6. Cerebrovascular and cardiorespiratory variables before and following Naproxen during hyper and hypocapnia

	Hypercapnia					Hypocapnia				
	Baseline	+3 mmHg	+6 mmHg	+9 mmHg	Baseline	-3 mmHg	-6 mmHg	-9 mmHg		
$Q_{ICA}$ (ml min <sup>-1</sup> )	Pre	277.67 ± 53.45	308.44 ± 41.18*	400.49 ± 81.82*	468.87 ± 78.80*	269.23 ± 31.17	245.91 ± 31.75	211.66 ± 25.84*	195.48 ± 24.42*	
	Post	271.45 ± 52.39	325.67 ± 66.86*	388.25 ± 79.00*	459.64 ± 92.25*	267.59 ± 48.09	260.39 ± 43.68	237.43 ± 56.30*	222.89 ± 55.85*	
ICAV (cm s <sup>-1</sup> )	Pre	47.33 ± 4.17	51.44 ± 4.24*	62.92 ± 7.45*	72.43 ± 7.47*	43.21 ± 4.44	40.19 ± 2.63*	36.17 ± 3.11*	33.25 ± 3.89*	
	Post	46.98 ± 3.84	53.99 ± 4.86*	61.51 ± 4.88*	71.28 ± 7.92*	43.60 ± 2.94	42.37 ± 1.93*	38.86 ± 3.80*	37.65 ± 4.17*	
Diameter (mm)	Pre	4.97 ± 0.41	5.03 ± 0.37*	5.16 ± 0.40*	5.23 ± 0.40*	5.14 ± 0.33	5.07 ± 0.43	4.98 ± 0.48	4.99 ± 0.49	
	Post	4.91 ± 0.39	5.02 ± 0.43*	5.14 ± 0.43*	5.20 ± 0.41*	5.06 ± 0.36	5.08 ± 0.42	5.05 ± 0.51	4.97 ± 0.51	
MCAv (cm s <sup>-1</sup> )	Pre	57.44 ± 10.26	62.75 ± 13.28*	70.00 ± 11.71*	81.80 ± 13.17*	55.88 ± 8.15	51.85 ± 6.77*	45.66 ± 6.65*	43.74 ± 6.29*	
	Post	55.60 ± 8.58	62.07 ± 12.33*	68.08 ± 13.48*	79.26 ± 16.40*	55.30 ± 8.31	50.83 ± 7.29*	47.28 ± 8.71*	44.63 ± 7.19*	
MAP (mmHg)	Pre	76.82 ± 6.81	77.75 ± 4.48	80.26 ± 5.54	84.71 ± 5.61*	78.54 ± 2.41	78.08 ± 4.29	75.64 ± 3.83	78.18 ± 2.69	
	Post	78.01 ± 5.04	81.28 ± 3.99	79.79 ± 3.87	81.05 ± 3.80*	74.25 ± 3.70	75.97 ± 4.10	77.88 ± 2.42	77.29 ± 2.95	
CVC (ml min <sup>-1</sup> mmHg <sup>-1</sup> )	Pre	3.59 ± 0.37	3.95 ± 0.32*	4.96 ± 0.69*	5.52 ± 0.66*	3.43 ± 0.34	3.15 ± 0.42	2.80 ± 0.37*	2.50 ± 0.28*	
	Post	3.50 ± 0.80	4.01 ± 0.83*	4.86 ± 0.89*	5.66 ± 1.00*	3.59 ± 0.50	3.44 ± 0.60	3.06 ± 0.77*	2.90 ± 0.82*	
HR (beats min <sup>-1</sup> )	Pre	55.09 ± 7.56	58.26 ± 8.10	64.39 ± 8.78*	67.85 ± 12.74*	53.23 ± 9.30	55.94 ± 8.56	54.52 ± 8.69	57.58 ± 10.12*	
	Post	55.59 ± 8.59	58.95 ± 8.44	62.50 ± 8.70*	68.51 ± 10.55*	54.53 ± 9.30	55.17 ± 8.78	56.20 ± 8.36	56.46 ± 8.53	
$V_E$ (l min <sup>-1</sup> )	Pre	11.80 ± 1.90	21.02 ± 6.39	32.77 ± 11.35*	42.99 ± 11.39*	13.27 ± 1.77	25.62 ± 6.74*	24.23 ± 4.70*	27.06 ± 5.43*	
	Post	14.97 ± 2.84	23.91 ± 7.42	30.85 ± 11.90*	43.20 ± 16.81*	14.78 ± 3.32	26.49 ± 6.64*	30.97 ± 4.22*	30.19 ± 4.13*	
$P_{ETCO_2}$ (mmHg)	Pre	41.20 ± 1.78	44.52 ± 1.54*	48.03 ± 1.73*	51.19 ± 1.81*	41.19 ± 2.07	38.43 ± 1.83*	35.50 ± 1.84*	33.03 ± 1.54*	
	Post	41.01 ± 1.50	44.26 ± 1.68*	47.31 ± 1.82*	50.43 ± 1.76*	41.06 ± 1.73	38.79 ± 1.56*	36.13 ± 1.73*	33.40 ± 1.72*	
$P_{ETO_2}$ (mmHg)	Pre	94.58 ± 2.78	95.14 ± 2.24	94.46 ± 2.30	93.26 ± 2.41	95.30 ± 2.17	94.42 ± 2.93	93.30 ± 3.22*	92.52 ± 2.44*	
	Post	95.70 ± 2.58	95.10 ± 2.83	95.11 ± 3.4	94.71 ± 2.97	98.38 ± 5.52	93.80 ± 3.04	92.33 ± 2.75*	92.07 ± 2.87*	

There was no main effect of naproxen on any variable. \*Significant difference from baseline. †Significant interaction between pre and post.  $V_E$ , expiratory tidal volume.

**Table 7. Cerebrovascular and blood pressure responses to CO<sub>2</sub> before and following naproxen**

Units		Absolute reactivity		Relative reactivity		
		Pre-naproxen	Naproxen	Pre-naproxen	Naproxen	
<b>Hypercapnia</b>						
<b>Q<sub>ICA</sub></b>	(ml min <sup>-1</sup> mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	19.9 ± 3.2	19.9 ± 3.5	(% mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	7.2 ± 0.7	7.4 ± 1.1
<b>MCAv</b>	(cm s <sup>-1</sup> mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	2.3 ± 0.6	2.0 ± 0.7	(% mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	4.3 ± 1.1	4.1 ± 1.0
<b>MAP</b>	(mmHg mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	0.8 ± 0.4	0.2 ± 0.6	(% mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	1.1 ± 0.6	0.3 ± 0.9
<b>V<sub>E</sub></b>	(l min <sup>-1</sup> mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	3.1 ± 1.1	2.9 ± 1.6	(% mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	28.6 ± 1.8	18.9 ± 7.8 <sup>†</sup>
<b>Hypocapnia</b>						
<b>Q<sub>ICA</sub></b>	(ml min <sup>-1</sup> mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	9.3 ± 3.7	6.0 ± 1.9	(% mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	3.4 ± 1.1	2.3 ± 0.9
<b>MCAv</b>	(cm s <sup>-1</sup> mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	1.6 ± 0.4	1.1 ± 0.4	(% mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	2.8 ± 0.5	2.7 ± 0.7
<b>MAP</b>	(mmHg mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	0.2 ± 0.5	-0.4 ± 0.8	(% mmHg P <sub>ETCO<sub>2</sub></sub> <sup>-1</sup> )	0.2 ± 0.7	-0.6 ± 1.1

<sup>†</sup>Significant difference between hypercapnic and hypocapnic reactivities within an experimental trial,  $P < 0.05$ . V<sub>E</sub>, expiratory tidal volume.

until approximately +9 mmHg P<sub>ETCO<sub>2</sub></sub> (Coverdale *et al.* 2014).

### Comparisons between studies

Conflicting data exist as to whether CO<sub>2</sub>-mediated vasomotion of the ICA does (Willie *et al.* 2012; Hoiland *et al.* 2014) or does not occur (Coverdale *et al.* 2015). The present study aimed to resolve these conflicting data, and corroborates the data exemplifying that vasomotion of the ICA does occur. Differences between studies are probably due to several methodological and analytical differences. These differences include the method of manipulating P<sub>aCO<sub>2</sub></sub>, and determination of vessel diameter. For example, Coverdale *et al.* (2015) utilized elevations in fractional inspired CO<sub>2</sub> (0.06) to elicit a ~10 mmHg increase in P<sub>ETCO<sub>2</sub></sub>. This technique is limited in that P<sub>ETCO<sub>2</sub></sub> tends to overestimate P<sub>aCO<sub>2</sub></sub> by ~4–6 mmHg at this level of hypercapnia (Peebles *et al.* 2007). In contrast, our approach of end-tidal forcing results in P<sub>ETCO<sub>2</sub></sub> over-estimating P<sub>aCO<sub>2</sub></sub> by only ~1–2 mmHg (Tymko *et al.* 2015, 2016). Therefore, it is possible that the hypercapnic P<sub>aCO<sub>2</sub></sub> (not P<sub>ETCO<sub>2</sub></sub>) stimulus applied by Coverdale *et al.* (2015) was only around ~5–6 mmHg above baseline whereas the P<sub>ETCO<sub>2</sub></sub> stimulus in the current study is representative of the true P<sub>aCO<sub>2</sub></sub> stimulus (Tymko *et al.* 2016). As the current study shows very modest changes in diameter with mild hypercapnia, this provides an explanation, in part, for the lack of ICA dilatation observed by Coverdale *et al.* (2015). Second, discrepancies between studies may be a reflection of the use of caliper-based manual diameter measures (quantified over three cardiac cycles) rather than our automated approach (quantified over a minimum of 12 consecutive cardiac cycles). The latter approach is affected less by artifacts, and by the potential influences of respiration and blood pressure variability. As such, using edge-detection software not only provides a more

robust and sensitive assessment of vessel diameter and velocity (Woodman *et al.* 2001), it also limits subjectivity and bias during data analysis (see Methods for details).

### COX inhibition

It has been previously established that the dose of INDO in the current study is sufficient to effectively inhibit PG synthesis (Eriksson *et al.* 1983). Inhibition of COX with INDO resulted in a significant reduction in both resting CBF and cerebrovascular CO<sub>2</sub> reactivity in both the hypo- and the hyper-capnic range. This result is in agreement with previous studies using various measurement techniques to quantify CBF (Wennmalm *et al.* 1981; Bruhn *et al.* 2001; St Lawrence *et al.* 2002; Xie *et al.* 2006; Hoiland *et al.* 2015). However, COX inhibition with ketorolac and naproxen did not affect resting CBF, cerebrovascular CO<sub>2</sub> reactivity or the vasomotor responsiveness of the ICA. Some previous data, comparing INDO to other COX inhibitors, have indicated that INDO reduces CBF and cerebrovascular CO<sub>2</sub> reactivity via a 'COX inhibition independent' mechanism (Eriksson *et al.* 1983; Markus *et al.* 1994); however, these studies provided limited suggestion of alternative mechanisms and did not directly assess cerebral artery vasomotion. On the basis of previous studies *in vitro* and in highly controlled animal models, we reason here that the selectivity of INDO is related to either the inhibition of cAMP-dependent protein kinase (Kantor & Hampton, 1978; Goueli & Ahmed, 1980), inhibition of PG (i.e. prostacyclin) receptor binding-mediated increases in vascular smooth muscle cAMP (Parfenova *et al.* 1995) or both (see *Pharmacological interventions* below). Thus, it appears PGs may not play an appreciable (or detectable) role in the regulation of extra-cranial cerebral artery vasomotion during alterations in P<sub>aCO<sub>2</sub></sub>.

## Pharmacological interventions

Several factors have supported INDO as a useful pharmacological agent to assess the effects of COX inhibition on CBF: cardiac output is minimally affected by the administration of INDO (Nowak & Wennmalm, 1978; Wennmalm *et al.* 1984) and INDO does not appear to alter cerebral metabolism (Kraaier *et al.* 1992), resting ventilation (Hoiland *et al.* 2015), peripheral chemosensitivity (Xie *et al.* 2006) or plasma catecholamines (Staessen *et al.* 1984; Green *et al.* 1987). Thus, INDO has been extensively used to assess the influence of PG on cerebrovascular CO<sub>2</sub> reactivity (Wennmalm *et al.* 1981; Eriksson *et al.* 1983; Bruhn *et al.* 2001; St Lawrence *et al.* 2002) in addition to many other experimental paradigms. That other COX-inhibiting drugs such as aspirin and naproxen (Eriksson *et al.* 1983; Markus *et al.* 1994) yield divergent findings compared to INDO, however, suggests that INDO reduces cerebrovascular CO<sub>2</sub> reactivity through a PG-independent mechanism. Yet, it is still continually used to assess PG-mediated responses. For example, extremely low doses of INDO have been reported to inhibit cAMP-dependent protein kinase activity (Kantor & Hampton, 1978; Goueli & Ahmed, 1980). As illustrated in Fig. 1, this inhibition will directly affect vascular smooth muscle cell calcium sensitivity (Adelstein & Conti, 1978), and thus vasomotor tone (Kerrick & Hoar, 1981). Moreover, in newborn pigs, which also demonstrate reductions in resting CBF and cerebrovascular CO<sub>2</sub> reactivity to INDO but not other COX inhibitors (e.g. aspirin, ibuprofen, naproxen; Chemtob *et al.* 1991), INDO blocks prostacyclin receptor-mediated signal transduction during hypercapnia (Parfenova *et al.* 1995), specifically downstream increases in cAMP (see Fig. 1). However, aspirin does not inhibit prostacyclin receptor-mediated signalling and subsequent increases in smooth muscle cell cAMP (Parfenova *et al.* 1995), highlighting that this is a unique effect of INDO. Therefore, although the primary purpose of this study was to characterize the effect of INDO on CO<sub>2</sub>-mediated vasomotor responses of the ICA, we also aimed to test the effect of ketorolac and naproxen on such vasomotor responses. These additional drug interventions were used to determine if INDO acts differently from other COX inhibitors in its ability to blunt CO<sub>2</sub>-mediated vasomotion (in addition to flow reactivity) of large extra-cranial cerebral arteries. With ketorolac and naproxen we showed no difference in the vasomotor response (i.e. magnitude of dilatation), consistent with the lack of reductions in flow and velocity reactivity observed in previous studies (Eriksson *et al.* 1983; Markus *et al.* 1994), indicating that INDO is affecting the vasomotor tone of larger cerebral arteries in a permissive (i.e. independent of COX inhibition) manner.

The possibility remains that only low levels of PG are required to induce vasomotion (through downstream increases in cAMP) during CO<sub>2</sub> perturbations. This provides an explanation for the lack of effect of COX inhibitors – with the exception of INDO – on cerebrovascular CO<sub>2</sub> reactivity, consistent with the lack of detectable levels of PG during hypercapnia (Eriksson *et al.* 1983). For example, near complete inhibition of PG synthesis with aspirin does not inhibit PG receptor agonism (i.e. via iloprost) or hypercapnia-mediated increases in cAMP and vascular diameter in newborn pigs (Parfenova *et al.* 1995). If large doses of PGs were necessary to produce dilatation one may expect a dose-dependent relationship between prostacyclin receptor agonism and vasodilatation; however, this is not the case during hypercapnia (Leffler *et al.* 1999). Collectively, these data provide a possible explanation (i.e. inhibition of prostacyclin receptor-mediated signal transduction) as to how INDO inhibits cerebrovascular CO<sub>2</sub> reactivity both independent of COX inhibition and in a manner unique from other COX inhibitors.

## Limitations

It should be acknowledged that the use of CVC does not account for the complicated interactions of CO<sub>2</sub>-induced MAP and vasomotor changes. In keeping with this, in the current study the increase in MAP during hypercapnia poses as a potential confound to the flow and vasomotor response of the ICA (Willie *et al.* 2012; Regan *et al.* 2014). As the MAP increase between trials (i.e. pre- vs. post-INDO) was not different and there was no interaction effect between MAP and INDO (Table 1) it is quite unlikely that MAP is a contributing factor to the reduced vasomotion observed post-INDO. However, the influence of MAP in the regulation of CBF during hypercapnia still warrants consideration. Increases in MAP will increase CBF through direct pressure effects (Lucas *et al.* 2010; Numan *et al.* 2014) despite activation of autoregulatory mechanisms aimed to counteract MAP-induced increases in perfusion (Fog, 1939; Kontos *et al.* 1978). While this study did not assess potential autoregulatory effects, it is possible that pressure-induced vasoconstriction may have, to some extent, counteracted CO<sub>2</sub>-induced vasodilatation leading to underestimation of ICA vasomotion in all trials.

## Implications

For the last 30 years, assessment of cerebrovascular responses has been dominated by the use of TCD. However, assessment of CO<sub>2</sub> reactivity with TCD operates on the assumption (also its primary limitation) that the diameter of the MCA does not change in response to CO<sub>2</sub> – an assumption previously thought to be true

(Serrador *et al.* 2000). Recently, studies using higher resolution magnetic resonance imaging (Verbree *et al.* 2014; Coverdale *et al.* 2014, 2015) have reported MCA vasomotion in response to changes in CO<sub>2</sub> and consequent underestimation of reactivity by TCD measures of velocity. Other studies assessing volumetric flow reactivity through the extra-cranial cerebral arteries (Willie *et al.* 2012; Hoiland *et al.* 2015) provide further evidence that TCD is limited in its ability to accurately measure cerebrovascular CO<sub>2</sub> reactivity.

Reduced cerebrovascular CO<sub>2</sub> reactivity is indicative of an increased risk for all-cause and cardiovascular (inclusive of stroke) mortality (Portegies *et al.* 2014). As it seems that changes in diameter can contribute to nearly half of the increase in flow observed during elevations in  $P_{\text{ETCO}_2}$ , we speculate that the magnitude of the vasomotor response (i.e. diameter change) in response to  $P_{\text{ETCO}_2}$  perturbations may be indicative of cerebrovascular health (i.e. endothelial function), much like peripheral flow-mediated dilatation is indicative of cardiovascular risk (Inaba *et al.* 2010; Green *et al.* 2011). The incorporation of diameter measures into the prediction of cerebrovascular events and related mortality should be considered.

While INDO is exemplary in its ability to reduce cerebrovascular reactivity, and is thus an attractive tool for the assessment of physiological function associated with cerebrovascular reactivity (i.e. control of breathing; Xie *et al.* 2006; Hoiland *et al.* 2015), its utility for investigating the role of PG in mediating cerebrovascular responses should be cautioned. As we and others (Eriksson *et al.* 1983; Markus *et al.* 1994) have identified, INDO reduces cerebrovascular CO<sub>2</sub> reactivity and CO<sub>2</sub>-mediated vasomotion in a manner that is unique from other COX inhibitors. It is clear that INDO is acting in a PG-independent manner, probably through the inhibition of cAMP-dependent protein kinase (Kantor & Hampton, 1978; Goueli & Ahmed, 1980), and reductions in PG receptor-mediated increases in smooth muscle cAMP (Parfenova *et al.* 1995) to affect cerebrovascular reactivity to CO<sub>2</sub>.

## Conclusions

We demonstrate significant vasodilatation and vasoconstriction of the ICA during alterations in  $P_{\text{ETCO}_2}$ . Moreover, this study demonstrates that INDO reduces the vasomotor response of the ICA to changes in  $P_{\text{ETCO}_2}$  and provides evidence that it is independent of COX inhibition. Future studies should aim to determine the mechanism(s) underlying INDO's unique ability to reduce CO<sub>2</sub>-mediated cerebrovascular vasomotion and to determine other regulatory mechanisms governing cerebrovascular vasomotion in healthy humans.

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## Additional information

### Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

### Author contributions

Conception and design of experiments: R.L.H., P.N.A. Data collection: R.L.H., M.M.T., K.W.W., A.R.B., B.M., P.N.A. Data analysis and interpretation: R.L.H., P.N.A. Manuscript first draft: R.L.H., P.N.A. Critical revisions of manuscript for important intellectual content: R.L.H., M.M.T., K.W.W., A.R.B., B.M., P.N.A. Approval of final draft: R.L.H., P.N.A.

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