

Cyclooxygenases 1 and 2 Differentially Regulate Blood Pressure and Cerebrovascular Responses to Acute and Chronic Intermittent Hypoxia: Implications for Sleep Apnea

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Background—Obstructive sleep apnea (OSA) is associated with increased risk of cardiovascular and cerebrovascular disease resulting from intermittent hypoxia (IH)-induced inflammation. Cyclooxygenase (COX)-formed prostanoids mediate the inflammatory response, and regulate blood pressure and cerebral blood flow (CBF), but their role in blood pressure and CBF responses to IH is unknown. Therefore, this study's objective was to determine the role of prostanoids in cardiovascular and cerebrovascular responses to IH.

Methods and Results—Twelve healthy, male participants underwent three, 6-hour IH exposures. For 4 days before each IH exposure, participants ingested a placebo, indomethacin (nonselective COX inhibitor), or Celebrex[®] (selective COX-2 inhibitor) in a double-blind, randomized, crossover study design. Pre- and post-IH blood pressure, CBF, and urinary prostanoids were assessed. Additionally, blood pressure and urinary prostanoids were assessed in newly diagnosed, untreated OSA patients (n=33). Nonselective COX inhibition increased pre-IH blood pressure ($P \leq 0.04$) and decreased pre-IH CBF ($P = 0.04$) while neither physiological variable was affected by COX-2 inhibition ($P \geq 0.90$). Post-IH, MAP was elevated ($P \leq 0.05$) and CBF was unchanged with placebo and nonselective COX inhibition. Selective COX-2 inhibition abrogated the IH-induced MAP increase ($P = 0.19$), but resulted in lower post-IH CBF ($P = 0.01$). Prostanoids were unaffected by IH, except prostaglandin E₂ was elevated with the placebo ($P = 0.02$). Finally, OSA patients had elevated blood pressure ($P \leq 0.4$) and COX-1 formed thromboxane A₂ concentrations ($P = 0.02$).

Conclusions—COX-2 and COX-1 have divergent roles in modulating vascular responses to acute and chronic IH. Moreover, COX-1 inhibition may mitigate cardiovascular and cerebrovascular morbidity in OSA.

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Obstructive sleep apnea (OSA) is a chronic medical condition affecting 3% to 7% of the population¹ and is associated with an increased risk of cardiovascular and

cerebrovascular disease.² OSA is characterized by repetitive cessation of breathing during sleep due to recurrent closure of the pharynx, resulting in chronic exposure to intermittent hypoxia (IH). In healthy humans, exposure to experimental IH disrupts peripheral and cerebral vascular regulation, manifested as an increased resting blood pressure^{3,4} and a smaller decrease in cerebrovascular resistance during hypoxia.⁵ While the mechanisms implicated in vascular dysfunction with OSA are multifactorial, IH-induced inflammation is a fundamental component leading to increased cardiovascular morbidity.^{6,7}

Prostanoids, ubiquitous molecules formed via the catabolism of arachidonic acid by cyclooxygenase (COX) isoenzymes COX-1 and COX-2, are of particular interest because they are both, important mediators of the inflammatory response,⁸ and vital regulators of the peripheral⁹ and cerebral vasculatures.^{10,11} The increased risk of peripheral and cerebral vascular disease associated with nonselective COX inhibiting nonsteroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibiting coxibs^{12,13} emphasize the importance of

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prostanoids in vascular regulation. Additionally, although nonselective and COX-2 selective inhibition increase the risk of cardiovascular and cerebrovascular disease,¹² there is still considerable controversy regarding the importance of COX-1 and COX-2 formed prostanoids for vascular regulation.^{13–16}

With IH, prostanoid concentrations are shifted towards vasoconstriction and atherogenesis.^{17,18} However, whether this concentration shift is involved in IH-induced increases in blood pressure, and altered cerebral blood flow (CBF) regulation, is not known. Thus, using an experimental model of IH in healthy humans, the objective of this study was to determine the role of COX-1 and COX-2 formed prostanoids in modulating the vascular responses to an acute (6 hours) IH exposure. Furthermore, the impact of chronic IH exposure on prostanoid concentrations was explored through comparison with a clinical population of newly diagnosed (untreated) OSA patients.

Methods

Approvals

This study was performed according to the Declaration of Helsinki, was approved by the Conjoint Health Research Ethics Board at the University of Calgary, and is registered as a clinical trial at www.clinicaltrials.gov (NCT01280006). After initial contact, volunteers were provided a familiarization session where they were introduced to the experimental set-up, instrumentation, and provided with an informed consent. Volunteers were then given a minimum of 24 hours to reflect on the information provided prior to signing the informed consent.

Healthy Participants

Fifteen male volunteers were assessed for eligibility. Immediate exclusion criteria included residence in Calgary, Alberta (altitude ≈ 1101 m) for <1 year, a body mass index (BMI) ≥ 35 kg·m⁻², cigarette smoking within the past year, and any active inflammatory or musculoskeletal condition for which volunteers were currently taking any NSAIDs. Two volunteers declined to participate. The remaining 13 volunteers underwent additional screening.

Screening

Screening started with a medical history, measurement of resting blood pressure, and a 12-lead ECG. Volunteers with a history of cardiorespiratory disease, gastrointestinal bleeding, gastritis, inflammatory bowel disease, peptic ulcers, had a sulfa allergy, had an irregular ECG, or were hypertensive (ie, systolic/diastolic blood pressure $>140/90$) were excluded.

Next, the presence of diabetes and liver and/or kidney dysfunction were assessed via fasting venous blood and urine samples. Venous blood samples were collected from the antecubital fossa into evacuated blood collection tubes (BD Vacutainers[®]; 1×4.5 mL sodium citrate tube, 1×5 mL serum separator tube [SST], 1×4 mL ethylenediaminetetraacetic acid [EDTA]). Samples were analyzed for glucose with a benchtop blood gas analyzer (ABL 827 Flex; Radiometer) and alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), the international normalization ratio, plasma creatinine, and complete blood counts by Calgary Laboratory Services. Urine samples were collected into sterile specimen containers (LeakBuster, Starplex Scientific) and aliquoted into 2×10 mL vials for sodium and creatinine determination by Calgary Laboratory Services and the remainder of the sample was used for urinalysis (Chemstrip 10; Roche Diagnostics). Volunteers were excluded if there was evidence of diabetes (fasting glucose >7.0 mmol·L⁻¹), liver dysfunction (ie, ALT, AST, ALP, and INR outside normal ranges), and/or renal dysfunction (estimated glomerular filtration rate (GFR) ≤ 60 mL·min⁻¹·1.73 m⁻², urinary protein excretion >150 mg·24 h⁻¹). Finally, volunteers were screened for sleep apnea and nocturnal hypoxia with a level 3 diagnostic sleep study (Remmers Sleep Recorder Model 4.2; Sagatech Electronics). The Remmers Sleep Recorder records arterial oxyhemoglobin saturation (SaO₂) and heart rate via a finger pulse oximeter, nasal airflow via a nasal cannula connected to a pressure transducer, snoring via a microphone placed on the suprasternal notch, and body position (supine/non-supine) with a body position sensor within the microphone housing. The SaO₂ signal is recorded at 1 Hz and analyzed using a proprietary scoring algorithm. The respiratory disturbance index (RDI) is calculated as the number of times SaO₂ decreased by $\geq 4\%$, divided by the total recording time. This system has been validated against polysomnography, the gold standard diagnostic test for OSA.^{19,20} Raw data from the sleep recorder (ie, SaO₂, nasal airflow, snoring, body position, and heart rate) was reviewed by a sleep medicine physician (P.J.H.) to confirm the absence of OSA and nocturnal hypoxia. Volunteers were excluded if they had a RDI >5 events·h⁻¹ and/or a mean SaO₂ during sleep $<90\%$.

Volunteers who met all inclusion criteria were randomized into the experimental protocol. Figure 1 is a CONSORT diagram showing the flow of participants through the study.

Experimental protocol

The study used a double-blind, placebo-controlled, randomized, cross-over experimental design consisting of 3 experimental IH exposures. For 4 days prior to each IH exposure, each participant ingested one of the following: a lactose placebo, the nonselective cyclooxygenase (COX)

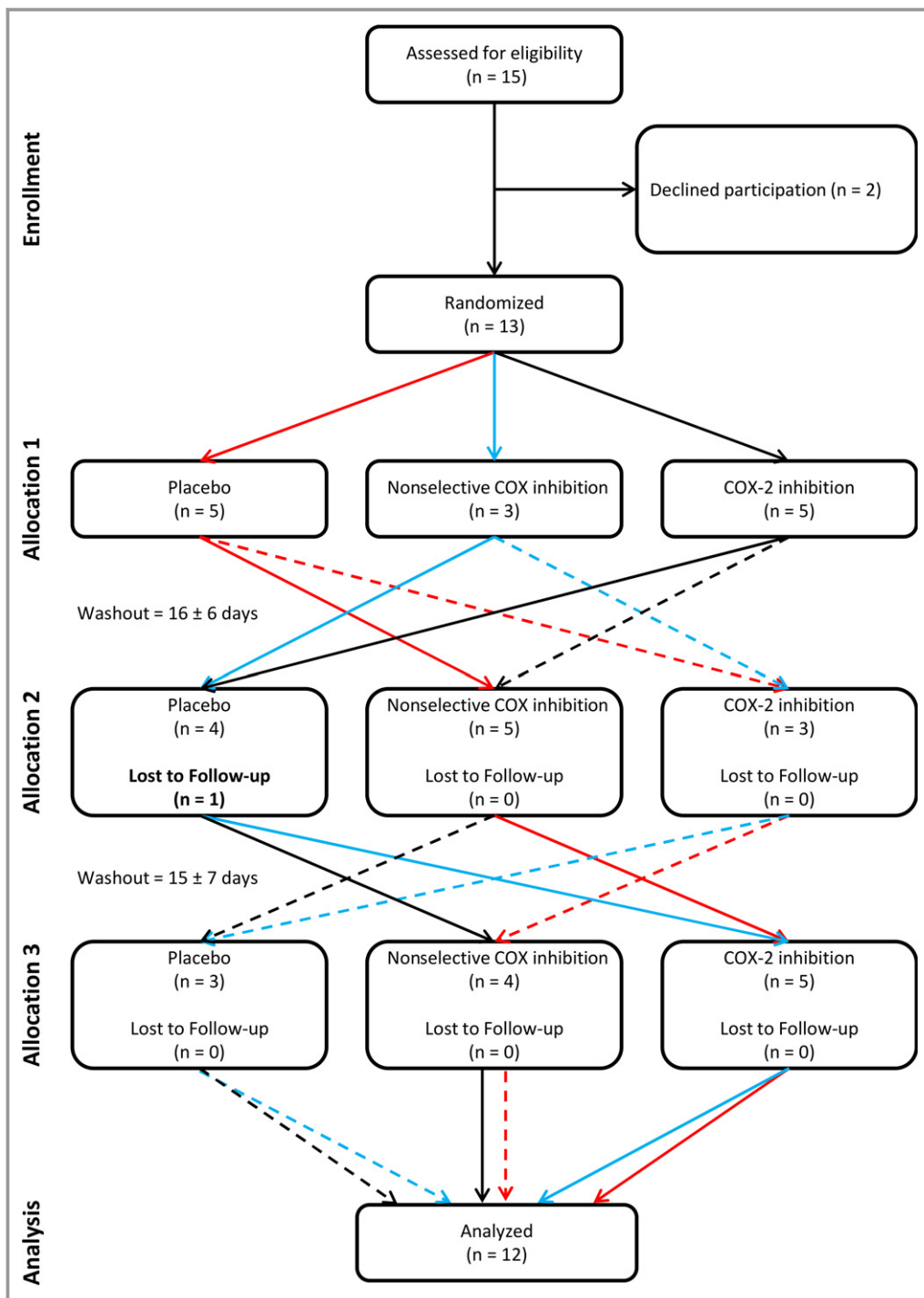


Figure 1. CONSORT diagram showing the flow of healthy participants exposed to intermittent hypoxia (IH).

inhibitor indomethacin, or the selective COX-2 inhibitor Celebrex[®]. On IH exposure days, participants were instructed to have a light breakfast prior to taking the morning dose of medication. The participant arrived in the laboratory at ≈0800 hour and provided a urine sample shortly after arrival. Next, resting brachial blood pressure and cerebral blood flow were assessed. Subsequently, the

participant was exposed to 6 hours of IH. After the IH exposure, the participant provided another urine sample and resting blood pressure and cerebral blood flow measurements were repeated. A minimum of 4 days was provided between the conclusion of an IH exposure and the start of the next medication. Figure 2 shows a schematic of experimental protocol.

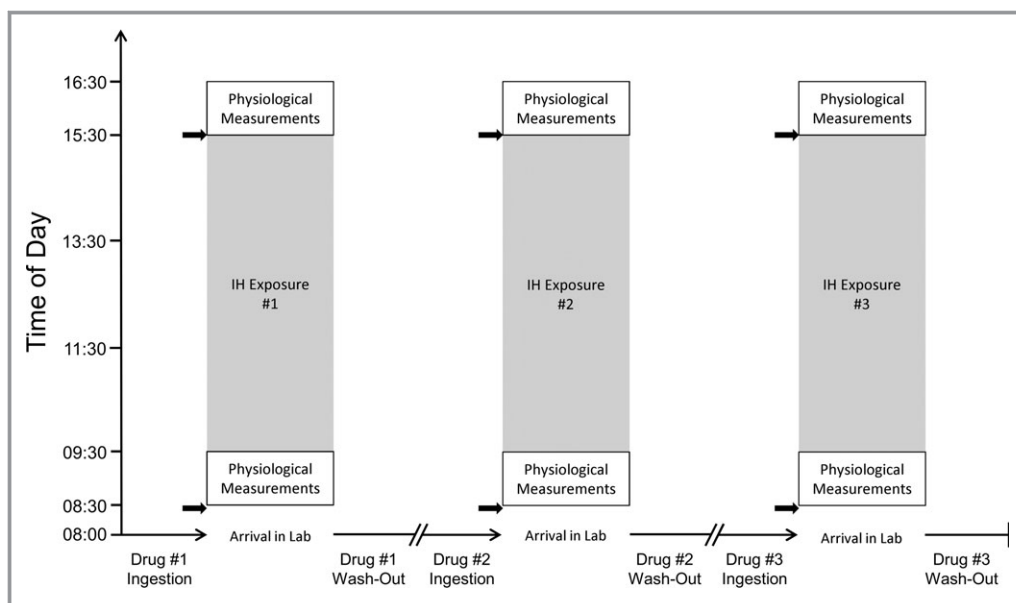


Figure 2. Healthy male participants were exposed to 6 hours of isocapnic intermittent hypoxia on 3 separate occasions (IH Exposure #1 to 3). For 4 days before each IH exposure, participants ingested either 100 mg lactose placebo (3 times/day), 50 mg (3 times/day) of the nonselective cyclooxygenase (COX) inhibitor indomethacin, or 200 mg (2 times/day) of the selective COX-2 inhibitor Celebrex[®] (Drug Ingestion #1 to 3), administered in a random order. For each IH Exposure, participants arrived at ≈0800 hours and immediately provided a urine sample (➡) and were instrumented for Physiological Measurements of blood pressure and cerebral blood flow. Next, each participant was exposed to 6 hours of IH that consisted of cycling their end-tidal partial pressure of oxygen (PET_{O₂}) between 88 mm Hg (normal value for the altitude (≈1101 m) at which the laboratory is located) and 45 mm Hg every 60 seconds. After each IH exposure, participants provided another urine sample (➡) and the Physiological Measurements (ie, blood pressure and cerebral blood flow) were repeated. Each IH exposure was separated by at least 4 days to allow washout of the medication from their system (Drug Washout #1 to 3).

Medication dosage

The placebo (100 mg lactose placebo) was ingested 3 times a day at 0800, 1400, and 2000 hours. Similarly, the nonselective COX inhibitor indomethacin (50 mg) was also ingested 3 times a day at 0800, 1400, and 2000 hours. The selective COX-2 inhibitor Celebrex[®] (200 mg) was ingested 2 times a day at 0800 and 2000 hours.²¹ The double-blind study was maintained by adding a placebo as the second pill at 1400 hours when participants were taking Celebrex[®]. On IH exposure days (ie, day 5), the dosage regimen was maintained through the end of physiological sampling and measurement (ie, only the final dose scheduled for 2000 hour was omitted in all conditions).

Intermittent hypoxia exposure

Exposure to IH was performed in a custom-built normobaric hypoxic room⁴ and consisted of cycling between 1 minute of hypoxia and 1 minute of normoxia for 6 hours, thus replicating an RDI of 30 events·h⁻¹, which is seen in patients with moderate-to-severe OSA. Hypoxia was induced by maintaining the fraction of O₂ within the room at a level sufficient to

decrease the end-tidal partial pressure of O₂ (PET_{O₂}) to 45 mm Hg within 60 seconds and normoxia was established by administering 100% O₂ to the participant at a flow rate sufficient to return PET_{O₂} to 88 mm Hg (normal PET_{O₂} for the altitude at which the laboratory is located [≈1101 m]) within 60 seconds. The end-tidal partial pressure of CO₂ (PET_{CO₂}) was maintained at normal levels by administering 100% CO₂ to the participant during each hypoxic cycle.

Briefly, during all IH exposures the participants were instrumented with a nasal cannula and a non-vented, full-face respiratory mask (Mirage NV Full Face Mask Series 2; Resmed) attached to a 2-way non-rebreathing valve (2600 series; Hans Rudolph). Connected to the inspired side of the non-rebreathing valve was a 25 cm long, wide-bore tubing acting as a mixing chamber through which the participant could breathe the hypoxic gas within the chamber and 100% O₂ and/or CO₂ could be delivered to the participant as required. Respired gases were sampled continuously at a rate 150 mL·min⁻¹ from the nasal cannula and the fractions of O₂ and CO₂ were measured by a dual O₂ and CO₂ gas analyzers (NormcapOxy; Datex-Ohmeda); allowing for breath-by-breath determination of PET_{O₂} and PET_{CO₂}. Finally, a pulse oximeter

(3900p; Datex-Ohmeda) was attached to the earlobe for monitoring of SaO_2 .

Blood pressure, heart rate, and cerebral blood flow measurements

Before and immediately after IH exposure participants rested for 10 minutes in a semi-reclined position for assessment of brachial artery blood pressure, heart rate, and CBF. Brachial blood pressure was measured using an automated oscillometric blood pressure monitor (Dinamap Compact S; Critikon Inc) and the mean of at least 2 measurements was recorded. Heart rate was monitored via a 3-lead ECG (Micromon 7142 B; Kontron Medical) and CBF was assessed by monitoring the velocity of blood travelling through the middle cerebral artery with transcranial Doppler ultrasonography.^{4,5} Resting heart rate and CBF were recorded as the mean rate and velocity, respectively, over the last 5 minutes of the resting period.

Urine sampling

Midstream urine samples were collected into sterile specimen containers (LeakBuster, Starplex Scientific) immediately upon arrival in the laboratory in the morning and immediately after the 6 hours of IH exposure. This permitted assessment of the urinary prostanoid production across the 6-hour interval of IH. Each urine sample was immediately aliquoted into pre-frozen, sterile centrifuge tubes as approximately eight 10 mL samples and promptly stored at -80°C for future prostanoid analyses.

OSA Patients

To extend observations from our acute model of IH to a clinical model of chronic IH, 33 newly diagnosed OSA patients were recruited from the Foothills Medical Centre Sleep Centre and a respiratory homecare company (Healthy Heart Sleep Company) between June 2011 and May 2012. Men and women, aged 18 to 70, with moderate-to-severe OSA and significant nocturnal hypoxia, were eligible to participate in the study. All participants underwent a medical history, physical examination, and laboratory screening. Exclusion criteria included cardiovascular disease, cerebrovascular disease, kidney disease, uncontrolled hypertension (blood pressure $>140/90$ despite maximal use of antihypertensive medications), diabetes, severe lung disease, current smoking, pregnancy, use of NSAIDs or exogenous sex hormones. These criteria deliberately excluded many of the co-morbidities commonly associated with OSA.²

Determination of OSA severity

Similar to healthy participants, OSA participants performed an unattended, level 3 diagnostic sleep study (Remmers Sleep

Recorder Model 4.2; Sagatech Electronics) following current guidelines and recommendations.²² Sleep apnea was defined as a RDI ≥ 15 as this reflects moderate-to-severe sleep apnea which is likely to be clinically significant.²⁰ Significant nocturnal hypoxia was defined as $\text{SaO}_2 \leq 90\%$ for $\geq 12\%$ of the total monitoring time as used within the Sleep Heart Health Study.²³ The raw data from the Remmers Sleep Recorder was reviewed by a sleep medicine physician (P.J.H.) to confirm the presence and severity of OSA.

Experimental protocol

OSA patients were participating in a larger study assessing the impact of OSA on the renin-angiotensin system (RAS) and instructed to consume >200 mmol of sodium per day for 3 days before each study day to ensure maximum suppression of the RAS.²⁴ Subjects were subsequently studied while awake in the supine position in a temperature-controlled, quiet room after an 8-hour fast. All patients provided a second morning midstream urine sample immediately upon arrival. Premenopausal female OSA patients were studied 14 days after the first day of the last menstrual period, determined by counting days.²⁵ Finally, patients on hypertensive medications that interfere with RAS activity were switched to a calcium-channel blocker (amlodipine) to achieve adequate blood pressure control 2 weeks prior to the study day, as these agents are considered to have a neutral effect on the RAS.²⁶

Blood pressure measurements

Similar to healthy participants, brachial blood pressure was measured while patients rested in a semi-reclined position using an automated oscillometric blood pressure monitor (Critikon Dinamap Pro Care; GE Healthcare). The mean of at least 2 measurements was recorded.

Urine sampling

The second morning midstream urine sample was collected into sterile specimen containers (LeakBuster, Starplex Scientific) and immediately put on ice and subsequently stored in -80°C until analyzed for prostanoid concentrations. Thus, urine samples from the OSA patients were collected by similar methodology to the IH healthy participants.

Urinary Prostanoids

Urine samples collected from healthy participants before, and after, IH exposures and from OSA patients were thawed completely in a chilled water bath, centrifuged at 3000 rpm, and the supernatant from each sample was analyzed via enzyme immunoassays for the stable urinary metabolites of prostacyclin (PGI_2 ; Enzo Life Sciences), prostaglandin E_2 (PGE_2), thromboxane A_2 (TXA_2), and prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$;

Cayman Chemical Company). To reduce quantitation error intrinsic to the assay methodology, all samples were analyzed in triplicate, with the mean value carried forward into subsequent analyses. All assays were performed according to the manufacturer's instructions and all inter- and intra-assay measures of variability, as well as quality control pools, were within expected limits as established by the manufacturer.

Statistical Analyses

The sample size of healthy participants was determined based upon previous findings from our group showing a 6.6 ± 6.3 mm Hg (mean \pm SD) increase in mean arterial pressure using the same 6-hour exposure to isocapnic IH protocol employed in the current study.⁴ In order to achieve a power of at least 0.85 for a 1-tailed, paired *t* test with an alpha of 0.05 a sample size of 10 was predicted to be required. Considering the potential for a 20% dropout rate, 15 volunteers were assessed for participation in the study.

Dependent variables were assessed for a normal distribution using the Wilk-Shapiro test. In healthy participants most dependent variables were found to have a normal distribution with 1 variable (PGE₂) exhibiting a slight departure from normality at 2 (out of 6) time points analyzed and 2 variables (TXA₂ and the PGI₂:TXA₂ ratio) showing a slight departure from normality at 1 time point (out of 6) analyzed. As previous research has demonstrated that analyses of variance are robust to departures from the assumption of normality even under small sample sizes,^{27–29} changes in blood pressure, heart rate, cerebral blood flow, and prostanoid concentrations before, and after, IH were analyzed using a 3-by-2 repeated measures analysis of variance (RM ANOVA) with the factors of medication (placebo, nonselective, and selective COX-2 inhibition) and IH (pre- and post-IH). Furthermore, if the assumption of Sphericity was violated, the Greenhouse-Geisser corrected *P* value was reported. Finally, if there was a significant main effect, post hoc comparisons were performed incorporating a Bonferroni correction for multiple comparisons.

For OSA patients, age, BMI, diastolic blood pressure (DBP), and urinary TXA₂ concentrations were normally distributed, while weight, mean arterial (MAP) and systolic blood pressure (SBP), and urinary concentrations of PGI₂, PGE₂, and PGF_{2 α} , and the PGI₂:TXA₂ ratio were not normally distributed. Therefore, comparisons between healthy participants and OSA patients for normally distributed variables were performed using independent sample Student *t* tests while comparisons of non-normally distributed variables were performed using the Mann-Whitney U test. For all comparisons, a Bonferroni correction for multiple comparisons was incorporated into the analyses. All results are provided as the mean \pm standard deviation and alpha was set a priori at 0.05.

Results

One healthy participant was removed from the study because of an adverse reaction to the first medication to which they were allocated (Figure 1). The remaining 12 participants completed all IH exposures and none were excluded on the basis of physiological measures. Table shows the characteristics of the healthy participants and newly diagnosed OSA patients.

Healthy Participants

Blood pressure, heart rate, and cerebral blood flow before intermittent hypoxia

Ingestion of the lactose placebo for 4 days did not change resting MAP, SBP or DBP ($P \geq 0.46$), while ingestion of the nonselective COX inhibitor indomethacin for 4 days resulted in higher MAP, SBP, and DBP, and a lower heart rate, compared with the placebo ($P \leq 0.05$; Figure 3). Blood pressures and heart rate were not impacted by 4 days of ingestion of the selective COX-2 inhibitor Celebrex[®] as blood pressures and heart rate were similar to placebo ($P \geq 0.56$; Figure 3). The blood pressure findings are consistent with the acute effects of each medication (ie, 2 to 3 hours after ingesting a single dose) on resting blood pressure (Figure 4). As salt balance may influence blood pressure,³⁰ 24-hour sodium excretion was estimated³¹ from urine samples collected before each IH exposure. Sodium excretion was similar among the 3

Table. Characteristics of Healthy Participants and Obstructive Sleep Apnea (OSA) Patients

	Healthy Participants	OSA Patients
Sample size (n)	12	33
Gender	12 male	23 male
Age, y	25.8 \pm 5.1	51.7 \pm 10.3*
BMI, kg m ⁻²	24.9 \pm 2.5	34.9 \pm 6.8*
MAP, mm Hg	83.0 \pm 8.3	96.2 \pm 10.3*
SBP, mm Hg	117.2 \pm 12.4	132.1 \pm 18.1*
DBP, mm Hg	65.9 \pm 8.7	78.2 \pm 8.9*
RDI, events h ⁻¹	1.8 \pm 1.1	47.4 \pm 21.3*
Mean SaO ₂ , %	94.8 \pm 1.1	88.8 \pm 3.9*
Minimum SaO ₂ , %	89.2 \pm 4.6	69.9 \pm 8.6*
Time SaO ₂ <90% (%)	0.0 \pm 0.1	41.1 \pm 25.5*

BMI, body mass index; MAP, mean arterial blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; RDI, respiratory disturbance index; Mean SaO₂, mean arterial oxyhemoglobin saturation (SaO₂) for entire duration of monitoring during a level 3 sleep diagnostic test; Minimum SaO₂, lowest SaO₂ recorded during a level 3 diagnostic sleep test; and Time SaO₂ < 90%, percentage of total monitoring time that SaO₂ was less than 90% during a level 3 diagnostic sleep test. All data provided as mean \pm SD.

* $P \leq 0.05$ versus healthy participants.

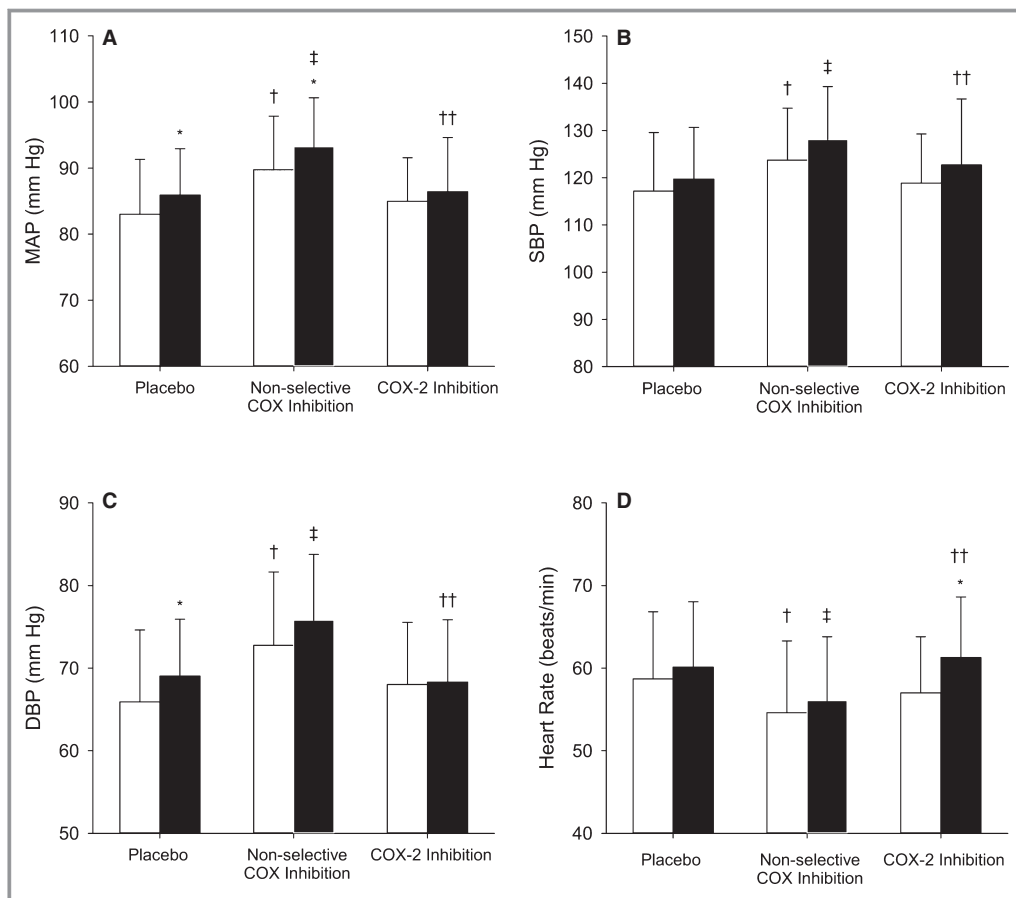


Figure 3. Resting mean (MAP), systolic (SBP) and diastolic (DBP) brachial blood pressures and heart rate of healthy participants before (□), and after (■) 6 hours of IH. † indicates significantly different from placebo before-IH values with $P \leq 0.05$; * indicates significant effect of IH within each condition $P \leq 0.05$; ‡ significantly different from after-IH placebo values with $P \leq 0.05$; and †† indicates significant difference from nonselective COX inhibition after-IH values. IH indicates intermittent hypoxia.

conditions ($P \geq 0.73$; Figure 5), and thus does not explain the observed blood pressures differences.

Cerebral blood flow and cerebrovascular resistance ($CVR = MAP/CBF$) prior to IH are shown in Figure 6. CBF was lower and CVR was higher after 4 days of nonselective COX inhibition compared with the placebo, ($P \leq 0.04$). There was no difference in CBF and CVR between the selective COX-2 inhibition and placebo conditions ($P \geq 0.92$). Again, these results are similar to the acute effects of each medication, except the acute effects of nonselective COX inhibition were much greater— $\approx 26\%$ decrease in CBF and $\approx 42\%$ increase in CVR versus $\approx 9\%$ decrease in CBF and $\approx 14\%$ increase in CVR (Figure 7).

Blood pressure and cerebral blood flow after intermittent hypoxia

After exposure to IH, MAP was increased within the placebo ($P = 0.03$) and nonselective COX inhibition conditions ($P = 0.05$) to a similar degree, but was unchanged within the selective COX-2 inhibition condition ($P = 0.19$; Figure 3A). There was a

trend for SBP to be increased in all conditions (placebo: $P = 0.09$; nonselective COX inhibition: $P = 0.08$; selective COX-2 inhibition: $P = 0.08$; Figure 3B) while DBP was increased with the placebo ($P = 0.03$), showed a trend ($P = 0.07$) to be elevated within the nonselective COX inhibition condition, but was unchanged from the pre-IH level with selective COX-2 inhibition ($P = 0.41$; Figure 3C). As a result, MAP, SBP, and DBP with nonselective COX inhibition remained significantly higher than within the placebo condition ($P \leq 0.01$) and was higher than observed within the selective COX-2 inhibition condition ($P \leq 0.03$) after IH. Furthermore, after the IH exposure, heart rate was similar to the pre-IH level within the placebo ($P = 0.28$) and nonselective inhibition conditions ($P = 0.39$), but was increased in the selective COX-2 inhibition condition ($P = 0.01$; Figure 3D). Moreover, post-IH heart rate was lower with nonselective COX inhibition compared to the placebo ($P = 0.01$) and selective COX-2 inhibition ($P < 0.01$) conditions. Additionally, although IH elevated estimated 24-hour sodium excretion³² within all conditions ($P = 0.05$; IH main effect) the elevation was similar across all 3

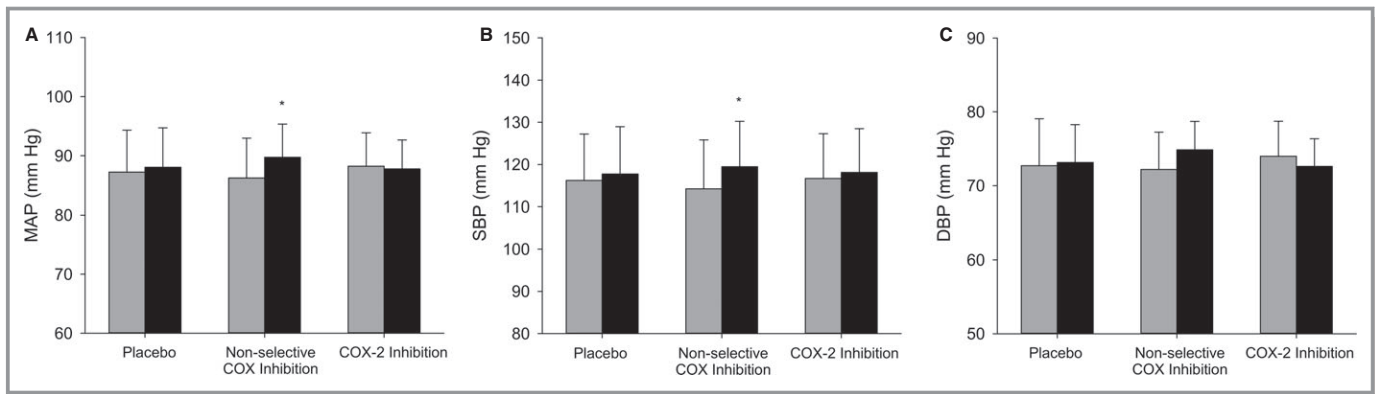


Figure 4. Using a single-blind, placebo-controlled, randomized, cross-over experimental design, the acute blood pressure responses to placebo, nonselective COX inhibitor, and selective COX-2 inhibitor medications were assessed in 10 healthy participants (9 overlap with the 12 from the IH study). Participants ingested a single oral dose of placebo (100 mg lactose), the nonselective COX inhibitor indomethacin (50 mg), and the selective COX-2 inhibitor Celebrex® (200 mg). Mean (MAP; A), systolic (SBP; B) and diastolic blood pressures (DBP; C) brachial blood pressures were measured before (□), and either 2 hours (placebo and non-selective COX inhibition) or 3 hours (COX-2 inhibition) after ingestion of medications (■). The mean of 3 separate blood pressure measurements taken over 10 minutes while participants were resting in a seated position was recorded. Participants ate a standardized breakfast prior to ingesting the medications. After ingestion, the participant remained in the seated, resting position watching television until post-drug measurements were performed. The time to post-drug measurements was based upon the pharmacokinetics of each medication and the time required to reach peak serum levels. The 3 experimental days were separated by at least 4 days. Pre-drug blood pressures were not different between the 3 conditions ($P \geq 0.26$). MAP and SBP were significantly elevated after ingestion of the non-selective COX inhibitor ($P \leq 0.01$). These findings support the conclusion that prostanoids formed via COX-1 are the primary regulator of resting blood pressure in healthy individuals. Results provided as mean \pm SD. * significantly different from Pre-drug values. COX indicates cyclooxygenase; IH, intermittent hypoxia.

conditions ($P=0.54$). As a result, estimated 24-hour sodium excretion was similar between all conditions after IH ($P=0.45$; Figure 5).

Exposure to 6 hours of IH, did not alter CBF within the placebo and nonselective COX inhibition conditions ($P \geq 0.54$), but resulted in a significantly decreased CBF ($\approx 10\%$) when COX-2 was selectively inhibited ($P=0.01$; Figure 6A). Combined with the MAP changes, CVR was unchanged within the

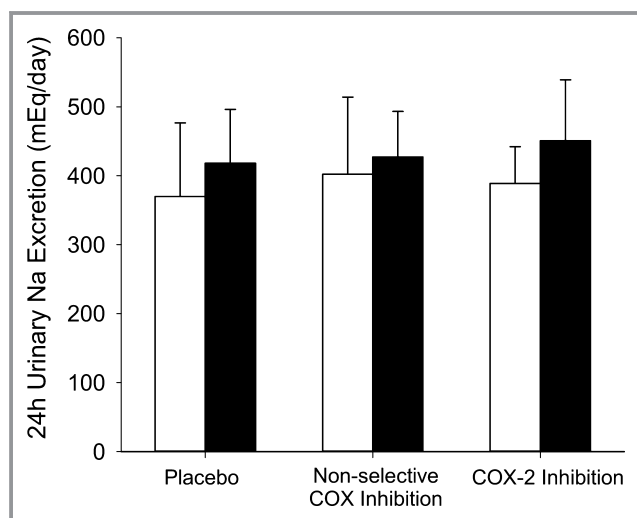


Figure 5. Estimated 24-hour sodium excretion before (□), and after (■), intermittent hypoxia exposure.

placebo and nonselective COX inhibition conditions ($P \geq 0.29$), but was elevated with selective COX-2 inhibition ($P < 0.01$; Figure 6B).

Urinary prostanoids

Urinary prostanoids concentrations before, and after, IH exposure are shown in Figure 8. Compared with placebo concentrations (Figure 8A), nonselective COX inhibition (Figure 8B) significantly decreased the vasodilatory prostanoids prostacyclin (PGI_2) and prostaglandin E_2 (PGE_2 ; $P < 0.01$) along with the vasoconstrictor prostanoids thromboxane A_2 (TXA_2) and prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$; $P \leq 0.01$). Similarly, selective COX-2 inhibition (Figure 8C) decreased the vasodilatory prostanoids PGI_2 and PGE_2 ($P \leq 0.04$), but in contrast to nonselective COX inhibition, did not change concentrations of the vasoconstrictor prostanoids TXA_2 and $\text{PGF}_{2\alpha}$ ($P=1.0$ for both prostanoids). Accordingly, the PGI_2 : TXA_2 ratio was higher after 4 days of nonselective COX inhibition and lower after 4 days of selective COX-2 inhibition compared with the placebo ($P < 0.01$).

Within the placebo condition, IH increased PGE_2 ($P=0.02$), but did not alter any of the other measured prostanoids ($P \geq 0.76$) or the PGI_2 : TXA_2 ratio ($P=0.25$). This increase in PGE_2 following IH was prevented by both, nonselective COX inhibition ($P=0.45$), and selective COX-2 inhibition ($P=0.13$). Furthermore, similar to the placebo condition, IH did not alter any of the additional measured prostanoids within the

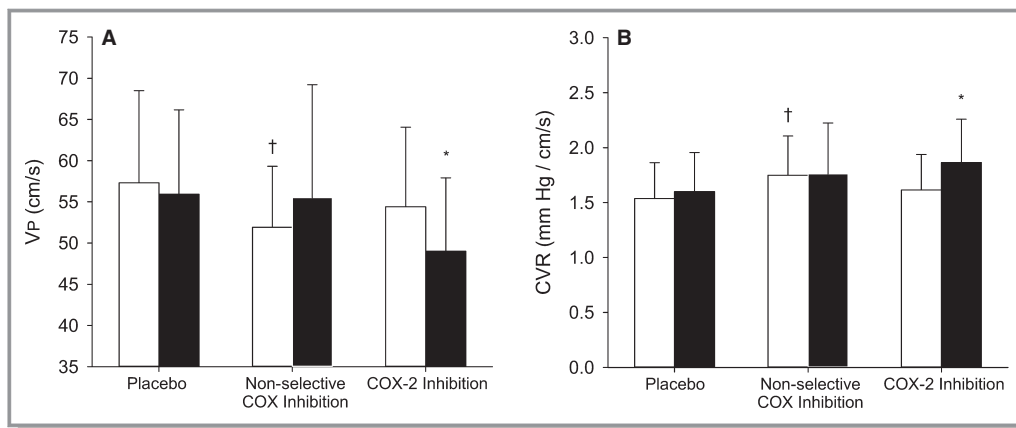


Figure 6. Cerebral blood velocity through the middle cerebral artery (\bar{V}_P) and cerebrovascular resistance (CVR) of healthy participants before (\square), and after (\blacksquare) 6 hours of IH. † indicates significantly different from placebo before-IH values with $P \leq 0.05$; * indicates significant effect of IH within each condition $P \leq 0.05$. IH indicates intermittent hypoxia.

nonselective COX and selective COX-2 inhibition conditions ($P \geq 0.16$).

OSA Patients

Untreated OSA patients had significantly higher MAP, SBP, and DBP compared with healthy participants before IH exposure within the placebo condition (Table). Moreover, OSA patients had a lower urinary concentration of PGE₂ ($P=0.03$) and a higher urinary concentration of the vasoconstrictor TXA₂ ($P<0.01$; Figure 9) compared with healthy participants before IH exposure within the placebo condition. In contrast, OSA patients had similar concentrations of the vasodilator PGI₂, and the vasoconstrictor PGF_{2 α} , as well as the PGI₂:TXA₂ ratio to the healthy participants before IH exposure within the placebo condition ($P \geq 0.20$).

Compared with the prostanoid concentrations of healthy participants after IH, PGI₂, and PGF_{2 α} concentrations remained similar between OSA patients and healthy participants ($P \geq 0.28$), while TXA₂ remained significantly higher ($P<0.01$) and PGE₂ concentrations remained lower ($P<0.01$) in OSA patients. Additionally, the PGI₂:TXA₂ ratio for OSA patients was lower ($P=0.04$) than the post-IH value of healthy participants.

Discussion

The principal findings of this double-blind, randomized, placebo-controlled, cross-over study were (1) in healthy participants, 4 days of nonselective COX inhibition elevated resting blood pressure and decreased CBF, while 4 days of selective COX-2 inhibition did not change either physiological variable; (2) in the placebo and nonselective COX inhibition conditions, IH-mediated similar elevations in blood pressure, and selective COX-2 inhibition prevented this increase;

(3) 6 hours of IH did not change resting CBF when COX isoenzymes were uninhibited (placebo) or when both COX-1 and COX-2 were inhibited (nonselective inhibition), but significantly decreased CBF and increased CVR when COX-2 was selectively inhibited; and (4) untreated OSA patients had higher blood pressures and urinary concentrations of the vasoconstrictor TXA₂ compared with healthy participants.

Nonselective COX inhibition and selective COX-2 inhibition are associated with increased risk of cardiovascular and cerebrovascular disease.¹² Based upon decreases in urinary concentrations of the vasodilator, antithrombotic prostacyclin (PGI₂),⁸ but no alteration in the vasoconstrictor, thrombotic thromboxane A₂ (TXA₂)⁸ concentrations with selective COX-2 inhibitors and COX-2 knockout mice,^{33,34} COX-2 has been proposed as the primary COX isoenzyme responsible for endothelial production of PGI₂. Accordingly, inhibition of COX-2 with traditional NSAIDs (non-selective COX inhibitors) is thought to be responsible for the increased cardiovascular and cerebrovascular risks associated with these medications, but this is not without controversy.^{14–16} A recent meta-analysis has enhanced this debate by concluding certain nonselective NSAID medications have similar vascular risks as selective COX-2 inhibitors.¹² In our healthy participants, 4 days of nonselective COX inhibition increased blood pressure and decreased CBF. Although we also observed significantly lower urinary PGI₂, but maintained TXA₂ concentrations with selective COX-2 inhibition,^{33,35} these did not translate into elevated blood pressure or altered CBF. The lack of change in blood pressure is similar to what has been reported previously.³³ Together, these findings do not support the contention that inhibition of COX-2 derived PGI₂ is responsible for the increased cardiovascular and cerebrovascular risks with nonselective COX inhibitors. In conjunction with the observed acute effects of nonselective and COX-2 selective inhibition on

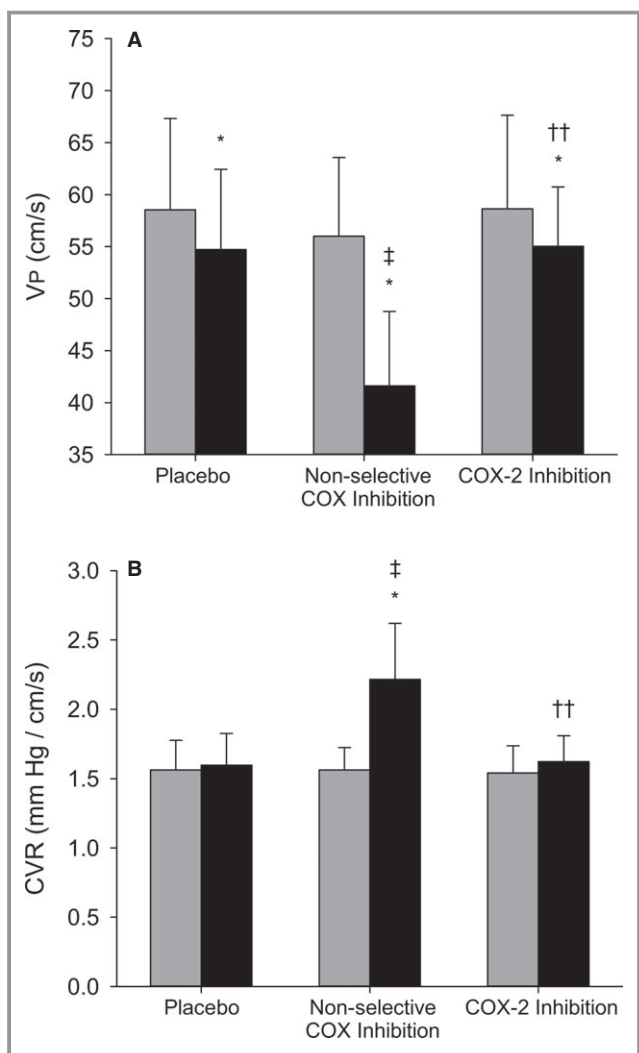


Figure 7. Acute cerebral blood velocity through the middle cerebral artery (\bar{V}_p) and cerebrovascular resistance (CVR) responses to a single dose of placebo (100 mg lactose), nonselective COX inhibitor (50 mg indomethacin), and selective COX-2 inhibitor (200 mg Celebrex[®]) medications. \bar{V}_p and CVR were assessed before (■), and either 2 hours (placebo (100 mg lactose) and nonselective COX inhibition) or 3 hours (COX-2 inhibition) after ingesting medications (■). Pre-drug \bar{V}_p and CVR were not different between the 3 drug conditions ($P \geq 0.24$). At the post-drug time point, \bar{V}_p was significantly lower within all 3 drug conditions ($P \leq 0.05$) while CVR was significantly increased after ingestion of only the non-selective COX inhibitor ($P < 0.01$). The magnitude of the decrease in \bar{V}_p from the pre-drug to post-drug condition was significantly greater with nonselective COX inhibition compared to placebo ($P < 0.01$) and selective COX-2 inhibition ($P < 0.01$). These findings support the conclusion that prostanoids formed via COX-1 are the primary regulators of resting cerebral blood flow in healthy individuals. Results provided as mean \pm SD. * indicates significant difference from pre-drug values with $P \leq 0.05$; ‡ significantly different from post-drug placebo values with $P \leq 0.05$; and †† indicates significant difference from nonselective COX inhibition post-drug values with $P \leq 0.05$. COX indicates cyclooxygenase.

blood pressure and CBF, these findings indicate prostanoids formed via COX-1 are the primary regulators of resting blood pressure¹⁶ and CBF in healthy humans.

Systemic inflammation induced by IH is an important mechanism for the cardiovascular and cerebrovascular sequelae of OSA by contributing to the development of endothelial dysfunction.^{6,7} Currently, there is mounting evidence that implicates alterations in prostanoids with IH as part of the pathway leading to vascular dysfunction.^{17,18} Nacher et al¹⁷ showed that rats subjected to OSA for 3 hours (60 apneas/h; 15-second apneas) or 3 hours of IH (15-second hypoxia and 15-second normoxia) had decreased plasma PGI₂ metabolites and elevated TXA₂ metabolites compared to a control group. Regrettably, no physiological responses were reported and, consequently, the impact of these prostanoid changes on blood pressure and CBF is unknown. More recently, Gautier-Veyret et al¹⁸ reported COX-1 mRNA was elevated by $\approx 70\%$ and COX-2 mRNA was increased by $\approx 25\%$ in ApoE knockout mice exposed to chronic IH (8 weeks; 60-second IH cycles; 8 hours/day). In addition, IH-induced atherosclerotic lesion size correlated with COX-1 and thromboxane synthase (downstream enzyme from COX-1 responsible for TXA₂ formation) mRNA, and selective COX-1 inhibition reduced atherosclerotic lesion size following IH exposure. Although the elevated COX mRNA did not translate into enhanced secretion capacity (ie, stimulated release) of PGI₂ and TXA₂ and basal PGI₂ and TXA₂ concentrations were not assessed, this study supports a greater role for COX-1 in the etiology cardiovascular sequelae of chronic IH exposure.

Unlike previous reports, the current study specifically investigated the relationship between prostanoids derived from both COX-1 and COX-2, and modifications in resting blood pressure and CBF with acute IH exposure in healthy humans. In the placebo condition, both MAP and DBP were elevated after 6 hours of IH, consistent with previous findings from our group using the same model of IH.⁴ Although nonselective COX inhibition elevated blood pressure prior to IH exposure, the IH-induced elevation in blood pressure was similar to the placebo condition. In contrast, selective COX-2 inhibition prevented the increase in blood pressure with IH. This protective effect of selective COX-2 inhibition is potentially the result of altering the interaction between COX-2 activity and the renin angiotensin system (RAS).

Upregulation of the RAS via enhanced sympathetic activation is intimately involved in IH-mediated elevations in blood pressure with renal denervation³⁶ and suppression of the RAS via salt loading³⁷ in rats preventing IH-induced increases in blood pressure. More recently, in a pig model of OSA, renal denervation reduced post-apneic rises in blood pressure and blunted the increase in circulating RAS components (plasma renin activity (PRA) and plasma aldosterone) associated with 4 hours of obstructive apneas (2-minute apneas, 4/hour).^{38,39}

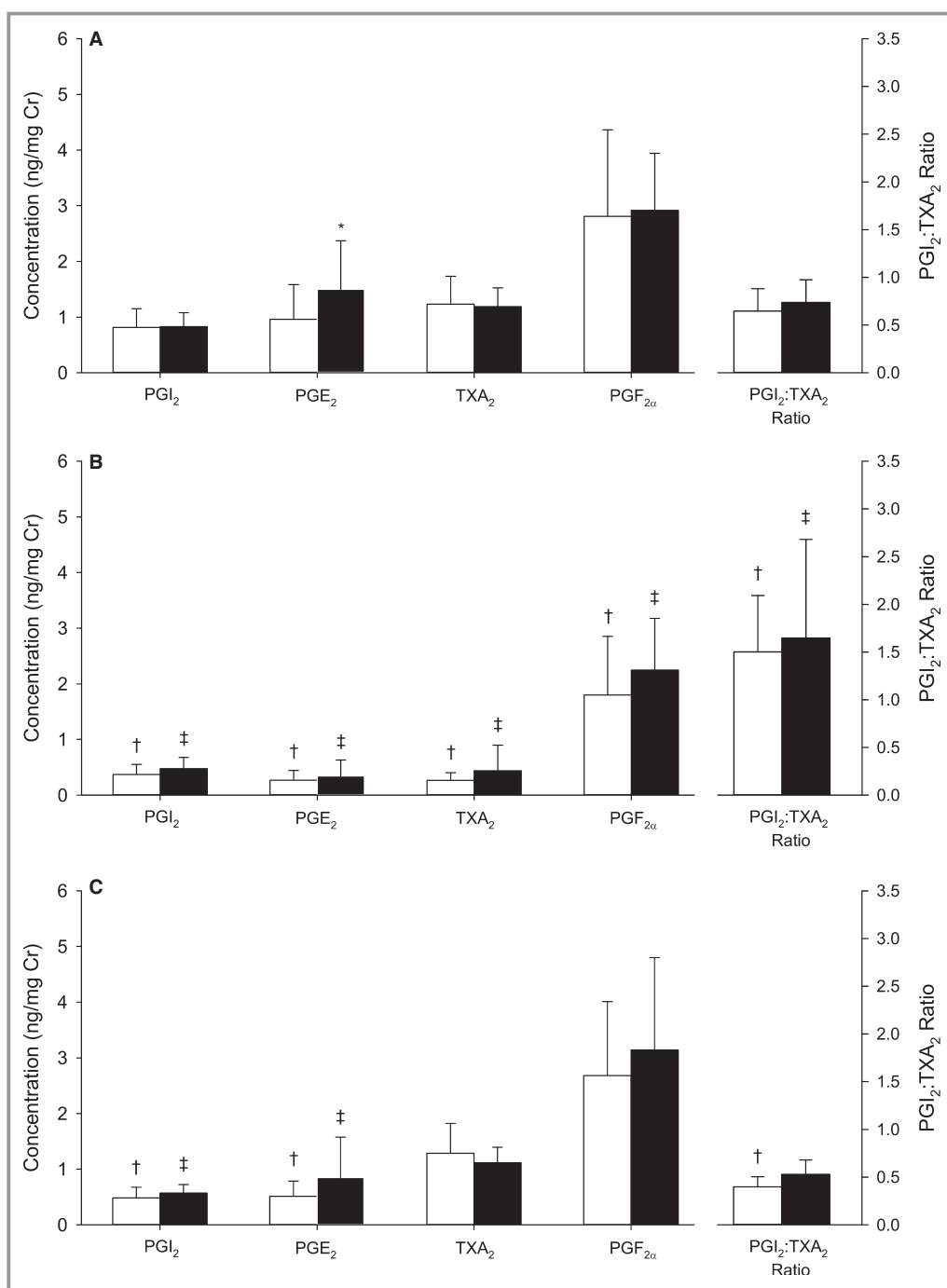


Figure 8. Urinary concentrations (normalized to creatinine) of prostacyclin (PGI₂), prostaglandin E₂ (PGE₂), thromboxane A₂ (TXA₂), and prostaglandin F_{2α} (PGF_{2α}) and the PGI₂:TXA₂ ratio in healthy participants before (□), and after (■), IH exposures within the placebo (A), nonselective COX inhibition (B) and the selective COX-2 inhibition (C) conditions. † indicates significantly different from placebo before-IH values with $P \leq 0.05$; * indicates significant effect of IH within each condition $P \leq 0.05$; and ‡ indicates significant difference from after-IH placebo values with $P \leq 0.05$. COX indicates cyclooxygenase; IH, intermittent hypoxia.

In addition, we recently showed, using the same acute IH paradigm as in the present study, blockade of type 1 angiotensin-II receptors (AT₁Rs) prevents the increase in blood pressure associated with IH⁴ by blunting increases in

oxidative stress and decreases in nitric oxide bioavailability.⁴⁰ In addition to increasing superoxide generation,⁴¹ angiotensin-II also increases COX-2 expression in vascular smooth muscle via binding to AT₁Rs, and the increased COX-2 activity

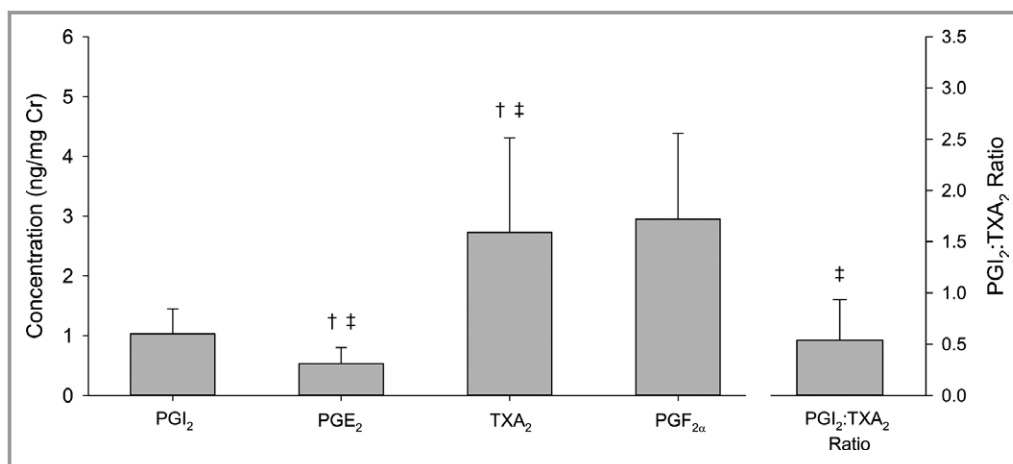


Figure 9. Urinary prostanoid concentrations (normalized to creatinine) in newly diagnosed OSA patients. † indicates significantly different from placebo pre-IH values with $P \leq 0.05$ and ‡ significantly different from post-IH placebo values with $P \leq 0.05$. IH indicates intermittent hypoxia; OSA, obstructive sleep apnea; PGE₂, prostaglandin E₂; PGF_{2α}, prostaglandin F_{2α}; PGI₂, prostacyclin; TXA₂, thromboxane A₂.

magnifies the actions of angiotensin-II on vascular smooth muscle cells.^{42,43} Moreover, prostanoids mediate renin release from the kidneys in response to sympathetic activation⁴⁴ and this appears to be COX-2 dependent²¹ as renin release is decreased with COX-2 inhibition.⁴⁵ Therefore, our results indicate RAS-induced increases in blood pressure with IH may be COX-2 dependent.

Although we did not assess components of the circulating RAS in the current study, our group has previously reported, using the identical model of isocapnic-IH during wakefulness (ie, IH exposure between $\approx 09:30$ and $15:30$ hours), that plasma renin activity (PRA) and plasma aldosterone concentration are lower after the 6 hours of IH. This decrease reflected the diurnal variation in PRA and plasma aldosterone as the decrease observed was similar to a 6-hour sham-IH (ie, euoxia) condition.⁴ Therefore, there is the potential the observed effects of IH on blood pressure may have been enhanced if the IH was administered during sleep when PRA and aldosterone concentrations are typically higher.⁴⁶

Following acute IH exposure, resting CBF is typically maintained or increased^{4,5} despite decreases in regulators of basal CBF such as nitric oxide.^{4,5,40,47} Since selective COX-2 inhibition resulted in a decreased CBF after IH exposure, the maintenance of CBF following IH may be dependent upon increased COX-2 production of vasodilatory prostanoids (PGI₂ and PGE₂) involved in basal CBF regulation.⁴⁷ Although speculative, an increase in PGI₂ and PGE₂ may be due to an augmented expression and activity of endothelial COX-2 stimulated by greater liberation of NF- κ B, and concentrations of IL-1 β and TNF α ^{48–50} with IH exposure.^{17,51}

The divergent responses to IH observed between nonselective COX and selective COX-2 inhibition are the likely result of indomethacin having ≈ 50 times greater selectivity for COX-

1 compared with COX-2.^{52,53} Thus, the contrasting responses of the two COX inhibitors reflect the greater role of COX-2 in the blood pressure and CBF responses to acute IH. Figure 10 outlines putative pathways through which COX-2 inhibition may have prevented the expected increase in blood pressure and maintained CBF with acute IH exposure.

To our knowledge, this is the first study to assess the impact of acute IH on prostanoid formation in healthy humans. The lack of change in urinary prostanoid formation with IH, except for an elevated PGE₂ within the placebo condition, contrasts to an animal study reporting decreased PGI₂ and elevated TXA₂ metabolites after only 3 hours of IH.¹⁷ These conflicting findings may be due to differences in the body fluid analyzed as well as species differences. In our study, we measured urinary prostanoids as a measure of systemic prostanoid production,⁵⁴ whereas the previous study¹⁷ assessed plasma prostanoid concentrations in rats.

In contrast to acute IH, chronic IH exposure (eg, untreated OSA) was associated with increased urinary TXA₂ and decreased PGE₂ concentrations. Three prior studies have compared urinary prostanoid concentrations between healthy controls and OSA patients,^{18,55,56} and all focused on PGI₂ and TXA₂. All studies report an elevated TXA₂ in untreated OSA patients with one study reporting an elevated TXA₂ in only OSA patients with cardiovascular risk factors such as obesity, hypertension, dyslipidemia, smoking, and metabolic syndrome.¹⁸ Thromboxane A₂ is preferentially formed via platelet COX-1,⁸ indicating a greater influence of chronic IH on COX-1 activity and derived prostanoids. The direct mechanism underlying the increased TXA₂ and decreased PGE₂ concentrations with chronic IH is unknown, but in addition to IH-induced inflammation, it may also be related to the chronic sympatho-excitation associated with untreated OSA.⁵⁷ Acute

because the female sex may be protective against cardiovascular consequences of IH.⁶³ Consequently, results may not be generalized to the female population. Third, our healthy controls were younger and lighter than the OSA patients. Although these differences may confound some of our results, neither age nor BMI were related to MAP or TXA₂ concentration within the healthy participants ($P \geq 0.412$) and OSA patients ($P \geq 0.349$). Fourth, prostanoid-stable metabolites were assessed in urine as validated markers of systemic prostanoid production,⁵⁴ but this provides an integrated measure over the time of urine production, rather than an instantaneous measure of vascular prostanoid concentrations.⁶⁴ Fifth, a potential limitation of the sample size of 12 healthy participants is a lack of statistical power. An a priori power calculation based upon previous findings⁴ indicated this sample size would provide a power of ≈ 0.85 to observe a ≈ 6.6 mm Hg increase in MAP following IH within the placebo condition. Although, the 2.9 ± 4.9 mm Hg increase in MAP after IH exposure within placebo condition is smaller than expected and a post hoc power calculation revealed the current study had a power of 0.61, this increase in MAP was significant ($P = 0.03$), indicating the study still had sufficient power to observe the enhancing effect of IH on MAP.

In conclusion, this study showed that, in healthy, male individuals, COX-1 formed prostanoids are the principal regulators of resting blood pressure and cerebral blood flow. Conversely, as outlined in Figure 10, COX-2 appears to be the primary COX isoenzyme contributing to the increase in blood pressure and maintenance of CBF following acute IH exposure. Furthermore, OSA is associated with elevated levels of the predominantly COX-1 derived vasoconstrictor TXA₂. Hence, COX-2 and COX-1 appear to have divergent roles in modulating vascular responses to acute versus chronic IH. These findings indicate COX-2 inhibition may be beneficial for individuals exposed to acute IH (eg, altitude training), while traditional nonselective COX inhibiting NSAIDs may help prevent cardiovascular and cerebrovascular morbidity and mortality in OSA via inhibition of COX-1,¹⁸ although enhanced patient monitoring may be required as a result of the cardiovascular risks associated with nonselective COX inhibitors.¹²

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Author Contributions

The work outlined in this manuscript was led by a multi-group collaboration of senior team members including Hanly (obstructive

sleep apnea; phanly@ucalgary.ca), Wynne-Edwards (prostanoids; k.wynne-edwards@ucalgary.ca), Ahmed (renal function and the renin angiotensin system; sofia.ahmed@albertainhealthservices.ca), and Poulin (human integrative physiology and intermittent hypoxia; poulin@ucalgary.ca). Beaudin, Pun, Slater, Ahmed, Hanly, and Poulin conceived the experimental design of studies conducted in healthy humans. Nicholl, Ahmed, Hanly, and Poulin conceived the experimental design of studies conducted in patients with obstructive sleep apnea (OSA). Wynne-Edwards led, guided and oversaw the analytical (development, validation, final analyses) work on prostanoid quantification, which was conducted by Beaudin, Pun, and Yang. Primary supervision for Beaudin was provided by Hanly and Poulin; primary supervision for Pun by Ahmed and Poulin; primary supervision for Yang by Poulin; primary supervision for Nicholl by Ahmed and Hanly. The experiments in healthy humans were performed by Beaudin, Pun, Yang, and Steinback while the experiments in patients with OSA were performed by Nicholl. All co-authors contributed to the interpretation of the data. Beaudin wrote the first draft of the manuscript and Pun, Steinback, Slater, Hanly, Wynne-Edwards, Ahmed, and Poulin edited the manuscript.

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Disclosures

None.

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