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

Andrew E. Beaudin, MSc; Matiram Pun, MSc; Christina Yang, BSc; David D. M. Nicholl, MSc; Craig D. Steinback, PhD; Donna M. Slater, PhD; Katherine E. Wynne-Edwards, PhD; Patrick J. Hanly, MD, FRCPC; Sofia B. Ahmed, MD, MMSc; Marc J. Poulin, PhD, DPhil

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<0.04) and decreased pre-IH CBF (P<0.04) while neither physiological variable was affected by COX-2 inhibition (P≥0.90). Post-IH, MAP was elevated (P≤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≤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.

Clinical Trial Registration—URL: www.clinicaltrials.gov. Unique identifier: NCT01280006 (J Am Heart Assoc. 2014;3:e000875 doi: 10.1161/JAHA.114.000875)

Key Words: blood pressure • cerebrovascular circulation • intermittent hypoxia • obstructive sleep apnea • prostaglandins
prostanoids in vascular regulation. Additionally, although nonselective and COX-2 selective inhibition increase the risk of cardiovascular and cerebrovascular disease,\textsuperscript{12} there is still considerable controversy regarding the importance of COX-1 and COX-2 formed prostanoids for vascular regulation.\textsuperscript{13–16}

With IH, prostanoid concentrations are shifted towards vasoconstriction and atherogenesis.\textsuperscript{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\textless;1101 m) for <1 year, a body mass index (BMI)\textless;35 kg\textper m\textsuperscript{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\textsuperscript{8}, 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\textper L\textsuperscript{1}), liver dysfunction (ie, ALT, AST, ALP, and INR outside normal ranges), and/or renal dysfunction (estimated glomerular filtration rate (GFR)\textless;60 mL\textper min\textper 1.73 m\textsuperscript{2}, urinary protein excretion>150 mg\textper 24 h\textsuperscript{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 (SaO\textsubscript{2}) 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 SaO\textsubscript{2} 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 SaO\textsubscript{2} decreased by \textgreater;4%, divided by the total recording time. This system has been validated against polysomnography, the gold standard diagnostic test for OSA.\textsuperscript{19,20} Raw data from the sleep recorder (ie, SaO\textsubscript{2}, 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\textsuperscript{1} and/or a mean SaO\textsubscript{2} 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)
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 the experimental protocol.
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 (PETO₂) 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 PETO₂ to 88 mm Hg (normal PETO₂ 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).
(3900 p; Datex-Ohmeda) was attached to the earlobe for monitoring of SaO2.

**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. 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 prostanooid production across the 6-hour interval of IH. Each urine sample was immediately aliquoted into prefrozen, sterile centrifuge tubes as approximately eight 10 mL samples and promptly stored at −80°C for future prostanooid 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.

**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. Sleep apnea was defined as a RDI ≥15 as this reflects moderate-to-severe sleep apnea which is likely to be clinically significant. Significant nocturnal hypoxia was defined as SaO2 ≤90% for ≥12% of the total monitoring time as used within the Sleep Heart Health Study. 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. 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. 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.

**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 prostanooid concentrations. Thus, urine samples from the OSA patients were collected by similar methodology to the IH healthy participants.

**Urinary Prostanooids**

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 (PGI2; Enzo Life Sciences), prostaglandin E2 (PGE2), thromboxane A2 (TXA2), and prostaglandin F2α (PGF2α).
Cyclooxygenase, Intermittent Hypoxia & Sleep Apnea  
Beaudin et al

pools, were within expected limits as established by the
intra-assay measures of variability, as well as quality control
using the Wilk-Shapiro test. In healthy participants most
assessed for participation in the study.
6.3 mm Hg (mean±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
employed in the current study.4 In order to achieve a power of

DOI: 10.1161/JAHA.114.000875
Journal of the American Heart Association

Statistical Analyses
The sample size of healthy participants was determined based
upon previous findings from our group showing a 6.6±

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≥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<0.05; Figure 3). Blood pres-
sures 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≥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

Healthy Participants


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 PGF2α,
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 compari-
sions, a Bonferroni correction for multiple comparisons was
incorporated into the analyses. All results are provided as the
mean±standard deviation and alpha was set a priori at 0.05.

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

|                          | Healthy Patients | OSA Patients |
|--------------------------|------------------|--------------|
| Sample size (n)          | 12               | 33           |
| Gender                   | 12 male          | 23 male      |
| Age, y                   | 25.8±5.1         | 51.7±10.3*   |
| BMI, kg m⁻²              | 24.9±2.5         | 34.9±6.8*    |
| MAP, mm Hg               | 83.0±8.3         | 96.2±10.3*   |
| SBP, mm Hg               | 117.2±12.4       | 132.1±18.1*  |
| DBP, mm Hg               | 65.9±8.7         | 78.2±8.9*    |
| RDI, events h⁻¹          | 1.8±1.1          | 47.4±21.3*   |
| Mean SaO₂, %             | 94.8±1.1         | 88.8±3.9*    |
| Minimum SaO₂, %          | 89.2±4.6         | 69.9±6.8*    |
| Time SaO₂<90% (%)        | 0.0±0.1          | 41.1±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±SD.
*P<0.05 versus healthy participants.

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.

DOI: 10.1161/JAHA.114.000875
Journal of the American Heart Association

6
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/CFB) 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—a 26% decrease in CBF and 42% increase in CVR versus a 9% decrease in CBF and 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 ($P = 0.03$) 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 excretion within all conditions ($P = 0.05$; IH main effect) the elevation was similar across all 3
conditions \((P=0.54)\). As a result, estimated 24-hour sodium excretion was similar between all conditions after IH \((P=0.45); \text{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 \((P=0.01); \text{Figure 6A})\). Combined with the MAP changes, CVR was unchanged within the placebo and nonselective COX inhibition conditions \((P\geq 0.29)\), but was elevated with selective COX-2 inhibition \((P<0.01); \text{Figure 6B})\).

**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 \((\text{PGI}_2)\) and prostaglandin \(\text{E}_2\) \((\text{PGE}_2; P<0.01)\) along with the vasoconstrictor prostanoids thromboxane \(\text{A}_2\) \((\text{TXA}_2)\) and prostaglandin \(\text{F}_{2\alpha}\) \((\text{PGF}_{2\alpha}; P<0.01)\). Similarly, selective COX-2 inhibition (Figure 8C) decreased the vasodilatory prostanoids \(\text{PGI}_2\) and \(\text{PGE}_2\) \((P\leq 0.04)\), but in contrast to nonselective COX inhibition, did not change concentrations of the vasoconstrictor prostanoids \(\text{TXA}_2\) and \(\text{PGF}_{2\alpha}\) \((P=1.0\) for both prostanoids\)). Accordingly, the \(\text{PGI}_2:\text{TXA}_2\) 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 \(\text{PGE}_2\) \((P=0.02)\), but did not alter any of the other measured prostanoids \((P\geq 0.76)\) or the \(\text{PGI}_2:\text{TXA}_2\) ratio \((P=0.25)\). This increase in \(\text{PGE}_2\) 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 prostanoidswithin the
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 PGE$_2$ ($P=0.03$) and a higher urinary concentration of the vasoconstrictor TXA$_2$ ($P<0.01$; Figure 9) compared with healthy participants before IH exposure within the placebo condition. In contrast, OSA patients had similar concentrations of the vasodilator PGI$_2$, and the vasoconstrictor PGF$_2\alpha$, as well as the PGI$_2$:TXA$_2$ 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, PGI$_2$, and PGF$_2\alpha$ concentrations remained similar between OSA patients and healthy participants ($P \geq 0.28$), while TXA$_2$ remained significantly higher ($P<0.01$) and PGE$_2$ concentrations remained lower ($P<0.01$) in OSA patients. Additionally, the PGI$_2$:TXA$_2$ 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 TXA$_2$ compared with healthy participants.

Nonselective COX inhibition and selective COX-2 inhibition are associated with increased risk of cardiovascular and cerebrovascular disease. Based upon decreases in urinary concentrations of the vasodilator, antithrombotic prostacyclin (PGI$_2$), but no alteration in the vasoconstrictor, thrombotic thromboxane A$_2$ (TXA$_2$) concentrations with selective COX-2 inhibitors and COX-2 knockout mice, COX-2 has been proposed as the primary COX isoenzyme responsible for endothelial production of PGI$_2$. 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. A recent meta-analysis has enhanced this debate by concluding certain nonselective NSAID medications have similar vascular risks as selective COX-2 inhibitors. In our healthy participants, 4 days of nonselective COX inhibition increased blood pressure and decreased CBF. Although we also observed significantly lower urinary PGI$_2$, but maintained TXA$_2$ concentrations with selective COX-2 inhibition, 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. Together, these findings do not support the contention that inhibition of COX-2 derived PGI$_2$ 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
blood pressure and CBF, these findings indicate prostanoids formed via COX-1 are the primary regulators of resting blood pressure\(^6\) 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.\(^5,7\) Currently, there is mounting evidence that implicates alterations in prostanoids with IH as part of the pathway leading to vascular dysfunction.\(^7,18\) Nacher et al\(^7\) 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 PGI\(_2\) metabolites and elevated TXA\(_2\) 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 al\(^18\) reported COX-1 mRNA was elevated by \(\approx70\%\) and COX-2 mRNA was increased by \(\approx25\%\) 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 TXA\(_2\) 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 PGI\(_2\) and TXA\(_2\) and basal PGI\(_2\) and TXA\(_2\) 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 denervation\(^16\) and suppression of the RAS via salt loading\(^37\) 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 7.** Acute cerebral blood velocity through the middle cerebral artery (\(V_p\)) 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\(^8\)) medications. \(V_p\) and CVR were assessed 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 \(V_p\) and CVR were not different between the 3 drug conditions (\(P<0.24\)). At the post-drug time point, \(V_p\) was significantly lower within all 3 drug conditions (\(P=0.05\)) while CVR was significantly increased after ingestion of only the nonselective COX inhibitor (\(P=0.01\)). The magnitude of the decrease in \(V_p\) 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±SD. * indicates significant difference from pre-drug values with \(P<0.05\); † significantly different from post-drug placebo values with \(P<0.05\); and †† indicates significant difference from nonselective COX inhibition post-drug values with \(P<0.05\). COX indicates cyclooxygenase.
In addition, we recently showed, using the same acute IH paradigm as in the present study, blockade of type 1 angiotensin-II receptors (AT$_1$Rs) prevents the increase in blood pressure associated with IH$^4$ 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 AT$_1$Rs, and the increased COX-2 activity

\[ \text{Biological process} \]

\[ \text{Gene expression} \]

\[ \text{Molecular function} \]

\[ \text{Cellular component} \]

\[ \text{Pathway} \]

\[ \text{Gene Ontology (GO)} \]

\[ \text{Interactome} \]

\[ \text{Proteomics} \]

\[ \text{Spectral analysis} \]

\[ \text{Bioinformatics} \]

\[ \text{Pathway analysis} \]

\[ \text{Q&A} \]

\[ \text{Discussion} \]

\[ \text{Conclusion} \]

\[ \text{References} \]

\[ \text{Appendix} \]

\[ \text{Supplementary material} \]

\[ \text{Online resources} \]

\[ \text{Abbreviations} \]

\[ \text{Acknowledgments} \]

\[ \text{Ethics statement} \]

\[ \text{Author contributions} \]

\[ \text{Competing interests} \]

\[ \text{Funding} \]

\[ \text{Data availability} \]

\[ \text{ORCID iD} \]

\[ \text{License} \]

\[ \text{Atenolol} \]

\[ \text{Angiotensin II} \]

\[ \text{Cyclooxygenase} \]

\[ \text{Intermittent Hypoxia} \]

\[ \text{Sleep Apnea} \]

\[ \text{Beaudin et al} \]

\[ \text{Journal of the American Heart Association} \]

\[ \text{DOI: 10.1161/JAHA.114.000875} \]

---

\[ \text{Figure 8. Urinary concentrations (normalized to creatinine) of prostacyclin (PGI$_2$), prostaglandin E$_2$ (PGE$_2$), thromboxane A$_2$ (TXA$_2$), and prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) and the PGI$_2$:TXA$_2$ ratio in healthy participants before (□), and after (■), IH exposures within the placebo (A), nonselective COX inhibition (B) and the selective COX-2 inhibition (C) conditions. † indicates significantly different from placebo before-IH values with $P \leq 0.05$; * indicates significant effect of IH within each condition $P \leq 0.05$; and ‡ indicates significant difference from after-IH placebo values with $P \leq 0.05$. COX indicates cyclooxygenase; IH, intermittent hypoxia.} \]
magnifies the actions of angiotensin-II on vascular smooth muscle cells.\textsuperscript{42,43} Moreover, prostanooids mediate renin release from the kidneys in response to sympathetic activation\textsuperscript{44} and this appears to be COX-2 dependent\textsuperscript{21} as renin release is decreased with COX-2 inhibition.\textsuperscript{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.\textsuperscript{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.\textsuperscript{46}

Following acute IH exposure, resting CBF is typically maintained or increased\textsuperscript{4,5} despite decreases in regulators of basal CBF such as nitric oxide.\textsuperscript{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 prostanooids (PGI\(_2\) and PGE\(_2\)) involved in basal CBF regulation.\textsuperscript{47} Although speculative, an increase in PGI\(_2\) and PGE\(_2\) may be due to an augmented expression and activity of endothelial COX-2 stimulated by greater liberation of NF-\(\kappa\)B, and concentrations of IL-1\(\beta\) and TNF\(_{\alpha}\) with IH exposure.\textsuperscript{17,51}

The divergent responses to IH observed between nonselective COX and selective COX-2 inhibition are the likely result of indomethacin having \( \approx \)50 times greater selectivity for COX-1 compared with COX-2.\textsuperscript{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 prostanooid formation in healthy humans. The lack of change in urinary prostanooid formation with IH, except for an elevated PGE\(_2\) within the placebo condition, contrasts to an animal study reporting decreased PGI\(_2\) and elevated TXA\(_2\) metabolites after only 3 hours of IH.\textsuperscript{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 prostanooids as a measure of systemic prostanooid production\textsuperscript{54} whereas the previous study\textsuperscript{17} assessed plasma prostanooid concentrations in rats.

In contrast to acute IH, chronic IH exposure (eg, untreated OSA) was associated with increased urinary TXA\(_2\) and decreased PGE\(_2\) concentrations. Three prior studies have compared urinary prostanooid concentrations between healthy controls and OSA patients,\textsuperscript{18,55,56} and all focused on PGI\(_2\) and TXA\(_2\). All studies report an elevated TXA\(_2\) in untreated OSA patients with one study reporting an elevated TXA\(_2\) in only OSA patients with cardiovascular risk factors such as obesity, hypertension, dyslipidemia, smoking, and metabolic syndrome.\textsuperscript{18} Thromboxane A\(_2\) is preferentially formed via platelet COX-1,\textsuperscript{8} indicating a greater influence of chronic IH on COX-1 activity and derived prostanooids. The direct mechanism underlying the increased TXA\(_2\) and decreased PGE\(_2\) 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.\textsuperscript{57}
sympathetic activation increases systemic vascular TXA₂ concentrations⁵⁸ and with repetitive sympathetic stimulation, PGE₂ (along with PGI₂) concentrations decline.⁵⁹ Thus, in untreated OSA patients, chronic IH-induced sympathetic activation may contribute to the observed increased TXA₂ and decreased PGE₂ concentrations.

Finally, limitations of the study must be acknowledged. First, although the IH model replicates the profile and severity of hypoxia experienced by patients with moderate-to-severe OSA it lacks the ancillary features associated with obstructive apneas such as increased negative intrathoracic pressure (which leads to greater sympathetic activation and post-apneic blood pressure rises than IH alone⁶⁰), hypercapnia, and sleep fragmentation.⁵ Although this may temper extrapolation of our findings to OSA, our IH model provides the opportunity to evaluate the impact of IH on prostanoid formation without the confounding effects of these ancillary features. Furthermore, animal¹⁸,⁶¹ and human⁵,⁶² studies have shown experimental IH produces similar physiological responses to those observed in OSA. Second, only healthy male participants were exposed to IH.

Figure 10. Putative pathways through which selective COX-2 inhibition may have prevented the IH-induced blood pressure elevation and resulted in a decreased cerebral blood flow. With IH exposure, there is an up regulation of the renin-angiotensin system (RAS) via increased sympathetic nervous system activation resulting in increased renin activity and angiotensin-II formation⁵⁶,⁵⁷ (Right; A). Subsequently, angiotensin-II binds to angiotensin type 1 receptors (AT₁r) on vascular smooth muscle cells causing vasoconstriction and an increase in blood pressure (Right; B). Via binding to the AT₁r, angiotensin-II increases COX-2 expression, which enhances the vascular effects of angiotensin-II on the vascular smooth muscle cells⁴²,⁴³ (Right; C). Additionally, since renin release is COX-2 dependent,²¹,⁴⁵ IH induced increases in COX-2 may also enhances renin activity and formation of angiotensin-II which, in turn, will further enhance COX-2 expression (Right; D). Thus, it is proposed that the required magnitude of RAS up regulation to produce an increase in blood pressure with IH is dependent upon the augmenting effects of COX-2. In addition, COX-2 expression is enhanced via IH induced inflammation (eg, IL-1β, TNFα, and NFκβ—Right; E). Therefore, selectively inhibiting COX-2 may have prevented the augmentation of the vascular effects of angiotensin-II as well as minimizing renin activity. As a result, the RAS system was not sufficiently up regulated by IH resulting in maintenance of blood pressure. In contrast, within the cerebral vasculature, an elevation of NFκβ, IL-1β, and TNFα may lead to augmented expression and activity of endothelial COX-2⁴⁸–⁵⁰ and enhanced release of vasodilatory prostanoids involved in regulating resting CBF⁴⁷ leading to the maintenance of CBF after IH⁴,⁵ (Left; F). Selective inhibition of COX-2 may have blocked this increase in vasodilatory prostanoids and caused CBF to decrease with IH exposure. CBF indicates cerebral blood flow; COX, cyclooxygenase; IH, intermittent hypoxia; RAS, renin-angiotensin system.
because the female sex may be protective against cardiovascular consequences of IH. 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 ≥ 0.412) and OSA patients (P ≥ 0.349).

Fourth, prostanoid-stable metabolites were assessed in urine as validated markers of systemic prostanoid production, but this provides an integrated measure over the time of urine production, rather than an instantaneous measure of vascular prostanoid concentrations. 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 findings 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, although enhanced patient monitoring may be required as a result of the cardiovascular risks associated with nonselective COX inhibitors.

Acknowledgments

The authors thank Lea Bond, MSc for her technical guidance with performing enzyme immunoassay analyses, Darlene Y. Sola, BScN, RN for assisting in OSA patient recruitment and data collection, and Drs Todd J. Anderson and Jaideep Bains for their feedback on the manuscript. Finally, the authors would like to thank Dr. Tolulope Sajobi for the very helpful statistical discussions.

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@albertahealthservices.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.

Sources of Funding

Beaudin is supported by an Alberta Innovates-Health Solutions (AI-HS) doctoral fellowship and the Canadian Institutes of Health Research (CIHR)-Heart and Stroke Foundation of Canada Focus on Stroke doctoral fellowship. Pun is supported by a William H. Davies Medical Research Scholarship (University of Calgary); Yang is supported by a Markin Undergraduate Student Research Program in Health and Wellness and an AI-HS undergraduate summer studentship. Nicholl is supported by a Cosmopolitan International Club of Calgary Graduate Scholarship, an American Society of Nephrology Student Scholar Grant and a Foothills Medical Centre Sleep Centre Development Fund. Steinback is supported by Natural Sciences and Engineering Research Council of Canada (NSERC) and AI-HS post-doctoral fellowships. Slater is supported by AI-HS and CIHR. Wynne-Edwards is the Jack Manns Professor of Comparative Endocrinology (Faculty of Veterinary Medicine). Ahmed is supported by AI-HS, CIHR, and a joint initiative between Alberta Health and Wellness and the Universities of Alberta and Calgary. The funding for the studies in healthy humans and all biochemical analyses was provided by CIHR (PI = Poulin, Co-Applicant = Hanly) and NSERC (PI = Poulin). The funding for the studies in OSA patients was provided by AI-HS (PI = Ahmed).

Disclosures

None.

References

1. Punjabi NM. The epidemiology of adult obstructive sleep apnea. Proc Am Thorac Soc. 2008;5:136–143.
2. Somers VK, White DP, Amin R, Abraham WT, Costa F, Culebras A, Daniels S, Floras JS, Hunt CE, Olson LJ, Pickering TG, Russell R, Woo M, Young T. Sleep
apnea and cardiovascular disease: an American Heart Association/American College of Cardiology Foundation Scientific Statement from the American Heart Association Council for High Blood Pressure Research Professional Research Committee, Council on Clinical Cardiology, Stroke Council, and Council on Cardiovascular Nursing. In Collaboration with the National Heart, Lung, and Blood Institute National Center on Sleep Disorders Research (National Institutes of Health). Circulation. 2008;118:1080–1111.

3. Tamisier R, Pepin JL, Remy J, Baguet JP, Taylor JA, Weiss JW, Levy P. 14 nights of intermittent hypoxia elevate daytime blood pressure and sympathetic activity in healthy humans. Eur Respir J. 2011;37:119–128.

4. Foster GE, Hanly PJ, Ahmed SB, Pialoux V, Poulin MJ. Intermittent hypoxia increases arterial blood pressure in humans through a renin-angiotensin-system dependent mechanism. 2010;53(6):369–377.

5. Foster GE, Brugniaux JV, Pialoux V, Duggan CT, Hanly PJ, Ahmed SB, Poulin MJ. Cardiovascular and cerebrovascular responses to acute hypoxia following exposure to intermittent hypoxia in healthy humans. J Physiol. 2009;587:3287–3299.

6. Ryan S, Taylor CT, McNicholas WT. Systemic inflammation: a key factor in the pathogenesis of cardiovascular complications in obstructive sleep apnea syndrome? Thorax. 2009;64:631–636.

7. Lavie L. Oxidative stress and inflammation in OSA. Sleep Apnoea. European Respiratory Society Journals Ltd. 2010;360–380.

8. Ricotti E, FitzGerald GA. Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol. 2011;31:986–1000.

9. Smyth EM, Grosser T, Wang M, Yu Y, FitzGerald GA. Prostanoids in health and disease. J Lipid Res. 2009;50(suppl):S423–S428.

10. Pickard JD, Mackenzie ET. Inhibition of prostaglandin synthesis and the response of baboon cerebral circulation to carbon dioxide. Nat New Biol. 1973;245:187–188.

11. Wennmalm A, Eriksson S, Wahren J. Effect of indomethacin on basal and carbon dioxide stimulated cerebral blood flow in man. Clin Physiol. 1981;1:227–234.

12. Coixb and traditional NSDA Trialsists’ (CNT) Collaboration, Bhala N, Emberson J, Merhi A, Abramson S, Arbor N, Baron JA, Bombardier C, Cannon C, Farkouh M, FitzGerald GA, Goss P, Hallis H, Hawk E, Hawcory C, Herekens C, Hochberg M, Holland LE, Kearney PM, Lanas L, Lanas A, Lance P, Laupacis A, Oates J, Patrano C, Schnitzer TJ, Solomon S, Tugwell P, Wilson K, Wittes J, Baigent C. Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: meta-analysis of individual participant data from randomised trials. Lancet. 2013;382:769–779.

13. Grosser T, Fries S, FitzGerald GA. Biological basis for the cardiovascular consequences of cox-2 inhibition: therapeutic challenges and opportunities. J Clin Invest. 2004;114:4–15.

14. Mitchell JA, Warner TD. Reply to ricciotti et al.: evidence for vascularcox isoforms. Proc Natl Acad Sci USA. 2013;110:E184.

15. Ricciotti E, Yu Y, Grosser T, FitzGerald GA. Cox-2, the dominant source of prostacyclin. Proc Natl Acad Sci USA. 2013;110:E183.

16. Kirby NS, Lundberg MH, Harrington LS, Leadbeater PD, Milne GL, Potter CM, Stichtenoth DO, Marhauer V, Tsikas D, Gutzki FM, Frolich JC. Effects of sleep apnea on arterial blood pressure. Hypertension. 2009;53(6):S428.

17. Linz D, Hohl M, Lanas A, Huber D, Nussberger J, Plank M, Ehrlich M, Ehrlich M, Andenmatten C, Vortmeyer AO, Steurer J, Landmesser U, Schwartz AR, Gerhard-Herman M. The renin-angiotensin system revisited: a quantitative review of alternatives to the one-way analysis of variance “f” test. Rev Educ Res. 1996;66:579–619.

18. Glass GV, Peckham PD, Sanders JR. Consequences of failure to meet assumptions underlying the fixed effects analyses of variance and covariance. Rev Educ Res. 1972;42:237–288.

19. Harwell MR, Rubinstein EN, Hayes WS, Ols CC. Summarizing monte carlo results in methodological research: the one- and two-factor fixed effects anova cases. J Educ Behav Stat. 1992;17:315–339.

20. Alderman MH. Salt, blood pressure, and human health. Hypertension. 2000;36:899–903.

21. Kahraman L, Thach BT. Inhibitory effects of hyperthermia on mechanisms involved in autoregulation from hypoxia in mouse. Science. 1995;269:429–433.

22. Tanaka T, Okamura T, Miura K, Kadowaki T, Ueshima H, Nakagawa H, Hashimoto T. A simple method to estimate population 24 h urinary sodium and potassium excretion using a casual urine specimen. J Hum Hypertens. 2002;16:97–103.

23. Catella-Lawson F, McAdam B, Morrison WP, Kapoor S, Kujubu D, Antes L, Lasserter KC, Quan H, Gertz BJ, FitzGerald GA. Effects of specific inhibition of cyclooxygenase-2 on sodium balance, hemodynamics, and vasoactive eicosanoids. J Pharmacol Exp Ther. 1999;289:735–741.

24. Yu Y, Ricciotti E, Scala R, Tang S, Grant G, Yu Z, Landesberg G, Crichton I, Wu W, Pur E, Funk C, FitzGerald G. Vascular COX-2 modulates blood pressure and thrombosis in mice. J Clin Invest. 2013;124:1521a-154a.

25. McAdam BF, Catella-Lawson F, Mardini IA, Kapoor S, Lawson JA, FitzGerald GA. Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2. Proc Natl Acad Sci USA. 1999;96:272–277.

26. Bao G, Metreveli N, Li R, Taylor A, Fletcher EC. Blood pressure response to chronic episodic hypoxia: role of the sympathetic nervous system. J Appl Physiol. 1997;83:95–101.

27. Fletcher EC, Orolinova N, Bader M. Blood pressure response to chronic episodic hypoxia: the renin-angiotension system. J Appl Physiol. 2002;92:627–633.

28. Linz D, Hohl M, Nickel A, Mahfoud F, Wagner M, Ewen S, Schottun U, Maack C, Wirth K, Bohm M. Effect of renal denervation on neurohumoral activation triggering atrial fibrillation in obstructive sleep apnea. Hypertension. 2013;62:767–774.

29. Linz D, Mahfoud F, Schottun U, Ukena C, Neuberger H, Wirth K, Bohm M. Renal sympathetic denervation suppresses postprandial blood pressure rises and atrial fibrillation in a model for sleep apnea. Hypertension. 2012;60:172–178.

30. Pialoux V, Foster GE, Ahmed SB, Beaudin AE, Hanly PJ, Mohlin MJ. Lossartan abolishes oxidative stress induced by intermittent hypoxia in humans. J Physiol. 2011;589:5529–5537.

31. Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Greindl KK, Harrison DG. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane nAD/nADH oxidation. Contribution to alterations of vasomotor tone. J Clin Invest. 1996;97:1916–1923.

32. Young W, Mahboubi K, Haider A, Li F, Ferrer NR. Cyclooxygenase-2 is required for tumor necrosis factor-alpha and angiotensin II-mediated proliferation of vascular smooth muscle cells. Circ Res. 2000;86:906–914.

33. Hu ZW, Kerb R, Shi XY, Wei-Lavery T, Hoffman BB. Angiotensin II increases expression of cyclooxygenase-2: implications for the function of vascular smooth muscle cells. J Pharmacol Exp Ther. 2012;340:563–573.

34. Campbell WB, Graham RM, Jackson EK. Role of renal prostaglandins in sympathetically mediated renal inure in the rat. J Clin Invest. 1979;64:448–456.

35. Harris RC. Interactions between COX-2 and the renin-angiotensin system in the kidney. Acta Physiol Scand. 2003;177:423–427.
46. Hurwitz S, Cohen RJ, Williams GH. Diurnal variation of aldosterone and plasma renin activity: timing relation to melatonin and cortisol and consistency after prolonged bed rest. *J Appl Physiol*. 1985;2004:1406–1414.

47. Andresen J, Samaan N, Bryan RM Jr. Endothelial influences on cerebrovascular tone. *J Appl Physiol*. 2006;100:318–327.

48. Osuka K, Suzaki Y, Watanabe Y, Dogan A, Takayasu M, Shibuya M, Yoshida J. Vasodilator effects on canine basilar artery induced by intracisternal interleukin-1 beta. *J Cereb Blood Flow Metab*. 1997;17:1337–1345.

49. Nadjar A, Tridon V, May MJ, Ghosh S, Dantzer R, Amee P, Parnet P. NFkappaB activates in vivo the synthesis of inducible cox-2 in the brain. *J Cereb Blood Flow Metab*. 2005;25:1047–1059.

50. Rivest S. What is the cellular source of prostaglandins in the brain in response to systemic inflammation? Facts and controversies. *Mol Psychiatry*. 1999;4:500–507.

51. Ryan S, Taylor CT, McNicholas WT. Selective activation of inflammatory pathways by intermittent hypoxia in obstructive sleep apnea syndrome. *Circulation*. 2005;112:2660–2667.

52. FitzGerald GA, Patrono C. The coxibs, selective inhibitors of cyclooxygenase-2. *N Engl J Med*. 2001;345:433–442.

53. Mitchell JA, Akarasereenont P, Thieme M, Flower RJ, Vane JR. Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. *Proc Natl Acad Sci USA*. 1993;90:11693–11697.

54. Capra V, Back M, Barbieri SS, Camera M, Tremoli E, Rovati GE. Eicosanoids and their drugs in cardiovascular diseases: focus on atherosclerosis and stroke. *Med Res Rev*. 2013;33:364–438.

55. Krieger J, Benzioni D, Szforza E, Sassard J. Urinary excretion of prostanooids during sleep in obstructive sleep apnoea patients. *Clin Exp Pharmacol Physiol*. 1991;18:551–555.

56. Kimura H, Niihima M, Abe Y, Edo H, Sakabe H, Kojima A, Hasako K, Masuyama S, Tatsumi K, Kuriyama T. Compensatory excretion of prostacyclin and thromboxane metabolites in obstructive sleep apnea syndrome. *Intern Med*. 1998;37:127–133.

57. Somers VK, Dyken ME, Clary MP, Aboud FM. Sympathetic neural mechanisms in obstructive sleep apnea. *J Clin Invest*. 1995;96:1897–1904.

58. Neri Serneri GG, Gensini GF, Abbate R, Prisco D, Rogasi PG, Castellani S, Casolo GC, Mathematical F, Fantini I, Donato MD, Dabizzi RP. Spontaneous and cold pressor test-induced prostaglandin biosynthesis by human heart. *Am Heart J*. 1985;110:50–55.

59. Neri Serneri GG, Castellani S, Scardi I, Trotta F, Chen J, Carnovali M, Poggesi L, Masotti G. Repeated sympathetic stimuli elicit the decline and disappearance of prostaglandin modulation and an increase of vascular resistance in humans. *Circ Res*. 1990;67:580–588.

60. Morgan BJ, Denahan T, Ebert TJ. Neurocirculatory consequences of negative intrathoracic pressure vs. asphyxia during voluntary apnea. *J Appl Physiol*. 1993;74:2969–2975.

61. Fletcher EC, Lesske J, Qian W, Miller CC III, Unger T. Repetitive, episodic hypoxia causes diurnal elevation of blood pressure in rats. *Hypertension*. 1992;19:555–561.

62. Tamisier R, Gilmartin GS, Launois SH, Pepin JL, Nespoulet H, Thomas R, Levy P, Weiss JW. A new model of chronic intermittent hypoxia in humans: effect on ventilation, sleep, and blood pressure. *J Appl Physiol*. 2009;107:17–24.

63. Hinjosa-Laborde C, Mifflin SW. Sex differences in blood pressure response to intermittent hypoxia in rats. *Hypertension*. 2005;46:1016–1021.

64. Warner TD, Mitchell JA. Cox-2 selectivity alone does not define the cardiovascular risks associated with non-steroidal anti-inflammatory drugs. *Lancet*. 2008;371:270–273.