# Increasing cerebral blood flow reduces the severity of central sleep apnea at high altitude

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*DOI:*
10.1152/japplphysiol.00799.2017

*Document Version*
Peer reviewed version

*Citation for published version (Harvard):*
Burgess, KR, Lucas, SJE, Burgess, KM, Sprecher, KE, Donnelly, J, Basnet, AS, Tymko, MM, Day, TA, Smith, KJ, Lewis, NCS & Ainslie, P 2018, 'Increasing cerebral blood flow reduces the severity of central sleep apnea at high altitude', *Journal of Applied Physiology*. https://doi.org/10.1152/japplphysiol.00799.2017

# TITLE PAGE

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**RUNNING HEADING**: Increasing cerebral blood flow improves CSA

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**TOTAL NUMBER OF FIGURES:** 5

**TOTAL NUMBER OF TABLES:** 2

**TOTAL NUMBER OF PAGES:** 28

## ABSTRACT

Earlier studies have indicated an important role for cerebral blood flow in the pathophysiology of central sleep apnea (CSA) at high altitude, but were not decisive. To test the hypothesis that pharmacologically altering cerebral blood flow (CBF) without altering arterial blood gas (ABGs) values would alter the severity of CSA at high altitude, we studied 11 healthy volunteers. (8M, 3F; 31±7 years) in a randomized placebo-controlled single-blind study at 5,050 metres in Nepal. CBF was increased by intravenous (iv) acetazolamide (Az; 10mg/kg) plus iv dobutamine (Dob) infusion (2-5 ug/kg/min) and reduced by oral indomethacin (Indo; 100mg). ABG samples were collected and ventilatory responses to hypercapnia (HCVR) and hypoxia (HVR) were measured by rebreathing and steady-state techniques before and after drug/placebo. Duplex ultrasound of blood flow in the internal carotid and vertebral arteries was used to measure global CBF. The initial 3-4 hours of sleep were recorded by full polysomnography. Iv Az+Dob increased global CBF by 37±15% compared to placebo ($P<0.001$), whereas it was reduced by 21±8% by oral Indo ($P<0.001$). ABGs and HVR were unchanged in both interventions. HCVR was reduced by 28%±43% ($P=0.1$) during iv Az±Dob administration and was elevated by 23%±30% ($P=0.05$) by Indomethacin. During iv Az+Dob, the CSA index fell from 140±45 (control night) to 48±37 events/hour of sleep ($P<0.001$). Oral Indo had no significant effect on CSA. We conclude that increasing cerebral blood flow reduced the severity of CSA at high altitude; the likely mechanism is via a reduction in the background stimulation of central chemoreceptors.



**Key Words:** Central sleep apnea**;** Cerebral blood flow; Ventilatory responses; High altitude.

## NEW AND NOTEWORTHY

This work is significant because it shows convincingly for the first time in healthy volunteers, that increasing cerebral blood flow will reduce the severity of CSA in a high altitude model, without the potentially confounding effects of altering $PaCO_2$ or the ventilatory response to hypoxia.

The proposed mechanism of action is that of increasing the removal of locally produced $CO_2$ from the central chemoreceptors, causing the reduction in hypercapnic ventilatory response, hence reducing loop gain.

## INTRODUCTION

Following ascent to high altitude by otherwise healthy individuals, CSA during sleep is almost universal, occurring in >90% of people above 5,000m.(7) Experiments at high altitude provide insight into the mechanisms underlying the pathogenesis of CSA, as well as potential therapeutic opportunities. The common trigger to both CSA in heart failure and high altitude exposure is transient reduction in the partial pressure of arterial carbon dioxide ($PaCO_2$) (12) below the apneic threshold during light sleep.(11) The magnitude of the required $PaCO_2$ reduction to initiate the CSA depends on the awake values, the ventilatory response to $PaCO_2$ below eupnea and the position of the iso-metabolic line.(11, 30) Other possible contributing factors, which have not been investigated extensively, especially following ascent to high altitude, are breathing pattern and cerebral blood flow (CBF), which are closely linked by the $PaCO_2$.(11, 32) The effects of $PaCO_2$ on CBF provide an important protective mechanism which serves to minimize changes in brain $[H^+]$, thereby stabilizing the breathing pattern in the face of perturbations in $PaCO_2$.(18, 32)

Hypocapnia normally causes marked cerebral vasoconstriction and reduces CBF, thus attenuating the fall in brain tissue $PCO_2$ relative to that of $PaCO_2$(16). Accordingly, ventilatory inhibition in response to reduced $PCO_2$ will be lessened, because of the attenuated decrease in $[H^+]$ stimulus to central chemoreceptors. In addition, ascent to high altitude increases ventilatory responses to hypercapnia and hypoxia (6), which will likely cause greater breathing instability due to increases in ventilatory 'loop gain'.(3) This has even greater significance during sleep, when $PaCO_2$ becomes critical in regulating the breathing pattern in the absence of the wakefulness drive to breathe.(13)

The $PCO_2$ in the brain is higher than $PaCO_2$; thus perfusion at the level of central chemoreceptors affects the strength of the locally produced ($CO_2$/H+) stimulus.

It is established that CBF falls at sleep onset in healthy individuals.(18) In a previous study, in a small number of subjects, we found an association between the degree of reduction of CBF at sleep onset and the development of CSA during sleep at high altitude (3900m).(6) In subsequent experiments at 5050m, we demonstrated a significant association between the reduction of CBF by oral indomethacin (Indo) and the increase in CSA severity. In the same series of experiments we were able to increase CBF by administering intravenous (iv) acetazolamide (Az), which markedly reduced the severity of CSA. Unfortunately the interpretation of those results was complicated by a concomitant rise in $PaCO_2$ of 3 mmHg.(10) Those observations generated our current hypothesis that changes in CBF play an important role in the pathophysiology of CSA at high altitude by altering the background stimulation of the central chemoreceptors. Although we clearly acknowledge the important role of the peripheral chemoreceptors(26), the main aim of this experiment was to test this hypothesis via the pharmacological manipulation of CBF in normal volunteers and assess its importance in the pathophysiology of CSA at high altitude.

## MATERIALS AND METHODS

Eleven healthy Caucasian adults usually residing at sea level (eight males and three females), with a mean age of 31 ± 7 years (mean ± SD) and body mass index of 25.6 ± 3.6 kg/m$^2$ completed the study, which was approved by the University of British Columbia Ethics Committee and the Nepal Health Medical Research Council and conformed to the standards set by the *Declaration of Helsinki.* Written informed consent was obtained. Other experiments were conducted on the same expedition before and after these experiments, hence the subject numbers are not continuous but are identical in all experiments from the same expedition. However, there was no overlap with the sleep experiments or any confounding pharmacological manipulation or exercise.

### Experimental design and ascent profile

High altitude exposure was chosen as a model for investigating the pathophysiology of CSA, because it is reproducible, relatively stable over at least one month, and can accommodate a large number of subjects in and around a stable laboratory site over a period of several weeks.

All participants underwent full medical screening, including 12-lead ECG and echo-cardiography assessment. Participants were not taking any medication, all were non-smokers, and none had any history of cardiovascular, cerebrovascular, or respiratory disease. In addition, only two participants had previous high altitude experience, which was >4 years previous to this expedition. 15 subjects were recruited initially to these experiments. All by general invitation to graduate students within the Dept of Physiology, University of British Columbia, Kelowna. Two withdrew during the course of the experiments due to illnesses unrelated to the

158 experimental methods, one subject had incomplete data collections and one
159 withdrew to accompany another subject during an aeromedical evacuation. (3m/1F
160 – mean age 31, BMI =23.3).

161 All studies were conducted at 5050m (Pb = 413). However, familiarisation
162 was conducted one month earlier at low altitude (in Kelowna, BC, Canada; 344 m
163 above sea level) with the protocols completed one-month before arriving in Nepal.
164 There was no evidence of abnormal central or obstructive sleep apnea evident in
165 their sleep studies at 334m. Participants spent seven-days at Kathmandu (~1400 m)
166 before flying to Lukla (2860 m). Participants then trekked to the Ev-K2-cnr Pyramid
167 Laboratory over a nine-day period, which included rest days at Namche Bazar (3450
168 m) and Pheriche (4252 m). During the first seven days, all participants used a small
169 dose (125mg) of **oral** Acetazolamide(25) twice daily during the trek to help speed
170 acclimatization (4) and limit altitude illness. Importantly, treatment was discontinued
171 >24 h before reaching 5050m to allow sufficient clearance time. The reported half-
172 life for **oral** acetazolamide is 10 h and this low-dose quantity has been reported to be
173 90–100% excreted within 24 h of administration (22); this approach, therefore, was
174 unlikely to confound our findings. Furthermore, to avoid any confounding influence
175 of initial AMS, experimental sessions were carried out between days 4-14 after
176 arrival to 5,050 m.

177 *Pharmacological manipulation of cerebral blood flow:* Cerebral blood flow (CBF) was
178 altered by the administration of licensed medications: oral indomethacin (Indo)
179 100mg; to reduce CBF, and intravenous acetazolamide (Az 10mg/kg) (31) followed
180 by an infusion of dobutamine (Dob) at 2-5 ug/kg/min to increase CBF. The
181 combination of one dose of intravenous Az followed by an infusion of Dob is an
182 original one which was arrived at by trial and error in Australia in 2011, which

involved testing several agents alone and in combination on the investigators before settling on Az+Dob. The theory is that Az paralyses the central arteries, preventing auto-regulation of CBF, and the Dob by increasing cardiac output increases CBF. Why $PaCO_2$ does not change with the combination is not known, but it might be that the slight metabolic acidosis seen with the combination (table 1) caused additional hyperventilation, which reduced $PaCO_2$ to the placebo value.

Indomethacin, at a dose of 100 mg orally, reduces CBF and its reactivity by 20-40% within 90 minutes, for up to 4 hours.(31) **Intravenous** Az can increase CBF by 20-50% within 30 minutes, for up to 8 hours (10). It has very different effects to oral Az. For example, when administered intravenously the effects are predominantly on CBF and **extra renal** carbonic anhydrase, and it does not induce measurable metabolic acidosis within this time (eg., <5 hours). Using these pharmacological agents on different days, in a randomized fashion (toss of coin for first drug allocation, then alternate allocation), we altered CBF in both directions, and examined the result of altering CBF on the severity of CSA and the potential underlying mechanisms (eg. alterations in ventilatory responses and blood gases). Indomethacin or placebo was administered orally approximately 90 minutes before testing began with 20 ml of an antacid solution, and Az+Dob or 0.9% saline was administered intravenously 30 minutes before testing began. The data were collected and analyzed as “control”, “drug 1” or “drug 2”.

Figure 1 shows the overview of the experimental design; it should be noted that there was a 2 day “washout” after the first drug administration before the control night studies were performed. There was then another one day until the second drug

was administered (i.e., a minimum of three days between pharmacological interventions). In addition, placebo controls were used to account for possible indirect effects of the medications. The placebo for Indo was an empty "indomethacin" gelatin capsule refilled with sugar, while normal saline was used as the intravenous Az+Dob placebo.

*Sleep studies*

All sleep studies were carried out with a Compumedics portable system (Somté PSG; Melbourne, Australia). Participants were set up for the polysomnogram by experienced polysomnography technologists according to standard format, as described in detail elsewhere (7, 8). Four studies were carried out simultaneously with real time data acquisition and monitoring. All studies were scored post hoc by the same certified polysomnography technologist, who was not part of the expedition and who was blinded as to the nature of the study, using standard definitions.(1, 2) The first three to four hours of sleep were used for analysis of the drug effects because the duration of action of Indo may be only four hours after onset (tested during pilot work). It was intended to use the first 4 hrs of sleep, however some subjects woke after 3 hrs complaining of discomfort, (equipment or beds), and were unable to return to sleep before the 4hr time limit.

**Experimental procedures**

The ventilatory response (VR) testing was performed in the afternoons and the sleep studies commenced approximately six hours later. All procedures were performed with participants lying in a supine position.

Following 10-15 min of quiet rest, each experimental testing session comprised of: a) an arterial blood gas sample, b) instrumentation, c) 5-min resting

baseline, including measurement of volumetric CBF, d) modified hyperoxic hypercapnic rebreathing (HCVR) and poikilocapnic hypoxia (HVR; see details of methods below), e) drug intervention / placebo, f) 90 min rest, g) repeat testing of a-d. After a delay of approximately six-hours, subjects received another dose of drug and placebo 90 and 30 minutes prior to being put to bed for a night of full polysomnographic monitored sleep (figure 1).

For the central chemoreflex magnitude (HCVR), hyperoxic hypercapnia was intentionally used in order to eliminate the influence of hypoxic-induced peripheral chemoreceptor activation at high altitude and acutely remove the influence of hypoxia on cerebrovascular tone. The modified hypercapnia rebreathing protocol was preceded by a 5-min period of voluntary hyperventilation, in accordance with the standardized protocol of Duffin (14). For the peripheral chemoreflex magnitude, the HVR was assessed by a two-point steady-state test which measured ventilation at ambient air and after breathing an $FIO_2$ = 0.38 for 10 minutes (approximately equivalent to the inspired $PO_2$ in Kelowna). The order of the steady-state (HVR) and modified rebreathing tests (HCVR) was randomized between participants, but was consistent within participants across all trials and pre and post intervention, and full recovery (5-min) was permitted between each trial to restore end-tidal gases to baseline resting values.

Due to equipment limitations, only 4 participants were studied each night. Therefore, it took 3 consecutive nights to study all 11 participants at each time point. All ventilatory testing was completed in the afternoon, and participants were instructed to avoid caffeine, alcohol and exercise in the 12 hours prior to experimental testing.

**Extracranial ultrasound of blood flow in conduit vessels**

Continuous diameter and blood flow recordings in the left internal carotid artery (ICA), and right vertebral artery (VA) were obtained using a 10-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (Terason 3000$^{TM}$, Teratech, Burlington, MA). Imaging of the extracranial arteries was conducted during the 5-min resting baseline period. The ICA blood flow measures were recorded at least 2 cm from the carotid bifurcation, whilst ensuring there was no evidence of turbulent or retrograde flow. The VA was measured within 1 cm either proximal or distal (but at the same location within each subject) to the transverse process of C3. Average diameter and blood flow recordings were made from a minimum of 10 cardiac cycles (see below), and care was taken to ensure probe position was stable so that the angle of insonation did not vary from 60º. The sample volume was positioned in the centre of the vessel and adjusted to cover the width of the vessel diameter. Measurement settings for each extracranial artery within an individual were standardised for each VR test and all within individual measures were done by the same sonographer (i.e., pre and post for both interventions).

All extracranial vascular images were directly stored as a DICOM file for offline analysis. As described in depth elsewhere (27), analysis involved continuous measurements of arterial diameter synchronous with measurements of blood velocity at 30 Hz performed using an off-line custom-designed edge-detection and wall tracking software. Reproducibility of diameter measurements using this software is significantly better than manual methods as it reduces observer error significantly(27). Volumetric global cerebral blood flow (gCBF) was calculated by:

$$gCBF\ (ml.min^{-1}) = (QICA\ .\ 2) + (QVA\ .\ 2)$$

Where QICA is the blood flow from the ICA and QVA is the blood flow in the VA. The combined total of QICA and QVA therefore is the estimated global CBF assuming a symmetrical blood flow of contralateral ICA and VA arteries (18, 27).

The measurements were made by experienced sonographers blinded to the drug administration (MHT, KS, NL).

**Ventilatory response testing**

*Modified hyperoxic rebreathing method (HCVR):* Participants wore a nose clip and breathed through a mouthpiece connected to a T-valve, which allowed switching from room air to a 8-L rebreathing bag filled with 7% $CO_2$ and 93% $O_2$. Following baseline data collection, participants were instructed to hyperventilate for 5 minutes to lower and then maintain a partial pressure of end-tidal $CO_2$ ($P_{ET}CO_2$) at 22 ± 2 mm Hg (at low altitude), and 17 ± 2 mm Hg (at high-altitude). Participants were then switched to the rebreathing bag at the end of expiration and were instructed to take three deep breaths to ensure rapid equalization of $PCO_2$ in the rebreathing circuit. The rebreathing test was terminated when either: i) $P_{ET}CO_2$ reached 60 mm Hg; ii) partial pressure of end-tidal $O_2$ ($P_{ET}O_2$) dropped below 160 mm Hg; iii) ventilation ($V_E$) exceeded 100 L $min^{-1}$, or iv) the participant reached the end of their tolerance.

The rebreathing data were analyzed on a breath-by-breath basis using a specially-designed programme (Full Fit Rebreathing programme, Version 3.1, University of Toronto, Toronto, Canada). In brief, the initial 3-breath equilibration, sighs, swallows and aberrant breaths were excluded from analysis. Next, the breath-by-breath $P_{ET}CO_2$ values were plotted against time and fitted with a least squares regression line to minimise inter-breath variability (27). Subsequently, $V_E$ was plotted against the predicted $P_{ET}CO_2$ obtained by the regression analysis.

The $\dot{V}_E$ plot was fitted with a model made up of the sum of two segments separated by a breakpoint. (27) The first segment was taken from resting $\dot{V}_E$ following equilibration with the rebreathing circuit. Thereafter, $\dot{V}_E$ increased in conjunction with the predicted $P_{ET}CO_2$. Since hyperoxia ($PaO_2 \geq 150$ mm Hg) diminishes peripheral chemoreceptors output (9), the observed breakpoint was taken as the ventilatory recruitment threshold of the central chemoreflex, while the slope of the second segment was assumed to be the ventilatory $CO_2$ sensitivity (or gain) attributed primarily to the central chemoreflex.

*Poikilocapnic hypoxia (HVR):* Participants wore a nose clip and breathed through a mouthpiece connected to a two-way, T-shaped non-rebreathing valve that allowed switching from room air to a circuit consisting of a 200 L Douglas bag containing 38% oxygen. The protocol began with baseline room air breathing for five-minutes, before participants were switched to the 38% oxygen circuit for 10-minutes. The 38% oxygen was used to passively normalize inspired $PO_2$ back to sea level values. This was done to allow comparison with earlier sea level studies, (data in preparation).

The mean $V_E$ over the last five-minutes of oxygen breathing was used as one data point and the mean resting (room air) ventilation as the other. The slope of the delta $V_E$ vs. delta $SpO_2$ joining line was taken as the HVR.

*Respiratory variables:* Inspiratory flow was measured using a heated pneumotach (Hans-Rudolph 3813), attached to the mouthpiece (via a disposal filter). Partial pressures of end-tidal $CO_2$ and $O_2$ were sampled from a needle inserted into the mouthpiece, dried with nafion tubing and dessicant, and measured using a dual $CO_2$ and $O_2$ gas analyzer (ML206, ADInstruments, Australia). Gases were measured in percent and converted to mm Hg (BTPS) using the ambient atmospheric pressure.

Minute ventilation and gas values were displayed in real time during testing (PowerLab, ADInstruments). Prior to each testing session, the pneumotachometer was calibrated using a 3-L syringe (Hans-Rudolph 5530) and the gas analyzers were calibrated using known concentrations of $CO_2$ and $O_2$.

Cardiovascular and respiratory variables were measured continuously at 200 Hz using an analog-to-digital converter (Powerlab 16/30 ML880; ADInstruments), interfaced with a computer, and were subsequently analyzed using commercially available software, (LabChart v7, ADInstruments).

***Blood gases***. Arterial blood variables [pH, partial pressure of arterial $O_2$ ($PaO_2$), partial pressure of arterial $CO_2$ ($PaCO_2$), arterial $O_2$ saturation ($SaO_2$), bicarbonate concentration [$HCO_3^-$], and haematocrit (Hct)] from the radial artery (occasionally femoral artery) were obtained after 10-min supine rest using a 23 or 25-gauge needle into a preheparinised syringe. Following standardized calibration, all blood samples were analyzed using an arterial blood-gas analyzing system (ABL-90 Co-Ox, Radiometer, Copenhagen, Denmark).

**Statistical Analysis**

*Data Sets:* There were complete data sets for the collected variables for CBF, ABGs and PSG data; however the ventilatory response test data was incomplete. There were 2 empty cells from 44 in the HVR and HCVR results before and after Indo. All results were analyzed using SPSS software (v23. IBM Corp. Ireland). The Shapiro-Wilks test was used to test for distribution normality in each data set. Data sets that were normally distributed were analyzed by paired t-test (most data). Data sets not normally distributed (ie. Pre Az/Dob CBF, Pre Az/Dob $PaO_2$, Post Indo BE, Mean control AHI, and all HCVR data), were analyzed with their data pairs by a non-

parametric test (Wilcoxon Sign Rank Test)(23). The AHI data were analyzed by repeated measures ANOVA with post hoc Bonferroni tests between conditions.

The correlations shown in Table 2 were performed using Pearson's and Spearman's methods (23) in SPSS v23. Pearson's correlation method was used for the normally distributed data. Spearman's method was used when any of the input data was not normally distributed, [all correlations with hypercapnic ventilatory responses (HCVR)] and those correlations using data from baseline cerebral blood flow (CBF) prior to Az+Dob, [ie change in cerebral blood flow (Δ CBF post Acetazolamide)].

## RESULTS

### EFFECTS OF ACETAZOLAMIDE+DOBUTAMINE AND INDOMETHACIN

*Acetazolamide+dobutamine*

Acute **intravenous** administration of acetazolamide (Az) followed by a continuous intravenous infusion of dobutamine (Dob) (2-5 ug/kg/min) increased awake resting CBF by 37% (95%CI: 28-46%; P< 0.001; Table 1. figure 2A), while the apnea-hypopnoea index (AHI) that night was 65% (-80% to -50%)(figure 3A) lower than control (P=0.001; table 1). During Az+Dob administration, $PaCO_2$ was unchanged from pre administration. However there was a non-significant fall in pH (P>0.05; table 1) due to the development of a slight metabolic acidosis. Base excess (BE) increased from -4.8±1.7 to -7.0±2.8 (P<0.05).

The HVR, did not significantly change after the administration of Az+Dob (figure 5A). The slope of the HCVR fell from 5.9 ± 2.7 to 4.2 ± 2.8 l/min/mmHg. (P= 0.1; table 1, figure 4A).

The arousal index was reduced from 68 ± 47/hr on the control night to 22 ± 10/hr (P < 0.01 table 1). There was no change in sleep efficiency, or total sleep time.

*Indomethacin*

Ninety minutes following the **oral** administration of indomethacin (Indo), awake resting CBF was reduced by 21% (95%CI: 16-26%), while the mean AHI during sleep was not significantly altered (see table 1, figures 2B and 3B). The $PaCO_2$ did not change from 26 $\pm$ 3 mm Hg (see table 1); yet metabolic alkalosis was still observed, with the pH rising slightly from 7.46±02 to 7.48±02 (P=NS; table 1).

Although the HVR did not increase significantly following Indo (figure 5B), the HCVR was increased by 1.5 l/min/mmHg (P=0.05; table 1, figure 4B). The mean % increase was 23% (95%CI: 2-44%).

There was no change in sleep efficiency, nor total sleep time.

*Correlations*

Table 2 shows the correlation co-efficients for the relevant respiratory variables following the administration of the two drugs and the potential influence that each had with the severity of AHI.

## DISCUSSION

Herein, we report the results of what we believe to be only the second attempt to artificially manipulate CBF in the field, in the midst of two weeks of acclimatization to an altitude of 5,050 m above sea level, in a group of otherwise healthy volunteers. Both drug interventions were effective in altering CBF. The novel combination of intravenous acetazolamide plus dobutamine infusion significantly reduced the severity of CSA, but on this occasion was not associated with a significant change in $PaCO_2$, as occurred in our previous study(10) that confounded interpretation of those data. The Indo administration on the other hand, appears to have had only one unintended effect; CSA severity was unaltered, probably because the AHI was already at, or near, its theoretical maximum. The mean CSA index in these experiments was 140/hr compared to 89/hr for the 'control night' comparison used in the previous study (10). The other findings, and relevant methodological considerations, are outlined below.

We recognized that acclimatization would be ongoing throughout the duration of our study(9), and adjusting for its effects would be important in the conduct of experiments and in the interpretation of the results of the current study. This was achieved by obtaining new arterial blood gas samples, ventilatory response and CBF

414 measurements immediately prior to each drug intervention, and randomly allocating
415 the order of the drug administration to either side of a control night study. Each drug
416 was equally administered pre and post the control night.
417 Central sleep apnea at high altitude occurs during light sleep (Stages 1 and 2
418 NREM sleep), in the presence of relative hypocapnia and alkalosis at sleep onset
419 (12). Although many studies cite the classic Lahiri study (17) to provide evidence of
420 the relationship between the magnitude of HVR and periodic breathing, this
421 relationship was largely created by the inclusion of a Sherpa group with a blunted
422 HVR. However, there was no obvious relationship between HVR and periodic
423 breathing within the lowlander population. This absence of a relationship between
424 HVR was further confirmed, albeit in a subgroup (n=5), at 6300 and 8050 m (29).
425 These findings are consistent with Masuyama et al (20), who found that two of nine
426 mountaineers did not develop CSA at altitude despite normal values for HVR (20).
427 More recently, we have also reported an absence of a relationship between HVR
428 and periodic breathing at 5050 m (9). In contrast, at 4400 m in a small sample size
429 (n=4) it was shown that the respiratory stimulant almitrine doubled the HVR and
430 elevated periodic breathing compared with Az or placebo (15). A number of potential
431 explanations exist for these discrepant and variable findings, including: (a) evidence
432 that the hypoxic and $CO_2$ response are not always similar above and below eupnea
433 (11), (b) differences in awake vs. sleep respiratory control, (c) variable acid-base
434 status, and (d) methodological differences (e.g., chemoreflex testing, natural vs.
435 simulated altitude, etc.). Nevertheless, collectively these findings highlight the multi-
436 factorial complexity of periodic breathing at high altitude.
437
438 *Influence of cerebral blood flow on CSA severity and ventilatory responses*

Intravenous Az+Dob caused a 37% increase in global CBF. This increase was associated with a 65% reduction in AHI. Our hypothesis was that this would be due to a reduction in central chemoreceptor stimulation by locally produced $CO_2$, because of increased clearance caused by the higher CBF. Mean HCVR was lowered by the Az+Dob by 28% (P=0.1). In support of a putative link between chemoreflex drive and CBF, correlational analysis revealed a modest correlation (r=0.41 P=0.054) between the change in HCVR compared to the change in CBF after intravenous Az+Dob, and change in HCVR and change in CBF (r=0.48, P=0.19) after Indo. (see table 2). Crucially, with our combined pharmacological interventions to increase CBF there was no change in $PaCO_2$, or pH, in contrast to our previous study (10).

Oral Indo administration resulted in a 21% (95%CI: 16-26%) reduction in CBF and increased HCVR by 23% (95%CI: 2-44% P=0.05). This was associated with no significant change in AHI, unlike our earlier study (10) at the same altitude. On this occasion, there was no change in $PaCO_2$ or pH. Most subjects had little or no change from their very high values for AHI prior to drug administration (AHI >100/hr), which suggests that they were perhaps already close to their maximum values for AHI. (10) These experiments were conducted after a longer period of acclimatization at 5,050 metres, leading to a markedly elevated central AHI.

Theoretically a reduction in the length of the apneas below 10 seconds in duration, could cause a reduction in the scored events and hence CSA index. Similarly, because CSA occurs predominantly in stage 2 NREM sleep, an increase in stable breathing could also cause a reduction in CSA index. Those mechanisms were not present in these experiments: the reduction in CSA index was due to a marked reduction in events not a shortening of apneas to below the 10 second

scoring threshold. The percentages of stable breathing [Slow Wave Sleep (NREM3) together with REM sleep] were not altered.

The increase in CBF using intravenous Az plus Dob infusion dramatically reduced CSA. In these experiments, as compared to our earlier experiments where CBF was increased by iv acetazolamide only, the interpretation of that outcome has not been confounded by an increase in $PaCO_2$ (and presumably brain $PCO_2$), so the interpretation can be made more confidently.

*Limitations*

The major limitation of this study was that the study group comprised only 11 subjects; however, our data are broadly consistent with recent data from our earlier studies at this altitude(10), as well as Block et al (5) and earlier data from Salvaggio et al(24). Other limitations included: The inclusion of subjects in the study group with generally lower ventilatory responses and low control AHI values increased the variability in the data, especially ventilatory response data. Due to time constraints there was no true control group in our study. Instead, approximately in the middle of the two weeks acclimatization at 5050m, in randomized order, CBF was artificially increased and decreased by drug administration. *Post hoc* analysis revealed exactly equal dispersion over time, between the two interventions within the recorded acclimatization period.

We studied only the first three to four hours of sleep because of the limited duration of effect of the indomethacin, which is approximately 4 hours(31). We have previously confirmed this time course by *post hoc* observation on other subjects (10) and during pilot testing in our laboratory.

While there are a number of meaningful ways to assess the HVR at sea level using steady-state (isocapnic hypoxia) or rebreathing methods (hyperoxic vs hypoxic rebreathing), at high altitude the methodological approach becomes even more complex (14, 21, 26), and consensus on the best approach has not been reached. Further, it is known that steady-state techniques produce higher values for HVR than non steady-state techniques (19). Nevertheless, we chose a steady-state test so that we could match inspired $PO_2$ values between the low altitude control and high altitude studies. As this was a within-subjects design we did not need to correct HVR for vital capacity or FEV1(28), which has been suggested by others to improve the test.

## CONCLUSION

The findings of the present study highlight an important role for CBF in CSA severity at high altitude, although the mechanisms of action cannot be ascertained from our data. There was a highly significant reduction in CSA severity with Acetazolamide+Dobutamine administration, and a suggestion of a relationship between the reduction in HCVR and the increase in CBF with the same intervention, however, there was no significant correlation between change in either CBF or HCVR and AHI with Az-Dob. That may be due to a type 2 error due to the reduced subject numbers. Reducing CBF with indomethacin did not affect AHI in this study, probably because the AHI was already at or near its maximal possible value.

## ACKNOWLEDGEMENTS

This study was carried out within the framework of the Ev-K2-CNR Project in collaboration with the Nepal Academy of Science and Technology as foreseen by the Memorandum of Understanding between Nepal and Italy, and thanks to contributions from the Italian National Research Council and the Italian Ministry of Foreign Affairs. We extend our thanks to Compumedics Ltd for the use of their laboratory equipment. We also thank Ms M Cheong for scoring all the sleep studies, and Ms Sue Coulson for manuscript preparation.

## ACKNOWLEDGEMENT OF FINANCIAL SUPPORT STATEMENT / GRANTS

This study was supported by The Peninsula Health Care P/L, NSERC, CRC, MRU Petro-Canada Young Innovator Award, Lottery Health NZ and the University of Otago.

## DISCLOSURES

Intravenous acetazolamide use was off label.

All authors disclose the absence of any conflicts of interest.

529 **Table 1**: The effects of intravenous Acetazolamide + Dobutamine and oral

530 Indomethacin on the key sleep and respiratory variables.

| | **Pre Acetazolamide + Dobutamine** | **Post Acetazolamide + Dobutamine** | **Pre Indomethacin** | **Post Indomethacin** |
|---|---|---|---|---|
| **Global CBF (ml/min)** | 526±110 | 718± 120** | 546±64 | 430±51 *** |
| **AHI (event/hr)** | 140± 45 | 48 ± 37*** | 140 ± 45 | 123 ± 30 |
| **Arousal Index (event/hr)** | 68 ± 47 | 22 ± 10** | 68 ± 47 | 60 ± 36 |
| **$PaO_2$ (mmHg)** | 42 ± 2 | 44 ± 4 | 42 ± 4 | 44 ± 4 |
| **$PaCO_2$ (mmHg)** | 25 ± 3 | 25 ± 3 | 26 ± 2 | 26 ± 3 |
| **pH** | 7.48 ± .02 | 7.45 ± .03 | 7.46 ± .02 | 7.48 ± .02 |
| **BE** | -4.8 ± 1.7 | -7.0 ± 2.8* | -5.2 ± 1.7 | -4.5 ± 1.8 |
| **HCVR (L/min/mmHg)** | 5.9 ± 2.7<br>n=11 | 4.2 ± 2.8[#]<br>n=11 | 6.4 ± 4.2<br>n=11 | 7.9 ± 6.0*<br>n=11 |
| **HVR (L/min/%$SpO_2$)** | 0.3 ± 0.16<br>n=11 | 0.3 ± 0.20<br>n=11 | 0.31 ± 0.14<br>n=10 | 0.33 ± 0.20<br>n=10 |

531

532 Pre drug value for AHI are from the control night sleep studies. All other control

533 values recorded immediately before intervention.

534 * P<0.05; **P<0.01; ***P ≤ 0.001; [#] P = 0.1

535

536 **Table 2:** The correlations between key Cerebral Blood Flow, sleep and respiratory

537 variables.

| **Inputs** | **Post Acetazolamide** | | **Post Indomethacin** | |
|---|---|---|---|---|
| | r value | P value | r value | P value |
| **AHI / CBF** | -0.27 | 0.48 | 0.05 | 0.90 |
| **AHI / HCVR*** | -0.30 | 0.37 | -0.39 | 0.24 |
| **AHI / $PaCO_2$** | -0.16 | 0.64 | -0.21 | 0.55 |
| **AHI / HVR** | -0.55 | 0.08 | 0.23 | 0.52 |
| **AHI / pH** | -0.10 | 0.77 | -0.25 | 0.45 |
| **AHI / $PaO_2$** | -0.20 | 0.55 | -0.02 | 0.96 |
| **Δ AHI / Δ HVR** | -0.04 | 0.92 | 0.22 | 0.55 |
| **Δ AHI / Δ HCVR*** | -0.20 | 0.56 | 0.17 | 0.76 |
| **Δ HCVR / Δ CBF*** | 0.41 | 0.054 | 0.48 | 0.19 |
| **Δ HVR / Δ CBF*** | -0.01 | 0.78 | 0.66 | 0.07 |
| **Δ AHI / Δ CBF*** | 0.14 | 0.98 | -0.20 | 0.60 |

538 AHI = Apnea-Hypopnoea Index (events/hr sleep)

539 HCVR = Hypercapnic Ventilatory Response (L/min/mmHg)

540 HVR = Hypoxic Ventilatory Response (L/min/%$SpO_2$)

541 Δ AHI = Change in Apnea-Hyperpnea Index

542 Δ HVR = Change in Hypoxic Ventilatory Response

543 Δ HCVR = Change in Hypercapnic Ventilatory Response

544 Δ CBF = Change in Cerebral Blood

545 r-value = Pearson or Spearman correlation co-efficient

546 * = Spearman correlation method. All other correlations tested by Pearson method.

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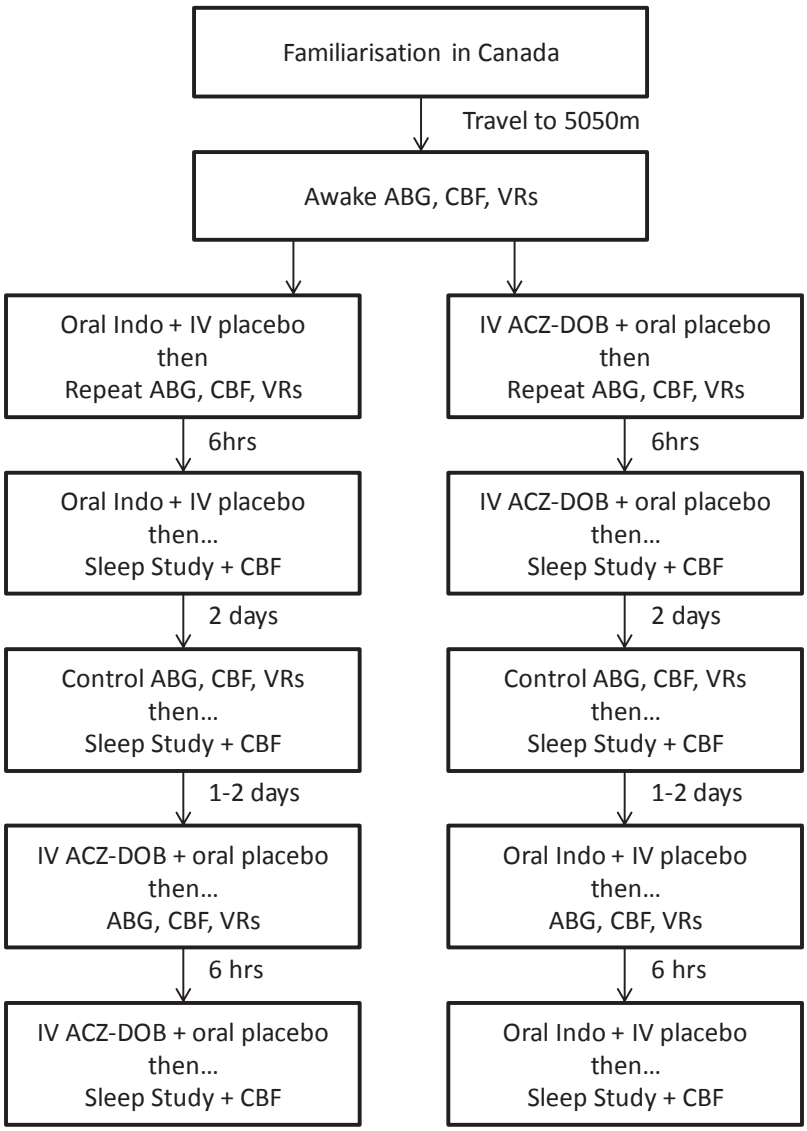
**Figure 1:** An overview of the experimental design indicating the sequence of testing




INDO = Indomethacin

ACZ = Acetazolamide

DOB = Dobutamine

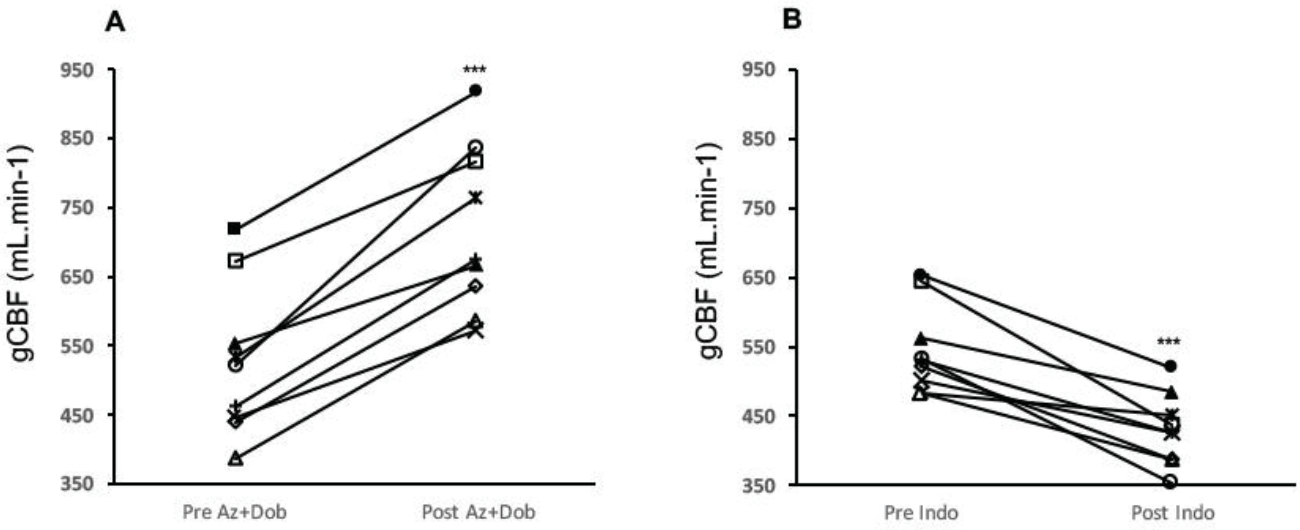
ABG = arterial blood gas measurement

CBF = cerebral blood flow

VRs = ventilatory response testing

**Figure 2**: Panel A: The effect of intravenous Az+Dob on CBF.

Panel B: The effect of oral Indo on CBF.




*** = P < 0.001

gCBF = global Cerebral Blood Flow

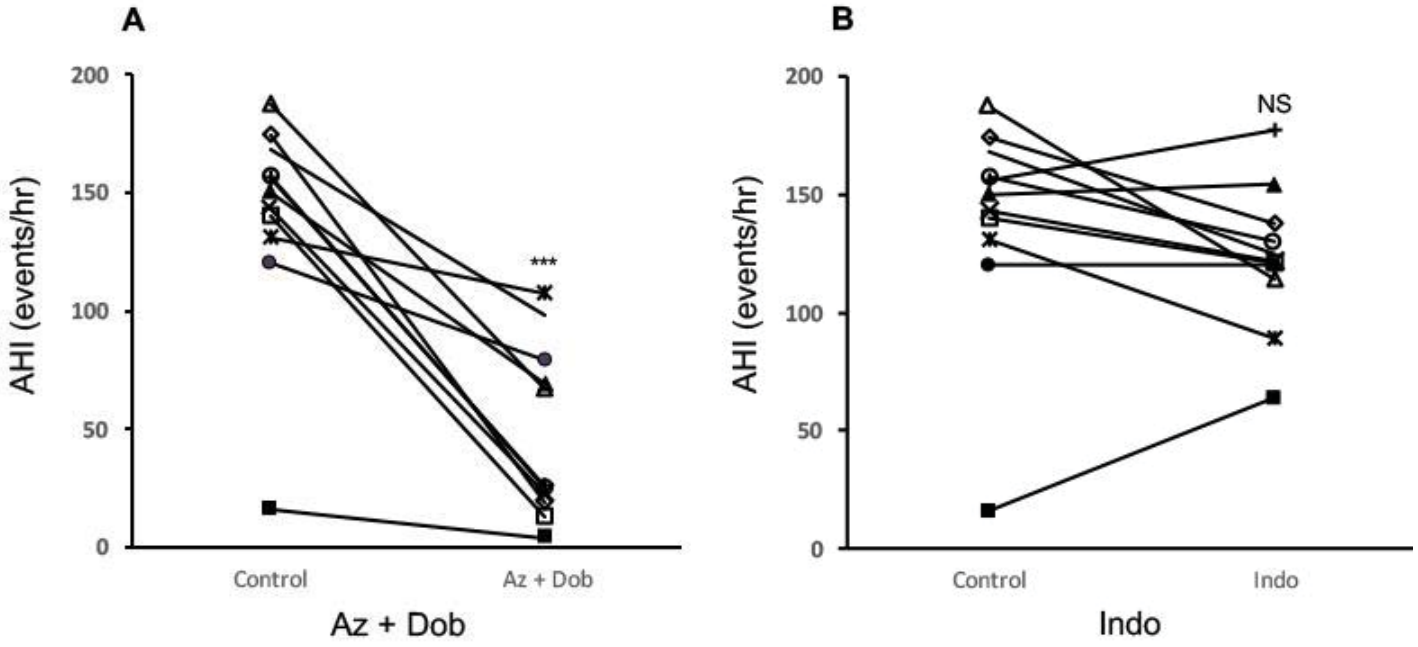
Az = Acetazolamide

Dob = Dobutamine

Indo = Indomethacin

**Figure 3**: Panel A: The effect of intravenous Az+Dob on apnea-hypopnea index.

Panel B: The effect of oral Indo on apnea-hypopnea index.




*** = P < 0.001

NS = Non significant

AHI = Apnea-hypopnea index

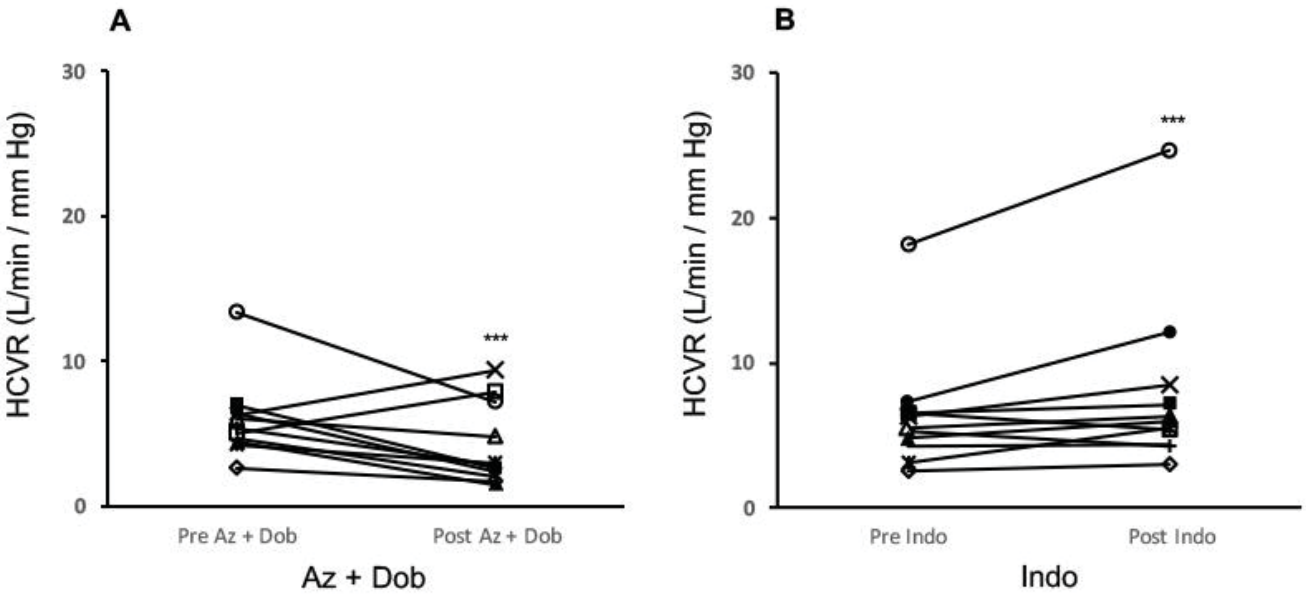
Az = Acetazolamide

Dob = Dobutamine

Indo = Indomethacin

**Figure 4**: Panel A. The effect of intravenous Az+Dob on HCVR.

Panel B: The effect of oral Indo on HCVR.




*** = P < 0.001

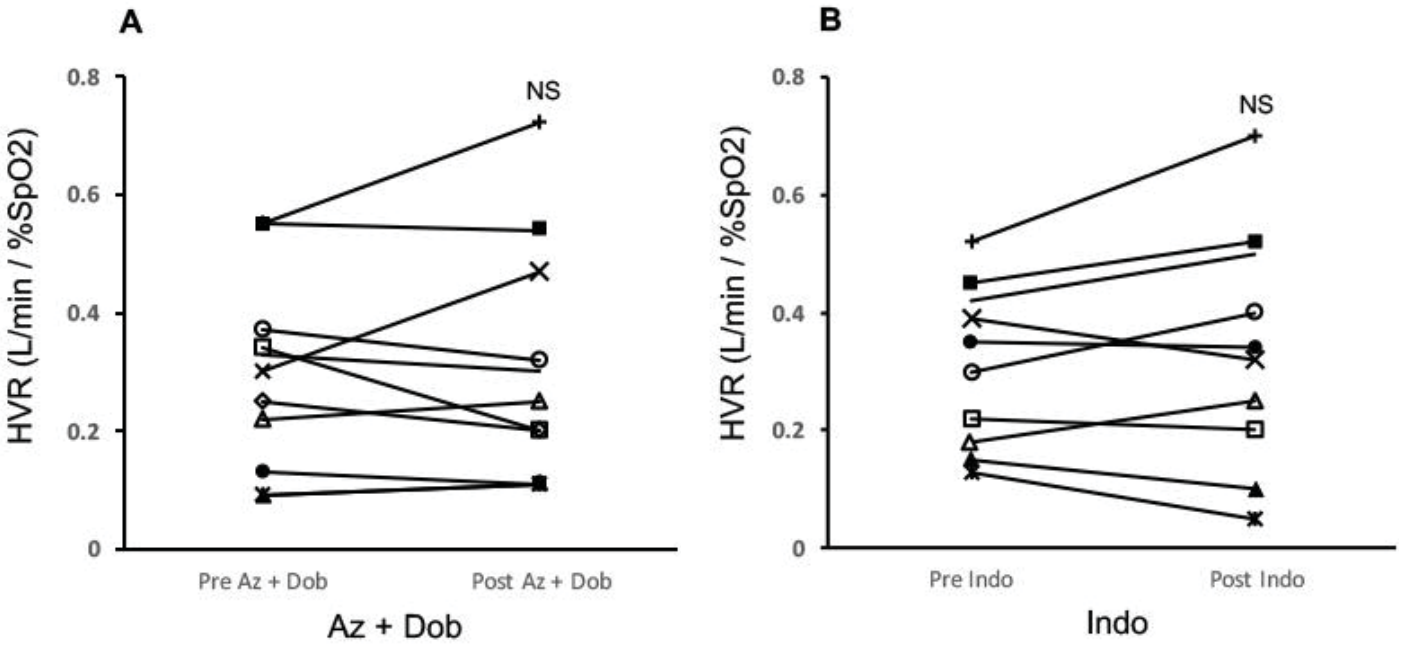
Az = Acetazolamide

Dob = Dobutamine

Indo = Indomethacin

**Figure 5**: Panel A: The effect of intravenous Az+Dob on HVR.

Panel B: The effect of oral Indo on HVR.




NS = Non significant

Az = Acetazolamide

Dob = Dobutamine

Indo = Indomethacin