

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

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DOI:

[10.1152/jappphysiol.00799.2017](https://doi.org/10.1152/jappphysiol.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/jappphysiol.00799.2017>

[Link to publication on Research at Birmingham portal](#)

Publisher Rights Statement:

Checked for eligibility: 14/05/2018
<https://doi.org/10.1152/jappphysiol.00799.2017>

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1 **TITLE PAGE**

2

3 **TITLE:** Increasing cerebral blood flow reduces the severity of central sleep apnea at

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

39

40

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51 **TOTAL NUMBER OF FIGURES: 5**

52 **TOTAL NUMBER OF TABLES: 2**

53 **TOTAL NUMBER OF PAGES: 28**

54

55 **ABSTRACT**

56 Earlier studies have indicated an important role for cerebral blood flow in the
57 pathophysiology of central sleep apnea (CSA) at high altitude, but were not decisive.
58 To test the hypothesis that pharmacologically altering cerebral blood flow (CBF)
59 without altering arterial blood gas (ABGs) values would alter the severity of CSA at
60 high altitude, we studied 11 healthy volunteers. (8M, 3F; 31±7 years) in a
61 randomized placebo-controlled single-blind study at 5,050 metres in Nepal.
62 CBF was increased by intravenous (iv) acetazolamide (Az; 10mg/kg) plus iv
63 dobutamine (Dob) infusion (2-5 ug/kg/min) and reduced by oral indomethacin (Indo;
64 100mg). ABG samples were collected and ventilatory responses to hypercapnia
65 (HCVR) and hypoxia (HVR) were measured by rebreathing and steady-state
66 techniques before and after drug/placebo. Duplex ultrasound of blood flow in the
67 internal carotid and vertebral arteries was used to measure global CBF. The initial 3-
68 4 hours of sleep were recorded by full polysomnography. Iv Az+Dob increased
69 global CBF by 37±15% compared to placebo (P<0.001), whereas it was reduced by
70 21±8% by oral Indo (P<0.001). ABGs and HVR were unchanged in both
71 interventions. HCVR was reduced by 28%±43% (P=0.1) during iv Az±Dob
72 administration and was elevated by 23%±30% (P=0.05) by Indomethacin. During iv
73 Az+Dob, the CSA index fell from 140±45 (control night) to 48±37 events/hour of
74 sleep (P<0.001). Oral Indo had no significant effect on CSA. We conclude that
75 increasing cerebral blood flow reduced the severity of CSA at high altitude; the likely
76 mechanism is via a reduction in the background stimulation of central
77 chemoreceptors.

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

81 **NEW AND NOTEWORTHY**

82 This work is significant because it shows convincingly for the first time in healthy
83 volunteers, that increasing cerebral blood flow will reduce the severity of CSA in a
84 high altitude model, without the potentially confounding effects of altering PaCO₂ or
85 the ventilatory response to hypoxia.

86 The proposed mechanism of action is that of increasing the removal of locally
87 produced CO₂ from the central chemoreceptors, causing the reduction in
88 hypercapnic ventilatory response, hence reducing loop gain.

89

90 INTRODUCTION

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

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

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

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

133

134 **MATERIALS AND METHODS**

135 Eleven healthy Caucasian adults usually residing at sea level (eight males
136 and three females), with a mean age of 31 ± 7 years (mean \pm SD) and body mass
137 index of 25.6 ± 3.6 kg/m² completed the study, which was approved by the University
138 of British Columbia Ethics Committee and the Nepal Health Medical Research
139 Council and conformed to the standards set by the *Declaration of Helsinki*. Written
140 informed consent was obtained. Other experiments were conducted on the same
141 expedition before and after these experiments, hence the subject numbers are not
142 continuous but are identical in all experiments from the same expedition. However,
143 there was no overlap with the sleep experiments or any confounding
144 pharmacological manipulation or exercise.

145 **Experimental design and ascent profile**

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

150 All participants underwent full medical screening, including 12-lead ECG and
151 echo-cardiography assessment. Participants were not taking any medication, all
152 were non-smokers, and none had any history of cardiovascular, cerebrovascular, or
153 respiratory disease. In addition, only two participants had previous high altitude
154 experience, which was >4 years previous to this expedition. 15 subjects were
155 recruited initially to these experiments. All by general invitation to graduate students
156 within the Dept of Physiology, University of British Columbia, Kelowna. Two
157 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

183 involved testing several agents alone and in combination on the investigators before
184 settling on Az+Dob. The theory is that Az paralyzes the central arteries, preventing
185 auto-regulation of CBF, and the Dob by increasing cardiac output increases CBF.
186 Why PaCO₂ does not change with the combination is not known, but it might be that
187 the slight metabolic acidosis seen with the combination (table 1) caused additional
188 hyperventilation, which reduced PaCO₂ to the placebo value.

189

190 Indomethacin, at a dose of 100 mg orally, reduces CBF and its reactivity by 20-40%
191 within 90 minutes, for up to 4 hours.(31) **Intravenous** Az can increase CBF by 20-
192 50% within 30 minutes, for up to 8 hours (10). It has very different effects to oral Az.
193 For example, when administered intravenously the effects are predominantly on CBF
194 and **extra renal** carbonic anhydrase, and it does not induce measurable metabolic
195 acidosis within this time (eg., <5 hours). Using these pharmacological agents on
196 different days, in a randomized fashion (toss of coin for first drug allocation, then
197 alternate allocation), we altered CBF in both directions, and examined the result of
198 altering CBF on the severity of CSA and the potential underlying mechanisms (eg.
199 alterations in ventilatory responses and blood gases). Indomethacin or placebo was
200 administered orally approximately 90 minutes before testing began with 20 ml of an
201 antacid solution, and Az+Dob or 0.9% saline was administered intravenously 30
202 minutes before testing began. The data were collected and analyzed as “control”,
203 “drug 1” or “drug 2”.

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

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

212 *Sleep studies*

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

225

226 **Experimental procedures**

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

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

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

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

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

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

257 Continuous diameter and blood flow recordings in the left internal carotid
258 artery (ICA), and right vertebral artery (VA) were obtained using a 10-MHz
259 multifrequency linear array probe attached to a high-resolution ultrasound machine
260 (Terason 3000TM, Teratech, Burlington, MA). Imaging of the extracranial arteries
261 was conducted during the 5-min resting baseline period. The ICA blood flow
262 measures were recorded at least 2 cm from the carotid bifurcation, whilst ensuring
263 there was no evidence of turbulent or retrograde flow. The VA was measured within
264 1 cm either proximal or distal (but at the same location within each subject) to the
265 transverse process of C3. Average diameter and blood flow recordings were made
266 from a minimum of 10 cardiac cycles (see below), and care was taken to ensure
267 probe position was stable so that the angle of insonation did not vary from 60°. The
268 sample volume was positioned in the centre of the vessel and adjusted to cover the
269 width of the vessel diameter. Measurement settings for each extracranial artery
270 within an individual were standardised for each VR test and all within individual
271 measures were done by the same sonographer (i.e., pre and post for both
272 interventions).

273 All extracranial vascular images were directly stored as a DICOM file for
274 offline analysis. As described in depth elsewhere (27), analysis involved continuous
275 measurements of arterial diameter synchronous with measurements of blood velocity
276 at 30 Hz performed using an off-line custom-designed edge-detection and wall
277 tracking software. Reproducibility of diameter measurements using this software is
278 significantly better than manual methods as it reduces observer error
279 significantly(27). Volumetric global cerebral blood flow (gCBF) was calculated by:
280 $gCBF (ml.min^{-1}) = (QICA \cdot 2) + (QVA \cdot 2)$

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

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

286 **Ventilatory response testing**

287 *Modified hyperoxic rebreathing method (HCVR):* Participants wore a nose clip and
288 breathed through a mouthpiece connected to a T-valve, which allowed switching
289 from room air to a 8-L rebreathing bag filled with 7% CO₂ and 93% O₂. Following
290 baseline data collection, participants were instructed to hyperventilate for 5 minutes
291 to lower and then maintain a partial pressure of end-tidal CO₂ (P_{ET}CO₂) at 22 ± 2 mm
292 Hg (at low altitude), and 17 ± 2 mm Hg (at high-altitude). Participants were then
293 switched to the rebreathing bag at the end of expiration and were instructed to take
294 three deep breaths to ensure rapid equalization of PCO₂ in the rebreathing circuit.
295 The rebreathing test was terminated when either: i) P_{ET}CO₂ reached 60 mm Hg; ii)
296 partial pressure of end-tidal O₂ (P_{ET}O₂) dropped below 160 mm Hg; iii) ventilation (V_E)
297 exceeded 100 L min⁻¹, or iv) the participant reached the end of their tolerance.

298 The rebreathing data were analyzed on a breath-by-breath basis using a
299 specially-designed programme (Full Fit Rebreathing programme, Version 3.1,
300 University of Toronto, Toronto, Canada). In brief, the initial 3-breath equilibration,
301 sighs, swallows and aberrant breaths were excluded from analysis. Next, the
302 breath-by-breath P_{ET}CO₂ values were plotted against time and fitted with a least
303 squares regression line to minimise inter-breath variability (27). Subsequently, V_E
304 was plotted against the predicted P_{ET}CO₂ obtained by the regression analysis.

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

313 *Poikilocapnic hypoxia (HVR):* Participants wore a nose clip and breathed through a
314 mouthpiece connected to a two-way, T-shaped non-rebreathing valve that allowed
315 switching from room air to a circuit consisting of a 200 L Douglas bag containing
316 38% oxygen. The protocol began with baseline room air breathing for five-minutes,
317 before participants were switched to the 38% oxygen circuit for 10-minutes. The
318 38% oxygen was used to passively normalize inspired PO_2 back to sea level values.
319 This was done to allow comparison with earlier sea level studies⁷ (data in
320 preparation).

321 The mean V_E over the last five-minutes of oxygen breathing was used as one
322 data point and the mean resting (room air) ventilation as the other. The slope of the
323 ΔV_E vs. ΔSpO_2 joining line was taken as the HVR.

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

330 Minute ventilation and gas values were displayed in real time during testing
331 (PowerLab, ADInstruments). Prior to each testing session, the pneumotachometer
332 was calibrated using a 3-L syringe (Hans-Rudolph 5530) and the gas analyzers were
333 calibrated using known concentrations of CO₂ and O₂.

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

338 **Blood gases.** Arterial blood variables [pH, partial pressure of arterial O₂ (PaO₂),
339 partial pressure of arterial CO₂ (PaCO₂), arterial O₂ saturation (SaO₂), bicarbonate
340 concentration [HCO₃⁻], and haematocrit (Hct)] from the radial artery (occasionally
341 femoral artery) were obtained after 10-min supine rest using a 23 or 25-gauge
342 needle into a preheparinised syringe. Following standardized calibration, all blood
343 samples were analyzed using an arterial blood-gas analyzing system (ABL-90 Co-
344 Ox, Radiometer, Copenhagen, Denmark).

345 **Statistical Analysis**

346 *Data Sets:* There were complete data sets for the collected variables for CBF, ABGs
347 and PSG data; however the ventilatory response test data was incomplete. There
348 were 2 empty cells from 44 in the HVR and HCVR results before and after Indo. All
349 results were analyzed using SPSS software (v23. IBM Corp. Ireland). The Shapiro-
350 Wilks test was used to test for distribution normality in each data set. Data sets that
351 were normally distributed were analyzed by paired t-test (most data). Data sets not
352 normally distributed (ie. Pre Az/Dob CBF, Pre Az/Dob PaO₂, Post Indo BE, Mean
353 control AHI, and all HCVR data), were analyzed with their data pairs by a non-

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

356

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

363

364 **RESULTS**

365 **EFFECTS OF ACETAZOLAMIDE+DOBUTAMINE AND INDOMETHACIN**

366 *Acetazolamide+dobutamine*

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

375 The HVR, did not significantly change after the administration of Az+Dob
376 (figure 5A). The slope of the HCVR fell from 5.9 ± 2.7 to 4.2 ± 2.8 l/min/mmHg. ($P=$
377 0.1 ; table 1, figure 4A).

378 The arousal index was reduced from 68 ± 47 /hr on the control night to $22 \pm$
379 10 /hr ($P < 0.01$ table 1). There was no change in sleep efficiency, or total sleep time.

380 *Indomethacin*

381 Ninety minutes following the **oral** administration of indomethacin (Indo),
382 awake resting CBF was reduced by 21% (95%CI: 16-26%), while the mean AHI
383 during sleep was not significantly altered (see table 1, figures 2B and 3B). The
384 PaCO_2 did not change from 26 ± 3 mm Hg (see table 1); yet metabolic alkalosis was
385 still observed, with the pH rising slightly from 7.46 ± 0.02 to 7.48 ± 0.02 ($P=NS$; table 1).

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

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

390 *Correlations*

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

394

395 **DISCUSSION**

396 Herein, we report the results of what we believe to be only the second attempt
397 to artificially manipulate CBF in the field, in the midst of two weeks of acclimatization
398 to an altitude of 5,050 m above sea level, in a group of otherwise healthy volunteers.
399 Both drug interventions were effective in altering CBF. The novel combination of
400 intravenous acetazolamide plus dobutamine infusion significantly reduced the
401 severity of CSA, but on this occasion was not associated with a significant change in
402 PaCO₂, as occurred in our previous study(10) that confounded interpretation of those
403 data. The Indo administration on the other hand, appears to have had only one
404 unintended effect; CSA severity was unaltered, probably because the AHI was
405 already at, or near, its theoretical maximum. The mean CSA index in these
406 experiments was 140/hr compared to 89/hr for the 'control night' comparison used in
407 the previous study (10). The other findings, and relevant methodological
408 considerations, are outlined below.

409

410 We recognized that acclimatization would be ongoing throughout the duration
411 of our study(9), and adjusting for its effects would be important in the conduct of
412 experiments and in the interpretation of the results of the current study. This was
413 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 CO₂ 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*

439 Intravenous Az+Dob caused a 37% increase in global CBF. This increase
440 was associated with a 65% reduction in AHI. Our hypothesis was that this would be
441 due to a reduction in central chemoreceptor stimulation by locally produced CO₂,
442 because of increased clearance caused by the higher CBF. Mean HCVR was
443 lowered by the Az+Dob by 28% (P=0.1). In support of a putative link between
444 chemoreflex drive and CBF, correlational analysis revealed a modest correlation
445 (r=0.41 P=0.054) between the change in HCVR compared to the change in CBF
446 after intravenous Az+Dob, and change in HCVR and change in CBF (r=0.48,
447 P=0.19) after Indo. (see table 2). Crucially, with our combined pharmacological
448 interventions to increase CBF there was no change in PaCO₂, or pH, in contrast to
449 our previous study (10).

450 Oral Indo administration resulted in a 21% (95%CI: 16-26%) reduction in CBF
451 and increased HCVR by 23% (95%CI: 2-44% P=0.05). This was associated with no
452 significant change in AHI, unlike our earlier study (10) at the same altitude. On this
453 occasion, there was no change in PaCO₂ or pH. Most subjects had little or no
454 change from their very high values for AHI prior to drug administration (AHI >100/hr),
455 which suggests that they were perhaps already close to their maximum values for
456 AHI. (10) These experiments were conducted after a longer period of acclimatization
457 at 5,050 metres, leading to a markedly elevated central AHI.

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

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

466 The increase in CBF using intravenous Az plus Dob infusion dramatically
467 reduced CSA. In these experiments, as compared to our earlier experiments where
468 CBF was increased by iv acetazolamide only, the interpretation of that outcome has
469 not been confounded by an increase in PaCO₂ (and presumably brain PCO₂), so the
470 interpretation can be made more confidently.

471

472 *Limitations*

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

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

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

498

499 **CONCLUSION**

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

509

510 **ACKNOWLEDGEMENTS**

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

518

519 **ACKNOWLEDGEMENT OF FINANCIAL SUPPORT STATEMENT / GRANTS**

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

523

524 **DISCLOSURES**

525 Intravenous acetazolamide use was off label.
526 All authors disclose the absence of any conflicts of interest.

527

528

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
PaO₂ (mmHg)	42 ± 2	44 ± 4	42 ± 4	44 ± 4
PaCO₂ (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 n=11	4.2 ± 2.8 [#] n=11	6.4 ± 4.2 n=11	7.9 ± 6.0* n=11
HVR (L/min/%SpO₂)	0.3 ± 0.16 n=11	0.3 ± 0.20 n=11	0.31 ± 0.14 n=10	0.33 ± 0.20 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 / PaCO₂	-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 / PaO₂	-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/%SpO₂)

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.

547
548

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550

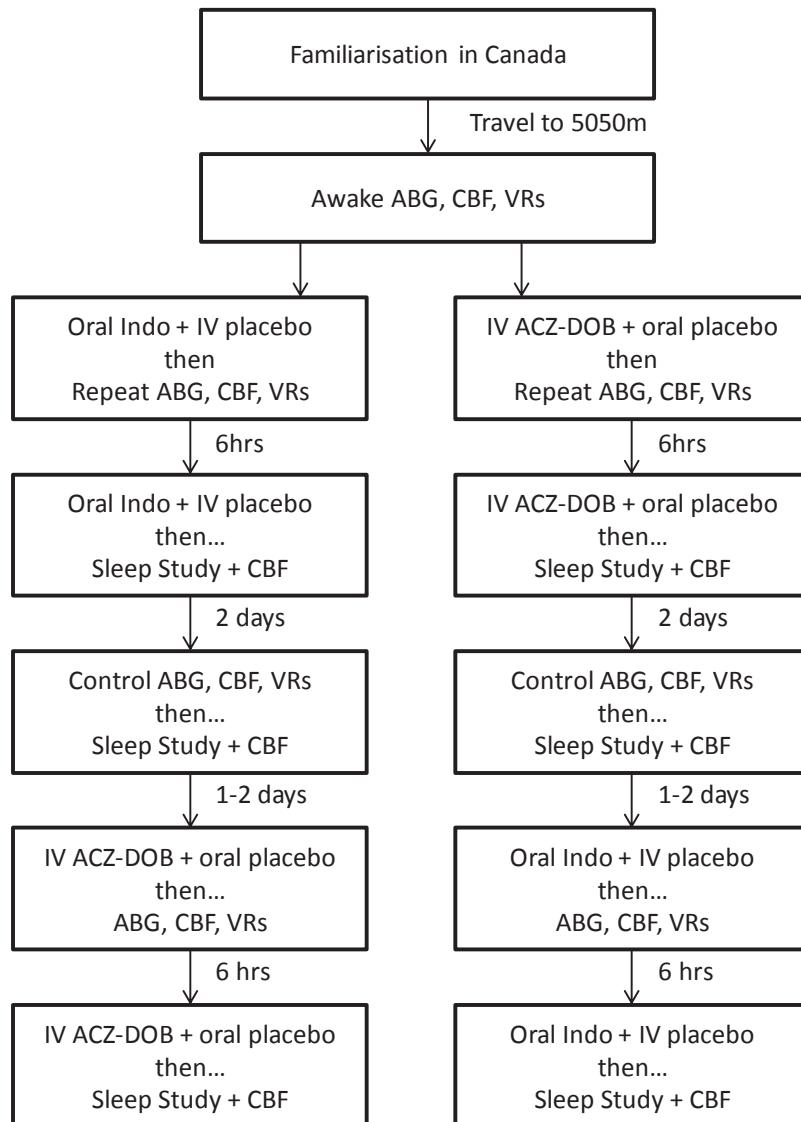
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643

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



INDO = Indomethacin

ACZ = Acetazolamide

DOB = Dobutamine

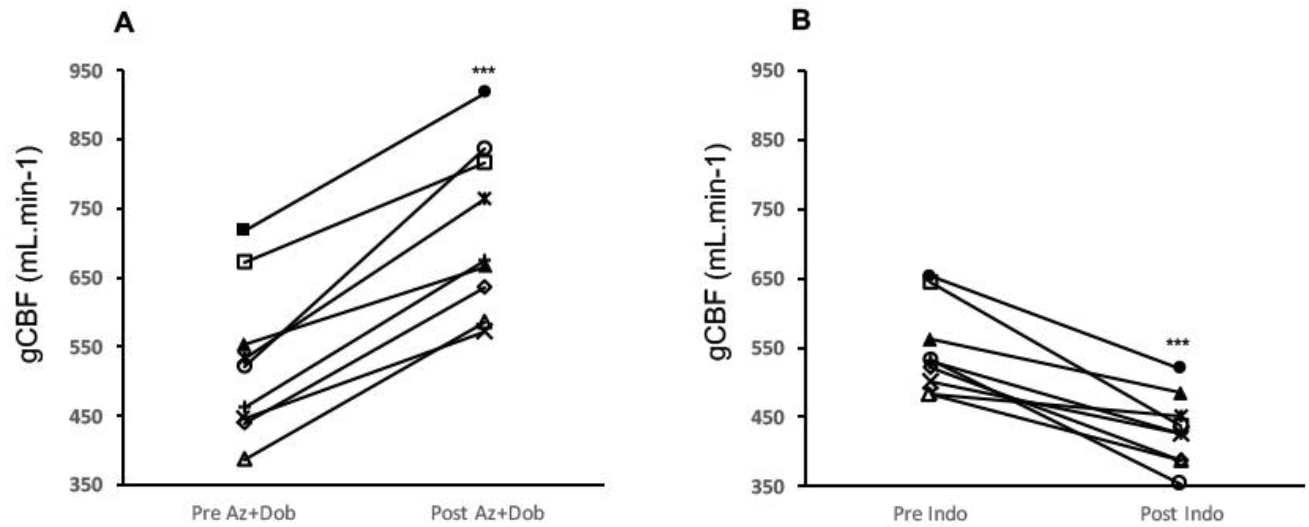
ABG = arterial blood gas measurement

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

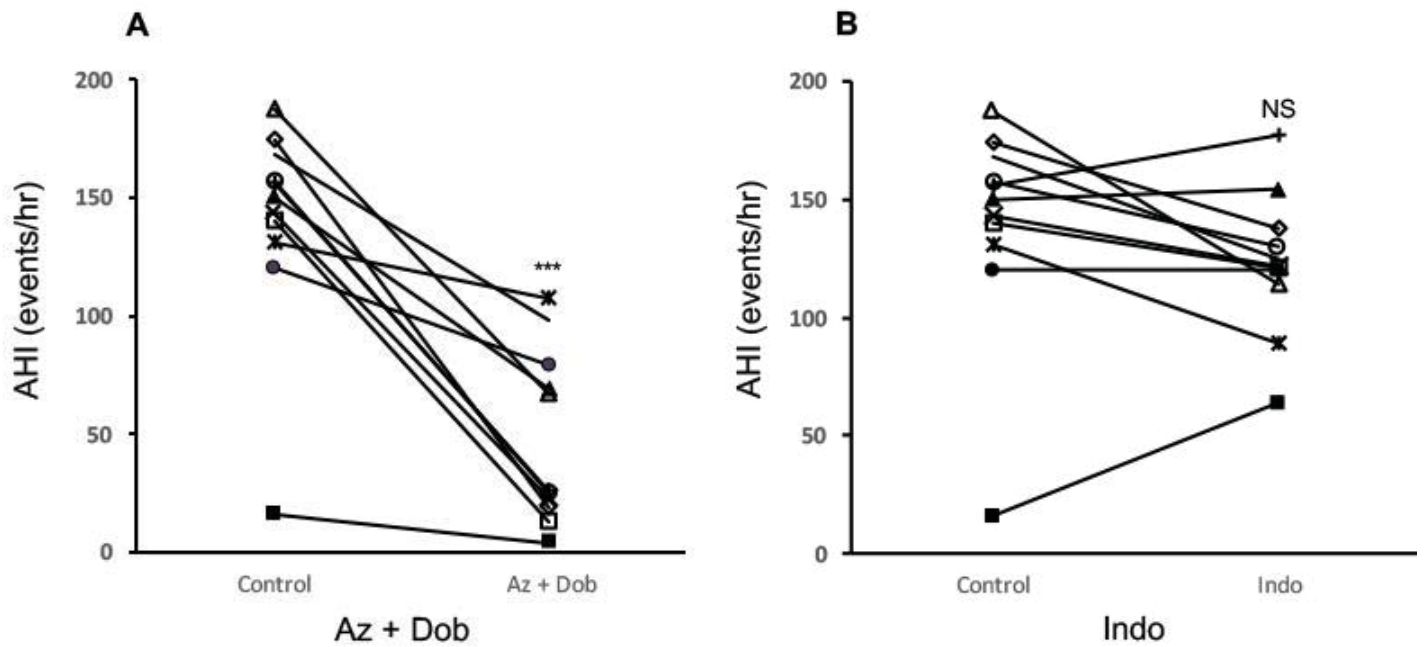
Az = Acetazolamide

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

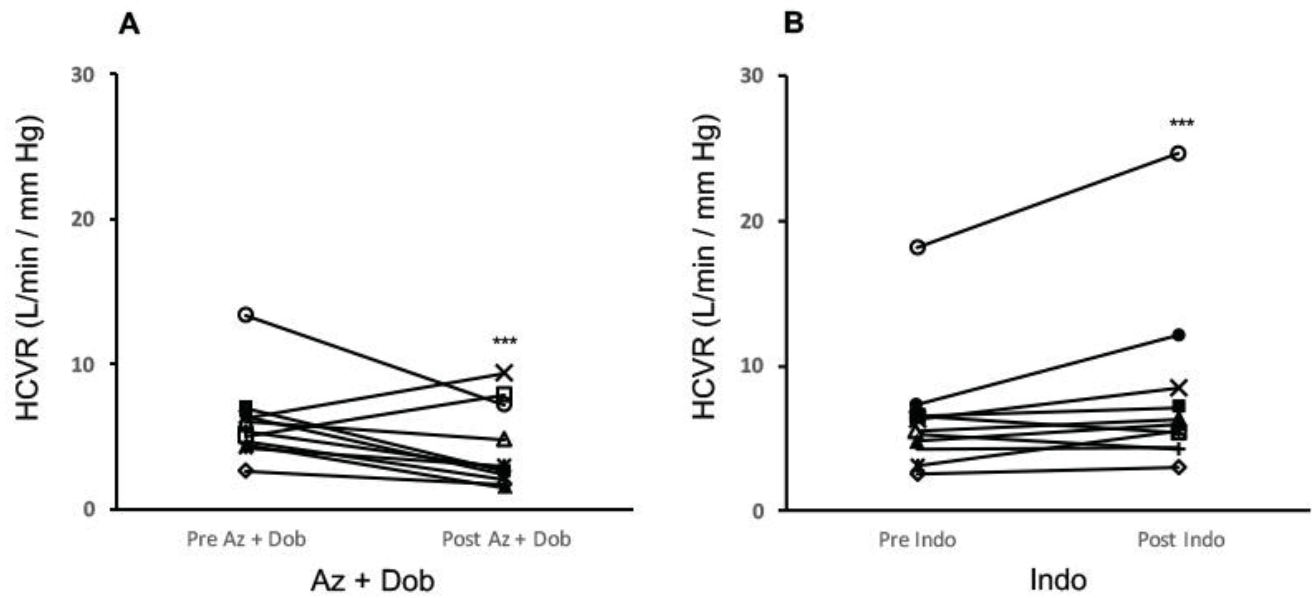
Az = Acetazolamide

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.



*** = $P < 0.001$

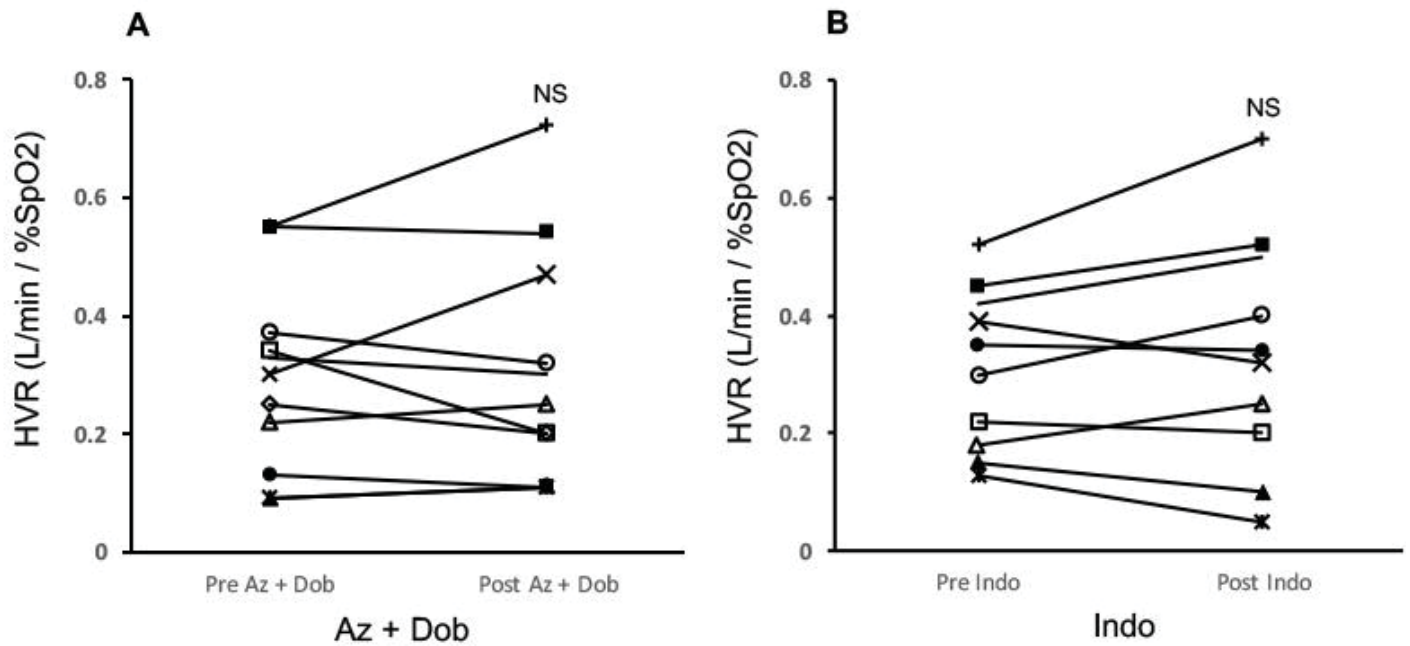
Az = Acetazolamide

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