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Title: Acute hypoxemia and vascular function in healthy humans

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Running Title: Acute hypoxic exposure and the NO-vasodilator system

Abstract: Vascular function is impaired at high altitude and following one hour of comparably severe normobaric hypoxia ($\sim FIO_2=0.11$). Whether vascular function is impaired during milder hypoxia is unknown. We examined the hypothesis that vascular function would be impaired following acute exposure to mild (74 ± 2 mmHg $P_{ET}O_2$) and moderate (50 ± 3 mmHg $P_{ET}O_2$) normobaric hypoxia. Brachial endothelium-dependent flow mediated dilation (FMD) was assessed at baseline and following 30-minutes of hypoxia ($n=12$) or normoxia (time control trial; $n=10$). Endothelium-independent dilation (via glyceryl trinitrate; GTN) was assessed following the hypoxic FMD test, and in normoxia on a separate control day ($n=8$). Compared to normoxic baseline, allometrically correcting for baseline diameter and FMD shear rate under the curve, FMD and GTN-induced dilation were reduced following mild hypoxia (FMD: 6.4 ± 1.0 vs. $5.9 \pm 1.0\%$; GTN: 16.4 ± 4.0 vs. $14.3 \pm 4.0\%$; $P \leq 0.02$) and moderate hypoxia (FMD: 6.6 ± 1.0 vs. $4.5 \pm 1.0\%$; GTN: 16.4 ± 4.0 vs. $12.9 \pm 4.0\%$; $P \leq 0.02$). The

Disclaimer: This is a confidential document.

normoxic time-control data, however, revealed a ~8% decline in FMD (comparable with the FMD decline during mild hypoxia), indicating that 30 minutes of recovery for repeated FMD assessments is insufficient. Considering the methodological effects of repetitive FMD testing, endothelial dilation is unaltered following mild hypoxia exposure, yet it is significantly impaired during more moderate hypoxia. Graded impairments in smooth muscle function is evident following mild and moderate hypoxia, and this has implications for individuals acutely exposed to hypoxia.

New Findings: Endothelial dilation is impaired following an acute moderate hypoxia stimulus; therefore, the central question of this study is to investigate whether this impairment in endothelial dilation is evident following a mild hypoxic exposure, and if smooth muscle dilation is impaired following acute hypoxic exposure. Vascular smooth muscle cells sensitivity to a NO is impaired following mild and moderate hypoxia equivalent to ~2000m and ~5000m respectively. Unlike following moderate hypoxia exposure, it appears endothelial dysfunction is not impaired following mild hypoxia. These findings have important implications for individuals with pre-existing medical conditions, especially those who are rapidly exposed to hypoxia.

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1 **Acute hypoxemia and vascular function in healthy humans**

2

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9

10 **Running Title:** Acute hypoxic exposure and the NO-vasodilator system

11

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13

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23

24 **New Findings (100 words)**

25 **What is the central question of this study?**

26 Endothelial dilation is impaired following an acute moderate hypoxia stimulus; therefore, the
27 central question of this study is to investigate whether this impairment in endothelial dilation is
28 evident following a mild hypoxic exposure, and if smooth muscle dilation is impaired following
29 acute hypoxic exposure.

30 **What is the main findings and its importance?**

31 Vascular smooth muscle cells sensitivity to a NO is impaired following mild and moderate
32 hypoxia equivalent to ~2000m and ~5000m respectively. Unlike following moderate hypoxia
33 exposure, it appears endothelial dysfunction is not impaired following mild hypoxia. These
34 findings have important implications for individuals with pre-existing medical conditions,
35 especially those who are rapidly exposed to hypoxia.

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47 **Abstract (250)**

48

49 Vascular function is impaired at high altitude and following one hour of comparably severe
50 normobaric hypoxia ($\sim F_{I}O_2=0.11$). Whether vascular function is impaired during milder hypoxia
51 is unknown. We examined the hypothesis that vascular function would be impaired following
52 acute exposure to mild (74 ± 2 mmHg $P_{ET}O_2$) and moderate (50 ± 3 mmHg $P_{ET}O_2$) normobaric
53 hypoxia. Brachial endothelium-dependent flow mediated dilation (FMD) was assessed at
54 baseline and following 30-minutes of hypoxia ($n=12$) or normoxia (time control trial; $n=10$).
55 Endothelium-independent dilation (via glyceryl trinitrate; GTN) was assessed following the
56 hypoxic FMD test, and in normoxia on a separate control day ($n=8$). Compared to normoxic
57 baseline, allometrically correcting for baseline diameter and FMD shear rate under the curve,
58 FMD and GTN-induced dilation were reduced following mild hypoxia (FMD: 6.4 ± 1.0 vs.
59 $5.9\pm 1.0\%$; GTN: 16.4 ± 4.0 vs. $14.3\pm 4.0\%$; $P\leq 0.02$) and moderate hypoxia (FMD: 6.6 ± 1.0 vs.
60 $4.5\pm 1.0\%$; GTN: 16.4 ± 4.0 vs. $12.9\pm 4.0\%$; ≤ 0.02). The normoxic time-control data, however,
61 revealed a $\sim 8\%$ decline in FMD (comparable with the FMD decline during mild hypoxia),
62 indicating that 30 minutes of recovery for repeated FMD assessments is insufficient. Considering
63 the methodological effects of repetitive FMD testing, endothelial dilation is unaltered following
64 mild hypoxia exposure, yet it is significantly impaired during more moderate hypoxia. Graded
65 impairments in smooth muscle function is evident following mild and moderate hypoxia, and this
66 has implications for individuals acutely exposed to hypoxia.

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69

70 **Introduction**

71 The nitric oxide (NO)-vasodilator system is important in the maintenance of vasoregulation and
72 vascular health and its function is a marker of cardiovascular risk. Endothelium-dependent flow
73 mediated dilation (FMD) assesses conduit artery vasodilatory capacity following a reactive
74 hyperemia stimulus (shear stress). The latter component of the NO-dilator cascade endothelium-
75 independent NO-mediated smooth muscle relaxation can be assessed by administering glyceryl
76 trinitrate (GTN)(Corretti *et al.*, 2002). Therefore, the assessment of both FMD and GTN
77 measures within subjects provides complimentary information regarding the locus of change in
78 vascular function *in vivo* (Celermajer *et al.*, 1992).

79

80 The effect of acute normobaric or hypobaric hypoxia on basal FMD and GTN-induced dilation is
81 unclear. Some studies have reported a significant decline following hypoxia (Bailey *et al.*, 2013;
82 Lewis *et al.*, 2014), others an absence of change (Frick *et al.*, 2006; Frobert *et al.*, 2008; Bailey
83 *et al.*, 2013). These discrepancies are perhaps not surprising, since studies are often confounded
84 by 1) pathology such as metabolic syndrome (Frick *et al.*, 2006), chronic mountain sickness
85 (Bailey *et al.*, 2013) and cardiovascular disease (Frobert *et al.*, 2008); or 2) methodological
86 limitations e.g., different definition of acute hypoxic exposure (5 minutes vs. 1 hour vs. 3 days),
87 different acute hypoxic stimuli (hypobaric hypoxia vs. normobaric hypoxia) (Frobert *et al.*, 2008;
88 Lewis *et al.*, 2014), different population groups (native highlanders vs. lowlanders; (Bailey *et al.*,
89 2013), and/or inappropriate FMD and GTN data collection and analysis protocols [cuff
90 placement, period of data collection, non-use of edge detection software; (Frobert *et al.*, 2008)].

91

92 By employing international guidelines for the assessment of FMD and endothelium-independent
93 NO-mediated smooth muscle relaxation (Thijssen *et al.*, 2011), we recently documented a 14%
94 decline in both FMD and GTN-induced dilation following three days of hypobaric hypoxia
95 (5050m) in healthy individuals (Lewis *et al.*, 2014). These findings suggest that endothelial and
96 vascular smooth muscle dysfunction both contribute to a decline in the NO-vasodilator system
97 with hypobaric hypoxia. In a follow-up study, we discovered that a substantial larger and
98 sustained decline in FMD (~28%) occurs as a result of 60-minutes of exposure to normobaric
99 hypoxia (FIO₂=0.11; a hypoxic level stimulating ~5000m). These marked reductions in FMD
100 were abolished following sympathetic nerve activity (SNA) blockade (Lewis *et al.*, 2014).
101 However, it is currently unclear whether GTN-induced dilation is impaired to the same degree
102 within 60-minutes following normobaric hypoxia. Furthermore, it is unknown whether the
103 impairment in FMD and potential decline in GTN-induced dilation following acute (<60-
104 minutes) normobaric hypoxia is sensitive to distinct levels of hypoxia.

105

106 The primary purpose of this study was to examine the effect of acute (<60 minutes) exposure to
107 mild (end-tidal oxygen P_{ET}O₂ = 75 mm Hg; ~2000m) and moderate (end-tidal P_{ET}O₂ = 50 mm
108 Hg; ~4600m) isocapnic hypoxia on brachial FMD and GTN-induced dilation. We hypothesized
109 that FMD and GTN-induced dilation would be impaired following mild hypoxia and more so
110 following moderate hypoxia. We intentionally chose this mild exposure as a comparable PO₂ to
111 that encountered during commercial air travel (Smith *et al.*, 2012), during trekking, and ski
112 vacation sites in North America. To ensure that there were no repetitive influences of the FMD
113 testing, we conducted a normoxic time-control study to quantify the effect of 30 minutes of
114 supine normoxic rest on FMD. Based on previously published guidelines (Corretti *et al.*, 2002;

115 Barton *et al.*, 2011), we reasoned that FMD and related hemodynamic variables would be
116 unaltered following 30 minutes of normoxic supine rest.

117

118 **Materials and Methods**

119 **Participants:** Twelve healthy normotensive volunteers (7 men, 5 women; mean \pm SD: age, $26 \pm$
120 6 years; body mass, 71 ± 12 kg; height, 176 ± 8 cm; body mass index, 23 ± 3 kg/m²) participated
121 in this randomized counter-balanced experiment. The study was approved by the Human Ethics
122 Committee of the University of British Columbia and conformed to the standards set by the
123 Declaration of Helsinki. All volunteers provided written informed consent. Participants were
124 non-smokers, had no previous history of cardiovascular, cerebrovascular, or respiratory diseases,
125 and were not taking any medications, other than the contraceptive pill. Females were tested
126 during the either the pill withdrawal/placebo phase, or in the earlier follicular phase of the
127 menstrual cycle of consecutive cycles. All experimental testing took place at the University of
128 British Columbia (altitude 344 m).

129

130 **Study design:** Participants attended the laboratory on four occasions (one familiarisation session
131 and three experimental session). The experimental sessions were separated by >7 day and each
132 session commenced between 8:00-9:00 A.M. Experimental testing followed a minimum of 12 h
133 abstinence from alcohol, caffeine, and strenuous exercise, and an overnight fast. Experimental
134 session one and two consisted of 20 minutes of supine rest following which, cardiorespiratory
135 measures were monitored for 5 minutes and the assessment of FMD was undertaken under
136 normoxic conditions. Participants were then rapidly exposed to isocapnic hypoxia. Following 30
137 minutes of isocapnic hypoxia exposure cardiorespiratory measures and the assessment of FMD

138 were repeated and following 60 minutes of isocapnic hypoxia GTN-induced dilation assessed.
139 The level of isocapnic hypoxia experience in each session was randomized and counter-
140 balanced. Using end-tidal forcing, in the separate visits, the participant's end-tidal oxygen
141 ($P_{ET}O_2$) was rapidly reduced down to 75 mm Hg (mild-hypoxia) or 50 mm Hg (moderate-
142 hypoxia) following baseline assessments. End-tidal carbon dioxide ($P_{ET}CO_2$) was clamped as
143 baseline levels.

144
145 During experimental session three, following 20 minutes of supine rest the assessment of GTN-
146 induced dilation was made in normoxic conditions, in eight of the twelve participants who
147 completed experimental sessions one and two. The GTN-dilation assessment was made on a
148 separate day from the two hypoxic tests due to the half-life of GTN being approximately four
149 hours and the potential interference with other measures if conducted at normoxia in
150 experimental sessions one or two.

151
152 **Experimental Measures and Data Analysis**

153 **Brachial artery vascular function:** A 10 MHz multifrequency linear array probe attached to a
154 high-resolution ultrasound machine (Terason 3000, Teratech) was used to image the brachial
155 artery in the right arm. Blood flow velocity was measured as peak blood flow velocity of the
156 Doppler shift, with the sample gate begin placed in the centre of the lumen.

157 **Endothelium-dependent FMD.** FMD was assessed according to international guidelines
158 (Thijssen *et al.*, 2011). With the occluding cuff placed distal to the ultrasound probe, 1 minute of
159 brachial diameter and blood flow velocity recordings preceded forearm cuff inflation to 220

160 mmHg for 5 minutes. Brachial diameter and blood flow velocity recordings resumed 30 s prior to
161 cuff deflation and continued for 3 minutes thereafter.

162 ***Endothelium-independent FMD (GTN)***. Following 20 minutes of rest, brachial diameter and
163 blood flow velocity recordings were made for 1 minute prior to participants receiving a
164 sublingual dose of glyceryl trinitrate (GTN; 400 μ g spray). Brachial diameter and blood flow
165 velocity recordings were taken continuously for a 10-minute period thereafter.

166

167 Custom-designed edge-detection and wall-tracking software, which is largely independent of
168 investigator bias, was utilised for the analysis of FMD and GTN (Woodman *et al.*, 2001; Black
169 *et al.*, 2008; Thijssen *et al.*, 2011). This software provides continuous and simultaneous
170 diameter, blood flow velocity at 30Hz. From this synchronized diameter and velocity data, blood
171 flow (the product of lumen cross-sectional area and Doppler velocity and shear rate (SR [4 times
172 velocity divided by diameter]) (Pyke *et al.*, 2004; Pyke & Tschakovsky, 2007) are calculated at
173 30 Hz. This semi-automated software provides higher reproducibility of diameter measurements
174 and reduces both observer error and bias with a reported intra-observer CV for FMD% of 6.7%
175 (Woodman *et al.*, 2001). Baseline diameter, blood flow, and SR patterns were calculated as the
176 mean of data acquired across the minute preceding the cuff inflation period. Peak diameter after
177 cuff deflation was automatically detected according to an algorithm that identified the maximum
178 bracket of data, and FMD% was calculated as the percentage rise of this peak diameter from the
179 preceding baseline diameter. The time to peak diameter (in seconds) was calculated from the
180 point of cuff deflation to the maximum post-deflation diameter and SR area under curve (SR_{AUC})
181 was calculated for the FMD stimulus up to peak diameter (Black *et al.*, 2008). Recent evidence
182 has highlighted that FMD% can under some circumstances fail to consider the difference in

183 baseline artery diameter following an intervention or between groups (Atkinson & Batterham,
184 2013; Atkinson *et al.*, 2013). Therefore, as outlined in detail (Atkinson & Batterham, 2013;
185 Atkinson *et al.*, 2013), we adopted an allometric scaling approach to adjust for baseline diameter
186 in the calculation of FMD and GTN-induced dilation. Also, where necessary we also adjusted the
187 FMD and GTN dilation for changes in SR_{AUC} . These results are presented as ‘allometrically
188 corrected’ FMD%. Oscillatory shear index, an indicator of the magnitude of shear oscillation,
189 was defined as: $(|retrograde\ SR|) / (|antegrade\ SR| + |retrograde\ SR|)$. We also calculated
190 FMD/GTN ratio, to correct the FMD for potential differences in GTN-induced dilation.

191

192 **Cardiorespiratory Measures:** Beat-to-beat blood pressure (BP) was measured by finger
193 photoplethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, Netherlands)
194 and normalized to manual cuff measurements of the brachial artery. Stroke volume (SV) and
195 cardiac output (CO) were calculated from the BP waveform obtained from the finger
196 photoplethysmography using the Modelflow method, incorporating age, sex, height, and weight
197 (BeatScope 1.0 software; TNO TPD; Biomedical Instruments). Heart rate was measured (HR)
198 via three-lead electrocardiogram (ML132; ADInstruments, Colorado Springs CO). All measures
199 were monitored for 5 minutes prior to FMD assessment, where minute 4 to 5 was used as a
200 representation of baseline values. Peripheral oxygen saturation (SpO_2 ; Pulse Oximeter
201 MD300K1; Vacumed, Ventura, CA) was measured immediately prior to the FMD assessment.

202

203 For measurement of $P_{ET}CO_2$ and $P_{ET}O_2$, subjects breathed through a mouthpiece connected to a
204 two-way non-rebreathing valve. Respired gas pressures were sampled at the mouth by securing a
205 sample line connected to a calibrated online gas analyzer (model ML206, AD Instruments,

206 Colorado Springs, CO) into the mouthpiece. Respiratory flow was measured at the mouth using a
207 pneumotachograph (model HR 800L, HansRudolph, Shawnee, KS). $P_{ET}CO_2$, $P_{ET}O_2$ and
208 inspiratory and expiratory tidal volume were determined for each breath online using specifically
209 designed software (LabView, Austin, TX). $P_{ET}CO_2$ and $P_{ET}O_2$ were controlled via end-tidal
210 forcing system (Tymko *et al.*, 2015). This system uses independent gas solenoid valves for
211 oxygen, carbon dioxide and nitrogen and controls the volume of each gas delivered to the
212 inspiratory reservoir through a mixing-and-humidification chamber. With use of feedback
213 information regarding $P_{ET}CO_2$, $P_{ET}O_2$, and inspiratory and expiratory tidal volume, the system
214 prospectively targets the inspire to bring end-tidal gas to the desired level. Gas control was
215 fine-tuned using a feedback control and error reduction algorithm. Clamped $P_{ET}CO_2$ levels were
216 determined as the values measured during the last 5-minutes of normoxic measurements.

217

218 **Normoxic Time-Control Study**

219 **Participants:** Ten healthy normotensive volunteers, (9 men, 1 women; mean \pm SD: age, 27 ± 2
220 years; body mass, 77 ± 8 kg; height, 180 ± 1 cm; body mass index, 23 ± 2 kg/m²) participated in
221 this study. All participant pre-experimental considerations were the same as described for the
222 hypoxia studies.

223

224 **Experimental Design and Methods:** All participants were familiarised with the FMD protocol,
225 and attended the laboratory for one experimental session. Here, FMD was assessed in normoxic
226 conditions prior to (FMD one) and following 30 minutes of supine rest (FMD two). All
227 methodological and data analysis procedures were performed as outlined for the hypoxia studies.

228

229 **Statistical Analysis:** All data were analysed using SPSS (version 21, IBM, Surrey, UK) and
230 expressed as mean \pm SD. Statistical significance was defined as $P\leq 0.05$ and distribution normality
231 confirmed using repeated Shapiro–Wilk W tests.

232 *Study 1:* To examine the interaction between the experimental intervention (normoxia vs. hypoxic
233 stimuli) and the experimental condition ($P_{ET}O_2$ 75 mmHg (mild-hypoxia) trial vs. $P_{ET}O_2$ 50
234 mmHg (moderate-hypoxia)) a two-way repeated measures ANOVA was used; to further
235 exposure any significant interaction effect, two-tailed paired t tests were used to quantify the
236 effect of the hypoxic stimuli on the measures of interest. For the assessment of GTN-induced
237 dilation and related variables, a one-way repeated measures ANOVA was used to compare the
238 trial difference between normoxia and the two hypoxic stimuli. Pearson’s correlation analysis
239 was used to examine the relationship between selected measures. *Normoxic time-control study* To
240 examine the interaction between normoxic baseline and following 30 minutes of supine rest a
241 two-tailed paired t tests were used, unless stated otherwise. *Hypoxia and Normoxic time-control*
242 *studies:* A linear mixed model for repeated measures was used to allometrically correct FMD and
243 GTN-dilation for baseline diameter and SR_{AUC} as covariates.

244

245 **Results**

246 **Effect of hypoxia on cardiorespiratory variables**

247 Per the study design, a significant interaction between the experimental intervention (normoxia
248 vs. hypoxia) and experimental condition ($P_{ET}O_2$ 75 mmHg trial vs. $P_{ET}O_2$ 50 mmHg trial) was
249 evident for $P_{ET}O_2$ ($P<0.01$). No difference in baseline (normoxia) $P_{ET}O_2$ was evident between
250 experimental conditions; however, as desired, $P_{ET}O_2$ was lower following hypoxia exposure in
251 the $P_{ET}O_2$ 50 mmHg (50 ± 3 mm Hg) versus the $P_{ET}O_2$ 75 mmHg trial (74 ± 2 mm Hg; $P<0.01$;

252 Table 1). There were no differences in $P_{ET}CO_2$ during the experimental intervention or condition
253 (Table 1).

254

255 A significant interaction between the experimental intervention and experimental condition was
256 evident for ventilation ($P<0.01$; Table 1). Compared to normoxia, ventilation was increased
257 following exposure to $P_{ET}O_2$ 75 mmHg ($+3.1 \pm 3.5$ %; $P=0.01$) and $P_{ET}O_2$ 50 mmHg ($+10.0 \pm$
258 5.8 %; $P<0.01$); the increase in ventilation was greater following exposure to $P_{ET}O_2$ 50 mmHg
259 ($P<0.01$). Likewise, a significant interaction between the experimental intervention and
260 experimental condition was evident for SaO_2 ($P=0.001$; Table 1). Compared to normoxia, the
261 reductions in SaO_2 were greater at $P_{ET}O_2$ 50 mm Hg compared with 75 mmHg (-15 ± 3 % vs $-5 \pm$
262 2 %; $P<0.01$).

263

264 A main effect for the experimental intervention was evident for mean arterial blood pressure
265 (MAP) independent of hypoxic stimulus; compared to normoxia, MAP increased in both hypoxic
266 trials by 6 ± 2 mmHg ($P=0.001$, Table 1), respectively. An interaction between experimental
267 intervention and experimental condition was evident for HR ($P=0.01$) and CO ($P=0.03$; Table 1).
268 The increase in HR was greater (6 ± 4 beats \cdot min $^{-1}$; $P<0.01$; Table 1) in the $P_{ET}O$ 50 mmHg trial
269 compared with the 75 mmHg trial. Likewise, the increase in CO was greater in the $P_{ET}O_2$ 50
270 mmHg trial (0.6 ± 0.7 L \cdot min $^{-1}$; $P=0.03$; Table 1). No difference was evident in the SV response
271 following exposure to hypoxia ($n= 10$).

272

273 **Effect of hypoxia on brachial artery baseline measures**

274 A significant interaction between the experimental intervention (normoxia vs. hypoxia) and
275 experimental condition ($P_{ET}O_2$ 75 mmHg trial vs. $P_{ET}O_2$ 50 mmHg trial) was evident for baseline
276 brachial arterial diameter ($P=0.01$). Following exposure to $P_{ET}O_2$ 50 mmHg, arterial diameter
277 increased by 0.02 ± 0.02 cm (relative 4%; $P=0.01$). In contrast, no diameter changes were
278 evident following exposure to $P_{ET}O_2$ of 75 mmHg ($P=0.80$; Table 2).

279
280 Compared to normoxia, independent of the level of hypoxic stimulus, significant main effects
281 were evident for reductions in baseline peak blood velocity (-3.6 ± 2.9 $cm \cdot s^{-1}$; [relative $\sim -42\%$]
282 $P<0.01$), baseline peak blood flow (-13.2 ± 9.3 $ml \cdot min^{-1}$ [relative, $\sim -39\%$; $P=0.01$], baseline
283 mean SR (-37 ± 12 s [relative, $\sim -43\%$; $P<0.01$], baseline antegrade SR (-24 ± 7 s [relative, $\sim -$
284 21% ; $P=0.01$], and an increase in baseline retrograde SR ($+ -13 \pm 5$ s [relative, $\sim +48\%$; $P=0.01$)
285 and oscillatory shear index ($+ 0.1 \pm 0.0$ [relative, $\sim +54\%$; $P=0.01$; Table 2 and Figure 1). A
286 significant interaction between intervention (normoxia vs. hypoxia) and experimental condition
287 ($P_{ET}O_2$ 75 mmHg trial vs. $P_{ET}O_2$ 50 mmHg trial) was evident for baseline retrograde SR and
288 oscillatory shear index ($P \leq 0.01$); the intervention change following exposure to $P_{ET}O_2$ 50 mmHg
289 was greater (retrograde SR: $+ -18 \pm 9$ s; oscillatory shear index $+0.12 \pm 0.0$) than exposure to
290 $P_{ET}O_2$ 75 mm Hg (retrograde SR: $+ -8 \pm 2$ s; oscillatory shear index: 0.07 ± 0.0 ; $P \leq 0.03$; Figure
291 1).

292

293 **Effect of hypoxia on brachial artery FMD (n=12)**

294 A significant interaction between the experimental intervention and experimental condition was
295 evident for FMD ($P<0.01$; Figure 2 A). Compared to normoxia, FMD was significantly reduced

296 following exposure to $P_{ET}O_2$ 75 mmHg ($-1.1 \pm 1.1\%$ [relative, $\sim -17\%$]; $P=0.005$) and $P_{ET}O_2$ 50
297 mmHg ($-3.1 \pm 1.7\%$ [relative 45%]; $P<0.01$); the decline in FMD was greater following exposure
298 to $P_{ET}O_2$ 50 mmHg by $2 \pm 1\%$ [relative 63%; $P<0.01$]. A significant main effect for intervention
299 was evident for SR_{AUC} ($P=0.01$). Here, compared to normoxia, SR_{AUC} (25855 ± 9699 AUC) was
300 reduced following hypoxia exposure (19441 ± 10386 AUC) independent of hypoxic stimulus
301 (Table 2). Following allometric scaling of FMD and accounting for the decline in SR_{AUC} as a
302 covariate, a significant interaction between experimental intervention and experimental condition
303 was evident ($P<0.01$). Compared to normoxia, FMD was significantly reduced following
304 exposure to $P_{ET}O_2$ 75 mmHg (-0.5% [relative -8%]), but the decline following the 30 minutes
305 exposure to $P_{ET}O_2$ 50 mmHg was greater (-2.1% [relative, $\sim -32\%$]; Figure 2B).

306

307 **Effect of hypoxia on brachial artery GTN ($n=8$; Table 3)**

308 One-way ANOVA revealed that GTN-induced dilation was reduced following hypoxic exposure
309 ($P=0.01$); Figure 3A). Compared with normoxia, GTN-induced dilation was significantly
310 decreased following exposure to $P_{ET}O_2$ 75 mmHg ($-2.1 \pm 2.5\%$ [relative -12%] and $P_{ET}O_2$ 50
311 mmHg ($-4.2 \pm 4.0\%$ [relative -25%]). The decline with $P_{ET}O_2$ 50 mmHg was greater than the
312 decline observed with $P_{ET}O_2$ 75 mmHg by $2.1 \pm 2.6\%$; [relative 14%; $P=0.06$; Figure 3).
313 Following allometric scaling for baseline diameter, GTN-dilation was still significantly
314 decreased following exposure to $P_{ET}O_2$ 75 mmHg (-2.1% [relative -13%] and even more so
315 following $P_{ET}O_2$ 50 mmHg (-3.5% [relative -22%]; $P=0.02$; Figure 3B). Compared to normoxia,
316 the FMD:GTN ratio was significantly decreased following exposure to $P_{ET}O_2$ 75 mmHg ($-0.05 \pm$
317 0.03% [relative 11%] and $P_{ET}O_2$ 50 mmHg ($-0.16 \pm 0.04\%$ [relative 35%] $P<0.01$). The decline

318 in the FMD:GTN ratio was significantly greater following exposure to $P_{ET}O_2$ 50 mmHg than
319 $P_{ET}O_2$ 75 mmHg ($P=0.02$; Table 3).

320

321 **Normoxic time-control study**

322 Baseline MAP, HR, and SaO_2 % were 80 ± 6 mmHg, 56 ± 8 beats \cdot min $^{-1}$, 98 ± 1 %. No
323 significant difference in baseline diameter was evident following 30 minutes of supine rest
324 (Table 4). Compared to baseline (pre-FMD one), however, reductions in baseline peak blood
325 flow velocity (-5.8 ± 1.2 cm \cdot s $^{-1}$; [relative $\sim 36\%$] $P=0.01$), peak blood flow (-0.98 ± 0.32 ml \cdot min $^{-1}$
326 [relative, $\sim 37\%$; <0.01]), were evident following 30 minutes of supine rest (Table 4). Reduction
327 in baseline mean SR ($\sim 37 \pm 8$ s [relative, $\sim 34\%$; $P<0.04$) and baseline anterograde SR (-32 ± 8
328 s [relative, $\sim -27\%$; $P=0.06$) were evident following FMD one and 30 minutes of supine rest,
329 retrograde SR and oscillatory SR index were not significantly changed ($P=0.19$; Table 4).
330 Compared to FMD one, FMD was significantly reduced following 30 minutes of supine rest by -
331 0.62 ± 0.28 % (relative, $\sim -8.4\%$; $P=0.02$). No significant difference in FMD SR_{AUC} was evident
332 between the two FMDs ($P=0.13$; Table 4). Following allometric scaling of FMD, where baseline
333 diameter and SR_{AUC} were considered as a covariate, the decline in FMD following 30 minutes of
334 supine rest still evident ($P=0.05$, Figure 4).

335

336 **Discussion**

337 The primary aim of study one was to examine the acute effects (<60 minutes) of a mild ($P_{ET}O_2$
338 75 mm Hg; ~ 2000 m) and moderate ($P_{ET}O_2$ 50 mm Hg; ~ 5000 m) isocapnic normobaric hypoxic
339 stimulus on the NO-vasodilator system via the assessment of FMD and GTN-induced dilation in
340 the brachial artery. The novel findings were: 1) Compared to normoxia, FMD and GTN-induced

341 dilation were reduced following moderate hypoxia and, to a lesser extent, following mild
342 hypoxia. 2) FMD SR_{AUC} was reduced during both moderate and mild hypoxic conditions;
343 however, when the decline in FMD was corrected for the decline in SR_{AUC} , the decline in FMD
344 with mild and moderate hypoxia were attenuated. 3) Following exposure to both mild and
345 moderate hypoxia there was a decline in baseline blood flow and anterograde SR, and an
346 increase in retrograde SR. The increase in retrograde SR was greater during moderate hypoxia.
347 The main findings of the normoxic time-control study were that baseline blood flow and blood
348 flow velocity along with FMD were all reduced following 30 minutes of supine normoxic rest.
349 Such findings indicate that 30 minutes of recovery time for repeated FMD assessments is
350 insufficient. Based on these findings, the following discussion outlines putative mechanisms that
351 likely underpin hypoxia-induced declines in vascular function, including: 1) methodological
352 considerations of repetitive FMD testing and data interpretation; 2) hypoxic-induced declines in
353 FMD SR_{AUC} ; 3) an increase in oscillatory shear; and 4) impaired endothelial function and
354 smooth muscle vasodilation.

355

356 ***Methodological considerations of repetitive FMD testing:*** The initial finding of this study
357 revealed an acute decline in FMD following 30-minutes of isocapnic hypoxia, which appears to
358 be dependent on the severity of the hypoxic stimulus. FMD was reduced by (relative) ~17% and
359 ~45% following 30 minutes of mild ($P_{ET}O_2$ 75 mm Hg, SaO_2 93%) and moderate ($P_{ET}O_2$ 50 mm
360 Hg, SaO_2 83%) hypoxia, respectively. The SR_{AUC} component of the FMD provides an estimation
361 of the shear stress stimulus created upon cuff release, which ultimately provokes the production
362 and release of NO from the endothelium. In both hypoxic trials, FMD SR_{AUC} decreased by
363 ~25%, a finding which was not evident in our normoxic time-control trial. Although not

364 statistically significant, SR_{AUC} has previously been reported to be reduced by ~21% following
365 60-minutes of hypoxia, and appears to recover to pre-hypoxic levels following ~6-hours of
366 hypoxic exposure (Lewis *et al.*, 2014). When we accounted for the decline in FMD SR_{AUC} in our
367 covariate analyses, we found the relative decline in FMD with mild hypoxia (-17% to -8%) and
368 moderate hypoxia (-45% to -32%) was attenuated by ~9%. These results suggest the decline in
369 FMD with hypoxia is partly due to a decline in FMD SR_{AUC} . Although the mechanisms
370 influencing the decline in FMD SR_{AUC} with acute hypoxia are unknown, we speculate the
371 possibility that forearm sympathetic constraint following hypoxic (Weisbrod *et al.*, 2001)
372 exposure may have hindered the ischemic response to FMD cuff occlusion, and resulted in a
373 lower reactive hyperemic response on cuff release. Nevertheless, as discussed next, other
374 mechanism(s) also appear to affect the decline in FMD with moderate hypoxia.

375

376 A strength of our study was that we conducted a normoxic time-control trial to rule out any
377 influence of repetitive FMD testing. Had we not have done this control, we would have falsely
378 concluded a major influence of mild hypoxia on FMD. Our data indicates that a repeated
379 assessment of FMD following 30 minutes of supine rest is reduced by 8%. Although not
380 mentioned in the recent FMD guidelines (Thijssen *et al.*, 2011), the original International
381 Brachial Artery Reactivity Task Force (Corretti *et al.*, 2002) states that at least 10 minutes of
382 supine rest is needed after reactive hyperemia before another assessment is conducted. More
383 recently, it was reported that repeated measures of FMD in the brachial artery may be taken after
384 a minimum of 5 minutes or as soon as the vessel has returned to its baseline diameter (Barton *et*
385 *al.*, 2011). In light of these studies, 30 minutes of supine rest between FMD assessment in the
386 current studies should have been conservative recovery period, especially since baseline arterial

387 diameter was unchanged in the time-control study or following mild hypoxia exposure. Arterial
388 diameter was larger following moderate hypoxia exposure; however, this was likely due to the
389 effect of moderate hypoxia and has been accounted for in our interpretations of the data
390 (allometric scaling). In summary, at least in our experimental study with a highly experienced
391 (>1000 FMD tests and established high reducibility) vascular scanner it appears 30 minutes of
392 recovery for repeated FMD assessments is insufficient, and should be considered in future
393 research.

394

395 Given that the relative decline in FMD following mild hypoxia exposure (-8%) was comparable
396 to that seen in the time-control study it is likely that the decline in FMD following acute mild
397 exposure was due to lasting effect of the baseline (normoxic) FMD assessment. Nevertheless,
398 even if we consider the 8% decline in FMD due to the negative impact of repeated measures, a
399 relative decline of 24% in FMD is still present following moderate hypoxia. We have previously
400 (Lewis *et al.*, 2014) documented a ~28% decline in FMD following 60-minutes of normobaric
401 hypoxia ($FIO_2=0.11$; SaO_2 79%), supporting the current findings of an acute impairment in FMD
402 following moderate hypoxia.

403

404 **Alterations in baseline blood flow and oscillatory shear patterns:** Pre-FMD baseline blood
405 flow velocity and blood flow were reduced by ~42% and ~39%, respectively, following hypoxic
406 exposure. Declines in brachial artery blood flow (-11%) and blood flow velocity (-2%) have
407 previously been reported following 10 minutes of moderate hypoxia (FIO_2 0.12) (Iwamoto *et al.*,
408 2015). Given that the decline in blood flow velocity / blood flow was comparably reduced
409 following hypoxic exposure (39-42%) and in our normoxic time control trial (~36-37%), it is

410 possible long lasting effects of forearm ischemia from the baseline FMD may have altered blood
411 velocity hemodynamics and explain this decline in blood flow prior to repeated assessment of
412 FMD. The topics require further investigation as it clearly have important methodological
413 considerations in the design of related vascular function experiments.

414
415 Significant change in baseline SR patterns were evident with hypoxia, with a decrease in
416 antegrade SR (~21%) and an increase in retrograde SR (~48%), and oscillatory shear index
417 (~54%). No significant changes in SR patterns were evident in the normoxic time-control study,
418 therefore, it appears the alteration in SR were an effect of hypoxia. This is supported by others
419 who have reported an increase in retrograde SR (>39%) and oscillatory shear index (>35%)
420 following 10 minutes of hypoxia (FIO₂ 0.12) (Iwamoto *et al.*, 2015; Katayama *et al.*, 2016).
421 Although hypoxia causes net vasodilation (Heistad & Wheeler, 1970), sympathetic excitation
422 within 5-10 minutes of isocapnic hypoxia (SaO₂ 85%) exposure has been shown to mask the
423 vasodilation effects of hypoxia in the resistant vessels of the forearm (Weisbrod *et al.*, 2001;
424 Weisbrod *et al.*, 2004). Additionally, acute excitation and elimination of sympathetic nerve
425 activity on forearm vascular resistant has been shown to increase and reduce retrograde and
426 oscillatory SR patterns respectively, in the brachial artery (Thijssen *et al.*, 2009; Padilla *et al.*,
427 2010; Casey *et al.*, 2012; Padilla *et al.*, 2014). Therefore, it is possible that heightened
428 sympathetic vasoconstrictor activity with acute hypoxia (Dinenno *et al.*, 2003) and subsequently
429 hypoxic vasodilation constraint in the forearm (Weisbrod *et al.*, 2001) could have increased
430 downstream resistance vessel tone, and altered SR blood flow patters.

431

432 The increase in retrograde and oscillatory SR patterns in the current study was significantly
433 larger following exposure to moderate vs. mild hypoxic exposure. Acute and progressive
434 increases in baseline retrograde and oscillatory SR patterns in the brachial artery have been
435 shown to elicit a dose-dependent impairment in brachial FMD (Thijssen *et al.*, 2009).
436 Furthermore, graded reductions in hypoxia have been shown to elicit a graded increase in MSNA
437 (Rowell *et al.*, 1989), and graded increase in MSNA have been was associated with an
438 incremental increase in retrograde and oscillatory SR patterns (Padilla *et al.*, 2010). Therefore, it
439 is possible that a greater increase in SNA with moderate hypoxic exposure possibly explains the
440 larger increase in retrograde and oscillatory SR patterns in this condition and the significant
441 impairment in FMD, this concept warrant future investigation.

442

443 **Impaired vascular smooth muscle and endothelial vasodilation:** The GTN-induced dilation in
444 the current study was reduced by (relative) ~13% and ~22% following 60-minutes of mild and
445 moderate hypoxia, respectively. We have previously reported a decline (relative: ~14%) in GTN-
446 induced dilation following 3-days at 5050m (Lewis *et al.*, 2014); however, as far as we are
447 aware, this is the first report of acute effects of hypoxia on GTN-induced vasodilation. Given
448 that the assessment GTN-induced dilation represents vascular smooth muscle cell sensitivity to
449 NO (Corretti *et al.*, 2002; Maruhashi *et al.*, 2013), the findings of the current study supports the
450 notion of impairment in vascular smooth muscle function following hypoxic exposure (Lewis *et*
451 *al.*, 2014). This reduction in GTN-induced dilation undoubtedly influence the impairment
452 observed in FMD responses, especially following moderate hypoxia. However, currently what
453 level of impairment in smooth muscle function is required to hinder upon FMD measures is
454 currently unknown.

455 The acute decline in FMD following 60-minutes of normobaric hypoxia has previously been
456 shown to be partially reversed following an α 1-adrenoreceptor blockade, suggesting
457 sympathoexcitation is one of the mechanisms by which FMD is impaired following acute
458 hypoxic exposure (Weisbrod *et al.*, 2004; Lewis *et al.*, 2014). Although the effect of hypoxic-
459 induced sympathoexcitation on the acute impairment in GTN-induced dilation has not been
460 reported, it is likely a key mechanism for reductions in FMD (Saito *et al.*, 1988; Rowell *et al.*,
461 1989) i.e., via increasing vascular smooth muscle tone and impairing vascular smooth muscle
462 cell ability to relax in response to NO.

463

464 Previous work has reported a ~20% increase in muscle sympathetic nerve activity (MSNA)
465 following 5-minutes of isocapnic hypoxia ($FIO_2=0.10$; SaO_2 82%) (Somers *et al.*, 1988).
466 Moreover, Rowell *et al.*, (1989) reported an inverse relationship between graded reductions in
467 FIO_2 and elevations in MSNA. For example, after 20-minutes of hypoxia at FIO_2 0.12 and FIO_2
468 0.10, MSNA was elevated by ~90% and 250%, respectively (Rowell *et al.*, 1989). The duration
469 that MSNA remains elevated during an acute hypoxic insult, and its potential effect on GTN-
470 induced dilation and FMD are currently unknown and warrant investigation. Furthermore, since
471 the magnitude of hypoxic-induced elevations in MSNA seems to be dependent on the severity of
472 the hypoxic stimulus, this could also explain why the degree of FMD and GTN-induced dilation
473 impairment were larger following the moderate hypoxic exposure compared to the mild hypoxic
474 exposure in the current study. Future studies combining MSNA measures with and without SNA
475 blockade are needed to clearly test this hypothesis.

476

477 When we corrected our assessments of FMD with the changes in GTN induced dilation with
478 hypoxia, we found that FMD-to-GTN% was decreased by ~ 35% from normoxia moderate
479 hypoxic exposure. The FMD-to-GTN ratio represents global NO-dependent vasodilator function
480 (Spence *et al.*, 2013; Lewis *et al.*, 2014); thus, following 30-minutes of moderate hypoxia it
481 appears the decline in FMD is partly due to endothelial dysfunction in addition to vascular
482 smooth muscle dysfunction.

483

484 **Implications**

485 It has been estimated that a 1% absolute reduction in FMD is associated with a 9% increase in
486 cardiovascular disease risk (Green *et al.*, 2012); thus, 2% absolute decline in FMD with
487 moderate isocapnic hypoxia in the current study is potentially associated with an elevation
488 in cardiovascular disease risk. This may potentially have some health implications for
489 individuals acutely exposed to moderate hypoxia, such as Heli hikes / skiing activities. Acute
490 impairment in smooth muscle dilation may potentially have implications for individuals exposed
491 to mild and moderate hypoxia during air travel. Medical issues during air travel are estimated at
492 about 350 per day worldwide, and currently aircraft carrying passengers are pressurized and
493 maintain a cabin altitude between 1525m to 2438 m (Sohail & Fischer, 2005). One study
494 investigated the change in SpO₂ levels in healthy flight-crew members during 22 scheduled
495 flights, and found mean SpO₂ nadir levels fell from 97% (preflight) to 88.6% at cruising altitude
496 (Cottrell *et al.*, 1995). Therefore, air travel has the potential to exacerbate risk for passengers
497 with underlying cardiovascular conditions, and increase the risk of medical events. Furthermore,
498 although factors in addition to the mild hypoxemia likely also play a role (e.g. diet, shift work,

499 sleep patterns) flight attendants have a 3.5 fold increase risk of developing cardiovascular disease
500 compared to the general public (McNeely *et al.*, 2014).

501

502 **Conclusion**

503 In light of the methodological effects of repetitive FMD testing, there does not appear to be an
504 impairment in endothelial function following mild hypoxia exposure. However, there is
505 significant endothelial impairment following moderate hypoxic exposure and our data indicate
506 that this impairment is potentially influenced by adverse changes in SR patters and increase in
507 oscillatory SR with graded increases in hypoxia. Graded impairments in smooth muscle cell
508 sensitivity to NO is evident following mild and moderate hypoxia, and has important
509 implications for individuals acutely exposed to mild and moderate hypoxia, especially those with
510 cardiovascular risk factors.

511

512

513 **Reference**

514 Atkinson G & Batterham AM (2013). Allometric scaling of diameter change in the original flow-
515 mediated dilation protocol. *Atherosclerosis* 226, 425-427.

516

517 Atkinson G, Batterham AM, Thijssen DH & Green DJ (2013). A new approach to improve the
518 specificity of flow-mediated dilation for indicating endothelial function in cardiovascular
519 research. *J Hypertens* 31, 287-291.

520

- 521 Bailey DM, Rimoldi SF, Rexhaj E, Pratali L, Salinas Salmon C, Villena M, McEneny J, Young
522 IS, Nicod P, Allemann Y, Scherrer U & Sartori C (2013). Oxidative-nitrosative stress and
523 systemic vascular function in highlanders with and without exaggerated hypoxemia.
524 Chest 143, 444-451.
- 525
- 526 Barton M, Turner AT, Newens KJ, Williams CM & Thompson AK (2011). Minimum recovery
527 time between reactive hyperemia stimulus in the repeated measurement of brachial flow-
528 mediated dilatation. Ultrasound Med Biol 37, 879-883.
- 529
- 530 Black MA, Cable NT, Thijssen DH & Green DJ (2008). Importance of measuring the time
531 course of flow-mediated dilatation in humans. Hypertension 51, 203-210.
- 532
- 533 Casey DP, Padilla J & Joyner MJ (2012). alpha-adrenergic vasoconstriction contributes to the
534 age-related increase in conduit artery retrograde and oscillatory shear. Hypertension 60,
535 1016-1022.
- 536
- 537 Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK &
538 Deanfield JE (1992). Non-invasive detection of endothelial dysfunction in children and
539 adults at risk of atherosclerosis. Lancet 340, 1111-1115.
- 540
- 541 Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield
542 J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J & Vogel R (2002).
543 Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated

544 vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity
545 Task Force. *J Am Coll Cardiol* 39, 257-265.

546
547 Cottrell JJ, Lebovitz BL, Fennell RG & Kohn GM (1995). Inflight arterial saturation: continuous
548 monitoring by pulse oximetry. *Aviat Space Environ Med* 66, 126-130.

549
550 Dinunno FA, Joyner MJ & Halliwill JR (2003). Failure of systemic hypoxia to blunt alpha-
551 adrenergic vasoconstriction in the human forearm. *J Physiol* 549, 985-994.

552
553 Frick M, Rinner A, Mair J, Alber HF, Mittermayr M, Pachinger O, Humpeler E, Schobersberger
554 W & Weidinger F (2006). Transient impairment of flow-mediated vasodilation in patients
555 with metabolic syndrome at moderate altitude (1,700 m). *Int J Cardiol* 109, 82-87.

556
557 Frobert O, Holmager P, Jensen KM, Schmidt EB & Simonsen U (2008). Effect of acute changes
558 in oxygen tension on flow-mediated dilation. Relation to cardiovascular risk. *Scand*
559 *Cardiovasc J* 42, 38-47.

560
561 Green DJ, Jones H, Thijssen D, Cable NT & Atkinson G (2012). Flow-mediated dilation and
562 cardiovascular event prediction: does nitric oxide matter? *Hypertension* 57, 363-369.

563
564 Heistad DD & Wheeler RC (1970). Effect of acute hypoxia on vascular responsiveness in man. I.
565 Responsiveness to lower body negative pressure and ice on the forehead. II. Responses to

- 566 norepinephrine and angiotensin. 3. Effect of hypoxia and hypocapnia. *J Clin Invest* 49,
567 1252-1265.
- 568
- 569 Iwamoto E, Katayama K & Ishida K (2015). Exercise intensity modulates brachial artery
570 retrograde blood flow and shear rate during leg cycling in hypoxia. *Physiol Rep* 3.
571
- 572 Katayama K, Yamashita S, Iwamoto E & Ishida K (2016). Flow-mediated dilation in the inactive
573 limb following acute hypoxic exercise. *Clin Physiol Funct Imaging* 36, 60-69.
574
- 575 Lewis NC, Bailey DM, Dumanoir GR, Messinger L, Lucas SJ, Cotter JD, Donnelly J, McEneny
576 J, Young IS, Stembridge M, Burgess KR, Basnet AS & Ainslie PN (2014). Conduit
577 artery structure and function in lowlanders and native highlanders: relationships with
578 oxidative stress and role of sympathoexcitation. *J Physiol* 592, 1009-1024.
579
- 580 Maruhashi T, Soga J, Fujimura N, Idei N, Mikami S, Iwamoto Y, Kajikawa M, Matsumoto T,
581 Hidaka T, Kihara Y, Chayama K, Noma K, Nakashima A, Goto C & Higashi Y (2013).
582 Nitroglycerine-induced vasodilation for assessment of vascular function: a comparison
583 with flow-mediated vasodilation. *Arterioscler Thromb Vasc Biol* 33, 1401-1408.
584
- 585 McNeely E, Gale S, Tager I, Kincl L, Bradley J, Coull B & Hecker S (2014). The self-reported
586 health of U.S. flight attendants compared to the general population. *Environ Health* 13,
587 13.
588

589 Padilla J, Jenkins NT, Laughlin MH & Fadel PJ (2014). Blood pressure regulation VIII:
590 resistance vessel tone and implications for a pro-atherogenic conduit artery endothelial
591 cell phenotype. *Eur J Appl Physiol* 114, 531-544.

592

593 Padilla J, Young CN, Simmons GH, Deo SH, Newcomer SC, Sullivan JP, Laughlin MH & Fadel
594 PJ (2010). Increased muscle sympathetic nerve activity acutely alters conduit artery shear
595 rate patterns. *Am J Physiol Heart Circ Physiol* 298, H1128-1135.

596

597 Pyke KE, Dwyer EM & Tschakovsky ME (2004). Impact of controlling shear rate on flow-
598 mediated dilation responses in the brachial artery of humans. *J Appl Physiol* (1985) 97,
599 499-508.

600

601 Pyke KE & Tschakovsky ME (2007). Peak vs. total reactive hyperemia: which determines the
602 magnitude of flow-mediated dilation? *J Appl Physiol* 102, 1510-1519.

603

604 Rowell LB, Johnson DG, Chase PB, Comess KA & Seals DR (1989). Hypoxemia raises muscle
605 sympathetic activity but not norepinephrine in resting humans. *J Appl Physiol* (1985) 66,
606 1736-1743.

607

608 Saito M, Mano T, Iwase S, Koga K, Abe H & Yamazaki Y (1988). Responses in muscle
609 sympathetic activity to acute hypoxia in humans. *J Appl Physiol* (1985) 65, 1548-1552.

610

- 611 Smith TG, Talbot NP, Chang RW, Wilkinson E, Nickol AH, Newman DG, Robbins PA &
612 Dorrington KL (2012). Pulmonary artery pressure increases during commercial air travel
613 in healthy passengers. *Aviat Space Environ Med* 83, 673-676.
614
- 615 Sohail MR & Fischer PR (2005). Health risks to air travelers. *Infect Dis Clin North Am* 19, 67-
616 84.
617
- 618 Somers VK, Mark AL & Abboud FM (1988). Potentiation of sympathetic nerve responses to
619 hypoxia in borderline hypertensive subjects. *Hypertension* 11, 608-612.
620
- 621 Spence AL, Carter HH, Naylor LH & Green DJ (2013). A prospective randomized longitudinal
622 study involving 6 months of endurance or resistance exercise. Conduit artery adaptation
623 in humans. *J Physiol* 591, 1265-1275.
624
- 625 Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, Parker B, Widlansky ME,
626 Tschakovsky ME & Green DJ (2011). Assessment of flow-mediated dilation in humans:
627 a methodological and physiological guideline. *Am J Physiol Heart Circ Physiol* 300, H2-
628 12.
629
- 630 Thijssen DH, Dawson EA, Tinken TM, Cable NT & Green DJ (2009). Retrograde flow and
631 shear rate acutely impair endothelial function in humans. *Hypertension* 53, 986-992.
632

633 Tymko MM, Ainslie PN, MacLeod DB, Willie CK & Foster GE (2015). End tidal-to-arterial
634 CO₂ and O₂ gas gradients at low- and high-altitude during dynamic end-tidal forcing.
635 Am J Physiol Regul Integr Comp Physiol 308, R895-906.

636
637 Weisbrod CJ, Eastwood PR, O'Driscoll G, Walsh JH, Best M, Halliwill JR & Green DJ (2004).
638 Vasomotor responses to hypoxia in type 2 diabetes. Diabetes 53, 2073-2078.

639
640 Weisbrod CJ, Minson CT, Joyner MJ & Halliwill JR (2001). Effects of regional phentolamine on
641 hypoxic vasodilatation in healthy humans. J Physiol 537, 613-621.

642
643 Woodman RJ, Playford DA, Watts GF, Cheetham C, Reed C, Taylor RR, Puddey IB, Beilin LJ,
644 Burke V, Mori TA & Green D (2001). Improved analysis of brachial artery ultrasound
645 using a novel edge-detection software system. J Appl Physiol 91, 929-937.

646

647 **Additionally Information**

648 **Author Contribution:** 1) Conceived and designed research; 2) Performed experiments,
649 Analyzed data, Interpreted results of experiment; 3) Drafted manuscript, Edited and revised
650 manuscript for important intellectual content; 4) Approved final version of Manuscript. 5)
651 Agreed to be accountable for all aspects of the work. 6) Qualify for authorship.

652
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655

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661

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666 **Figure Captions**

667

668 **Figure 1:** Effect of normoxia (baseline) and acute isocapnic hypoxia ($P_{ET}O_2$ 75 mmHg and
669 $P_{ET}O_2$ 50 mmHg) on pre-FMD baseline shear rate (SR) patterns and oscillatory shear index. *
670 Significant main effect for intervention (normoxia vs hypoxia), $P=0.01$. † Significant interaction
671 between intervention and condition, $P=0.04$, the increase from normoxic baseline in retrograde
672 SR and oscillatory shear index with hypoxia was greater in the $P_{ET}O_2$ 50 mmHg trial compared
673 to $P_{ET}O_2$ 75 mmHg.

674

675 **Figure 2:** The effect of normoxia and acute isocapnic hypoxia ($P_{ET}O_2$ 75 mmHg and $P_{ET}O_2$ 50
676 mmHg) on FMD. A) Uncorrected and B) corrected for significant changes in baseline arterial
677 diameter and shear rate area under the curve. * Significant main effect for intervention

678 (normoxia vs hypoxia), $P < 0.01$. † Significant main effect for condition ($P_{ET}O_2$ 75 mmHg vs
679 $P_{ET}O_2$ 50 mmHg), $P < 0.01$. ‡ Significant interaction between intervention and condition, $P < 0.01$.

680

681 **Figure 3:** The effect of normoxia and acute isocapnic hypoxia ($P_{ET}O_2$ 75 mmHg and $P_{ET}O_2$ 50
682 mmHg) on GTN dilation; A) uncorrected and B) corrected for significant changes in pre-GTN
683 arterial diameter. * Significant main effect for intervention (normoxia vs hypoxia), $P = 0.05$. †
684 Significant main effect for condition ($P_{ET}O_2$ 75 mmHg vs $P_{ET}O_2$ 50 mmHg), $P < 0.01$. ‡
685 Significant interaction between intervention and condition, $P = 0.01$.

686

687 **Figure 4:** Mean and SD uncorrected and corrected (for baseline arterial diameter and shear rate
688 area under the curve). * (paired t-test) † (linear mix model) Post 30-min significantly different
689 from baseline; $P \leq 0.05$.

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705 **Tables**

706

707 **Table 1: Effect of normoxia and acute isocapnic hypoxia (P_{ET}O₂ 75 mmHg and P_{ET}O₂ 50**
708 **mmHg) on cardiorespiratory variables.**

709

Experimental Condition	P _{ET} O ₂ 75 mmHg		P _{ET} O ₂ 50 mmHg		
Experimental Intervention	Normoxia	Hypoxia	Normoxia	Hypoxia	
Ventilation (L·min)	12.4 ± 2.7	15.1 ± 3.8	12.8 ± 2.1	22.3 ± 6.7	* † ‡
P _{ET} O ₂ (mmHg)	92.0 ± 4.7	74.0 ± 1.6	92.6 ± 5.8	50.0 ± 2.8	* † ‡
P _{ET} CO ₂ (mmHg)	40.8 ± 2.2	40.9 ± 2.0	41.3 ± 2.4	41.0 ± 2.4	
MAP (mmHg)	85 ± 14	93 ± 16	85 ± 13	90 ± 15	†
SBP (mmHg)	106 ± 22	108 ± 27	107 ± 27	117 ± 25	†
DBP (mmHg)	64 ± 8	69 ± 8	64 ± 9	65 ± 11	†
HR (beats·min ⁻¹)	58 ± 13	57 ± 12	64 ± 13	64 ± 11	* † ‡
SV (ml)	103 ± 27	106 ± 30	107 ± 21	106 ± 23	
CO (L·min)	5.5 ± 1.1	6.0 ± 1.3	5.6 ± 1.1	6.7 ± 1.4	† ‡
SaO ₂ (%)	97 ± 1	93 ± 2	97 ± 1	83 ± 3	* † ‡

710

711 Values expressed as mean ± SD: End-tidal oxygen (P_{ET}O₂), end-tidal carbon dioxide (P_{ET}CO₂),
712 mean arterial blood pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure
713 (DBP), heart rate (HR), stroke volume (SV, n=10), cardiac output (CO, n=10), oxygen saturation
714 (SaO₂). * Significant main effect for intervention (normoxia vs hypoxia), P≤0.04. † Significant
715 main effect for condition (P_{ET}O₂ 75 mmHg vs. P_{ET}O₂ 50 mmHg), P≤0.04. ‡ Significant
716 interaction between intervention and condition, P≤0.02.

Table 2: Effect of normoxia (baseline) and acute isocapnic hypoxia on FMD related variables.

Experimental Condition	$P_{ET}O_2$ 75 mmHg		$P_{ET}O_2$ 50 mmHg		
Experimental Intervention	Normoxia	Hypoxia	Normoxia	Hypoxia	
FMD					
Baseline diameter (mm)	3.86 ± 0.73	3.85 ± 0.73	3.94 ± 0.70	4.12 ± 0.77	†‡
Baseline peak blood flow velocity (cm·s ⁻¹)	8.4 ± 4.4	5.5 ± 2.8	8.6 ± 4.9	4.4 ± 2.6	*
Baseline peak blood flow (ml·min)	66.2 ± 52.9	43.5 ± 32.5	70.7 ± 57.1	40.5 ± 34.4	*
Peak diameter (mm)	4.12 ± 0.73	4.07 ± 0.75	4.21 ± 0.71	4.25 ± 0.78	
Time to peak diameter (s)	62 ± 27	46 ± 13	57 ± 27	56 ± 31	
SR _{AUC} (AUC)	26546 ± 10249	20199 ± 9886	25163 ± 11096	18683 ± 11947	*

Values expressed as mean ± SD: Shear rate area under the curve (SR_{AUC}); Flow mediated dilation (FMD). * Significant main effect for intervention (normoxia vs hypoxia), P<0.01. † Significant main effect for hypoxic condition (75 mm Hg vs. 50 mm Hg), P=0.01. ‡ Significant interaction between intervention and condition, P<0.001.

Table 3: Effect of normoxia and acute isocapnic hypoxia on GTN related variables

Experimental Condition	Normoxia	P _{ET} O ₂ 75 mmHg	P _{ET} O ₂ 50 mmHg	
GTN				
Baseline diameter (mm)	3.95 ± 0.69	3.99 ± 0.77	4.13 ± 0.72	*
Peak diameter (mm)	4.61 ± 0.73	4.54 ± 0.77	4.63 ± 0.72	
Time to peak diameter (s)	450 ± 95	472 ± 82	453 ± 62	
FMD:GTN ratio	0.45 ± 0.20	0.40 ± 0.18	0.29 ± 0.17	*

Values expressed as mean ± SD: Shear rate area under the curve (SR_{AUC}); Flow mediated dilation (FMD); Endothelium-independent FMD (GTN). * Significant main effect for intervention (normoxia vs hypoxia), P<0.0

Table 4: FMD related variables prior to and following 30-minutes of supine normoxic rest.

	Baseline	Post 30-min	
Baseline diameter (mm)	4.46 ± 0.42	4.39 ± 0.39	
Baseline blood flow velocity (cm·s ⁻¹)	16.0 ± 7.1	10.2 ± 5.9	*
Baseline peak blood flow (ml·min)	162 ± 106	96 ± 70	*
Baseline Mean SR (s)	111 ± 49	74 ± 42	†
Baseline Anterograde SR (s)	120 ± 46	87 ± 38	
Baseline Retrograde SR (s)	-8 ± 8	-13 ± 13	
Baseline Oscillatory SR Index	0.08 ± 0.09	0.13 ± 0.12	
Peak diameter (cm)	4.79 ± 0.35	4.69 ± 0.33	*
Time to peak diameter (s)	61 ± 22	64 ± 30	
SR _{AUC} (AUC)	30948 ± 10303	27768 ± 11846	

Values expressed as mean ± SD: Shear rate area under the curve (SR_{AUC}); Flow mediated dilation (FMD); Shear rate (SR). * †(Wilcoxon test) Post 30-min significantly different from baseline; P<0.03.

Figure 1

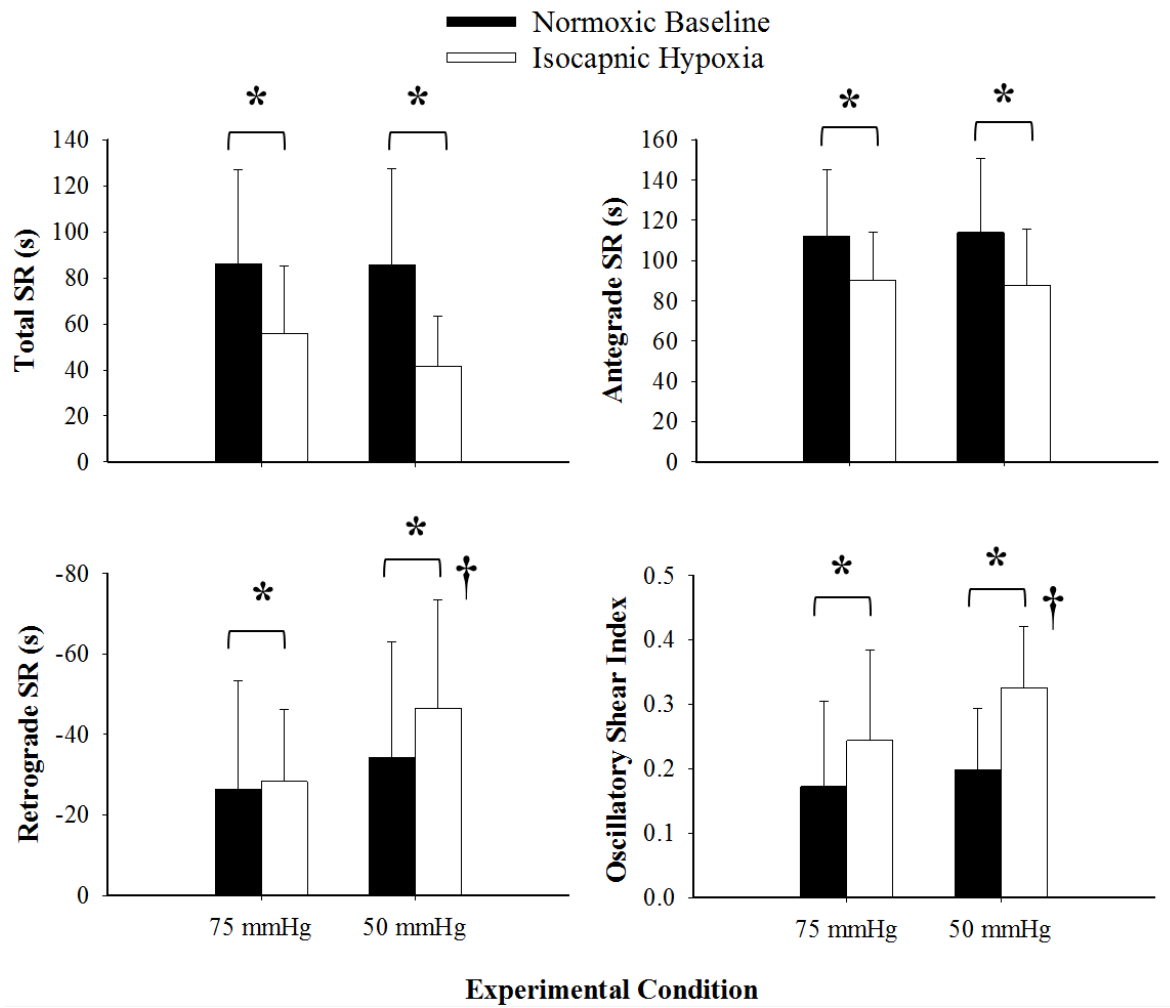


Figure 2

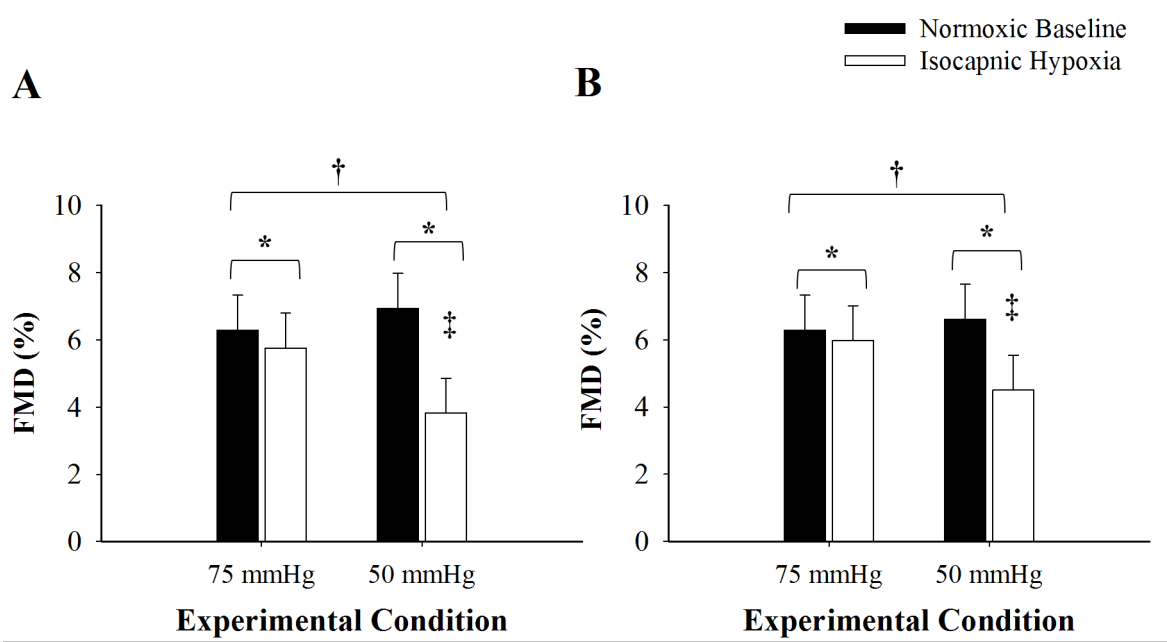


Figure 3

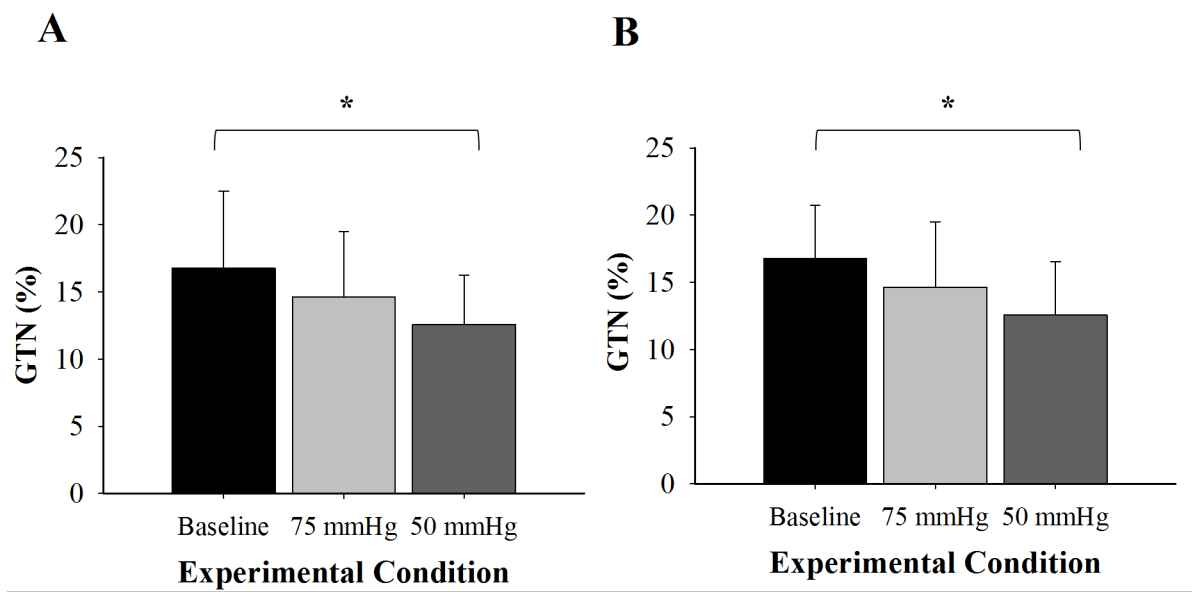


Figure 4

