

Carotid chemoreceptor control of muscle sympathetic nerve activity in hypobaric hypoxia

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3 **Carotid chemoreceptor control of muscle sympathetic nerve activity in**
4 **hypobaric hypoxia**

5
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31 **NEW FINDINGS**

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

33 High altitude hypoxia increases muscle sympathetic nerve activity (MSNA), but whether
34 intravenous infusion of dopamine, to blunt the responsiveness of the carotid chemoreceptors,
35 reduces MSNA at high altitude is not known.

36

37 **What is the main finding and its importance?**

38 MSNA was elevated after 15-17 days of high altitude hypoxia (3,454 m) compared to sea level
39 (432 m) values. However, intravenous dopamine infusion to blunt the responsiveness of the
40 carotid chemoreceptors did not significantly decrease MSNA either at sea level or high altitude,
41 suggesting that high altitude sympathoexcitation arises via a different mechanism.

42 **ABSTRACT**

43 High altitude hypoxia causes pronounced sympathoexcitation but the underlying
44 mechanisms remain unclear. We tested the hypothesis that intravenous infusion of dopamine to
45 attenuate carotid chemoreceptor responsiveness would reduce muscle sympathetic nerve activity
46 (MSNA) at high altitude. Nine healthy individuals (mean [SD]; 26 [4] yr) were studied at sea
47 level (SL, Zurich) and at high altitude (ALT, 3454 m, 15-17 days after arrival), both while
48 breathing the ambient air and during an acute incremental hypoxia test (8 x 3 min stages, $P_{ET}O_2$
49 90-45 mmHg). Intravenous infusion of dopamine ($3 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) and placebo (saline) were
50 administered on both study days, according to a single blind randomized cross-over design.
51 Sojourn to high altitude decreased $P_{ET}O_2$ (to ≈ 60 mmHg) and increased minute ventilation (V_E ;
52 mean \pm SE; saline [SL, ALT], 8.6 ± 0.5 to 11.3 ± 0.6 ; dopamine, 8.2 ± 0.5 to 10.6 ± 0.8 $\text{L}\cdot\text{min}^{-1}$;
53 $P<0.05$) and MSNA burst frequency by $\approx 80\%$ (saline [SL, ALT], 16 ± 3 to 28 ± 4 ; dopamine, 16 ± 4
54 to 31 ± 4 bursts $\cdot\text{min}^{-1}$; $P<0.05$) when breathing the ambient air, but were not different with
55 dopamine. Increases in MSNA burst frequency and V_E during the acute incremental hypoxia test
56 were greater at ALT than SL ($P<0.05$). Dopamine did not affect the magnitude of the MSNA
57 burst frequency response to acute incremental hypoxia at either SL or ALT. However, V_E was
58 lower with dopamine than saline administration throughout the acute incremental hypoxia test at
59 ALT. These data indicate that intravenous infusion of low-dose dopamine to blunt the
60 responsiveness of the carotid chemoreceptors does not significantly decrease MSNA at high
61 altitude.

62

63 **Keywords:** autonomic nervous system, high altitude, microneurography

64

65

66 INTRODUCTION

67 Hypoxia increases the afferent discharge of the carotid chemoreceptors causing reflex
68 increases in ventilatory drive and efferent sympathetic nerve activity directed towards the heart,
69 kidneys and peripheral vasculature (Guyenet, 2000; Kumar & Prabhakar, 2012). In humans, the
70 use of the microneurography technique to directly record sympathetic nerve activity to skeletal
71 muscle vasculature (MSNA) reveals that acute hypoxic exposure elicits variable but typically
72 dose dependent sympathoexcitation once SpO₂ reaches <85% (breathing hypoxic gas mixtures
73 with an of F_iO₂ 0.11-0.13%) (Saito *et al.*, 1988; Rowell *et al.*, 1989; Somers *et al.*, 1989; Seals *et*
74 *al.*, 1991; Duplain *et al.*, 1999). However, such increases in MSNA are dwarfed by those elicited
75 by chronic hypoxic exposure which can reach ≈300% above sea level values, despite reductions
76 in SpO₂ being equivalent (Hansen & Sander, 2003). The mechanism for this difference is
77 unclear, which is unfortunate because similar mechanisms may be important for the
78 pathophysiology of a variety of disease states characterized by chronic sympathoexcitation and
79 chronic intermittent or sustained hypoxaemia (e.g., sleep apnoea related hypertension (Carlson *et*
80 *al.*, 1993; Narkiewicz & Somers, 1999), chronic obstructive pulmonary disease (Heindl *et al.*,
81 2001) and chronic heart failure (Leimbach *et al.*, 1986; Narkiewicz *et al.*, 1999)).

82 Following acclimatization to high altitude there is an augmentation of the ventilatory
83 response to hypoxia that has been ascribed to a sensitization of peripheral chemoreceptors
84 (Forster *et al.*, 1971). Ventilatory and sympathetic chemoreflexes share common afferent
85 pathways and the central neurocircuitry responsible for the efferent activation of the phrenic and
86 sympathetic nerves act in parallel (Guyenet, 2000; Kumar & Prabhakar, 2012). For example,
87 denervation of the carotid body markedly reduces the increases in ventilation and renal
88 sympathetic nerve activity induced by hypoxia in rabbits with pacing-induced congestive heart
89 failure (Marcus *et al.*, 2014). However, it has been suggested that a peripheral chemoreceptor

90 mechanism only modestly contributes to increase in MSNA accompanying chronic exposure to
91 high-altitude hypoxia. Indeed, Hansen and Sander (2003) observed that 100% oxygen breathing
92 following 4 weeks at 5,260 m slightly reduced MSNA (by 7 bursts·min⁻¹), but it still remained
93 robustly elevated (41 bursts·min⁻¹) compared with sea level values (16 bursts·min⁻¹). As
94 acknowledged by the investigators, oxygen administration may have led to a fall in ventilation
95 and an increase in arterial CO₂, which in turn could attenuated the sympathoinhibitory effects of
96 pulmonary stretch reflex engagement and increase central chemoreflex activation. Hyperoxia
97 also has non-specific effects and can cause peripheral vasoconstriction in some individuals
98 (Crawford *et al.*, 1997). Taken together these factors suggest that the contribution of the
99 peripheral chemoreceptors to the control of MSNA in hypoxia warrants further consideration.

100 Chemoreceptor signalling within the carotid and aortic bodies involves a plethora of
101 excitatory (e.g., adenosine, ATP, acetylcholine and endothelin) and inhibitory neurotransmitters
102 (Lazarov *et al.*, 2009). Dopamine is one of these primary signalling molecules and has an
103 inhibitory effect on high-affinity D₂ autoreceptors (D₂R) located on Type 1 glomus cells
104 (Gonzalez *et al.*, 1994). Intracarotid infusion of dopamine inhibits chemoreceptor afferent
105 activity in dogs (Bisgard *et al.*, 1979), while systemic administration of low-dose dopamine (i.e.,
106 <3 µg·kg⁻¹·min⁻¹) is an established method of acutely reducing the responsiveness of the carotid
107 chemoreceptors in humans (Boetger & Ward, 1986; Dahan *et al.*, 1996; Limberg *et al.*, 2016).
108 One study suggests that the suppressive effects of dopamine on the hypoxic ventilatory response
109 are unaltered after individuals have been exposed to isocapnic hypoxia for 8 h (Pedersen *et al.*,
110 1999). However, ventilatory acclimatization is not complete in humans after 8 h (Dempsey &
111 Forster, 1982) and the effect of low-dose dopamine on the ventilatory response to acute hypoxia
112 following more prolonged high altitude exposure in humans remains unexamined.

113 The purpose of the present study was to determine whether elevations in steady-state
114 MSNA and ventilation are reduced following 15-17 days of exposure to high altitude hypoxia
115 (3,454 m) (i.e., ambient air breathing) by intravenous infusion of low-dose dopamine (*Aim 1*).
116 We also determined whether the MSNA and ventilatory responses to an acutely administered
117 incremental hypoxia test were attenuated following intravenous dopamine infusion (*Aim 2*) and
118 whether the magnitude of any such inhibitory effect was altered following 15-17 days of
119 exposure to high altitude hypoxia (*Aim 3*). We tested the hypothesis that intravenous dopamine
120 would reduce MSNA and ventilation both at high altitude with ambient air breathing and during
121 an acute incremental hypoxia test, and that the inhibitory effects of dopamine during the
122 incremental hypoxia test would be augmented at high altitude.

123

124

125

126 **METHODS**

127 *Ethical Approval.*

128 The experiments were undertaken in accordance with the Declaration of Helsinki, except
129 for registration in a database, and were approved by the Ethical Committee of the Swiss Federal
130 Institute of Technology Zurich (EK 2011-N-51). Written informed consent to take part was
131 obtained from all participants after they had received a detailed verbal and written explanation of
132 the study procedures.

133

134 *Participant characteristics.*

135 Nine healthy individuals (mean (SD); 26 (4) yr, 179 (9) cm, 75 (10) kg, 1 woman)
136 participated in this study. No participant had a medical history of cardiovascular, respiratory or
137 neurological disease and no participant slept >2,500 m in the 3 months prior to the start of the
138 study. Abstinence from caffeine, alcohol and exercise was requested for the 12 h before
139 experimental sessions.

140

141 *Experimental measures.*

142 Participants rested in semi-recumbent position while continuous recordings of MSNA,
143 respiratory and cardiovascular variables were made. Heart rate (HR) was monitored using a lead
144 II electrocardiogram (ECG, BioAmp, ADInstruments, Bella Vista, Australia). Mean arterial
145 pressure (MAP) and stroke volume (SV) were recorded on a beat-to-beat basis via finger
146 photoplethysmography (Nexfin, BMEYE B.V, Amsterdam, the Netherlands)(Bogert *et al.*,
147 2010). Peripheral capillary oxygen saturation (SpO₂) was determined using finger pulse
148 oximetry. However, due to technical issues data steady-state SpO₂ data are presented for n=6

149 participants and acute incremental hypoxia test SpO₂ data are presented for n=7 participants.
150 Participants breathed through a mouthpiece whilst wearing a nose clip and minute ventilation
151 (V_E), tidal volume (T_V), respiratory frequency (R_f), and the partial pressure of end-tidal oxygen
152 (P_{ET}O₂) and carbon dioxide (P_{ET}CO₂) were measured breath-by-breath (Cosmed Quark b2,
153 Rome, Italy). Multi-unit recordings of MSNA were obtained (FE185 NeuroAmp EX,
154 ADInstruments, Bella Vista, Australia) from the peroneal nerve using tungsten microelectrodes
155 (FHC, Bowdoin, USA) (Adlan *et al.*, 2017). A reference electrode was inserted subcutaneously 2
156 to 3 cm away from the recording electrode which was selectively inserted into a sympathetic
157 nerve fascicle. Neural signals were amplified (x100k), filtered (100 Hz high pass, 2,000 Hz low
158 pass), rectified and integrated (absolute value, time constant decay 0.1 s) to obtain a mean
159 voltage sympathetic neurogram. An acceptable MSNA recording exhibited the following
160 characteristics: displayed a pulse-synchronous bursts pattern, had a signal-to-noise ratio of >3:1,
161 was increased during an end-expiratory breath-hold or Valsalva manoeuvre, and was
162 unresponsive to an unexpected loud noise or skin stroking.

163

164 *Experimental protocol.*

165 Each individual participated in two experimental sessions, the first was conducted in
166 Zurich, Switzerland (SL, 432 m) and the other at the high altitude Jungfrauoch research station
167 (ALT, 3,454 m), 15-17 days after arrival. Participants were familiarized with the study
168 procedures before collection of study data. At both research sites, following instrumentation and
169 acquisition of an acceptable MSNA signal the stability of the recording was verified for ≈10
170 mins. The experimental protocol then commenced with the collection of 5 min of eupnoea
171 baseline data (SL-baseline, ALT-baseline) (i.e., ambient air breathing). The SL-baseline was then

172 followed by the addition of supplemental CO₂ to the inspired air in order to raise P_{ET}CO₂ by 2
173 mmHg (Altitrainer, SMTEC, Nyon, Switzerland). A 3-min period was permitted to allow a new
174 steady-state to be established (stage 1) following which the incremental hypoxia test commenced
175 (stages 2-8). First, P_{ET}O₂ was reduced to 75 mmHg for 3 min, and then incrementally reduced by
176 a further 5 mmHg every 3 min until it reached 45 mmHg, while P_{ET}CO₂ remained clamped at +2
177 mmHg throughout, following the modified methods of Mou *et al.* (1995) (Altitrainer, SMTEC,
178 Nyon, Switzerland). At high-altitude, the ALT-baseline was followed by the addition of
179 supplemental CO₂ and O₂ to the inspired air to raise P_{ET}O₂ and P_{ET}CO₂ to the SL-baseline levels
180 (stage 1). A 3-min period was permitted to allow a new steady-state to be established following
181 which the incremental hypoxia test (stages 2-8) was repeated using the P_{ET}O₂ and P_{ET}CO₂ levels
182 observed at SL as a target.

183 Both at SL and high altitude the protocols described above were repeated during the
184 continuous infusion of dopamine into the antebrachial vein at a rate of 3 µg·kg⁻¹·min⁻¹ in
185 accordance with several previous studies in humans (Boetger & Ward, 1986; Dahan *et al.*, 1996;
186 Limberg *et al.*, 2016). Dopamine infusion was commenced a minimum of 10 minutes prior to
187 any data collection. Termination criteria for dopamine infusions were: signs of poor perfusion
188 (cyanosis or pallor), technical difficulties in monitoring ECG or systolic blood pressure, subject's
189 desire to stop, ST elevation (≥ 1.0 mm, in leads other than V1 or aVR), sustained ventricular
190 tachycardia, arrhythmias other than sustained ventricular tachycardia (including multifocal
191 premature ventricular complexes, triplets of premature ventricular complexes, supraventricular
192 tachycardia, heart block, or bradyarrhythmias), chest pain, systolic blood pressure > 250 mmHg.
193 Termination criteria were not met on any occasion.

194

195 *Data analysis.*

196 Data was acquired using the Powerlab 16/35 data acquisition system and Labchart Pro
197 software (ADInstruments, Bella Vista, Australia). ECG, MAP, SV, and SpO₂ were sampled at
198 1,000 Hz and raw MSNA was sampled at 20,000 Hz and stored for offline analysis (LabChart 7
199 Pro v7.3.5 and Powerlab, ADInstruments, Bella Vista, NSW, Australia). Cardiac output (CO)
200 was calculated as SV x HR, and total peripheral resistance (TPR) as MAP / CO. Sympathetic
201 bursts were identified by a single observer (JPF) using a semi-automated scoring system created
202 using Spike 2 (Cambridge Electronic Design, Cambridge, UK). MSNA was characterised in
203 terms of burst incidence (bursts·100 heartbeats⁻¹) and burst frequency (bursts·min⁻¹). In one
204 individual microneurography was unsuccessful, and in another individual the MSNA recording
205 was lost during the final stages of the acute incremental hypoxia test. As a consequence, the
206 steady-state MSNA data are presented for n=8 participants and acute incremental hypoxia test
207 MSNA data are presented for n=7 participants.

208

209 *Statistics.*

210 Statistical analysis was performed using SPSS software, version 19 (SPSS Inc, Chicago,
211 Illinois). Physiological data were statistically analyzed using repeated measures analysis of
212 variance (ANOVA), with Greenhouse-Geisser corrections applied where significant violations of
213 the sphericity assumption were detected. More specifically, to determine whether dopamine
214 lowers steady-state MSNA and ventilation at high altitude (SL-baseline vs. ALT-baseline; Aim
215 1) a two-way repeated measures ANOVA was used, in which the factors were altitude (SL vs.
216 ALT) and infusion (saline vs. dopamine), as well as the interaction between them. To determine
217 whether dopamine lowers MSNA and ventilation during an acutely administered hypoxic test

218 (Aim 2), and whether the magnitude of this inhibitory test is augmented at high altitude (Aim 3),
219 this model was extended to a three-way repeated measures ANOVA, additionally including the
220 incremental hypoxia test stage (stages 1-8), as well as all two- and three-way interactions. Where
221 the three-way interaction (altitude x infusion x stage) was not found to be significant, the
222 approach was simplified by dividing the analysis into separate models for each altitude, each
223 containing the infusion, hypoxia test stage and an interaction as factors. Post hoc analysis was
224 employed using Student's t tests with Bonferroni correction to investigate significant main
225 effects and interactions. Data expressed as mean (standard deviation) unless otherwise stated.
226 $P < 0.05$ was considered statistically significant.

227 **RESULTS**228 *High altitude hypoxia, ventilation and MSNA with ambient air breathing.*

229 Sojourn to high altitude decreased $P_{ET}O_2$ (saline [SL, ALT], 93 (2) to 60 (4); dopamine
230 [SL, ALT], 90 (5) to 57 (2) mmHg. $P<0.001$), $P_{ET}CO_2$ (saline [SL, ALT], 40 (2) to 31 (1);
231 dopamine [SL, ALT], 41 (3) to 32 (2) mmHg. $P<0.001$) and SpO_2 (saline [SL, ALT], 97 (1) to 92
232 (2); dopamine [SL, ALT], 97 (1) to 89 (2) %. $P<0.001$) and increased V_E (by $\approx 2.5 \text{ L}\cdot\text{min}^{-1}$,
233 $P<0.002$. Figure 1.) With dopamine, $P_{ET}O_2$ was slightly lower ($P=0.023$) and $P_{ET}CO_2$ slightly
234 higher ($P=0.003$) compared to saline, but no altitude x infusion interaction was observed. SpO_2
235 was not different with dopamine at SL ($P=0.789$), whereas it was lower with dopamine at ALT
236 ($P=0.028$). V_E was not different with dopamine ($P=0.186$), and no altitude x infusion interaction
237 was noted for any respiratory variable (Figure 1).

238 ALT increased MSNA burst frequency (by $\approx 80\%$, $P=0.019$), MAP (by $\approx 12\%$, $P=0.002$)
239 and HR, while MSNA burst incidence (saline [SL, ALT], 25 ± 16) to 38 ± 11); dopamine [SL,
240 ALT], 26 (21) to 40 (12) bursts $\cdot 100$ heartbeats $^{-1}$. $P=0.088$) tended to increase (Figures 1 and 2).
241 However, CO ($P<0.646$), SV and TPR ($P<0.100$), were not different at ALT (Figure 3).
242 Dopamine infusion increased HR ($P=0.001$) and CO ($P<0.001$), decreased TPR ($P=0.035$), but
243 had no effect on MSNA burst frequency ($P=0.289$), MSNA burst incidence ($P=0.555$), MAP
244 ($P=0.837$) and SV ($P=0.119$). No altitude x infusion interaction was noted for any MSNA or
245 cardiorespiratory variable.

246

247 *Acute incremental hypoxia at SL and ALT: ventilation and MSNA.*

248 During the acute incremental hypoxia test, $P_{ET}O_2$ and SpO_2 were decreased ($P<0.001$) in
249 the same stepwise manner under all conditions (Table 1, 2 and 3). At SL, $P_{ET}CO_2$ remained

250 stable throughout the incremental hypoxia test ($P=0.177$) and there were no differences between
251 the saline and dopamine conditions ($P=0.523$). $P_{ET}CO_2$ was ≈ 3 mmHg lower ($P<0.001$) at ALT
252 than at SL during the test, and although no differences were observed between the saline and
253 dopamine conditions ($P=0.177$), $P_{ET}CO_2$ fell during stages 3 and 4 ($P<0.05$ vs. stage 1).

254 V_E , T_V , and R_f increased ($P<0.001$) with acute incremental hypoxia at both SL and ALT,
255 but the magnitude of this increase was greater at altitude ($P<0.001$. Figure 4, Tables 2 and 3). At
256 SL, dopamine did not affect the increase in V_E ($P=0.298$), T_V ($P=0.120$), and R_f ($P=0.922$) with
257 incremental hypoxia, however at ALT V_E ($P=0.023$), T_V ($P=0.047$), and R_f ($P=0.050$) were lower
258 with dopamine. For V_E , T_V , and R_f , no interactions were noted between infusion and incremental
259 hypoxia test stage for either the SL or ALT conditions.

260 MSNA burst frequency increased similarly during the acute incremental hypoxia test at
261 SL ($P=0.028$) and ALT ($P=0.023$) (Figures 5 and 6, Tables 2 and 3). MSNA burst frequency was
262 higher during the incremental hypoxia test with dopamine at both SL ($P=0.051$) and ALT
263 ($P=0.015$). MAP and CO increased progressively during the incremental hypoxia test at both SL
264 and ALT ($P<0.01$), but the magnitude of this increase was greater at altitude ($P<0.001$).

265 Dopamine did not affect MAP at either SL ($P=0.590$) or ALT ($P=0.308$), but it did increase CO
266 at SL ($P=0.041$). TPR was progressively decreased ($P<0.001$) with acute incremental hypoxia
267 both at SL and ALT. For MSNA burst frequency, MAP, CO and TPR no interactions were noted
268 between infusion and incremental hypoxia test stage for either the SL or ALT conditions.

269

270 **DISCUSSION**

271 We sought to ascertain whether the sympathoexcitation and hyperventilation associated
272 with hypoxia are lowered at high altitude by the intravenous infusion of low-dose dopamine to
273 attenuate carotid chemoreceptor responsiveness. The major novel finding of the present study
274 are; 1) the elevations in MSNA and ventilation observed after 15-17 days of high altitude
275 hypoxia (3,454 m) were not reduced by intravenous dopamine infusion when participants were
276 breathing ambient air, 2) the magnitude of the increase in MSNA during an acute incremental
277 hypoxia test performed at sea level and high altitude was not affected by dopamine, and 3)
278 ventilation was elevated during acute incremental hypoxia at high altitude compared to sea level,
279 but was lower at high altitude with dopamine. In the following paragraphs a context will be
280 provided to these findings in light of the relevant literature and several important methodological
281 considerations relating to our experimental design will be discussed.

282

283 *MSNA, hypoxia and dopamine*

284 The carotid chemoreceptors are classically recognized for their oxygen sensing function
285 and consummate reflex increase in ventilation upon activation, however they also possess
286 important autonomic cardiovascular effects with relevance for health and disease (Guyenet,
287 2000; Kumar & Prabhakar, 2012). Acute hypoxia increases the afferent discharge of the carotid
288 chemoreceptors causing an increase sympathetic nerve activity to several regions (Guyenet,
289 2000; Kumar & Prabhakar, 2012). However, the contribution of the carotid chemoreceptors to
290 the sympathoexcitatory effects of chronic hypoxia is more controversial. Indeed, in the present
291 study sojourn to 3,454 m for 15-17 days markedly increased steady-state MSNA, however this
292 was not attenuated with dopamine administration. This supports the findings of Hansen and

293 Sander (2003) who observed that 100% oxygen breathing after 4 weeks at 5,260 m only
294 minimally reduced MSNA (from 48 to 41 bursts·min⁻¹). What is more, we observed MSNA
295 responses to acute incremental hypoxia at altitude were also unaltered with intravenous
296 dopamine infusion. At present the mechanisms underlying such high altitude sympathetic
297 hyperactivity remain obscure and no satisfactory explanation exists. Hansen and Sander (2003)
298 furthermore demonstrated that cardiopulmonary baroreceptor loading at altitude only has a minor
299 effect on MSNA. Remaining possibilities include central changes in the long-term potentiation
300 of sympathetic outflow (Xie *et al.*, 2001), attenuated central sympathoinhibitory pathways such
301 as nitric oxide (Ogawa *et al.*, 1995) and alterations in other reflex control mechanisms.

302

303 *Ventilation, hypoxia and dopamine*

304 D₂-receptor blockade in rats and cats increases carotid chemoreceptor afferent activity
305 and ventilation (Tatsumi *et al.*, 1995; Huey *et al.*, 2003). Moreover, in the same species, 24-48 h
306 of chronic hypoxia decreased carotid body dopaminergic inhibition (Tatsumi *et al.*, 1995; Huey
307 *et al.*, 2003). Domperidone infusion to block D₂-receptors similarly augmented the hypoxic
308 ventilatory response before and after 4 h of isocapnic hypoxia in goats (Janssen *et al.*, 1998) and
309 8 h of isocapnic hypoxia in humans (Pedersen *et al.*, 1999), suggesting that dopaminergic
310 inhibitory mechanisms are preserved. It has been suggested that the magnitude of the reduction
311 in chemosensitivity with dopamine is reflective of the baseline chemosensitivity, and thus the
312 endogenous dopamine concentration (Ward, 1984). As such, our finding that ventilation was
313 lower with dopamine compared to saline administration during an acute hypoxia test following
314 15-17 days at high altitude could suggest that endogenous dopamine levels at the carotid
315 chemoreceptor are decreased at altitude in humans. However, as we did not administer a D₂-

316 receptor blockade (e.g., domperidone) we cannot provide a definitive insight into this issue. Our
317 findings are however compatible with the view that dopamine is an important inhibitory
318 neurotransmitter in the human carotid body and the inhibitory effects of its endogenous provision
319 evoke a more pronounced effect on ventilation during an acute hypoxia test following 15-17 days
320 of high altitude exposure compared to that observed at sea level. The differential effects of
321 dopamine on the ventilatory and MSNA responses described may be attributable to the actions of
322 distinct populations of glomus cells (Paton *et al.*, 2013).

323

324 *Experimental considerations*

325 The results and conclusions of the present study must be viewed in light of several
326 experimental considerations. Contrary to previous reports (Welsh *et al.*, 1978; Pedersen *et al.*,
327 1999), low-dose dopamine was not found to suppress the ventilation under conditions of
328 normoxia and acute hypoxia at SL ($P=0.186$), however $P_{ET}CO_2$ was increased and $P_{ET}O_2$ was
329 decreased with dopamine, consistent with a mild ventilatory suppression (Welsh *et al.*, 1978).
330 The differences between studies may be attributable to the marked inter-individual differences in
331 the ventilatory response to dopamine *per se* (Limberg *et al.*, 2016). In a recent report, 30% of
332 individuals were shown to have an increase rather than a decrease in the ventilatory response to
333 acute hypoxia with dopamine infusion at $3 \mu g \cdot kg^{-1} \cdot min^{-1}$ (Limberg *et al.*, 2016). Differences in
334 the administration of hypoxia and the analytical approaches used to assess the physiological
335 effects of hypoxia, also makes it challenging to directly compare studies employing low-dose
336 dopamine to inhibit the chemoreflex. We utilized an acute incremental hypoxia test that was
337 administered in the form of sequential stepwise reductions in the target $P_{ET}O_2$, following a
338 modification of the methods of Mou *et al.* (1995). An alternative approach would have been to

339 employ short discrete discontinuous bouts of hypoxia, either in a repeated or stepwise manner.
340 This would have perhaps better circumvented issues associated with potential carry-over effects
341 between the stages of hypoxia and any hypoxic ventilatory depression (Teppema & Dahan,
342 2010). It is also acknowledged that the hypoxic ventilatory response in humans can be expressed
343 relative to SpO₂, but due to technical issues this data was not acquired in all participants.
344 Nevertheless, the approach we employed enabled to consistently control the stepwise reductions
345 to the target P_{ET}O₂ under all conditions.

346 High doses of dopamine (i.e., >3 µg·kg⁻¹·min⁻¹) may activate α- and β-adrenoreceptors
347 with well-defined cardiovascular actions and can result in hypertension (Stickland *et al.*, 2011).
348 At a low-dose (<3 µg·kg⁻¹·min⁻¹), dopamine infusion can however cause vasodilatation and
349 increased blood flow through several regions by activation of postsynaptic D₁-receptors in
350 coronary, renal, mesenteric and cerebral circulations and presynaptic D₂-receptors in the
351 peripheral and kidney vasculature (Clark & Menninger, 1980). As mentioned above, the
352 peripheral chemoreceptors also exert effects on reflex cardiovascular control (Guyenet, 2000;
353 Kumar & Prabhakar, 2012). In agreement with other studies (Eugene, 2016; Limberg *et al.*,
354 2016), low-dose dopamine infusion decreased TPR in the present study. Such vasodilatory
355 actions of dopamine likely contributed to the elevation of HR and CO under steady-state
356 conditions and during the acute incremental hypoxia test, and the elevated MSNA burst
357 frequency during the acute incremental hypoxia test. This likely occurred via baroreflex
358 mechanism in order to preserve MAP, which was largely unchanged. It is acknowledged that the
359 occurrence of such secondary compensatory hemodynamic adjustments arguably constrains the
360 interpretation of the data generated. In addition, dopamine has been shown to have direct cardiac
361 effects (Holmes & Fowler, 1962), which may have contributed to the elevated HR and CO

362 observed with dopamine infusion. The systemic administration of low-dose dopamine (i.e., 3
363 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was undertaken in accordance with several previous studies in humans (Boetger
364 & Ward, 1986; Dahan *et al.*, 1996; Limberg *et al.*, 2016). However, it is important to note that
365 despite a change in the prevailing MSNA with dopamine, the responses to the acute incremental
366 hypoxia test were unchanged (i.e., no infusion x stage interaction, noted either at SL or ALT).
367 An alternative approach would have been to administer dopamine directly into the carotid artery
368 and/or record carotid chemoreceptor afferent nerve discharge to verify carotid body inhibition, as
369 has been performed in dogs (Bisgard *et al.*, 1979; Stickland *et al.*, 2007), but this extremely
370 invasive technique was unfeasible. The hypoxic pressor response was augmented at altitude, but
371 rather than occurring via a sympathetic vasoconstrictor effect, appeared to occur secondary to an
372 augmented increase in CO. Whether this relates to a difference in autonomic cardiac control
373 relating to chemoreflex activation *per se* warrants further investigation.

374 We attempted to control $P_{\text{ET}}\text{CO}_2$ such that it remained at SL isocapnic conditions
375 throughout the acute incremental hypoxia test, however it was lower (≈ 3 mmHg) at altitude.
376 Therefore, it is possible that the sympathoexcitatory and hyperventilatory responses to the test
377 were underestimated at ALT compared to SL. However, no differences in $P_{\text{ET}}\text{CO}_2$ were noted
378 between the saline and dopamine conditions. Ventilation was higher at high altitude when
379 participants were breathing air with $P_{\text{ET}}\text{CO}_2$ and $P_{\text{ET}}\text{O}_2$ maintained at sea level values (Figure
380 4A) compared to when they were breathing the ambient air (i.e., poikilocapnic hypoxia). A
381 potential explanation for this is that the supplemental CO_2 provided to the inspired air to return it
382 to sea level values stimulated the chemoreceptors at high-altitude (e.g., due to central acid-base
383 balance alterations) (Ainslie *et al.*, 2013). We observed subtle differences in the MSNA
384 responses to altitude and acute incremental hypoxia, when expressed as burst frequency

385 (bursts·min⁻¹) or burst incidence (bursts·100 heartbeats⁻¹). For example, steady-state MSNA
386 frequency and burst incidence were both robustly elevated at altitude, but likely due to a
387 concomitantly elevated (P=0.088) HR. When interpreting sympathetic effects of altitude and
388 dopamine in the present study we have principally relied upon burst frequency data (bursts per
389 unit time). SV and CO were monitored using finger photoplethysmography, and although this
390 approach can reliably track changes in these parameters during laboratory-based manoeuvres
391 (Bogert *et al.*, 2010), the indirect nature of this method is a potential limitation. Finally, the small
392 sample size is a potential limitation of our study. Although the number of participants is similar
393 to earlier work employing a within subject design to examine the influence of high altitude on
394 MSNA (Hansen & Sander, 2003), we acknowledge the potential for a type II error to have
395 occurred.

396 In this study, we examined the effects of intravenous low-dose dopamine on neural
397 cardiovascular control following chronic hypobaric hypoxia (15-17 days at 3,454 m).
398 Intravenous dopamine infusion did not lower the increases in MSNA at high altitude when
399 ambient air was breathed, furthermore the MSNA response to an acute incremental hypoxia test
400 was not affected by dopamine infusion either at sea level and high altitude. These findings
401 support the view that intravenous low-dose dopamine to attenuate the responsiveness of the
402 carotid chemoreceptors does not diminish the sympathoexcitation of high altitude, but should be
403 viewed in light of the methodological considerations relating to our experimental design that are
404 discussed above.

405

406 **CONFLICTS OF INTERESTS/COMPETING INTERESTS**

407 The authors have no conflicts of interest/competing interests.

408

409 AUTHOR CONTRIBUTIONS

410 JPF was involved with the conception and design of the experiments, the collection,
411 analysis and interpretation of data, and drafting the first version of the article. DF and MPH were
412 involved in collection, analysis and interpretation of data, and revising the article critically for
413 important intellectual content. CL was involved in the conception and design of the experiments,
414 collection and interpretation of data, and revising the article critically for important intellectual
415 content. All authors have approved the final manuscript and agree to be accountable for all
416 aspects of the work in ensuring that questions related to the accuracy or integrity of any part of
417 the work are appropriate. All persons designated as authors qualify for authorship and all those
418 who qualify for authorship are listed.

419

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422

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425

426

Table 1. Selected cardiorespiratory responses to the acute incremental hypoxia test at Zurich (SL, 408 m) and Jungfrauoch research station (ALT, 3,454 m) during infusion of saline or dopamine.

	Stage of incremental hypoxia test							
	1	2	3	4	5	6	7	8
P_{ET}O₂ (mmHg)								
SL saline	96.7 (2.5)	74.5 (1.4)	69.9 (1.5)	64.8 (0.7)	59.0 (1.4)	55.0 (0.9)	49.8 (0.7)	45.0 (1.3)
SL dopamine	95.4 (4.4)	73.6 (1.0)	70.9 (1.8)	64.9 (1.6)	60.0 (1.4)	54.9 (0.8)	49.9 (1.5)	45.5 (1.0)
ALT saline	98.7 (12.7)	79.2 (10.8)	70.1 (0.9)	63.9 (1.7)	59.7 (0.6)	54.6 (2.2)	50.1 (0.8)	44.7 (0.6)
ALT dopamine	95.5 (4.6)	75.7 (1.5)	69.1 (1.2)	64.7 (1.8)	59.5 (1.0)	55.1 (0.7)	50.0 (1.2)	44.7 (0.6)
P_{ET}CO₂ (mmHg)								
SL saline	41.5 (2.1)	41.7 (2.1)	41.7 (2.0)	41.5 (2.0)	41.7 (2.3)	41.7 (2.2)	41.6 (2.2)	41.6 (2.2)
SL dopamine	42.9 (2.9)	42.9 (2.6)	43.1 (2.8)	43.2 (3.0)	43.3 (3.0)	43.1 (3.1)	43.0 (2.9)	43.0 (3.1)
ALT saline	39.5 (1.8)	37.8 (2.8)	35.3 (2.7)	35.3 (2.1)	38.8 (1.9)	39.9 (1.8)	40.2 (1.6)	39.9 (1.1)
ALT dopamine	39.7 (2.1)	39.4 (2.1)	36.0 (2.6)	36.1 (2.5)	37.7 (2.2)	40.5 (1.8)	40.5 (1.8)	40.6 (2.1)
SpO₂ (%)								
SL saline	98.0 (0.8)	96.2 (0.9)	95.5 (0.9)	94.6 (1.0)	93.0 (1.2)	91.3 (1.8)	88.0 (2.5)	83.6 (3.7)

SL dopamine	97.9 (1.2)	95.8 (1.1)	95.6 (1.2)	94.2 (1.5)	92.9 (2.1)	90.7 (2.5)	87.6 (2.6)	84.1 (3.0)
ALT saline	98.5 (0.9)	96.7 (1.4)	95.7 (0.8)	94.4 (1.1)	92.8 (1.5)	90.2 (1.9)	87.2 (2.7)	81.7 (3.5)
ALT dopamine	97.9 (1.0)	95.9 (1.2)	95.1 (1.2)	93.7 (1.2)	92.3 (1.3)	90.0 (1.2)	87.5 (3.7)	81.4 (4.2)
MSNA incidence (bursts·100 heartbeats ⁻¹)								
SL saline	26 (18)	26 (19)	27 (19)	25 (20)	21 (16)	23 (12)	22 (13)	23 (11)
SL dopamine	29 (20)	32 (20)	33 (20)	30 (16)	27 (14)	27 (16)	35 (14)	31 (12)
ALT saline	37 (16)	35 (16)	32 (16)	33 (16)	36 (16)	33 (16)	34 (16)	34 (16)
ALT dopamine	40 (16)	35 (14)	40 (14)	42 (12)	37 (15)	38 (12)	38 (13)	35 (9)
HR (beats·min ⁻¹)								
SL saline	65 (10)	66 (10)	70 (11)	72 (11)	74 (11)	76 (13)	80 (10)	82 (12)
SL dopamine	69 (10)	71 (10)	73 (11)	75 (12)	76 (13)	78 (12)	83 (11)	87 (10)
ALT saline	74 (8)	76 (9)	77 (9)	80 (11)	84 (13)	87 (10)	90 (12)	95 (14)
ALT dopamine	77 (8)	83 (7)	84 (10)	84 (11)	86 (12)	90 (10)	95 (9)	99 (10)
SV (ml)								
SL saline	115 (12)	115 (11)	114 (13)	113 (13)	114 (14)	114 (13)	113 (13)	113 (13)
SL dopamine	120 (11)	121 (10)	120 (10)	120 (11)	118 (9)	120 (10)	118 (12)	118 (13)

ALT saline	113 (7)	111 (5)	112 (6)	110 (6)	110 (6)	112 (9)	115 (9)	116 (8)
ALT dopamine	117 (11)	116 (10)	114 (12)	114 (11)	114 (8)	114 (8)	113 (10)	114 (9)

$P_{ET}O_2$, partial pressure of end-tidal oxygen; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; MSNA, muscle sympathetic nerve activity; HR, heart rate; SV, stroke volume. Data expressed as mean (standard deviation).

Table 2. P values derived from repeated measures ANOVA in which the factors of altitude (SL vs. ALT), infusion (saline vs. dopamine) and incremental hypoxia test stage (stages 1-8) were considered, as well as all two- and three-way interactions.

	Altitude	Infusion	Stage	Altitude x Infusion	Altitude x Stage	Infusion x Stage	Altitude x Infusion x Stage
P_{ET}O₂	0.562	0.461	0.000	0.347	0.291	0.297	0.668
P_{ET}CO₂	0.000	0.015	0.000	0.141	0.000	0.479	0.260
SpO₂	0.331	0.626	0.000	0.729	0.243	0.629	0.617
V_E	0.000	0.019	0.000	0.034	0.000	0.085	0.072
V_T	0.000	0.022	0.000	0.184	0.000	0.343	0.236
R_f	0.001	0.089	0.000	0.061	0.001	0.657	0.035
MSNA frequency	0.025	0.034	0.002	0.961	0.236	0.065	0.109
MSNA incidence	0.174	0.081	0.375	0.106	0.353	0.133	0.092
MAP	0.000	0.372	0.000	0.371	0.000	0.227	0.128
CO	0.033	0.010	0.000	0.798	0.036	0.205	0.517
TPR	0.030	0.046	0.000	0.805	0.176	0.279	0.678
HR	0.002	0.101	0.000	0.840	0.218	0.449	0.712

SV	0.286	0.107	0.249	0.323	0.266	0.109	0.026
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$P_{ET}O_2$, partial pressure of end-tidal oxygen; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; V_E , minute ventilation; V_T , tidal volume; R_f , respiratory frequency; MSNA, muscle sympathetic nerve activity; MAP, mean arterial pressure; CO, cardiac output; TPR, total peripheral resistance; HR, heart rate; SV, stroke volume.

Table 3. P values derived from repeated measures ANOVA in which the factors of infusion (saline vs. dopamine) and incremental hypoxia test stage (stages 1-8) and their two-way interaction were considered separately at SL and ALT.

	SL			ALT		
	Infusion	Stage	Infusion x Stage	Infusion	Stage	Infusion x Stage
P_{ET}O₂	0.809	0.000	0.310	0.376	0.000	0.457
P_{ET}CO₂	0.025	0.523	0.637	0.177	0.000	0.351
SpO₂	0.743	0.000	0.603	0.417	0.000	0.841
V_E	0.298	0.000	0.517	0.023	0.000	0.073
V_T	0.120	0.001	0.259	0.047	0.000	0.283
MSNA frequency	0.051	0.028	0.091	0.015	0.023	0.042
MSNA incidence	0.053	0.332	0.201	0.063	0.313	0.034
MAP	0.590	0.011	0.565	0.308	0.000	0.133
CO	0.041	0.000	0.650	0.135	0.000	0.206
TPR	0.228	0.000	0.113	0.175	0.000	0.473
HR	0.273	0.000	0.682	0.218	0.000	0.515

$P_{ET}O_2$, partial pressure of end-tidal oxygen; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; V_E , minute ventilation; V_T , tidal volume; R_f , respiratory frequency; MSNA, muscle sympathetic nerve activity; MAP, mean arterial pressure; CO, cardiac output; TPR, total peripheral resistance; HR, heart rate; SV, stroke volume.

Figure legends

Figure 1. Intravenous infusion of dopamine did not significantly modify steady-state respiration at Zurich (SL, 432 m) and the Jungfrauoch research station (ALT, 3,454 m) while participants breathed the ambient air. VE, minute ventilation; VT, tidal volume; Rf, respiratory frequency. Data expressed as individual values and means with (standard deviation). ANOVA P values are displayed.

Figure 2. Original sympathetic neurograms obtained at sea level and high altitude with infusion of saline and dopamine. In this individual, a low level of MSNA was present at sea level, but the recording site was verified with a pronounced MSNA response to a breath hold. Note the minimal response to dopamine, but pronounced sympathoexcitation at altitude.

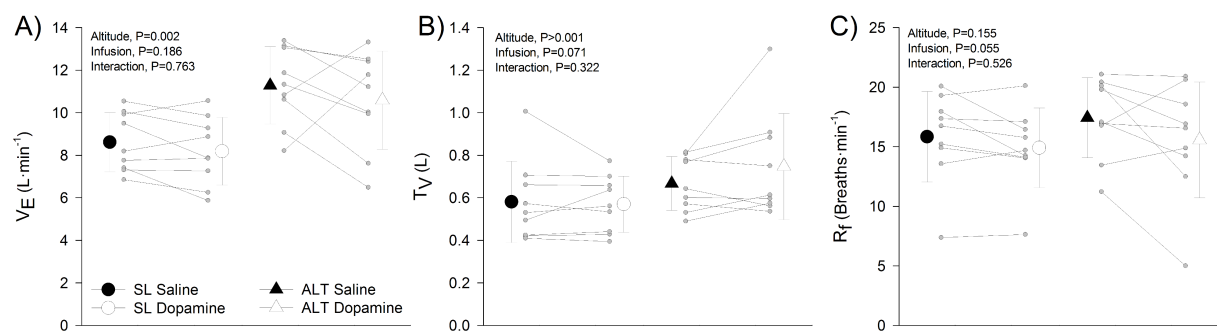
Figure 3. Cardiovascular variables at Zurich (SL) and the Jungfrauoch research station (ALT) during infusion of saline and dopamine with participants breathing ambient air. MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; TPR, total peripheral resistance; HR, heart rate; SV, stroke volume. Data expressed as individual values and means with (standard deviation). ANOVA P values are displayed.

Figure 4. Respiratory responses to acute incremental hypoxia at Zurich (SL) and Jungfrauoch research station (ALT) during infusion of saline or dopamine. VE, minute ventilation; VT, tidal volume; Rf, respiratory frequency. Data expressed as individual values and means with (standard deviation). ANOVA P values are displayed.

Figure 5. Original sympathetic neurograms obtained during the initial (1) and final (8) stages of the acute incremental hypoxia test at sea level and high altitude with infusion of saline and dopamine. Note the modest increase in MSNA in response with either acute incremental hypoxia or dopamine, and the pronounced sympathoexcitation at ALT.

Figure 6. Cardiovascular responses to acute incremental hypoxia at Zurich (SL, 432 m) and Jungfrauoch research station (ALT) during infusion of saline or dopamine.

MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; TPR, total peripheral resistance. Data expressed as individual values and means \pm standard error.

**Figure 1**

A) Sea Level (Zurich, 408 m)

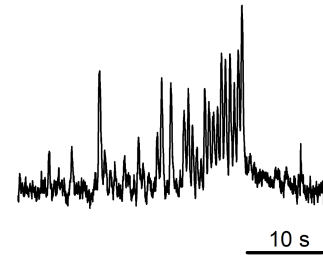
i) Saline



ii) Dopamine

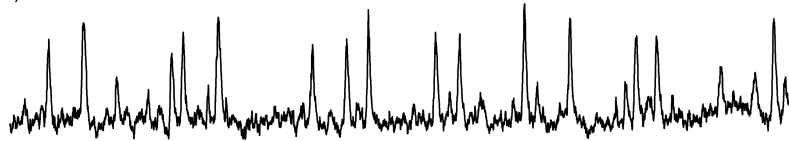


iii) Breath hold

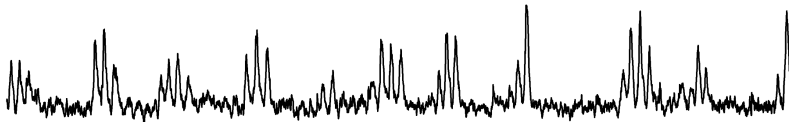


B) Altitude (Jungfrauoch, 3454 m)

iv) Saline



v) Dopamine

**Figure 2**

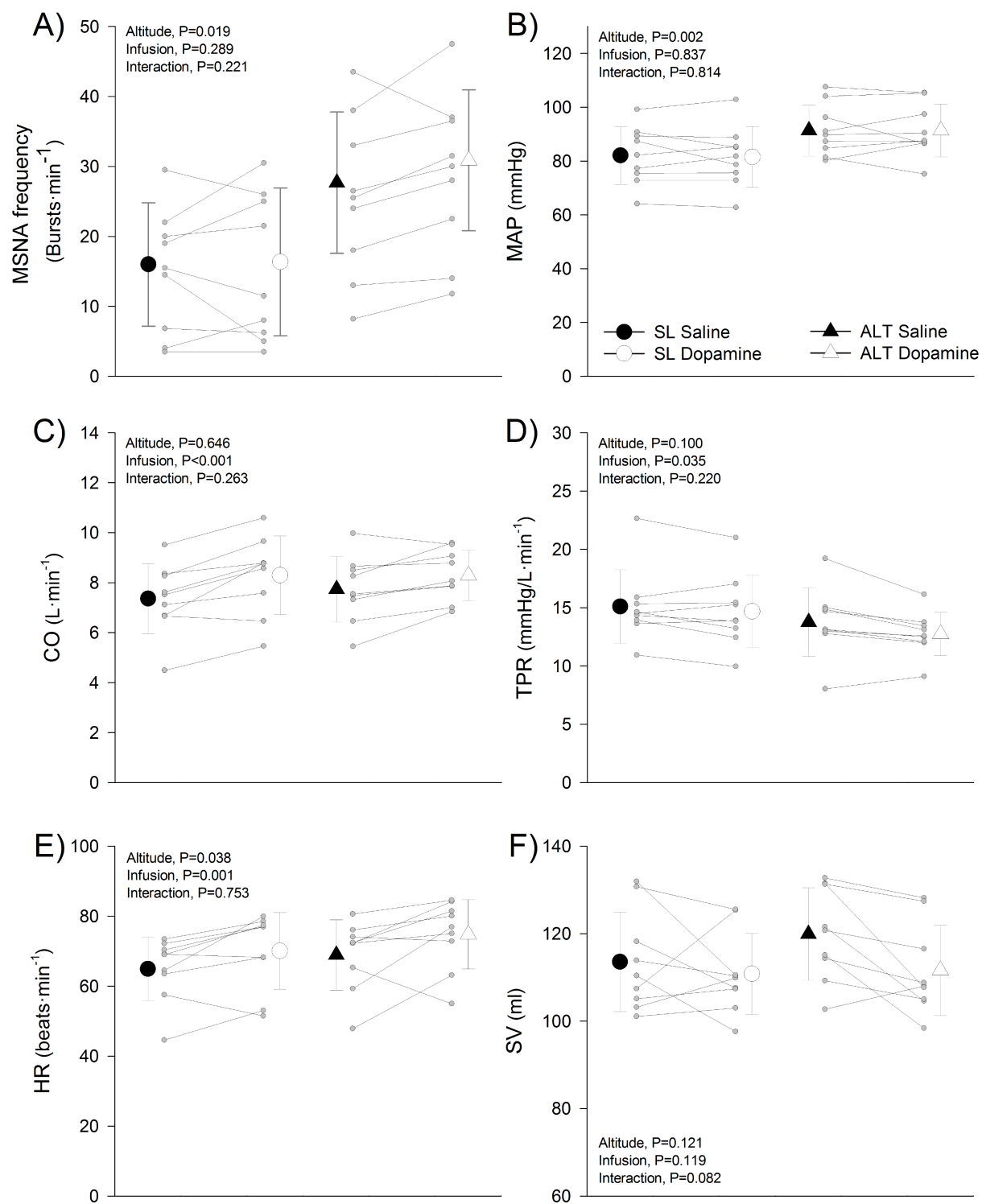
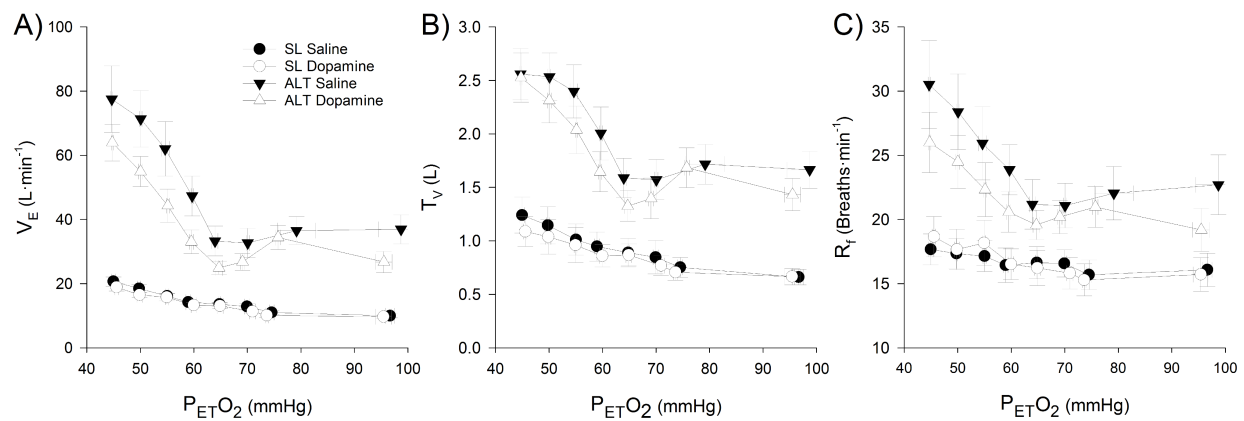


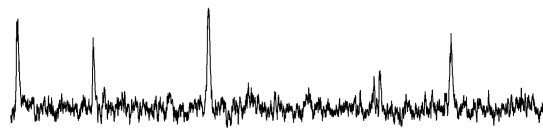
Figure 3

**Figure 4**

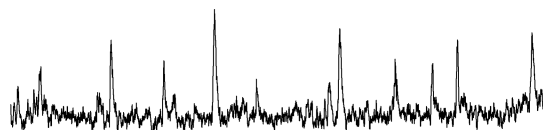
A) Sea Level (Zurich, 408 m)

Stage 1 (Target $P_{ET}O_2$ 95 mmHg)Stage 8 (Target $P_{ET}O_2$ 45 mmHg)

i) Saline



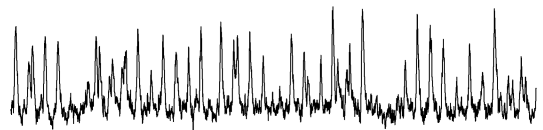
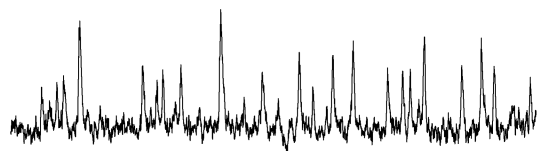
ii) Dopamine



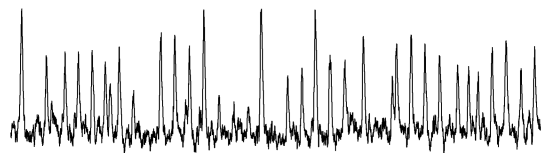
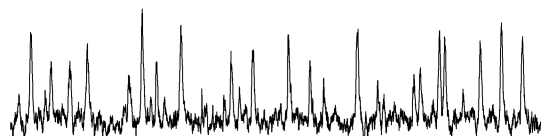
B) Altitude (Jungfrauoch, 3454 m)

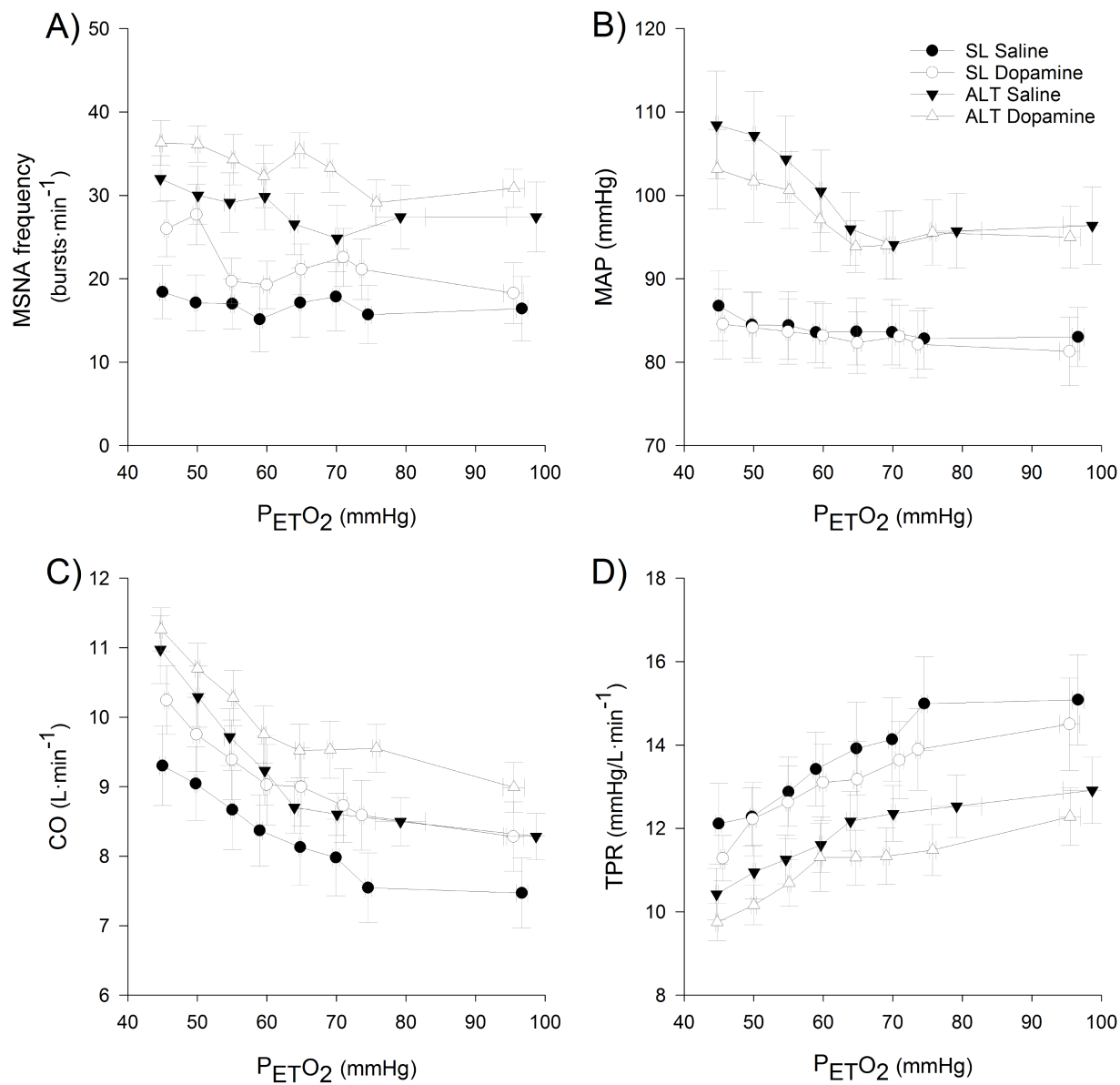
Stage 1 (Target $P_{ET}O_2$ 95 mmHg)Stage 8 (Target $P_{ET}O_2$ 45 mmHg)

iv) Saline



v) Dopamine

**Figure 5**

**Figure 6**

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