

1 **TITLE**


2 Effects of Normobaric Hypoxia on Oxygen Saturation Variability

3

4 **RUNNING TITLE**

5 Hypoxia and SpO<sub>2</sub> entropy

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22

23 **Abstract:** The study is the first to evaluate the effects of graded normobaric hypoxia  
24 on SpO<sub>2</sub> variability in healthy individuals. Twelve healthy males (mean (SD) age 22  
25 (4) years) were exposed to four simulated environments (FiO<sub>2</sub>: 0.12, 0.145, 0.17 and  
26 0.21) for 45-min, in a balanced cross-over design. Sample entropy, a tool that  
27 quantifies the irregularity of pulse oximetry fluctuations, was used as a measure of  
28 SpO<sub>2</sub> variability. SpO<sub>2</sub> entropy increased as the FiO<sub>2</sub> decreased, and there was a  
29 strong significant negative correlation between mean SpO<sub>2</sub> and its entropy during  
30 hypoxic exposure ( $r = -0.841$  to  $-0.896$ ,  $P < 0.001$ ). In addition, SpO<sub>2</sub> sample entropy,  
31 but not mean SpO<sub>2</sub>, was correlated ( $r = 0.630$  to  $0.760$ ,  $P < 0.05$ ) with dyspnoea in  
32 FiO<sub>2</sub> 0.17, 0.145, and 0.12 and importantly, SpO<sub>2</sub> sample entropy at FiO<sub>2</sub> 0.17 was  
33 correlated with dyspnoea at FiO<sub>2</sub> 0.145 ( $r = 0.811$ ,  $P < 0.01$ ). These findings suggest  
34 that SpO<sub>2</sub> variability analysis may have the potential to be used in a clinical setting as  
35 a non-invasive measure to identify the negative sequelae of hypoxaemia.

36

37 **Key words**

38 Dyspnoea, Oxygen saturation, Pulse oximetry, Sample entropy, SpO<sub>2</sub> variability

39 **Introduction**

40 Tissue hypoxia is a fundamental consequence not only of high-altitude exposure but  
41 also of critical illness, where it may occur either as a cause, or as a result of, various  
42 pathologies (Berger and Grocott, 2017). Hypoxia also causes a concomitant decrease  
43 in SpO<sub>2</sub> through its effects on the arterial partial pressure of oxygen (PaO<sub>2</sub>), in  
44 accordance with the alveolar gas equation and the oxyhemoglobin dissociation curve.  
45 For example, SpO<sub>2</sub> on arrival at terrestrial altitude of 3800m can reach ~90%, and  
46 further decline to ~81% after a trek to 5200m (Mellor *et al.* 2015). Similarly, SpO<sub>2</sub>  
47 values below 80% are regularly observed in patients in intensive care (Wilson *et al.*  
48 2010; Van de Louw *et al.* 2001). Following the stimulation of aortic-arch  
49 chemoreceptors and carotid bodies, the physiological response to hypoxemia is  
50 characterized by an increase in cardiac output, ventilation, and haemoglobin  
51 concentration (Berger and Grocott, 2017; Wilson *et al.* 2005).

52  
53 Accumulating evidence indicates that SpO<sub>2</sub> variability analysis is more insightful than  
54 mean SpO<sub>2</sub> (Garde *et al.* 2016; Bhogal and Mani, 2017). Using mean or time averaged  
55 physiological data does not illuminate the pattern, complexity, and irregularity which is  
56 observed in most biological systems, and in the cardiovascular system in particular  
57 (Bhogal and Mani, 2017). The majority of oscillations in physiological time-series data  
58 are not linear, and recent evidence suggests that these oscillations can provide a  
59 useful insight into the activity of the underlying control network (i.e. the cardiovascular  
60 system) (Wagner and Persson, 1998). Sample entropy is one method of describing  
61 these nonlinear data, and is commonly used to study the dynamics of the  
62 cardiovascular system (e.g. heart rate and respiratory rate) (Richman and Moorman,  
63 2000). Briefly, entropy describes the unpredictability and irregularity of time-series

64 data and allows physiological signals (e.g. heart rate and SpO<sub>2</sub>) to be classified over  
65 time (Wagner and Persson, 1998; Moorman et al., 2011). Although there are a variety  
66 of techniques used when assessing fluctuations in time-series data, entropy is often  
67 selected as an index of variability due to its link to information theory (Pincus, 1994).  
68 Information theory is the mathematical study of the coding of information in the form  
69 of sequences of impulses, and can potentially quantify data within a complex system  
70 (Mitchell, 2009, Pincus 1994) (e.g. the cardiovascular system).

71

72 This nonlinear analysis may provide useful information on the integrity of the  
73 cardiovascular system in both health and disease. Heart rate and respiratory rate  
74 variability analysis have previously been used extensively to study the integrity of the  
75 cardio-respiratory system with promising applications (Shirazi et al., 2013; Tipton et  
76 al., 2017). Recently, Garde et al. (2016) reported that SpO<sub>2</sub> variability data improved  
77 the identification of children who were admitted to hospital. Further, Bhogal and Mani  
78 (2017) and others (Pham, 2018) have demonstrated that SpO<sub>2</sub> entropy decreases with  
79 age and that this can differentiate healthy individuals aged over 35 from their younger  
80 counterparts. Increasingly, it appears that variability analysis provides more  
81 information about physiological systems compared to absolute or mean values  
82 (Garrido et al., 2017). These findings suggest that variability indices have the potential  
83 to predict mortality both in healthy individuals and in clinical populations (Tsuji et al.,  
84 1994; Mani et al., 2009; Bhogal et al., 2019). However, to our knowledge the use of  
85 SpO<sub>2</sub> variability analysis has not been studied empirically within the field of high-  
86 altitude physiology and pathophysiology (e.g., Acute Mountain Sickness).

87

88 Therefore, the present study sought to characterize the effects of graded normobaric  
89 hypoxia on SpO<sub>2</sub> variability in healthy individuals for the first time. Any non-invasive  
90 measurement that offer insight into the state of an individual when hypoxic is valuable  
91 in multiple clinical settings. Reduced entropy in a physiological setting can be  
92 interpreted as less engagement of the components within a control system (Pincus,  
93 1994). In healthy physiological systems, more information processing (i.e.  
94 engagement of the regulatory components) in response to environmental challenges  
95 such as hypoxia would be expected. As entropy is a measure of information content  
96 in complex physiologic time-series, we hypothesised that normobaric hypoxia would  
97 increase the entropy of SpO<sub>2</sub> signal in healthy individuals and that SpO<sub>2</sub> entropy and  
98 mean SpO<sub>2</sub> would be negatively correlated.

99

## 100 **Methods**

101

### 102 **Ethical approval**

103 Before providing their written informed consent, all participants were informed of the  
104 requirements and potential risks of the study. The experimental procedures adhered  
105 to the standards set by the latest revision of the Declaration of Helsinki, except for  
106 registration in a database, and were approved by the Science Faculty Ethics  
107 Committee of The University of Portsmouth (project number 2017-025).

108

### 109 **Experimental design**

110 This study was part of a larger project investigating effects of normobaric hypoxia on  
111 physiological and cognitive function and the experimental design has been described  
112 in detail elsewhere (Williams et al., 2019). A convenience sample of twelve healthy

113 males, (mean [SD] age 22 [4] years, height 1.78 [0.05] m, mass 75 [9] kg, FEV<sub>1</sub>/FVC  
114 ratio 85 [5] %) volunteered to participate in this study. All participants were non-  
115 smokers, free of any cardiovascular, respiratory and cerebrovascular diseases, were  
116 not diabetic and were not taking any prescription drugs at the time of or before  
117 participation. All participants resided at <1000 m and had not spent time at altitude for  
118 at least 1 month prior to commencement of the study, including commercial flights.  
119 Participants were instructed to refrain from any strenuous exercise, caffeine or alcohol  
120 in the 24 h preceding each visit to the laboratory. In addition, participants were  
121 requested to record their dietary intake for 24 h prior to their first visit and to replicate  
122 their eating habits for each visit thereafter.

123

124 A within participant, balanced cross-over design was employed. Participants were  
125 required to visit the laboratory on 5 occasions (one health screening and four  
126 experimental trials). For each experimental trial participants were exposed to  
127 normobaric hypoxia for 45 minutes in a purpose-built hypoxic chamber (Sporting  
128 Edge, Sheffield on Loddon, UK). The fraction of inspired oxygen (FiO<sub>2</sub>) values were  
129 0.2093 (sea-level), 0.17 (equivalent to ~1600 m), 0.145 (~3000 m), and 0.12 (4500  
130 m). If end-tidal O<sub>2</sub> (P<sub>ET</sub>O<sub>2</sub>) or end-tidal CO<sub>2</sub> (P<sub>ET</sub>CO<sub>2</sub>) fell below 45 mmHg and 25  
131 mmHg respectively, for three consecutive breaths, or if SpO<sub>2</sub> went below 65 %,   
132 participants were given a supply of normoxic air and subsequently removed from the  
133 chamber. Participants were also blinded to which condition they were in. The ambient  
134 temperature was maintained at 25 °C and the relative humidity was controlled at 50 %  
135 throughout. Experimental trials were separated by a minimum of 48 h and conducted  
136 at the same time of day.

137

138 **Cardiorespiratory responses**

139 Minute ventilation ( $\dot{V}_E$ ), respiratory frequency ( $f_R$ ), tidal volume ( $V_T$ ), end-tidal pressure  
140 of CO<sub>2</sub> ( $P_{ET}CO_2$ ) and O<sub>2</sub> ( $P_{ET}O_2$ ), and heart rate were measured breath by breath using  
141 a metabolic cart (Quark CPET, Cosmed, Rome, Italy) and appropriate calibration  
142 procedures were performed according to the manufacturer's instructions.

143

144 **SpO<sub>2</sub> and SpO<sub>2</sub> variability**

145 SpO<sub>2</sub> was recorded using pulse oximetry on the index finger of the right hand (Nonin  
146 7500, US). Data were continually recorded using an analogue to digital acquisition  
147 system with a sampling rate of 1000 Hz using a PowerLab system (ADInstruments,  
148 Castle Hill, Australia). The recorded data were extracted using LabChart software and  
149 down-sampled by 1000 to 1.s<sup>-1</sup>. Data were subsequently down sampled as pulse  
150 oximeter readings are not sampled at such a high rate, and thus at that resolution, the  
151 variability presented would not reflect true SpO<sub>2</sub> variation. This method is commonly  
152 used when assessing SpO<sub>2</sub> entropy (Bhoghal and Mani, 2017, Lazareck and  
153 Tarassenko, 2006). The data were visually scanned and any obvious artefacts (e.g.  
154 missed or spurious SpO<sub>2</sub> data) were removed (less than 1%). From the recording there  
155 were 4 X 8-minute segments of data that were used for analysis. A reading prior to  
156 exposure, a recording once exposed to the altered FiO<sub>2</sub>, a third reading at 30-min of  
157 exposure, and finally one after 45-min of exposure.

158

159 The oxygen saturation data were analysed using linear (e.g. standard deviation) and  
160 non-linear methods (e.g. entropy measures) written in MATLAB (MathWorks,  
161 R2017a). For each 8-min segment the mean SpO<sub>2</sub> and standard deviation were  
162 calculated as tools to understand the overall variability. We also employed sample

163 entropy, detrended fluctuation analysis (DFA) and multiscale entropy (MSE) as  
164 measures of complexity in SpO<sub>2</sub> fluctuations (Richman and Moorman, 2000; Costa et  
165 al., 2005). Sample entropy is a tool that quantifies the degree of irregularity present in  
166 a dataset by calculating the probability that an event with window length,  $m$ , and  
167 degree of tolerance,  $r$ , will be repeated at later time. In present study  $m$  was set at 2  
168 and  $r$  at 0.2 as described by Richman and Moorman (2000). Many physiological time-  
169 series (e.g. heart rate, respiratory rate and SpO<sub>2</sub>) show a fractal-like pattern of  
170 fluctuations (Raoufy et al., 2016; Bhoghal and Mani., 2017; Bhogal et al., 2019).  
171 Fractals exhibit similar patterns at increasingly small scale. A variety of methods have  
172 been developed to quantify fluctuation of physiological signals at different time scales.  
173 Detrended fluctuation analysis examines the self-similarity of a time series to  
174 determine the structural integrity of the signal at different time scales (Peng et al.,  
175 1995). In this analysis the data are split into boxes of various lengths ( $n$ ) and this is  
176 plotted against the  $F(n)$ , which is the variability of detrended signals in different scales  
177 ( $n$ ). The slope of the resulting log-log graph is known as “scaling exponent” which  
178 indicates the type of fractal-like dynamics present in the physiological signal (Peng et  
179 al., 1995). Another method which takes scaling into account is multiscale entropy.  
180 Multiscale entropy is an extension of sample entropy and fractal analysis, as it  
181 examines the sample entropy at different time scales (Costa et al., 2005). The data  
182 are averaged within window length consisting of a number of data points to create a  
183 coarse-grained time-series (Costa et al., 2005). The sample entropy of this is then  
184 calculated and plotted against the window length (Costa et al., 2005). The trend of  
185 changes in entropy in different scales gives information about complexity of a data set.  
186 Compared to a previous report we used multiscale entropy to five scales due to the  
187 shorter nature of the collected data (Bhogal and Mani, 2017).



188

## 189 **Dyspnoea**

190 Dyspnoea was recorded using a modified Borg scale (0, 'Nothing at all' to 10,  
191 'Shortness of breath so severe you need to stop', Mahler et al, 1987) before and after  
192 30-min of exposure.

193

## 194 **Statistical analyses**

195 The distribution of data was assessed using descriptive methods (skewness, outliers,  
196 and distribution plots) and inferential statistics (Shapiro–Wilk test).  $\dot{V}_E$ ,  $f_R$ ,  $V_T$ ,  $P_{ET}CO_2$ ,  
197  $P_{ET}O_2$ , and heart rate data were 5-min averaged. All data were analysed by either a  
198 one-way or a two-way repeated measures ANOVA and post-hoc comparisons were  
199 completed using a Tukey test. Spearman's correlation coefficients were used to  
200 examine the relationship between  $SpO_2$  variability and dyspnoea. Repeated measure  
201 correlation coefficients ( $r_{tm}$ ) were computed for the correlations of  $SpO_2$  entropy and  
202 mean  $SpO_2$  using the method described by Bland and Altman (1995) and the software  
203 developed by Bakdash and Marusich (2017). Statistical analyses were performed  
204 using SPSS (Statistical Package for the Social Sciences), version 22.0 (SPSS Inc,  
205 Chicago, IL, USA) or R (R Core Team, 2007). Statistical significance was accepted at  
206  $P < 0.05$ . All data are expressed as means  $\pm$  standard deviation (SD) unless otherwise  
207 stated.

208

## 209 **Results**

210 One participant was removed from the chamber in  $FIO_2$  0.12 ( $P_{ET}O_2$  fell below 45  
211 mmHg). Therefore, the following analyses are for the 12 participants in  $FIO_2$  0.2093,  
212 0.17, and 0.145 and 11 participants in  $FIO_2$  0.12.

213

## 214 **Cardiorespiratory responses**

215 Minute ventilation ( $\dot{V}_E$ ), respiratory frequency ( $f_R$ ), tidal volume ( $V_T$ ), end-tidal pressure  
216 of CO<sub>2</sub> ( $P_{ETCO_2}$ ) and O<sub>2</sub> ( $P_{ETO_2}$ ), and heart rate data are displayed across the four  
217 environments in Figure 1.

218

219 << INSERT FIGURE 1 ABOUT HERE >>

220

## 221 **SpO<sub>2</sub> and SpO<sub>2</sub> variability**

222 An example of SpO<sub>2</sub> signals at different FiO<sub>2</sub> is displayed in Figure 2A. The oxygen  
223 saturation readings exhibit more fluctuations with lower FiO<sub>2</sub>. Figures 2 also depict  
224 SpO<sub>2</sub> (Figure 2B), SpO<sub>2</sub> standard deviation (Figure 2C), and sample entropy (Figure  
225 2D). An increase in standard deviation of SpO<sub>2</sub> fluctuations and a concomitant  
226 increase in sample entropy was observed when FiO<sub>2</sub> was decreased (Figures 2C and  
227 D). Detrended fluctuation analysis demonstrates that the scaling exponent ( $\alpha$ ) was  
228 consistent across all FiO<sub>2</sub> conditions and no significant differences were observed  
229 (Figure 3A). Finally, the relationship between multiscale entropy and FiO<sub>2</sub>, a measure  
230 of complexity, is displayed in Figure 3B. SpO<sub>2</sub> entropy increases as the scale  
231 increases. This indicates that the pattern of SpO<sub>2</sub> fluctuations is not random (Costa et  
232 al., 2005). Multiscale entropy increased following exposure to the lowest level of  
233 inspired oxygen. Two-way ANOVA indicated that effect of FiO<sub>2</sub> ( $P < 0.0001$ ) and scale  
234 ( $P < 0.0001$ ) were both statistically significant. Interestingly, multiscale entropy can  
235 characterise and separate the groups better in scale 5 than scale 1 (Figure 3B).

236

237 << INSERT FIGURE 2 ABOUT HERE >>

238

239 << INSERT FIGURE 3 ABOUT HERE >>

240

### 241 **Intra time-series analysis**

242 Figures 4A-D demonstrate the temporal changes of SpO<sub>2</sub> and SpO<sub>2</sub> variability.

243 Sample entropy is more responsive to the hypoxic stimulus when compared to the

244 mean oxygen saturation. The sample entropy plateaus ~20 minutes before mean

245 oxygen saturation in the three hypoxic environments. No significant correlations

246 between SpO<sub>2</sub> variability at FiO<sub>2</sub> 0.2093 and mean SpO<sub>2</sub> at FiO<sub>2</sub> 0.17, 0.145, or 0.12

247 were observed. Similarly, no significant correlation between SpO<sub>2</sub> variability at FiO<sub>2</sub>

248 0.17 and mean SpO<sub>2</sub> at FiO<sub>2</sub> 0.145 or 0.12 was observed.

249 << INSERT FIGURE 4 ABOUT HERE >>

250

### 251 **The relationship between mean SpO<sub>2</sub> and SpO<sub>2</sub> variability**

252 Linear regression analysis demonstrated that the relationships between mean SpO<sub>2</sub>  
253 and SpO<sub>2</sub> standard deviation or sample entropy were strongly correlated (Figure 5).

254 For the correlation between mean SpO<sub>2</sub> and its standard deviation, the repeated

255 measures correlation coefficients ( $r_m$ ) were -0.833 after 10-min, and -0.757 after 30-

256 min of exposure ( $p < 0.0001$ , Figure 5A and B). The  $r_m$  were -0.841 after 10-min, and

257 -0.896 after 30-min of exposure for correlation between SpO<sub>2</sub> and its sample entropy

258 ( $p < 0.0001$ , Figure 5C and D).

259

260 << INSERT FIGURE 5 ABOUT HERE >>

261

### 262 **Correlation between SpO<sub>2</sub> variability and dyspnoea**

263 No significant change in dyspnoea was observed in any of the environments (FiO<sub>2</sub>  
264 0.2093, 0.3±0.9 (range: 0.0-3.0), 0.17, 0.3±0.6 (range: 0.0-2.0), 0.145, 0.8±1.5 (range:  
265 0.0-4.0), and 0.12, 1.1±1.2 (range: 0.0-3.0); p > 0.05). However, a significant  
266 correlation between sample entropy and dyspnoea (measured using a modified Borg  
267 scale) was observed in FiO<sub>2</sub> 0.17, 0.145 and 0.12 (see Table 1). Interestingly, sample  
268 entropy at FiO<sub>2</sub> 0.17 was significantly correlated with dyspnoea at FiO<sub>2</sub> 0.145 and  
269 approached significance in FiO<sub>2</sub> 0.12 (r = 0.577, p = 0.063). Mean SpO<sub>2</sub> was not  
270 correlated (p > 0.05) with dyspnoea in any environment.

271

272 <<INSERT TABLE 1 ABOUT HERE>>

273

## 274 **Discussion**

275 The current study is the first to systematically evaluate the effects of graded  
276 normobaric hypoxia on SpO<sub>2</sub> variability in healthy individuals. In support of our initial  
277 hypotheses the main findings of this investigation, are as follows: (1) a strong inverse  
278 correlation between SpO<sub>2</sub> entropy and mean SpO<sub>2</sub> during hypoxia was observed, (2)  
279 SpO<sub>2</sub> sample entropy, but not mean SpO<sub>2</sub>, was correlated with modest levels of  
280 dyspnoea, and (3) SpO<sub>2</sub> sample entropy at FiO<sub>2</sub> 0.17 was correlated with dyspnoea  
281 at FiO<sub>2</sub> 0.145, but not FiO<sub>2</sub> 0.12. This suggests that SpO<sub>2</sub> sample entropy during  
282 moderate levels of hypoxic exposure may be able to provide an insight into an  
283 individual response to a more severe hypoxic challenge.

284 These findings extend our previous work in healthy individuals in a normoxic  
285 environment (where SpO<sub>2</sub> averaged 98 ± 1 %) (Bhogal and Mani, 2017), to a more  
286 severe hypoxic state where SpO<sub>2</sub> values of 79.6 ± 3.6% were recorded in FiO<sub>2</sub> 0.12.

287 Interestingly, we observed a strong inverse linear relationship between sample  
288 entropy and SpO<sub>2</sub> (Figure 5). We have previously reported an inverse relationship  
289 between these two variables, however, these earlier finding were limited to SpO<sub>2</sub>  
290 values >94% (Bhogal and Mani, 2017). Given the importance of maintaining  
291 homeostatic function of arterial oxygenation, it is plausible that SpO<sub>2</sub> variability may  
292 provide an index of central regulation ventilation in adults during hypoxic exposure.  
293 However, it remains unknown if SpO<sub>2</sub> entropy can provide useful diagnostic  
294 information in high altitude medicine and physiology. For example, future research  
295 should consider the relationship between SpO<sub>2</sub> entropy and hypoxic maladaptation  
296 (e.g. low hypoxic ventilator response) and the pathophysiology of acute mountain  
297 sickness during prolonged or more severe hypoxic exposures.

298 Although the precise mechanism(s) for this relationship is currently unknown, we  
299 speculated that this relationship might be explained by the sigmoidal oxyhaemoglobin  
300 saturation curve. Any perturbation or change at a different point of pO<sub>2</sub> (x-axis) would  
301 result in a different corresponding range of haemoglobin saturation (y-axis). Using the  
302 Hill's equation, we generated pO<sub>2</sub> values for further exploratory analysis (<<see  
303 supplementary data>>). Based on this simulation, the plot of mean haemoglobin  
304 saturation plotted against the standard deviation of the SpO<sub>2</sub> data, demonstrated a  
305 linear inverse relationship, which corroborates with our experimental findings.  
306 However, no correlation was found between mean haemoglobin saturation and  
307 sample entropy. Therefore, this exploratory analysis suggests oxyhaemoglobin  
308 saturation curve alone does not explain the SpO<sub>2</sub> entropy data (data not presented).

309 We speculated that the increase in SpO<sub>2</sub> entropy was indicative of the  
310 signal/fluctuations becoming more informative, and not more random. To address this

311 hypothesis, we used multiscale entropy analysis, which calculates sample entropy  
312 after averaging data at different time scales. In a random process (e.g. white noise) a  
313 reduction in entropy in larger scales would be expected, as random fluctuations cancel  
314 out each other during the scaling process (Costa et al., 2005). However, a positive  
315 slope was observed in multiscale entropy analysis (Figure 3B) in the current study and  
316 in our previous work (Bhogal and Mani, 2017). This indicates that the hypoxia-induced  
317 increase in SpO<sub>2</sub> entropy did not deviate to a random process, but rather that the  
318 higher entropy was associated with increased structural richness/information from the  
319 pulse oximetry data. Furthermore, the scaling exponent of the detrended fluctuation  
320 analysis demonstrated that the scaling exponent is close to  $\alpha=1.2$  (Figure 3A) in all  
321 experimental conditions which is markedly higher from than that observed in random  
322 noise ( $\alpha=0.5$ ) (Peng et al., 1995).

323 In addition to the potential application of SpO<sub>2</sub> entropy as a screening tool for those  
324 exposed to extreme environments (e.g. high-altitude medicine), entropy analysis may  
325 have some usefulness in clinical medicine. However, oxygen saturation variability is  
326 typically measured using standard deviation or detrended fluctuation analysis of  
327 oxygen saturation signals in the existing literature (Garde et al., 2016; Vaquerizo-Villar  
328 et al., 2018). Data from this study suggest that entropy is a more effective method of  
329 studying oxygen saturation variability (Figure 2). Although the calculation of standard  
330 deviation is easier than entropy, entropy may provide more insightful information on  
331 the complexity of SpO<sub>2</sub> fluctuations in our data for two reasons: (a) sample entropy  
332 was the only variability index that demonstrated a significant correlation with  
333 dyspnoea, and (b) entropy analysis can distinguish random time-series from complex  
334 time-series

335 To our knowledge, this study is the first to demonstrate that SpO<sub>2</sub> entropy at FiO<sub>2</sub> 0.17,  
336 0.145, and 0.12 was significantly correlated with dyspnoea (all  $p < 0.05$ , Table 1).  
337 Moreover, sample entropy at FiO<sub>2</sub> 0.17 was significantly correlated with dyspnoea at  
338 FiO<sub>2</sub> 0.145 ( $r = 0.811$ ,  $p < 0.01$ ) and approached significance in FiO<sub>2</sub> 0.12 ( $r = 0.577$ ,  
339  $p = 0.063$ ). Interestingly, no such correlations were observed with mean SpO<sub>2</sub> and  
340 dyspnoea. These data suggest that SpO<sub>2</sub> entropy may provide more useful  
341 information, compared to absolute/mean values of oxygen saturation, for predicting  
342 dyspnoea in response to a more severe hypoxic challenge. However, we must  
343 acknowledge that the mean dyspnoea ratings across the four environmental  
344 conditions was relatively modest, where the highest value recorded was four out of  
345 ten, corresponding to 'somewhat severe'. Therefore, future research examining this  
346 relationship when participants experience greater levels of dyspnoea is required.

347 The present study was not without limitation. Firstly, the current findings are limited to  
348 a small sample of healthy male volunteers exposed to normobaric hypoxia. Future  
349 research is required to expand these findings to females and older individuals.  
350 Moreover, future investigations are also required to establish the utility of these novel  
351 insights, for example, the relationship between SpO<sub>2</sub> entropy and clinical outcomes,  
352 when monitoring patients in critical care or those with chronic respiratory diseases  
353 (e.g. COPD). Secondly, the duration of recording physiological variability data is  
354 typically greater than 8-min (e.g. 60-min). Due to methodological constraints this was  
355 not possible in the current study. This information is of practical importance as a  
356 shorter timeframe, i.e.  $\leq 8$ -min as opposed to 60-min, of data recording is feasible in  
357 both a clinical setting and in the field (e.g. at terrestrial high altitude). Finally, despite  
358 elucidating an interesting phenomenon, with multiple potential applications, we  
359 considered that attempting to explain the mechanism(s) of association between mean

360 SpO<sub>2</sub> and entropy outside the scope of the current investigation. However, it is  
361 plausible that increased SpO<sub>2</sub> entropy in response to hypoxia may be related to altered  
362 ventilation. Alternatively, changes in SpO<sub>2</sub> entropy might indicate the degree of  
363 heterogeneity of haemoglobin molecules at different saturations. Detailed  
364 molecular/electrophysiological research on respiratory control centres are therefore  
365 warranted to help improve our mechanistic understanding of the observed effect.

366 In conclusion, this is the first study to systematically evaluate the effects of simulated  
367 graded normobaric hypoxia on SpO<sub>2</sub> variability in healthy individuals. This study is the  
368 first to suggest that that sample entropy may convey valuable, and prompt, predictive  
369 information about the level of hypoxemia and dyspnoea experienced. Further research  
370 is warranted to establish if SpO<sub>2</sub> sample entropy has potential as a non-invasive  
371 outcome measure in clinical settings.

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376

### 377 **Conflicts of interest**

378 The authors have no conflicts of interest.

379

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382

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471

472 **Tables and Figures**

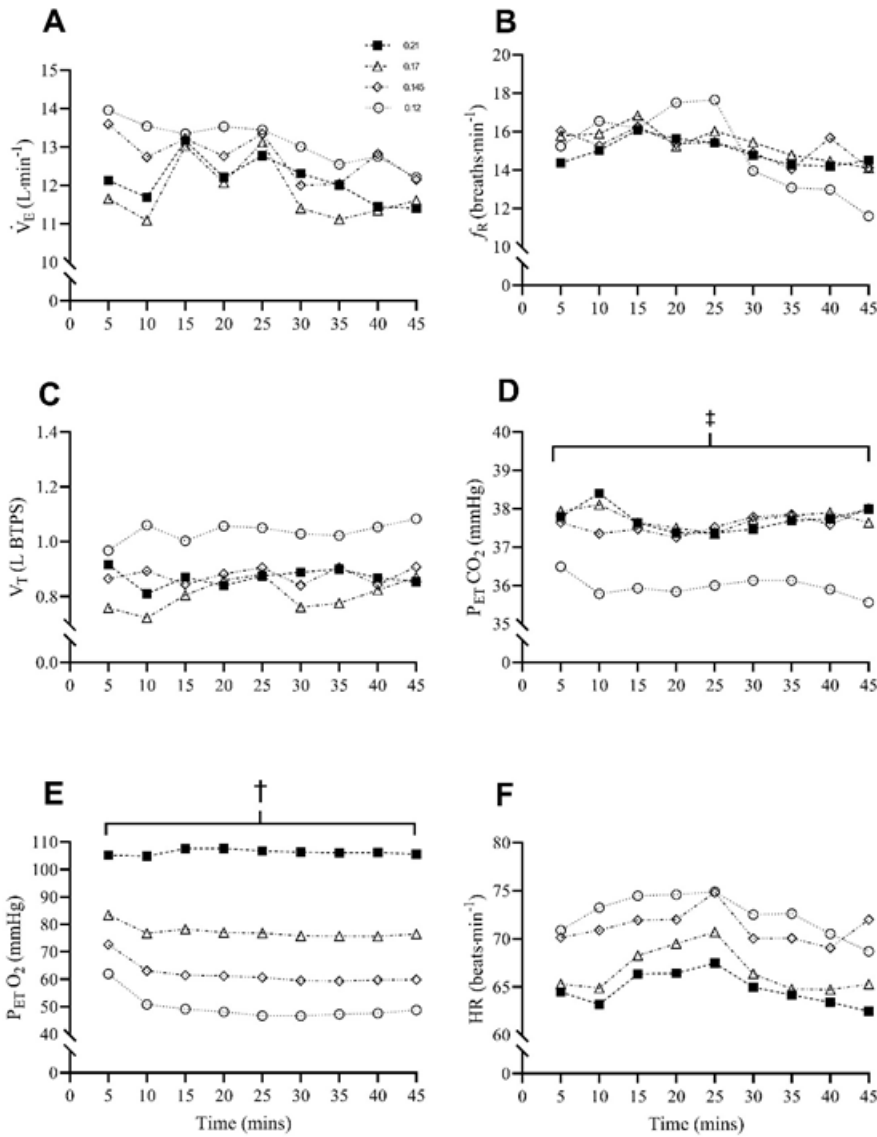
473

474 **Table 1.** Correlation between mean SpO<sub>2</sub> and SpO<sub>2</sub> sample entropy with dyspnoea.

475 The values represent Spearman's correlation coefficient (r). \* P<0.05, \*\* P<0.01.

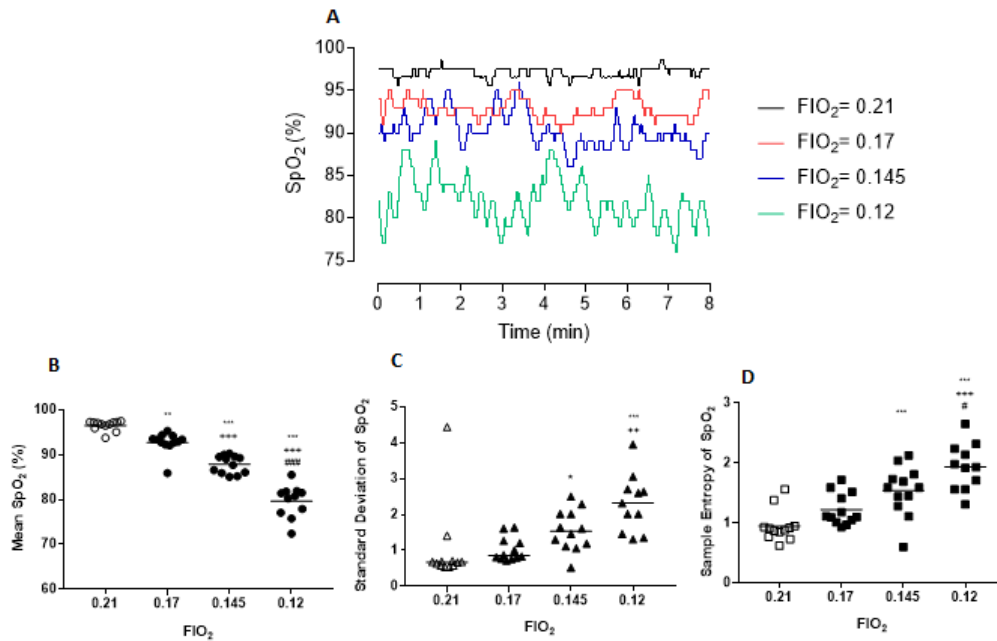
	Dyspnoea (FiO <sub>2</sub> 0.17)	Dyspnoea (FiO <sub>2</sub> 0.145)	Dyspnoea (FiO <sub>2</sub> 0.12)
Mean SpO <sub>2</sub> (FiO <sub>2</sub> 0.17)	-0.261	-0.194	0.044
SpO <sub>2</sub> Sample Entropy (FiO <sub>2</sub> 0.17)	<b>0.760**</b>	<b>0.811**</b>	0.577
Mean SpO <sub>2</sub> (FiO <sub>2</sub> 0.145)	0.083	0.059	0.023
SpO <sub>2</sub> Sample Entropy (FiO <sub>2</sub> 0.145)	0.367	<b>0.636*</b>	0.455
Mean SpO <sub>2</sub> (FiO <sub>2</sub> 0.12)	-0.012	-0.021	-0.279
SpO <sub>2</sub> Sample Entropy (FiO <sub>2</sub> 0.12)	0.320	0.344	<b>0.630*</b>

476



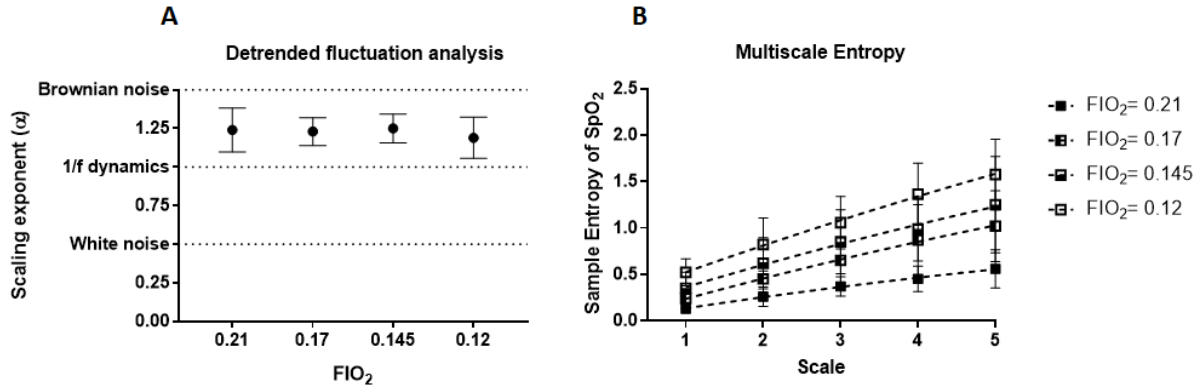
477

478 **Figure 1.** Mean ( $n=12$ ) minute ventilation ( $\dot{V}_E$ ) (A), respiratory frequency ( $f_R$ ) (B), tidal  
 479 volume ( $V_T$ ) (C), end-tidal pressure of  $CO_2$  ( $P_{ET}CO_2$ ) (D) and  $O_2$  ( $P_{ET}O_2$ ) (E), and heart  
 480 rate (F) in  $F_{iO_2}$  0.21 (filled squares), 0.17 (open triangles), 0.145 (open diamonds) and  
 481 0.12 (open circles;  $n=11$ ). SD are omitted for clarity. ‡  $P < 0.03$  for all environments  
 482 compared to  $F_{iO_2}$  0.12. †  $P < 0.001$  for all conditions  $F_{iO_2}$  0.21  $<$  0.17  $<$  0.145  $<$  0.12.



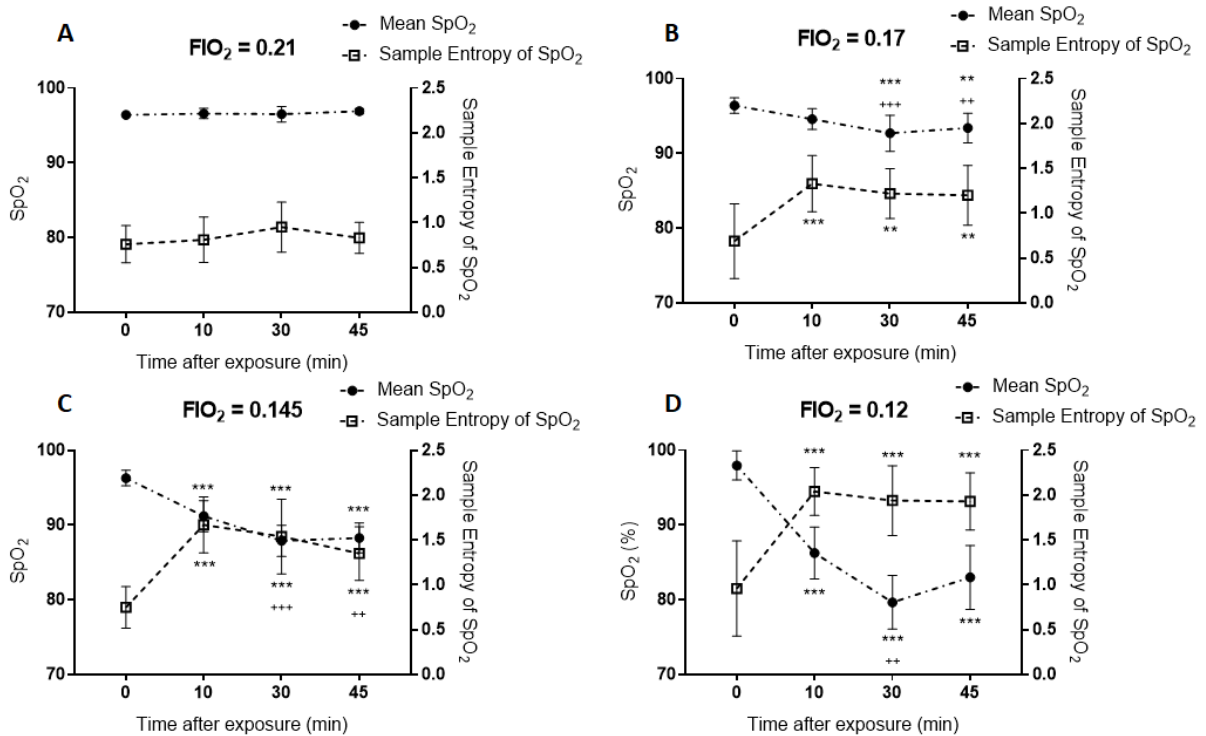
483

484 **Figure 2. A.** Sample SpO<sub>2</sub> signals in a healthy volunteer collected over 8 min after  
485 exposure to normobaric hypoxia. **B.** Changes in mean SpO<sub>2</sub> following 30 min exposure  
486 to different fraction of inspired oxygen (FiO<sub>2</sub>). \*\* P<0.01 in comparison with FiO<sub>2</sub> 0.21,  
487 \*\*\* P<0.001 in comparison with FiO<sub>2</sub> 0.21, +++ P<0.001 in comparison with FiO<sub>2</sub> 0.17,  
488 #### P<0.01 in comparison with FiO<sub>2</sub> 0.145. **C.** Changes in standard deviation of SpO<sub>2</sub>  
489 fluctuations following 30 min exposure to different fraction of inspired oxygen (FiO<sub>2</sub>).  
490 \*\* P<0.01 in comparison with FiO<sub>2</sub> 0.21, ++ P<0.01 in comparison with FiO<sub>2</sub> 0.17. **D.**  
491 Changes in Sample Entropy of SpO<sub>2</sub> fluctuations following 30 min exposure to different  
492 fraction of inspired oxygen (FiO<sub>2</sub>). \*\* P<0.01 in comparison with FiO<sub>2</sub> 0.21, ++ P<0.01  
493 in comparison with FiO<sub>2</sub> 0.17. \*\*\* P<0.001 in comparison with FiO<sub>2</sub> 0.21, +++ P<0.001  
494 in comparison with FiO<sub>2</sub> 0.17.



495

496 **Figure 3. A.** Detrended fluctuation analysis (DFA) of SpO<sub>2</sub> fluctuations after 30 min  
 497 exposure to different fractions of inspired oxygen (FiO<sub>2</sub>). No statistical significance  
 498 between the different conditions. **B.** Multiscale Entropy (MSE) analysis of SpO<sub>2</sub>  
 499 fluctuations after 30 min exposure to different fraction of inspired oxygen (FiO<sub>2</sub>). Two-  
 500 way ANOVA indicated that effect of FiO<sub>2</sub> and scale are both statistically significant  
 501 ( $F_{scale}=19.46, P<0.0001$ ;  $F_{FiO_2}=26.05, P<0.0001$ ).

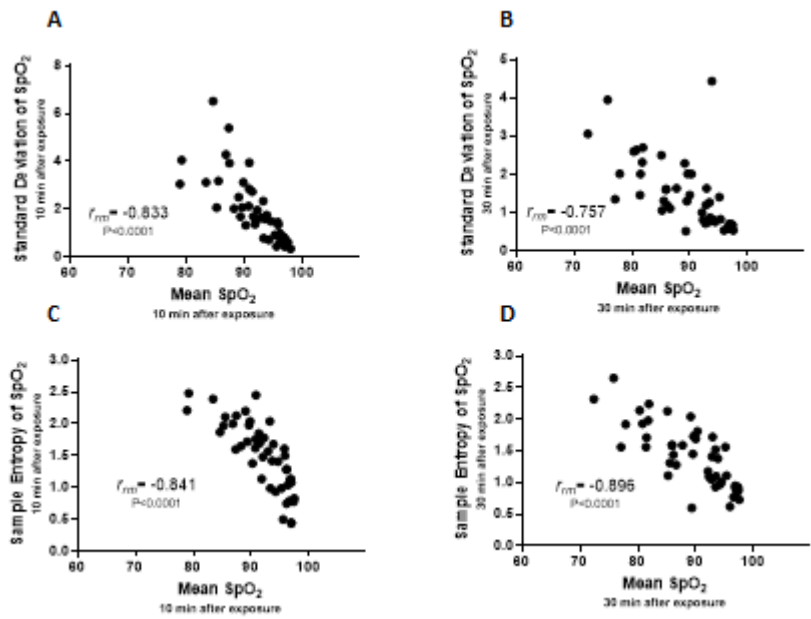


502

503 **Figure 4.** Comparison of the trend of changes in mean SpO<sub>2</sub> and Sample Entropy of  
 504 SpO<sub>2</sub> fluctuations during 45 min exposure to different FIO<sub>2</sub> (A-D). \*\*\* P<0.001 in



505 comparison with time = 0, ++ P<0.01 in comparison with time = 10 min, +++ P<0.001  
506 in comparison with time = 10 min.



507

508 **Figure 5.** The correlation between mean SpO<sub>2</sub> and its variability 10 and 30 min after  
509 exposure to different FIO<sub>2</sub>. **A** and **B.** The relationship between mean SpO<sub>2</sub> and SpO<sub>2</sub>  
510 Standard Deviation (the linear regression equations are  $y = -0.228x + 22.87$  and  $y = -$   
511  $0.087x + 9.275$  for 10 and 30 min respectively). **C** and **D.** The relationship between  
512 mean SpO<sub>2</sub> and SpO<sub>2</sub> Sample Entropy (the linear regression equations are  $y = -$   
513  $0.091x + 9.878$  and  $y = -0.058x + 6.595$  for 10 and 30 min respectively). The  $r_{rm}$  values  
514 represent repeated measure correlation coefficient.

515

516 **Appendix 1.** Simulation of the effect of haemoglobin saturation curve on the relationship between  
517 mean SpO<sub>2</sub> and its variability (i.e. standard deviation and sample entropy)

518

519 **Introduction**

520

521 SpO<sub>2</sub> is a measure of haemoglobin oxygen saturation. We wondered if the relationship between a  
522 decrease in SpO<sub>2</sub> correlating with an increase in SpO<sub>2</sub> variability may be explained by haemoglobin  
523 saturation curve. The haemoglobin saturation curve is nonlinear and is often described by Hill's  
524 equation (Fig S1):

525

526 
$$Hb\text{ Saturation} = \frac{pO_2^n}{k_d + pO_2^n}$$

527

528

529

530 **Figure 1S.** Haemoglobin saturation  
531 curve based on the Hill's equation  
532 with n=2.8 and k<sub>d</sub>=4 kPa.

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549 The sigmoidal shape is due to the binding capacity behaviour of haemoglobin and the nature of the  
550 dissociation curve. This is related co-operative binding behaviour and the requirement for  
551 haemoglobin to release oxygen at low oxygen saturation but bind oxygen at higher oxygen (pO<sub>2</sub>)  
552 concentrations.

553

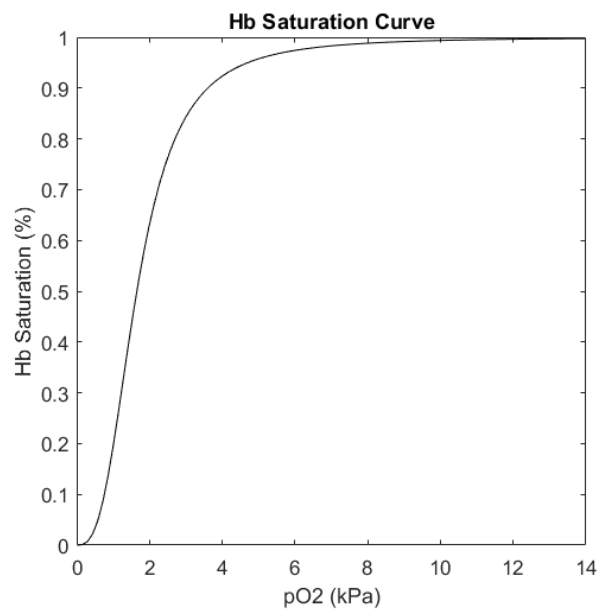
554 Taking this into account, a small perturbation or incremental change at a different point of the x-axis  
555 (pO<sub>2</sub>) would result in a different corresponding range of haemoglobin saturation values. i.e. the  
556 same change in x-values at lower pO<sub>2</sub> values would result in a larger range in y values due to the  
557 changing gradient of the slope, according the equation of the curve. Given this reasoning a  
558 simulation using the Hill's equation and generated pO<sub>2</sub> values were used for further analysis.

559

560 **Method**

561

562 MATLAB programming language was used to generate simulated data and implementation of the  
563 algorithms. Hundred independent normally distributed random pO<sub>2</sub> time-series with 480 data points  
564 were generated to have mean values between 3 and 14 kPa (with standard deviation of 0.1 kPa).  
565 Haemoglobin saturation values were calculated in these hundred pO<sub>2</sub> time-series based on the Hill's

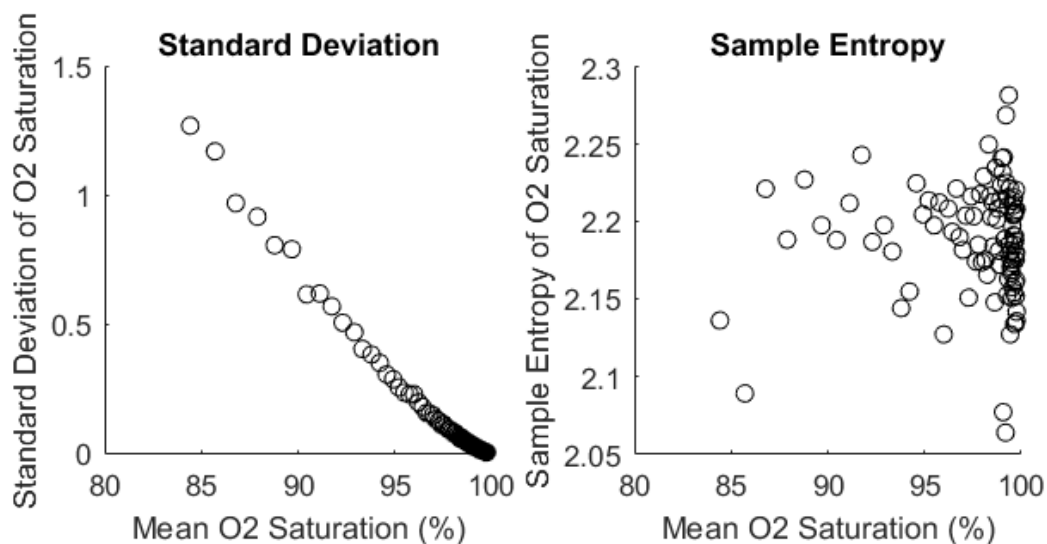


566 equation (with  $n = 2.8$  and  $k_d=4$  kPa as parameters). These values were used to calculate mean,  
567 standard deviation and sample entropy. Mean Haemoglobin saturation was then plotted against  
568 standard deviation and sample entropy.  
569

## 570 Results

571  
572 The plot of mean Haemoglobin Saturation vs Standard deviation showed a linear inverse relationship  
573 with a correlation coefficient of 0.993 ( $p<0.0001$ ) (Figure S). This result supports the trend seen from  
574 the experimental data - a decrease in  $SpO_2$  correlates an increase in variability.  
575

576 However, there was no inverse correlation between Mean Haemoglobin Saturation and its Sample  
577 entropy (Figure S). The plot of Mean Haemoglobin Saturation vs Entropy had a correlation  
578 coefficient of 0.02 ( $p= 0.801$ ). Therefore, this simulation shows that the model of haemoglobin does  
579 not explain the experimental data that we observed.  
580  
581



582  
583  
584 **Figure S.** Correlation between mean  $O_2$  saturation ( $SpO_2$ ) and its Standard deviation (left) or Sample  
585 entropy (right) in a simulation experiment where random fluctuation of  $pO_2$  and the Hill Function  
586 were considered as the only factors influencing  $SpO_2$  variability.  
587

## 588 Interpretation / Limitations

589  
590 The inverse relationship between mean hemoglobin saturation and its standard deviation  
591 corroborates well with the sigmoidal shape of hemoglobin saturation curve. However, it does not  
592 explain the relationship between mean  $SpO_2$  and the pattern (entropy) of hemoglobin saturation. A

593 more complex model may be required to explain the relationship entropy. The model does not take  
 594 into account the influence of chemoreceptors, changes in respiration for example and more broadly  
 595 the network of processes that regulates the highly regulated physiological state. Considering the  
 596 amount of information processing that is exhibited in a human body we felt that global information  
 597 processing may play a larger role. In addition, multiple different models investigating different  
 598 parameters may be required to explain this relationship.

599  
 600

601 The scripts in MATLAB used for simulation of the effect of haemoglobin saturation curve on the  
 602 relationship between mean SpO<sub>2</sub> and its variability.

603

```

604 close all
605 clc
606 clear all
607
608 n=2.8; % a Hill's function parameter
609 Kd =4; % a Hill's function parameter
610 B=linspace(3,14,100);B=B';% different oxygen concertation in kPa
611 T=480; % T is the length of each simulated time-series (480 corresponds to
612 % 8 min recording with a sampling rate of 1/s)
613
614 Y = NaN(480,100);
615 A = NaN(480,100);
616
617 % generation of random fluctuation in [O2] (oxygen concentration)
618
619 for j=1:100
620 for i=1:T
621
622     A (i,j)= B(j,1) + 0.1*randn; % generation of random variation with
623 % standard deviation of 0.1 kPa
624
625 end
626
627 end
628
629 % calculation of haemoglobin saturation using Hill's equation
630 for j=1:100
631 for i=1:T
632
633     Y(i,j) = 100*(A(i,j)^n)/(Kd+A(i,j)^n);
634
635 end
636 end
637
638 % Calculation of sample entropy using sampen function based on m=2 and
639 % r=0.2.
640 % To use this code, you need to have access to sampen function and WFDB
641 % toolbox. sampen is a function to calculate sample entropy and can be
642 % accessed using the following link:
643 % https://www.physionet.org/physiotools/sampen/matlab/1.1/
644 % WFDB toolbox for MATLAB (wfdb-app-toolbox-0-10-0) can be accessed at the
645 % following link: https://physionet.org/physiotools/matlab/wfdb-app-matlab/
646
647
648
649 sam = NaN(100,1);
650 for i=1:100
651     se= sampen (Y(1:T,i),2,0.2);

```

```

652     sam(i,1)=se(2,1);
653
654     end
655
656     m=mean(Y);
657     s=std(Y);
658
659     subplot(1,2,1)
660     scatter(m,s,'ko')
661     axis square
662     title('Standard Deviation')
663     xlabel('Mean O2 Saturation (%)')
664     ylabel('Standard Deviation of O2 Saturation')
665     [r1,p1] = corrcoef(m,s)
666
667     subplot(1,2,2)
668     scatter(m,sam,'ko')
669     axis square
670     title('Sample Entropy')
671     xlabel('Mean O2 Saturation (%)')
672     ylabel('Sample Entropy of O2 Saturation')
673     [r2,p2] = corrcoef(m,sam)
674
675
676
677
678
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683
684

```