TITLE

Effects of Normobaric Hypoxia on Oxygen Saturation Variability

RUNNING TITLE

Hypoxia and SpO_2 entropy

AUTHORS

Joseph T. Costello[1]✉, Amar S. Bhogal[2], Thomas B. Williams[1], Richard Bekoe[2], Amin Sabir[2], Michael J. Tipton[1], Jo Corbett[1], Ali R. Mani[2]

AFFILIATION

[1] Extreme Environments Laboratory, Department of Sport and Exercise Science, University of Portsmouth, Spinnaker Building, Cambridge Road, Portsmouth PO1 2ER, UK

[2] UCL Division of Medicine, University College London, London, United Kingdom

CORRESPONDING AUTHOR

Dr Joseph T Costello, Ph.D.

Address: Department of Sport and Exercise Science, University of Portsmouth, PO1 2ER, United Kingdom.

Email: joe.costello@port.ac.uk

Phone: +44 23 9284 5366

Abstract: The study is the first to evaluate the effects of graded normobaric hypoxia on SpO_2 variability in healthy individuals. Twelve healthy males (mean (SD) age 22 (4) years) were exposed to four simulated environments (FiO_2: 0.12, 0.145, 0.17 and 0.21) for 45-min, in a balanced cross-over design. Sample entropy, a tool that quantifies the irregularity of pulse oximetry fluctuations, was used as a measure of SpO_2 variability. SpO_2 entropy increased as the FiO_2 decreased, and there was a strong significant negative correlation between mean SpO_2 and its entropy during hypoxic exposure ($r = -0.841$ to -0.896, $P < 0.001$). In addition, SpO_2 sample entropy, but not mean SpO_2, was correlated ($r = 0.630$ to 0.760, $P < 0.05$) with dyspnoea in FiO_2 0.17, 0.145, and 0.12 and importantly, SpO_2 sample entropy at FiO_2 0.17 was correlated with dyspnoea at FiO_2 0.145 ($r = 0.811$, $P < 0.01$). These findings suggest that SpO_2 variability analysis may have the potential to be used in a clinical setting as a non-invasive measure to identify the negative sequalae of hypoxaemia.

Key words

Dyspnoea, Oxygen saturation, Pulse oximetry, Sample entropy, SpO_2 variability

Introduction

Tissue hypoxia is a fundamental consequence not only of high-altitude exposure but also of critical illness, where it may occur either as a cause, or as a result of, various pathologies (Berger and Grocott, 2017). Hypoxia also causes a concomitant decrease in SpO_2 through its effects on the arterial partial pressure of oxygen (PaO_2), in accordance with the alveolar gas equation and the oxyhemoglobin dissociation curve. For example, SpO_2 on arrival at terrestrial altitude of 3800m can reach ~90%, and further decline to ~81% after a trek to 5200m (Mellor *et al.* 2015). Similarly, SpO_2 values below 80% are regularly observed in patients in intensive care (Wilson *et al.* 2010; Van de Louw *et al.* 2001). Following the stimulation of aortic-arch chemoreceptors and carotid bodies, the physiological response to hypoxemia is characterized by an increase in cardiac output, ventilation, and haemoglobin concentration (Berger and Grocott, 2017; Wilson *et al.* 2005).

Accumulating evidence indicates that SpO_2 variability analysis is more insightful than mean SpO_2 (Garde *et al.* 2016; Bhogal and Mani, 2017). Using mean or time averaged physiological data does not illuminate the pattern, complexity, and irregularity which is observed in most biological systems, and in the cardiovascular system in particular (Bhogal and Mani, 2017). The majority of oscillations in physiological time-series data are not linear, and recent evidence suggests that these oscillations can provide a useful insight into the activity of the underlying control network (i.e. the cardiovascular system) (Wagner and Persson, 1998). Sample entropy is one method of describing these nonlinear data, and is commonly used to study the dynamics of the cardiovascular system (e.g. heart rate and respiratory rate) (Richman and Moorman, 2000). Briefly, entropy describes the unpredictability and irregularity of time-series

data and allows physiological signals (e.g. heart rate and SpO_2) to be classified over time (Wagner and Persson, 1998; Moorman et al., 2011). Although there are a variety of techniques used when assessing fluctuations in time-series data, entropy is often selected as an index of variability due to its link to information theory (Pincus, 1994). Information theory is the mathematical study of the coding of information in the form of sequences of impulses, and can potentially quantify data within a complex system (Mitchell, 2009, Pincus 1994) (e.g. the cardiovascular system).

This nonlinear analysis may provide useful information on the integrity of the cardiovascular system in both health and disease. Heart rate and respiratory rate variability analysis have previously been used extensively to study the integrity of the cardio-respiratory system with promising applications (Shirazi et al., 2013; Tipton et al., 2017). Recently, Garde et al. (2016) reported that SpO_2 variability data improved the identification of children who were admitted to hospital. Further, Bhogal and Mani (2017) and others (Pham, 2018) have demonstrated that SpO_2 entropy decreases with age and that this can differentiate healthy individuals aged over 35 from their younger counterparts. Increasingly, it appears that variability analysis provides more information about physiological systems compared to absolute or mean values (Garrido et al., 2017). These findings suggest that variability indices have the potential to predict mortality both in healthy individuals and in clinical populations (Tsuji et al., 1994; Mani et al., 2009; Bhogal et al., 2019). However, to our knowledge the use of SpO_2 variability analysis has not been studied empirically within the field of high-altitude physiology and pathophysiology (e.g., Acute Mountain Sickness).

Therefore, the present study sought to characterize the effects of graded normobaric hypoxia on SpO_2 variability in healthy individuals for the first time. Any non-invasive measurement that offer insight into the state of an individual when hypoxic is valuable in multiple clinical settings. Reduced entropy in a physiological setting can be interpreted as less engagement of the components within a control system (Pincus, 1994). In healthy physiological systems, more information processing (i.e. engagement of the regulatory components) in response to environmental challenges such as hypoxia would be expected. As entropy is a measure of information content in complex physiologic time-series, we hypothesised that normobaric hypoxia would increase the entropy of SpO_2 signal in healthy individuals and that SpO_2 entropy and mean SpO_2 would be negatively correlated.

Methods

Ethical approval

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

Experimental design

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

males, (mean [SD] age 22 [4] years, height 1.78 [0.05] m, mass 75 [9] kg, FEV_1/FVC ratio 85 [5] %) volunteered to participate in this study. All participants were non-smokers, free of any cardiovascular, respiratory and cerebrovascular diseases, were not diabetic and were not taking any prescription drugs at the time of or before participation. All participants resided at <1000 m and had not spent time at altitude for at least 1 month prior to commencement of the study, including commercial flights. Participants were instructed to refrain from any strenuous exercise, caffeine or alcohol in the 24 h preceding each visit to the laboratory. In addition, participants were requested to record their dietary intake for 24 h prior to their first visit and to replicate their eating habits for each visit thereafter.

A within participant, balanced cross-over design was employed. Participants were required to visit the laboratory on 5 occasions (one health screening and four experimental trials). For each experimental trial participants were exposed to normobaric hypoxia for 45 minutes in a purpose-built hypoxic chamber (Sporting Edge, Sherfield on Loddon, UK). The fraction of inspired oxygen (FiO_2) values were 0.2093 (sea-level), 0.17 (equivalent to ~1600 m), 0.145 (~3000 m), and 0.12 (4500 m). If end-tidal O_2 ($P_{ET}O_2$) or end-tidal CO_2 ($P_{ET}CO_2$) fell below 45 mmHg and 25 mmHg respectively, for three consecutive breaths, or if SpO_2 went below 65 %, participants were given a supply of normoxic air and subsequently removed from the chamber. Participants were also blinded to which condition they were in. The ambient temperature was maintained at 25 °C and the relative humidity was controlled at 50 % throughout. Experimental trials were separated by a minimum of 48 h and conducted at the same time of day.

Cardiorespiratory responses

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

SpO_2 and SpO_2 variability

SpO_2 was recorded using pulse oximetry on the index finger of the right hand (Nonin 7500, US). Data were continually recorded using an analogue to digital acquisition system with a sampling rate of 1000 Hz using a PowerLab system (ADInstruments, Castle Hill, Australia). The recorded data were extracted using LabChart software and down-sampled by 1000 to $1.s^{-1}$. Data were subsequently down sampled as pulse oximeter readings are not sampled at such a high rate, and thus at that resolution, the variability presented would not reflect true SpO_2 variation. This method is commonly used when assessing SpO_2 entropy (Bhoghal and Mani, 2017, Lazareck and Tarassenko, 2006). The data were visually scanned and any obvious artefacts (e.g. missed or spurious SpO_2 data) were removed (less than 1%). From the recording there were 4 X 8-minute segments of data that were used for analysis. A reading prior to exposure, a recording once exposed to the altered FiO_2, a third reading at 30-min of exposure, and finally one after 45-min of exposure.

The oxygen saturation data were analysed using linear (e.g. standard deviation) and non-linear methods (e.g. entropy measures) written in MATLAB (MathWorks, R2017a). For each 8-min segment the mean SpO_2 and standard deviation were calculated as tools to understand the overall variability. We also employed sample

entropy, detrended fluctuation analysis (DFA) and multiscale entropy (MSE) as measures of complexity in SpO_2 fluctuations (Richman and Moorman, 2000; Costa et al., 2005). Sample entropy is a tool that quantifies the degree of irregularity present in a dataset by calculating the probability that an event with window length, m, and degree of tolerance, r, will be repeated at later time. In present study m was set at 2 and r at 0.2 as described by Richman and Moorman (2000). Many physiological time-series (e.g. heart rate, respiratory rate and SpO_2) show a fractal-like pattern of fluctuations (Raoufy et al., 2016; Bhoghal and Mani., 2017; Bhogal et al., 2019). Fractals exhibit similar patterns at increasingly small scale. A variety of methods have been developed to quantify fluctuation of physiological signals at different time scales. Detrended fluctuation analysis examines the self-similarity of a time series to determine the structural integrity of the signal at different time scales (Peng et al., 1995). In this analysis the data are split into boxes of various lengths (n) and this is plotted against the F(n), which is the variability of detrended signals in different scales (n). The slope of the resulting log-log graph is known as “scaling exponent” which indicates the type of fractal-like dynamics present in the physiological signal (Peng et al., 1995). Another method which takes scaling into account is multiscale entropy. Multiscale entropy is an extension of sample entropy and fractal analysis, as it examines the sample entropy at different time scales (Costa et al., 2005). The data are averaged within window length consisting of a number of data points to create a coarse-grained time-series (Costa et al., 2005). The sample entropy of this is then calculated and plotted against the window length (Costa et al., 2005). The trend of changes in entropy in different scales gives information about complexity of a data set. Compared to a previous report we used multiscale entropy to five scales due to the 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_{rm}) 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 ± standard deviation (SD) unless otherwise
207 stated.

208

209 **Results**

210 One participant was removed from the chamber in F_IO_2 0.12 ($P_{ET}O2$ fell below 45
211 mmHg). Therefore, the following analyses are for the 12 participants in F_IO_2 0.2093,
212 0.17, and 0.145 and 11 participants in F_IO_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_2 ($P_{ET}CO_2$) and O_2 ($P_{ET}O_2$), and heart rate data are displayed across the four
217 environments in Figure 1.

218

219 <u><< INSERT FIGURE 1 ABOUT HERE>></u>

220

221 **SpO_2 and SpO_2 variability**

222 An example of SpO_2 signals at different FiO_2 is displayed in Figure 2A. The oxygen
223 saturation readings exhibit more fluctuations with lower FiO_2. Figures 2 also depict
224 SpO_2 (Figure 2B), SpO_2 standard deviation (Figure 2C), and sample entropy (Figure
225 2D). An increase in standard deviation of SpO_2 fluctuations and a concomitant
226 increase in sample entropy was observed when FiO_2 was decreased (Figures 2C and
227 D). Detrended fluctuation analysis demonstrates that the scaling exponent (α) was
228 consistent across all FiO_2 conditions and no significant differences were observed
229 (Figure 3A). Finally, the relationship between multiscale entropy and FiO_2, a measure
230 of complexity, is displayed in Figure 3B. SpO_2 entropy increases as the scale
231 increases. This indicates that the pattern of SpO_2 fluctuations in 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_2 ($P<0.0001$) 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 <u><< INSERT FIGURE 2 ABOUT HERE>></u>

<< INSERT FIGURE 3 ABOUT HERE>>

Intra time-series analysis

Figures 4A-D demonstrate the temporal changes of SpO_2 and SpO_2 variability. Sample entropy is more responsive to the hypoxic stimulus when compared to the mean oxygen saturation. The sample entropy plateaus ~20 minutes before mean oxygen saturation in the three hypoxic environments. No significant correlations between SpO_2 variability at FiO_2 0.2093 and mean SpO_2 at FiO_2 0.17, 0.145, or 0.12 were observed. Similarly, no significant correlation between SpO_2 variability at FiO_2 0.17 and mean SpO_2 at FiO_2 0.145 or 0.12 was observed.

<< INSERT FIGURE 4 ABOUT HERE>>

The relationship between mean SpO_2 and SpO_2 variability

Linear regression analysis demonstrated that the relationships between mean SpO_2 and SpO_2 standard deviation or sample entropy were strongly correlated (Figure 5). For the correlation between mean SpO2 and its standard deviation, the repeated measures correlation coefficients (r_{rm}) were -0.833 after 10-min, and -0.757 after 30-min of exposure ($p<0.0001$, Figure 5A and B). The r_{rm} were -0.841 after 10-min, and -0.896 after 30-min of exposure for correlation between SpO_2 and its sample entropy ($p<0.0001$, Figure 5C and D).

<< INSERT FIGURE 5 ABOUT HERE>>

Correlation between SpO_2 variability and dyspnoea

No significant change in dyspnoea was observed in any of the environments (FiO_2 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: 0.0-4.0), and 0.12, 1.1±1.2 (range: 0.0-3.0); $p > 0.05$). However, a significant correlation between sample entropy and dyspnoea (measured using a modified Borg scale) was observed in FiO_2 0.17, 0.145 and 0.12 (see Table 1). Interestingly, sample entropy at FiO_2 0.17 was significantly correlated with dyspnoea at FiO_2 0.145 and approached significance in FiO_2 0.12 ($r = 0.577$, $p = 0.063$). Mean SpO_2 was not correlated ($p > 0.05$) with dyspnoea in any environment.

<<INSERT TABLE 1 ABOUT HERE>>

Discussion

The current study is the first to systematically evaluate the effects of graded normobaric hypoxia on SpO_2 variability in healthy individuals. In support of our initial hypotheses the main findings of this investigation, are as follows: (1) a strong inverse correlation between SpO_2 entropy and mean SpO_2 during hypoxia was observed, (2) SpO_2 sample entropy, but not mean SpO_2, was correlated with modest levels of dyspnoea, and (3) SpO_2 sample entropy at FiO_2 0.17 was correlated with dyspnoea at FiO_2 0.145, but not FiO_2 0.12. This suggests that SpO_2 sample entropy during moderate levels of hypoxic exposure may be able to provide an insight into an individual response to a more severe hypoxic challenge.

These findings extend our previous work in healthy individuals in a normoxic environment (where SpO_2 averaged 98 ± 1 %) (Bhogal and Mani, 2017), to a more severe hypoxic state where SpO_2 values of 79.6 ± 3.6% were recorded in FiO_2 0.12.

Interestingly, we observed a strong inverse linear relationship between sample entropy and SpO_2 (Figure 5). We have previously reported an inverse relationship between these two variables, however, these earlier finding were limited to SpO_2 values >94% (Bhogal and Mani, 2017). Given the importance of maintaining homeostatic function of arterial oxygenation, it is plausible that SpO_2 variability may provide an index of central regulation ventilation in adults during hypoxic exposure. However, it remains unknown if SpO_2 entropy can provide useful diagnostic information in high altitude medicine and physiology. For example, future research should consider the relationship between SpO_2 entropy and hypoxic maladaptation (e.g. low hypoxic ventilator response) and the pathophysiology of acute mountain sickness during prolonged or more severe hypoxic exposures.

Although the precise mechanism(s) for this relationship is currently unknown, we speculated that this relationship might be explained by the sigmoidal oxyhaemoglobin saturation curve. Any perturbation or change at a different point of pO_2 (x-axis) would result in a different corresponding range of haemoglobin saturation (y-axis). Using the Hill's equation, we generated pO_2 values for further exploratory analysis (<<see supplementary data>>). Based on this simulation, the plot of mean haemoglobin saturation plotted against the standard deviation of the SpO_2 data, demonstrated a linear inverse relationship, which corroborates with our experimental findings. However, no correlation was found between mean haemoglobin saturation and sample entropy. Therefore, this exploratory analysis suggests oxyhaemoglobin saturation curve alone does not explain the SpO_2 entropy data (data not presented).

We speculated that the increase in SpO_2 entropy was indicative of the 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_2 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_2 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_2 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

To our knowledge, this study is the first to demonstrate that SpO_2 entropy at FiO_2 0.17, 0.145, and 0.12 was significantly correlated with dyspnoea (all $p < 0.05$, Table 1). Moreover, sample entropy at FiO_2 0.17 was significantly correlated with dyspnoea at FiO_2 0.145 ($r = 0.811$, $p < 0.01$) and approached significance in FiO_2 0.12 ($r = 0.577$, $p = 0.063$). Interestingly, no such correlations were observed with mean SpO_2 and dyspnoea. These data suggest that SpO_2 entropy may provide more useful information, compared to absolute/mean values of oxygen saturation, for predicting dyspnoea in response to a more severe hypoxic challenge. However, we must acknowledge that the mean dyspnoea ratings across the four environmental conditions was relatively modest, where the highest value recorded was four out of ten, corresponding to 'somewhat severe'. Therefore, future research examining this relationship when participants experience greater levels of dyspnoea is required.

The present study was not without limitation. Firstly, the current findings are limited to a small sample of healthy male volunteers exposed to normobaric hypoxia. Future research is required to expand these findings to females and older individuals. Moreover, future investigations are also required to establish the utility of these novel insights, for example, the relationship between SpO_2 entropy and clinical outcomes, when monitoring patients in critical care or those with chronic respiratory diseases (e.g. COPD). Secondly, the duration of recording physiological variability data is typically greater than 8-min (e.g. 60-min). Due to methodological constraints this was not possible in the current study. This information is of practical importance as a shorter timeframe, i.e. ≤ 8-min as opposed to 60-min, of data recording is feasible in both a clinical setting and in the field (e.g. at terrestrial high altitude). Finally, despite elucidating an interesting phenomenon, with multiple potential applications, we considered that attempting to explain the mechanism(s) of association between mean

SpO_2 and entropy outside the scope of the current investigation. However, it is plausible that increased SpO_2 entropy in response to hypoxia may be related to altered ventilation. Alternatively, changes in SpO_2 entropy might indicate the degree of heterogeneity of haemoglobin molecules at different saturations. Detailed molecular/electrophysiological research on respiratory control centres are therefore warranted to help improve our mechanistic understanding of the observed effect.

In conclusion, this is the first study to systematically evaluate the effects of simulated graded normobaric hypoxia on SpO_2 variability in healthy individuals. This study is the first to suggest that that sample entropy may convey valuable, and prompt, predictive information about the level of hypoxemia and dyspnoea experienced. Further research is warranted to establish if SpO_2 sample entropy has potential as a non-invasive outcome measure in clinical settings.

Acknowledgements

We would like to thank the participants for volunteering for this study. We also wish to thank Danny White, Geoff Long and Harry Mayes for their technical assistance and Christopher Richards for his help with data collection.

Conflicts of interest

The authors have no conflicts of interest.

FUNDING

The present study received no external funding.

References

384 Bakdash JZ and Marusich LR (2017). Repeated measures correlation. Front Psychol
385 8:456.
386 Berger MM, Grocott MPW (2017). Facing acute hypoxia: from the mountains to critical
387 care medicine. Br J Anaesth 118:283–6.
388 Bhogal AS, Mani AR (2017). Pattern analysis of oxygen saturation variability in healthy
389 individuals: entropy of pulse oximetry signals carries information about mean oxygen
390 saturation. Front Physiol 8:555.
391 Bhogal AS, De Rui M, Pavanello D, El-Azizi I, Rowshan S, Amodio P, Montagnese S,
392 Mani AR (2019). Which heart rate variability index is an independent predictor of
393 mortality in cirrhosis. Dig Liver Dis 5:695-702.
394 Bland JM, and Altman DG (1995). Calculating correlation coefficients with repeated
395 observations. Part 1—correlation within subjects. Br Med J 310:446.
396 Costa M, Goldberger AL, Peng CK (2005). Multiscale entropy analysis of biological
397 signals. Phys Rev E 89:1–8.
398 Dipietro JA, Caughy MO, Cusson R, Fox NA (1994). Cardiorespiratory functioning of
399 preterm infants: Stability and risk associations for measures of heart rate variability
400 and oxygen saturation. Dev Psychobiol 27:137–52.
401 Garde A, Zhou G, Raihana S, Dunsmuir D, Karlen W, Dekhordi P, ... Ansermino JM
402 (2016). Respiratory rate and pulse oximetry derived information as predictors of
403 hospital admission in young children in Bangladesh: a prospective observational
404 study. BMJ Open 6:e011094.
405 Garrido M, Saccardo D, De Rui M, Vettore E, Verardo A, Carraro P, ... Montagnese S
406 (2017). Abnormalities in the 24-hour rhythm of skin temperature in cirrhosis: Sleep-
407 wake and general clinical implications. Liver Int 37:1833-1842.

Gholami M, Mazaheri P, Mohamadi A, Dehpour T, Safari F, Hajizadeh S … Mani AR (2012). Endotoxemia is associated with partial uncoupling of cardiac pacemaker from cholinergic neural control in rats. Shock 37:219-27.

Lazareck L and Tarassenko L. Detection of apnoeic and breathing activity through pole-zero analysis of the SpO_2 signal," (2006); in 28th IEEE EMBS Ann. Int. Conf., New York City, USA, 3879-3882.

Mani AR, Montagnese S, Jackson CD, Jenkins CW, Head IM, Stephens RC ... Morgan MY (2009). Decreased heart rate variability in patients with cirrhosis relates to the presence and degree of hepatic encephalopathy. Am J Physiol Gastrointest Liver Physiol 296:G330-8.

Mellor AJ, Boos CJ, Ball S, Burnett A, Pattman S, … and Woods DR (2015). Copeptin and arginine vasopressin at high altitude: relationship to plasma osmolality and perceived exertion. Eur J Appl Physiol 115:91–98.

Mitchell M. Complexity: A guided tour. (2009) New York: Oxford University Press.

Moorman JR, Delos JB, Flower AA, Cao H, Kovatchev BP, Richman JS, Lake DE (2011). Cardiovascular oscillations at the bedside: early diagnosis of neonatal sepsis using heart rate characteristics monitoring. Physiol Meas 32:1821–32.

Paggiaro P (2004). Does early treatment of exacerbation improve outcome in chronic obstructive pulmonary disease? Am J Respir Crit Care Med 169:1267–8.

Peng CK, Havlin S, Stanley HE, Goldberger AL (1995). Quantification of scaling exponents and crossover phenomena in nonstationary heartbeat time series. Chaos 5:82–7.

Pham TD (2018). Pattern analysis and classification of blood oxygen saturation signals with nonlinear dynamics features. International Conference on Biomedical and Health Informatics 112–5.

433 Pincus SM (1994). Greater signal regularity may indicate increased system isolation.
434 Math Biosci 122:161–81.
435 Raoufy MR, Ghafari T, Darooei R, Nazari M, Mahdaviani SA, Eslaminejad AR,
436 Almasnia M, Gharibzadeh S, Mani AR, Hajizadeh S (2016). Classification of Asthma
437 Based on Nonlinear Analysis of Breathing Pattern. PLoS One 11:e0147976.
438
439 Richman JS, Moorman JR (2000). Physiological time-series analysis using
440 approximate entropy and sample entropy. Am J Physiol Heart Circ Physiol
441 278:H2039–49.
442 Shirazi AH, Raoufy MR, Ebadi H, De Rui M, Schiff S, Mazloom R, … Mani AR (2013).
443 Quantifying Memory in Complex Physiological Time-Series. PLoS One 8:e72854.
444 Tipton M, Harper A, Paton JFR, and Costello JT (2017). The human ventilatory
445 response to stress: rate or depth? J Physiol 595:5729-5752.
446 Tsuji H, Venditti FJ Jr, Manders ES, Evans JC, Larson MG, … and Levy D (1994).
447 Reduced heart rate variability and mortality risk in an elderly cohort. The Framingham
448 Heart Study. Circulation 90:878-83.
449 Vaquerizo-Villar F, Álvarez D, Kheirandish-Gozal L, Gutiérrez-Tobal GC, Barroso-
450 García V, Crespo A, Del Campo F, Gozal D, Hornero R (2018). Detrended fluctuation
451 analysis of the oximetry signal to assist in paediatric sleep apnoea-hypopnoea
452 syndrome diagnosis. Physiol Meas 39:114006.
453 Van de Louw A, Cracco C, Cerf C, Harf A, Duvaldestin P, … and Brochard L (2001).
454 Accuracy of pulse oximetry in the intensive care unit. Intensive Care Med 27:1606–
455 1613.
456 Wagner CD, and Persson PB (1998). Chaos in the cardiovascular system: an update.
457 Cardiovascular Res 40:257–264.

458 Williams TB, Corbett J, McMorris T, Young JS, Dicks M, Ando S, Thelwell RC, Tipton
459 MJ, Costello JT (2019). Cognitive performance is associated with cerebral
460 oxygenation and peripheral oxygen saturation, but not plasma catecholamines, during
461 graded normobaric hypoxia. Exp Physiol 1–14. https://doi.org/10.1113/
462 EP087647
463 Wilson BJ, Cowan HJ, Lord JA, Zuege DJ, and Zygun DA. The accuracy of pulse
464 oximetry in emergency department patients with severe sepsis and septic shock: a
465 retrospective cohort study. BMC Emerg Medicine (2010); 10: 9.
466 Wilson RC, & Jones PW. A comparison of the visual analogue scale and modified
467 Borg scale for the measurement of dyspnoea during exercise. Clinical Science.
468 (1989); 76(3): 277–282.
469 Wilson DF, Roy A, and Lahiri S. Immediate and long-term responses of the carotid
470 body to high altitude. High Alt Med Biol (2005); 6: 97-111.
471

Tables and Figures

Table 1. Correlation between mean SpO_2 and SpO_2 sample entropy with dyspnoea. The values represent Spearman's correlation coefficient (r). * P<0.05, ** P<0.01.

	Dyspnoea (FiO_2 0.17)	Dyspnoea (FiO_2 0.145)	Dyspnoea (FiO_2 0.12)
Mean SpO_2 (FiO_2 0.17)	-0.261	-0.194	0.044
SpO_2 Sample Entropy (FiO_2 0.17)	**0.760****	**0.811****	0.577
Mean SpO_2 (FiO_2 0.145)	0.083	0.059	0.023
SpO_2 Sample Entropy (FiO_2 0.145)	0.367	**0.636***	0.455
Mean SpO_2 (FiO_2 0.12)	-0.012	-0.021	-0.279
SpO_2 Sample Entropy (FiO_2 0.12)	0.320	0.344	**0.630***

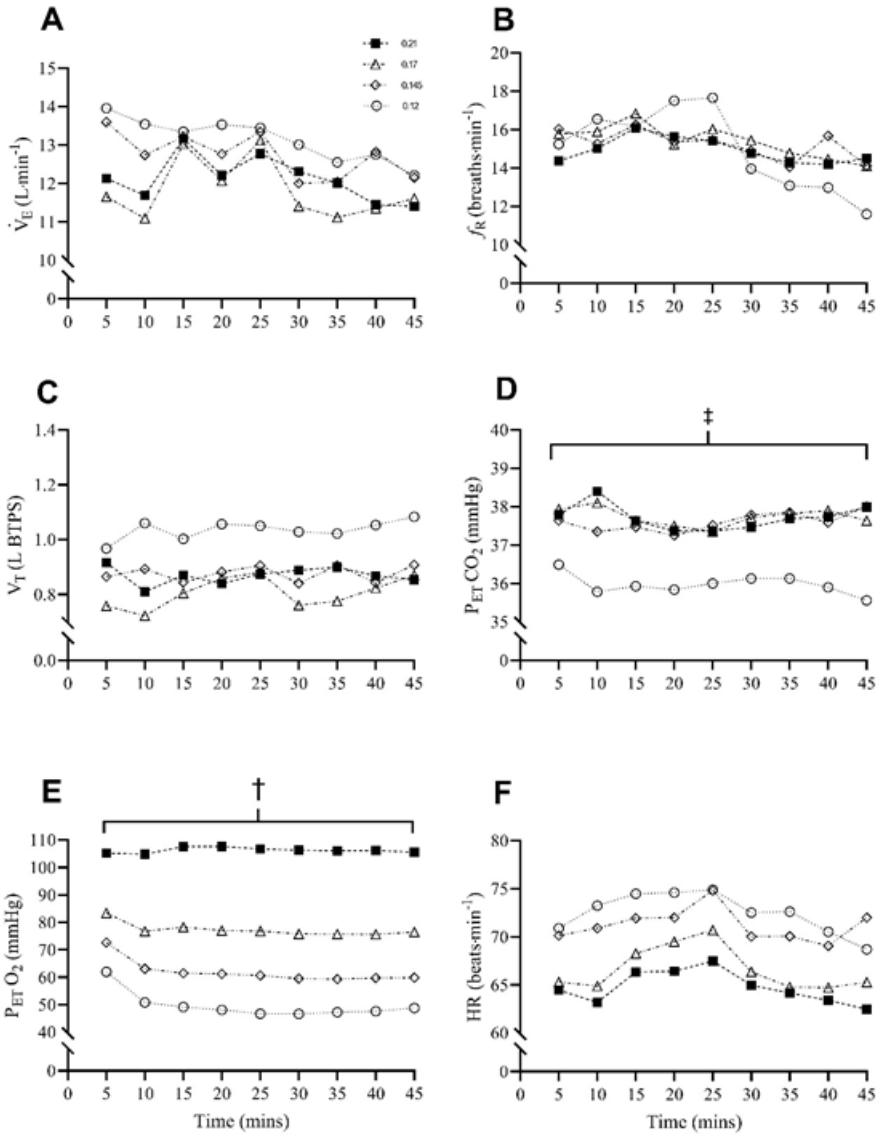
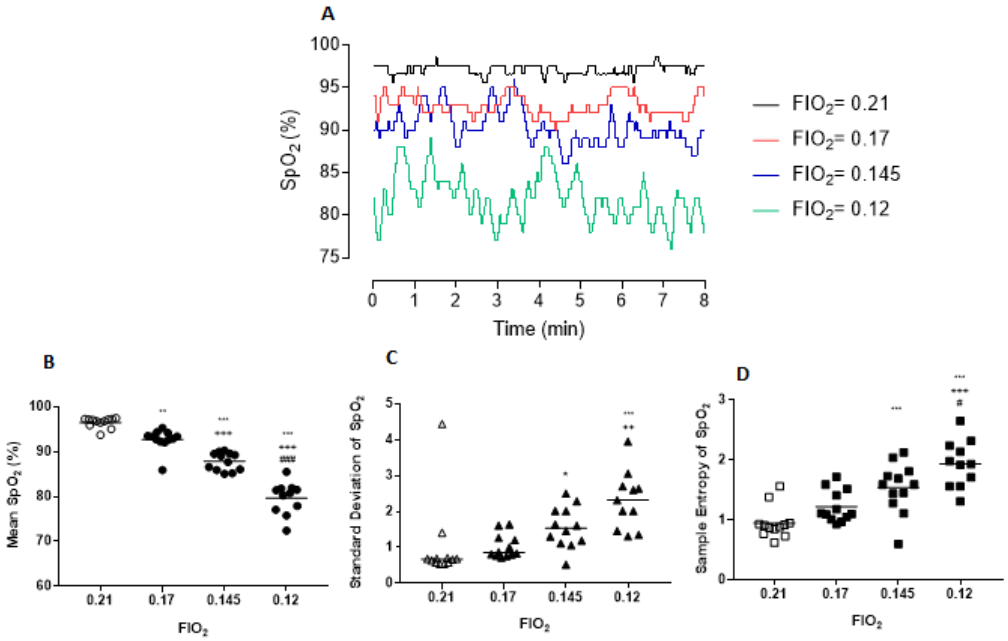


Figure 1. Mean (n=12) minute ventilation ($\dot{V}_E$) (A), respiratory frequency (f_R) (B), tidal volume (V_T) (C), end-tidal pressure of CO_2 ($P_{ET}CO_2$) (D) and O_2 ($P_{ET}O_2$) (E), and heart rate (F) in FiO_2 0.21 (filled squares), 0.17 (open triangles), 0.145 (open diamonds) and 0.12 (open circles; n=11). SD are omitted for clarity. ‡ P<0.03 for all environments compared to FiO_2 0.12. † P <0.001 for all conditions FiO_2 0.21<0.17<0.145<0.12.



483

484 **Figure 2. A.** Sample SpO_2 signals in a healthy volunteer collected over 8 min after
485 exposure to nomobaric hypoxia. **B.** Changes in mean SpO_2 following 30 min exposure
486 to different fraction of inspired oxygen (FiO_2). ** P<0.01 in comparison with FiO_2 0.21,
487 *** P<0.001 in comparison with FiO_2 0.21, +++ P<0.001 in comparison with FiO_2 0.17,
488 ### P<0.01 in comparison with FiO_2 0.145. **C**. Changes in standard deviation of SpO_2
489 fluctuations following 30 min exposure to different fraction of inspired oxygen (FiO_2).
490 ** P<0.01 in comparison with FiO_2 0.21, ++ P<0.01 in comparison with FiO_2 0.17. **D.**
491 Changes in Sample Entropy of SpO_2 fluctuations following 30 min exposure to different
492 fraction of inspired oxygen (FiO_2). ** P<0.01 in comparison with FiO_2 0.21, ++ P<0.01
493 in comparison with FiO_2 0.17. *** P<0.001 in comparison with FiO_2 0.21, +++ P<0.001
494 in comparison with FiO_2 0.17.

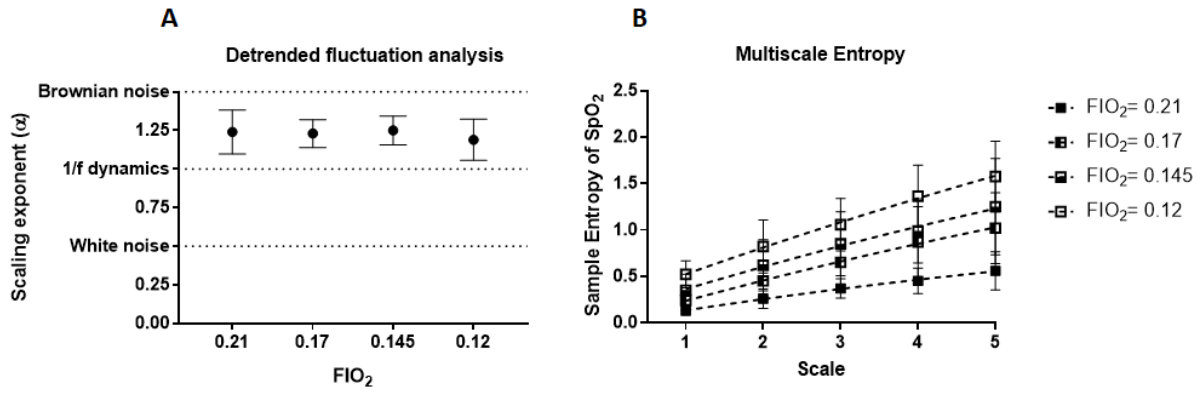


Figure 3. **A**. Detrended fluctuation analysis (DFA) of SpO_2 fluctuations after 30 min exposure to different fractions of inspired oxygen (FiO_2). No statistical significance between the different conditions. **B**. Multiscale Entropy (MSE) analysis of SpO_2 fluctuations after 30 min exposure to different fraction of inspired oxygen (FiO_2). Two-way ANOVA indicated that effect of FiO_2 and scale are both statistically significant (F_{scale}=19.46, P<0.0001; F_{FiO2}=26.05, P<0.0001).

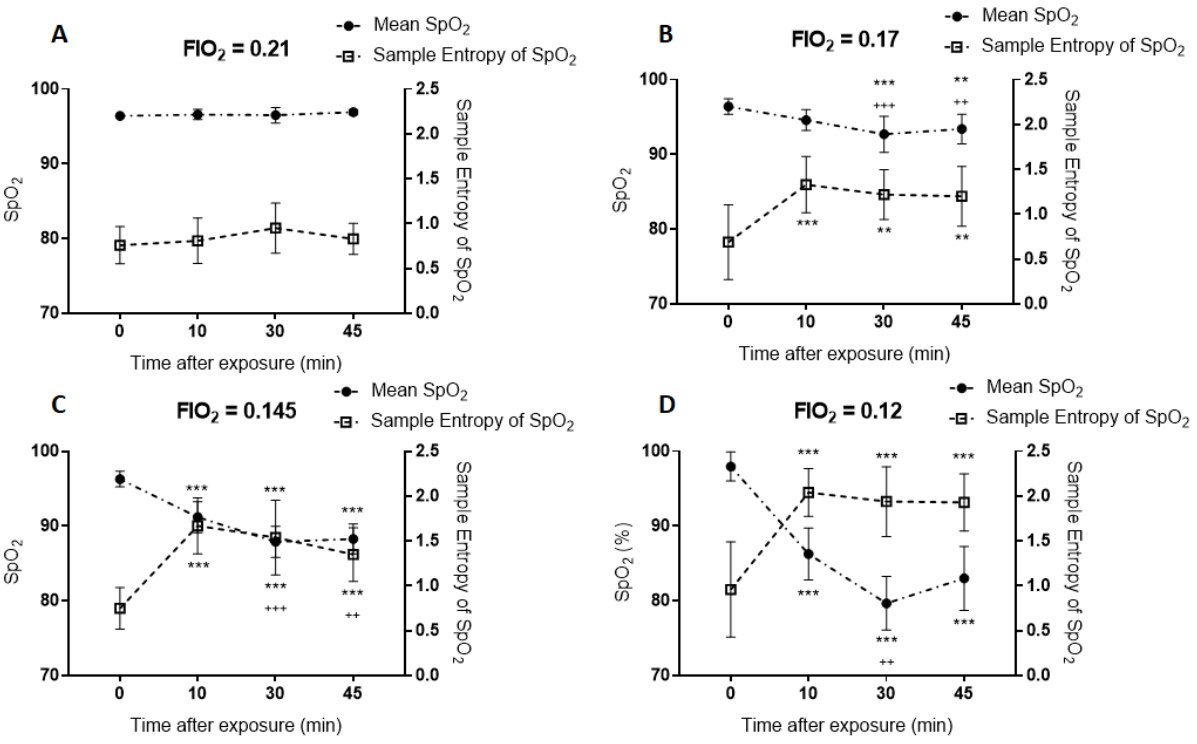
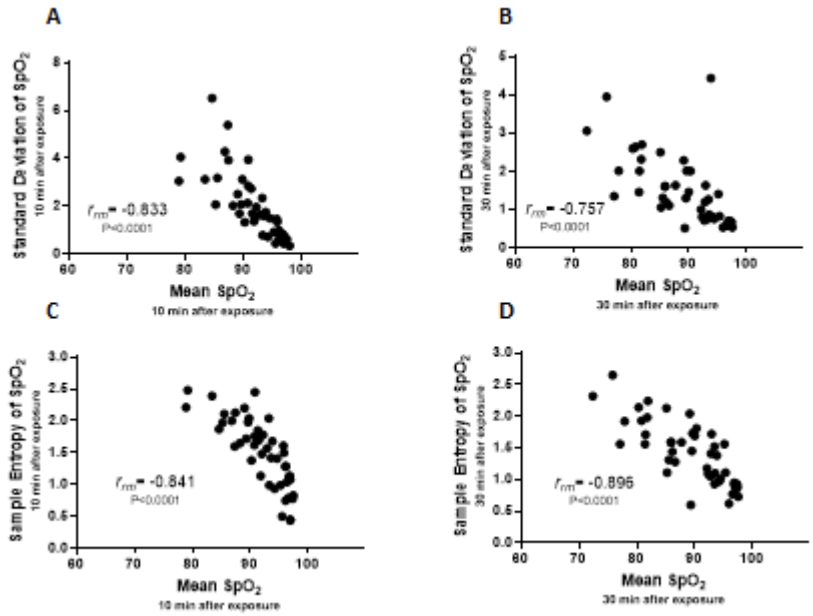


Figure 4. Comparison of the trend of changes in mean SpO_2 and Sample Entropy of SpO_2 fluctuations during 45 min exposure to different FIO_2 (A-D). *** P<0.001 in

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_2 and its variability 10 and 30 min after
509 exposure to different FIO_2. **A** and **B**. The relationship between mean SpO_2 and SpO_2
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_2 and SpO_2 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

Appendix 1. Simulation of the effect of haemoglobin saturation curve on the relationship between mean SpO_2 and its variability (i.e. standard deviation and sample entropy)

Introduction

SpO_2 is a measure of haemoglobin oxygen saturation. We wondered if the relationship between a decrease in SpO_2 correlating with an increase in SpO_2 variability may be explained by haemoglobin saturation curve. The haemoglobin saturation curve is nonlinear and is often described by Hill's equation (Fig S1):

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

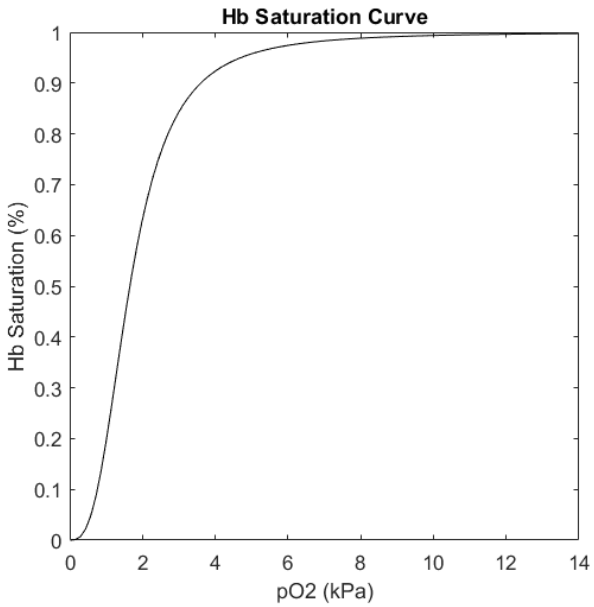


Figure 1S. Haemoglobin saturation curve based on the Hill's equation with n=2.8 and k_d=4 kPa.

The sigmoidal shape is due to the binding capacity behaviour of haemoglobin and the nature of the dissociation curve. This is related co-operative binding behaviour and the requirement for haemoglobin to release oxygen at low oxygen saturation but bind oxygen at higher oxygen (pO_2) concentrations.

Taking this into account, a small perturbation or incremental change at a different point of the x-axis (pO_2) would result in a different corresponding range of haemoglobin saturation values. i.e. the same change in x-values at lower pO_2 values would result in a larger range in y values due to the changing gradient of the slope, according the equation of the curve. Given this reasoning a simulation using the Hill's equation and generated pO_2 values were used for further analysis.

Method

MATLAB programming language was used to generate simulated data and implementation of the algorithms. Hundred independent normally distributed random pO_2 time-series with 480 data points were generated to have mean values between 3 and 14 kPa (with standard deviation of 0.1 kPa). Haemoglobin saturation values were calculated in these hundred pO_2 time-series based on the Hill's

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

Results

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

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

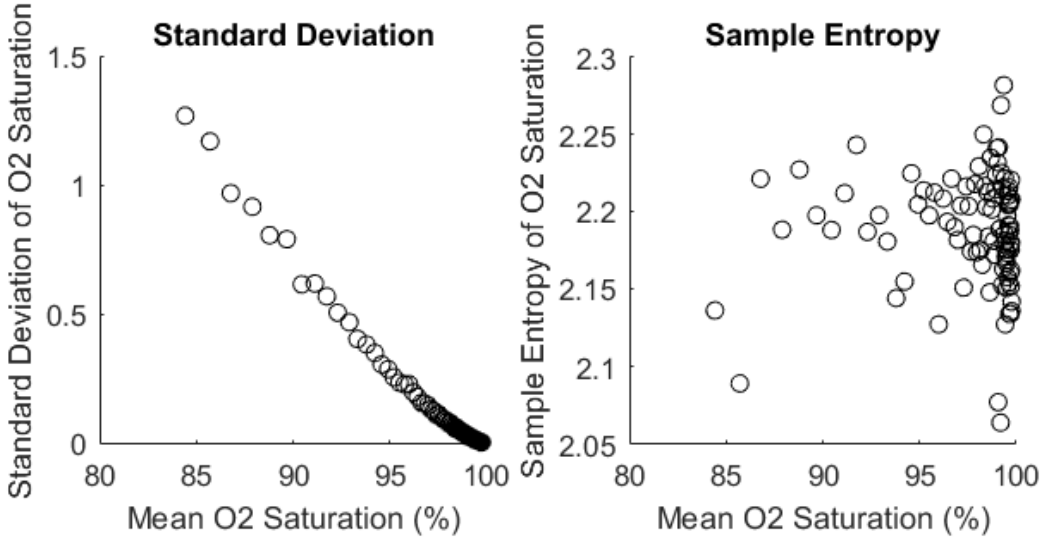


Figure S. Correlation between mean O_2 saturation (SpO_2) and its Standard deviation (left) or Sample entropy (right) in a simulation experiment where random fluctuation of pO_2 and the Hill Function were considered as the only factors influencing SpO2 variability.

Interpretation / Limitations

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

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

The scripts in MATLAB used for simulation of the effect of haemoglobin saturation curve on the relationship between mean SpO_2 and its variability.

```
close all
clc
clear all

n=2.8; % a Hill's function parameter
Kd =4; % a Hill's function parameter
B=linspace(3,14,100);B=B';% different oxygen concertation in kPa
T=480; % T is the length of each simulated time-series (480 corresponds to
% 8 min recording with a sampling rate of 1/s)

Y = NaN(480,100);
A = NaN(480,100);

% generation of random fluctuation in [O2] (oxygen concentration)

for j=1:100
for i=1:T

    A (i,j)= B(j,1) + 0.1*randn; % generation of random variation with
% standard deviation of 0.1 kPa

end

end

% calculation of haemoglobin saturation using Hill's equation
for j=1:100
for i=1:T

    Y(i,j) = 100*(A(i,j)^n)/(Kd+A(i,j)^n);

end
end

% Calculation of sample entropy using sampen function based on m=2 and
% r=0.2.
% To use this code, you need to have access to sampen function and WFDB
% toolboox. sampen is a function to calculate sample entropy and can be
% accessed using the following link:
% https://www.physionet.org/physiotools/sampen/matlab/1.1/
% WFDB toolboox for MATLAB (wfdb-app-toolbox-0-10-0) can be accessed at the
% following link: https://physionet.org/physiotools/matlab/wfdb-app-matlab/



sam = NaN(100,1);
for i=1:100
   se= sampen (Y(1:T,i),2,0.2);
```

```
    sam(i,1)=se(2,1);

    end

m=mean(Y);
s=std(Y);

subplot(1,2,1)
scatter(m,s,'ko')
axis square
title('Standard Deviation')
xlabel('Mean O2 Saturation (%)')
ylabel('Standard Deviation of O2 Saturation')
[r1,p1] = corrcoef(m,s)

subplot(1,2,2)
scatter(m,sam, 'ko')
axis square
title('Sample Entropy')
xlabel('Mean O2 Saturation (%)')
ylabel('Sample Entropy of O2 Saturation')
[r2,p2] = corrcoef(m,sam)
```