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SHORT COMMUNICATION

A network physiology approach to oxygen saturation variability during normobaric hypoxia

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Abstract

Peripheral capillary oxygen saturation (S_{pO_2}) exhibits a complex pattern of fluctuations during hypoxia. The physiological interpretation of S_{pO_2} variability is not well understood. In this study, we tested the hypothesis that S_{pO_2} fluctuation carries information about integrated cardio-respiratory control in healthy individuals using a network physiology approach. We explored the use of transfer entropy in order to compute the flow of information between cardio-respiratory signals during hypoxia. Twelve healthy males (mean (SD) age 22 (4) years) were exposed to four simulated environments (fraction of inspired oxygen (F_{IO_2}): 0.12, 0.145, 0.17, and 0.2093) for 45 min, in a single blind randomized controlled design. The flow of information between different physiological parameters (S_{pO_2}, respiratory frequency, tidal volume, minute ventilation, heart rate, end-tidal pressure of O_2 and CO_2) were analysed using transfer entropy. Normobaric hypoxia was associated with a significant increase in entropy of the S_{pO_2} time series. The transfer entropy analysis showed that, particularly at F_{IO_2} 0.145 and 0.12, the flow of information between S_{pO_2} and other physiological variables exhibits a bidirectional relationship. While reciprocal interactions were observed between different cardio-respiratory parameters during hypoxia, S_{pO_2} remained the main hub of this network. S_{pO_2} fluctuations during graded hypoxia exposure carry information about cardio-respiratory control. Therefore, S_{pO_2} entropy analysis has the potential for non-invasive assessment of the functional connectivity of respiratory control system in various healthcare settings.

KEYWORDS
altitude, hypoxic, sample entropy, S_{pO_2}, transfer entropy

1 | INTRODUCTION

Peripheral capillary oxygen saturation (S_{pO_2}) is measured non-invasively and is extensively used for monitoring patients in clinical settings. Although the absolute value of S_{pO_2} is currently used by clinicians, S_{pO_2} time series exhibit a complex pattern of fluctuations which may carry useful information (Bhogal & Mani, 2017). For example, it has previously been reported that the inclusion of

S_{pO_2} variability data into routine clinical practice can predict the requirement of hospitalization in children with acute illness (Garde et al., 2016). Likewise a recent study has demonstrated that S_{pO_2} fluctuation exhibits a fractal-like pattern which can assist in the diagnosis of sleep apnoea (Vaquerizo-Villar et al., 2018).

The complexity of physiological time series data can be measured by computing the degree of irregularity of the signal (i.e. entropy) (Pincus, 1991). By applying entropy analysis to S_{pO_2} time series data, we have previously demonstrated that S_{pO_2} entropy, and not its absolute value (or mean), can distinguish older healthy individuals from their younger counterparts (Bhogal & Mani, 2017). More recently, we have demonstrated that as the concentration of inspired oxygen (F_{IO_2}) is decreased, S_{pO_2} entropy increases, and there is a strong significant negative correlation between mean S_{pO_2} and its entropy during normobaric hypoxic exposure (Costello et al., 2020). In addition, S_{pO_2} entropy, but not mean S_{pO_2}, was correlated with perception of breathlessness in otherwise healthy individuals when hypoxic (Costello et al., 2020).

The entropy of a physiological time series is a measure of how much total information the signal contains (Mitchell, 2009; Pincus, 1994). Thus, it is reasonable to suggest that S_{pO_2} fluctuations may carry information about the integrity of the respiratory system. Although this hypothesis is plausible, it has not been systematically investigated using empirical data. Respiration is tightly controlled and requires transfer of information from chemoreceptors, which are centrally and peripherally located. Tissue oxygenation involves a complex network of feedback loops that integrate different components of both the cardiovascular and respiratory systems. Thus, respiratory rate, tidal volume, heart rate and partial pressure of oxygen and carbon dioxide interact in a non-linear fashion to maintain respiratory homeostasis (Tipton, Harper, Paton, & Costello, 2017). We have recently described the physiological response to graded normobaric hypoxia in healthy individuals and found that while S_{pO_2} variability is significantly increased, mean respiratory rate, tidal volume and heart rate were not markedly altered during graded hypoxia when resting (Costello et al., 2020). After completing these analyses and publishing the findings (Costello et al., 2020), we subsequently became curious to discover if useful information is present in the cardio-respiratory time series which is not reflected in their mean values. An ideal method for assessing the complex interactions in a control system is to measure the causal relationship between physiological signals. This can be achieved by measuring the flow of information between parallel time series. Schreiber developed an analytical tool (i.e. transfer entropy) to detect the directed exchange of information between two systems (Schreiber, 2000). Several groups have extended this concept and demonstrated that measures of information transfer may serve as proxies for causal interactions (Barnett, Barrett, & Seth, 2009; Wibral, Vicente, & Lindner, 2014). This motivated us to look at the interaction between S_{pO_2} and other cardio-respiratory time series during a hypoxic challenge using transfer entropy.

Network physiology is an emerging field that reveals the topology of functional interactions between different components of physiological systems (Bashan, Bartsch, Kantelhardt, Havlin, & Ivanov, 2012; Kanter

New Findings

- **What is the central question of this study?**
  What is the physiological interpretation of S_{pO_2} fluctuations observed during normobaric hypoxia in healthy individuals?

- **What is the main finding and its importance?**
  There is a significant flow of information between S_{pO_2} and other cardio-respiratory time series during graded hypoxia. Analysis of the pattern of S_{pO_2} variations has potential for non-invasive assessment of the engagement of respiratory control system in health and disease.

et al., 2015). By measuring the information that is exchanged between different physiological parameters, we could potentially assess the connectivity of physiological control (Buchman, 2002; Pincus, 1994). Transfer entropy has the potential to compute bidirectional interaction between cardio-respiratory time series and thus reveal the network of interaction between different physiological parameters (Faes, Marinazzo, Montalto, & Nollo, 2014; Marzbanrad, Kimura, Palaniswami, & Khandoker, 2015). Therefore, in this study, we tested the hypothesis that S_{pO_2} fluctuation carries information about cardio-respiratory control in healthy individuals using a network physiology approach. In order to address this hypothesis, the engagement of different physiological parameters, i.e. respiratory frequency (f_R), tidal volume (V_T), minute ventilation ($\dot{V}_E$), heart rate (HR), S_{pO_2}, end-tidal pressure of O_2 (P_{ETO_2}) and CO_2 (P_{ETCO_2}) were analysed using transfer entropy in healthy volunteers who were exposed to normobaric hypoxia.

2 | METHOD

2.1 | Ethics

All participants provided their written informed consent before taking part in this 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).

2.2 | 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 (Costello

et al., 2020; Williams et al., 2019). A convenience sample of 12 healthy males participated in this study, with mean (SD) age 22 (4) years, height 1.78 (0.05) m, mass 75 (9) kg, FEV1/FVC ratio 85 (5)%. 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. A within-participant, single blind randomized controlled design was employed. Participants were required to visit the laboratory on five occasions (one health screening and four experimental conditions). For each experimental condition, participants were exposed to normobaric hypoxia for 45 min in a purpose-built hypoxic chamber (Sporting Edge, Sherfield on Loddon, UK). The fraction of inspired oxygen (F_{IO_2}) values were 0.2093 ($\approx$ sea level), 0.17 (equivalent to ~1600 m), 0.145 (~3000 m), and 0.12 (~4500 m). If P_{ETO_2} or P_{ETCO_2} fell below 45 and 25 mmHg, respectively, for three consecutive breaths, or if S_{pO_2} went below 65%, participants were given a supply of normoxic air and subsequently removed from the chamber. Experimental conditions were separated by a minimum of 48 h and conducted at the same time of day.

2.3 | Physiological recording

Participants' cardiorespiratory parameters, including f_R, V_T, $\dot{V}_E$, HR, S_{pO_2} (using an ear clip), P_{ETO_2} and P_{ETCO_2}, were measured and monitored non-invasively for 45 min using a metabolic cart (Quark CPET, Cosmed, Rome, Italy). During each experimental condition participants wore an oro-nasal facemask for complete breath collection (7400 series Vmask, Hans Rudolph, Shawnee, KS, USA). The sampling rate was one sample per respiratory cycle. We have previously demonstrated that it takes ~15 min for participants to acclimate with the experimental setting during each condition (Costello et al., 2020). Thus, time series taken in the last 30 min were used for analysis in this study (i.e. minutes 15–45). A digital filter was developed in MATLAB (The MathWorks Inc., Natick, MA, USA, R2019b) and applied to remove any missing data and replace them with the overall average value of the data thread. Data were discarded if missing data equated to more than 5% of total length of the time series. Accordingly, one participant had more than 5% missing data and was subsequently discarded from the analyses.

2.3.1 | Sample entropy calculation

Sample entropy of physiological time series was calculated using an algorithm developed in MATLAB (Goldberger et al., 2000). Sample entropy is a measure of irregularity of a time series by calculating the logarithmic likelihood that a sequence with window length, m, and degree of tolerance, r, will be repeated at a later time. In the present analysis, m and r were set at 2 (window length) and 0.2 (0.2 × SD) as described previously (Bhogal & Mani, 2017; Richman & Moorman, 2000).

2.3.2 | Transfer entropy

Transfer entropy reflects the measures of causal relationship between two parallel time series (Barnett et al., 2009; Schreiber, 2000). In this study, it was employed to quantify the level of directed influence and information transfer that a data segment of one physiological time series can have on the future progress of another in a different time series. We used an open source function in MATALB to compute the transfer entropy between two parallel time series (Lee et al., 2012; code of this function can be found at PhysioNet: https://www.physionet.org/content/tewp/1.0.0/; Goldberger et al., 2000). Probability density estimation was based on Gaussian kernel density estimation. Calculation of transfer entropy requires parameters such as the time lag, which was set to 1. The number of equally spaced points along each dimension where probabilities were estimated was set to 10 (Lee et al., 2012).

2.3.3 | Statistical analysis

Data are presented as the mean (SD), unless otherwise stated. The distribution of data was assessed using descriptive methods (skewness, outliers and distribution plots) and inferential statistics (Shapiro–Wilk test). A one-way ANOVA followed by Tukey's *post hoc* test was used to compare the physiological indices at different F_{IO_2} values. Statistical analyses were carried out using MATLAB and GraphPad Prism (version 7, GraphPad Software Inc., San Diego, CA USA). $P < 0.05$ was considered statistically significant.

2.4 | Network visualization

The directed transfer entropy values that physiological time series (i.e. f_R, V_T, $\dot{V}_E$, HR, S_{pO_2}, P_{ETO_2} and P_{ETCO_2}) exerted on each other following exposure to an F_{IO_2} of 0.17, 0.145, 0.12 were compared against those at sea level (F_{IO_2}: ~0.2093–0.21). With the values obtained, any significant value in transfer entropy calculation was then compiled to form an adjacency matrix for each F_{IO_2}. If there was no statistically significant difference in transfer entropy in comparison of with F_{IO_2} 0.21, a transfer entropy of zero was considered in the adjacency matrix. This matrix was used to plot a directed graph. The codes for calculation of transfer entropy and plotting the network were written in MATLAB.

3 | RESULTS

Eleven participants completed the study (45 min conditions × 4 sessions). One participant was removed from the chamber in F_{IO_2} 0.12 (P_{ETO_2} fell below 45 mmHg). A sample representing 30 min recording of physiological signals during hypoxia is demonstrated in Figure 1.

Table 1 displays the changes in mean f_R, V_T, $\dot{V}_E$, HR, S_{pO_2}, P_{ETO_2} and P_{ETCO_2} at the various F_{IO_2} values. While mean f_R, V_T, $\dot{V}_E$ and HR did not
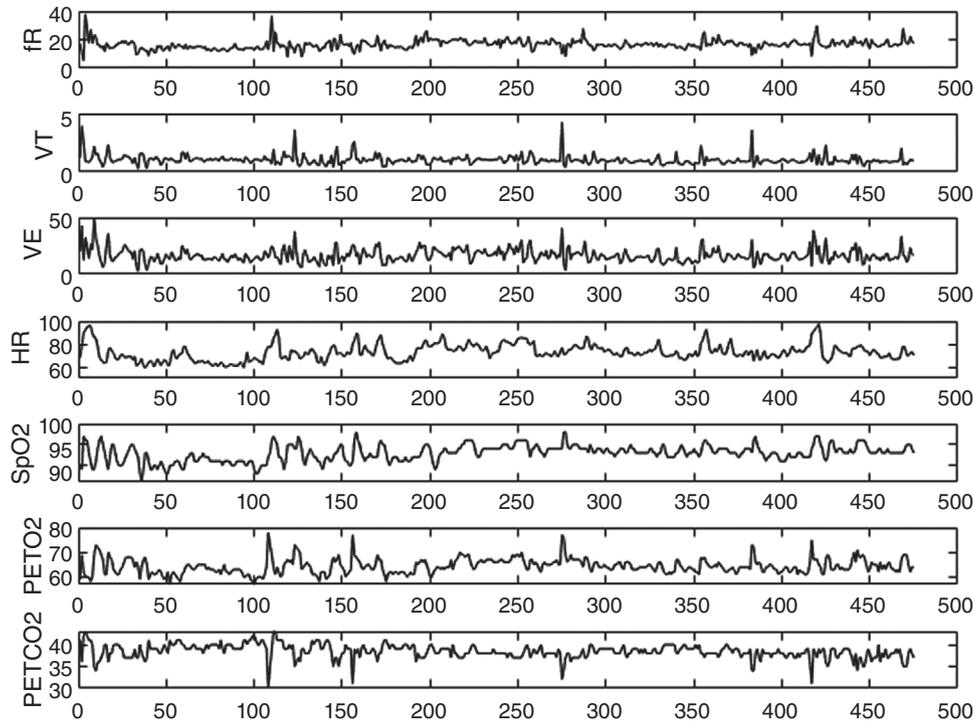
FIGURE 1 A sample representing fluctuations of physiological signals during normobaric hypoxia (length of recording 30 min, F_{IO_2}: 0.145). f_R, respiratory frequency; HR, heart rate; P_{ETCO_2}, end-tidal pressure of CO_2; P_{ETO_2}, end-tidal pressure of O_2; S_{pO_2}, peripheral capillary oxygen saturation; $\dot{V}_E$, minute ventilation; V_T, tidal volume

TABLE 1 Comparison of mean value of physiological parameters during graded normobaric hypoxia

	F_{IO_2}				
	0.21	**0.17**	**0.145**	**0.12**	**P (ANOVA)**
f_R (breath min^{-1})	16.68 ± 2.73	17.24 ± 2.64	17.63 ± 3.67	17.08 ± 5.01	0.943
V_T (l)	0.88 ± 0.28	0.82 ± 0.20	0.88 ± 0.16	0.97 ± 0.31	0.588
$\dot{V}_E$ (l)	12.78 ± 1.89	12.86 ± 2.37	14.17 ± 2.78	14.58 ± 4.01	0.355
HR (beat min^{-1})	66.01 ± 8.66	69.89 ± 11.77	74.14 ± 10.02	76.20 ± 11.18	0.120
S_{pO_2} (%)	99.51 ± 0.55^c	98.22 ± 1.00^c	93.94 ± 1.85^a	$86.22 \pm 2.80^{a,c}$	**<0.0001**
P_{ETO_2} (mmHg)	106.87 ± 1.78^b	77.31 ± 3.37^a	$61.61 \pm 5.31^{a,b}$	$49.53 \pm 3.07^{a,b,c}$	**<0.0001**
P_{ETCO_2} (mmHg)	37.24 ± 1.69	37.00 ± 1.70	36.39 ± 2.41	$34.22 \pm 3.34^{a,b}$	**0.0198**

Data are presented as means $\pm$ SD ($n = 11$). *Post hoc* analysis: [a]$P < 0.05$ in comparison with $F_{IO_2} = 0.21$, [b]$P < 0.05$ in comparison with $F_{IO_2} = 0.17$, [c]$P < 0.05$ in comparison with $F_{IO_2} = 0.145$. f_R, respiratory frequency; HR, heart rate; P_{ETCO_2}, end-tidal pressure of CO_2; P_{ETO_2}, end-tidal pressure of O_2; S_{pO_2}, peripheral capillary oxygen saturation; $\dot{V}_E$, minute ventilation; V_T, tidal volume.

change during the hypoxic challenge, there were significant changes in mean S_{pO_2}, P_{ETO_2} and P_{ETCO_2} during normobaric hypoxia.

Sample entropy of the physiological time series are detailed in Table 2. S_{pO_2} sample entropy, calculated from S_{pO_2} signals using an ear clip oximeter with a resolution of one sample per respiratory cycle, increased as the concentration of inspired oxygen decreased ($P < 0.0001$). This finding is in agreement with our previous report where S_{pO_2} was recorded using a finger pulse oximeter with a sampling rate of 1 Hz (Costello et al., 2020). None of the other measured physiological time series exhibited any alteration in their sample entropy following the hypoxic challenge.

Graphical presentation of directed transfer entropy between different physiological parameters is shown in Figure 2. These networks were plotted based on the adjacency matrices of transfer entropy at different F_{IO_2} values in comparison with normoxia (Tables 3 and 4). At an inspired oxygen concentration of 0.17, there was a significant increase in transfer entropy for $S_{pO_2} \rightarrow \dot{V}_E$ and $S_{pO_2} \rightarrow V_T$ in comparison with transfer entropy in F_{IO_2} 0.21 (Figure 2a). At lower F_{IO_2} exposures (i.e. 0.145 and 0.12) transfer entropy between S_{pO_2} and other physiological time series increased markedly in comparison to transfer entropy in F_{IO_2} 0.21 (Figures 2b,c). The highest information flow was detected for $P_{ETO_2} \rightarrow S_{pO_2}$, which were 0.23 and 0.22 bits

TABLE 2 Comparison of sample entropy of physiological parameters during graded normobaric hypoxia

	F_{IO_2}				
	0.21	0.17	0.145	0.12	P (ANOVA)
f_R entropy	1.62 ± 0.19	1.64 ± 0.16	1.71 ± 0.19	1.77 ± 0.22	0.234
V_T entropy	1.12 ± 0.37	1.24 ± 0.31	1.32 ± 0.28	1.48 ± 0.38	0.095
$\dot{V}_E$ entropy	1.57 ± 0.27	1.65 ± 0.18	1.74 ± 0.21	1.72 ± 0.33	0.394
HR entropy	1.21 ± 0.30	1.10 ± 0.30	1.06 ± 0.32	1.16 ± 0.27	0.652
S_{pO_2} entropy	0.09 ± 0.17^b	0.42 ± 0.27^b	0.98 ± 0.28^a	1.33 ± 0.47^a	**<0.0001**
P_{ETO_2} entropy	1.48 ± 0.43	1.58 ± 0.41	1.55 ± 0.45	1.57 ± 0.40	0.946
P_{ETCO_2} entropy	1.24 ± 0.41	1.24 ± 0.39	1.21 ± 0.35	1.43 ± 0.35	0.498

Data are presented as means $\pm$ SD ($n = 11$). *Post hoc* analysis: [a]$P < 0.05$ in comparison with $F_{IO_2} = 0.21$, [b]$P < 0.05$ in comparison with $F_{IO_2} = 0.145$. f_R, respiratory frequency; HR, heart rate; P_{ETCO_2}, end-tidal pressure of CO_2; P_{ETO_2}, end-tidal pressure of O_2; S_{pO_2}, peripheral capillary oxygen saturation; $\dot{V}_E$, minute ventilation; V_T, tidal volume.
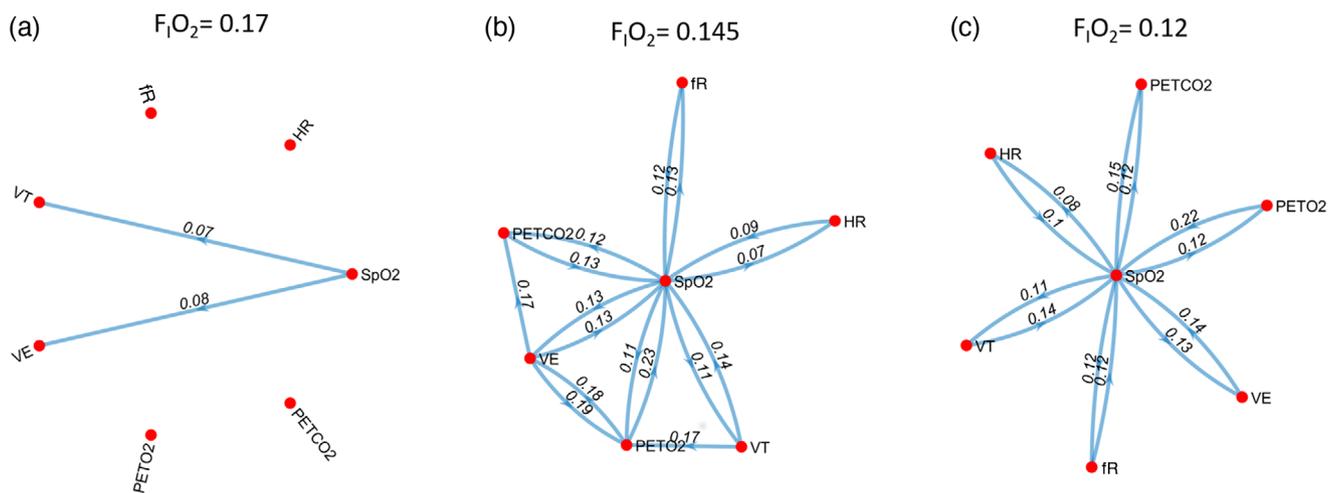

FIGURE 2 Graphical presentation of directed transfer entropy between different physiological parameters as the concentration of inspired oxygen decreases ($n = 11$). (a) $F_{IO_2} = 0.17$, (b) $F_{IO_2} = 0.145$, and (c) $F_{IO_2} = 0.12$. Each node represents a physiological time series. Network edges (links) represent the link between two variables if there is a statistically significant difference in transfer entropy in comparison with $F_{IO_2} = 0.21$. The number on each edge represent transfer entropy (bits). f_R, respiratory frequency; HR, heart rate; P_{ETCO_2}, end-tidal pressure of CO_2; P_{ETO_2}, end-tidal pressure of O_2; S_{pO_2}, peripheral capillary oxygen saturation; $\dot{V}_E$, minute ventilation; V_T, tidal volume

TABLE 3 Transfer entropy between physiological variables (horizontal → vertical) at $F_{IO_2} = 0.21$

	S_{pO_2}	HR	f_R	V_T	$\dot{V}_E$	P_{ETO_2}	P_{ETCO_2}
S_{pO_2}		0.029 ± 0.009	0.024 ± 0.005	0.022 ± 0.009	0.024 ± 0.006	0.052 ± 0.018	0.037 ± 0.028
HR	$0.025 + 0.021$		0.103 ± 0.030	0.084 ± 0.031	0.092 ± 0.027	0.081 ± 0.022	0.075 ± 0.020
f_R	0.036 ± 0.041	0.140 ± 0.049		0.103 ± 0.030	0.129 ± 0.039	0.139 ± 0.032	0.122 ± 0.033
V_T	0.029 ± 0.035	0.087 ± 0.033	0.089 ± 0.029		0.081 ± 0.037	0.096 ± 0.024	0.090 ± 0.032
$\dot{V}_E$	0.028 ± 0.026	0.128 ± 0.040	0.143 ± 0.046	0.083 ± 0.047		0.128 ± 0.039	0.114 ± 0.029
P_{ETO_2}	0.021 ± 0.026	0.105 ± 0.026	0.103 ± 0.022	0.102 ± 0.027	0.123 ± 0.040		0.060 ± 0.013
P_{ETCO_2}	0.043 ± 0.043	0.125 ± 0.031	0.123 ± 0.026	0.114 ± 0.032	0.121 ± 0.051	0.092 ± 0.019	

Data are shown as means $\pm$ SD. f_R, respiratory frequency; HR, heart rate; P_{ETCO_2}, end-tidal pressure of CO_2; P_{ETO_2}, end-tidal pressure of O_2; S_{pO_2}, peripheral capillary oxygen saturation; $\dot{V}_E$, minute ventilation; V_T, tidal volume.

TABLE 4 Adjacency matrices representing transfer entropy of physiological signals at different fraction of inspired oxygen (F_{IO_2})

A. $F_{IO_2}=0.17$

	S_{pO_2}	HR	f_R	V_T	$\dot{V}_E$	P_{ETO_2}	P_{ETCO_2}
S_{pO_2}	0	0	0	0.07	0.08	0	0
HR	0	0	0	0	0	0	0
f_R	0	0	0	0	0	0	0
V_T	0	0	0	0	0	0	0
$\dot{V}_E$	0	0	0	0	0	0	0
P_{ETO_2}	0	0	0	0	0	0	0
P_{ETCO_2}	0	0	0	0	0	0	0

B. $F_{IO_2}=0.145$

	S_{pO_2}	HR	f_R	V_T	$\dot{V}_E$	P_{ETO_2}	P_{ETCO_2}
S_{pO_2}	0	0.07	0.13	0.11	0.13	0.11	0.12
HR	0.09	0	0	0	0	0	0
f_R	0.12	0	0	0	0	0	0
V_T	0.14	0	0	0	0	0.17	0
$\dot{V}_E$	0.13	0	0	0	0	0.19	0.17
P_{ETO_2}	0.23	0	0	0	0.18	0	0
P_{ETCO_2}	0.13	0	0	0	0	0	0

C. $F_{IO_2}=0.12$

	S_{pO_2}	HR	f_R	V_T	$\dot{V}_E$	P_{ETO_2}	P_{ETCO_2}
S_{pO_2}	0	0.08	0.12	0.11	0.13	0.12	0.12
HR	0.10	0	0	0	0	0	0
f_R	0.12	0	0	0	0	0	0
V_T	0.14	0	0	0	0	0	0
$\dot{V}_E$	0.14	0	0	0	0	0	0
P_{ETO_2}	0.22	0	0	0	0	0	0
P_{ETCO_2}	0.15	0	0	0	0	0	0

A: F_{IO_2} = 0.17; B: F_{IO_2} = 0.145; C: F_{IO_2} = 0.12. Zero value means that there is no statistically significant difference in transfer entropy in comparison with F_{IO_2} = 0.21. f_R, respiratory frequency; HR, heart rate; P_{ETCO_2}, end-tidal pressure of CO_2; P_{ETO_2}, end-tidal pressure of O_2; S_{pO_2}, peripheral capillary oxygen saturation; $\dot{V}_E$, minute ventilation; V_T, tidal volume.

in F_{IO_2} of 0.145 and 0.12 bits respectively (Table 4). Moreover, this enhanced connectivity displayed a bidirectional flow of information as shown in Figure 2. While, bidirectional interactions were observed between various cardio-respiratory parameters at an F_{IO_2} of 0.145, S_{pO_2} exhibited the highest connectivity in the network (Figure 2b). In F_{IO_2} 0.12, S_{pO_2} remained the main *hub* of the network as shown in Figure 2c.

4 | DISCUSSION

The main finding of this study, in support of the hypothesis, was that S_{pO_2} fluctuations during graded normobaric hypoxia exposure carry information about cardio-respiratory control. Specifically, during lower F_{IO_2} exposures (0.145 and 0.12), transfer entropy between S_{pO_2} and other time series increased in comparison to normoxia. Moreover, this enhanced connectivity showed bidirectional flow of information which supports the existence of multiple feedback loops within the autonomic control of the cardio-respiratory system. These findings imply that pattern analysis of S_{pO_2} fluctuations has the potential for assessing the integrity of an individual's cardio-respiratory system in response to physiological challenges (e.g. hypoxia).

Control of respiration requires transfer of information from central and peripheral chemoreceptors to ensure a rigorous balance between supply and use of oxygen. If oxygen availability is reduced, the respiratory centres in the brainstem respond to this flow of information by changing their firing pattern to alter breathing rate and volume (Jubran & Tobin, 2000). Such physiological responses require optimum transfer of information between the different components involved in the homeostatic control of tissue oxygenation (e.g. respiratory centres in the brainstem, autonomic and cardiovascular centres, cardiac pacemaker, vasculature, airways, respiratory muscles, etc.). Previous

reports have indicated that hypoxia leads to the development of subtle ventilatory oscillations which have not been thoroughly examined in the existing literature (Jubran & Tobin, 2000). Some investigators have suggested that the engagement of the respiratory control system in the response to environmental or pathological challenges can be quantified by studying the pattern of respiratory rhythm and volume (Jubran & Tobin, 2000; Satti et al., 2019; Shirazi et al., 2013; Tipton et al., 2017). The current findings have further extended our understanding of this process by demonstrating that there is a higher degree of information transfer between different components of the respiratory control system, despite the absence of a significant change, or increase, in *average* f_R, V_T, or $\dot{V}_E$. Our findings also highlight that this bidirectional flow of information can be quantified by transfer entropy, a tool which shows merit in network visualization of the respiratory control during hypoxia.

A novel finding in the present study was the existence of a causal relationship between S_{pO_2} fluctuations and respiratory control. Although previous observational and experimental studied have shown that S_{pO_2} instability is associated with pathophysiological and environmental challenges, the interpretation of such observations had remained speculative (Costello et al., 2020; Dipietro, Caughy, Cusson, & Fox, 1994; Garde et al., 2016; Roe & Jones, 1993). In general, investigating the causal relationship between physiological signals has largely been limited to reductionistic approaches (i.e. *in vitro*, *ex vivo* studies) (Altimiras, 1999). However, novel analytical methods have provided reliable tools to assess causal inference in multivariate time series data. Barnett and colleagues showed that the transfer entropy between two time series is equivalent to the Granger causality and can be used for data-driven causal inference (Barnett et al., 2009). Thus, transfer entropy has potential for the assessment of the respiratory control system based on multivariable recording of physiological signals, which is often available in acute clinical settings (e.g. intensive care units). Alternative methods that measure flow of information between parallel time series (e.g. mutual information and cross-entropy), do not reveal bidirectional relationship between time series (Raoufy et al., 2016; Richman & Moorman, 2000). This makes transfer entropy a unique method to non-invasively study the feedback loops responsible for autonomic control of the cardio-respiratory system.

In the present study, we observed that during exposure to F_{IO_2} 0.17, S_{pO_2} had a causal influence on V_T and $\dot{V}_E$, but not f_R (Figure 2a). This corroborates previous literature showing that the ventilatory response to normobaric hypoxia is influence by V_T more than f_R (Tipton et al., 2017). However, at a lower F_{IO_2}, the flow of information between S_{pO_2} and other variables (f_R, V_T, $\dot{V}_E$, P_{ETO_2}, P_{ETCO_2}) exhibits a bidirectional relationship. This may indicate tighter regulation as the physiological challenge or stimulus is increased. The presence of bidirectional relationships in the network suggests that S_{pO_2} is not only influenced by ventilatory parameters, but also has an impact on respiratory variables, possibly through feedback regulation. As expected, the strongest causal link was related to $P_{ETO_2} \rightarrow S_{pO_2}$ (Figure 2b,c). This corroborates a recent observation on mechanically ventilated pigs, which demonstrated that variations in the arterial

partial oxygen pressure (P_{aO_2}) is related to cyclic fluctuations of alveolar oxygen tension during respiratory cycles (Formenti et al., 2017). Thus, the information derived from physiological fluctuations in alveolar oxygen tension can be transmitted to the systemic arteries and hence be reflected in haemoglobin saturation and its fluctuations. We also observed a reciprocal association between HR and S_{pO_2} time series in F_{IO_2} 0.145 and 0.12 (Figure 2). This relationship was weaker than that observed between S_{pO_2} and the respiratory variables, and is consistent with previous reports on the relationship between S_{pO_2} and HR variability at high altitude or during normobaric hypoxia (Krejčí, Botek, & McKune, 2018; Saito, Tanobe, Yamada, & Nishihara, 2005).

Understanding integrated physiological function is the focus of network physiology (Bartsch, Liu, Bashan, & Ivanov, 2015). Here using a network approach, we have demonstrated that the degree of connectivity in the cardio-respiratory network was markedly higher during normobaric hypoxia compared to normoxia. This finding supports what we already knew: that the functional connectivity of different physiological systems is crucial for the effective response to environmental (or pathological) challenges, and hence survival. Likewise, recent evidence suggests that patients are more likely to survive critical illnesses as long as the physiological systems remain part of a connected interactive network (Asada et al., 2016). According to these studies, despite being matched for the severity of their illnesses, non-surviving patients exhibit significantly less functional physiological system (network) connectivity in comparison with survivors (Asada et al., 2016). Although we have only studied the integrity of the cardio-respiratory network in healthy individuals, our observations have made us speculate that the functional connectivity between respiratory parameters might be disrupted in patients with illnesses where responses/adaptation to hypoxia plays an important role in their survival (e.g. chronic obstructive pulmonary disease acute respiratory distress syndrome, and possibly COVID-19). Thus, a network approach may help understand the pathophysiology of such complex illnesses beyond what has already been found using traditional reductionistic methods.

While reciprocal interactions were observed between different cardio-respiratory parameters during hypoxia, S_{pO_2} remained the main *hub* of the network (Figure 2). This may indicate that indices derived from S_{pO_2} variability analysis have potential for non-invasive field and clinical studies to measure the connectivity of the respiratory control system. For example, application of fractal analysis of S_{pO_2} signals has been suggested as an alternative in paediatric sleep apnoea (Vaquerizo-Villar et al., 2018). Future studies are therefore warranted to investigate the application of non-invasive S_{pO_2} fluctuation analysis in monitoring individuals exposed to terrestrial altitude or patients who are susceptible to respiratory failure (e.g. patients with COVID-19). Despite extensive literature describing the physiology of respiration in health and disease, our understanding is not complete (Bunn & Poyton, 1996; Tipton et al., 2017; West, 2004). Recent observations of individuals displaying symptoms of 'happy' or 'silent' hypoxia with COVID-19 have not been thoroughly explained (Couzin-Frankel, 2020; Wilkerson, Adler, Shah, & Brown, 2020). A

network physiology approach, using information transfer between cardio-respiratory variables, may help to decipher these observations in the future.

4.1 | Study limitations and future perspectives

This study uses a convenient sample of healthy young males to test the feasibility of the proposed method. First, in order to generalize the findings, future research is required to expand these findings to females and older individuals. Second, all of the participants were healthy and did not have any comorbidities that would affect the dynamics of the cardio-respiratory network. Therefore, the application of S_{pO_2} fluctuation analysis to the general population may show different results. For example, we have previously shown that S_{pO_2} entropy is affected by ageing (Bhogal & Mani, 2017). Other underlying conditions (e.g. respiratory diseases) are likely to impact the network and require attention in future research.

Pulse oximeter technology has recently expanded beyond measurement of S_{pO_2} to other applications, including detection of pulsus paradoxus and fluid responsiveness based on variability analysis of the plethysmography waveform (Hess, 2016). However, the potential application of analysing the variability in the S_{pO_2} signal *per se* has only recently been appreciated (Costello et al., 2020; McGrath, Perreard, MacKenzie, & Blike, 2020). To the best of our knowledge, this was the first time that the transfer of information between S_{pO_2}, f_R, V_T, $\dot{V}_E$, P_{ETO_2}, P_{ETCO_2} and HR time series during graded normobaric hypoxia has been demonstrated.

It appears that S_{pO_2} fluctuations during graded normobaric hypoxia exposure carries information about cardio-respiratory control. Implementations of the algorithms developed in the present study could be utilized as physiological signal predictors incorporated into smart devices and fitness equipment, making them suitable for monitoring changes in aerobic fitness and physical health beyond the infrequent monitoring of patients during clinical interventions and rehabilitation programmes. Future studies should also examine if S_{pO_2} variability analysis could be used to improve monitoring in intensive care settings (e.g. need for or step-down from mechanical ventilation) in a similar way that is described for respiratory rate variability analysis (Seely et al., 2014).

4.2 | Conclusion

S_{pO_2} fluctuations during graded hypoxia exposure carry information about cardio-respiratory control. S_{pO_2} entropy analysis has potential for non-invasive assessment of the engagement of the respiratory control system.

AUTHOR CONTRIBUTIONS
Conception or design of the work: J.T.C., M.J.T., J.C. and A.R.M.; acquisition, analysis, or interpretation of data for the work: Y.J., T.B.W., N.P., A.B. and A.R.M.; drafting of the work or revising it critically for important intellectual content: Y.J, J.T.C., T.B.W., N.P., A.B., M.J.T., J.C. and A.R.M. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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