TITLE

Alterations in Autonomic Cardiac Modulation in Response to Normobaric Hypoxia

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ABSTRACT

Purpose. The present study aimed to determine if autonomic cardiac modulation was influenced by acute exposure to normobaric hypoxia. **Method.** Ten healthy male lowland dwellers completed five block-randomised single-blinded, crossed-over acute exposures to a normobaric hypoxic environment, each separated by 24 hours' recovery (20.3, 17.4, 14.5, 12.0 and 9.8% F_IO_2). Supine recordings were made of arterial oxygen saturation and electrocardiogram (ECG). RR intervals from the ECG trace were analysed for time (SDNN, lnRMSSD), frequency (lnVLF, lnLF, lnHF, lnTP, LFnu & HFnu) and nonlinear (DFA-α1 and SampEn) heart rate variability components. **Results.** A significant reduction in arterial SaO_2 occurred with reduced F_IO_2, along with a rise in heart rate (Cohen's d = 1.16, 95% Confidence Interval [2.64 to 6.46]), significant at 9.8% F_IO_2. A decrease in autonomic cardiac modulation was also found as shown by a statistically significant (at 9.8% F_IO_2) decrease lnTP (d = 1.84 [1.74 to 1.94]), and SampEn (d = 0.98 [0.83 to 1.12]) and an increase in DFA-α_1 (d = 0.72 [0.60 to 0.84]) from normoxia at 9.8% F_IO_2. **Conclusion.** The decrease in variability indicated a reduction in autonomic cardiac modulation. There appears to be a threshold ~ 9.8% F_IO_2 (~6000 m equiv.), below which significant alterations in autonomic control occur.

KEYWORDS

Normobaric Hypoxia, Autonomic Nervous System, Heart Rate Variability, Sample Entropy, Cardiac Function

ABBREVIATIONS

AMS	Acute Mountain Sickness
ANOVA	Analysis of Variance
ANS	Autonomic Nervous System
DFA-α_1	Detrended Fluctuation Analysis – Level α_1
F_IO_2	Fraction of Inspired Oxygen
HRV	Heart Rate Variability
PO_2	Oxygen Partial Pressure
SampEn	Sample Entropy
SaO_2	Arterial Oxygen Saturation
SDNN	Standard Deviation of the Normal to Normal Intervals
TP	Total Power
rMSSD	Root Mean Square of the Successive Differences
VLF	Very Low Frequency
LF	Low Frequency
HF	High Frequency
ln	Log Transformed Variable

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INTRODUCTION

Heart rate variability (HRV) is a non-invasive physiological measure that provides valuable information about the body's capacity to function effectively in complex environments (Thayer, Ahs, Fredrikson, Sollers, & Wager, 2012). Elevated HRV is thought to reflect a healthy autonomic nervous system (ANS) that can efficiently respond to changing environmental conditions (Thayer et al., 2012). Conversely, suppressed HRV has been demonstrated with pre-arrhythmias, in those with sleep apnoea, ventricular dysfunction, and mortality risk post myocardial infarction (Colhoun, Francis, Rubens, Underwood, & Fuller, 2001; Seely & Macklem, 2004). In a hypoxic environment, reductions in ANS responsiveness and the body's ability to adapt have been observed (Chen, Lin, Shiao, & Chang, 2008; Jun et al., 2008). Further, it has been suggested that suppressed ANS responsiveness may be associated with acute mountain sickness (AMS) (Chen et al., 2008). The relationship between hypoxia and HRV is mediated by the severity of the hypoxic exposure, which is determined by the relative reduction in oxygen partial pressure (hypobaric hypoxia), or alterations in the fraction of inspired oxygen (F_IO_2; normobaric hypoxia), both of which result in a subsequent decrease in arterial oxygen saturation (SaO_2; see Millet, Faiss, and Pialoux (2012) for a discussion of hypobaric hypoxia vs. normobaric hypoxia). With an increase in the severity of hypoxia, reduced total spectral HRV power has been found, representing a decrease in autonomic cardiac modulation, and is seen to occur in the majority of HRV hypoxia research (Chen et al., 2008; Cornolo, Mollard, Brugniaux, Robach, & Richalet, 2004; Jun et al., 2008; Millet et al., 2012; Vigo et al., 2010). However, research, exploring changes in nonlinear dynamics of HRV, with alterations in hypoxia, are more limited and less conclusive.

Nonlinear HRV data analysis techniques describe the qualitative properties of RR data, rather than the magnitude of the signal, complementing time and frequency HRV measures (Goldberger, 1997; Huikuri, Perkiömäki, Maestri, & Pinna, 2009). Nonlinear fluctuations of the sinus rhythm are determined by interactions of electrophysiological, haemodynamic and humoral variables, along with autonomic and central nervous system regulation (Seely & Macklem, 2004). Methodologically speaking, nonlinear variables, particularly DFA, are less dependent on changes in HR and display less inter- and intra- individual variation (Huikuri et al., 2009), are not as sensitive to missing RR intervals (Yuanyuan, Zhengtao, et al., 2013), and do not appear to be directly associated with fluctuations in HR, particularly elevated HR (Vigo et al., 2010). Changes in nonlinear HRV have been related to physical stress (Javorka, Zila, Balharek, & Javorka, 2002), psychological stress (Mateo, Blasco-Lafarga, Martínez-Navarro, Guzmán, & Zabala, 2012), and a large number of pathological conditions (Francesco et al., 2012). Significantly, in a clinical setting Goldberger (1997) proposed that health may be characterised as organised variability and disease as a decomplexification, with increased regularity and a reduction in variability. In a hypoxia research context nonlinear HRV methods provide a means of quantifying acute responses, with reduced variability potentially indicating an inability for the body to adapt, as they are in response to traumatic incidents, stress and pathological conditions (Colhoun et al., 2001; Huikuri et al., 2009).

Research exploring nonlinear HRV and hypoxia is limited, relative to that completed using time and frequency based analysis techniques. From the present research that has examined nonlinear dynamics of HR, it is not clear whether there is a reduction or increase in complexity or differences in the threshold at which significance alterations in variability occur (Taralov et al., 2015; Vigo et al., 2010; Zhang, She, Zhang, & Yu, 2014). As such, the aim of the current study was to determine how autonomic cardiac modulation was influenced by the severity of an acute hypoxic insult, as measured by time, frequency and nonlinear HRV parameters.

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MATERIALS AND METHODS

Participants. Eleven male, physically fit, non-smoking, lowland dwellers volunteered to participate in this study (age 21.8 ± 0.9 years; height 1.8 ± 0.1 m; mass 81.1 ± 7.4 Kg). Written informed consent and medical health questionnaires were completed prior to taking part in the study. Exclusion criteria included recent travel to altitude (4 weeks), current or recent smoker, a diagnosis of, or receiving medications for cardiac or cardiovascular disease, or autonomic disorders such as anxiety or depression. Institutional ethical approval was granted prior to data collection and conformed to the principles of the Declaration of Helsinki.

Study design. For this single-blinded, block randomised crossover trial, participants presented to the laboratory on five occasions. Each visit was separated by 24 ± 0.5 hours. During each visit, participants completed an acute exposure to a hypoxic environment, created through manipulation of the F_IO_2 within an environmental chamber (TISS Model 201003-1), located at 20 m above sea-level. The following F_IO_2 (equivalent PO_2 and height above sea level) were selected: 20.3 ± 0.3% F_IO_2 (152.1 mmHg PO_2; 0 meters), 17.4 ± 0.1% F_IO_2 (130.8 mmHg PO_2; 1500 meters), 14.5 ± 0.1% F_IO_2 (109.5 mmHg PO_2; 3000 meters), 12.0 ± 0.0% F_IO_2 (90.6 mmHg PO_2; 4500 meters) and 9.8 ± 0.1% F_IO_2 (74.2 mmHg PO_2; 6000 meters). Environmental conditions were maintained at 20.0 °C and 50% relative humidity.

During each exposure three lead electrocardiographic recordings (ECG; Powerlab, running Chart 5 Pro Version 5.5.1) were made in a supine position. Participants were kept in silence, without moving, and with limited audible and visual stimulation; recordings were made after 10-minutes of hypoxic exposure, once a stable HR was observed, and lasted for five minutes. The mean respiratory rate over one minute ($bf.min^{-1}$) was computed using a custom chest strap force transducer (Powerlab, running Chart 5 Pro, Version 5.5.1). Respiratory rate was manually assessed from the recordings, noting the frequency of inspiration peaks over a five-minute period. Arterial oxygen saturation was recorded every fifteen seconds for the duration of the protocol (pulse oximeter, %, Datex-Ohmeda 3800). If a participant's SaO_2 dropped below 70%, or complained of pre-syncope, or wished to withdraw voluntarily, testing was stopped, and the participant immediately removed from the chamber. One participant's data was excluded, following being withdrawn at 9.8% F_IO_2, due to becoming symptomatic.

Heart rate variability data analysis. R-wave peaks were detected automatically in Chart 5 Pro; the difference between each successive interval was recorded as RR data. Prior to spectral analysis, the ECG trace was analysed visually for missing or ectopic beats in Chart 5 pro, if an error was detected a beat was deleted, or inserted retrospectively (n = 3), following correction the data was considered normal to normal (NN). Mean heart rate ($b.min^{-1}$) was calculated from the mean NN data.

Heart rate variability analysis was performed on 256 NN interval segments, recorded during the last 4-minutes of rest, to ensure the stability of the data. The analysis was conducted using Kubios HRV software (Version 2.2; Tarvainen, Niskanen, Lipponen, Ranta-Aho, & Karjalainen, 2014). The following HRV parameters were calculated: (1) Time domain, the mean NN interval, the standard deviation of NN intervals (SDNN) and the root-mean-square difference of successive normal NN intervals (rMSSD). (2) Frequency domain, prior to the calculation of to power frequency analysis, NN data were detrended (Smooth priors, k = 500; Tarvainen, Ranta-Aho, & Karjalainen, 2002) and resampled at 4 Hz. The Fast Fourier Transform spectrum was then calculated using a Welch's periodogram method. Total power (TP, ≤0.4 Hz), very low frequency (VLF, 0 – 0.04), low-frequency power (LF, 0.04 – 0.15 Hz), high-frequency power (HF, 0.15 – 0.4 Hz) were calculated as integrals of the respective power spectral density curve, along with LF:HF power ratio and normalized values of LF and HF

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power (LFnu and HFnu). (3) Poincare´ scattergrams were plotted, in which each R-R interval is plotted as a function of the previous one. The standard deviation of the instantaneous beat-to-beat variability data (SD1), were calculated from each tacogram (Seely & Macklem, 2004). (4) Nonlinear dynamics of NN interval data were assessed with the short-term fractal component DFA-α1, to quantify self-similarity correlations, and sample entropy (SampEn) to provide an indication of the general predictability of the NN interval time series (Seely & Macklem, 2004).

Statistical analysis. Descriptive statistics were first calculated for all variables (Mean ± SD). Normal distribution and homogeneity of variance were assessed through visual inspection of the frequency histogram, and with a Shapiro-Wilk test; significance was found for rMSSD, TP, VLF, LF, HF, and LF:HF ratio. The non-normally distributed variables were log transformed; following transformation, the variables were normally distributed. One-way repeated measures ANOVAs were calculated for each dependent variable to assess differences between the five hypoxic conditions. A significance level of $p < 0.05$ was used for all ANOVAs. Bonferroni corrected posthoc-paired sample *t-tests* were calculated to further investigate differences found between the five levels (4 comparisons, significance level $p < 0.0125$). The magnitude of the difference of the significant parameters was calculated by determining the Cohen *d* effect size (ES), representing the mean difference over the pooled standard deviation of the difference (Thomas, Nelson, & Silverman, 2010); the difference was considered trivial when ES < 0.2, small when ES 0.2 – 0.6, moderate when ES 0.6 – 1.2 and large when ES 1.2 – 2.0 (Hopkins, Marshall, Batterham, & Hanin, 2009). All data was analysed using SPSS (Version 22).

RESULTS

The randomised order of exposure of the participants to hypoxia did not elicit any significant effect on any measures (two-way ANOVA; Order x F_IO_2). A statistically significant 19.1% (absolute %) decrease in mean arterial SaO_2 was observed between 20.3% and 9.8% F_IO_2 (Table I). Further Bonferroni-corrected *t*-tests revealed significance in SaO_2 from 20.3% at 14.5% F_IO_2 and below; the effect at 14.5% F_IO_2 was 2.88 (95 % confidence interval, 2.09 to 3.68; Table II), 12.0% F_IO_2 was 4.23 (2.91 to 5.56) and at 9.8% F_IO_2 was 4.65 (2.84 to 6.46). With the reduction in F_IO_2 and SaO_2, there was also a significant 16.1 $b.min^{-1}$ elevation in mean HR; HR did not reach statistical significance from 20.3% until 9.8% F_IO_2, at this level the effect was 1.16 (0.85 to 2.34).

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In comparison to 20.3% F_IO_2, the decrease in F_IO_2 resulted in a non-significant reduction in lnVLF, lnLF and lnHF spectral components, and a statistically significant reduction in lnTP; Bonferroni corrected *t*-tests revealed lnTP to be significant at 9.8% F_IO_2, with an effect of 1.84 (1.74 to 1.94). LFnu saw a small non-significant increase (HFnu, the inverse, decreased proportionally) with the decrease in F_IO_2. Statistically significant changes were also observed in both DFA-α_1 and SampEn, increasing and decreasing respectively (Fig 1.). Further Bonferroni-corrected *t*-tests revealed significance from 20.3% at 9.8% F_IO_2 for both DFA-α_1, effect 0.72 (0.60 to 0.84) and SampEn with an effect of 0.98 (0.83 to 1.12). While SDNN decreased and showed a moderate effect and lnrMSSD increased with a moderate effect, neither reached statistical significance.

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DISCUSSION

The present study aimed to determine how autonomic cardiac modulation was influenced by the severity of an acute hypoxic insult, as measured by time, frequency and nonlinear HRV parameters. The main finding was a moderate and statistically significant increase in HR, accompanied by a large decrease lnTP, a moderate decrease in SampEn, and a moderate increase in DFA-α_1 from normoxia at 9.8% F_IO_2 (Table I and II). These findings are suggestive of a reduction in the autonomic cardiac modulation of the ANS and possibly an inability for the body to adapt to an acute reduction in arterial SaO_2. Further, in agreement with the conclusions of Iwasaki et al. (2006), our results suggest a threshold may exist, below which significant alterations in autonomic control may be observed; although, the critical point occurred later than previous research (Iwasaki et al., 2006; Saito, Tanobe, Yamada, & Nishihara, 2005; Taralov et al., 2015), between 14.5% and 9.8% F_IO_2, with significance found in the present study at 9.8% F_IO_2 (~6000 m equiv.).

Total power, along with log-transformed linear HRV indices, decreased with the increased hypoxic exposure. Decreases in TP are common to the majority of research, with few exceptions (Zhang et al., 2014); however, in contrast to a number of studies, the large decrease in lnTP only resulted in significance at 9.8% F_IO_2. In line with the present study, on acute exposure to normoxic hypoxia Taralov et al. (2015) observed no significant changes in TP at the beginning of exposure to 12.3% F_IO_2. Further, also in agreement with the results of the present study, Mairer, Wille, Grander, and Burtscher (2013) reported a significant decrease in TP with normoxic hypoxia at 11% F_IO_2 and Vigo et al. (2010) reduced linear HRV indices at all frequency levels using a hypobaric chamber, at an equivalent of 8230 m. Conversely, the lack of significance in lnTP in the present study, until 9.8% F_IO_2, contrasts statistically significant changes observed in hypobaric hypoxia studies at both 3180 and 3675 m (Chen et al., 2008; Jun et al., 2008). Differences in the point at which previous research and the present study found significance are likely to be related to methodological differences, in particular between hypobaric hypoxia (Chen et al., 2008; Jun et al., 2008), and normobaric hypoxia (Taralov et al., 2015; Vigo et al., 2010). It is possible that, as with Taralov et al. (2015) and Vigo et al. (2010), the present study did not find significance in earlier stages due to differences in the means of eliciting hypoxia, as discussed by Millet et al. (2012).

Nonlinear HRV measures of DFA-α_1 and SampEn became increasingly regular with the reduction in SaO_2. Although, as with lnTP, significance in both SampEn and DFA-α_1 was only observed at 9.8% F_IO_2; the difference in the mean values at this level demonstrated a moderate effect. SampEn, which quantifies the complexity/ irregularity of heartbeat series (lower values representing a more regular, less complex, signal) decreased significantly from normoxia at 9.8% F_IO_2; conversely, DFA-α_1 increased significantly, reflecting pathological alteration in the underlying system and evidenced a loss of fractality toward a strongly correlated signal (Seely & Macklem, 2004). Similarly, increased periodicity and small cycle-to-cycle variations were also found by Yamamoto et al. (1993) in recordings over 6000 m, during a long-term hypobaric study (40 days). Saito et al. (2005), Yuanyuan, Zhengtao, et al. (2013) and Taralov et al. (2015) also reported decreased irregularity in HRV signal, however, in contrast to the present study, these were significant at 3456 m, 3000/4000 m and 4000 m, respectively. Interestingly, following the decrease in irregularity, Yuanyuan, Zhengtao, et al. (2013) reported an increase in entropy over time, possibly due to acclimation. In contrast to the above findings, and those of the present study, Zhang et al. (2014) reported increased complexity and irregularity in the RR intervals, with

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increased sample entropy at 4000 m in a hypobaric chamber; they postulated that this indicated that acute hypoxia enhanced autonomic modulation of heartbeat irregularity. Similarly, Vigo et al. (2010) speculated that their reported decrease in DFA-α_1 and increase in SampEn on acute exposure to the equivalent of 8230 m in a hypobaric chamber were the result of compensatory physiological mechanisms induced by hypoxia. While Goldberger (1997) proposed health as organised variability and disease as a decomplexification, it has also been suggested that disease may manifest with either increased or decreased variability depending on underlying dimensions (Vaillancourt & Newell, 2002). Rather than a single directional response, disease may occur when the distance from equilibrium is either too close with too little variation and low entropy, or too far with increased variation, representing pathological alterations (Seely & Macklem, 2004). It is possible that a similar bi-directional effect is observed in the present study, as while non-linear HRV measures became increasingly regular with the reduction in SaO_2, there were also large amounts of inter-individual variation with large confidence intervals (Table II) and SD (Table I); however, this variation may also be attributed to sample size of this preliminary study.

A reduction in lnTP and non-linear measures of HRV are postulated to represent a decrease in the responsiveness of the ANS (Sztajzerl, 2004). It appears that a reduction in F_IO_2 beyond a critical threshold elicits a significant decrease in HRV, perhaps reflecting a decrease in autonomic cardiovascular modulation (Thayer et al., 2012). Previously Iwasaki et al. (2006) speculated that this threshold was to be found at around 15% F_IO_2 (~3000 m equiv.). While our study supports the notion of a threshold, the critical point occurred later than previous research (Iwasaki et al., 2006; Saito et al., 2005; Taralov et al., 2015), between 14.5% and 9.8% F_IO_2. Significance was found in the present study at 9.8% F_IO_2 (~6000 m equiv.; Fig 1.); although moderate to large effects were observed at 12.0% F_IO_2 (Table II). Differences between the size of effects and statistical significance, with moderate effects occurring at earlier stage than the statistical significance seen at 9.8% F_IO_2, are inherent to the techniques used, particularly as effect size is independent to the sample size (Sullivan & Feinn, 2012). It is thought that an increase in the severity of hypoxic insult beyond a critical point induces vagal withdrawal and/or a reduction in spontaneous arterial-cardiac baroreflex function (Iwasaki et al., 2006). The large confidence intervals seen in the effects (Table II) and SD (Table I) are indicative of inter-individual variation in response to hypoxic exposure, and as previously mentioned, the smaller sample size of this preliminary study. Interestingly, in support of the findings of Huikuri et al. (2009), the non-linear HRV analysis methods displayed less inter- and intra-individual variation (and significance), which appeared to be responsible for the lack of significant seen in SDNN and lnLF:HF measures, despite the moderate effect sizes observed in SDNN. Further, a larger sample size would likely demonstrate significance at 17.5%, in line with previous research.

Differences in alterations in TP and entropy between studies may, at least partially, be explained by methodological differences in exposure duration, severity and type (hypobaric hypoxia vs. normobaric hypoxia) (Vigo et al., 2010; Zhang et al., 2014). For example, in contrast to our study, both Zhang et al. (2014) and Vigo et al. (2010) used a hypobaric hypoxic chamber in order to induce alterations in partial pressure; while, Saito et al. (2005) used a high altitude lab and normobaric hypoxia to elicit alterations in arterial SaO_2, taking participants to 3456 m, which involved both a car journey to 2100 m and a 4 hour walk to 3456 m. Millet et al. (2012) presented a compelling argument for hypobaric hypoxic being more severe than normoxic hypoxia, with differences in fluid balance, AMS symptoms, NO metabolism, ventilatory response and performance identified. Similarly, small differences in the duration may also play a critical role. Taralov et al. (2015) observed no significant changes in TP at the beginning of an exposure equivalent to 4200 m, while significance changes in TP occurred over the

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duration of the exposure. This is especially pertinent when comparing studies, for example, Vigo et al. (2010) exposed participants immediately to equivalent partial pressure of 8230 m, while Yuanyuan, Binhua, Chengyu, Jun, and Zhengtao (2013) protocol included a stepwise exposure over 120 minutes and Yamamoto et al. (1993) chronic exposure, with 40 days of exposure to a hypoxic environment, with participants reaching a peak simulated altitude of 8840 m following a lengthy aclimation period.

Alterations in HRV, and the underlying changes cardiovascular modulation that they quantify, play a role in understanding individual's response to hypoxia and, eventually, adaptation to the environment. Saito et al. (2005) suggested that reduced modulation and HRV could indicate an inability for the body to adapt to the challenging conditions of acute hypoxia, as they are in response to traumatic incidents (Colhoun et al., 2001; Huikuri et al., 2009). In support of this Chen et al. (2008) and Karinen et al. (2012) found that alterations in spectral HRV components were more commonly associated with those suffering from symptoms of AMS. Furthermore, it has been shown that reductions in TP are significantly associated with risk of cardiac events (Tsuji et al., 1996). Interestingly, the use of non-linear HRV analysis and AMS has not seen research attention. As nonlinear methods are less dependent on changes in HR and display less inter- and intra-individual variation (Huikuri et al., 2009), are not as sensitive to missing RR intervals (Yuanyuan, Zhengtao, et al., 2013), and do not appear to be directly associated with fluctuations in HR, particularly elevated HR (Vigo et al., 2010) they would present a compelling avenue for future research.

Our research presents a preliminary insight into alterations in autonomic cardiac modulation with changes in F_IO_2, and has made several advancements on previous research: 1) with a decrease in the inspired oxygen fraction there is also a decrease in autonomic cardiac modulation, as shown by the large and moderate to large effects reported; and 2) there appears to be a threshold, below 10% F_IO_2, that greater changes from normoxia are found. However, it is acknowledged that the sample size limits conclusions that may be drawn from the findings. Future studies could explore inter-individual variation in responses to acute hypoxia, in particular concentrating on individual's level of arterial desaturation, respiratory response and concurrent changes in cardiac modulation. Further research may enable HRV to be used to assess and quantify individual's susceptibility to AMS, and response to hypoxic exposure. Especially as AMS has been shown to be the result of a blunted response of the ANS to hypoxia, it may be possible that nonlinear HRV could be used prior to the presentation of clinical signs (Chen et al., 2008).

CONCLUSIONS

The analysis of time, frequency and nonlinear HRV components identified a statistically significant decrease in the overall variability of the ANS, as shown by a significant reduction in lnTP and SampEn and an increase in DFA-α_1. Therefore, whilst preliminary, the major finding of the study is a decrease in variability, indicative of a reduction autonomic cardiac modulation. Further, there appears to be a threshold below which significant alterations in autonomic control may be observed, occurring between 14.5 and 9.8% F_IO_2, significant at 9.8% F_IO_2 (~6000 m equiv.). The threshold may depend on a number of factors, including the means of eliciting hypoxia, duration of exposure and inter-individual responsiveness.

CONFLICT OF INTEREST

No funding was received for the purposes of this study. The authors declare no conflicts of interest.

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FIGURE CAPTIONS

Fig 1: SampEn and DFA-α_1 behaviour with a reduction in the fraction of inspired oxygen (F_IO_2), overlapping standard deviations omitted for clarity. (*Denotes significance ($p < 0.0125$) from 20.3% F_IO_2).

Fig 2: Tachograms, power spectrums and Poincare´ scattergrams during supine HRV recordings in one representative participant at 20.3%, 17.4%, 14.5%, 12.0% and 9.8% F_IO_2.

Sensitivity: Internal