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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% FIO₂). Supine recordings were made of arterial oxygen saturation and electrocardiogram (ECG). RR intervals from the ECG trace were analysed for time (SDNN, lnRMSD), frequency (lnVLF, lnLF, lnHF, lnTP, LFnu, and HFnu), and nonlinear (DFA-α1 and SampEn) heart rate variability components.

Results: A significant reduction in arterial SaO₂ occurred with reduced FIO₂, along with a rise in heart rate (Cohen’s d = 1.16, 95% Confidence Interval [2.64–6.46]), significant at 9.8% FIO₂. A decrease in autonomic cardiac modulation was also found as shown by a statistically significant (at 9.8% FIO₂) decrease in lnTP (d = 1.84 [1.74–1.94]), and SampEn (d = 0.98 [0.83–1.12]) and an increase in DFA-α1 (d = 0.72 [0.60–0.84]) from normoxia at 9.8% FIO₂. Conclusion: The decrease in variability indicated a reduction in autonomic cardiac modulation. There appears to be a threshold ∼9.8% FIO₂ (∼6000 m equiv.), below which significant alterations in autonomic control occur.

Keywords: Normobaric hypoxia, autonomic nervous system, heart rate variability, sample entropy, cardiac function

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-arhythmic events, 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\textsubscript{I}O\textsubscript{2}; normobaric hypoxia), both of which result in a subsequent decrease in arterial oxygen saturation (SaO\textsubscript{2}; see Millet, Faiss, and Pialoux, 2012 for a discussion of hypobaric hypoxia versus 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, & Richard, 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, is 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.

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; and 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\textsubscript{I}O\textsubscript{2} within an environmental chamber (TISS Model 201003-1), located at 20 m above sea level. The following F\textsubscript{I}O\textsubscript{2} (equivalent PO\textsubscript{2} and height above sea level) were selected: 20.3 ± 0.3% F\textsubscript{I}O\textsubscript{2} (152.1 mmHg PO\textsubscript{2}; 0 m), 17.4 ± 0.1% F\textsubscript{I}O\textsubscript{2} (130.8 mmHg PO\textsubscript{2}; 1500 m), 14.5 ± 0.1% F\textsubscript{I}O\textsubscript{2} (109.5 mmHg PO\textsubscript{2}; 3000 m), 12.0 ± 0.0% F\textsubscript{I}O\textsubscript{2} (90.6 mmHg PO\textsubscript{2}; 4500 m), and 9.8 ± 0.1% F\textsubscript{I}O\textsubscript{2} (74.2 mmHg PO\textsubscript{2}; 6000 m). Environmental conditions were maintained at 20.0°C and 50% relative humidity.

During each exposure three lead electrocardiographic recordings (ECG; Powerlab, and 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). The 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 15 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 were excluded, following being withdrawn at 9.8% F\(_{1}\)O\(_2\), due to becoming symptomatic.

**HRV 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 the 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 were considered normal to normal (NN). The mean heart rate (b min\(^{-1}\)) was calculated from the mean NN data. The HRV analysis was performed on 256 NN interval segments, recorded during the last four 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: \(\leq 0.4\) Hz), very low frequency (VLF: 0–0.04), low-frequency power (LF: 0.04–0.15 Hz), and high-frequency power (HF: 0.15–0.4 Hz) were calculated as integrals of the respective power spectral density curve, along with the LF:HF power ratio and normalised values of LF and HF power (LFnu and HFnu). (3) Poincare’ scattergrams were plotted, in which each RR interval is plotted as a function of the previous one. The standard deviation of the instantaneous beat-to-beat variability data (SD1) was calculated from each tachogram (Seely & Macklem, 2004). (4) Nonlinear dynamics of NN interval data were assessed with the short-term fractal component DFA-\(alpha\), 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 \(\pm\) 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 \(p < .05\) was used for all ANOVAs. Bonferroni-corrected *post hoc* paired sample *t*-tests were used to further investigate differences found between the five levels (four comparisons, significance level \(p < .0125\)). 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 were 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 \(\times\) F\(_{1}\)O\(_2\)). A statistically significant 19.1% (absolute %) decrease in mean arterial SaO\(_2\) was observed between 20.3% and 9.8% F\(_{1}\)O\(_2\) (Table I). Further Bonferroni-corrected *t*-tests revealed significance in SaO\(_2\) from 20.3% at 14.5% F\(_{1}\)O\(_2\) and below; the effect at 14.5% F\(_{1}\)O\(_2\) was 2.88 (95% confidence interval, 2.09–3.68; Table II), 12.0% F\(_{1}\)O\(_2\) was 4.23 (2.91–5.56), and at 9.8% F\(_{1}\)O\(_2\) was 4.65 (2.84–6.46). With the reduction in F\(_{1}\)O\(_2\) and SaO\(_2\), there was also a significant 16.1 b min\(^{-1}\) elevation in the mean HR; the HR did not reach statistical significance from 20.3% until 9.8% F\(_{1}\)O\(_2\), at this level the effect was 1.16 (0.85–2.34).
In comparison to 20.3% FIO₂, the decrease in FIO₂ 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% FIO₂, with an effect of 1.84 (1.74–1.94). LFnu saw a small non-significant increase (HFnu, the inverse, decreased proportionally) with the decrease in FIO₂. Statistically significant changes were also observed in both DFA-α₁ and SampEn, increasing and decreasing respectively (Figure 1). Further Bonferroni-corrected t-tests revealed significance from 20.3% at 9.8% FIO₂ for both DFA-α₁, effect 0.72 (0.60–0.84) and SampEn with an effect of 0.98 (0.83–1.12). While SDNN decreased and showed a moderate effect and lnMSSD increased with a moderate effect, neither reached statistical significance.

### 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 (Figure 2). The main finding was a moderate and statistically significant increase in HR, accompanied by a large decrease in lnTP, a moderate decrease in SampEn, and a moderate increase in DFA-α₁ from normoxia at 9.8% FIO₂ (Tables 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 SaO₂. Further, in agreement with the conclusions of Iwasaki et al. (2006), our results suggest that a threshold may exist, below which significant alterations in autonomic control may be observed; although, the critical point occurred later than

### Table I. Alterations in physiological and HR variability measures with the decrease in the fraction of inspired oxygen (FIO₂)

| FIO₂ (%) | 20.3 ± 0.3 | 17.4 ± 0.1 | 14.5 ± 0.1 | 12.0 ± 0.0 | 9.8 ± 0.1 |
|----------|------------|------------|------------|------------|------------|
| SaO₂ (%) | 96.8 ± 2.1 | 95.8 ± 1.7 | 91.6 ± 1.7*| 84.1 ± 4.0*| 77.7 ± 5.8*|
| HR (b min⁻¹) | 67.3 ± 13.5 | 71.1 ± 10.0 | 70.8 ± 10.5 | 77.1 ± 11.6 | 81.7 ± 12.8*|
| Respiratory rate (bf min⁻¹) | 15.6 ± 3.4 | 16.2 ± 2.7 | 14.8 ± 3.0 | 12.4 ± 4.2 | 12.9 ± 4.2 |
| SDNN (ms) | 68.3 ± 32 | 49.5 ± 26.1 | 52.8 ± 18.2 | 54.0 ± 21.1 | 46.2 ± 15.7 |
| lnMSSD (ms) | 1.6 ± 0.4 | 1.5 ± 0.3 | 1.4 ± 0.3 | 1.4 ± 0.3 | 1.4 ± 0.2 |
| lnTP (ms²) | 3.6 ± 0.2 | 3.4 ± 0.3 | 3.3 ± 0.3 | 3.2 ± 0.3 | 3.1 ± 0.3* |
| lnVLF (ms²) | 3.1 ± 0.5 | 3.0 ± 0.5 | 3.1 ± 0.5 | 3.0 ± 0.5 | 2.8 ± 0.5 |
| lnLF (ms²) | 3.0 ± 0.4 | 2.8 ± 0.3 | 2.8 ± 0.4 | 2.9 ± 0.4 | 2.8 ± 0.4 |
| lnHF (ms²) | 2.7 ± 0.7 | 2.5 ± 0.6 | 2.5 ± 0.6 | 2.5 ± 0.5 | 2.3 ± 0.5 |
| lnLF/HF (ms²) | 0.3 ± 0.4 | 0.3 ± 0.5 | 0.3 ± 0.3 | 0.4 ± 0.3 | 0.5 ± 0.5 |
| lnLF/HF | 37.6 ± 20.6 | 38.8 ± 20.4 | 33.8 ± 13.6 | 28.2 ± 12.5 | 30.6 ± 16 |
| lnHF (ms²) | 62.4 ± 20.6 | 61.2 ± 20.4 | 66.2 ± 13.6 | 71.8 ± 12.5 | 69.3 ± 16 |
| SD1 | 38.0 ± 31.8 | 27.1 ± 22.8 | 23.2 ± 12.4 | 23.2 ± 14.5 | 18.1 ± 9.2 |
| SampEn | 1.45 ± 0.43 | 1.48 ± 0.34 | 1.43 ± 0.22 | 1.22 ± 0.23 | 1.13 ± 0.25* |
| DFA 1 | 1.15 ± 0.35 | 1.12 ± 0.31 | 1.26 ± 0.17 | 1.34 ± 0.25 | 1.35 ± 0.22* |

* Denotes significance (p < .0125) from 20.3% FIO₂.

### Table II. Effect sizes [95% CI] for 17.4%, 14.5%, 12.0%, and 9.8% FIO₂ when compared to 20.3% FIO₂

| FIO₂ (%) | 20.3–17.4% | 20.3–14.5% | 20.3–12.0% | 20.3–9.8% |
|----------|------------|------------|------------|------------|
| SaO₂ (%) | 0.57 [−0.21 to 1.36] | 2.88 [2.09 to 3.68]‡ | 4.23 [2.91 to 5.56]‡ | 4.65 [2.84 to 6.46]‡ |
| HR | 0.34 [−4.61 to 5.28] | 0.31 [−4.73 to 5.34] | 0.82 [−4.41 to 6.06] | 1.16 [−4.31 to 6.62] |
| Respiratory rate | 0.21 [−1.07 to 1.49] | 0.27 [−1.05 to 1.59] | 0.89 [−0.71 to 2.48] | 0.74 [−0.85 to 2.34] |
| SDNN | 0.68 [−11.47 to 12.83] | 0.63 [−10.19 to 11.45] | 0.56 [−10.71 to 11.82] | 0.90 [−9.58 to 11.38] |
| lnMSSD | 0.41 [−0.27 to 0.55] | 0.51 [−0.37 to 0.64] | 0.54 [−0.41 to 0.68] | 0.84 [−0.71 to 0.97] |
| lnTP | 0.81 [0.72 to 0.91] | 0.80 [0.66 to 0.93] | 1.11 [0.93 to 1.20] | 1.84 [1.74 to 1.94] |
| lnVLF | 0.37 [0.17 to 0.57] | 0.06 [−0.14 to 0.25] | 0.27 [0.10 to 0.43] | 0.76 [0.56 to 0.96] |
| lnLF | 0.72 [−0.37 to 0.87] | 0.56 [−0.40 to 0.72] | 0.25 [0.08 to 0.41] | 0.60 [0.45 to 0.76] |
| lnHF | 0.36 [−0.08 to 0.64] | 0.45 [−0.18 to 0.72] | 0.47 [0.21 to 0.73] | 0.67 [0.41 to 0.94] |
| lnLF/HF | 0.03 [−0.16 to 0.21] | 0.18 [−0.02 to 0.34] | 0.51 [−0.35 to 0.67] | 0.42 [0.22 to 0.62] |
| LnHnu | 0.06 [−8.46 to 8.59] | 0.23 [−7.02 to 7.48] | 0.58 [−6.50 to 7.66] | 0.40 [−7.26 to 8.05] |
| Hfnu | 0.06 [−8.46 to 8.59] | 0.23 [−7.02 to 7.48] | 0.58 [−6.50 to 7.66] | 0.40 [−7.26 to 8.05] |
| SD1 | 0.41 [−11.09 to 11.92] | 0.65 [−9.38 to 10.68] | 0.63 [−9.64 to 10.9] | 0.90 [−8.83 to 10.62] |
| SampEn | 0.09 [−0.07 to 0.25] | 0.07 [−0.07 to 0.22] | 0.70 [0.56 to 0.85] | 0.98 [0.83 to 1.12] |
| DFA 1 | 0.10 [−0.04 to 0.24] | 0.41 [0.30 to 0.53] | 0.64 [0.52 to 0.77] | 0.72 [0.60 to 0.84] |

‡ Denotes a moderate effect.

‡ Denotes a large effect.
previous research (Iwasaki et al., 2006; Saito, Tanobe, Yamada, & Nishihara, 2005; Taralov et al., 2015), between 14.5% and 9.8% FIO2, with significance found in the present study at 9.8% FIO2 (∼6000 m equiv.).

TP, 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% FIO2. 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% FIO2. 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% FIO2 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% FIO2, 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 earlier due to differences in the means of eliciting hypoxia, as discussed by Millet et al. (2012).

Nonlinear HRV measures of DFA-α1 and SampEn became increasingly regular with the reduction in SaO2. Although, as with lnTP, significance in both SampEn and DFA-α1 was only observed at 9.8% FIO2; 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% FIO2; conversely, DFA-α1 increased significantly, reflecting pathological alterations in the underlying system and evidenced a loss of fractality towards 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, 3000/4000 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 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-α1 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 nonlinear HRV measures became increasingly regular with the reduction in SaO2, 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 nonlinear measures of HRV are postulated to represent a decrease in the responsiveness of the ANS (Sztajzerl, 2004). It appears that a reduction in FIO2 beyond a critical threshold elicits a significant decrease in HRV,
Figure 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% $F_O2$. 
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\textsubscript{2}O\textsubscript{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\textsubscript{2}O\textsubscript{2}. Significance was found in the present study at 9.8% F\textsubscript{2}O\textsubscript{2} (~6000 m equiv.; Figure 1), although moderate to large effects were observed at 12.0% F\textsubscript{2}O\textsubscript{2} (Table II). Differences between the size of effects and statistical significance, with moderate effects occurring at an earlier stage than the statistical significance seen at 9.8% F\textsubscript{2}O\textsubscript{2}, are inherent to the techniques used, particularly as the 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 nonlinear 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 versus 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 laboratory and normobaric hypoxia to elicit alterations in arterial Sa\textsubscript{O\textsubscript{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 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 the 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 nonlinear HRV analysis and AMS has not grabbed 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\textsubscript{2}O\textsubscript{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\textsubscript{2}O\textsubscript{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 the AMS, and response
to hypoxic exposure. Especially as the 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-α1. Therefore, while 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% F2O2, significant at 9.8% F2O2 (~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.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Chen, Y. C., Lin, F. C., Shiao, G. M., & Chang, S. C. (2008). Effects of rapid ascent to high altitude on autonomic cardiovascular modulation. The American Journal of the Medical Sciences, 336(3), 248–253. doi:10.1097/MAJ.0b013e3181629a32

Colhoun, H. M., Francis, D. P., Rubens, M. B., Underwood, S. R., & Fuller, J. H. (2001). The association of heart-rate variability with cardiovascular risk factors and coronary artery calcification: A study in type 1 diabetic patients and the general population. Diabetes Care, 24(6), 1108–1114. doi:10.2337/ diacare.24.6.1108

Cornolo, J., Mollard, P., Brugniaux, J. V., Robach, P., & Richealet, J. P. (2004). Autonomic control of the cardiovascular system during acclimatization to high altitude: Effects of sildenafil. Journal of Applied Physiology, 97(3), 935–940. doi:10.1152/ japplphysiol.00239.2004

Francesco, B., Maria Grazia, B., Emanuele, G., Valentina, F., Sara, C., Chiara, F., ... Francesco, F. (2012). Linear and nonlinear heart rate variability indexes in clinical practice. Computational and Mathematical Methods in Medicine, 2012. Article ID 219080. doi:10.1155/2012/219080

Goldberger, A. L. (1997). Fractal variability versus pathologic periodicity: Complexity loss and stereotypy in disease. Perspectives in Biology and Medicine, 40(4), 543–561. doi:10.1353/pbm.1997.0063

Hopkins, W., Marshall, S., Batterham, A., & Hanin, J. (2009). Progressive statistics for studies in sports medicine and exercise science. Medicine and Science in Sports Exercise, 41(1), 3–13. doi:10.1249/MSS.0b013e31818cb278

Huikuri, H. V., Perkiömäki, J. S., Maestri, R., & Pinna, G. D. (2009). Clinical impact of evaluation of cardiovascular control by novel methods of heart rate dynamics. Philosophical Transactions of the Royal Society of London A: Mathematical, Physical and Engineering Sciences, 367(1892), 1223–1238. doi:10.1098/rsta.2008.0294

Iwasaki, K.-I., Ogawa, Y., Aoki, K., Saitoh, T., Otsubo, A., & Shibata, S. (2006). Cardiovascular regulation response to hypoxia during stepwise decreases from 21% to 15% inhaled oxygen. Aviation, Space, and Environmental Medicine, 77(10), 1015–1019.

Javorka, M., Zila, I., Balharek, T., & Javorka, K. (2002). Heart rate recovery after exercise: Relations to heart rate variability and complexity. Brazilian Journal of Medical and Biological Research, 35(8), 991–1000. doi:10.1590/S0100-879X20020000100018

Jun, Q., Lan, H., Kaixin, T., Shiyong, Y., Yang, Y., & Min, L. (2008). Changes in autonomic nervous system function in healthy young men during initial phase at acute high-altitude exposure. Journal of Medical Colleges of PLA, 23, 270–275. doi:10.1016/S1000-1948(08)60053-2

Karinen, H. M., Uusitalo, A., Vähä-Ypyä, H., Kähönén, M., Peltonen, J. E., Stein, P. K., ... Tikkanen, H. O. (2012). Heart rate variability changes at 2400 m altitude predicts acute mountain sickness on further ascent at 3000–4300 m altitudes. Frontiers in Physiology, 3, 1–15. doi:10.3389/fphys.2012.00336

Maier, K., Wille, M., Grander, W., & Burtscher, M. (2013). Effects of exercise and hypoxia on heart rate variability and acute mountain sickness. International Journal of Sports Medicine, 34(8), 700–706. doi:10.1055/s-0032-1327577

Mateo, M., Blasco-Lafarga, C., Martínez-Navarro, I., Guzmán, J. F., & Zabala, M. (2012). Heart rate variability and pre-competitive anxiety in BMX discipline. European Journal of Applied Physiology, 112(1), 113–123. doi:10.1007/s00421-011-1962-8

Millet, G. P., Fais, R., & Pialoux, V. (2012). Point: Counterpoint: Hypobaric hypoxia induces/does not induce different responses from normobaric hypoxia. Journal of Applied Physiology, 112(10), 1783–1784. doi:10.1152/japplphysiol.00607.2012

Saito, S., Tanobe, K., Yamada, M., & Nishihara, F. (2005). Relationship between arterial oxygen saturation and heart rate variability at high altitudes. The American Journal of Emergency Medicine, 23(1), 8–12. doi:10.1016/j.ajem.2004.09.023

Selye, A., & Macklem, P. T. (2004). Complex systems and the technology of variability analysis. Critical Care, 8(6), R367–R384. doi:10.1186/cc2948

Sullivan, G. M., & Feinn, R. (2012). Using effect size—or why the P value is not enough. Journal of Graduate Medical Education, 4(3), 279–282. doi:10.4300/JGME-D-12-00156.1

Sztajerl, J. (2004). Heart rate variability: A non-invasive electrocardiographic method to measure the autonomic nervous system. Swiss Medical Weekly, 134, 514–521.

Taralov, Z., Terziyski, K., Dimov, P., Marinov, B., Tarvainen, M. P., Perini, R., & Kostianev, S. (2015). Assessment of the acute impact of normobaric hypoxia as a part of an intermittent hypoxic training on heart rate variability. Cor et Vasa, 57, e251–e256. doi:10.1016/j.crvasa.2015.05.010

Tarvainen, M. P., Niskanen, J.-P., Lipponen, J. A., Ranta-Aho, P. O., & Karjalanen, P. A. (2014). Kubios HRV – heart rate variability analysis software. Computer Methods and Programs in Biomedicine, 113(1), 210–220. doi:10.1016/j.cmpb.2013.07.024

Tarvainen, M. P., Ranta-Aho, P. O., & Karjalanen, P. A. (2002). An advanced detrending method with application to HRV analysis. IEEE Transactions on Biomedical Engineering, 49(2), 172–175.

Thayer, J. F., Ahs, F., Fredrikson, M., Sollers, J. J., & Wager, T. D. (2012). A meta-analysis of heart rate variability and
neuroimaging studies: Implications for heart rate variability as a marker of stress and health. *Neuroscience & Biobehavioral Reviews, 36*(2), 747–756. doi:10.1016/j.neubiorev.2011.11.009

Thomas, J. R., Nelson, J. K., & Silverman, S. J. (2010). *Research methods in physical activity*. Champaign, IL: Human Kinetics.

Tsuji, H., Larson, M. G., Venditti, F. J Jr., Manders, E. S., Evans, J. C., Feldman, C. L., & Levy, D. (1996). Impact of reduced heart rate variability on risk for cardiac events: The Framingham heart study. *Circulation, 94*(11), 2850–2855.

Vaillancourt, D. E., & Newell, K. M. (2002). Changing complexity in human behavior and physiology through aging and disease. *Neurobiology of Aging, 23*(1), 1–11. doi:10.1016/S0197-4580(01)00247-0

Vigo, D. E., Lloret, S. P., Videla, A. J., Chada, D. P., Hünicken, H. M., Mercuri, J., … Cardinali, D. P. (2010). Heart rate nonlinear dynamics during sudden hypoxia at 8230 m simulated altitude. *Wilderness & Environmental Medicine, 21*(1), 4–10. doi:10.1016/j.wem.2009.12.022

Yamamoto, Y., Hughson, R. L., Sutton, J. R., Houston, C. S., Cymerman, A., Fallen, E. L., & Kamath, M. V. (1993). Operation Everest II: An indication of deterministic chaos in human heart rate variability at simulated extreme altitude. *Biological Cybernetics, 69*(3), 205–212. doi:10.1007/BF00198960

Yuanyuan, L., Binhua, W., Chengyu, L., Jun, Y., & Zhongtao, C. (2013). Impact of hypoxia on heart rate variability based on sample entropy. *Journal of Theoretical and Applied Information Technology, 48*(2), 1265–1269.

Yuanyuan, L., Zhongtao, C., Jun, Y., Mengsun, Y., Binhua, W., Yanyan, W., & Chengyu, L. (2013). Heart rate variability analysis during stepwise hypoxia from 3000 m to 4500 m. *Life Science Journal, 10*(3), 1127–1131.

Zhang, D., She, J., Zhang, Z., & Yu, M. (2014). Effects of acute hypoxia on heart rate variability, sample entropy and cardiopulmonary phase synchronization. *Biomedical Engineering Online, 13*(1), 73. doi:10.1186/1475-925x-13-73