

RELIABILITY OF MEASURES OF AUTONOMIC ACTIVITY DURING ACUTE HYPOXIC EXPOSURES

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INTRODUCTION

Power spectral analysis of heart rate variability (HRV) has been widely used for assessing autonomic nervous system (ANS) activity (Akselrod *et al.*, 1981, Pagani *et al.*, 1986, Malliani *et al.*, 1991). The power spectra can be divided into low frequency (LF 0.04-0.15 Hz) and high frequency (HF, 0.15-0.4 Hz) components. HF power mainly reflects the parasympathetic influence (Pomeranz *et al.*, 1985, Malliani *et al.*, 1991), whereas it appears that LF power may be modulated by both sympathetic and parasympathetic activity (Pagani *et al.*, 1986, Pomeranz *et al.*, 1985) and, as such, should be interpreted cautiously (Houle and Billman, 1999, Jokkel *et al.*, 1995).

Hypoxia is suggested to be a potent stimulator of the sympathetic nervous system (Rostrup, 1998). Exposure to hypoxic environments decreases arterial oxygen saturation and, as a result, autonomic balance is suggested to change. This has been determined indirectly from measurements of heart rate variability (HRV) (Povea *et al.*, 2005, Bernardi *et al.*, 1998, Bernardi *et al.*, 2001). Recent evidence suggests that repeated bouts of hypoxia increases chemosensitivity (Katayama *et al.*, 2001, Foster *et al.*, 2005) and that sympathetic activity may remain elevated after the termination of an acute hypoxic exposure (Wadhwa *et al.*, 2008, Xie *et al.*, 2001, Mitchell *et al.*, 2001). Therefore, the first acute hypoxic exposure may influence the measures of HRV during a second acute hypoxic episode.

The aim of the present study was to establish the reliability of measures of HRV (when participants rested and exercised) during two acute hypoxic exposures separated by 96 hours. It was hypothesised that there would be no differences in the autonomic responses to the two acute hypoxic exposures.

METHODS

Twelve male volunteers gave their written informed consent to participate in the ethically approved study (University of Portsmouth Biosciences Research Ethics Committee). Their average (SD) age, height, mass and sum of 8 skinfolds were: 23 (4) years, 1.80 (0.09) m, 80.4 (13.0) kg and 73.7 (27.5) mm, respectively.

The subjects were familiarised thoroughly prior to the experiment and completed the experiment during two sessions separated by 96 hours. Participants performed the following conditions twice during each testing session, once normoxic (N, $F_{I}O_2 = 0.2093$) and once whilst hypoxic (H, $F_{I}O_2 = 0.15$):

1. Supine rest (10 min)
2. Seated unloaded cycling (10 min) at 60 revs min⁻¹
3. Seated cycling at 100 Watts of external work for (10 min) at 60 revs min⁻¹.

The six conditions were performed in a balanced order, with each ten-minute bout separated by a recovery period for f_C and \dot{V}_E to return to baseline levels. The recovery periods were approximately 10 minutes in duration.

During each testing session ambient data were recorded and subjects were instrumented with a 3-lead ECG (Lifepulse HME, UK) (for the measurement of f_C and HRV), a pulse oximeter finger probe (to estimate oxygen saturation) (Nonin 7500, US) and an oro-nasal mask for the collection of expired air measurements (V_T , f_R , $F_{E}O_2$ and $F_{E}CO_2$). The gas concentrations and volume were measured using a gas analyser (Hi-tech Instruments limited, Bedfordshire, UK), and pneumotach (Hans Rudolph, US). All of these systems were recorded onto laptop computer via a Powerlab data acquisition system (AD Instruments, Australia). The \dot{V}_E was calculated from the product of f_R and V_T , and the $\dot{V}O_2$ was computed using \dot{V}_E , $F_{E}O_2$ and $F_{E}CO_2$.

The ECG waveform was analysed using HRV analysis software (KubiosHRV version 2.0, Biosignal Analysis and Medical Imaging Group, University of Kuopio, Finland) to provide time (R-R interval and SDNN (standard deviation of N-N intervals) and frequency domain (LF power, HF power, LF:HF ratio and total power) measures of HRV following the parameters recommended by The Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology (1996). The last five minute portion of each ten minute condition was analysed using Fast Fourier Transforms. The spectral power analyses are presented in absolute terms (ms^2) and log transformed using the natural logarithm (Ln).

The participants performed the experimental sessions clothed in shorts, a t-shirt and trainers. The room was maintained at an ambient temperature of $22.0(0.7)^\circ C$ and relative humidity 48 (10)% during all trials.

Statistical Analysis

All statistical analyses for measures of difference were performed using SPSS version 15. A factorial ANOVA with repeated measures (Day x Oxygen level x Posture), was used to investigate between conditions differences *post-hoc* by pairwise comparisons. Statistical significance was accepted at $P < 0.05$ and a trend was defined as $P < 0.1$, with data presented as means (SD). Coefficients of variation were also calculated using the method of Hopkins (2000).

RESULTS

There were no significant differences between experimental days in any of the variables measured. However, the reliability of the measurements calculated by the coefficient of variation ranged from 10.8-24.4% for the respiratory measures, from 3.3-27.0% for time domain measures of HRV and from 21.0-107.2% for frequency domain measures of HRV (Table 1).

The measures of HRV, SpO₂ and respiratory parameters during all conditions from the first experimental day are shown in Table 1. With respect to time domain measures, f_C was higher during all H exposures compared to N. Consequently, the R-R interval was reduced significantly by the H environment compared to N, however, SDNN did not differ between N and H during supine rest, unloaded or 100 W of cycling.

Table 1. Coefficients of variation (%) of measures of heart rate variability, respiratory parameters and estimated oxygen saturation.

	Supine		Unloaded		100 W	
	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia
f_c (beats.min ⁻¹)	4.7	4.9	7.4	4.6	4.1	5.0
R-R interval(ms)	3.5	4.0	6.6	5.0	3.3	4.6
SDNN (ms)	15.8	11.9	25.0	27.0	20.1	17.3
HF (ms ²)	31.2	54.5	107.2	96.2	68.5	80.1
Ln HF (ms ²)	3.7	7.0	13.4	12.7	30.1	48.2
Total power	21.0	36.0	73.4	64.2	52.9	61.3
LF:HF	53.3	54.5	47.1	39.6	35.4	35.7
\dot{V}_E (L.min ⁻¹)	16.1	22.2	20.2	18.0	11.4	11.1
$\dot{V}O_2$ (L.min ⁻¹)	15.7	24.4	19.2	19.2	11.1	10.8
SpO ₂ (%)	0.5	1.1	0.8	1.1	0.6	0.8

The mean total power declined during the unloaded and 100 W cycling conditions compared to supine rest, despite the large SD in the supine and unloaded cycling conditions. Total power was lower when cycling at 100 W in H compared to N, but no differences in total power between N and H were observed during unloaded cycling or supine rest, which again may be a consequence of the large SD. Similarly, differences in HF power (ms²) during N and H were found during 100 W of cycling, but not during supine rest or unloaded cycling conditions, whereas, Ln HF power was significantly reduced in H compared to N during the unloaded cycling and 100 W of cycling. Both HF and Ln HF power were significantly reduced between the supine rest and unloaded cycling conditions and between the unloaded cycling and cycling at 100 W. Whereas, the LF:HF ratio was significantly greater when cycling at 100 W during H compared to the N environment.

Table 2. Mean (SD) of heart rate variability measures when subjects were breathing normoxic or hypoxic gas, whilst resting in a supine position, performing unloaded recumbent cycling and 100 W on the recumbent cycle ergometer ($n=12$).

	Supine		Unloaded		100w	
	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia
HR (beats.min ⁻¹)	65(9) ‡	69(10) ‡	77(10)*a‡	83(7)*a‡	113(17)*a‡	123(14)*a‡
R-R interval (ms)	948(132) ‡	890(159) ‡	765(107) *a‡	723(63) *a‡	497(64) *a‡	465(66) *a‡
SDNN (ms)	80(50)	71(33)	49(17)*a	43(17)*a	13(6)*a	11(7)*a
HF (ms ²)	1889(2539)	1008(993)	381(385)*a	297(495)*a	21(29) *a‡	9(15)*a‡
Ln HF	6.6(1.4)	6.3(1.1)	5.4(1.0)*a ‡	5.0(0.9)*a ‡	2.3(1.2)*a ‡	1.4(1.2)*a ‡
Total power	3626(3804)	2461(922)	1037(609)*a	922(861)*a	63(66)*a‡	24(25)*a ‡
LF:HF ratio	1.3(1.1)	1.9(2.1)	1.8(1.3)	2.3(1.9)	1.6(0.9) ‡	2.5(1.6) ‡
\dot{V}_E (L.min ⁻¹)	9.6(2.7)	10.5(2.9)	15.3(2.7)*a‡	17.0(5.0) *a‡	41.5(7.5)*a‡	49.2(14.6) *a‡
$\dot{V}O_2$ (L.min ⁻¹)	0.4(0.1)	0.4(0.1)	0.6(0.1)*a	0.7(0.2)*a	2.0(0.4)*a	2.2(0.6)*a
SpO ₂ (%)	98.0(2.2)‡	92.7(2.8) ‡	98.4(2.4)a ‡	91.9(3.2) a‡	92.3(2.8)*a ‡	89.6(3.4) *a‡

*P<0.05 diff to supine; a P<0.05 diff between unload and 100W; ‡P<0.05 diff between N and H.

The respiratory measures recorded during N and H environments when participants were resting in a supine posture, performing unloaded and 100 W of recumbent cycling are shown in Table 2. \dot{V}_E and $\dot{V}O_2$ were significantly different in all three conditions, with values increasing from supine rest to unloaded cycling to 100 W of cycling. In addition, \dot{V}_E was also elevated during the unloaded cycling and whilst cycling at 100 W in the H environment compared to cycling in the N conditions. However, $\dot{V}O_2$ was not different between N and H conditions at any stage. The SpO₂ was lower during 100 W of cycling compared to the supine and unloaded cycling conditions, and was also lower during all H conditions compared to N.

DISCUSSION

The results of this study suggest that measures of HRV were not significantly different between the two experimental days. Therefore, the first acute hypoxic exposure did not influence the autonomic activity during the second. In addition, the hypoxic environment did affect measures of HRV. Frequency domain measures of HRV indicated that parasympathetic withdrawal occurs when exposed to hypoxic environments or whilst performing during light cycling exercise (indicated by a reduced total power, HF power and elevated in LF:HF ratio). However, elevated sympathetic activity (indicated by a increase in LF power) was not found when participants were hypoxic in the present study. This suggests that there was no change in sympathetic activity, this is in contrast to previous studies which found an elevated LF power during and after the acute hypoxic exposure (Wadhwa et al., 2008, Xie et al., 2001). However, both previous studies exposed volunteers to lower levels of hypoxia than the present study. Therefore, the hypoxic exposure in the present study may not have been stressful enough to stimulate an increase in sympathetic activity compared to normoxia.

When assessing the reliability of HRV, previous research suggests that the frequency domain measures have moderate to poor reliability (Sandercock *et al.*, 2005) and should not be used as the sole measure of autonomic activity (Jauregui-Renaud *et al.*, 2001), but combined with other measures to corroborate the findings. The present study confirms this view, as the time domain measures had a moderate degree of reproducibility, whereas frequency domain measures showed poor reliability between repeated measures which was improved when the absolute HF power values were log transformed (Ln HF).

Whilst participants were exercising or breathing the hypoxic environment, f_C and \dot{V}_E were elevated, the largest increase occurred when cycling at 100 W in the hypoxic environment. The elevations in f_C and \dot{V}_E were in proportion to the O₂ demand and supply pathway, the greater changes in f_C and \dot{V}_E were observed when the O₂ demand in the respiring tissues was greatest (during exercise in a hypoxic environment), at this point the level of parasympathetic activity was reduced in order to elevate f_C .

It is concluded that light cycling exercise in a hypoxic environment (F_IO₂ = 0.15) results in a greater parasympathetic withdrawal than cycling in a normoxic environment, however there is no elevation in sympathetic activity. In addition, acute hypoxic exposures separated by 96 hours have no effect on measures of HRV therefore the hypothesis is accepted. However, the reliability of the measures of HRV, particularly frequency domain measures (which are indicative of

autonomic activity) is questionable and should not be used as the sole measure of autonomic activity.

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