RELIABILITY OF NON-INVASIVE MEASURES OF AUTONOMIC MODULATION IN DIFFERENT POSTURES AND EXERCISE INTENSITIES

Heather Lunt, Martin J. Barwood, Jo Corbett, Michael J. Tipton
Department of Sport and Exercise Sciences, University of Portsmouth, Cambridge Road, Portsmouth, PO1 2ER
Contact person: heather.lunt@port.ac.uk

INTRODUCTION

The effectiveness of any technique that maybe used to detect differences within or between individuals on separate occasions is dependant upon its reliability. Repeated heart rate variability (HRV) measures are performed in clinical settings to predict mortality of patients (Kleiger et al., 1987) and the general population (Kleiger et al., 1991). In addition, HRV provides an index of autonomic balance; sympathetic and parasympathetic activity (Akselrod et al., 1981) and can be used to monitor autonomic activity during exposures to different environmental conditions (Hughson et al., 1994, Westerlund et al., 2006). For these reasons, the reliability of HRV measures is important if valid conclusions are to be derived from its use.

Numerous studies have investigated HRV in both healthy and diseased human populations, (approximately 500-600 published articles a year) (Pinna *et al.*, 2007) but there is a paucity of data on the reliability of HRV measurement. From the published evidence, several conclusions have been reached (Pinna et al., 2007): firstly, measures of HRV taken during rest maybe more reliable than those made during interventions such as tilt or cold pressor testing. Secondly, healthy subjects provided more reliable HRV data than clinical populations. Consequently, normative values of HRV measures may depend upon the population tested and postures or activities being performed during data recording. Studies have performed repeated measures of HRV on healthy subjects at rest and during cold pressor tests (Jauregui-Renaud *et al.*, 2001), it was concluded that HRV measurements, although reproducible, have large differences between and with-in subjects. The author recommended that measures of HRV should not be used as the sole measure of autonomic response.

The aim of this study was to establish the reliability of HRV as a non-invasive measure of autonomic nervous function at rest and during light recumbent cycling exercise. It was hypothesised that measures of HRV would not differ between repeated recordings when at rest and during moderate recumbent cycling (H_0).

METHODS

Following receipt of ethical approval, eight males gave their written informed consent to participate. Their average (SD) age, height, mass and sum of 8 skinfolds were: 19 (1) years, 1.81 (0.08) m, 83.2 (8.0) kg and 75.2 (18.1) mm. Each participant was familiarised with the surroundings and equipment used during the study. The study consisted of two test sessions separated by 96 hours. Ten-minutes prior to the start of the experimental session the participants were instrumented with ECG electrodes and leads (HME, UK). This phase was followed by four periods of ten-minutes during which the following postures and exercise work rates were adopted by participants:

1. Supine rest with collection of expired air measurements, subjects wore the respiratory mask for all subsequent measurement periods. (Supine)
2. Paced breathing whilst in a supine posture (Paced),
3. Unloaded recumbent cycling (Unloaded Cycling),
4. Loaded recumbent cycling at 100 Watts (100 W of Cycling).

All conditions were performed in a balanced order on the same day; each condition was separated by a recovery period during which *fc* and $\dot{V}_E$ recovered to baseline values. During the paced condition, participants were asked to voluntarily control their respiratory frequency (f_R) in time to an audible signal (SQ50, Seiko, China) (paced breathing at 10 breaths.min^{-1}, 0.17 Hz), throughout the other conditions they breathed spontaneously.

The participants performed the experimental sessions clothed in shorts, a t-shirt and trainers. Ambient temperature (Ta; °C) was monitored via wet and dry bulb thermometers and maintained at 23 .8 (0.7) °C and relative humidity (RH, %) at 38.1 (4.1) % for the experimental sessions.

Data Analysis

During each testing session ambient data were recorded and subjects were instrumented with a 3-lead ECG (Lifepulse HME, UK) for analysis of HRV, and an oro-nasal mask for the collection of expired air measurements (V_T, f_R,). 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 through 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_EO_2 and F_ECO_2.

The ECG waveform was analysed using HRV analysis software (KubiosHRV version 2.0, Biosignal Analysis and Medical Imaging Group, University of Kulpio, 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 frequency domain HRV measures were presented in log transformed terms using the natural logarithm (Ln). The log transformation of the raw HRV values was recommended due to the heteroscadastic nature of the measurement (Bland and Altman, 1996).

Statistical Analysis

A one-way repeated measures ANOVA was used to investigate between conditions differences in time and frequency domain measures of HRV. Significant differences were examined *post-hoc* by a Tukey test. Statistical significance was accepted at $P<0.05$ with data presented as means (SD). The reliability of the measures was calculated using the technical error of measurement coefficient of variation calculated at a percentage (TEM CV%) described by Hopkins (2000).

RESULTS

Reliability of Measures of HRV

No significant differences in any of the measures of HRV were observed between the first and second experimental days (Table 1). However, the reliability of the measure cannot be determined from tests of statistical difference. When a reliability statistic (CV) was utilised for time domain measures of HRV (R-R interval) the CVs between the first and second measurements were small to moderate (Table 1). This suggests that these measures were reproducible. In contrast, frequency domain measures of total power, HF (ms^2) and LF:HF ratio show large variations in values between the first and second measurement. Normalising or log transforming the raw data reduced the CVs (Table 2).

Table 1. Mean (SD) of time and frequency domain measures of HRV and respiratory variables during supine rest, during paced breathing in a supine position, seated unloaded cycling and 100 W of cycling, *n*=8

	Supine		Paced		Unloaded		100 W	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
R-R interval (ms)	870(130)	920(142)	879(85)	953(87)	724(110)	766(173)	503(52)*	509(57)*
Total power (ms^2)	1717(1292)	1614(1323)	1656(789)	2804(2040)	512(471)	495(484)	18(13)a	30(27)a
Ln Total Power	7.2(0.8)	7.1(0.9)	7.3(0.6)	7.6(0.8)	5.7(1.3)	5.7(1.2)	2.6(0.8)*ab	2.7(1.3)*ab
HF (ms^2)	814(732)	1077(1048)	975(593)	822(581)	133(117)	124(57)	3(2)*	5(4)*
Ln HF	6.3(0.9)	6.4(1.2)	6.7(0.6)	6.7(0.6)	4.5(1.1)	4.7(0.6)	0.9(0.9)*	1.3(1.0)*
LF:HF ratio	0.8(0.3)	1.1(0.8)	0.6(0.6)	0.5(0.3)	3.8(2.1)*	2.1(1.8)*	2.8(1.2)*	2.8(1.2)*

Key * Different from Supine $P<0.05$, a Different to Paced $P<0.05$, b Different to Paced $P<0.05$

Table 2. Coefficients of variation (%) for time and frequency domain measures of HRV and respiratory variables during supine rest, during paced breathing in a supine position, seated unloaded cycling and 100 W of cycling

	Supine	Paced	Unloaded	100 W
R-R interval(ms)	13.5	9.1	12.3	3.6
HF (ms^2)	297.4	87.0	120.4	196.3
Ln HF(ms^2)	32.0	9.2	11.6	17.4
HF (nu)	63.1	20.3	62.8	36.6
Total power	120.0	62.3	129.0	158.1
Ln Total Power	11.7	6.6	14.4	16.8
LF:HF ratio	188.3	55.2	107.9	96.2

The Effect of Condition on Measures of HRV

Statistically significant reductions in R-R interval, HF(ms^2) and Ln HF were observed between cycling at 100 W and supine conditions; the values for paced and unloaded cycling conditions were not different to those seen the supine condition. Whereas the total power (ms^2) was found to be greater during the paced breathing condition in comparison to 100 W of cycling. However, significantly larger values of log transformed total power were observed during the supine, paced and unloaded conditions in comparison to 100 W of cycling exercise. Finally, in both the unloaded cycling and 100 W of cycling, the LF:HF ratio was significantly larger than during the supine or during paced breathing conditions.

DISCUSSION

The primary aim of this study was to establish if repeated measurements HRV were reliable at rest and during moderate recumbent cycling. No significant differences between the two trial days were observed for any of the measures of HRV (Table 1). Cycling at 100 W resulted in a withdrawal of parasympathetic activity (indicated by a decline in HF power variables) in comparison to the supine condition. The paced breathing condition resulted in the greatest parasympathetic activity (indicated by elevated total power and HF power). This agrees with the findings of previous studies (Hirsch and Bishop, 1981, Blain et al., 2005).

Statistical tests of difference establish the probability that a change in a measure is not due to chance. They do not indicate the reliability of the data, for this a measure of reliability is required, such as the CV. Coefficients of variation for the raw frequency domain measures of total power, HF (ms^2) power and LF:HF ratio were large in all conditions, indicating poor reliability. However, normalised values reduced the CV, and further reductions in the CVs were found when the raw data were log transformed, which suggests that log transformed measures of HRV had moderate reliability. Consequently, HRV should not be used as a single measure for determining autonomic activity; other methods of monitoring autonomic function should be employed to confirm the log transformed HRV data. Therefore, this study supports the findings of Jauregui-Renaud and colleagues (2001).

The smallest CVs in the present study were found during the paced breathing condition (6.6-87%) (which also resulted in the greatest level of parasympathetic activity). Similar CV values to those observed in this study have been previously reported, from 25-139% for time domain and 45-114% for frequency domain measures (Ponikowski et al., 1996, Lord et al., 2001). However, smaller CVs were found by Sinnerich *et al.* (1998) when subjects were familiarised with the study, this increased familiarity with the protocol and allows any learning effects to occur prior to the measurement phase of the study, thus resulting in more reliable results. In addition, smaller CVs were also found when R-R interval editing was carried out both automatically and reviewed manually by a skilled operator. Both of these processes were performed in this study.

During the exercising conditions, the lowest CVs were observed during unloaded cycling, with CVs of the R-R interval further declining during the 100 W of exercise as parasympathetic withdrawal occurs (indicated by a reduction in Ln HF), Whereas CV values for frequency domain measures were larger during 100 w of cycling than during the unloaded cycling condition. The frequency domain signals during the 100 W of exercise were small in comparison to the other conditions, therefore small biological variations in either the first or second trial would have resulted in the larger CVs.

It is concluded that CVs of raw frequency domain measures of HRV have poor reliability during rest and exercise when recordings are separated by 96 hours. However, log transformation of raw frequency domain measures of HRV reduces the CVs. Therefore, the hypothesis can be accepted, as R-R interval and log transformed frequency domain measures of HRV had moderate reliability between repeated measurements. However, it is recommended that an additional measure of autonomic activity should be used to corroborate the findings of the HRV data.

ACKNOWLEDGEMENTS

Thanks to the subjects for their patience and Geoff Long, Nicola Fergusson, Amanda Ward and Christine Balch for their technical support.

REFERENCES

Akselrod, S., D, G., Ubel, F., Shannon , D., Berger, A. & Cohen, R. 1981. Power spectrum analysis of heart rate fluctuations: a quantitative probe of beat-to-beat cardiovascular control. *Science,* **213,** 220-222.

Blain, G., Meste, O. & Bermon, S. 2005. Influences of breathing patterns on respiratory sinus arrythymia in humans during exercise. *American Journal Heart Circulatory Physiology,* **23,** H887-H895.

Bland, J. M. & Altman, D. G. 1996. Statistics Notes: Logarithms. *British Medical Journal,* **312,** 700.

Hirsch, J. & Bishop, B. 1981. Respiratory sinus arrhythmia in humans: how breathing pattern modulates heart rate. *American Journal of Physiology,* **241,** H620-H629.

Hughson, R. L., Yamamoto, Y., McCullough, R. E., Sutton, J. R. & Reeves, J. T. 1994. Sympathetic and parasympathetic indicators of heart rate control at altitude studied by spectral analysis. *Journal Applied Physiology,* **77,** 2537-2542.

Jauregui-Renaud, K., Hermosillo, A., Marquez, M., Ramos-Aguilar, F., Hernandez-Goribar, M. & Cardenas, M. 2001. Repeatability of heart rate variability during simple cardiovascular reflex tests on healthy subjects. *Archives of Medical Research,* **32,** 21-26.

Kleiger, R., Bigger, T., Bosner, M., Chung, M., Cook, J., Rolnitzky, L.*, et al.* 1991. Stability over time of variables measuring heart rate variability in normal subjects. *The American Journal of cardiology,* **68,** 626-630.

Kleiger, R. E., Miller, J. P., Bigger, T. & Moss, A. J. 1987. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *The American Journal of cardiology,* **59,** 256-262.

Lord, S. W., Senior, R. R., Das, M., Whittam, A. M., Murray, A. & McComb, J. M. 2001. Low-frequency heart rate variability: reproducibility in cardiac transplant recipients and normal subjects. *Clinical Science,* **100,** 43-46.

Pinna, G., Maestri, R., Torunski, A., Danilowicz-Szymanowicz, L., Szwoch, M., La Rovere, M.*, et al.* 2007. Heart rate variability measures: a fresh look at reliability. *Clinical Science,* **113,** 131-140.

Ponikowski, P., Piepoli, M., Amadi, A. A., Chua, T. P., Harrington, D., Volterrani, M.*, et al.* 1996. Reproducibility of heart rate variability measures in patients with chronic heart failure. *Clinical Science,* **91,** 391-398.

Sinnreich, R., Kark, J. D., Friedlander, Y., Sapoznikov, D. & Luria, M. H. 1998. Five minute recordings of heart rate variability for population studies: repeatability and age-sex characteristics. *British Medical Journal,* **80,** 156-162.

The Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology 1996. Heart Rate Variability: Standards of measurement, Physiological Interpretation and clinical use. *Circulation,* **93,** 1043-1065.

Westerlund, T., Uusitalo, A., Smolander, J. & Mikkelsson, M. 2006. Heart rate variability in women exposed to very cold air (110^{o}C) during whole-body cryotherapy. *Journal of Thermal Biology,* **31,** 342-346.