Reliability of heart rate variability in healthy older women at rest and during orthostatic testing

Sylvia Reland[1], Nathalie S. Ville[2,3], Sara Wong[4], Guy Carrault[4], and François Carré[1,3]

[1]Groupe de Recherche Cardiovasculaire, Université de Rennes 1, Rennes cedex, Unité de Biologie et Médecine du Sport, CHU Pontchaillou, Rennes, [2]Laboratoire de Physiologie et Biomécanique de l'Exercice Musculaire, UFR STAPS Université de Rennes 2, Rennes, [3]GIS Sciences du Mouvement, Université de Rennes 2, Rennes, [4]Laboratoire Traitement du Signal et de l'Image, INSERM Université de Rennes 1, Rennes cedex, France

ABSTRACT. ***Background and aims:*** *In the older population, the reliability of heart rate variability (HRV) has only been evaluated in a few studies, in the supine position, and covering a broad sample of age and patients of both sexes. To document the relevance of using HRV analysis in healthy older women, the aim of this study was to evaluate the reliability of HRV indexes during three classical tests.* ***Methods:*** *33 healthy women (66.9±0.7 years old) performed two test sessions. Each session consisted of an ECG recorded in the supine position, first with free breathing (Test 1), then with controlled breathing (Test 2), and in the upright position (Test 3). Time and frequency HRV indexes were obtained by processing the ECG signals. Reliability was assessed between sessions using Student's paired* t*-test, intraclass correlation coefficient (ICC) and coefficient of variation (CV).* ***Results:*** *There were no differences between the sessions. ICC showed good reliability for all HRV indexes. CV was low for absolute HRV indexes, except in Test 3 for parasympathetic indexes with modest CV. The CV of HRV ratio indexes were modest to high in all three tests.* ***Conclusions:*** *Time and absolute frequency HRV indexes are reliable when testing healthy older women. Our results support the use of such indexes in gerontology research, to assess the effects of clinical or pharmacological interventions on the autonomic nervous system.*

(Aging Clin Exp Res 2005; 17: 316-321)

INTRODUCTION

Spontaneous fluctuations around the mean heart rate, called heart rate variability (HRV) can be studied non-invasively and reflect the sinus node response to parasympathetic and sympathetic inputs of the autonomic nervous system (1). HRV is used in a wide variety of clinical research settings (2-5), particularly in gerontology (3-5). Indeed, as HRV decreases with age, it can be used as a biological marker of the aging process (4). Low HRV has been identified as a validated cardiovascular risk marker, and represents a useful prognostic value in clinical cardiology (6). Since coronary heart diseases and the incidence of sudden cardiac death increase in women after menopause (7), it is important to study HRV in older women.

Various physiological stresses, such as controlled breathing or active standing, are used to assess autonomic cardiovascular modulations (8, 9). HRV index reliability depends on the condition in which it is measured (2, 10, 11). Thus, before these various stresses can be used to evaluate the effects of clinical intervention (i.e. drug therapy) on HRV, their reliability must be studied.

In healthy mixed young and older populations, the results of HRV reliability are controversial (10-14). Since age and gender have been demonstrated to influence HRV (15, 16), these factors can be considered to contribute to these discrepancies. In aging subjects, to the best of our knowledge, HRV reliability has only been tested in the supine position, and is still poorly documented (13, 17). Indeed, its reliability has only been evaluated in a few studies, concerning a broad sample of age and patients of both sexes.

The aim of the present study was therefore to evaluate the reliability of HRV indexes during three classical clinical tests in healthy older women. HRV analysis was performed at rest in the supine position, with either free or controlled breathing, and in active standing posture.

Key words: Autonomic nervous system, heart rate variability, orthostatic testing, post-menopausal women.

Correspondence: S. Reland, PhD, Unité de Biologie et Médecine du Sport, CHU Pontchaillou, 2 rue Henri Le Guilloux, 35000 Rennes, France.
E-mail: sylvia_reland@yahoo.fr

Received May 14, 2004; accepted in revised form November 10, 2004.

MATERIALS AND METHODS

Subjects

After the study protocol had been explained to them, 35 post-menopausal women involved in leisure association activities volunteered to participate in this study. The nature, purpose and risks of the study were clearly explained to each subject and their individual written informed consent was obtained. The women were non-smokers, normotensive, and free of cardiovascular disease according to history and clinical examination, including resting and maximal exercise blood pressure measurements and electrocardiograms (ECG). None were on cardioactive medication. Some were receiving hormone replacement therapy, which remained unchanged throughout the research protocol.

Experimental procedure

Subjects were first familiarized with the controlled breathing test without ECG recording.

Then two experimental sessions were performed at a one-week interval. Subjects were instructed to refrain from any excessive physical activity and from drinking beverages containing caffeine or alcohol for at least 24 h before testing. Both sessions were held in the morning, 3 hours after a light meal in the same room with a low noise level, constant lighting, and a temperature of 20-22°C. The same technician (SR) performed all recordings.

Before recordings, skin electrodes were placed for a three-lead ECG, and then subjects rested for 10 min in a supine position. Each session included three test recordings: supine position with free breathing (Test 1), supine position with controlled frequency breathing (Test 2), and orthostatism with free breathing (Test 3). A previous pilot study had shown no significant difference in HRV data between 12, 15 and 20 breaths/min controlled breathing frequency. The 20 breaths/min rate was retained because it seemed the easiest for the subjects to maintain. To achieve 20 breaths/min, subjects inspired (1.5 s) and expired (1.5 s) in synchrony with an audible signal. No attempt was made to influence tidal volume. After Test 2, the subjects remained in an undisturbed supine resting position for 10 minutes. Then, they were asked to stand up suddenly as quickly as possible, and to remain standing unsupported for 6 minutes. The ECG recording of Test 3 started from the beginning of the postural change. ECG and breathing frequency were recorded for a 6-min period in each test.

ECG data analysis

ECG was sampled at 1000 Hertz with the PowerLab® acquisition system (AD Instruments Pty Ltd, Castle Hill, Australia), installed in a Macintosh computer (Power Mac). Thus, the accuracy of measurements was 1 ms. The first minute of each ECG recording was disregarded to allow for stabilization of data prior to analysis. The QRS complex was detected using Gritzali's algorithm (18). The RR interval sequence was defined by the duration (in ms) between two consecutive R peaks. These data were edited to eliminate any glitches due to premature cardiac contraction, using the procedure reported by Bruggeman and Andersen (19). Each RR interval was visually validated by two experts (SR, SW) before time and frequency analysis. For each RR sequence, three classical time parameters were extracted (20): mean RR interval, which represents mean heart rate; standard deviation of the average of RR interval, which reflects all cyclic components responsible for variability in the recording period (SD RR); the square root of the mean squared differences between adjacent RR intervals (rMSSD), which is considered as an index of parasympathetic modulation in HR. Prior to power spectrum density estimation, the RR sequence, which is intrinsically non-evenly spaced data, was linearly interpolated in order to obtain a series of uniformly sampled data. Interpretation of the frequency contents of HRV is therefore possible, independent of the mean RR value. The sampling rate was then set at 2 Hz. Using a sliding window of 64 seconds duration, time-varying auto-regressive (TVAR) modeling of the interpolated RR sequence was performed to estimate its power spectrum (in ms^2) in order to eliminate the slight non-stationarities of the sequence. On the basis of the well-know Akaike criteria, the order of the TVAR model was set at 12 (21). Low frequency (LF) was defined between 0.04-0.15 Hz, high frequency (HF) between 0.15-0.4 Hz, and total power (TP) as the sum of HF+LF (22). The spectral power of the RR series in these frequency bands was then calculated and averaged on the last five minutes of each recording (23). HF and LF are expressed in absolute terms (ms^2) and normalized units (n.u.), which represent the relative value of each power component in proportion to the TP (%). HF corresponds to respiratory sinus arrhythmia and represents vagally mediated modulations in heart rate. LF is influenced by both sympathetic and parasympathetic activity (20). LF/HF is a marker of sympatho-vagal balance (20).

Statistical analysis

Results are presented as means ± standard error of the means (SE). In the lack of normality, HRV values were natural log transformed for parametric statistical testing (14).

To verify HRV alterations induced by controlled breathing in the supine position (Test 2) and orthostatism (Test 3) in comparison with supine free breathing (Test 1), Student's paired *t*-test was applied and only the results observed during the last session are presented here.

The reliability of HRV indexes and breathing frequency between the two sessions was first assessed using Student's paired *t*-test, and then the intraclass cor-

Aging Clin Exp Res 17: 316-321, 2005

Table 1 - *Comparison between first and second sessions: intraclass correlation coefficients of HRV indexes calculated in each test.*

	RR (ms)	SDRR (ms)	rMSSD (ms)	HF (ms^2)	LF (ms^2)	TP (ms^2)	LF n.u. (%)	HF n.u. (%)	LF/HF
Test 1	0.91	0.85	0.84	0.83	0.88	0.87	0.85	0.85	0.73
Test 2	0.91	0.65	0.86	0.85	0.79	0.79	0.89	0.89	0.91
Test 3	0.88	0.77	0.79	0.82	0.76	0.78	0.79	0.79	0.88

Test 1: free breathing in supine position; Test 2: controlled frequency breathing (20 breaths/min) in supine position; Test 3: free breathing in upright position.

relation coefficients of the HRV indexes were calculated, as described by Baumgartner (23). Reliability was considered to be "good" if ICC ranged from 0.61 to 0.81 and "almost perfect" if it exceeded 0.81 (24). The conventional CV (25) was also calculated with a 95% confidence interval. A CV was considered as low when under 10%, modest when between 10 and 20%, and high when above 20% (26).

Statistical analyses were performed using Statistica software version 5.97 (StatSoft Inc., U.S.A.). A *p*-value <0.05 was considered as significant.

RESULTS

One subject did not complete the study for personal reasons, and another was excluded because of technical problems. Thus, 33 subjects completed the study. The anthropometric characteristics of the subjects were: 66.9±0.7 years old, weight= 58.1±1.2 kg, height= 159.4±0.9 cm, and BMI= 22.8±0.3 $kg.m^{-2}$.

The main HRV index alterations induced by controlled breathing and orthostatism during the second session are shown in Figure 1. From Test 1 to Test 2, RR, HF ($p<0.05$) and LF ($p<0.001$) decreased and LF/HF did not change. From Test 1 to Test 3, RR and HF decreased ($p<0.001$) and LF did not change, whereas LF/HF increased ($p<0.001$). Moreover, like HF, rMSSD decreased (20.8±1.8 in the supine position *vs* 16.0±1.7 ms in orthostatism, $p<0.001$).

As regards the reliability of HRV indexes and breathing frequency, no significant differences were observed when comparing each respective test between the two sessions.

The ICC values ranged from 0.65 to 0.91 (Table 1). During Test 1, the ICC were higher than 0.81, except for LF/HF, the value of which was 0.73. In Test 2, the ICC of the HRV indexes were higher than 0.81, except for SDRR (ICC 0.65) and LF and TP (ICC 0.79 for both parameters). Lastly, during Test 3, the ICC values ranged between 0.75 and 0.90.

Coefficients of variation with 95% confidence interval are listed in Table 2. They were lower than 10% for time and absolute frequency indexes, except for HF and rMSSD in Test 3 (range 10 to 20%). The CV for LF n.u. ranged from 10 to 20% in all three tests. For HF n.u., it was lower than 20% in Test 2, but higher than 20% in Tests 1 and 3. Lastly, coefficients of variation for LF/HF ranged from 31 to 35% in all three tests.

DISCUSSION

The present study, focusing on healthy older women, shows good reliability for most classical time and frequency HRV indexes, calculated from short ECG recordings obtained at rest in the supine position, with free and controlled breathing, and in orthostatism.

HRV indexes are influenced by a variety of physiological stimuli. Thus, before using these stresses to evaluate the effects of clinical interventions (i.e., drug therapy, physical training) on HRV, HRV index reliability must be confirmed in these conditions. In this study, HRV index reliability was therefore evaluated in three classical tests of au-

Table 2 - *Comparison between first and second sessions: coefficient of variation [95% confidence interval] of HRV indexes calculated in each test.*

	RR (ms)	SDRR (ms)	rMSSD (ms)	HF (ms^2)	LF (ms^2)	TP (ms^2)	LF n.u. (%)	HF n.u. (%)	LF/HF
Test 1	4.2 [2.9-5.4]	4.6 [3.2-6.0]	6.4 [4.0-8.8]	9.8 [6.5-13.1]	7.1 [4.9-9.2]	5.4 [3.4 -7.5]	12.1 [7.5-16.6]	21.0 [15.0-27.5]	31.0 [23.1-40.9]
Test 2	4.0 [3.0-5.1]	6.1 [4.6-7.7]	6.4 [4.4-8.3]	9.0 [5.5-12.5]	9.5 [6.8-12.2]	7.4 [5.0-9.8]	14.7 [9.8-19.6]	17.2 [10.8-23.6]	30.7 [21.9-39.5]
Test 3	5.0 [3.7-6.2]	5.9 [4.3-7.5]	10.0 [9.0-11.1]	14.4 [9.4-19.5]	9.8 [7.1-12.5]	8.9 [6.7-11.1]	11.5 [7.0-16.1]	25.2 [18.9-31.5]	34.9 [26.2-43.6]

Test 1: free breathing in supine position; Test 2: controlled frequency breathing (20 breaths/min) in supine position; Test 3: free breathing in upright position.

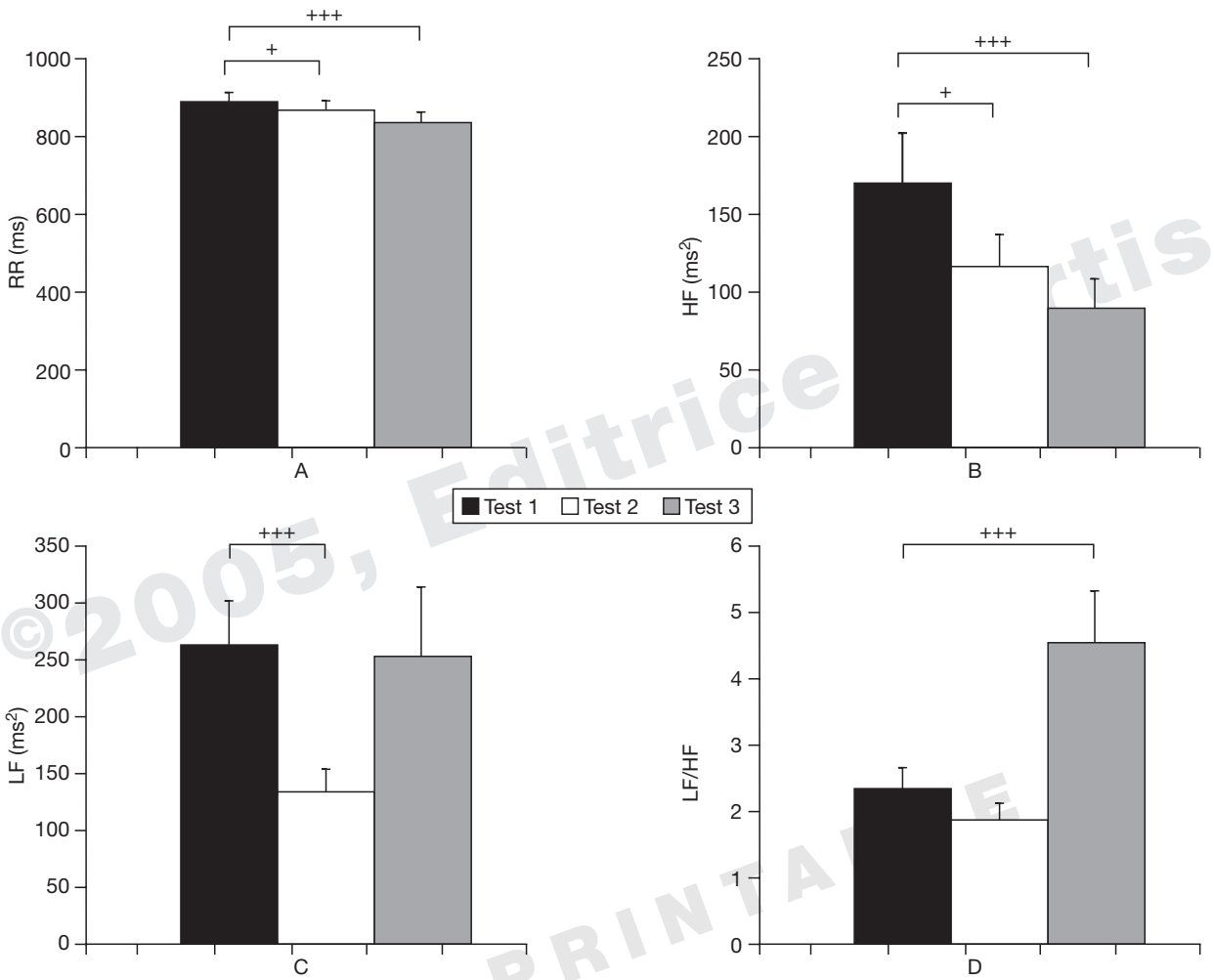

Fig. 1 - *Heart rate variability indexes alterations induced by controlled breathing (Test 2) and orthostatism (Test 3) in comparison with free breathing supine position (Test 1). A= RR, mean RR interval duration (ms); B= HF, high frequency spectral power (ms²); C= LF, low frequency spectral power (ms²); D= LF/HF.* $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$.

tonomic function (8, 9). The reliability of a measurement can be assessed in various and complementary ways. Of these, the intraclass correlation coefficient, a measure of intrasubject reliability, is recommended (27) and commonly used in HRV studies (10, 14). However, it should not be used alone, partly because it depends on the heterogeneity of the sample population (10, 27). As suggested in the literature, we calculated the conventional CV (28), and also its 95% confidence interval (26). The CV also quantifies variations between two measurements, which may be useful from a clinical point of view.

In the supine free breathing position, all absolute HRV indexes were highly reliable, with an ICC ranging from 0.83 to 0.91 and a CV lower than 10%. These results fit those previously published for middle-aged and aged subjects of both genders (13). RR is the highest reliable index, with an ICC of 0.91 and a CV of 4.2%, similar to previous data obtained from 24-hour recording in young adults (29).

Controlled breathing is recommended when studying parasympathetic modulations (20). Indeed, HF peak characteristics depend on respiratory frequency and tidal volume. In most published studies, including this one, only respiratory rate was controlled (30). In a mixed gender population, frequency indexes have been found to be reliable using a controlled breathing rate of 15 breaths/min (15). In our study, at controlled breathing at 20 breaths/min, all time and absolute frequency indexes were also reliable, with an ICC higher than 0.61 and a CV lower than 10%. However, SDRR, TP and LF are less re-

Aging Clin Exp Res 17: 316-321, 2005

liable in controlled *vs* free breathing tests. These results are in agreement with the low reliability of LF previously reported in young people in controlled breathing at 12 breaths/min (2, 11). They may be due to the numerous physiological factors represented by these indexes, as suggested by Amara and Wolfe (11).

Due to the higher risk of orthostatic intolerance, inducing dizziness and falls in the elderly, it is recommended to test orthostatic adaptations in this population (31). In orthostatism, absolute HRV indexes show good reliability with an ICC higher than 0.7 and a CV lower than 10%, except for rMSSD and HF which, following recommendations (26), express a modest CV (10%≤CV≤20%). However, this variability seems to be acceptable as, according to Low (8), one criterion for adequate testing of autonomic function is that the coefficient of variation must be lower than 20-25%. In the present work, as the HF and rMSSD decreased and LF was not altered, the increase in heart rate from supine to standing posture seemed principally due to vagal withdrawal. This result fits the attenuated LF alteration described during a tilt test in older subjects (32). In comparison with the classical tilt test, during the active standing posture test used here, several factors may vary (i.e., speed of posture change and control movements of the body). This may explain the relatively modest CV observed for the parasympathetic indexes. These results must be kept in mind when studying the effects of external factors upon parasympathetic indexes during orthostatic testing, since parasympathetic HRV indexes decrease from supine to orthostatic positions. However, fitting our CV results, in order to be physiologically relevant this decrease may exceed 20%, to avoid the bias of parasympathetic HRV index intrasubject variability noted in 60-70-year-old women.

Normalized unit indexes and LF/HF are also recommended when studying HRV (20). In our population, in no matter which test, the reliability of HF n.u., LF n.u., and LF/HF based on the CV result was lower than that of absolute HRV indexes. To our knowledge, few studies have focused on the reliability of these indexes (10, 26) and their results are not conclusive. This lower reliability found in our study may be due to the fact that these parameters represent the ratio of at least two absolute HRV indexes. Thus, the variability of each component further adds to the variability of the ratio.

Limitations. Some potential limitations of our study should be considered. A tilt test seems more standardized with control of speed and angle of rising, and may be more reliable when studying orthostatism effects. However, analyses of heart rate alterations in the elderly have shown active tests to be more informative than passive ones (31). During both sessions, our three tests were not randomized, the greatest stress being represented by the last one. Nevertheless, a 10-min resting period was respected between the three tests. For more information on the reliability of total autonomic cardiovascular control, blood pressure variability should be measured. However, HRV analysis is a well-accepted tool in investigating autonomic control of the heart (20).

CONCLUSIONS

Time and absolute frequency HRV indexes are reliable in healthy older women in a resting supine position with free or controlled breathing and during orthostatic stress. Our results therefore support the use of these HRV indexes in gerontology research, to assess the effects of clinical intervention on the autonomic nervous system.

ACKNOWLEDGEMENTS

We gratefully thank the technical staff of the Centre Cardio-Pneumologique and of the Unité de Biologie et de Médecine du Sport (CHU Pontchaillou, Rennes), volunteers for their generous cooperation with our project, J.Y. Bansard for statistical assistance, and D. James for English proof-reading and editing.

REFERENCES

1. Malik M, Camm AJ. Components of heart rate variability - what they really mean and what we really measure. Am J Cardiol 1993; 72: 821-2.
2. Dionne IJ, White MD, Tremblay A. The reproducibility of power spectrum analysis of heart rate variability before and after a standardized meal. Physiol Behav (brief communication) 2002; 75: 267-70.
3. Reland S, Ville NS, Wong S, Senhadji L, Carré F. Does the level of physical activity alter heart rate variability in healthy older women? Clin Sci 2004; 107: 29-35.
4. Schwartz JB, Gibb WJ, Tran T. Ageing effect on heart rate variation. J Gerontol 1991; 46A: M99-106.
5. Oida E, Kannagi T, Yamori Y. Aging alteration of cardiac vagosympathetic balance assessed through the tone-entropy analysis. J Gerontol 1999; 54A: M219-24.
6. Malik M, Camm AJ. Heart rate variability and clinical cardiology. Br Heart J 1994; 71: 3-6.
7. Eaker ED, Chesebro JH, Sacks FM, et al. Cardiovascular disease in women. Circulation 1993; 88: 1999-2009.
8. Low PA. Autonomic nervous system function. J Clin Neurophysiol 1993; 10: 14-27.
9. Guo XH, Yi G, Batchvarov V, et al. Effect of moderate physical exercise on noninvasive cardiac autonomic tests in healthy volunteers. Int J Cardiol 1999; 69: 155-68.
10. Pitzalis MV, Mastropasqua F, Massari F, et al. Short and long term reproducibility of time and frequency domain heart rate variability measurements in normal subjects. Cardiovasc Res 1996; 32: 226-33.
11. Amara CE, Wolfe LA. Reliability of non invasive methods to measure cardiac autonomic function. Can J Appl Physiol 1998; 23: 396-408.
12. Breuer HWM, Skyschally A, Wehr M, et al. Poor reproducibility of heart rate variability indices. Z Kardiol 1992; 81: 475-81.
13. Freed LA, Stein KM, Gordon M, et al. Reproducibility of power spectral measures of heart rate variability obtained from short-term sampling periods. Am J Cardiol 1994; 74: 972-3.
14. Melanson EL. Resting heart rate variability in men varying in habitual physical activity. Med Sci Sports Exerc 2000; 32: 1894-901.

15. Sinnreich R, Kark JD, Friedlander Y, et al. Five minute recordings of heart rate variability for population studies: repeatability and age-sex characteristics. Heart 1998; 80: 156-62.
16. Agelink MW, Malessa R, Baumann B, et al. Standardized tests of heart rate variability: normal ranges obtained from 309 healthy humans, and effects of age, gender and heart rate. Clin Auton Res 2001; 11: 99-108.
17. Kleiger RE, Bigger JT, Bosner MS, et al. Stability over time of variables measuring heart rate variability in normal subjects. Am J Cardiol 1991; 68: 626-30.
18. Gritzali F. Towards a generalized scheme for QRS detection in ECG waweforms. Signal Processing 1988; 15: 183-92.
19. Bruggeman D, Andersen D. Heart rate variability: how to exclude extrasystoles from the analysis. IEEE Computers in Cardiology 1993; 467-70.
20. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurements, physiological interpretation, and clinical use. Eur Heart J 1996; 17: 354-81.
21. Akaike H. Statistical predictor identification. Am Int Stat Math 1970; 22: 203-17.
22. Baumgartner TA. Norm referenced measurement: reliability. In Safrit MJ, Woods TM, eds. Measurement concepts in physical education and exercise science. Champaign, IL: Human Kinetics Books 1989: 45-72.
23. Reland S, Ville NS, Wong S, et al. Exercise heart rate variability of older women in relation to the level of physical activity. J Gerontol 2003; 58: B585-91.
24. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977; 33: 159-74.
25. Chambless LE, Mc Mahon RP, Brown SA, et al. Short term intraindividual variability in lipoprotein measurement: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Epidemiol 1992; 136: 1069-81.
26. Töyry J, Mäntysaari M, Hartikainen J, et al. Day to day variability of cardiac autonomic regulation parameters in normal subjects. Clin Physiol 1995; 15: 39-46.
27. Atkinson G, Nevill AM. Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. Sports Med 1998; 26: 217-38.
28. Hopkins WG. Measures of reliability in sports medicine and science. Sports Med 2000; 30: 1-15.
29. Huikuri HV, Kessler KM, Terracall E, et al. Reproducibility and circadian rhythm of heart rate variability in healthy subjects. Am J Cardiol 1990; 65: 391-3.
30. Brown TE, Beightol LA, Koh J, et al. Important influence of respiration on human RR interval power spectra is largely ignored. J Appl Physiol 1993; 75: 2310-7.
31. Jäntti PO. Orthostatic reactions in 85 year olds. Aging Clin Exp Res 1992; 4: 139-44.
32. Lipsitz LA, Mietus J, Moody GB, et al. Spectral characteristics of heart rate variability before and during postural tilt. Relation to aging and risk of syncope. Circulation 1990; 81: 1803-10.