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Mild-to-moderate intensity exercise improves cardiac autonomic drive in type 2 diabetes

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ABSTRACT

Aims/Introduction: The aim of the present study was to determine the effect of moderate aerobic exercise on cardiac autonomic function in type 2 diabetic patients.

Materials and Methods: Heart rate variability of 20 patients with type 2 diabetes was assessed. Resting electrocardiogram for the heart rate variability analysis at spontaneous respiration was recorded for 5 min in the supine position before and after 6 months of supervised aerobic training given three times per week.

Results: In time domain measures, the square root of the mean of the sum of the squares of differences between adjacent R-R intervals (RMSSD; 29.7 [26–34.5] vs 46.4 [29.8–52.2] ms, $P = 0.023$) and the percentage of consecutive RR intervals that differ by more than 50 ms (pNN50; 10.7 [5.5–12.7] vs 26.1 [6.6–37.2]%, $P = 0.025$] were significantly increased after exercise. In frequency domain measures, low frequency (62.4 [59.1–79.2] vs 37 [31.3–43.3] nu, $P = 0.003$) and low frequency/high frequency (1.67 [1.44–3.8] vs 0.58 [0.46–0.59]%, $P = 0.009$) were significantly decreased, whereas high frequency (95 [67–149] vs 229 [98–427] ms^2, $P = 0.006$) and high frequency (37.6 [20.8–40.9] vs 63 [56.7–68.7] normalized units, $P = 0.003$) were significantly increased after exercise. In a Poincaré plot, standard deviation perpendicular to the line of the Poincaré plot (SD1; 21.3 [18.5–24.8]–33.1 [21.5–37.2] ms, $P = 0.027$) was significantly increased after exercise.

Conclusions: These data suggest that three times per week moderate intensity aerobic exercise for 6 months improves cardiac rhythm regulation as measured by heart rate variability in type 2 diabetic patients.

INTRODUCTION

Several population-based studies have shown that regular physical activity is an important component of a healthy lifestyle and lack of activity is a predictor of cardiovascular mortality[1,2]. Low levels of physical fitness are closely related to several chronic diseases, including hypertension, coronary heart disease, depression and type 2 diabetes[3]. Many studies have suggested the beneficial effects of regular exercise in preventing sudden cardiac death in healthy individuals and in patients with cardiovascular disease[4].

It is commonly perceived that a regular heartbeat with sinus arrhythmia is a sign of a healthy heart. Thus, the rhythm of a healthy heart is characterized by significant beat-to-beat variability[5]. This heart rate variability (HRV) has been recognized as a powerful tool in the investigation of autonomic modulation of heart. Patients with type 2 diabetes show altered autonomic modulation of the heart as assessed by HRV[6]. Decreased HRV among patients with diabetes has been found to be predictive of cardiovascular morbidity and mortality[7–9].

Exercise therapy has been shown to improve autonomic nervous system modulation of HRV in healthy individuals[10–12]. Therefore, exercise training might improve cardiac autonomic regulation in a variety of clinical populations including type 2 diabetes. Thus, the main aim of the present study was to determine the effect of three times per week, 6-month, moderate aerobic exercise on cardiac autonomic function as measured by HRV in type 2 diabetic patients.

MATERIALS AND METHODS

Participants

A total of 20 type 2 diabetic male patients volunteered to participate in supervised three times per week aerobic training of

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moderate intensity for 6 months. The exclusion criteria were overt cardiovascular disease, diabetic complications, chronic heart failure, hypertension, arrhythmias, known neuropathy of any other etiology, comorbid conditions (e.g. cancer, immunodeficiency, autoimmune diseases) and current smoking. In the case of smokers and alcohol users, participants with low nicotine and alcohol dependence were included[13,14]. The participants with Ewing Score ≥3 and vibration perception threshold ≥6 V were also excluded from the study. Patients receiving dietary recommendations, insulin therapy, thiazolidinediones and angiotensin receptor blockers were also excluded. Ethical clearance was obtained from the ethics committee of Nepalgunj Medical College, Banke. Informed consent was obtained from all participants. The methods of measuring the parameters were entirely non-invasive, and blood was drawn only with a single use sterile syringe for biochemical parameters. The purpose, nature and potential risks of the study were explained to the participants before obtaining their written consent.

Clinical Examination

In a personal interview with the participants, a detailed history was obtained with special reference to age, duration, symptoms of neuropathy, diabetes-related complications and medication. All the participants were subjected to a clinical examination.

Each participant underwent the measurement of their weight and height recorded while wearing light indoor clothes, but no shoes. Using a measuring tape, waist circumference (midway between the lower rib margin and the top of the iliac crest) and hip circumference (the maximal circumference over the buttocks) were measured. Blood pressure was measured using the standard protocol. In addition, they underwent a detailed neurological examination. Non-invasive Ewing battery tests (Valsalva maneuver, heart rate response to standing up, heart rate response to deep breathing, blood pressure response to standing up, blood pressure response to sustained handgrip) were also carried out before the exercise program. Ewing battery tests are widely used for assessing cardiovascular autonomic neuropathy[15]. Ewing scores were assigned as follows: 0 for a normal test, 0.5 for a borderline value and 1 for an abnormal value[15]. Peripheral somatic neuropathy was assessed by a biothesiometer (Genesis Medical System, Hyderabad, India), measuring vibration perception threshold at toe, first metatarsal, third metatarsal, fifth metatarsal, instep and heel surfaces of each foot[16].

At the time of testing, all the participants were medication-free, stable in terms of cardiopulmonary function and showed no withdrawal symptoms. Possible diurnal variation was minimized by carrying out all tests in the same sequence between 09:00 and 11:00 hours, and maintaining the laboratory temperature at 26 ± 2°C.

Training Program

The participants were enrolled in a 6-month program of aerobic exercise. Aerobic exercise (also known as cardio) is physical exercise of relatively low intensity that depends primarily on the aerobic energy-generating process. Under the supervision by trained personnel, the participants carried out thrice-a-week sessions of physical activity. In order to produce the desired metabolic effects, each exercise session lasted 50 min; 10 min of warm-up, 30 min of activity (brisk walking, light running) and 10 min of cool-down. Considering the linear relationship between heart rate and % VO_2 reserve, exercise intensity was set between 60 and 85% of the maximum heart rate, which was calculated by the following formula: ([220 – age – resting heart rate] × % of maximum heart rate + resting heart rate)[17].

Laboratory Measurements

The electrocardiogram (ECG) signals for HRV were recorded using an ECG machine (Magic R Series; Maestros, Mumbai, India) after a supine rest of 15 min. The resting ECG at spontaneous respiration was recorded for 5 min in the supine position at chart speed 100 mm/s. From ECG, R-R intervals were measured manually with a ruler. Then these R-R intervals were saved as an ASCII file. This format was readable by software 'HRV analysis software 1.1'. This HRV analysis software, which calculates the time domain results, frequency domain results and non-linear measures of HRV, was developed by the Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Kuopio, Finland.

The time domain analysis of HRV consisted of the standard deviation of all R-R intervals (SDNN); the square root of the mean of the sum of the squares of differences between adjacent R-R intervals (RMSSD) and pNN50, which is the proportion of the total R-R intervals that have differences of R-R intervals greater than 50 ms[18].

The frequency-domain analysis of HRV consisted of power of high frequency (HF; 0.15–0.40 Hz), low frequency (LF; 0.04–0.15 Hz) and very low frequency (VLF; below 0.04 Hz) power ranges[18].

It has been speculated that analysis of HRV based on the methods of non-linear dynamics might elicit valuable information for the physiological interpretation of HRV. One non-linear method is Poincaré plot. The Poincaré plot is a scatterplot of the current R-R interval plotted against the preceding R-R interval. Using the method described by Brennan[19], these plots were used to extract indexes, such as length (SD2) and width (SD1) of the long and short axes of Poincaré plot images.

Venous blood was drawn in the morning after an overnight fast immediately before (baseline) and at the end of the training program (after 6 months). Plasma glucose was determined by standard laboratory procedures (BS-380; Diagnova Enzokit, RFCL, Haryana, India). Total cholesterol and triglycerides concentration were determined with a fully enzymatic analyzer (BS-380; Diagnova Enzokit, Haryana, India). Serum high-density lipoprotein cholesterol level was measured by using the phospho-tungstate precipitation method. Serum low-density lipoprotein cholesterol was calculated by the Friedewald's equation. Glycated hemoglobin was measured by ion exchange affinity chromatography (Kamineni Life Sciences, Hyderabad, India).

Statistical Analysis

Different anthropometric, cardiorespiratory and biochemical variables were compared before and after exercise using paired samples *t*-test, and data are presented as mean ± SD. However, non-parametric Wilcoxon signed-rank test was applied for comparisons of the HRV and the results are presented as median (interquartile range). A *P*-value of <0.05 was considered statistically significant. Data were analyzed with statistical software SPSS Statistics 21, (IBM, New York, United States).

RESULTS

Participant Characteristics

The clinical and biochemical characteristics of study participants are shown in Table 1. All these variables were significantly decreased after exercise. Figure 1 details the result from the 20 diabetic participants who completed all Ewing batteries tests before exercise.

Heart rate Variability Measures

Time domain variables

In the time domain variables, RMSSD and pNN50 were significantly increased, whereas SDNN was unaffected after exercise (Table 2).

Frequency domain variables

The variables analyzed in frequency domain measures included power of LF and HF in ms^2, and their normalized units (nu) and ratio of LF to HF (LF/HF). The HF power (ms^2) and HF

Table 1 | Clinical and biochemical characteristics of participants before and after 6 months of exercise

Variables	Before exercise, *n* = 20 (mean ± SD)	After exercise, *n* = 20 (mean ± SD)	*P*-value
Age (years)	42.2 ± 6.4		
Diabetes duration (years)	18.6 ± 4.6		
Height (cm)	165.46 ± 5.54		
Weight (kg)	72.44 ± 5.67	69.53 ± 3.9	0.013
Body mass index (kg/m^2)	24.46 ± 0.72	22.95 ± 0.64	0.021
Waist hip ratio	0.87 ± 0.27	0.84 ± 0.64	0.003
SBP (mmHg)	129.5 ± 6.84	122.13 ± 8.2	0.002
DBP (mmHg)	83.45 ± 2.4	79.23 ± 3.73	0.016
Pulse rate (b.p.m.)	75.65 ± 4.21	72.23 ± 3.88	0.032
Respiratory rate (/min)	15.78 ± 6.11	13.27 ± 7.66	0.011
Fasting glucose (mmol/L)	9.8 ± 3	8.6 ± 4	0.009
HbA_{1c} (%)	7.5 ± 1.1	7.3 ± 0.4	0.023
LDL cholesterol (mmol/L)	2.9 ± 1.1	2.6 ± 1.1	0.032
HDL cholesterol (mmol/L)	1.6 ± 0.4	1.3 ± 0.3	0.008
Triglycerides (mmol/L)	1.6 ± 0.3	1.2 ± 0.2	0.003

$P < 0.05$ was considered statistically significant. DBP, diastolic blood pressure; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; SD, standard deviation.

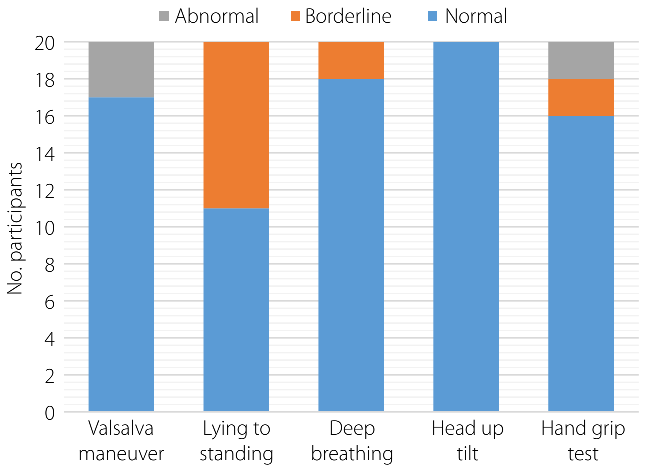

Figure 1 | Number of normal, borderline and abnormal test responses in 20 diabetic participants.

Table 2 | Time domain variables of participants before and after 6 months of exercise

Variables	Before exercise, *n* = 20 Median (interquartile range)	After exercise, *n* = 20 Median (interquartile range)	*P*-value
SDNN (ms)	37 (29–42)	41 (34– 51)	NS
RMSSD (ms)	29.7 (26–34.5)	46.4 (29.8–52.2)	0.023
pNN50 (%)	10.7 (5.5–12.7)	26.1 (6.6– 37.2)	0.025

$P < 0.05$ was considered statistically significant. NS, statistically non-significant; pNN50, percentage of consecutive R-R intervals that differ by more than 50 ms; RMSSD, the square root of the mean of the sum of the squares of differences between adjacent R-R intervals; SDNN, standard deviation of all R-R intervals.

(nu) were significantly increased, whereas LF (nu) and LF/HF ratio were significantly decreased after exercise. However, LF power (ms^2) was unaffected after exercise (Table 3).

Poincaré plot of HRV

In Poincare plot SD1 was significantly increased, whereas SD2 was unaffected after exercise (Table 4).

DISCUSSION

The present study was designed to determine long-term cardiovascular autonomic adaptation to a moderate aerobic exercise program in type 2 diabetic patients. The findings of the current study show predictable change in cardiac autonomic activity as measured by HRV among apparently sedentary, non-smoking, type 2 diabetic patients.

Cardiac autonomic function can be improved by better glycemic control, but also by physical training[20]. Several biological mechanisms have been proposed, but the relative importance of these exercise-related mechanisms is still unknown[4]. They

Table 3 | Frequency domain variables of participants before and after 6 months of exercise

Variables	Before exercise, $n = 20$ Median (interquartile range)	After exercise, $n = 20$ Median (interquartile range)	*P*-value
LF (ms^2)	199 (133–337)	158 (62–271)	NS
LF (nu)	62.4 (59.1–79.2)	37 (31.3–43.3)	0.003
HF (ms^2)	95 (67–149)	229 (98–427)	0.006
HF (nu)	37.6 (20.8–40.9)	63 (56.7–68.7)	0.003
LF/HF (%)	1.67 (1.44–3.8)	0.58 (0.46–0.59)	0.009

$P < 0.05$ was considered statistically significant. HF, high frequency; LF, low frequency; NS, statistically non-significant; nu, normalized unit.

Table 4 | Poincaré plot variables of participants before and after 6 months of exercise

Variables	Before exercise, $n = 20$ Median (interquartile range)	After exercise, $n = 20$ Median (interquartile range)	*P*-value
SD1 (ms)	21.3 (18.5–24.8)	33.1 (21.5–37.2)	0.027
SD2 (ms)	56.5 (34.4–64.8)	60.6 (47.8–79.7)	NS

$P < 0.05$ was considered statistically significant. NS, statistically non-significant; SD1, standard deviation perpendicular to line of entity in Poincaré plot; SD2, standard deviation along the line of entity in Poincaré plot.

could possibly be related to subsequent improvements in body fat distribution, atherogenic lipoprotein profiles and blood pressure, as well as beneficial effects on muscular capillary density and autonomic nervous system balance[21]. In diabetes mellitus, physical activity has beneficial effects on both glucose metabolism and insulin sensitivity. These include increased sensitivity to insulin, decreased production of glucose by liver, a large number of muscle cells that utilize more glucose than adipose tissue and reduced obesity[22]. One hypothesis is that regular exercise modulates cardiac autonomic control by enhancing vagal tone and lessening sympathetic influence[23]. This shift toward greater vagal modulation could positively affect the prognosis of individuals with a variety of morbidities[23].

Just a few prospective studies have assessed the effects of exercise training on HRV[20,24], and in agreement with the present study, those studies have found that HRV increased after training, although studies used either time domain measure or frequency domain measure for analysis of HRV. However, Loimaala *et al.*[25] reported no differences in the time domain or frequency domain HRV measures even after 12 months of training.

In the present study, RMSSD and pNN50, which reflect the vagal tone, were statistically increased after exercise; whereas SDNN, which reflects total variability and carries the strongest prognostic information in heart disease, was unaffected after exercise. The RMSSD and pNN50 also correlate highly with HF power, reflecting parasympathetic modulation[26]. Thus, the time domain analysis of HRV showed increased parasympathetic activity in type 2 diabetic patients after exercise.

By using more specific information obtained from frequency domain measures concentrated around respiratory frequency, HF (ms^2) and HF (nu) were significantly increased; LF (nu) and LF/HF (%) were significantly decreased, whereas LF (ms^2) was unaffected after exercise. The HF component of HRV is considered to represent the vagal control of heart rate[27–29]. Some authors have suggested that the LF component is a quantitative marker of sympathetic modulation[30,31], and others that it is a marker of both sympathetic and vagal modulation[32,33]. The LF/HF ratio is considered to reflect sympathovagal balance, and acts as an indicator for the sympathetic nervous activity[30,31,34,35]. The LF (nu) is also considered as a marker of sympathetic nervous function[18]. Thus, frequency domain analysis of HRV shows an increase in vagal activity and decrease in sympathetic activity in type 2 diabetic patients after exercise.

In Poincaré plot measures, SD1 was significantly increased, whereas SD2 was unaffected after exercise. SD1, which reflects the level of short-term HRV, is equivalent to the RMSSD; whereas SD2, which reflects the long-term HRV, is equivalent to SDNN[36,37]. SDNN (Poincaré length) correlates with both LF power and HF power; and RMSSD (Poincaré width) correlates with HF power and, to a lesser extent, LF power[38]. Thus, it also shows an increased vagal and decreased sympathetic influence in trained diabetic patients.

In conclusion, a three times per week, 6-month, moderate, supervised aerobic training program in type 2 diabetic patients who are clinically free of cardiovascular disease leads to significant improvements in cardiovascular autonomic function of HRV through increasing cardio-vagal tone and decreasing cardio-sympathetic tone. In addition, the present findings also suggest that thrice-a-week moderate intensity aerobic exercise is safe and could serve as a potential adjunct therapy in the management of people with type 2 diabetes. As the present study was limited by small sample size, further studies in a large number of type 2 diabetic patients are required to confirm that these beneficial effects observed in the biochemical and autonomic variables after the training period have favorable effects on the clinical outcome of the patients.

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REFERENCES

1. Chang JA, Froelicher VF. Clinical and exercise test markers of prognosis in patients with stable coronary artery disease. *Curr Probl Cardiol* 1994; 19: 533–587.
2. Pate RR, Pratt M, Blair SN, *et al*. Physical activity and public health: a recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. *JAMA* 1995; 273: 402–407.

3. Powell KE, Thompson PD, Caspersen CJ, *et al.* Physical activity and the incidence of coronary heart disease. *Annu Rev Public Health* 1987; 8: 253–287.
4. Zipes DP, Wellens HJ. Sudden cardiac death. *Circulation* 1998; 98: 2334–2351.
5. Routledge HC, Chowdhary S, Townend JN. Heart rate variability—a therapeutic target? *J Clin Pharm Ther* 2002; 27: 85–92.
6. Goit RK, Paudel BH, Sharma SK, *et al.* Heart rate variability and vibration perception threshold in type 2 diabetes mellitus. *Int J Diabetes Dev Ctries* 2013; 33: 134–139.
7. Astrup AS, Nielsen FS, Rossing P, *et al.* Predictors of mortality in patients with type 2 diabetes with or without diabetic nephropathy: a follow-up study. *J Hypertens* 2007; 25: 2479–2485.
8. Kataoka M, Ito C, Sasaki H, *et al.* Low heart rate variability is a risk factor for sudden cardiac death in type 2 diabetes. *Diabetes Res Clin Pract* 2004; 64: 51–58.
9. Wheeler SG, Ahroni JH, Boyko EJ. Prospective study of autonomic neuropathy as a predictor of mortality in patients with diabetes. *Diabetes Res Clin Pract* 2002; 58: 131–138.
10. Dixon EM, Kamath MV, McCartney N, *et al.* Neural regulation of heart rate variability in endurance athletes and sedentary controls. *Cardiovasc Res* 1992; 26: 713–719.
11. Furlan R, Piazza S, Dell'Orto S, *et al.* Early and late effects of exercise and athletic training on neural mechanisms controlling heart rate. *Cardiovasc Res* 1993; 27: 482–488.
12. Pichot V, Roche F, Denis C, *et al.* Interval training in elderly men increases both heart rate variability and baroreflex activity. *Clin Auton Res* 2005; 15: 107–115.
13. Heatherton TF, Kozlowski LT, Frecker RC, *et al.* The Fagerstrom test for nicotine dependence: a revision of the Fagerstrom Tolerance Questionnaire. *Br J Addict* 1991; 86: 1119–1127.
14. Baboor TF, Higgins-Biddle JC, Saunders JB, *et al.* AUDIT: The Alcohol Use Disorder Identification Test: Guidelines for Use in Primary Care, 2nd edn. World Health Organization, Geneva, 2001.
15. Ewing DJ, Martyn CN, Young RJ, *et al.* The value of cardiovascular autonomic function tests: 10 years experience in diabetes. *Diabetes Care* 1985; 8: 491–498.
16. Goit RK, Khadka R, Sharma SK, *et al.* Cardiovascular autonomic function and vibration perception threshold in type 2 diabetes mellitus. *J Diabetes Complications* 2012; 26: 339–342.
17. Swain DP, Leutholtz BC, King EM, *et al.* Relationship between% heart rate reserved and% VO_2 reserve in treadmill exercise. *Med Sci Sports Exerc* 1998; 30: 318–321.
18. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* 1996; 93: 1043–1065.
19. Brennan M, Palaniswami M, Kamen P. Do existing measures of Poincare plot geometry reflect nonlinear features of heart rate variability? *IEEE Trans Biomed Eng* 2001; 48: 1342–1347.
20. Howorka K, Pumprla J, Harber P, *et al.* Effects of physical training on heart rate variability in diabetic patients with various degrees of cardiovascular autonomic neuropathy. *Cardiovasc Res* 1997; 34: 206–214.
21. Shephard RJ, Balady GJ. Exercise as cardiovascular therapy. *Circulation* 1999; 99: 963–972.
22. Wasserman DH, Zinman B. Fuel homeostasis. In: Ruderman N, Devlin JT (eds). The Health Professional's Guide to Diabetes and Exercise. American Diabetes Association, Alexandria, VA, 1995; 29–47.
23. Malfatto G, Facchini M, Sala L, *et al.* Effects of cardiac rehabilitation and beta-blocker therapy on heart rate variability after first acute myocardial infarction. *Am J Cardiol* 1998; 81: 834–840.
24. Bhagyalakshmi S, Nagaraja H, Anupama B, *et al.* Effect of supervised integrated exercise on heart rate variability in type 2 diabetes mellitus. *Kardiol Pol* 2007; 65: 363–368.
25. Loimaala A, Huikuri HV, Kööbi T, *et al.* Exercise training improves baroreflex sensitivity in type 2 diabetes. *Diabetes* 2003; 52: 1837–1842.
26. Cowan MJ. Measurement of heart rate variability. *West J Nurs Res* 1995; 17: 32–48.
27. Fouad FM, Tarazi RC, Ferrario CM, *et al.* Assessment of parasympathetic control of heart rate by a noninvasive method. *Am J Physiol* 1984; 246: H838–H842.
28. Saeki Y, Atogami F, Takahashi K, *et al.* Reflex control of autonomic function induced by posture change during the menstrual cycle. *J Auton Nerv Syst* 1997; 66: 69–74.
29. Park MK, Watanuki S. Specific physiological responses in women with severe primary dysmenorrhea during the menstrual cycle. *J Physiol Anthropol Appl Human Sci* 2005; 24: 601–609.
30. Malliani A, Pagani M, Lombardi F, *et al.* Cardiovascular neural regulation explored in the frequency domain. *Circulation* 1991; 84: 482–492.
31. Montano N, Ruscone TG, Porta A, *et al.* Power spectral analysis of heart rate variability to assess the changes in sympathovagal balance during graded orthostatic tilt. *Circulation* 1994; 90: 1826–1831.
32. Akselrod S, Gordon D, Ubel FA, *et al.* Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science* 1981; 213: 220–222.
33. Appel ML, Berger RD, Saul JP, *et al.* Cohen RJ. Beat to beat variability in cardiovascular variables: noise or music? *J Am Coll Cardiol* 1989; 14: 1139–1148.
34. Pagani M, Montano N, Porta A, *et al.* Relationship between spectral components of cardiovascular variabilities and direct measures of muscle sympathetic nerve activity in humans. *Circulation* 1997; 95: 1441–1448.

35. Platisa MM, Gal V. Reflection of heart rate regulation on linear and nonlinear heart rate variability measures. *Physiol Meas* 2006; 27: 145–154.
36. Kamen PW, Tonkin AM. Application of the Poincare plot to heart rate variability: a new measure of functional status in heart failure. *Aust N Z J Med* 1995; 25: 18–26.
37. Brennan M, Palaniswami M, Kamen P. Poincare plot interpretation using a physiological model of HRV based on a network of oscillators. *Am J Physiol Heart Circ Physiol* 2002; 283: H1873–H1886.
38. Otzenberger H, Gronfier C, Simon C, *et al*. Dynamic heart rate variability: a tool for exploring sympathovagal balance continuously during sleep in men. *Am J Physiol* 1998; 275: H946–H950.