Quantitative beat-to-beat analysis of heart rate dynamics during exercise

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Tulppo, Mikko P., Timo H. Mäkikallio, Timo E. S. Takala, Tapio Seppänen, and Heikki V. Huikuri. Quantitative beat-to-beat analysis of heart rate dynamics during exercise. *Am. J. Physiol.* 271 (*Heart Circ. Physiol.* 40): H244–H252, 1996.—Beat-to-beat heart rate (HR) dynamics were studied by plotting each R-R interval as a function of the previous R-R interval (Poincaré plot) during incremental doses of atropine followed by exercise for 10 subjects and during exercise without autonomic blockade for 31 subjects. A quantitative two-dimensional vector analysis of a Poincaré plot was used by measuring separately the standard deviation of instantaneous beat-to-beat R-R interval variability (SD1) and the standard deviation of continuous long-term R-R interval variability (SD2) as well as the SD1/SD2 ratio. Quantitative Poincaré measures were compared with linear measures of HR variability (HRV) and with approximate entropy (ApEn) at rest and during exercise. A linear progressive reduction was observed in SD1 during atropine administration, and it remained almost at the zero level during exercise after a parasympathetic blockade. Atropine resulted in more variable changes in SD2 and the SD1/SD2 ratio, but during exercise after parasympathetic blockade, a progressive increase was observed in the SD1/SD2 ratio until the end of exercise. The SD1/SD2 ratio had no significant correlations with the frequency domain measures of HRV. However, the SD1/SD2 ratio had a modest correlation with ApEn at rest ($r = -0.69$, $P < 0.001$), but not during exercise ($r = 0.27$, $P =$ NS). All measures of vagal modulation of HR decreased progressively until the ventilatory threshold level was reached, when sympathetic activation was reflected as changes in the SD1/SD2 ratio. These results show that quantitative two-dimensional vector analysis of a Poincaré plot can provide useful information on vagal modulation of R-R interval dynamics during exercise that are not easily detected by linear summary measures of HRV or by ApEn.

cardiovascular regulation; Poincaré plot

THE REGULATION OF HEART RATE (HR) during exercise is a combination of at least three different physiological mechanisms: the Frank-Starling law of the heart, the humoral factors, and the autonomic nervous system. During dynamic exercise, the initial rise in the HR is mainly due to the withdrawal of vagal tone; then the increments are attributable to an increase in the activity of cardiac sympathetic nerves. The relative role of the two drives depends on exercise intensity (14, 16, 21). Humoral factors, such as circulating catecholamines, may have a more dominant role than the direct neural input in maintaining the tachycardia during steady-state exercise (6, 12).

Linear time domain and frequency domain measures of HR variability (HRV) have been used most commonly in the noninvasive assessment of the autonomic modulation of HR (1–4, 6, 12, 14–18, 29). The recent use of these methods to assess the autonomic modulation during exercise has produced inconsistent results with different spectral techniques or units and at different work rates (6, 7). Furthermore, the R-R interval time series is nonstationary during exercise, and the trends tend to dominate the features of power spectral calculations and conventional time domain calculations of HRV. From a statistical point of view, it is necessary to remove any trends before a reliable interpretation of HRV in these measurements. The assessment of HRV by means of the linear summary measures is also partly invalidated by the lack of knowledge of the temporal changes in beat-to-beat HR dynamics. Nonlinear methods have therefore been employed recently to quantify the beat-to-beat dynamics of R-R intervals under normal physiological conditions. A Poincaré plot analysis of HRV, which consists of each cardiac R-R interval plotted as a function of the previous intervals, is one such method that allows a calculation of changes in heart dynamics with trends. Approximate entropy (ApEn) is another recently described method that quantifies the randomness or predictability of R-R interval dynamics (19, 20). The purpose of this work was to study the applicability of quantitative Poincaré plot analysis to the assesment of autonomic modulation of R-R interval dynamics during exercise and to compare the quantitative Poincaré method with the conventional linear measures of HRV and with ApEn.

METHODS

Subjects. Thirty-one male subjects participated in the study. All the subjects were healthy, and none was taking medication. The subjects' characteristics are presented in Table 1. The study protocol was approved by the Ethics Committee of the Merikoski Rehabilitation and Research Center, and all subjects gave their informed consent.

Study protocol. The study was designed to evaluate the effects of parasympathetic blockade and exercise on HRV. The subjects were not allowed to eat or drink coffee for 3 h before the test. Vigorous exercise and alcohol were also forbidden for 48 h before the testing day (8). All subjects remained in a supine position in a quiet room for 0.5 h and then in a seated position for 10 min before the HRV baseline recordings. Ten of the subjects performed the exercise test after a complete parasympathetic blockade. Two of these subjects discontinued the exercise because of dryness of the mouth, and the final analyses during the exercise after atropine were conducted on eight subjects. Thereafter, a total of 31 subjects

Table 1. *Characteristics of the subjects*

	Mean ± SD	Minimum	Maximum
Age, yr	35.6 ± 6.3	27	48
Height, cm	178.7 ± 5.8	170.0	194.0
Weight, kg	81.6 ± 10.7	63.8	111.0
Fat, %	16.5 ± 5.4	8	28
Heart rate at rest, beats/min	67 ± 8	49	85
Blood pressure, mmHg			
Systolic	122 ± 13	102	150
Diastolic	79 ± 10	60	98
$\dot{V}O_{2max}$			
l/min	3.45 ± 0.58	2.56	5.39
$ml \cdot kg^{-1} \cdot min^{-1}$	42.6 ± 6.8	31.5	56.4
R	1.17 ± 0.08	1.01	1.41
Work, W	270 ± 28	225	325
Maximal heart rate, beats/min	188 ± 10	167	209
Maximal lactate, mmol/l	9.3 ± 2.0	6.2	13.2

n = 31. $\dot{V}O_{2max}$, maximal O_2 consumption; R, respiratory exchange ratio.

performed an incremental exercise test without autonomic blockade.

The R-R intervals of 10 subjects were measured in a seated position as a baseline of HRV data (10 min). Then fractionated doses (0.2 mg) of atropine sulfate were injected intravenously at 4-min intervals until two consecutive doses produced no further increase in the HR (9). During the parasympathetic blockade, the R-R intervals were recorded continuously, and immediately after the last atropine dose the subjects performed an incremental exercise test on a bicycle ergometer, where HRV and O_2 consumption ($\dot{V}O_2$) were measured throughout the test. During the exercise test, the R-R intervals of the last 100 beats of every load were recorded, and during the parasympathetic blockade 500 beats were used for ApEn calculations.

Measurement of maximal $\dot{V}O_2$ and ventilatory threshold. The subjects performed an incremental exercise test on a bicycle ergometer (model EL 400, Tunturi). It consisted of a 5-min warm-up period at 50 W followed by a ramp protocol, with the work rate increasing at a rate of 25 W every 2 min until exhaustion. A fingertip venous blood sample was taken 2 min after the exercise to analyze venous blood lactate (Analox LM3). The criteria used to document that the maximal $\dot{V}O_2$ ($\dot{V}O_{2max}$) had been attained during each test were *1*) a lack of increase in the $\dot{V}O_2$ upon an increase in work rate, *2*) a respiratory exchange ratio >1.1, and *3*) venous blood lactate >8.0. At least two of these criteria were met by every subject (24).

The ventilation ($\dot{V}E$), gas exchange (M909 ergospirometer, Medikro), and HR responses (Sport Tester Polar) were monitored continuously during the ramp protocol. The calculations for $\dot{V}E$ and gas exchange were performed on a breath-by-breath basis, but the mean values for 30 s were reported. The ventilatory threshold was considered to occur at the point at which $\dot{V}E$ and CO_2 output ($\dot{V}CO_2$) began to increase in a breaking fashion against $\dot{V}O_2$ and $\dot{V}E/\dot{V}O_2$ exhibited a systematic increase without a concomitant increase in $\dot{V}E/\dot{V}CO_2$ (25).

Measurement of HRV. The R-R intervals were recorded with a wireless HR monitoring system (Polar Electro Oy) and saved in a computer for further analysis of HRV by Heart Signal software (Kempele, Finland). The R-R interval series was passed through a filter that eliminates undesirable premature beats and noise. An R-R interval is interpreted as a premature beat if it deviates from the previous qualified interval value by >30%. All R-R intervals were edited automatically and thereafter manually with visual inspection. All undesirable beats were excluded, and these beats accounted for <1% in every subject. The details of this analysis and the filtering technique have been described previously (10, 11).

Spectral and time domain analysis of HRV. The mean of R-R intervals, the standard deviation of all R-R intervals (SDANN), and the standard deviation of successive differences of R-R intervals (SDsd) were calculated at each workload and during the atropine infusion. In the spectral analysis of HRV, the high-frequency (HF, 0.15–0.40 Hz) and low-frequency (LF, 0.04–0.15 Hz) components and the LF/HF ratio were calculated during every load and atropine infusion. An autoregressive method described in detail previously was used in the spectral analysis of HRV (10, 11).

Quantitative beat-to-beat analysis of HRV. The Poincaré plot is a diagram (scattergram) in which each R-R interval of a tachogram is plotted as a function of the previous R-R interval. The Poincaré plot gives a useful visual contact to the R-R data by representing qualitatively with graphical means the kind of R-R variations included in the recording. The shape of the plot can be used to classify the signal into one of the various classes (23, 27).

The Poincaré plots were also analyzed quantitatively. This quantitative method of analysis is based on the notion of different temporal effects of changes in the vagal and sympathetic modulation of the HR on the subsequent R-R intervals without a requirement for a stationary quality of the data (5, 22). The computerized analysis entails fitting an ellipse to the plot, with its center coinciding with the center point of the markings (Fig. 1). The line defined as *axis 2* shows the slope of the longitudinal axis, whereas *axis 1* defines the transverse slope, which is perpendicular to *axis 2*. In the computerized analysis, the Poincaré plot is first turned 45° clockwise, and the standard deviation of the plot data is then computed around the horizontal axis (*axis 2*), which passes through the data center (SD1). SD1 shows the instantaneous beat-to-beat variability of the data. The standard deviation of continuous long-term R-R intervals is quantified by turning the plot 45° counterclockwise (SD2) and by computing the data points around the horizontal axis (*axis 1*), which passes through the center of the data. In addition, the SD1/SD2 ratio was

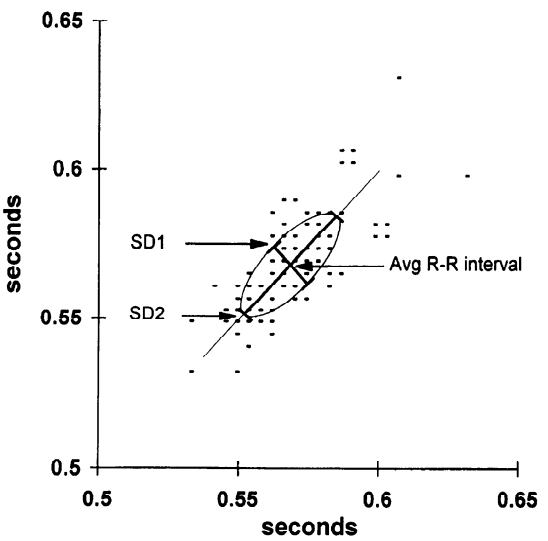

Fig. 1. Quantitative beat-to-beat analysis of heart rate dynamics during exercise at an intensity of 50 W: average R-R interval = 568 ms, standard deviation of instantaneous beat-to-beat R-R interval variability (SD1) = 8.4 ms, standard deviation of continuous beat-to-beat R-R interval variability (SD2) = 22.5 ms, and SD1/SD2 ratio = 0.37.

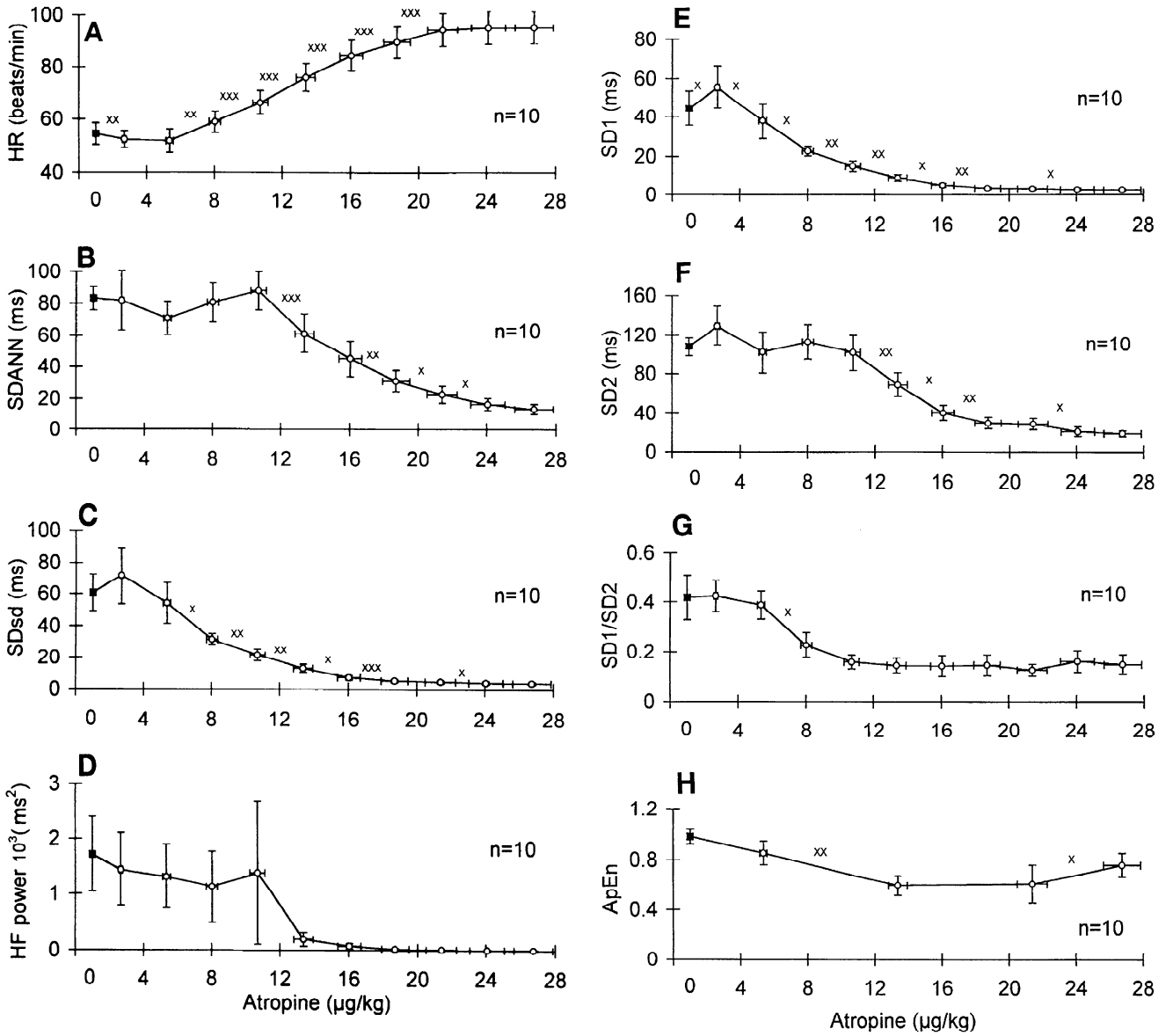

Fig. 2. Changes in heart rate (HR) variability variables during atropine infusion. *A*: changes in mean HR; *B*: changes in standard deviation of all R-R intervals (SDANN); *C*: changes in standard deviation of successive differences of R-R intervals (SDsd); *D*: changes in high-frequency power of spectral analysis (HF); *E*: changes in standard deviation of instantaneous beat-to-beat R-R interval variability (SD1); *F*: changes in standard deviation of continuous beat-to-beat R-R interval variability (SD2); *G*: changes in SD1/SD2 ratio; *H*: changes in approximate entropy (ApEn). ■, Seated position at rest. Significance levels with paired *t*-test between 2 successive measurement periods are as follows: $^{x}P < 0.05$, $^{xx}P < 0.01$, $^{xxx}P < 0.001$.

computed. An example of the quantitative analysis method of the Poincaré plot is shown in Fig. 1.

ApEn of R-R interval data. ApEn is a measure and a parameter that quantifies the amount of regularity or predictability in time series data. It measures the logarithmic likelihood that runs of patterns (beat-to-beat difference of R-R interval length) that are close to each other will remain close on the next incremental comparisons. A greater likelihood of remaining close (high regularity) produces smaller ApEn values, and conversely random data produce higher ApEn values. The details of this method have been described previously by Pincus and Goldberger (19, 20). The same algorithm was used for these analyses of ApEn. In this study, ApEn was calculated for 500 (n = 100 during exercise without parasympathetic blockade) consecutive HR data points in each segment. Two input variables, m and r, must be fixed to compute ApEn, and m = 2 and r = 20% of the standard deviation of the data sets were chosen on the basis of the previous studies showing good statistical validity for ApEn within these variable ranges (19, 20).

Statistical methods. Standard statistical methods were used for the calculation of means and standard deviations. For all variables, a new Δ variable was calculated (percent change between 2 successive points of measurement), and these Δ variables were tested using the Kolmogorov-Smirnov test (Z < 1.0). On the basis of these test results, the parametric tests were used on all the HRV variables. Analysis of variance of repeated measurements was used to determine the differences in the HRV parameters at different exercise intensities. The differences between the mean values were tested for significance using paired t-test. Pearson's correlation coefficients were used to estimate the univariate correla-

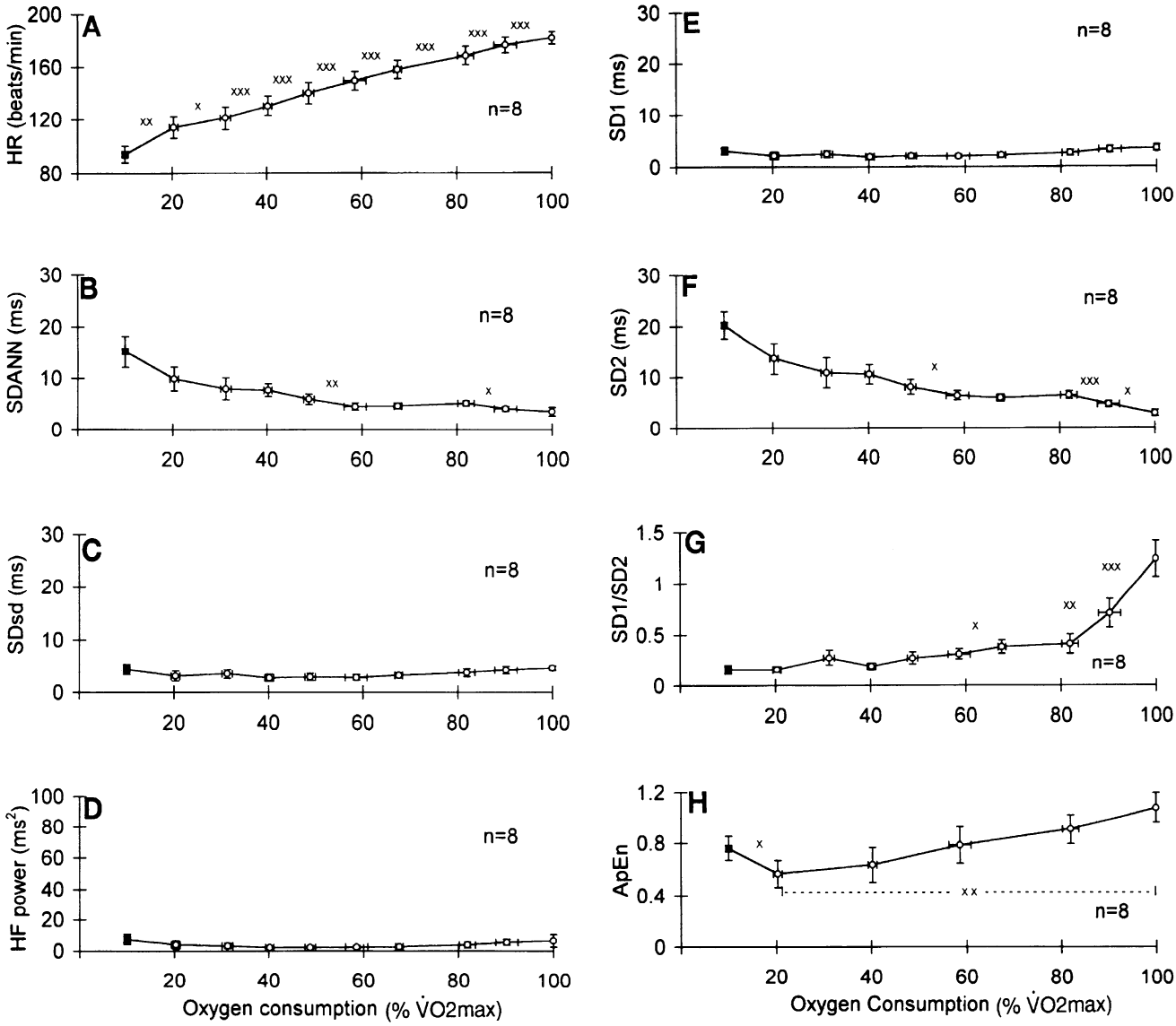

Fig. 3. Changes in HR variability variables during exercise after a complete parasympathetic blockade. ■, Seated position at rest after a complete parasympathetic blockade. See Fig. 2 legend for definition of abbreviations. Significance levels with paired t-test between 2 successive measurement periods are as follows: $^{x}P < 0.05$, $^{xx}P < 0.01$, $^{xxx}P < 0.001$.

tions between the different measures of HRV at rest and during exercise.

RESULTS

Changes in linear measures of HRV during parasympathetic blockade. The changes in HR and the different measures of HRV after incremental doses of atropine and during exercise after a parasympathetic blockade are shown in Figs. 2 and 3. During the atropine infusion, SDANN decreased progressively, and the reduction continued during the exercise up to 60% of $\dot{V}O_{2max}$ (Figs. 2*B* and 3*B*). A linear reduction was observed in SDsd during the atropine administration, and no change was seen during the exercise after a complete parasympathetic blockade (Figs. 2*C* and 3*C*). The mean value of the HF component achieved the zero level at the end of the atropine infusion and did not change during the exercise (Figs. 2*D* and 3*D*). The mean value of the LF component also decreased during the atropine administration, and the decrease continued during the exercise up to 80% of $\dot{V}O_{2max}$. The LF/HF ratio did not change significantly during the atropine infusion and showed a progressive decrease during the exercise up to 80% of $\dot{V}O_{2max}$.

Changes in nonlinear measures of HRV during parasympathetic blockade. In a quantitative beat-to-beat analysis of Poincaré plots, a linear progressive reduction was observed in SD1 during incremental doses of atropine. A gradual and more variable reduction was observed in SD2 during the atropine infusion. The SD1/SD2 ratio decreased after the first doses of atropine but remained stable thereafter (Fig. 2, *E–G*). During the exercise after a complete parasympathetic blockade, SD1 remained almost at the zero level and

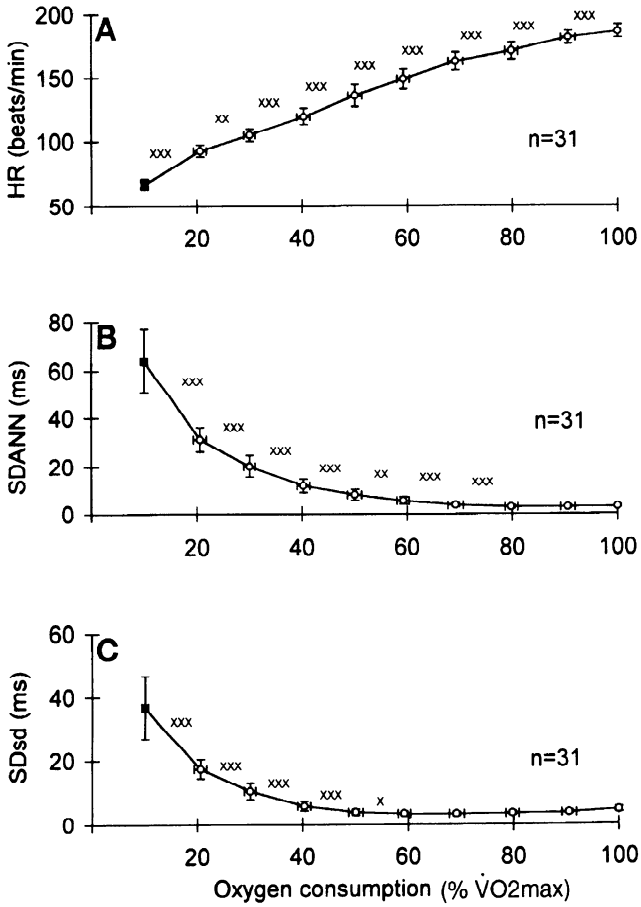

Fig. 4. Changes in time domain variables of HR variability during exercise compared with maximal O_2 consumption ($\dot{V}O_{2max}$). See Fig. 2 legend for definition of abbreviations. Values are means ± SD. ■, Seated position at rest. Significance levels with paired *t*-test between 2 successive measurement periods are as follows: $^{x}P < 0.05$, $^{xx}P < 0.01$, $^{xxx}P < 0.001$.

SD2 decreased progressively until the end of exercise. The SD1/SD2 ratio increased gradually during the exercise after a parasympathetic blockade, and a rapid increase was observed in this ratio from 80% of $\dot{V}O_{2max}$ to the end of the exercise (Fig. 3, *E–G*). The changes in ApEn were variable and small during the atropine administration, with a trend toward lower values after the first doses and a subsequent increase to the baseline level after a complete parasympathetic blockade. A progressive increase in ApEn was observed during the exercise after a parasympathetic blockade (Figs. 2*H* and 3*H*).

SD1 correlated strongly with SDsd at rest ($r = 0.99$, $P < 0.001$) and after atropine infusion ($r = 0.99$, $P < 0.001$), but this correlation became weaker at the end of the exercise after a complete parasympathetic blockade ($r = 0.65$, P = NS). SD2 correlated strongly with SDANN at rest ($r = 0.94$, $P < 0.001$) and at the end of the exercise after a complete parasympathetic blockade ($r = 0.91$, $P < 0.001$) but did not correlate after the atropine infusion ($r = 0.60$, P = NS). Furthermore, the SD1/SD2 ratio correlated significantly with the SDsd/SDANN ratio at rest ($r = 0.98$, $P < 0.001$) and after a complete parasympathetic blockade ($r = 0.85$, $P < 0.001$) but not at the end of the exercise ($r = 0.30$, P = NS).

Changes in HRV during exercise without parasympathetic blockade. The changes in HR and the different measures of HRV during exercise without parasympathetic blockade are shown in Figs. 4–6. The initial changes in all the linear measures of HRV, SD1, SD2, and the SD1/SD2 ratio during the exercise were comparable with the changes that took place during the atropine infusion. Similarly, the changes in the measures of HRV after the ventilatory threshold corresponded to those observed during exercise after a parasympathetic blockade. ApEn also increased at the end of the exercise in the same way as during the exercise after a parasympathetic blockade.

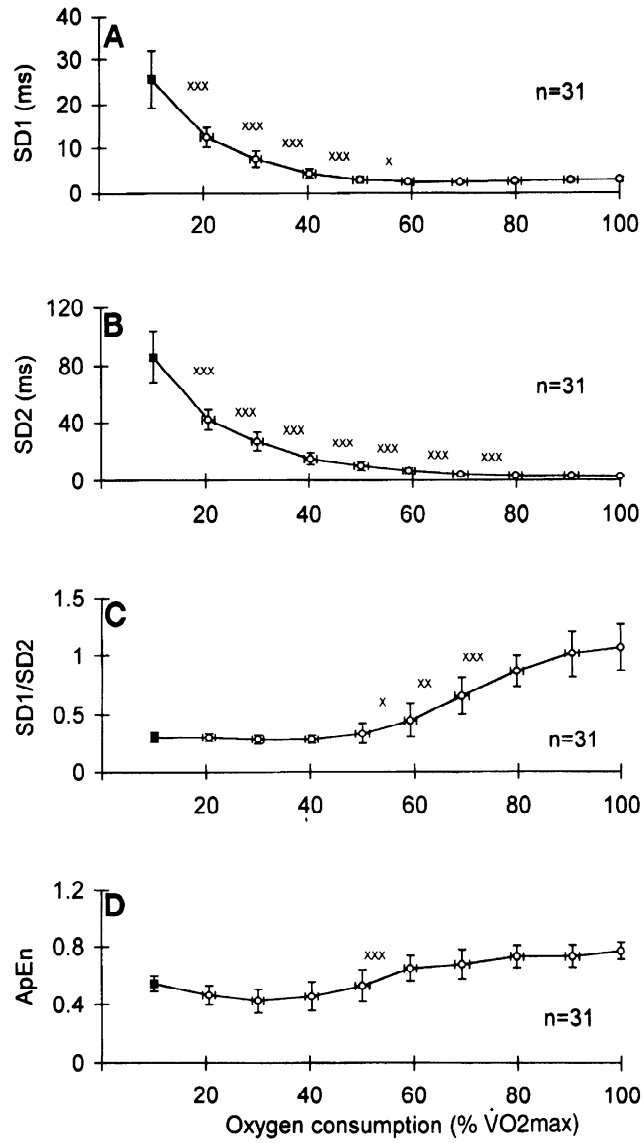

Fig. 5. Changes in nonlinear variables of HR variability during exercise compared with $\dot{V}O_{2max}$. Values are means ± SD. See Fig. 2 legend for definition of abbreviations. ■, Seated position at rest. Significance levels with paired *t*-test between 2 successive measurement periods are as follows: $^{x}P < 0.05$, $^{xx}P < 0.01$, $^{xxx}P < 0.001$.

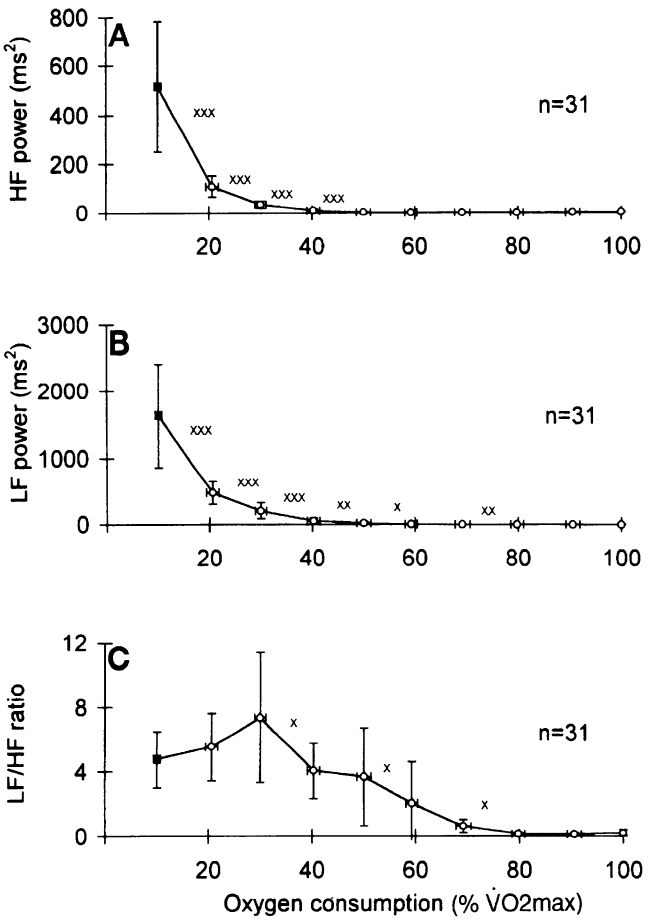

Fig. 6. Changes in spectral variables of HR variability during exercise compared with $\dot{V}O_{2max}$. *A*: changes in high-frequency power of spectral analysis (HF); *B*: changes in low-frequency power of spectral analysis (LF); *C*: changes in LF/HF ratio. Values are means ± SD. ■, Seated position at rest. Significance levels with paired *t*-test between 2 successive measurement periods are as follows: $^{x}P < 0.05$, $^{xx}P < 0.01$, $^{xxx}P < 0.001$.

Correlation between different measures of HRV at rest and at the ventilatory threshold during exercise. The univariate correlations among HR, the time and frequency domains, and the quantitative beat-to-beat analysis of HRV at rest and during exercise (at the ventilatory threshold level) are shown in Tables 2 and 3. SD1 correlated strongly ($r = 0.94$) with the HF power at rest, but at the ventilatory threshold level the correlation became weaker ($r = 0.70$). SD1 also correlated strongly with SDsd at rest and at the ventilatory threshold. Only a modest correlation existed between SD1 and the other summary measures of HRV during the exercise. SD2 correlated strongly with SDANN at rest and during the exercise. SD2 also shows a moderate correlation with the LF/HF ratio during the exercise but not at rest. The SD1/SD2 ratio correlated with the SDsd/SDANN ratio at rest ($r = 0.96$), but during the course of exercise the correlation became weaker ($r = 0.70$ at the end of the exercise). However, the SD1/SD2 ratio did not correlate noticeably with any spectral measures of HRV at rest or during the exercise. Furthermore, the SD1/SD2 ratio correlated moderately with ApEn at rest but not during the exercise.

Relation of HRV to ventilatory threshold. $\dot{V}O_2$ at the ventilatory threshold was 1.88 ± 0.34 l/min, and the mean HR was 141 ± 16 beats/min. In the time domain analysis, the mean values of SDANN decreased significantly after the ventilatory threshold. In the quantitative beat-to-beat analysis, the mean SD1 decreased significantly until the ventilatory threshold was reached and remained almost at the zero level thereafter. Similarly, the SDsd and HF components decreased progressively until the ventilatory threshold and remained stable thereafter. The SD1/SD2 ratio remained unchanged up to the ventilatory threshold (Fig. 7), then it began to increase toward the end of the exercise. The ApEn also started to increase gradually after the ventilatory threshold. The changes in the LF component or the LF/HF ratio had no relation to the ventilatory threshold.

DISCUSSION

The present data show that a quantitative beat-to-beat analysis of HRV can reveal patterns of HR dynamics during exercise that are not easily detected by conventional summary measures of HRV. The ratio of instantaneous to continuous R-R interval variance

Table 2. *Correlation coefficients among average HR, time and frequency domain measures, and quantitative beat-to-beat analysis of HR variability at rest*

	HR	SDANN	SDsd	SDsd/SDANN	HF	LF	LF/HF	SD1/SD2	SD2	SD1	ApEn
HR	1.0	0.51†	0.53†	0.27	0.53†	0.44*	−0.26	0.25	0.50†	0.59‡	−0.16
SDANN		1.0	0.80‡	0.07	0.75‡	0.72‡	−0.16	0.01	0.99‡	0.82‡	−0.16
SDsd			1.0	0.62‡	0.97‡	0.65‡	−0.37*	0.51†	0.78‡	0.98‡	−0.49†
SDsd/SDANN				1.0	0.62†	0.15	−0.43*	0.96‡	0.02	0.60‡	−0.68‡
HF					1.0	0.53†	−0.46†	0.51†	0.72‡	0.94‡	−0.50†
LF						1.0	0.11	0.16	0.70‡	0.72‡	−0.17
LF/HF							1.0	−0.40*	−0.14	−0.36*	0.13
SD1/SD2								1.0	−0.05	0.55‡	−0.69‡
SD2									1.0	0.78‡	−0.12
SD1										1.0	−0.49†
ApEn											1.0

Values are Pearson's correlation coefficients; $n = 31$. HR, heart rate; SDANN, standard deviation of all R-R intervals; SDsd, standard deviation of successive differences of R-R intervals; SD1, standard deviation of instantaneous beat-to-beat R-R interval variability; SD2, standard deviation of continuous beat-to-beat R-R interval variability; HF, high-frequency power; LF, low-frequency power; ApEn, approximate entropy. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

Table 3. *Correlation coefficients among average HR, time and frequency domain measures, and quantitative beat-to-beat analysis of HR variability at ventilatory threshold*

	HR	SDANN	SDsd	SDsd/SDANN	HF	LF	LF/HF	SD1/SD2	SD2	SD1	ApEn
HR	1.0	0.63‡	0.59‡	0.16	0.47†	0.58‡	0.51†	0.18	0.61‡	0.58‡	0.54†
SDANN		1.0	0.54†	−0.51†	0.29	0.73‡	0.78‡	0.46†	0.99‡	0.52†	−0.45*
SDsd			1.0	0.33	0.70†	0.63‡	0.48†	0.27	0.49†	0.99‡	−0.47†
SDsd/SDANN				1.0	0.20	−0.18	−0.42*	0.94‡	−0.56†	0.36*	0.23
HF					1.0	0.21	0.07	0.15	0.24	0.70‡	0.27
LF						1.0	0.90‡	−0.18	0.73‡	0.62‡	−0.54†
LF/HF							1.0	−0.21	0.79‡	0.49†	−0.33
SD1/SD2								1.0	−0.52†	0.31	0.27
SD2									1.0	0.47†	−0.45*
SD1										1.0	−0.48†
ApEn											1.0

Values are Pearson's correlation coefficients; $n = 31$. See Table 2 for definition of abbreviations. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

(SD1/SD2 ratio) correlated poorly with the linear summary measures of HRV during exercise. Furthermore, the relation between beat-to-beat measures and summary measures of HRV became weaker during the course of exercise than at rest. This is most probably due to the statistical problems in quantifying HRV by frequency domain methods during exercise with nonstationarity of the R-R interval time series, which makes the summary measures less useful in analyzing the changes in the autonomic modulation of HR during physical activity.

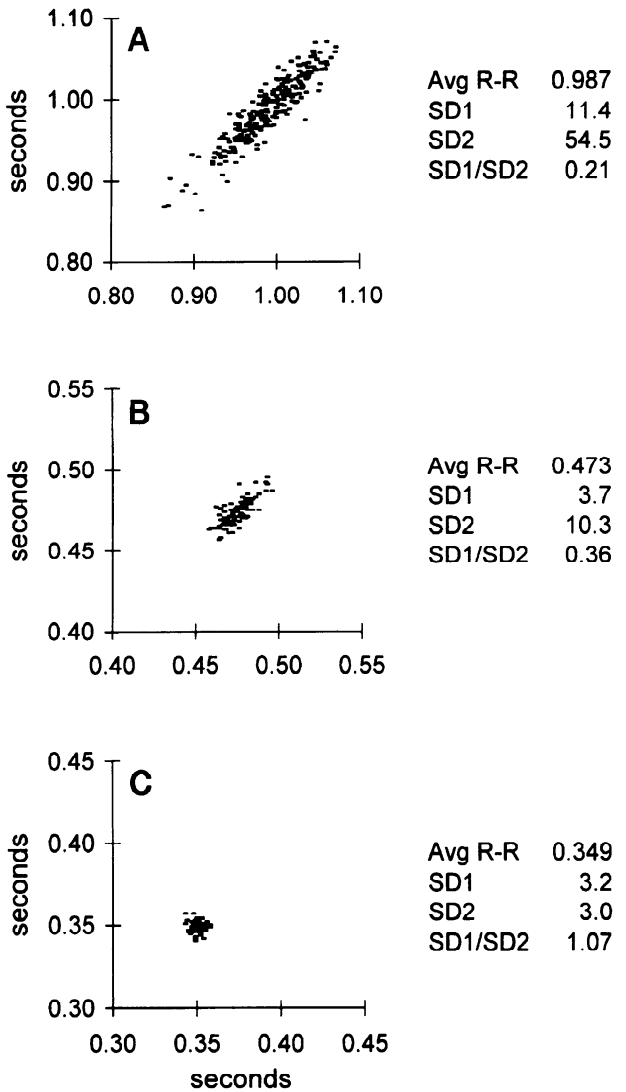

Fig. 7. Quantitative beat-to-beat analysis of HR dynamics at rest (*A*), at ventilatory threshold level (*B*), and at end of exercise (*C*). Note different scales on axes. Avg R-R, average R-R interval; see Fig. 2 legend for definition of other abbreviations.

Quantitative beat-to-beat analysis of Poincaré plots. The Poincaré plot method provides a beat-to-beat visual and quantitative analysis of R-R intervals. Visual interpretations of the shape of the plot and quantitative analysis of the dispersion of the R-R intervals at different HR have been used in some previous studies (23, 27, 30). The present quantitative analysis was planned to measure separately the instantaneous beat-to-beat variance of the R-R intervals (SD1), the long-term continuous variance of all R-R intervals (SD2), and the SD1/SD2 ratio. Instantaneous beat-to-beat changes in the R-R intervals are mediated by vagal efferent activity, because vagal effects on the sinus node are known to develop faster than sympathetically mediated effects (5, 22). The incremental doses of atropine resulted in linear progressive reduction in SD1, confirming that this index quantifies the vagal modulation of HR without invalidating the measurement by trends in the HR itself. Similar changes could be observed in SDsd, which had a strong correlation with SD1 measured from Poincaré plots at rest. This correlation, however, became weaker during the exercise, possibly because SDsd represents the differences between successive beats and SD1 quantifies the temporal differences between cycle lengths of more than two successive beats in relation to the average values in the neighborhood, and at the end of the exercise some alterations occurred in the dynamics of successive R-R intervals.

HR changes caused by alterations in sympathetic stimulation intensity have a more delayed and gradual onset. The increase of the average HR after the withdrawal of vagal tone mainly reflects the continuous increase in sympathetic activity during the course of exercise, but no measurement of the HR alone can define the point of vagal withdrawal. SD2 decreased and the SD1/SD2 ratio increased during the exercise after a complete parasympathetic blockade, suggesting

that sympathetic activation results in progressive reduction in the long-term oscillation of HR. No consistent changes were observed in the linear measures of HRV during the course of exercise after a parasympathetic blockade, which shows that these measures are poor indicators of changes in sympathetic tone. Furthermore, an abrupt increase in the SD1/SD2 ratio was observed during the last minutes of exercise, when circulating catecholamine levels are known to increase (6, 15). SDANN and the SDsd/SDANN ratio correlated with SD2 and the SD1/SD2 ratio, respectively, at rest but not at the end of the exercise, also suggesting that the quantitative Poincaré analysis method provides information about HR dynamics that is not detected by the conventional time domain methods.

ApEn during parasympathetic blockade and exercise. ApEn is a nonlinear measure of HRV that quantifies the amount of regularity and the logarithmic likelihood that patterns of beat-to-beat, successive R-R interval differences remain close on the next incremental comparison. We observed that a parasympathetic blockade caused only minimal changes in ApEn, suggesting that it reflects more closely the intrinsic complexity of the R-R interval dynamics independent of the fluctuation of vagal tone. On the other hand, ApEn had a modest gradual increase during the course of exercise after the ventilatory threshold level and during the exercise after atropine, suggesting that sympathetic activation will increase the values of ApEn. ApEn showed a moderate correlation with the SD1/SD2 ratio at rest, but during the exercise there was a poor correlation between ApEn and the SD1/SD2 ratio, which may be due to the confounding effects of nonstationarity on the interpretation of ApEn from data with significant trends, such as exercise or autonomic modulation (19, 20).

Relationship between ventilatory threshold and HRV. The quantitative beat-to-beat analysis of R-R intervals demonstrated that the vagal modulation of HR is nonexistent at the ventilatory threshold level; then the continuous R-R interval variability decreases progressively toward the end of the exercise. These data concur with the results of Yamamoto et al. (29), who also showed an association between the withdrawal of vagal activity and the ventilatory threshold level. Although there is some controversy as to the mechanisms responsible for the ventilatory threshold (26, 28), the present and the previous results suggest a link between the autonomic nervous system status and the ventilatory threshold. Further studies in larger groups of subjects with different exercise capacities may still be needed, however, to confirm the possible association between this exercise index and the autonomic modulation of the cardiovascular system.

Significance of beat-to-beat analysis of HRV during exercise. The exercise intensity above the level of nonexistent vagal modulation of the HR with increasing neurohumoral sympathetic activation may lead to an increased risk of cardiac vulnerability (13). The present data show that the measurement of the beat-to-beat dynamics of R-R intervals during exercise could be an easy and accurate noninvasive method to evaluate the cardiac autonomic modulation during exercise. The matters of obvious interest for future research include the practical applications of this method during exercise, i.e., the planning of training intensities of cardiac rehabilitation for patients with cardiac diseases as well as for healthy subjects.

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