Relationship between diabetic autonomic dysfunction and heart rate variability assessed by recurrence plot

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Mestivier, Denis, Nguyen Phong Chau, Xavier Chanudet, Bernard Bauduceau, and Pierre Larroque. Relationship between diabetic autonomic dysfunction and heart rate variability assessed by recurrence plot. Am. J. Physiol. 272 (Heart Circ. Physiol. 41): H1094–H1099, 1997.—Beat-to-beat heart rate (HR) and blood pressure were measured by the Finapres system in 44 healthy and 64 diabetic subjects in the at-rest condition. Autonomic control in diabetic subjects was assessed by the Ewing test. HR variability was explored by both linear and nonlinear methods. Linear methods used HR standard deviation and power spectrum. The percentage of the spectrum in the low frequencies was used to assess the sympathetic tone of the autonomic control. The nonlinear method used the “recurrence plot.” This method explored long parallel subsequences in the HR time series. These sequences characterize the dependence of the HR dynamics on initial values. The HR standard deviation was reduced in the diabetic subjects compared with the healthy subjects (2.80 ± 1.17 vs. 3.64 ± 1.45 beats/min; P < 0.001). In the diabetic subjects, the IIR standard deviation and the percentage of the spectrum in the low frequencies showed no correlations with the Ewing score (P > 0.10). In contrast, the longest length of the series at different frequencies (24). The diagnosis of neuropathy is usually performed by the Ewing test (6), which includes the Valsalva maneuver, the deep-breathing test, the standing-up test, and the isometric handgrip test. Blood pressure (BP) and heart rate (HR) are measured in these tests, which explore both the sympathetic and parasympathetic functions. However, the Ewing test is long to perform and requires good cooperation from the patient.

A simple measurement of HR might give some indication on a possible autonomic impairment in diabetic subjects. It is well established that HRV is reduced in diabetic neuropathy (14, 16, 20, 27, 29). However, the relationship between HRV (assessed by the HR standard deviation or by the power spectrum) and autonomic dysfunction is rather weak. In the present study, we questioned whether a different analysis of HRV, in particular using recent “nonlinear” approaches (19), might give a better insight into the autonomic status of diabetic subjects.

In the last decades, research on nonlinear dynamics has experienced an enormous growth. Ideas and methods from the theory have been applied to almost all branches of science. One novel idea is that many dynamic systems that are apparently very complex might be, in fact, governed by simple deterministic nonlinear mechanisms (12). Nonlinearity is the essential feature of these mechanisms. In physiology, erratic-looking time series (this is the case of heartbeats) are the rule, whereas regular (periodic or stable) time series are the exceptions. Mechanisms that regulate heartbeat might be nonlinear, and, therefore, several authors have used nonlinear techniques to analyze heartbeats in healthy subjects and in various pathologies (see, for example, 9, 13, 17, 26, 30–32).

HR is usually measured by the electrocardiograph. The recent Finapres system (21) enables one to measure simultaneously HR and BP and to explore the relationship between IIR and BP controls. This relationship is not well understood in diabetes mellitus.

The design of this study was as follows. We simultaneously measured HR and BP in subjects with diabetes mellitus (the subjects being at rest with normal breathing) with the Finapres system and performed Ewing tests in the same subjects. From HR values (in beats/min), R-R intervals were defined by the formula R-R (in s) = 60/HR. We analyzed the R-R intervals by nonlinear methods and looked for indexes that might be related to the score of Ewing.

METHODS

Subjects

Forty-four normal subjects (age 37 ± 17 yr, weight 64 ± 11 kg) and 64 diabetic subjects (age 56 ± 13 yr, weight 79 ± 14 kg; P < 0.01 for both variables) participated in this study. All subjects in the normal group were considered to be in good health, without any sign of cardiac, vascular, or neurological involvement. Patients with severe arthritis were not included. Informed consent was given by each subject after a

HEART RATE VARIABILITY (HRV) reflects the sympathetic and parasympathetic functions that control the autonomic nervous system. HRV has been intensively analyzed both in the time domain and in the frequency domain. In the time domain, the usual index of variability is the standard deviation of the measurements. In the frequency domain, the usual index is the Fourier power spectrum, which assesses the amount of variability of the series at different frequencies (24).

HRV is a variable of particular importance in diabetes mellitus because autonomic dysfunction is frequent in this group of patients (5, 6). The 5-yr mortality rate in diabetic patients with neuropathy is four to five times higher than in patients without neuropathy (5, 14). The diagnosis of neuropathy is usually performed by the Ewing test (6), which includes the Valsalva maneuver, the deep-breathing test, the standing-up test, and the isometric handgrip test. Blood pressure (BP) and heart rate (HR) are measured in these tests, which explore both the sympathetic and parasympathetic functions. However, the Ewing test is long to perform and requires good cooperation from the patient.
detailed description of the procedures. The protocol was approved by the Ethics Committee of Hôpital Bégin.

Measurement of BP and HR

After 10 min of rest, casual office BP was measured with a sphygmomanometer with the patients in the supine position. Beat-to-beat BP and HR were measured for ~0.5 h with the Finapres system. The device includes an infrared photoplethysmograph to record the arterial blood volume at the finger under an inflatable cuff. The continuous signal of volume changes was scaled by the system to a continuous signal of BP changes from which the systolic BP (SBP), diastolic BP (DBP), and pulse interval were determined. Beat-to-beat SBP, DBP, and HR were automatically stored in a computer file. The system has been validated in humans (15) by comparison of the data with simultaneous observations of intra arterial pressure in the same subjects. The calibration system was switched on during the measurement period to obtain a good estimate of BP changes on a beat-to-beat basis (3).

Cardiovascular Function Tests

To evaluate autonomic function, five cardiovascular function tests were used. The tests were performed only in diabetic subjects following the protocols described by Ewing et al. (6). We used the same normal limits as Ewing et al.

Valsalva maneuver. The subject sat quietly in a 20 ± 1°C temperature-controlled room and then blew into a mouthpiece to maintain a counterpressure of 40 mmHg for 15 s. HR increased during the maneuver, rebounding after release of the mouthpiece. The ratio of the longest to the shortest R-R interval during the maneuver (the Valsalva ratio) was calculated. A normal score is ~1.21.

HR response to standing up. The subject lay on a couch and then stood up unaided. HR increased during standing up, reaching a maximum at ~15 beats after standing up, and decreased to a minimum at about the 30th beat. The ratio of the longest to the shortest R-R interval during the maneuver (the Valsalva ratio) was calculated. A normal score is ≥1.04.

HR response to deep breathing. The subject sat quietly and then breathed deeply at a rate of ~6 breaths/min. The maximal and minimal HRs during each breath were measured, and the mean of the differences during three successive breaths was taken as the test score. A normal score is ≥15 beats.

SBP response to standing up. SBP was measured by a sphygmomanometer with the subjects lying down and then after standing up. The difference in SBP was taken as the test score. A normal score is ≥10 mmHg.

DBP response to sustained handgrip. Handgrip was maintained at 30% of the maximal voluntary contraction with a handgrip dynamometer (Vigorimeter, Martin Medizin Technik, Tuttlingen, Germany) up to a maximum of 5 min, and BP was measured each minute. The difference between DBP just before release of the handgrip and before starting the handgrip was taken as the test score. A normal response is ≥16 mmHg.

Each test was repeated three times, and the mean value of the three measurements was used.

Total score. Each of the two BP scores was classified as normal, borderline, or abnormal and coded by 0, 0.25, and 0.5, respectively. Each of the three HR scores was classified as normal, borderline, or abnormal and coded as 0, 0.5, and 1, respectively. The sum of the five codings gives the total score.

Analysis of the Data by Power Spectrum Analysis

Visual inspection of the HR and BP data was used to select intervals of ~600 beats that displayed neither artifacts nor trend. The means ± SD of BP and HR were estimated. The Fourier spectrum of the R-R intervals was calculated for the series. Because the data were measured at the times of each heartbeat, we interpolated R-R values at evenly spaced time intervals using the method of Berger et al. (2). A sample of 512 values was taken from this reconstructed series and submitted to the Fourier transform after linear detrending and with the Hanning window. Calculations were performed by our own software following Press et al. (18) and with explicit formulas given by Anderson (1). We calculated the total area under the Fourier spectrum (limited in practice to the frequencies between 0.02 and 0.40 Hz) and the percentage of this area in the low-frequency band (0.07-0.14 Hz; LF%). LF% is thought to reflect the sympathetic tone of the autonomic control (24).

Analysis of the Data by Recurrence Plot

Many methods are available to explore nonlinear time series, including the calculation of fractal dimension (7, 8, 13), Lyapunov exponents (13), forecasting (22), and entropies (17, 32). We selected in the present work the method of recurrence plot (4), which has been applied to biological data in previous reports (10, 28, 30, and the references therein).

To illustrate the method, Fig. 1A shows a series of 12 HR values (x; in beats/min), where i = 1, 2,..., 12. The recurrence plot looks for repeated sequences in the data. We consider that two HR values are the same if their difference is less than a small number (r), say, r = 2 beats/min. Starting from x1, we are interested to see whether the same value occurs later on in the series (i.e., for j > i). In Fig. 1A, the same values are found at j = 6 and j = 8. To mark these recurrences, we plot on a 12×12 square the points (1,6) and (1,8) (Fig. 1B). Then we start from x2 and plot the recurrent points (2,7) and (2,9). Figure 1B shows the recurrent points of the total series. Of particular interest are the diagonals. One example is the line (1,8)-(2,9)-(3,10). This means that when a recurrence is found, the two subsequences starting from these recurrent points remained close together for several subsequent beats: the trajectory x1=x2=x3 was parallel to the trajectory x5=x6=x7. If several such parallel subsequences are found, we have a sign...
of stability of the dynamic vs. initial values. We can count the number of diagonals of lengths \(1, 2, \ldots, k\) where \(k\) is the longest diagonal in the recurrence plot \((l_{\text{max}})\). In Fig. 1B, there were four diagonals of length 1, three of length 2, and one of length 3, and, therefore, \(l_{\text{max}} = 3\). The histogram of the number of diagonals with lengths \(1, 2, \ldots l_{\text{max}}\) is shown in Fig. 1C. The index used in the present study is \(l_{\text{max}}\).

In the original presentation of the method (4), all points \((i,j)\) with \(|x_i - x_j| < r\) were plotted. Because \(|x_i - x_j| < r\) implies \(|x_i - x_j| < r\), the plot is symmetrical vs. the \(l_{\text{max}}\). In this work, we have plotted only the upper half of Fig. 1 to avoid redundancy. A recurrence plot was performed for 1,000 readings for all subjects.

Some nonlinear dynamic systems have the property of “sensitivity to initial condition.” Starting from two points very close to each other, the systems may generate two sequences that diverge exponentially one from the other. The exponent of this exponential divergence is called the “Lyapunov exponent.” If a recurrence plot is performed in these cases, we will observe only diagonals of lengths that correspond to the time it takes for nearby trajectories to diverge. Therefore, the Lyapunov exponent of a series is inversely related to the \(l_{\text{max}}\) of its recurrence plots (4). A large value of \(l_{\text{max}}\) means that the HR dynamic is under stringent control. We will return to this point in DISCUSSION.

**Linear and Nonlinear Analyses**

To assess whether the observed \(l_{\text{max}}\) in the recurrence plot was due solely to linear correlations or to nonlinear dynamics, we used the method of surrogate data. Surrogate data were obtained as described by Theiler et al. (25). Take the Fourier transform to represent the signal as a sum of cosines of different frequencies. At each frequency, assign a random phase but keep the amplitude the same as the original. Take the inverse Fourier transform to convert back to the time domain. The result is a signal that has the same linear correlation coefficient and the same power spectrum, LF\%, and variance as the original data (1, 11) but has no nonlinear structure. An example of \(l_{\text{max}}\) calculated on observed data and on a large number of surrogate data will be shown in Surrogate Data, \(l_{\text{max}}\) and Nonlinearity.

**Statistical Analysis**

We used the classic Student’s \(t\)-test to compare observations in the normal and diabetic groups, the Pearson correlation coefficient to assess the relationship between two variables, and the partial correlation coefficient to account for the possible effects of confounders in a correlation study (11).

**RESULTS**

**BP, HR, and Score of Disautonomy**

SBP and DBP were slightly higher in the diabetic group compared with the normal group (systolic: 123 ± 11 vs. 113 ± 12 mmHg, \(P < 0.01\); diastolic: 71 ± 15 vs. 63 ± 10 mmHg; \(P < 0.01\)). The HR was slightly lower in the diabetic group compared with the normal group (70 ± 8 vs. 74 ± 9 beats/min; \(P < 0.05\)). The HRV in the diabetic subjects, assessed by its standard deviation, were, as expected, reduced compared with the normal subjects (2.80 ± 1.17 vs. 3.63 ± 1.45 beats/min; \(P < 0.001\)). The Ewing score ranged from 0 to 4. One-half of the diabetic subjects had moderate (Ewing score > 1) to severe autonomic neuropathy (Ewing score > 2).

**HRV in Diabetic Subjects Analyzed by Linear Methods**

Figure 2A shows the relationships between the Ewing score and HRV (assessed by its standard deviation). A negative correlation was apparent but was not significant (\(r = -0.21; P > 0.10\)). Figure 2B shows the Fourier spectra of the R-R intervals in the 64 diabetic subjects. The dominant terms were in the low-frequency domain. The LF\% was similar in the normal and diabetic groups (23.2 ± 3.8 vs. 22.4 ± 4.3%; not significant (NS)). Figure 2C shows the relationship between the Ewing score and this percentage. No significant correlation was found (\(r = 0.08\); NS).

**Recurrence Plot of HR and Ewing Score**

Figure 3 displays two examples of recurrence plots of HR, one for a normal subject (Fig. 3A) and one for a diabetic subject (Fig. 3B). The histograms of diagonals, including those of length 1, are given in Fig. 3, C and D. The \(l_{\text{max}}\) values were 32 and 44, respectively. The \(l_{\text{max}}\) was significantly higher in the diabetic subjects compared with the normal subjects (55 ± 21
DIABETIC AUTONOMIC NEUROPATHY AND HEARTBEAT Variability

vs. 37 ± 14; P < 0.0001). Figure 4 displays the relationship between $l_{\text{max}}$ and Ewing score in the diabetic subjects. The correlation was negative and very significant ($r = -0.60; P < 0.0001$).

The Ewing score and $l_{\text{max}}$ were both correlated with age in the diabetic subjects ($r = 0.41, P < 0.001$ and $r = 0.34, P < 0.01$, respectively). Also, the Ewing score and $l_{\text{max}}$ were correlated with the casual SBP ($r = 0.40, P < 0.002$ and $r = 0.30, P < 0.02$, respectively) and with the casual DBP ($r = 0.32, P < 0.01$ and $r = 0.27, P < 0.03$, respectively). After accounting for the age effect with a partial correlation, the $l_{\text{max}}$ and Ewing score were still highly correlated ($r = -0.53; P < 0.0001$). However, after accounting for the age effect, the Ewing score and $l_{\text{max}}$ were not related to SBP and DBP.

Recurrence Plot of BP

The same calculations were performed on SBP and DBP. The $l_{\text{max}}$ of the SBP was not correlated to the Ewing score, whereas the $l_{\text{max}}$ of the DBP was weakly related to the Ewing score ($r = 0.32; P < 0.03$). No relationship was observed between the standard deviation of the BPs and the $l_{\text{max}}$ of their recurrence plot.

Surrogate Data, $l_{\text{max}}$ and Nonlinearity

Figure 5 displays two series of heartbeats. In Fig. 5A, we display an observed series and its linear autocorrelation coefficients, correlation coefficients ($r_k$) of the linear regression ($x_i = ax_{i-k} + b$) with different delays ($k$), and Fourier power spectrum. In Fig. 5B, we display a series of surrogate data obtained from Fig. 5A and its autocorrelation coefficients, $r_k$, and power spectrum. The power spectrum (or, equivalently, the linear autocorrelation spectrum) and, therefore, the LF% cannot distinguish series such as in Fig. 5, A and B. We calculated $l_{\text{max}}$ in the series (Fig. 5A) and in 100 surrogate data obtained from this series. In the measured series, $l_{\text{max}} = 36$. In the 100 surrogate data, all $l_{\text{max}}$ were lower ($l_{\text{max}} = 23 ± 1$; see the histogram in Fig. 5C). This result indicates that $l_{\text{max}}$ is able to detect nonlinearity in the HR data.

DISCUSSION

An early diagnosis of possible neuropathy in diabetic subjects is of great clinical importance. HRV may mirror the autonomic function and has been extensively investigated but mostly by spectral methods. HR measurement is easy to perform and, if measured in standardized conditions with control of posture and respiration, is well reproducible (9). However, the variability in heartbeats is very complex and may reflect nonlinear interactions between the sympathetic and parasympathetic systems. Because new methods are available to explore nonlinear structures in a time series, it is natural to question whether these methods can disclose some internal structure of the heartbeat dynamic in diabetic subjects, a structure that might predict an autonomic neuropathy.
We measured the Ewing score in a large group of diabetic subjects and also a series of heartbeats in the same subjects in the at-rest condition. We assumed that the Ewing score is a good criterion of autonomic neuropathy. The question is whether some index derived from a nonlinear analysis of heartbeats at rest can explore the autonomic neuropathy assessed by the Ewing score.

Nonlinear methods have been applied to the analysis of heartbeats both in the healthy status and in different pathologies by many authors. These methods include the calculations of fractal correlation dimension (7, 13), information entropies (17, 30), forecasting (22), and recurrence plot (10, 28, 30). Among the different methods, recurrence plot is a simple one not requiring too long a series of data. Among different indexes deductible from the recurrence plot (3, 28, 30, 31), the $l_{\text{max}}$ index has a clear dynamic meaning, is inversely related to the well-known Lyapunov exponent (3), and appears the most promising for our purpose.

Most, if not all, methods of nonlinear dynamics data analysis require the selection of an "embedding dimension" (23), which reflects the number of independent variables needed to describe the dynamics. Problems in the interpretation of analysis arise when the results depend strongly on the embedding dimension. When the maximal norm is used, the numerical value of $l_{\text{max}}$ depends on the embedding dimension in a particularly simple way: a diagonal of length $k$ as calculated here would be a diagonal of length $k = p + 1$ in a $p$-dimensional embedding. In our series, all $l_{\text{max}} \geq 20$. Thus the correlations between $l_{\text{max}}$ and the other variables reported here do not depend on the embedding dimension for dimensions $< 20$, larger than the range of embedding dimensions typically found in the literature for HR data.

We calculated $l_{\text{max}}$ in a group of healthy young subjects to help in the interpretation of the results. A different $l_{\text{max}}$ is expected between healthy subjects and diabetic subjects. Because the Ewing score was available only in the diabetic subjects, our analysis was mainly focused on the diabetic group. The effect of age on HRV is well known (20). In the diabetic group, we took into account the role of age, using a partial correlation.

Our main result was the very significant relationship between the $l_{\text{max}}$ index and the Ewing score. Using classic indexes such as HR standard deviation or spectral power, we obtained only a weak relationship or no relationship of the indexes with the Ewing score. We recall that to assess the $l_{\text{max}}$ only a short measurement of heartbeats in the sitting position is needed. In contrast, to assess the Ewing score, several cardiac function tests are needed; this is time consuming for the medical staff and requires good cooperation from the patient. Therefore, from the clinical aspect, the $l_{\text{max}}$ might be a useful index to survey the autonomic function in diabetic subjects.

Our results suggest that, from healthy status to neuropathy, heartbeat is submitted to more and more stringent control. In the healthy status, the dynamics include a rich variety of cycles and therefore might adapt to energetic needs. This capacity is reduced in diabetes mellitus.

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REFERENCES