Heart Rate Variability: Short-Term Studies are as Useful as Holter to Differentiate Diabetic Patients from Healthy Subjects

Eduardo R. Migliaro, M.D., M.Sc., Rafael Canetti, Eng.,*
Paola Contreras, BSc., and Michel Hakas, Eng.*

*From the Departamento de Fisiología, Facultad de Medicina, and *Departamento de Control, Instituto de Ingeniería Eléctrica, Facultad de Ingeniería, Universidad de la República, Montevideo, Uruguay*

Background: The definitive incorporation of heart rate variability (HRV) as a clinical tool depends on the development of more confident techniques of measurement. The length of the studies is a critical issue. Whereas Holter studies allow the monitorization at different hours and activities, short-term recordings allow the control of environmental conditions. Recording length is also strongly related to the procedure of analysis; for instance, some time-domain indexes are strongly affected by the duration of the study. Meanwhile, spectral analyses require stationary conditions, only achieved in short-term studies. Our main goal was to determine if HRV indexes obtained from short-term analyses were as useful as those from Holter monitoring for diagnosis of reduced HRV in diabetes.

Methods: We studied two groups: one with impaired HRV (15 diabetic patients) and another with normal HRV (15 healthy subjects). HRV indexes obtained from 24-hour Holter recordings (SDNN, rMSSD, and the power of LF and HF bands), were correlated with analog indexes obtained from 10-minute digital acquired studies within each group. Besides, we compared the diabetic and control groups using the indexes obtained with both methodologies.

Results: The correlation was high ($0.70 \leq r \leq 0.85$, $P \leq 0.0032$) in the diabetic group, but was poor in the control group. HRV values were significantly lower in the diabetic group either for 24-hour or short-term studies ($P \leq 0.0113$).

Conclusion: We conclude that short-term studies are at least as powerful as Holter to differentiate the diabetic group (impaired HRV) from the control group. **A.N.E. 2003;8(4):313–320**

heart rate variability; short-term studies; diabetes

Heart rate variability (HRV) has become a valuable tool for heart physiology research. In addition, it is well known that HRV is useful for diagnostic or prognostic purposes in many pathologies such as coronary artery disease, diabetes mellitus, heart failure, and others.[1–5] The definitive incorporation of HRV as a clinical tool depends on the development of more confident techniques of measurement. In the last two decades different methods of analysis have been evaluated, among them, time- and frequency-domain methods have dominated the first years of the HRV research.[6–8] Recently, geometric and nonlinear methods have gained insight in this field.[9–11]

The best method has not yet been identified. Probably, a combination of two or more indexes would be the best choice, but certainly further research in this field is necessary.

One of the issues of the HRV technique that remains to be solved is the length of the studies. The recordings for HRV analysis can take few minutes (for instance, 5 or 10 minutes)[12,13] or last for many hours (usually 24 hours). Short-term recordings are commonly performed in controlled environmental conditions; meanwhile, when the study is

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Address for reprints: Dr. Eduardo R. Migliaro, Depto. de Fisiología, Facultad de Medicina, Av. Gral. Flores 2125, 11800 Montevideo, Uruguay. Fax: 598 2 9240395; E-mail: erm@fmed.edu.uy

maintained for many hours, environmental control is difficult.

Besides, the recording period is strongly related to the selected procedure of analysis. Time-domain methods provide good HRV evaluation in long-term recordings.[14] On the other hand, frequency-domain analyses require stationary conditions that are hardly achieved in long-lasting recordings.

This article was designed to answer the following question: Are the HRV indexes obtained from short-term analysis as useful as those from a standard Holter monitoring for diagnosis of reduced HRV in diabetic patients? To answer that question we studied HRV in two different groups: one supposed to have impaired HRV related to autonomic neuropathy (long-evolution insulin-dependent diabetic patients)[4,15–18] and another with presumed normal HRV (healthy subjects). The HRV measurements obtained from a commercial 24-hour Holter tape recording system were compared with those from 10-minute digital acquired studies.

We concluded that short-term studies are at least as powerful as 24-hour recordings to differentiate the group with impaired HRV from the control group.

METHODS

Thirty-two subjects were studied divided in two groups. The diabetic group was composed of 15 insulin-dependent diabetic patients (8 women and 7 men), who had had diabetes mellitus for a long time (mean diabetes duration ± SD: 38.3 ± 8.5 years). They were allowed to receive their usual medication (digitalis, calcium antagonists, converting enzyme inhibitors).

A group of 17 healthy nonsmokers, who had a normal body mass index (<30) and were not receiving any medication, were studied. Two men were excluded for arrhythmia in the ECG. Then, our control group was composed of 15 normal volunteers (9 women and 6 men). Age was similar in both groups as the control subjects were selected in the same age range as in the diabetic group (see Table 1).

After a detailed explanation of the routine and aims of the study, all subjects gave their consent. They were instructed to avoid caffeine, alcohol, and heavy exercise the day before the study. All tests started between 4 and 6 PM. Subjects were placed in a quiet room in supine position. After the preparation of the skin, seven self-adhesive electrodes were placed in the positions usually used for three-leads Holter monitoring. Three additional electrodes were placed in order to obtain a chest bipolar lead (plus ground) which was connected to an ECG recorder (Fukuda FJC 7110). The lead used was "V5 like,"[19] i.e., a positive electrode in V_5 position and the negative electrode on the right border of the sternum near the second rib. The final position of the positive electrode was chosen by visual control, seeking for high R waves. The Holter recordings were performed with a tape-based Scole Engineering equipment. After the initiation of the 24-hour recording, the subjects were allowed to relax for 20 minutes in order to stabilize their heart rate. The collection of data for the short-term study began after the rest interval. Respiration was not controlled in order to obtain a better relaxation of patients. The ECG signal was fed into a computer (Compaq Armada) by means of an A/D converter (National Instruments DAQ Card-1200) for 10 minutes. The sampling rate was 500 Hz. The heart rate measured during this period was considered as resting heart rate. Details of acquisition and processing are given in the Appendix.

Table 1. Comparison of Age and Heart Rate between Diabetic and Control Groups

	Diabetic Group	Control Group
Age (years)	53.8 ± 7.2	53.3 ± 8.8
Resting heart rate (bpm)	73.9 ± 9.5	69.4 ± 5.2

Values are means ± SD of all data. The Mann-Whitney test did not show differences between groups either in age or in resting heart rate values. N = 15 in each group.

DATA ANALYSIS

Holter Studies

For the Holter analysis Scole Engineering software was used. The first step was the qualification of R waves allowing the identification of premature contractions and artifacts. After that, the HRV measurements were obtained from the entire record, according to this software's routines. The indexes considered for this study were:

Time-domain indexes: Standard deviation of normal-to-normal intervals (SDNN), mean of the standard deviation of normal-to-normal intervals for 5-minute segments (SDNNIX) and root mean square successive differences (rMSSD).

Frequency-domain indexes: The commercial software used calculates the total power (TP, 0–0.5 Hz) of the tachogram, which is also divided in bands.. The very low frequency band (0.003–0.04 Hz: VLF), low frequency band (0.04–0.15 Hz: LF), and high frequency band (0.15–0.40 Hz: HF). Nevertheless, only LF and HF power will be considered in this work due to the limitations imposed by short-term analysis (see below).

Short-Term Analysis

A LabView®-based algorithm was used to detect R waves. After that, we used a routine that made possible the visual validation of the entire record and the correction of false positives (related to movements or extrasystolic beats) and negatives (lack of detection). Thus, we obtained very reliable data (see Appendix). Another software program, written in Matlab®, was used to measure R-R intervals and calculate the HRV statistical indexes: SDNN, rMSSD. The SDNNIX calculated from 24-hour recordings, does not have a short-term counterpart due to its definition. In the frequency-domain analysis, the total power of the spectrum and VLF band (0.003–0.04 Hz) could not be evaluated. The rationale is this: our 10-minute recording does not allow the evaluation of the power spectral density (PSD) below 0.003 Hz; in addition, the routine used for the calculation of the spectrum reduces the lower limit to 0.015 Hz (see Appendix). Therefore, only the power in LF (0.04–0.15 Hz) band and HF (0.15–0.4 Hz) band will be calculated for short-term analysis. Since the total power and VLF values were not taken into account, the corresponding indexes calculated for the Holter system could not be compared. Nevertheless, as we will see later, this limitation does not affect the conclusions of this work.

Comparison Between 24-Hour and Short–Term Analysis

We correlated the analog 24-hour and 10-minute indexes within each group. A nonparametric (Spearman) correlation coefficient (r) was calculated for each pair of indexes.

As another strategy, the indexes obtained with the same method were compared between the control and diabetic groups, using a nonparametric test (Mann-Whitney).

In all cases a two-tailed P value < 0.05 was considered as significant.

RESULTS

Correlation of Analog 10-Minute and 24-Hour Indexes

The values of HRV indexes, calculated from 24-hour Holter recordings for every subject within each group, were correlated with the values measured by short-term analysis. Table 2 shows the r values for all indexes in each group as well as their corresponding P values.

For diabetic patients, all the studied indexes show a high correlation of 24-hour and short-term studies. As can be seen in Table 2 all r values are ≥ 0.70 ($P \leq 0.0032$).

In the control group r values are lower than those of the diabetic group. Nevertheless, the P values are significant for rMSSD, LF, and HF (see discussion). In this case, the P value represents the random probability of obtaining a correlation coefficient as far from zero as observed if the two variables were not correlated.

Comparison Between Diabetic and Control Groups for Each Index

Resting heart rate is not significantly different between both groups (Table 1). The diabetic group shows significantly lower HRV indexes than the control group, either for 24-hour or 10-minute studies. Table 3 shows the mean HRV indexes and the P values from comparisons between both groups for 24-hour and short-term analysis (Mann-Whitney test). For Holter recordings, the worst

Table 2. Correlation of 10-Minute and 24-Hour Indexes in Both Groups

	SDNN	rMSSD	LF	HF
Diabetic patients 24-hour vs short-term studies				
r	0.70	0.83	0.79	0.85
P	0.0032	0.0001	0.0005	<0.0001
Control subjects 24 h vs short-term studies				
r	0.37	0.53	0.62	0.66
P	ns	0.044	0.014	0.008

Correlation coefficient (r) values in control and diabetic groups. The r values were calculated with a nonparametric test (Spearman).

Table 3. Comparison of HRV Indexes Between Control and Diabetic Groups for Both Methods (24-hour and 10-minute)

	24 h Holter Analysis					Short-Term Analysis				
HRV Index	Diabetic Mean	Group SD	Control Mean	Group SD	P value	Diabetic Mean	Group SD	Control Mean	Group SD	P value
SDNN (ms)	90.8	22.1	127.3	34.6	0.0045	22.9	12.0	44.3	10.4	<0.0001
RMSSD (ms)	18.3	8.3	26.1	8.1	0.009	14.5	13.0	23.7	9.2	0.0113
SDNNIX (ms)	34.3	12.0	53.0	12.3	0.0006	–	–	–	–	–
LF (ms^2)	306.8	264.7	852.1	366.8	<0.0001	125.7	172.7	546.4	398.8	<0.0001
HF (ms^2)	77.5	70.1	200.2	159.5	0.0023	83.3	117.6	278.7	292.1	0.0017

Analyses were performed using a nonparametric test (Mann-Whitney). All P values are significant. As can be seen P values are quite similar in both methods except for SDNN (see text for discussion).

P values are for rMSSD and SDNN. The same applies for rMSSD but not for SDNN in short-term studies. We would like to underline this difference in SDNN P values for 24-hour and 10-minute studies (see Fig. 1). We will analyze this point in the discussion.

DISCUSSION

As we pointed out before, this work was done to verify if the HRV indexes obtained from 10-minute analysis are as useful as those from a standard Holter monitoring for diagnosis of reduced HRV in diabetes.

Some previous reports have compared the results obtained with short-term and 24-hour recordings for other pathologies. Klingenheben et al.[20] compared the SDNN values with both methods with respect to risk stratification after myocardial infarction, they found that 24-hour SDNN is better for this purpose. For the same stratification, other authors[21,22] suggested that 24-hour studies could be limited to those patients preselected by depressed short-term HRV measures. It has to be pointed out that in these cases the comparisons were made using different indexes for 24-hour and short-term studies.

In diabetes, short-term studies were proved to be useful to assess autonomic dysfunction.[23,24] Nevertheless, as far as we know there are not comparisons between 24-hour and short-term studies in the same individual.

The main contribution of our work was the measurement of the heart intervals in the same subject by two different methods (24-hour tape recording and 10-minute digital acquisition). We would like to point out that the short-term study was performed simultaneously with a part of the 24-hour monitorization (see Methods). Starting from the heart intervals measured with each method, analog indexes of HRV were calculated and the two procedures were compared.

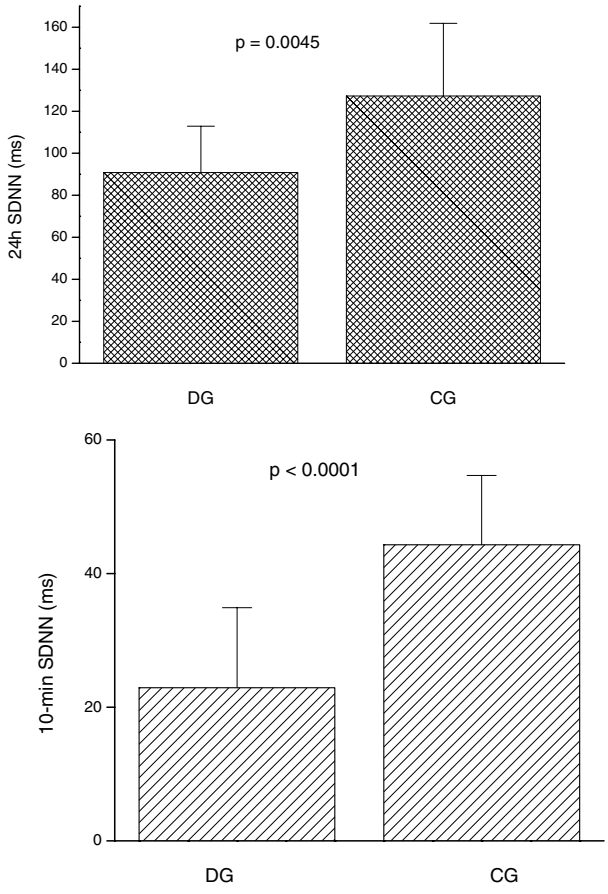

Figure 1. Comparison of SDNN differences between Diabetic Group (DG) and Control Group (CG) in 24-hour (above) and 10-minute (below) studies. Values are taken from Table 3. Note the different scales.

Correlation Between 10-Minute and 24-Hour HRV Indexes

In the diabetic group we found a very good correlation in all HRV indexes between short- and long-term recordings. As shown in Table 2, the diabetic group had correlation coefficients from 0.70 to 0.85 (all P values ≤ 0.0032). On the other hand, the control group had lower correlation coefficient values and higher P values for all HRV indexes. The SDNN index did not show a statistically significant P value (see Table 2).

This means that for the diabetic group, the same behavior is observed for 24-hour and 10-minute analog indexes, i.e, the same reported trends for 24-hour Holter analysis are expected in 10-minute studies.

The same applies for the control group, except SDNN that will be discussed below.

Comparison Between the Diabetic and Control Groups for Each Index

Both groups had the same age and resting heart rate (Table 1), two of the main determinants of HRV.[13,25,26] Then, the differences of HRV could be ascribed to the diabetes effect. Table 3 shows that the diabetic group had significantly lower HRV values than the control group either for 24-hour or short-term studies. Differences between both populations are very ($0.01 \geq P \geq 0.001$) or extremely ($P < 0.001$) significant, and in the same order of magnitude for both methodologies (except for SDNN); i.e., both methodologies are suitable for discriminating diabetic patients from healthy subjects.

In the case of SDNN, the differences of the medians of the two populations (Mann-Whitney test) are very significant in 24-hour analysis and extremely significant in short-term study. Indeed, the ratio between the associated P values is more than 45. The P value in the Mann-Whitney test can be interpreted as the probability of randomly having such large differences as measured, under the null hypothesis: both populations have the same median. This lower P value of SDNN in short-term analysis represents a better discrimination power of this index than its analog from 24-hour Holter recordings. As shown in Fig. 1, both groups are more clearly differentiated in short-term than in 24-hour study. The fact that 10-minute SDNN is better than its analog in 24-hour for diagnostic purposes implies that their correlation is impaired at least in one group as was previously pointed out for the Control Group. The SDNN values in short-term studies depend on fluctuations of a short period, mainly related to autonomic nervous system (ANS) modulation. The ANS-related rhythms are often impaired in diabetic patients.[4,14,27] Therefore, in such patients we can expect a stronger affectation of 10-minute SDNN than that from 24-hour studies. In fact, when long studies are divided in short (5 minute) segments and standard deviation for these rows is calculated, the average of such 5- minute periods, named SDNNIX, shows a similar performance than SDNN in 10-minute studies (see both P values in Table 3).

In the same line of reasoning, the rMSSD shows small differences between 24 hours and 10 minutes, because such an index evaluates short-term variations regardless of the total time of the study.

Our results for the frequency-domain indexes resemble those reported for ANS impairment; then it is easy to understand the very significant differences between diabetic patients and control subjects with both methodologies.

Consequently, according to our results, short-term analysis is, at least, as useful as 24-hour Holter recording for the diagnosis of reduced HRV in diabetes. Moreover, in the case of SDNN, its discrimination power is better in short-term studies than in longer-term studies.

Once this point has been proved, the advantages of the 10-minute recordings are manifest. Especially relevant are the short time for the study and the possibility of analyzing the subjects in similar environmental conditions.

Nevertheless, the widespread use of 24-hour recordings is easy to understand for many reasons, among them, Holter provides a long-lasting recording where changes in heart rate related to physiological rhythms (sleep/awake, circadian cycles or others) can be assessed. It can also be argued that the controlled conditions inhibit free-life activity, and this freedom must be a goal for patient evaluation.[17] However, we believe that the unrestricted activity that is essential for classical electrocardiographic evaluation (changes in ST segment or the onset of arrhythmias) is not appropriate for HRV analysis. For instance, values of HRV obtained when parasympathetic flow is physiologically enhanced (as happens during sleep) are processed together with the variability behavior seen during daily activities. As we mentioned before, Holter recordings can be divided into 5-minute segments,[14] and in such a case we can obtain

"samples" of short-term HRV studies from different hours and activities. Since the study of HRV could be considered as a picture of the sinus node regulation by ANS or other influences (fine tuning,[28,29] comparisons require the same environmental conditions. The control of such conditions is fairly better in short-term studies than in short rows obtained from a Holter recording.

Another advantage of the short-term studies is the possibility of being acquired directly into a computer with higher sampling rates than most Holter equipments (128 Hz).[14] Our short-term recording was performed with a sampling rate of 500 Hz, which improves the accuracy of the R wave peak detection.

Besides, digital recording avoids the use of tape recorders that introduce a new source of error related to the regularity of the tape motor.[16,30] As a conclusion, we can say that the 10-minute studies performed with an A/D converter are as useful as Holter recordings for low HRV diagnosis in diabetic patients.

Limitations of Our Study

Although the number of subjects is small, the clear differences seen in the results and the strong values of statistical analysis validate the conclusions, particularly, if we keep in mind that both types of studies were done on the same subjects and were synchronized.

Another design-limiting issue is the use of medication in the diabetic group that could affect HRV. Nevertheless, all the drugs used are reported to increase HRV. Therefore, the observed reduction in the diabetic group cannot be ascribed to medical treatment. Besides, the effect of medication in the Holter recordings and short-term studies is the same and does not have an influence in the comparison of methodologies.

APPENDIX

In this section we describe the main features of the hardware and software used to obtain 10-minute ECG recordings and to calculate R-R intervals and HRV indexes.

The ECG recordings were made with a Fukuda FJC-7110 electrocardiograph. This equipment has an electric output with values between −0, 5 V to +1, 5 V; its bandwidth is 22 Hz.

Signals were introduced into the computer through an acquisition card DAQ 1200. A specially designed software (programmed in LabView®) collected the entire ECG. The sampling rate was 500 Hz.

After that, a Matlab®-based software made possible the off-line recognition of R waves. Detection of R waves was performed in two steps: (1) an automatic (by software) "pre-detection," and (2) a manual inspection of each event, performed by an expert.

Predetection

The ECG signal was prefiltered in order to remove high frequency noise and low frequency baseline drift. This was done through a Butterworth fourth-order band-pass filter with 0.3–25 Hz passband. A filtered ECG signal named $x(t)$ was obtained. Predetection recognized the occurrence of an R wave, combining filtered ECG level and slope, i.e, when $x(t) >$ threshold1 and $dx(t)/dt >$ threshold2, an event was recognized, and the R wave occurrence was assigned to the maximum of the ECG signal.

Selecting the thresholds, a normal estimation, an underestimation, and an overestimation were obtained.

Manual Inspection

Each file containing the ECG and R wave detections was later processed with another LabView®-based software, in order to correct false detections. This was done through visual inspection, which enables the identification of false detections (both negative and positive) and makes any necessary corrections. In this visual inspection, the software displays simultaneously the ECG and the predetected hits in three sets: normal, under- and overestimated hits. Another row (output row) shows the validated hits.

When a false-positive related to an artifact was detected, the researcher manually deleted the spurious hit from the output row. Since only sinusal beats must be considered in HRV calculations, any nonsinusal beats had to be removed. Such action yielded a long interval that was replaced using the following algorithm: Starting from the last sinusal R wave, a new hit was added in order to obtain an interval equal to the average of the three preceding ones. From this "artificial detection" to the next

normal R wave, we usually found an interval that was within 20% of the average. Otherwise, the "artificial detection" was relocated to obtain two R-R intervals in the chosen range (±20%).

In the case of false negatives there were two possibilities: (a) When an R wave, which was easy to identify, had not been detected as normal estimation of hits. In such a case the visually identified R wave allowed the manual inclusion of a hit in the right place in the output row (usually determined through the overestimation series). (b) A false-negative, when an R wave could not be identified by visual inspection. In this case, a new hit was added following the algorithm described when a nonsinusal beat was deleted (see above). We would like to stress that the manual correction was used only in few cases of some recordings. After such a procedure we have two types of intervals: Most of them were truly sinusal intervals and we can certainly call them N-N (normal-to-normal intervals). Only a small fraction was obtained from the manual correction. Such "artificial" intervals ranging ± 20% from the mean N-N interval were also considered as N-N periods.

After that, another Matlab®-based software measured the duration of N-N intervals and calculated the indexes used in this work. Frequency-domain indexes were calculated using Welch's estimator[31,32] of the power spectral density (PSD). First the N-N interval sequence was interpolated with a cubic spline, uniformly resampled (Fs = 4 Hz), and detrended. The power spectral density of this signal was estimated by Welch's method, dividing the data in nonoverlapping intervals of length 512-samples each windowed with a Hanning window. Following this procedure the PSD can be estimated for frequencies greater than 0.015 Hz. Therefore, the power associated with ULF and VLF bands is not evaluated.

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