Impaired Circadian Modulation of Sympathovagal Activity in Diabetes

A Possible Explanation for Altered Temporal Onset of Cardiovascular Disease

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Background. **Diabetic subjects have a high incidence of cardiovascular accidents, with an altered circadian distribution. Abnormalities in the circadian rhythm of autonomic tone may be responsible for this altered temporal onset of cardiovascular disease.**

Methods and Results. **To assess circadian changes of sympathovagal balance in diabetes, we performed 24-hour power spectral analysis of RR interval fluctuations in 54 diabetic subjects (age, 44±2 years) with either normal autonomic function or mild to severe autonomic neuropathy and in 54 age-matched control subjects. The power in the low-frequency (LF, 0.03–0.15 Hz) and high-frequency (HF, 0.18–0.40 Hz) bands was considered an index of relative sympathetic and vagal activity, respectively. Diabetic subjects with autonomic abnormalities showed a reduction in LF compared with control subjects (5.95±0.12 ln-msec^2 versus 6.73±0.11, $p<0.001$) and an even greater reduction in LF, particularly during the night and the first hours after awakening (5.11±0.18 ln-msec^2 versus 6.52±0.14, $p<0.001$). Day–night rhythm in sympathovagal balance was reduced or absent in diabetic subjects compared with control subjects.**

Conclusions. **Diabetic subjects with or without signs of autonomic neuropathy have a decreased vagal activity (and hence a relatively higher sympathetic activity) during night hours and at the same time of the day, during which a higher frequency of cardiovascular accidents has been reported. These observations may provide insight into the increased cardiac risk of diabetic patients, particularly if autonomic neuropathy is present. (*Circulation* 1992;86:1443–1452)**

KEY WORDS • **diabetes mellitus** • **heart rate variability** • **Holter recordings** • **power spectrum analysis** • **autonomic neuropathy**

In the general population, autonomic activity shows a circadian rhythm with a prevalence of sympathetic tone during the day and the first hours after awakening and a marked relative increase in parasympathetic tone during the night.[1] Similarly, most acute cardiovascular diseases have a circadian rhythm, with greatest incidence during the morning.[2–11] Diabetic subjects, particularly those with autonomic abnormalities,[12] have a high incidence of acute cardiovascular accidents,[13–16] whereas epidemiological studies indicate that in diabetes the circadian distribution of myocardial infarction is altered.[17–19] The power spectral analysis of heart rate fluctuations has provided a new, powerful tool to assess the sympathovagal balance[20–22] by analysis of 24-hour ECG recordings.[1] Observations of the heart rate variability in diabetic patients, even when using power spectral analysis methods,[23–25] have usually been made over short time periods, whereas the simple observation of diurnal heart rate variations has been the object of a small number of studies[26] despite the frequently reported association between cardiovascular accidents and autonomic imbalance.[12,27–30] The aim of the present investigation was to assess the circadian variation in autonomic balance in patients with diabetes with or without autonomic abnormalities.

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Methods

Subjects

Fifty-four diabetic subjects aged 16–70 years (mean, 44±2 years, 34 men and 20 women, 34 with insulin-dependent type 1 diabetes and 20 with non–insulin-dependent type 2 diabetes) and 54 healthy subjects aged 43±2 years (31 men and 23 women) were recruited consecutively among those fulfilling the criteria for inclusion in the study. The diagnosis or exclusion of diabetes was made according to the criteria of National Diabetes Data Group.[31] Thirty-four diabetic patients were on insulin treatment, 16 were on oral hypoglycemic agents, and four were on special diets. The diabetic patients were grouped according to their response to a standardized battery of cardiovascular autonomic tests,

TABLE 1. Laboratory and Clinical Autonomic Abnormalities of the Diabetic Subjects Studied (D+ Group)

Patient	Age (years)	DB	VR	CC	ST	HDG	DZZ	ULC	IMP	DIA	UINC	SHYP
1	45	+		+		+						
2	62			+								
3	32	+		+			+					
4	45	+				+						
5	60	+	+	+		+				+		
6	45	+		+		+						
7	33	+		+		+		+				
8	63			+		+			+		+	
9	58	+		+								
10	66			+								
11	41	+		+								
12	54	+	+	+		+						
13	57			+				+				
14	64	+				+	+					
15	44	+		+	+			+				+
16	56			+		+	+					
17	19	+		+								
18	56	+		+				+				
19	38	+		+								
20	66			+	+	+		+				
21	44	+				+					+	
22	50	+		+			+					
23	20	+		+		+						
24	55	+		+		+						
25	50			+	+	+			+			
26	63	+	+	+	+		+					+
27	68			+	+	+			+			+
28	62			+			+					
29	47	+		+			+					
30	42		+									
31	70			+		+			+		+	

DB, deep breathing test; VR, Valsalva ratio; CC, cross-correlation between heart rate and respiration; ST, systolic blood pressure fall to standing; HDG, diastolic blood pressure rise to handgrip; DZZ, dizziness; ULC, foot ulcers; IMP, impotence; DIA, diarrhea; UINC, urinary incompetence; SHYP, symptomatic hypotension; +, abnormal test.

as previously described in detail[32]: RR interval variation to sustained deep breathing and to Valsalva maneuver, cross-correlation between heart rate and respiration,[23–25] and blood pressure response to standing and to handgrip. Twenty-three patients (age, 36±3 years; range, 16–58 years) had normal tests (D− group) and no symptoms indicative of autonomic dysfunction; 31 patients (age, 50±2 years; range, 19–70 years) had abnormalities in at least one autonomic function test (D+ group). In 18 of these patients, one or more clinical symptoms indicative of autonomic or peripheral neuropathy were also present. Table 1 summarizes the autonomic abnormalities found in the D+ group. Criteria of inclusion in the study was the absence of atrial fibrillation and other arrhythmias, cardiac failure or respiratory abnormalities, history of coronary heart disease, severe hypertension, or therapy with any drug known to influence the autonomic nervous system. Patients 1, 18, 26, and 27 of Table 1 had mild untreated hypertension (diastolic blood pressure at rest between 90 and 100 mm Hg) and were maintained in the study. The mean ages of these two subgroups were different ($p<0.001$), therefore comparisons were made by selecting two subgroups of control subjects of appropriate age (control group for D− group: age, 36±2 years; range, 16–58 years; 36 subjects; control group for D+ group: age, 50±2 years; range, 16–70 years; 37 subjects). The mean duration of diabetes was not significantly different in the D− group (120±19 months) compared with the D+ group (159±19, p=NS).

Holter Recordings

The 24-hour ambulatory tape recording was obtained using a TR1 two-channel Tracker Recorder (Reynolds Medical Ltd., Hertford, England) while the subject undertook his normal daily activities. Each recording was scrutinized for ventricular ectopic beats and other arrhythmias using a 6201 D3 Holter Cardiography System (ICR, Liverpool, N.Y.). There were no signs of myocardial ischemia or prior myocardial infarction in any of the recordings, nor were arrhythmias present. There were no significant differences in the lengths of time awake or asleep between the various groups analyzed.

Data Collection and Analysis

The software was written by our group in "C" (acquisition and editing) or in FORTRAN (spectral analysis) languages. The RR interval was measured by a timing circuit (NB-MIO-16H multifunction board, National Instruments, Austin, Tex.) inserted into a Macintosh II computer (Apple Inc., Cupertino, Calif.) and connected to the pulse output of the Holter analyzer, which provided a square wave synchronous with the peak of the R wave of the ECG. Premature beats were interactively identified and corrected by linear interpolation with the previous and following RR intervals. Spectral analysis of the RR sequences was applied by an autoregressive model, as previously described.[33–38] To obtain a robust coefficient estimate, each sequence was obtained at fixed hours on 1 whole hour of recording (2,000–9,000 RR intervals). It has been shown[21,22] that the spectrum of the RR interval sequence has two separate peaks. The one at higher frequency (HF) appears to be very close both in shape and center frequency to the peak of a respiratory signal (in the region between 0.18 and 0.40 Hz during spontaneous breathing). The low-frequency oscillations (LF) in the region between 0.03 and 0.15 Hz are not related to respiratory events; they increase after transition from supine to standing position and decrease after administration of acute β-adrenergic blocking drugs.[22] The different behavior of LF and HF oscillations seems thus to reflect the sympathovagal interaction, particularly if evaluated in relative terms (i.e., HF versus LF oscillations). LF and HF components of the RR interval spectrum were evaluated in both absolute and normalized units. Normalization was obtained by expressing the power of each component as the percentage of total oscillatory power. In addition, mean heart rate and RR interval variability (assessed by RR standard deviation) were also computed. For each of the variables considered, the average values for nighttime, daytime, and for the entire day were also obtained. Daytime was considered to be between 8 AM and 10 PM and nighttime between 11 PM and 7 AM, on the basis of the average time of waking up and going to bed of the subjects studied. Therefore, the terms "day" and "night" hours in the present study reflect the average time during which the subjects were awake/upright and supine/asleep, respectively.

Reproducibility of 24-Hour Power Spectral Analysis

Holter recordings were obtained in five diabetic subjects with autonomic neuropathy (mean age, 49 years; range, 36–61 years) and in five control subjects of similar age (mean age, 43 years; range, 24–54 years). Two recordings (day A and day B) were obtained 3 days apart for each subject and processed as described above. To compare the results of variables expressed in different units, the data were expressed as percent change from day A to day B using the formula $100 \cdot |\text{day A} - \text{day B}|/\text{day A}$. The absolute value of the difference was necessary to maintain the amount of change from one day to the other also in the average, otherwise the direction of the change would be casual with a near-zero average regardless of the variation.

Statistical Analysis

Data are expressed as mean±SEM. LF and HF oscillations, when expressed in absolute values, were evaluated after natural logarithm transformations, preliminary tests having shown a skewed distribution. The paired *t* test was used to assess differences between LF and HF within the same subject and to assess the prevalence of one oscillation over the other or a different value during night compared with daytime. The unpaired *t* test was used to assess difference in data obtained in diabetic versus control subjects.

Results

The 24-hour trends obtained in the various groups for each considered variable are presented in Figures 1, 2, and 3. Tables 2, 3, and 4 summarize the mean data obtained during night and daytime.

Normal Subjects

In normal subjects, the mean RR interval was highest during the night and lowest during the day ($p<0.001$), as was heart rate variability ($p<0.01$). The LF showed small but significant ($p<0.01$) changes between night and daytime, whereas the HF showed a larger difference ($p<0.001$). Figure 2 indicates that highly significant differences between LF and HF were present only during day and not during night. As a consequence, the LF/HF ratio was markedly lower during night than during day ($p<0.001$). Therefore, normal subjects showed a relatively higher sympathetic predominance during the daytime and an increase in parasympathetic tone during the night.

The HF and particularly the LF was slower (see Figure 3 and Tables 1, 2, and 3) during night than during day. The two subsets of normal subjects, although different in age, showed similar trends in all the variables considered (see Figures 1, 2, and 3, and Tables 2–4). Figure 4 shows an example of the power spectra obtained during the 24-hour period in one control subject.

Reproducibility of 24-Hour Power Spectral Analysis

Data are shown in Table 5. The change from day A to day B ranged from 0.6% (control subjects, LF during night hours) to 26.6% (diabetic subjects, HF during the entire day). Best reproducibility was obtained for the power of the LF and HF and for the normalized LF both in control and diabetic subjects, whereas the worst reproducibility was obtained for the HF normalized units, particularly for diabetic subjects in whom the HF normalized units were generally lower than in control subjects. Thus, the worst reproducibility was due to fluctuations in small percent values. For example, the percent variation of 26.6 in HF normalized units in diabetic subjects (the worst result obtained) was due to a change from 23.9 to 30.2, which resulted in a small overall variation.

Diabetic Subjects Without Autonomic Test Alterations

In the 23 diabetic subjects with no evidence of autonomic alterations, the mean RR interval was higher during night than during the day ($p<0.001$), and so was heart rate variability ($p<0.01$). These results were not different from those found in the age-matched subset of normal subjects. Both LF and HF showed changes between night and daytime ($p<0.01$ and $p<0.001$, respectively). Although their absolute values were not different from the age-matched subset of controls, the

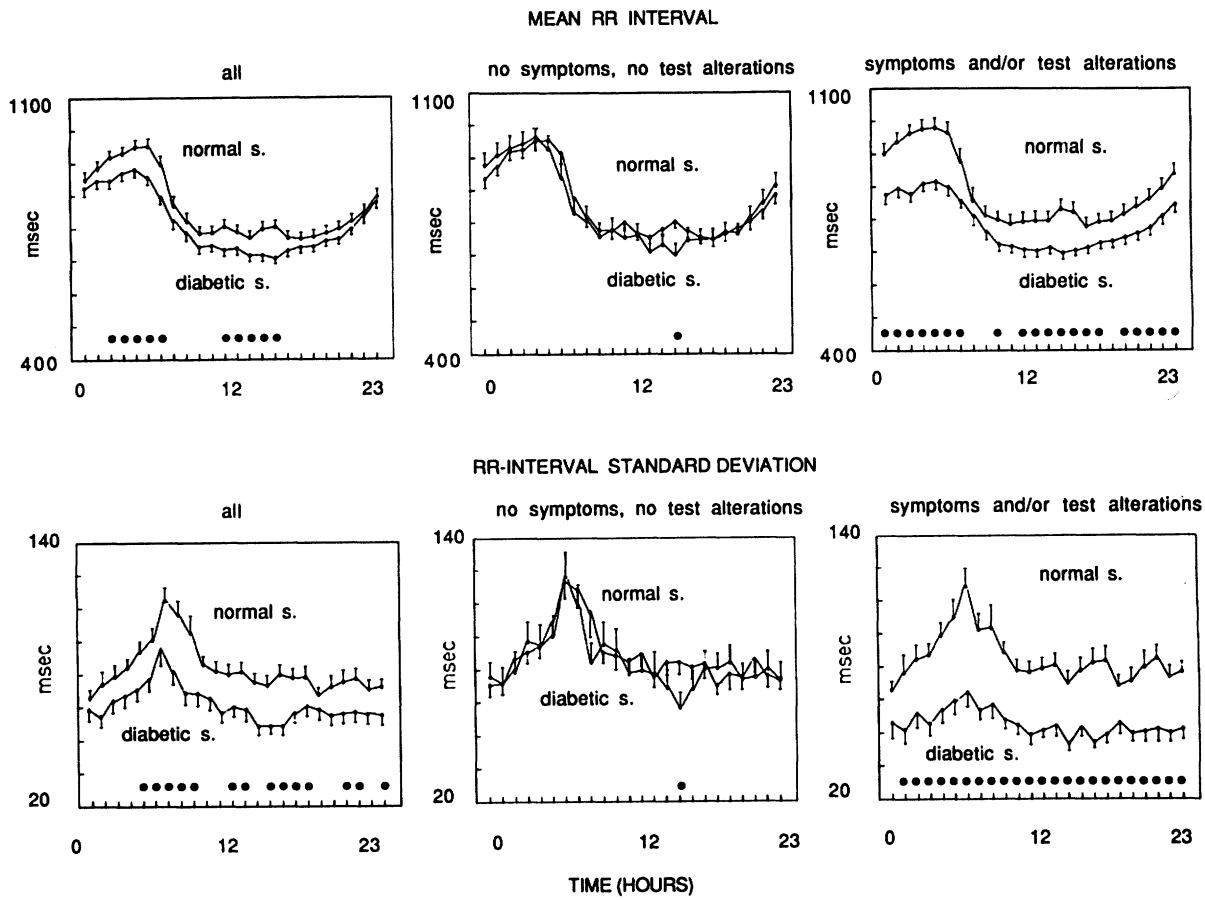

FIGURE 1. *Plots show hourly trends of mean RR interval* (top panels) *and RR interval standard deviation* (bottom panels) *in all control subjects (s) compared with all diabetic subjects, in diabetic subjects without symptoms and/or test alterations compared with age-matched control subjects, and in diabetic subjects with symptoms and/or test alterations compared with age-matched control subjects. Filled circles: significant differences* ($p<0.05$, *unpaired* t *test) between control and diabetic subjects.*

relative proportion of LF was higher than that of control subjects during nighttime ($p<0.01$), whereas the relative proportion of HF was decreased compared with normal subjects ($p<0.05$). Figure 2 indicates that there were significant differences between LF and HF during most of the 24 hours, including most of the night hours. As a consequence, the LF/HF ratio was increased during the night in these subjects compared with control subjects ($p<0.01$). Although the LF/HF ratio showed a day–night change ($p<0.01$), the day–night difference was lower than that observed in the age-matched control subjects because of greater ($p<0.01$, see Table 3) LF/HF ratio during night hours. Six of these 23 diabetic patients had LF/HF ratio during the night >2 SD from the mean of age-matched control subjects. Therefore, diabetic subjects without autonomic neuropathy showed a general trend similar to that of normals; however, the HF was slightly reduced, and the day–night difference appeared to be attenuated, indicating a lower relative parasympathetic tone during the night compared with control subjects. Figure 4 shows an example of the power spectra obtained during the 24-hour period in one diabetic subject of this group.

Also in these subjects the HF and particularly the LF was slower during the night than during the day ($p<0.001$). During night, the LF was significantly slower than in age-matched control subjects ($p<0.01$, see Figure 3 and Table 3).

Diabetic Subjects With Altered Test and/or Symptoms of Autonomic Neuropathy

In the 31 diabetic subjects with altered tests and/or symptoms of autonomic neuropathy, the mean RR interval was higher during the night than during the day ($p<0.001$). The heart rate variability showed a small but significant ($p<0.05$) night–day change, with higher values during night than during day. All these values were markedly ($p<0.001$) lower than those found in the age-matched subset of normal subjects. Both LF and HF showed small but significant ($p<0.01$) changes between night and daytime. All these values were significantly ($p<0.02$ or $p<0.001$) lower than those found in age-matched control subjects. When expressed in relative terms, a great reduction in HF during nighttime was observed compared with the age-matched control subjects ($p<0.001$). Figure 2 indicates that there were significant differences between LF and HF during all the 24 hours. As a consequence, the LF/HF ratio was markedly higher during nighttime in these subjects compared with normal subjects ($p<0.001$) and its value was not different from that observed during daytime. Sixteen of these 31 diabetic subjects (patients

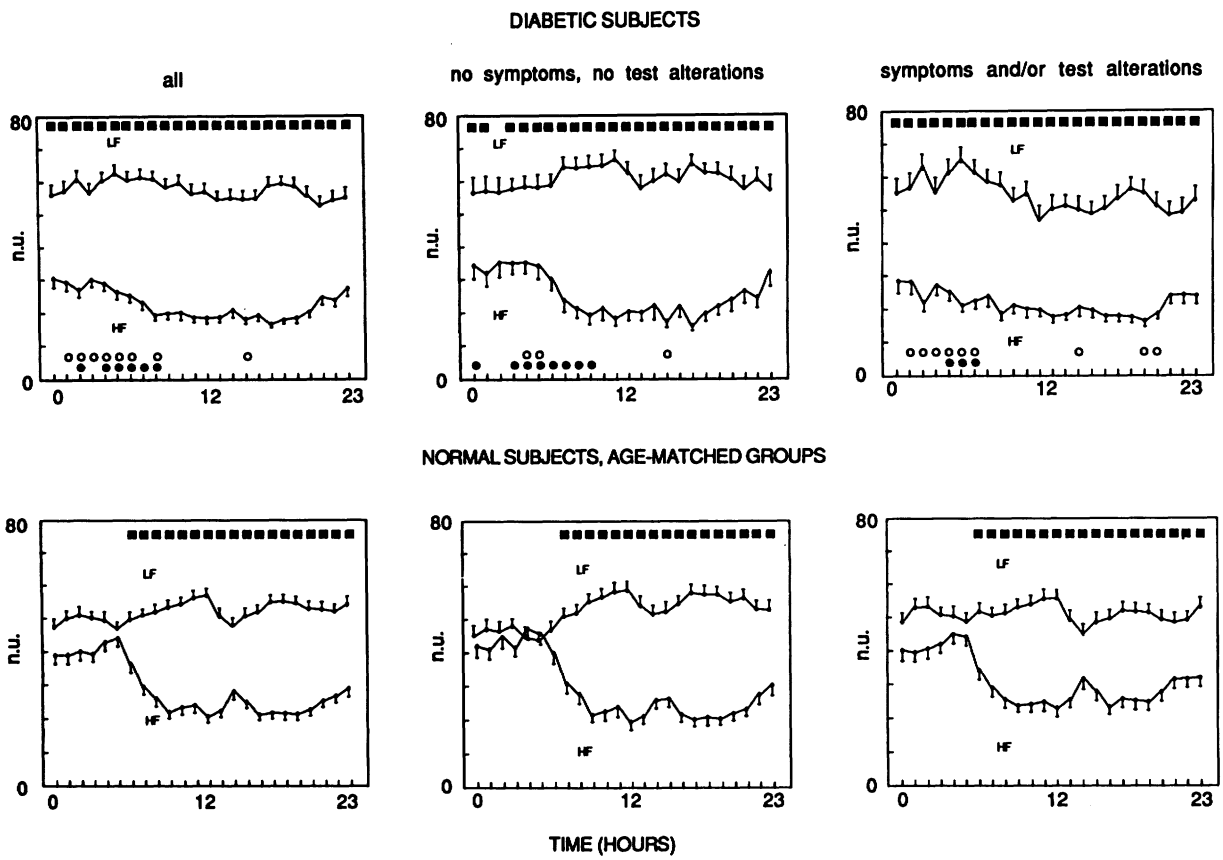

FIGURE 2. *Plots show hourly trends of relative prevalence (normalized units, n.u.) in spectral components (LF, low frequencies; HF, high frequencies) in diabetic* (top panels) *and control subjects* (bottom panels). *Filled circles: Significant differences* ($p<0.05$, *unpaired* t *test) in LF between control and diabetic subjects. Open circles: Significant differences* ($p<0.05$, *unpaired* t *test) in HF between control and diabetic subjects. Filled squares: Significant differences* ($p<0.05$, *unpaired* t *test) in LF vs. HF within each subset of subjects.*

2, 5, 6, 9, 10, 14, 16, 17, 20, 21, 22, 24, 26, 27, 29, and 31 of Table 1) had LF/HF ratio during the night higher than 2 SD from the mean of age-matched control subjects. Therefore, diabetic subjects with autonomic abnormalities showed a loss of the day–night changes in RR interval oscillations mainly because of a loss in HF during nighttime, indicating that these subjects have lost to a great extent their circadian variation in sympathovagal balance. Figure 4 shows an example of the power spectra obtained during the 24-hour period in one diabetic subject of this group.

Also in these subjects, the HF and particularly the LF was slower during night than during day ($p<0.01$). During both night and day, the LF was slower than in age-matched control subjects ($p<0.01$, see Figure 3 and Table 4).

Discussion

The importance of diabetes for the risk of cardiovascular burden has been repeatedly confirmed in both epidemiological[13–15,39] and clinical[12,16] studies. Although the unique contribution of diabetes to atherogenesis through its effects on blood clotting[40] is well documented, the cardiovascular risk is not entirely explicable in terms of the major cardiovascular risk factors,[14,39,41] suggesting that other abnormalities may contribute to the development of acute cardiovascular accidents.

No data on the autonomic nervous system function are available from these large epidemiological studies; however, several clinical observations suggest that diabetics with signs and/or symptoms of autonomic dysfunction have a high incidence of sudden death. Page and Watkins[12] reported 12 cardiorespiratory arrests in eight young diabetic patients with severe autonomic neuropathy. Subsequently, a number of clinical reports confirmed the association between autonomic neuropathy and unexplained cardiac arrest in diabetic subjects.[27–30] Ewing et al[42] reported a higher incidence of unexplained death in diabetic subjects with autonomic dysfunction in a 2.5-year follow-up in the Edinburgh population.

The diagnosis of autonomic dysfunction is currently made on the grounds of a simple and standardized battery of cardiovascular reflex tests[32] performed in a laboratory environment and requiring the collaboration of the patient; however, doubts have recently been forwarded[43] that this simple approach can help in identifying the subjects at risk for cardiovascular accidents. Outside the area of research in diabetes, it is now widely demonstrated that most cardiovascular accidents[4–11] have a circadian rhythm similar to that of the autonomic nervous system.[1] Specifically, the sympathetic tone is predominant in the general population during the daytime, when the frequency of cardiovascu-

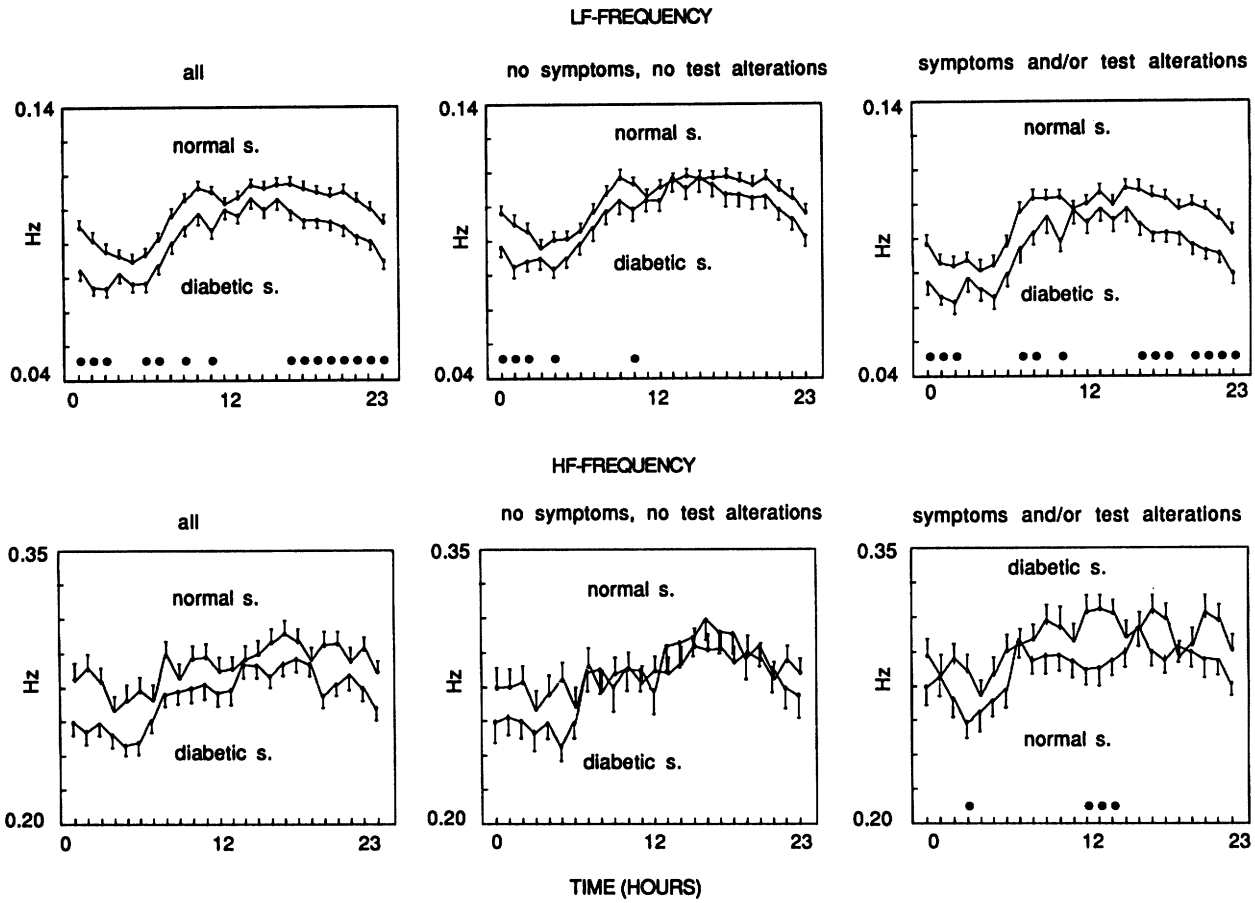

FIGURE 3. *Plots show hourly trends of frequency of RR interval fluctuations (LF, low frequencies;* top panels; *HF, high frequencies,* bottom panels*) in diabetic and control subjects (s). Filled circles: Significant differences* (p<*0.05, unpaired* t *test) between control and diabetic subjects.*

lar accidents is maximal,[2,3] and the treatment with propranolol reduced the mortality after myocardial infarction[44] during the morning surge of sympathetic activity.[45] Nevertheless, it has been found that the circadian distribution of onset of symptoms of acute myocardial infarction is different in a diabetic population of 767 subjects compared with nondiabetic subjects[18]: the morning peak is lower, there is a second peak

TABLE 2. Mean Results for All Subjects

	Diabetic subjects (n=54)			Control subjects (n=54)		
Time of day (hour)	23–7	8–22	24	23–7	8–22	24
RR interval						
Mean (msec)	861±17*	710±13*¶	767±14*	915±15	749±14¶	812±13
Standard deviation (msec)	73.4±4.5†	64.9±3.6†¶	68.3±3.9†	87.9±3.2	79.7±3.1¶	82.8±2.8
Low-frequency component						
Absolute power (ln-msec^2)	6.67±0.12*	6.16±0.11‡¶	6.33±0.11‡	7.02±0.09	6.75±0.09‖	6.85±0.08
Relative power (normalized units)	58.7±2.4*	56.3±2.1	57.2±2.1	50.7±1.7	53.7±1.7	52.5±1.4
Frequency (Hz)	0.080±0.002†	0.098±0.002†¶	0.091±0.002‡	0.090±0.002	0.107±0.001¶	0.101±0.001
High-frequency component						
Absolute power (ln-msec^2)	5.67±0.17‡	4.96±0.14‡¶	5.23±0.15‡	6.66±0.11	5.82±0.11¶	6.13±0.10
Relative power (normalized units	28.6±1.8‡	20.7±1.2¶	23.7±1.3*	37.8±1.6	23.6±1.3¶	28.9±1.2
Frequency (Hz)	0.276±0.004	0.300±0.004¶	0.291±0.003	0.278±0.005	0.294±0.004§	0.288±0.004
Low/high frequency	3.77±0.40‡	4.00±0.34	3.92±0.31†	2.09±0.17	3.37±0.26¶	2.88±0.20

Values are mean±SEM.
*p<0.05, †p<0.01, ‡p<0.001, diabetic subjects vs. age-matched control subjects, unpaired t test.
§p<0.05, ‖p<0.01, ¶p<0.001, day vs. night (23–7 vs. 8–22), paired t test.

TABLE 3. Mean Results for Diabetic Subjects With Normal Autonomic Function Versus Age-Matched Control Subjects

	Diabetic subjects (n=23)			Control subjects (n=36)		
Time of day (hour)	23–7	8–22	24	23–7	8–22	24
RR interval						
Mean (msec)	915±29	725±24¶	797±25	907±19	734±14¶	799±14
Standard deviation (msec)	91.9±6.8	80.1±6.1‖	84.6±6.1	90.9±4.3	82.8±4.3§	85.8±3.9
Low-frequency component						
Absolute power (ln-msec^2)	7.11±0.17	6.69±0.16‖	6.85±0.16	7.11±0.12	6.91±0.12§	6.99±0.11
Relative power (normalized units)	58.6±3.0†	62.2±2.5	60.8±2.5*	48.1±2.1	56.2±2.1¶	53.2±1.8
Frequency (Hz)	0.086±0.002†	0.106±0.003¶	0.098±0.003	0.095±0.001	0.111±0.002¶	0.105±0.001
High-frequency component						
Absolute power (ln-msec^2)	6.42±0.25	5.51±0.24¶	5.85±0.24	6.87±0.14	5.90±0.14¶	6.27±0.12
Relative power (normalized units)	33.1±3.0*	21.7±2.0¶	26.0±2.3	40.5±2.1	23.0±1.5¶	29.6±1.4
Frequency (Hz)	0.260±0.006	0.292±0.007¶	0.280±0.006	0.276±0.006	0.288±0.005	0.283±0.005
Low/high frequency	3.07±0.42†	4.51±0.64‖	3.97±0.52*	1.86±0.20	3.56±0.31¶	2.92±0.23

Values are mean±SEM.
*p<0.05, †p<0.01, ‡p<0.001, diabetic subjects vs. age-matched control subjects, unpaired t test.
§p<0.05, ‖p<0.01, ¶p<0.001, day vs. night (23–7 vs. 8–22), paired t test.

in evening hours, and during the night hours, the percentage of myocardial infarctions is higher. A circadian abnormality of the sympathovagal balance could thus be linked to the onset of cardiovascular accidents in diabetes, but to date no extensive studies exist on this matter.

The spectral analysis of heart rate variability[20–22] is a new, noninvasive tool to quantify the relative amount of sympathetic and vagal activity to the heart. Even when applied to ambulatory subjects, this method could successfully evaluate the circadian rhythm of the sympathovagal tone. The reproducibility of the method, as assessed in the present study for the 24-hour period and in previous studies for shorter time sequences,[23,25] is in the order of 10% for most of the variables considered (Table 5). The results obtained in normal subjects[1] confirm that the sympathetic tone prevails during the day, particularly during the first hours after awakening, whereas during the night the vagal tone becomes greater if not predominant.

In the present study, we have analyzed the circadian pattern of the sympathovagal balance in diabetic subjects either with normal autonomic function or with minimal to severe autonomic abnormalities and in a group of age-matched control subjects. The main finding obtained is a marked loss of the HF oscillations during the night and the first hours after awakening. Although to a lower extent, this was evident also in the group of diabetic subjects without even minimal autonomic involvement as assessed by standard cardiovascular tests. Therefore, the diabetic subjects examined have a loss of the parasympathetic tone during nighttime and hence a marked prevalence in sympathetic tone both during day and night without day–night changes in

TABLE 4. Mean Results for Diabetic Subjects With Autonomic Test Abnormalities and/or Symptoms of Autonomic Neuropathy Versus Age-Matched Control Subjects

	Diabetic subjects (n=31)			Control subjects (n=37)		
Time of day (hour)	23–7	8–22	24	23–7	8–22	24
RR interval						
Mean (msec)	821±17‡	699±15†¶	745±15†	942±21	770±20¶	834±20
Standard deviation (msec)	59.7±4.7‡	53.7±3.3‡§	56.1±3.8‡	88.0±4.1	78.8±4.0‖	82.2±3.7
Low-frequency component						
Absolute power (ln-msec^2)	6.32±0.15†	5.76±0.12‡‖	5.95±0.12‡	6.93±0.11	6.62±0.12‖	6.73±0.11
Relative power (normalized units)	58.8±3.5*	51.9±3.1‖	54.6±3.1	51.3±1.8	50.9±2.2	51.0±1.7
Frequency (Hz)	0.074±0.002†	0.092±0.003†¶	0.085±0.002†	0.085±0.002	0.104±0.002¶	0.097±0.002
High-frequency component						
Absolute power (ln-msec^2)	5.11±0.18‡	4.56±0.14‡‖	4.77±0.15‡	6.52±0.14	5.78±0.14¶	6.06±0.12
Relative power (normalized units)	25.2±2.1‡	20.0±1.5‖	21.9±1.5†	36.9±1.8	25.0±1.9¶	29.5±1.7
Frequency (Hz)	0.287±0.005	0.306±0.004‖	0.299±0.003	0.275±0.005	0.294±0.005‖	0.287±0.004
Low/high frequency	4.29±0.61‡	3.62±0.36	3.89±0.40†	2.07±0.19	3.05±0.30¶	2.67±0.22

Values are mean±SEM.
*p<0.05, †p<0.01, ‡p<0.001, diabetic subjects vs. age-matched control subjects, unpaired t test.
§p<0.05, ‖p<0.01, ¶p<0.001, day vs. night (23–7 vs. 8–22), paired t test.

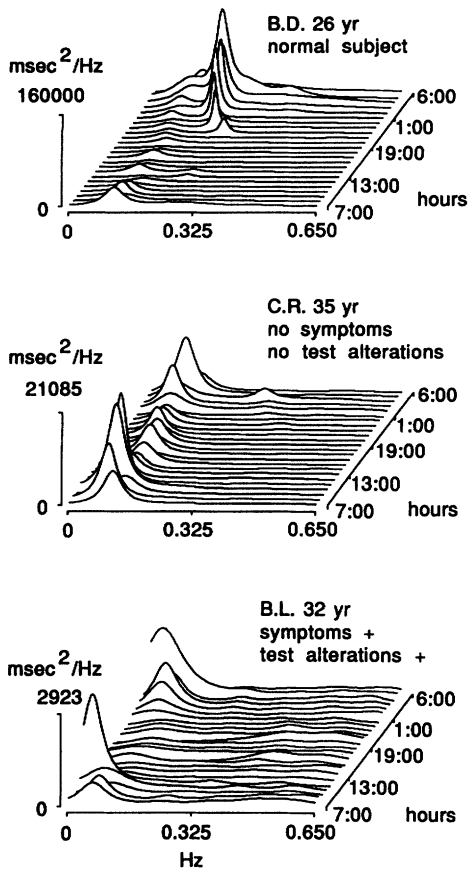

FIGURE 4. *Graphs of examples of 24-hour power spectral analysis obtained in a normal subject* (top panel), *in a diabetic subject without symptoms or alteration in autonomic function tests* (middle panel), *and in a diabetic subject with symptoms of autonomic dysfunction and alterations in autonomic function tests (*bottom panel, *subject 5 in Table 1).*

sympathovagal balance. If we assume that the parasympathetic tone may to some extent protect the cardiovascular system, as it can be suggested by the observation of the relatively low rate of cardiovascular accidents during the hours at which the parasympathetic tone is maximal, then it can be suggested that these patients are exposed for a longer period to the potentially dangerous effect of a sympathetic predominance. These findings agree with epidemiological data in diabetes showing an altered temporal onset of symptoms of myocardial infarction[18] characterized by an increase of episodes during night hours. In 22 of 54 of the diabetic subjects of the present study (six from the D− group and 16 from the D+ group), the LF/HF ratio was significantly increased during night hours compared with their age-matched control subjects.

Other factors, however, such as the increased baseline level of platelet aggregability documented in diabetic subjects, particularly those with neuropathy,[46,47] may contribute to reduce the morning peak (and to increase the risk during the night) of cardiovascular complications, because little change has been observed with assumption of upright posture in diabetics.[48] A relation between platelet aggregability and the sympathetic system,[49,50] as suggested by the assumption of upright posture, is still a matter of investigation. In the present study, no direct comparison could be made with blood pressure profile; however, our results seem to agree with recent preliminary reports showing that in diabetic subjects, particularly in those with autonomic neuropathy, the blood pressure is not decreased during night hours as occurs in normal subjects, the circadian rhythm of blood pressure is disrupted, and this is frequently associated with poor prognosis.[51,52] The loss of parasympathetic tone during night hours, together with a reduced heart rate variability, suggests a lower efficiency of the baroreceptor control of blood pressure, which is maximal during sleep[53] and hence during most of the night hours. Therefore, the information obtained so far from this and other studies suggests the hypothesis that a complex dysfunction of the autonomic nervous system, including the integrated control of heart rate and blood pressure possibly via the baroreceptor activity, may lead to an abnormal response to various trigger stimuli.

In the present study, we have found a reduction, particularly during nighttime, in the period of the LF oscillations in diabetic subjects. If these oscillations result from the transmission delay through the baroreceptor loop cyclically activated by respiratory changes,[54] this slowing could be the result of delayed neural transmission secondary to autonomic damage. Nevertheless, a slowing of this oscillation appears also in normal subjects during nighttime, when the barorecep-

TABLE 5. Reproducibility of 24-Hour Power Spectrum Data: Percent Change From Day A to Day B

	Control subjects (n=5)			Diabetic subjects (n=5)		
Time of day (hour)	23–7	8–22	24	23–7	8–22	24
Mean RR	7.5±3.0	7.0±1.4	5.6±1.9	2.1±0.8	2.9±0.4	2.5±0.6
RR (SD)	10.5±4.4	11.4±5.7	7.3±2.2	17.1±8.6	6.6±2.2	6.6±2.8
LF absolute power	0.6±0.3	1.9±1.6	1.7±0.8	4.1±2.2	1.9±0.8	2.3±1.6
LF relative power	10.1±2.2	0.7±0.2	3.4±1.0	9.4±3.8	4.7±3.5	4.3±2.0
LF frequency	5.4±1.9	7.5±1.0	4.1±1.3	10.6±5.2	6.7±4.2	6.0±3.7
HF absolute power	2.2±1.2	5.8±2.0	1.7±0.2	3.9±0.8	2.6±0.5	1.5±0.6
HF relative power	17.6±6.8	15.7±6.0	14.7±6.1	26.1±8.5	21.4±17.2	26.6±13.1
HF frequency	8.1±2.8	9.8±3.9	5.5±1.8	4.5±2.7	11.1±5.4	4.8±2.3
LF/HF ratio	13.6±7.2	22.4±3.4	9.4±0.9	25.1±13.5	8.8±3.9	15.0±7.5

Data are mean±SEM. Reproducibility data are calculated according to the formula 100 · |day A−day B|/day A. RR, RR interval; RR (SD), standard deviation of RR intervals; LF, low-frequency components; HF, high-frequency components.

tor activity is maximal. At present, this finding remains unexplained and deserves further investigation.

Conclusions

The data reported in the present study indicate that in diabetic subjects, there is a loss of day–night modulation of the autonomic tone. Not only is the sympathetic predominance observed during day hours more evident than in normal subjects, but it is also prolonged to most of the night instead of showing a relative increase in parasympathetic tone as occurs in normal subjects. These findings are in agreement with epidemiological data indicating an increased number of episodes during night hours in diabetic subjects compared with nondiabetic subjects.[18] If the predominance of sympathetic tone is facilitating the onset of cardiovascular accidents, then the present results might explain the increased susceptibility to cardiovascular accidents of these patients and might ultimately suggest a new, noninvasive tool to identify the subjects potentially at risk in clinical and epidemiological studies.

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