Title

Blood pressure regulation in diabetics with and without peripheral neuropathy

Authors

Siqi Wang¹, David C. Randall¹², Charles F. Knapp¹, Abhijit R. Patwardhan¹, Kevin R. Nelson³, Dennis G. Karounos⁴, Joyce M. Evans¹

Affiliation

¹Center for Biomedical Engineering, Wenner-Gren Research Laboratory, University of Kentucky, Lexington; ²Department of Physiology, University of Kentucky College of Medicine, Lexington; ³Department of Neurology, University of Kentucky College of Medicine, Lexington; ⁴Endocrinology Section, Veteran’s Administration Hospital, Lexington, KY and Department of Medicine, University of Kentucky College of Medicine, Lexington, KY

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Contact information

J. M. Evans, Center for Biomedical Engineering, Wenner-Gren Research Laboratory, Univ. of Kentucky, Lexington, KY 40506-0070 (email: jevans1@uky.edu)

Tel: +859-257-2685; fax: +859-257-1856
ABSTRACT

Cardiac and vascular dysfunctions resulting from autonomic neuropathy (AN) are complications of diabetes, often undiagnosed. Our objectives were to: 1) determine sympathetic and parasympathetic components of compromised blood pressure regulation in patients with peripheral neuropathy, and 2) rank noninvasive indexes for their sensitivity in diagnosing AN.

We continuously measured ECG, arterial blood pressure (BP) and respiration during supine rest and 70º head-up tilt in 12 able-bodied subjects (AB), 7 diabetics without, 7 diabetics with possible, and 8 diabetics with definite, sensory and/or motor neuropathy (D2). During the first 3 min of tilt, systolic BP (SBP) of D2 decreased (-10.9 ± 4.5 (SEM) mmHg), but increased in AB (+4.8 ± 5.4 mmHg). Compared to AB, D2 had smaller low frequency (0.04-0.15 Hz) spectral power of diastolic BP, lower baroreflex effectiveness index (BEI) and more SBP ramps. Except for low frequency power of SBP, D2 had greater SBP and smaller RR Interval harmonic and nonharmonic components at rest across the 0.003 - 0.45 Hz region. In addition, our results support previous findings of smaller HF RR interval power, smaller numbers of baroreflex sequences and lower baroreflex sensitivity in D2. We conclude that diabetic peripheral neuropathy is accompanied by diminished parasympathetic and sympathetic control of heart rate and peripheral vasomotion and diminished baroreflex regulation. A novel finding of this study lies in the sensitivity of BEI to detect autonomic neuropathy, presumably due to its combination of parameters that measure reductions in both sympathetic control of vasomotion and parasympathetic control of heart rate.

KEYWORDS

autonomic neuropathy, spectral power, baroreflex sensitivity, baroreflex effectiveness index, power spectral density
INTRODUCTION

Diabetic neuropathy is a common and serious complication of both type 1 and type 2 diabetes. In particular, autonomic neuropathy (AN), a common complication of diabetes, is associated with high mortality and morbidity including sudden cardiac death, ventricular arrhythmias, and orthostatic hypotension (53). Therefore, early detection of autonomic neuropathy leading to early medical intervention is of great clinical interest.

Current clinical methods used to diagnose diabetic autonomic neuropathy rely on a battery of reflex tests called "Ewing tests" (23). Although useful in diagnosing autonomic neuropathy, these tests require voluntary participation by patients and test sensitivity is limited. Therefore, other techniques are being examined for their potential to provide in depth, easy to administer, diagnostic tools. Comparisons of heart rate variability (HRV), low (LF) and high (HF) frequency components of HR oscillations have been correlated with abnormal results of Ewing tests (18, 27, 31, 60). Diabetic patients with abnormal Ewing results also demonstrated reduced LF spectral power of systolic BP (SBP) compared to able bodied controls or compared to diabetics with normal results during one hour supine or a short period of standing (18, 60). In diabetics with no signs of AN, the numbers of spontaneous baroreflex sequences were reduced and slopes of baroreflex sequences were smaller compared to controls (18, 26). The composite interpretation of these results points toward diabetic neuropathy-induced failure of both sympathetic and parasympathetic control of heart rate, baroreflex function and vasomotion.

Studies of sino-aortic denervated (SAD) animals (cats and dogs), a form of ‘dysautonomia’, showed that not only were harmonic components (LF or HF peaks) of HR and BP oscillations affected by SAD, but nonharmonic components were also altered over a wide frequency range (very low, low and high frequencies) (14, 56). In addition to the alteration in HR and BP variability over a wide frequency range, the baroreflex effectiveness index (BEI), which
represents the percentage of SBP ramps followed by reflex RR interval responses to all SBP ramps, was reduced by 89% in SAD cats (17). In humans with pure autonomic failure, HR and BP variability have been shown to be altered at all frequencies (45). Finally, we reported previously that spinal cord injury resulted in reduced baroreflex effectiveness index in tetraplegic patients compared to able bodied controls (1). Results of these previous human and animal studies indicated similarities between diabetic autonomic neuropathy, sinoaortic denervation and primary autonomic failure that appeared to be proportional to the level of neuropathy and observable in noninvasive indexes of autonomic function.

In the present study, we compare sympathetic, parasympathetic and baroreflex function of able-bodied individuals to that of individuals with diabetes with (a) absent, (b) possible or (c) definite peripheral sensory and/or motor neuropathy. We hypothesized that the presence of definite peripheral sensory and/or motor neuropathy would be associated with the greatest deficits in both cardiac and peripheral autonomic regulation and that broadband power of HR variability, BP variability, and baroreflex function, would be sensitive measures for early diagnosis of diabetic AN. Our tools included 1) spectral analysis techniques applied to heart rate and blood pressure over a wide range of frequencies and 2) spontaneous baroreflex sequence technique to determine numbers of blood pressure ramps, numbers of baroreflex sequences, baroreflex effectiveness and baroreflex sensitivity.

METHODS

Subjects

Twelve healthy able-bodied volunteers (AB) were recruited from the local community using word of mouth, newspaper advertisements and flyers mounted on boards across the university campus and hospital. Twenty-two diabetic subjects were recruited. The diabetic subjects were evaluated for distal symmetrical polyneuropathy using neuropathic symptoms, depressed ankle
reflexes, distal sensory loss, distal muscle weakness or atrophy, and nerve conduction studies (8). Subjects were then distinguished by consensus criteria that determined the likelihood of neuropathy using an ordinal scale from highest 4 to lowest 1 (20). ‘Possible neuropathy’ was defined by a likelihood ranking of two or three, and ‘definite neuropathy’ by the likelihood of 4. Diabetic subjects without neuropathy had no signs or symptoms of neuropathy and normal nerve conduction studies. Based upon the results of these evaluations the neuropathy of diabetic subjects was categorized as absent (D0), possible (D1) or definite (D2). In addition to the tests for peripheral neuropathy, and for fasting blood glucose levels on the day of study, all subjects filled out a comprehensive questionnaire to assist with classification of any potential neuropathy, and underwent a familiarization tilt and 12 lead ECG. Medications affecting autonomic function were discontinued for the day of study. Demographic characteristics of the participants is shown in Table 1.

Tilt Protocol

We utilized passive head-up tilt to challenge the autonomic regulation of arterial blood pressure. Before entering the study all subjects gave written informed consent as approved by the University of Kentucky Institutional Review Board. Prior to head-up tilt testing, subjects’ height, age, weight and diabetic status were recorded. Later, after blood glucose level had been determined, an IV cannula was placed in an antecubital vein. Subjects then rested in the supine position for 30 min when instrumentation was applied followed by 10 minutes of supine control and 30 minutes of 70-degree head-up tilt. If presyncopal symptoms developed during tilt, subjects were brought back to supine immediately. All subjects were followed for a supine recovery period of five minutes.

Measured Variables
Mixed venous blood samples were drawn from the antecubital vein catheter at the end of control, at three and seven min of tilt, and during the second minute of recovery. Results of blood assays are not reported here.

Three lead ECG (Pilot Colin), noninvasive continuous arterial BP (Portapres model-2), respiratory activity (Respirtrace) and tilt angle (Crossbow CXL 04LP3) were acquired continuously as described in detail elsewhere (1); recordings commenced during supine control, and continued uninterrupted during the 70 degree head-up tilt and supine recovery. The blood pressure sensor was maintained at heart level. Manual blood pressure using an arm cuff was acquired at the end of supine control, the beginning of tilt and at the end of recovery.

Data Acquisition and Analysis

BP, ECG, respiratory activity and tilt angle recordings were digitized at 1000 Hz by using a commercial data acquisition system (DATAQ).

Mean values. Heart rate and RR interval were computed by identifying R waves in the last 5 min of control, 0-3 min, 3-7 min, and 7-12 min of tilt and the first 3 min of recovery. Data were analyzed using custom written programs in C++ and MATLAB (the MathWorks, Natick, MA). Artifacts in HR and BP signals, including premature beats and Portapres servo adjustments (approximately 5% of the beats per time series), were manually removed. The cleaned signals were then aligned in time. Systolic (SBP) and diastolic (DBP) blood pressures were determined by computing the maximum and minimum values of clean arterial BP for each heart beat. Mean values of RR interval, SBP and DBP were computed in each time segment. All subsequent data analyses were performed in MATLAB.

Spectral analysis. RR interval, SBP, DBP and respiratory activity in control, tilt and recovery periods were resampled at 4 Hz using a cubic spline. Each segment was then linearly detrended. Power spectral densities of RR intervals, SBP, DBP and respiratory activity were
estimated using Welch’s method of averaged periodograms (480-point Hamming windows with 440-point overlap). Spectral powers in the LF (0.04-0.15 Hz) and HF (0.15-0.4 Hz) regions were obtained using trapezoidal integration over the specified frequency range. Spectral power of DBP was used as an index of sympathetic control of vasomotion because DBP correlated better than did SBP with vascular resistance (4). Power spectral density of SBP, however, was used as an index of baroreflex-mediated buffering of blood pressure. Power spectral densities were plotted in a log-power vs. log-frequency scale, and the slope of the linear regression of this plot within the LF range was calculated. This power law scaling relationship was quantified for RR interval, SBP and DBP to determine their harmonic and nonharmonic characteristics (19, 29).

**Baroreflex sequences.** We adopted the sequence method (3) to quantify the number of blood pressure ramps and baroreflex sequences, as well as baroreflex sensitivity. We identified sequences of three or more consecutive heartbeats, in which progressively increasing (or decreasing) SBP with at least 1 mmHg beat-to-beat change were followed within 1 heart beat by progressively lengthening (or shortening) of RR interval with at least 4 ms beat-to-beat change. A sequence was accepted as a baroreflex sequence if the correlation coefficient of the regression line between SBP and RR interval within the sequence was 0.85 or greater (3). The slope of the regression line for each sequence was taken as spontaneous baroreflex sensitivity (BRS). The ratio between the number of baroreflex sequences and the total number of increasing or decreasing SBP ramps determined the baroreflex effectiveness index (BEI) (17). Because the numbers of SBP ramps and baroreflex sequences depend on the number of analyzed heartbeats, which varied among and within subjects, the numbers of SBP ramps and baroreflex sequences were normalized by the number of analyzed heartbeats of each subject in each time segment.

**Arterial pulse transit time.** An index of arterial compliance, arterial pulse transit time (PTT), was measured by adapting the technique of Foo et al. (24). Arterial PTT was recorded as the
time interval between the R peak in ECG and the SBP peak in the arterial pressure measured at the finger. Average values from ten beats selected from clean data were taken in the last minute of supine control and the second minute of 70-degree head-up tilt.

**Statistical analysis.** We used SAS (The SAS Institute, Cary, NC) software to test a linear mixed model for differences within and among four groups (able-bodied, diabetic without neuropathy, diabetic with possible neuropathy, diabetic with definite neuropathy) during supine control, at three time segments during tilt, and in recovery. The group factor was used to test differences among the four groups. The time factor was used to test the tilt effect. The group × time factor was used to test for differences in tilt effects across groups. The changes from control to the first three minutes of tilt were tested with one-way ANOVA and two-tail t tests. For a priori hypothesized differences between variables, for which we predicted a specific difference (greater or less) before we analyzed the data, changes from control were analyzed using one-tail t tests. Data were transformed by using logarithm or square root if the residual plots showed heteroscedasticity. Outliers were identified if residuals were larger than two standard deviations and thus were not included in the statistical testing. Differences were considered significant if p ≤ 0.05. Results are presented as mean ± SEM.

**RESULTS**

Upon testing, one able-bodied subject was diagnosed with nondiabetic sensory and motor neuropathy; his data were not included in any group. Five able-bodied subjects, and three diabetics with possible neuropathy demonstrated symptoms of presyncope before finishing 30 min of head-up tilt and were returned to supine immediately. Typical RR interval, arterial BP and tilt angle of an asymptomatic able-bodied subject (left) and a diabetic patient with definite neuropathy (right) during supine and during 30 min head-up tilt are shown in Figure 1. Note the decrease of RR interval and the modest increase in BP during head-up tilt in the nonsyncopal,
able bodied subject and the decrease in BP and smaller change in RRI in the diabetic patient with definite neuropathy.

Mean Values

Figure 2 shows group averaged (± SEM) RR interval and SBP for able-bodied (AB; blue line) subjects, diabetics without neuropathy (D0; pink line), diabetics with possible neuropathy (D1; red line) and diabetics with definite neuropathy (D2; dark red line). Data are given for supine control, three stages of tilt and supine recovery. All groups decreased RR interval (Figure 2A) in response to head-up tilt. The magnitudes of SBP responses to the first three minutes of head-up tilt (Figure 2B2) were significantly different between able-bodied subjects and diabetics with definite neuropathy (one-tailed t-test); for able-bodied, SBP increased from 124 ± 5.8 mmHg in supine control to 132 ± 7.9 mmHg; for diabetics with definite neuropathy, SBP decreased from 128.7 ± 7.8 mmHg in supine control to 119.2 ± 10.7 mmHg. Supine control values of RR interval and SBP were not different between the groups.

Spectral Power

Figure 3 gives average high frequency spectral power of RR interval (HFRRI, Panel A, left) and low frequency spectral power of diastolic blood pressure (LFDBP, Panel B1, upper right) for the same four groups. In Figure 3A, HFRRI decreased during head-up tilt for all groups. In addition, able-bodied subjects and diabetics without neuropathy had higher HFRRI than did diabetics with definite neuropathy (group main effect). Figure 3B1 shows that diabetics with possible neuropathy and those with definite neuropathy tended to have lower LFDBP than did able-bodied subjects and diabetics without neuropathy during head-up tilt (group × time, p=0.06). The change in LFDBP during the first 3 min of tilt, Fig 3B2, differed significantly (p<0.05) between able-bodied (2.1 ± 0.4 mmHg² supine, to 4.9 ± 1.6 mmHg²) and diabetics with definite neuropathy (2.1 ± 0.5 mmHg² supine, to 1.6 ± 0.7 mmHg²).
Figure 4 shows group averaged log of power spectral density of SBP (PSD SBP; Figure 4A and 4B, top) and log of RR interval (PSD RRI; Figure 4D and 4E bottom) versus log of frequency for able-bodied subjects (left panels) and diabetics with definite peripheral neuropathy (middle panels). The analyses are based upon recordings taken during five minutes of supine control. Data shown are means (solid lines) ± SEM (shaded area above and below the mean). Spectral power of SBP was significantly greater (four fold, which is not immediately evident on logarithmic scales) in diabetics with definite neuropathy than in able-bodied subjects (Figure 4B vs. 4A) across the very low frequency (0.003 - 0.04 Hz) and high frequency (0.15 - 0.4 Hz) regions. However, the SBP spectral power of diabetics with definite neuropathy dipped at frequencies around 0.1 Hz (Figure 4B). In addition, the slope of the linear portion of this SBP curve in the low frequency region tended to be steeper in diabetics with definite neuropathy (-2.64 ± 0.44) than in able-bodied subjects (-1.54 ± 0.37) (Figure 4C). SBP powers for diabetics without, and those with, possible neuropathy, were similar to that of able-bodied subjects (not shown). In contrast to SBP, the variability of RR interval for diabetics with definite neuropathy was significantly (four fold) lower than that of able-bodied subjects over the range of frequencies (0.003 - 0.4 Hz) (Figure 4D vs. 4E). Log-log plots of RRI for diabetics without neuropathy and diabetics with possible neuropathy were intermediate between able-bodied subjects and diabetics with definite neuropathy (not shown). Slopes of the log-log RR interval/frequency curve were significantly steeper in the low frequency region (0.04 - 0.15Hz) in diabetics with possible (-2.19 ± 0.35) and definite neuropathy (-2.77 ± 0.24) than slopes of able-bodied (-1.05 ± 0.35) and diabetics without neuropathy (-1.15 ± 0.4), Figure 4F. In contrast to systolic pressure, the log-log plot of the power spectral density of diastolic blood pressure was not different between able-bodied subjects and diabetics with definite neuropathy (not shown).

Baroreflex Sequences
Figure 5 provides the average (± SEM) normalized number of SBP ramps (5A), normalized number of baroreflex sequences (5B), baroreflex effectiveness index (BEI; 5C), baroreflex slope (5D) and high frequency spectral power of systolic blood pressure (HFSBP; 5E) for each of the four groups. In supine control, both diabetics with possible, and those with definite, neuropathy had greater numbers of SBP ramps than did able-bodied subjects (Figure 5A). During head-up tilt, diabetics with definite neuropathy also had more SBP ramps than did diabetics without neuropathy during all three time segments, most clearly illustrated at 7-12 min of tilt (Figure 5A). However, diabetics with definite neuropathy had a significantly smaller number of baroreflex sequences than did able-bodied subjects and diabetics without neuropathy (group main effect; Figure 5B). As a result, BEI values for diabetics with possible, and for those with definite, neuropathy were significantly lower than those for able-bodied subjects and diabetics without neuropathy during head-up tilt (Figure 5C). One able-bodied subject had BEI residuals at all time segments larger than 2 standard deviations. When this subject's data were excluded from the analysis, the group x time interaction became significant. Other factors remained significant with or without exclusion of this subject, with no effect on the direction of the results. In addition, baroreflex slope significantly decreased in all groups in response to head-up tilt (Figure 5D). Diabetics with possible neuropathy had significantly lower baroreflex slopes than able-bodied subjects, and diabetics with definite neuropathy had significantly lower slopes than both able-bodied subjects and diabetics without neuropathy (group main effect). Finally, the diminution of baroreflex activity to buffer breathing frequency blood pressure oscillations in diabetics with definite neuropathy is illustrated in Figure 5E indicating a four fold greater magnitude of HFSBP oscillations in those subjects compared to the other groups (similar to the results illustrated in Figure 4 A and B).

Gender
Because of known gender differences in autonomic regulation of heart rate (parasympathetic dominance in high frequency power of heart rate in women compared to men) and peripheral vasculature (sympathetic dominance in control of vascular regulation in men) (2, 22, 30, 40), we examined heart rate, blood pressure and baroreflex variables for gender differences. The results of these analyses indicated that, for these groups, gender effects were obscured by effects of diabetes (data not shown).

*Arterial Pulse Transit Time (PTT)*

Arterial PTT was not significantly different among the four groups in supine or during the second minute of tilt, nor were the tilt-induced increases of PTT significantly different among the four groups.

**DISCUSSION**

This study tested noninvasive indexes of sympathetic, parasympathetic and baroreflex control of circulatory function for their ability to discriminate between able-bodied subjects, diabetics without peripheral neuropathy, diabetics with possible and diabetics with definite, length-dependent peripheral neuropathy. Peripheral neuropathy was determined by standard clinical neurological tests. We now report five major new findings. First, VLF systolic BP power spectral density in the supine state was four times greater in diabetic patients with length-dependent peripheral neuropathy as compared with the controls. Second, we also report an increase in HF SBP spectral power in supine patients suffering from diabetic neuropathy; as will be discussed below, this contrasts with other published findings. Third, the baroreflex effectiveness index was lower in diabetic patients with definite neuropathy, but without chronic renal failure, as compared to the control group and as compared to diabetic patients without length-dependent sensory or motor neuropathy. In fact, our data indicate that BEI is a more sensitive index of diabetic dysautonomia than is LF spectral power of SBP. Fourth, we found
that the baroreflex played an important role in regulating BP over a wide frequency range between 0.003 to 0.45 Hz, and that, as a consequence, RR interval spectral power in diabetic patients with definite neuropathy was one-fourth that of able bodied controls across the frequency range. Finally, the slope (i.e., “beta”) of the log-log relationship between the power spectral density of RR Interval in the low frequencies for recordings made during supine rest was significantly more negative in both the D1 and D2 patients compared to controls and there was a dip in LF SBP spectral power in the log-log plot centered around ~0.1 Hz in the D2 group. The latter finding supports the conclusion that baroreflex control of sympathetically mediated vasomotion is impaired in diabetic patients with neuropathy. In sum, the blood pressure regulatory capability of diabetics with neuropathy was significantly diminished, due to diminution of sympathetic and parasympathetic control of heart rate and peripheral vasculature and an almost total loss of baroreflex regulation of heart rate. As we explain below, the unique contributions of the present study lie in documenting mechanisms associated with failure of autonomic control and in identifying easily obtained, noninvasive measures to quantify those failures. In addition, results of our study support the hypothesis that patients with diabetic neuropathy resemble patients with primary autonomic failure (45) and SAD animals (14, 56). These findings potentially provide quantitative assessments of a serious complication in arterial pressure regulation in diabetic patients secondary to diabetic dysautonomia that go beyond standard clinical neurological tests, and could ultimately be of clinical use.

Mean Values

The inability of diabetics to increase, or even maintain, blood pressure during tilt (Figure 2), indicates that sympathetic pathways to the cardiovascular effectors were impaired in diabetics with peripheral neuropathy compared to able-bodied subjects. Moreover, the importance of the baroreflex in regulating blood pressure over longer time periods is illustrated in the very low frequency region of Figure 4. These results also support the comment made by many of the
patients that they had learned to move slowly into the upright position and if they move slowly they are okay. The slow speed (approximately 38 seconds to move from supine to 70 degrees head up) of our tilt was probably responsible for the fact that presyncopal symptoms were relatively rare in our diabetic subjects.

**RR Interval spectral power Indexes of Sympathetic and Parasympathetic Control of Heart Rate**

The magnitude of the RR interval harmonic component at respiratory frequencies (HFRRRI) is a marker of vagal control of the SA-node (47). The rapid decline of HFRRRI in response to head-up tilt and rapid recovery of HFRRRI when AB subjects were returned to supine (within a minute of the change in posture, Figure 3A) supports neural, reflex-mediated modulation of HR at respiratory frequencies in these people. During supine rest and head-up tilt, diabetics with definite neuropathy had lower HFRRRI than did able-bodied subjects or diabetics without neuropathy, indicating impaired parasympathetic control of HR in diabetics with signs of peripheral neuropathy (52). The reduced reserve of parasympathetic control of HR in response to head-up tilt in diabetics with possible or definite neuropathy also confirmed a diminished orthostatic response and progressive loss of parasympathetic control of heart rate with increasing neuropathy (45).

A previous study of sino-aortic denervated (SAD) cats determined that the wide band spectra of pulse interval was significantly lower in SAD cats than that in controls (15). In our study, the decreases of wide band RR interval spectra in diabetics with definite neuropathy may be due to diminished baroreflex function, or perhaps more specifically, to cardiac parasympathetic neuropathy. Moreover, the LF RRI component was different in diabetics with definite neuropathy: a steeper slope of the 1/f relationship was evident at the LF range of diabetics with definite neuropathy. This 1/f relationship of power spectrum exists in dynamic systems that have multiple control mechanisms with different time constants and the steeper
slope of the 1/f relationship suggests a less complex control system (6, 7, 29, 56) in diabetics with definite neuropathy. If so, the complexity of the sympathetic regulation of heart rate in diabetics with definite neuropathy was diminished, possibly reflecting a diminution of sympathetic regulation of the SA node, perhaps secondary to diabetic sympathetic neuropathy. In patients with Chagas disease, which damages autonomic neurons, the slope of the linear part of the log-log plot of PSD RRI was significantly steeper than that of controls, indicating dysautonomia in these patients (12, 50). Therefore, the slope of log-log plot of heart rate power spectral density of resting diabetic subjects calculated from a five-minute measurement appears to be a sensitive discriminator of autonomic neuropathy in the LF region.

**Blood Pressure Spectral Power as an Index of Sympathetic Control of Peripheral Vasculature**

Significant coherence between low frequency spectral power of diastolic blood pressure (LFDBP) and muscle sympathetic nerve activity has been reported in able-bodied humans (11). In particular, muscle sympathetic nerve activity increased with head-up tilt and remained elevated during tilt in able-bodied subjects (28). In our study, LFDBP increased in response to tilt and remained high in able-bodied and in diabetic subjects without neuropathy (Figure 3B). Conversely, in diabetics with possible, and those with definite, neuropathy, LFDBP did not change indicating that reflex-mediated sympathetic pathways to peripheral vasculature were impaired in diabetics, including those with milder symptoms of neuropathy.

The log-log plot (Figure 4A, B) of the power spectral density of systolic blood pressure (PSD SBP) taken during five minutes of supine rest, showed a significant elevation of harmonic and nonharmonic components of blood pressure variability in the VLF and the HF regions but not in the LF range (presumably because of a loss of the harmonic component) in diabetics with definite neuropathy compared to able bodied subjects. These findings confirm the previously
reported reduction of sympathetically mediated harmonic vasomotion in diabetic autonomic neuropathy (18, 60).

*Baroreflex*

The greater numbers of blood pressure ramps at rest and during tilt in diabetics with definite and suspected autonomic neuropathy (Fig 5a), was an unexpected finding of the present study. The combination of increased numbers of BP ramps with reduced numbers of baroreflex sequences (26, 60), suggests that diabetics with neuropathy experience diminished effectiveness of the baroreflex in driving the sinus node. As is clearly illustrated in Figure 5C: BEI was significantly increased compared to supine control in able-bodied subjects during all phases of head-up tilt and in diabetics without neuropathy during 7-12 min of tilt, but remained unchanged in diabetics with possible, or definite, neuropathy. Further, Figure 5 clearly illustrates the ability of this measure to differentiate diabetics with neuropathy (definite or possible) from diabetics without neuropathy. Therefore, in our study, BEI was the most sensitive discriminator of neuropathy. Other studies have proposed LF power of SBP (18) as a sensitive discriminator of AN, but for our study, BEI was significantly better at discriminating differences, perhaps because this measure combines loss of sympathetic modulation of vasomotion with loss of parasympathetic control of heart rate into one parameter. Previous studies showed a severe impairment of baroreflex effectiveness in diabetic chronic renal failure patients compared with non-diabetic patients at supine rest (33). However, as far as we can determine, the present study is the first to demonstrate a reduction in baroreflex effectiveness in diabetics with neuropathy, without the simultaneous complication of chronic renal failure.

Baroreflex sensitivity has been proposed to reflect the strength of the baroreflex when it is effective (17). In our study, all groups demonstrated decreased baroreflex slope in response to head-up tilt (Figure 5D) as expected (34, 43, 44). However during both supine control and
head-up tilt, diabetics with definite neuropathy had significantly smaller BRS than did able-bodied subjects and diabetics without neuropathy. During supine rest, diabetics without neuropathy tended to have smaller BRS than able-bodied subjects and larger BRS than diabetics with definite neuropathy. These findings support the widely held notion that diabetes reduces BRS and that reduced BRS is an early sign of AN (26, 49, 60). When we compared our significant reduction of BRS and BEI to the trend towards greater arterial pulse transit time in diabetics with definite neuropathy, we conclude that, for our study, baroreflex impairment was a more sensitive indicator of neuropathy than alterations in arterial stiffness (51).

Increased BP variability has been shown to predict nephropathy and retinopathy (36), and to correlate with endothelial and cardiovascular damage (13, 58) and higher mortality (35). Blood pressure variability, measured by standard deviation over 24 h, has been reported to be significantly higher in diabetic patients (25, 32, 41, 57) and was highest in those with cardiovascular autonomic neuropathy (10). In our study, we further explored the specific frequency ranges of increased BP variability and determined that diabetics with definite neuropathy had significantly higher VLF and HF power spectral density of SBP compared with able-bodied subjects. In addition, in all frequency ranges, our able bodied subjects demonstrated an ability to engage heart rate to buffer SBP fluctuations while diabetics with neuropathy demonstrated a reduced ability.

Arterial baroreflex buffering at respiratory frequencies may be more dominant in older persons. The fact that HF power of SBP was greater in our diabetics with neuropathy than in able-bodied subjects, but has been shown to be smaller in diabetic children and adolescents than in healthy controls (37), may be a matter of age, but may, more accurately, reflect the presence of neuropathy in our subjects. In addition, in middle-aged to older diabetics, HF power of SBP was reported to be smaller than in able-bodied during conditions of controlled breathing (18). The act of controlling breathing itself, however, may be responsible for this effect since we
previously determined that controlled breathing reduced cardiovascular parasympathetic influence (48).

An important indicator of impaired baroreflex-buffering of BP lies in the four-fold increase in the harmonic and nonharmonic components of VLF power spectral density of SBP in diabetics with definite neuropathy compared with able-bodied subjects (Figure 4A, B). Although enhanced VLF SBP has been reported to result from SAD in animals (9, 16), VLF SBP power in humans is believed to reflect renin-angiotensin system activity, endothelial factors, thermoregulation, etc (47). However, in patients with primary autonomic failure, enhanced VLF oscillations of SBP have been documented (45). Our study’s enhanced VLF SBP power and decreased VLF RR interval power in diabetics with definite neuropathy, indicated that intact baroreflex function is important for normal BP regulation in the VLF range for both harmonic and nonharmonic components in humans. Finally, in our study, VLF SBP was similar between able bodied and diabetics without neuropathy. This phenomenon has been reported previously in children and adolescents with Type 1 diabetes mellitus who have not developed other signs of neuropathy (37). These results suggest that in addition to BEI, increased VLF SBP spectral power may be a strong, early, indicator of diabetic neuropathy.

Our study did not show significantly increased standard deviation of SBP in diabetics with neuropathy. Similar to the finding of Frattola et al.(26), this may be due to the relatively short period of time over which the standard deviation was calculated (3 - 5 min in our study and 15 min in the study by Frattola et al.) compared to the greater standard deviation measured from 24h recordings (10, 25, 32, 41, 57).

At frequencies centered around 0.1 Hz (Figure 4B), the log-log power spectral density curve of SBP of diabetics with definite neuropathy demonstrated a dip, indicating that the loss of the harmonic component at LF (Fig 3B) is apparent in the log-log plot of SBP. This loss of power in
the LF region is similar to that previously reported following sino-aortic denervation in animals (15, 55) and total autonomic failure in humans (45). Sympathetic nerve activity (SNA) with a frequency around 0.1 Hz in man is similar to the 0.4 Hz rhythm in rat and, at this frequency, there is a tight coherence in unanesthetized rat between changes in SNA and changes in arterial BP (5). We conclude that the dip in oscillations of SBP in the LF region of diabetics with definite neuropathy is consistent with effects of neuropathy on sympathetically mediated vasomotion. This change is exposed by the corresponding loss of parasympathetically (baroreflex) -driven changes in heart rate in subjects with definite neuropathy. In addition, altered BP and RR interval power spectral densities over the range of frequencies between 0.003 and 0.45 Hz in diabetics with neuropathy indicate that an intact baroreflex is an important component of healthy human BP regulation at all frequencies. Each of these measures is relatively easy to determine from a short (five minute) recording of continuous blood pressure and heart rate from resting subjects.

**Limitations**

The time of onset, the prevalence and the development of diabetic neuropathy, and treatment effects have been reported to be different between Type 1 and Type 2 diabetes (54, 59). Because of our limited number of subjects, diabetic patients were not separated based on the type of diabetes. Therefore the relative ability of our indices of autonomic dysfunction to identify autonomic neuropathy in Type 1 vs. Type 2 diabetes is unknown. Direct measures of sympathetic autonomic function (like MIBG SPECT imaging) were not performed, thus comparisons between direct measures and our indices are not available. Again, because of the limited numbers of subjects in each group, we were not able to establish age effects on the autonomic indices we report. The D0 group was younger than the D2 group; however, major differences in this study lay between AB and D2 groups where there was no meaningful
difference in age. Greater numbers of subjects would be required to establish significant interactions of study variables with gender and age.

The rate of presyncope (5 of 11) in the able-bodied subjects of the present study was similar to able-bodied subjects we have reported in the past (39). In that study, 8 of 16 controls had symptoms within 30 minutes of head-up tilt. In another recent study of subjects who underwent 70-degree head-up tilt, 56% (9 of 16) had presyncopal symptoms during the 30 min tilt (21). Results from a third study of head-up tilt-mediated presyncope in healthy humans suggested that unexplained syncope was a result of altered cardiorespiratory interaction involving cerebral hemodynamics but with a normal neural control system (38). Similar to our results, other investigators have determined that the initial response to head-up tilt was similar between patients with vasovagal syncope and nonsyncopal subjects, and sympathetic nerve activity withdrawal did not occur until symptoms began (42). We do not believe that the incidence of presyncopal events in the AB subjects is remarkable, nor does it call into question any aspect of the interpretation of our findings.

Finally, abnormal findings in the battery of Ewing tests has been reported in 13% of the normal population (46). In our study, two able-bodied subjects with tingling of hands and/or feet were found to be free of peripheral neuropathy by standard clinical tests. Both subjects, however, decreased their BEI to near 0% in response to tilt while other able-bodied subjects increased or maintained their BEI at greater than 10%. In the group of diabetics with definite neuropathy, 6 of 8 subjects had BEI smaller than 10%. The similar behavior of the two able-bodied subjects to diabetics with definite peripheral neuropathy suggests that BEI may be an index of autonomic neuropathy that is more sensitive, and/or more selective for autonomic involvement than are standard sensory/motor neuropathy findings. Also for other variables, when we removed these two subjects from the able-bodied group, the statistical significance of the group differences reported here were dramatically increased, but without a physiological
reason to remove them, the results reported here include these subjects with the able-bodied group.

**Perspectives and Significance**

The much reduced ability to regulate blood pressure, characterized by decreased RRI buffering of an increased number of SBP ramps, the four-fold increases in VLF and HF SBP power spectral densities and the four-fold decrease in VLF, LF and HF RR interval power spectral densities indicate serious deficits in maintenance of cardiovascular homeostasis in diabetics with neuropathy. The major contributor to this loss of blood pressure regulation appeared to be the combination of a loss of sympathetically mediated control of vasomotion, with a reduced contribution from parasympathetically mediated responses of heart rate to buffer these changes in blood pressure. Our results indicate that preservation of baroreflex function needs to become a focus of diabetic neuropathy treatment. The present study also indicates that, in healthy subjects, baroreflex function is an important component of blood pressure regulation in all (very low, low and high) frequency regions and pertains to both self-similar as well as harmonic components. Finally, the results of our study indicate that noninvasive indexes of autonomic regulation were able to discriminate diabetics with autonomic neuropathy from subjects with reduced, or no, neuropathic damage.

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of Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation.


Table 1. **Demographic characteristics of participants**

<table>
<thead>
<tr>
<th></th>
<th>Able-bodied (AB)</th>
<th>No Peripheral Neuropathy (D0)</th>
<th>Possible Peripheral Neuropathy (D1)</th>
<th>Definite Peripheral Neuropathy (D2)</th>
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<tr>
<td>Age, yr</td>
<td>48.2 ± 3.9</td>
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<td>Weight, kg</td>
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<td>Height, cm</td>
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<td>164.6 ± 3.0</td>
<td>176.8 ± 4.6</td>
<td>174.2 ± 3.3</td>
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<tr>
<td>n (Male/Female)</td>
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<td>7 (1/6)</td>
<td>7 (5/2)</td>
<td>8 (5/3)</td>
</tr>
<tr>
<td>n of Type 1/Type 2</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Values are means ± SEM

**Figure legends:**

Figure 1. Illustrative data of RR interval, arterial blood pressure for one able-bodied (AB) subject and one diabetic patient with definite neuropathy (D2) undergoing shift from supine to 70 degree head-up tilt (HUT) for 30 min, followed by return to supine. RR interval decreased and arterial blood pressure was maintained during HUT in the AB subject but blood pressure decreased and changes in RRI were smaller in the D2 subject.

Figure 2. Average ± SEM RR interval (Figure A) and systolic blood pressure (Figure B1) for able-bodied subjects (AB; n=11), diabetics without neuropathy (D0; n=6), diabetics with possible neuropathy (D1; n=5) and diabetics with definite neuropathy (D2; n=8) in response to head up tilt. Figure B2 shows changes of SBP from supine control to the first 3 min of tilt. Figure A: All groups decreased RR interval during head up tilt. Figure B1: AB tended to increase SBP in response to head up tilt while D2 tended to decrease SBP in the first 3 min of tilt and recover during tilt 3-7 and 7-12 min. Figure B2: Changes of SBP from supine control to the first 3 min of tilt were significantly different between AB and D2. Δ, significantly different from control (same...
group); •, significantly different from AB (same time segment); *, significantly different from D0 (same time segment).

Figure 3. Panel A: Average ± SEM spectral power of RR interval in the high-frequency region for able-bodied subjects (AB; n = 11), diabetics without neuropathy (D0; n = 6), diabetics with possible neuropathy (D1; n=5) and diabetics with definite neuropathy (D2; n =8) at rest and in response to head up tilt. For all groups, the high frequency spectral power of RR interval decreased going from supine control to tilt with an immediate return during supine recovery. D2 had significantly smaller power than did AB and D0. Panel B1: The spectral power of DBP in the low-frequency region tended to increase in AB and D0 and not change in D1 and D2 in response to head up tilt. These group differences in response to the first 3 min of tilt are shown in Figure B2. •, significantly different from AB; *, significantly different from D0.

Figure 4. Average ± SEM logarithm of power spectral density of systolic blood pressure (PSD SBP) and RR interval (PSD RRI) taken from 5 min supine control (means shown in solid lines and SEM shown in shaded area above and below the mean in A, B, D and E). Plots A and B show log (PSD SBP) vs log (f) for AB (n = 11) and D2 (n=8) respectively. Values for the two other groups (D0 and D1) were similar to those of AB and are not shown. PSD SBP of D2 was significantly higher than that of AB across the very low frequency (0.003 - 0.04 Hz, VLF) and high frequency (0.15 - 0.4 Hz, HF) regions. Plot C shows the average value of the slopes of the linear portion of log (PSD SBP) vs log (f) curves in the low frequency (0.04 - 0.15 Hz, LF) region for all four groups. Plots D and E show log (PSD RRI) vs log (f) for AB and D2 respectively. PSD RRI of D2 was significantly lower than that of AB across all frequencies. Plot F shows the average value of the slopes of log (PSD RRI) vs log (f) curves in the low frequency region for all four groups. •, significantly different from AB; *, significantly different from D0.
Figure 5. Average ± SEM normalized number of SBP ramps (A), normalized number of baroreflex sequences (B), baroreflex effectiveness index (C), baroreflex slope (D), and high frequency spectral power of SBP (E) for AB (n = 11), D0 (n=6), D1 (n=5) and D2 (n=8) at rest and in response to head up tilt. Note diminished baroreflex activity in diabetics with neuropathy. Δ, significantly different from control (same group); •, significantly different from AB (same time segment); *, significantly different from D0 (same time segment).
Mean Values of RR Interval (A) And Systolic Blood Pressure (B)

Time: p < 0.0001
Group × time: p < 0.05

A.

B1.

B2.

Changes of Mean SBP from Control to The First 3 min of Tilt (mmHg)