Quantitative Evaluation of Cardiac Parasympathetic Activity in Normal and Diabetic Man

MICHAEL A. PFEIFER, DANIEL COOK, JOEL BRODSKY, DAVID TICE, A. REENAN, SALLY SWEDINE, JEFFREY B. HALTER, AND DANIEL PORTE, JR.

SUMMARY

Heart rate and RR variation (the standard deviation of the mean RR interval for a 5-min period) were evaluated as measurements of cardiac parasympathetic nervous system activity in fasting supine diabetic (N = 22) and comparable age normal (N = 22) subjects. The rate of breathing did not effect heart rate, but was inversely related to the RR variation ($r = 0.89$, $P < 0.01$). Heart rate was increased ($P < 0.0001$) and RR variation decreased ($P < 0.05$) during β-adenergic stimulation with isoproterenol and during parasympathetic blockade with atropine (both $P < 0.0001$). Hence, the cardiac effects of β-adrenergic stimulation may mimic the effects of diminished parasympathetic function. To evaluate parasympathetic control of RR variation, independently of possible effects of increased sympathetic activities, studies were performed during β-adrenergic blockade with propranolol. RR variation during propranolol was less both in 14 diabetic subjects without clinical symptoms of autonomic neuropathy ($P < 0.005$) and in 8 diabetics with clinical symptoms of autonomic neuropathy ($P < 0.001$) when compared with 22 age-comparable normal subjects. The measurement of RR variation was very reproducible with a day-to-day coefficient of variation of 9.7 ± 2.8% ($\bar{x}$ ± SEM) in diabetic subjects with stable hyperglycemia. It is concluded that supine RR variation during a deep respiratory rate and during β-adrenergic blockade is a sensitive, quantitive, and reproducible method to evaluate parasympathetic nervous activity in normal and diabetic subjects. Furthermore, cardiac parasympathetic activity may be diminished in diabetic subjects before clinical symptoms of autonomic neuropathy are evident. **DIABETES *31*: 339–345, April 1982.**

From the Departments of Medicine and Biostatistics, University of Washington School of Medicine, Division of Endocrinology and Metabolism, Diabetes Education and Research Center, and Geriatric Research, Education, and Clinical Center, Veterans Administration Medical Center, Seattle, Washington 98108.

Address reprint requests to Michael A. Pfeifer, M.D., Medical Research Service (151), VA Medical Center, 800 Zorn Ave., Louisville, Kentucky 40202.

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Autonomic neuropathy is a well-known complication of diabetes mellitus.[1,2] However, its clinical manifestations may be less obvious than peripheral somatosensory neuropathy. Although insidious in onset, autonomic neuropathy may be associated with substantial morbidity.[3] In fact, sudden death and cardiorespiratory arrest in diabetics has been attributed to cardiac autonomic dysfunction.[4–6] The mortality associated with this autonomic neuropathy after clinical diagnosis has been reported to be as high as 50% in three years,[7] and its presence has been suggested to serve as an indicator of poor prognosis.[5,7,8]

The diagnosis of autonomic neuropathy may be difficult to make because patients may be relatively asymptomatic and because of the lack of quantitative diagnostic tests. Many tests to assess cardiovascular autonomic function have been described in which blood pressure and heart rate are measured in response to a variety of maneuvers: immersion of face or hands into cold water,[9–12] hand grip,[13] exercise,[14–16] mental calculation,[17] changes in carotid pressure,[18] and administration of drugs such as amyl nitrate,[4] nitroglycerine,[19] phenylephrine.[19,20] These tests have been difficult to interpret because they often do not use a standard stimulus, and as they are reported in the literature, the measurements of the responses are usually not quantitative. Two of the most frequently used tests to assess autonomic nervous system integrity are postural change[21,22] and the Valsalva maneuver.[23–26] Postural changes have been shown to elicit a variety of responses,[21] and therefore interpretation of these results makes interpretation of the abnormality difficult. The Valsalva maneuver is a difficult test to perform accurately, and the results rely on an intact baroreceptor and the integrity of both the parasympathetic and sympathetic nervous system to both the heart and vascular bed. An abnormality in any of these could result in an abnormal Valsalva response.

Because of these difficulties, the diagnosis of autonomic neuropathy has been difficult to establish in diabetics with-

out classic symptoms such as impotence, postural hypotension, or diabetic diarrhea. However, methods which accurately measure heart rate variation during normal respiration may be potentially useful.[6,8] The studies in this article were designed to evaluate factors influencing potential quantitative methods which may be used to measure parasympathetic (PNS) function in nondiabetic man and to determine whether such methods are sufficiently sensitive to detect abnormalities of autonomic function in asymptomatic diabetic subjects.

MATERIALS AND METHODS

SUBJECTS

Twenty-two male diabetics participated in these studies. Their average age was 47 ± 3 yr ($\bar{x}$ ± SEM) with a range of 21–64 yr. According to the Metropolitan Life Insurance Co. Tables, 1959, their percentage of ideal body weight was 120 ± 4% (range: 78–148%). Other than insulin or oral hypoglycemic agents, none of the diabetics were using any medicines for at least 2 wk prior to any of these studies. Insulin and oral hypoglycemic agents were not allowed on the day of the study until after the studies were completed. There was no clinical evidence of intrinsic heart disease or significant diabetic retinopathy in any of these diabetics.

Fourteen of these diabetics had no clinical symptoms of neuropathy. This was determined by a score of zero for a subjective neurologic symptom scale as described by Dyck et al.[27] This scale has a range of 0–17, with a score of 0 relating to no symptoms and 17 suggesting symptoms of the motor, the sensory, and the autonomic nervous systems. Their characteristics are shown in Table 1. Four of these subjects were insulin-dependent diabetics and were on insulin therapy. Ten of these subjects were non-insulin-dependent diabetics. One of these subjects was being treated with chlorpropamide. Nine of these diabetics were untreated and had a fasting plasma glucose of 191 ± 21 mg/dl and a total glycosylated hemoglobin of 13.2 ± 1.3% of total hemoglobin. In our laboratory, normal glycosylated hemoglobin is less than 10%.[28,29]

Eight diabetics had clinical signs and/or symptoms of autonomic insufficiency. Patients were considered to have clinical signs and/or symptoms of autonomic insufficiency if: (1) there was a decrease in diastolic blood pressure of greater than 10 mm Hg within 2 min of assuming an upright position, (2) there was evidence of neurogenic impotence (a history consistent with organic impotence, flat line nocturnal penile tumescence studies, and normal plasma gonadotropin levels), and/or (3) diabetic diarrhea (greater than 20 bowel movements per day, nocturnal diarrhea, and stool weight greater than 225 g/day). Their characteristics are shown in Table 1. Seven of these subjects were insulin-dependent diabetics and were on insulin therapy. One of these diabetics with clinical symptoms of autonomic neuropathy was an untreated non-insulin-dependent diabetic (fasting plasma glucose: 356 mg/dl; total glycosylated hemoglobin: 14.6%).

Twenty-two normal male subjects participated in these studies. Not all of the subjects participated in every procedure. Their characteristics are shown in Table 1. None were taking medicines, including aspirin, during these studies.

STUDY PROTOCOLS: GENERAL PROCEDURES

Studies were performed in the Special Studies Unit at the Seattle Veterans Administration Medical Center. All patients were fasting from midnight the night before, and studies were performed at approximately 8 a.m. on the morning of the study. No medications (including insulin or oral hypoglycemic agents) were allowed until after the completion of the study. Patients were not allowed to smoke cigarettes on the day of the study.

CARDIAC STUDIES

General. These studies measured mean heart rate (expressed as mean RR interval) and heart rate variation in response to controlled breathing. Patients remained in a recumbent position, since standing diminishes these responses.[30,31] A 19-gauge butterfly needle was introduced into one antecubital vein and was kept patent by a slow infusion of 0.9% sodium chloride. The studies were performed ½ h after the placement of the i.v. lines.

By means of chest leads, the patient was attached to an EKG monitor (Electronics for Medicine, Inc., model PM-2) which identified each R-wave of the EKG complex and triggered a microprocessor. The microprocessor precisely measured each RR interval (to within 1 ms) and recorded the data in digital form on magnetic tape for subsequent analysis on a PDP-8 computer. This microprocessor was designed and built for these studies by Dr. William Moritz of the Department of Electrical Engineering at the University of Washington.

Control of the rate of breathing was aided by use of an oscilloscope that displayed a horizontal line oscillating vertically at a constant rate. The patient was instructed to match his rate of breathing (i.e., his chest wall) to the oscillating line. The subject was, therefore, constantly inspiring when

TABLE 1
Characteristics of subjects*

	Age (yr)	% Ideal body weight	Fasting plasma glucose (mg/dl)	Glycosylated hemoglobin (%)	Neurologic symptom score	Duration (mo)
Diabetics						
No clinical symptoms of neuropathy (N = 14)	49 ± 4	124 ± 5	205 ± 24	12.4 ± 0.9	0 ± 0	55 ± 25
Clinical symptoms of autonomic neuropathy (N = 8)	42 ± 4	112 ± 7	260 ± 46	12.9 ± 0.8	7 ± 2	169 ± 26
Normals (N = 22)	47 ± 3	112 ± 3	91 ± 2	7.9 ± 0.2	0 ± 0	

* All values $\bar{x}$ ± SEM.

the line was going up and continually expiring when the line was going down. For instance, if a subject was breathing at a rate of 5 times/min, the subject was inhaling for 6 s and then exhaling for 6 s. With practice, the patient was then able to breathe at the rate predetermined by the investigator.

Each cardiac study lasted for 6 min. Analysis was only done on the last 5 min of this 6-min period. This allowed an additional 1 min for the individual subject to become further acclimated to the rate of breathing for the study. The mean RR interval for the 5-min period was determined in each cardiac study. The magnitude of the change in RR interval seen during respiration (RR variation) was estimated by the standard deviation of this mean.[8,31]

Respiration rates. To determine if respiratory rate affects the heart rate or RR variation, measurements were made in eight normal individuals during several respiratory rates. These rates were 5, 8, 10, 12, 15, 18, and 20 times/min. The order in which the subject performed these rates was random, and there was a 15-min rest period between each rate. These rates were chosen because 5 times/min resulted in a maximal inspiration and expiration in subjects and greater than 20 times/min resulted in hyperventilation in some subjects.

Assessment of SNS and PNS involvement in the respiration-cardiac reflex. The effect of altered sympathetic nervous system activity was evaluated during i.v. infusion of three drugs: isoproterenol (β-adrenergic stimulation), phentolamine (α-adrenergic blockade), and propranolol (β-adrenergic blockade). After completing a saline control study, isoproterenol was infused in six normals at a rate of 2 μg/min. The cardiac studies were repeated after 30 min of the isoproterenol infusion. In separate studies, after the saline control study, six normal individuals received an infusion of phentolamine (10 mg intravenously followed by an infusion of 0.5 mg/min). The studies were repeated after 30 min of the phentolamine. After a saline control study, the 22 diabetics and the 22 normal individuals received propranolol (10 mg intravenously followed by an infusion of 0.1 mg/min). One-half hour after the 10-mg pulse of propranolol, the cardiac study was repeated. After the studies during propranolol alone, in eight normal subjects, atropine (1 mg intravenously plus 0.01 mg/min infusion) was added in order to determine if the effects of beta-adrenergic blockade could be altered by PNS blockade. One-half hour later the cardiac studies were repeated.

After a saline control study, the effects of parasympathetic nervous system blockade were evaluated in eight normal individuals by an administration of atropine (1 mg intravenously followed by an infusion of 0.01 mg/min). One-half hour after the pulse of atropine had been given, the cardiac studies were repeated. To determine if the effects of this parasympathetic PNS blockade could be altered by a change in sympathetic activity, propranolol (10 mg intravenously plus 0.1 mg/min) was then given in addition to the atropine. One-half hour later, the cardiac studies were again repeated.

ANALYTIC METHODS

Plasma glucose was measured with the Autoanalyzer glucose oxidase method (Technicon Inst. Corp., California). Glycosylated hemoglobin was measured by a colorimetric method previously described.[29] Statistical techniques included both paired and nonpaired *t* test, analysis of variance (ANOVA), and analysis of covariance with regression. The coefficient of variation (CV) for day-to-day variation in the same subject was based on the formula:

$$CV = (SD/\bar{x})\ (100)$$

where $\bar{x}$ (mean) and SD (standard deviation) are the $\bar{x}$ and SD of the two studies performed on separate days. A CV was calculated for each individual. The values expressed represent the average CV for all subjects.

RESULTS

NORMAL SUBJECTS

Effects of changes in respiration rates. In eight normals, the average RR interval and RR variation were determined at different respiratory rates. There was no change in the average RR interval (i.e., heart rate) over the range of respiratory rates tested (5–20 times/min). However, as shown in Figure 1, there was a linear decrease of RR variation with increasing respiration rate ($r = 0.89$, $P < 0.01$). As determined by analysis of variance, the greatest RR-variation occurred at a respiratory rate of 5/minute ($P < 0.01$). Therefore, all subsequent studies were performed at this respiratory rate.

Effects of sympathetic stimulation and blockade. The effects of β-adrenergic stimulation with isoproterenol in six normal individuals is summarized in Table 2. The average heart rate during saline of 56 beats/min ($\bar{x}$ RR interval = 1080 ± 79 ms) increased to 102 beats/min ($\bar{x}$ RR interval = 590 ± 47 ms, $P < 0.0001$) during the isoproterenol infusion.

FIGURE 1. The effects of respiratory rate on mean RR interval (heart rate) and RR variation. Eight normal subjects had mean RR interval (top panel) and RR variation (bottom panel) measured during different respiratory rates. The rate of respiration had no apparent effect on heart rate but had a significant effect on RR variation.

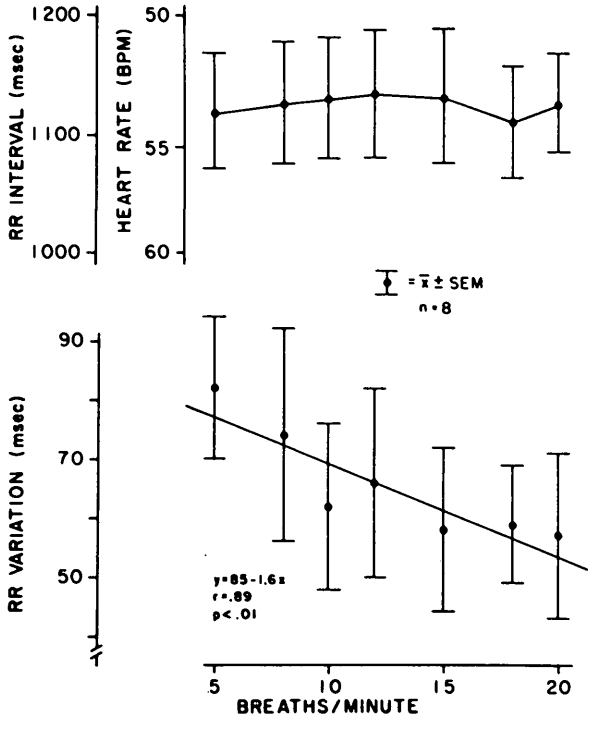


TABLE 2
Effects of autonomic drugs on mean RR interval and RR variation in normal man*

Protocol	x̄RR Interval (ms)	RR Variation (ms)
Sympathetic nervous system drugs		
Saline (N = 6)	1080 ± 79	83 ± 13
Isoproterenol	590 ± 47	29 ± 13
P (saline vs. iso)	0.0001	0.05
Saline (N = 6)	1120 ± 85	120 ± 12
Phentolamine	983 ± 100	109 ± 27
P (saline vs. phen)	0.05	NS
Saline (N = 22)	1003 ± 40	86 ± 9
Propranolol	1158 ± 33	94 ± 9
P (saline vs. prop)	0.0001	NS
Saline (N = 8)	1026 ± 57	82 ± 11
Propranolol	1148 ± 51	83 ± 14
Propranolol + Atropine	797 ± 19	17 ± 2
P (saline vs. prop + atrop)	0.005	0.0001
P (prop vs. prop + atrop)	0.0001	0.005
Parasympathetic nervous system drugs (N = 8)		
Saline	1032 ± 47	80 ± 11
Atropine	761 ± 24	17 ± 1
P (saline vs. atrop)	0.0001	0.0001
Atropine + Propranolol	847 ± 27	20 ± 3
P (saline vs. atrop + prop)	0.0001	0.0001
P (atrop vs. atrop + prop)	0.005	NS

* All values x ± SEM.
Iso = isoproterenol; phen = phenotolamine; prop = propranolol; Atrop = atropine; NS = nonsignificant (P > 0.05).

There was a simultaneous decrease in RR variation from 83 ± 13 ms to 29 ± 13 ms (P < 0.05).

During α-adrenergic blockade with phentolamine, there was an increase in heart rate from 54 beats/min (x̄ RR interval = 1120 ± 85 ms) during saline to 61 beats/min (x̄ RR interval = 983 ± 100 ms, N = 6, P < 0.05, Table 2). However, there was no significant effect of phentolamine on RR variation (120 ± 12 vs. 109 ± 27 ms, P = NS).

During β-adrenergic blockade with propranolol, there was a significant slowing of heart rate in normal individuals from 60 beats/min (x̄ RR interval = 1003 ± 40 ms) to 52 beats/min (x̄ RR interval = 1158 ± 33 ms, N = 22, P < 0.0001, Table 2). RR variation increased during propranolol but the effect was not significant (86 ± 9 vs. 94 ± 9 ms, P = NS). After the propranolol studies, an atropine pulse plus infusion was given to eight of these individuals (Table 2). This resulted in both an increase in heart rate from 52 beats/min (x̄ RR interval = 1148 ± 51 ms) during propranolol to 75 beats/min (x̄ RR interval = 797 ± 19 ms, P < 0.0001) and a decrease in RR variation (83 ± 14 ms vs. 17 ± 2 ms, P < 0.005).

Effect of parasympathetic blockade. When atropine was administered to achieve blockade of parasympathetic muscarinic receptors, there was an increase of heart rate from 59 beats/min during saline (x̄ RR interval = 1032 ± 47 ms) to 79 beats/min (x̄ RR interval = 761 ± 24 ms, N = 8, P < 0.0001, Table 2). There was also a decrease in RR variation during the atropine from 80 ± 11 to 17 ± 1 ms (P < 0.0001). After the addition of propranolol, there was a significant slowing of the heart rate to 71 beats/min (x̄ RR interval = 847 ± 27 ms, P < 0.005), but no change in RR variation (17 ± 1 vs. 20 ± 3 ms, P = NS, Table 2).

DIABETIC SUBJECTS

Effects of β-adrenergic blockade. The mean resting heart rate (HR) of 22 diabetics was 68 beats/min (x̄ RR interval = 884 ± 28 ms) during saline and slowed to 61 beats/min (x̄ RR interval = 985 ± 33 ms, P < 0.0001) during propranolol. When these diabetics (age: 47 ± 3 yr) were compared with normals of similar age (47 ± 3 yr, N = 22, P = NS), the heart rate was significantly faster in the diabetic subjects during both saline (normal: HR = 60; x̄ RR interval = 1003 ± 40 ms; P < 0.001) and propranolol (normal: HR = 52; x̄ RR interval = 1158 ± 33 ms, P < 0.001).

In the diabetics, RR interval during propranolol infusion varied less than during the saline control study (40 ± 9 ms vs. 45 ± 9 ms, N = 22, P < 0.01). The RR variation was significantly less in the diabetics than the normal subjects during either saline (45 ± 9 vs. 86 ± 9 ms, P < 0.005) or propranolol (40 ± 9 vs. 94 ± 9 ms, P < 0.001). Examples of respiratory RR variation in the presence of propranolol in a diabetic without clinical symptoms of autonomic neuropathy and an age-matched normal subject is shown in Figure 2.

Diabetic subjects without clinical symptoms of autonomic neuropathy. There were 14 diabetics (age: 49 ± 4 yr) without any clinical symptoms of autonomic neuropathy who had cardiac studies performed. Their cardiac responses during propranolol were compared with the 22 normal subjects (age: 47 ± 3 yr, P = NS). The diabetics had a faster heart rate during propranolol (59 beats/min, x̄ RR interval = 1013 ± 49 ms vs. 52 beats/min, x̄ RR interval = 1158 ± 33 ms; P < 0.02; Figure 3) when compared with the normal subjects. These diabetics also had less RR variation during propranolol (44 ± 14 ms vs. 94 ± 9 ms, P < 0.005; Figure 3).

Diabetic subjects with clinical symptoms of autonomic neuropathy. There were 8 diabetics (age: 42 ± 4 yr) with clinical symptoms of autonomic neuropathy. When compared with the 22 normal subjects (age: 47 ± 3 yr, P = NS), the diabetics had faster heart rates during propranolol (64 beats/min, x̄ RR interval = 936 ± 26 ms vs. 52 beats/min, x̄ RR interval = 1158 ± 33 ms, P < 0.001; Figure 3) and less RR variation (32 ± 4 ms vs. 94 ± 9 ms, P < 0.001; Figure 3).

Variation of the measurements in diabetics. Eleven diabetics without clinical symptoms of autonomic insufficiency had propranolol-cardiac studies done on two separate days. The average separation of these two measurements was 36 ± 7 days (range of 3–84 days). Diabetic control was not significantly different in the patients between day 1 and day 2 (fasting plasma glucose: day 1 = 208 ± 19 mg/dl vs. day 2 = 198 ± 21 mg/dl, P = NS; GHb: day 1 = 12.3 ± 0.9% vs. day 2 = 11.3 ± 0.6%, P = NS). The coefficient of variation (CV) for RR interval was 3.9 ± 0.9% with a range of 0.6–7.9%. For the RR variation, the CV was 9.7 ± 2.8% with a range of 0–28.3%.

DISCUSSION

Some of the factors affecting the assessment of this cardiac reflex have been previously evaluated.[6,8,30,31] It is apparent

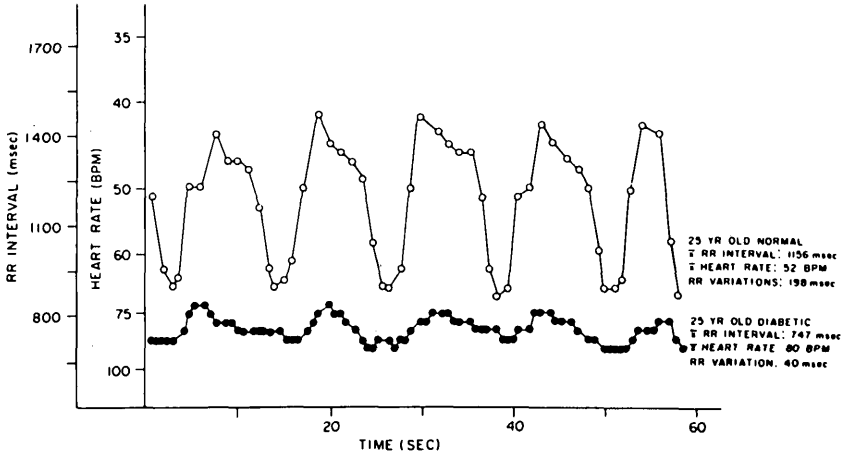


FIGURE 2. RR interval during propranolol and deep (5/min) supine respiration in a diabetic subject without clinical manifestations of autonomic neuropathy and in an age-matched normal. Note the decrease in RR interval (faster heart rate) during inspiration and the increase in RR interval (slower heart rate) during expiration. In the diabetic, there are both smaller RR intervals (faster heart rate) and smaller swings during respiration (RR variation).

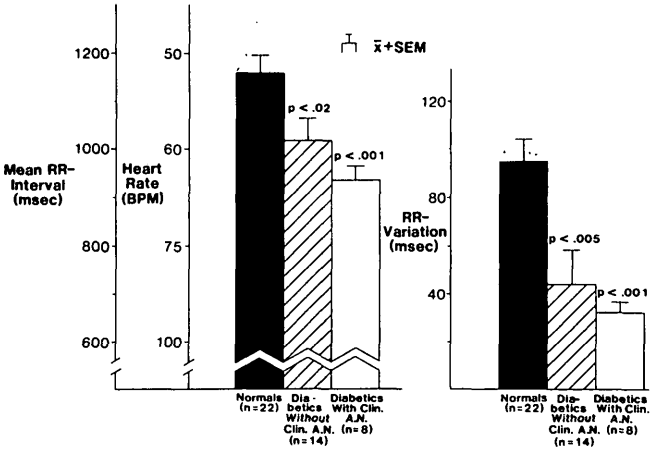
from these other studies that the position of the subject can affect both heart rate and RR variation.[30,31] Therefore, all our studies were done in the recumbent position. It has been suggested that a single deep inspiration may be a more sensitive index than the method we describe.[30] However, it is likely that the stimulus provided by a single inspiration would be very patient-dependent; that is, a single deep breath for one subject may be very much different than a single deep breath for another subject or even the same person at a different time. By having a subject breathe at 5 times/min for 6 min, a reproducible measure is achieved as indicated by the relatively low coefficients of variation observed (4 and 10%). This rate of breathing results in slow, probably near-maximal inspiration and expiration for each subject. However, since vital capacity was not measured, the depth of breath as a percentage of vital capacity may have varied.

Since many drugs and activities may affect the autonomic nervous system (ANS) function, use of all drugs and activities known to affect ANS function should be avoided, if possible, before performing these procedures. For example, both caffeine and cigarette smoking may affect the sympathetic nervous system (SNS) since they may cause an increase of plasma catecholamines.[32,33] These should, therefore, be avoided before the studies are performed. Since previous studies have suggested that there are direct cardiovascular effects of insulin in man,[34] we have performed all measurements in diabetics prior to insulin administration. Furthermore, there is an increase in parasympathetic nervous system (PNS) activity after eating; hence, the studies are probably most easily interpreted in a fasting patient.

In this study, we have found that a respiratory rate of 5 breaths/min results in the largest RR variation (Figure 1). Other studies have also found that 5–6 breaths/min yields the most consistent results and largest RR variation.[6,30] For these reasons, it seems reasonable to recommend a slow deep respiration of 5/min for 6 min with the fasting, nonsmoking subject in the supine position. However, shorter periods of time (e.g., 1–2 min) may also be acceptable.

Previous studies have implied that the SNS has little functional role in RR variation,[35] since propranolol did not significantly change this measure. However, in studies done in dogs, there is an increase in SNS activity to the heart during phrenic nerve activation (inspiration) and an increase in PNS activity to the heart during expiration.[36] This would imply that the SNS may play a role in respiratory heart rate variation in man. In normal subjects during the propranolol infusion, we also found no significant change in RR variation in spite of a small decrease in heart rate. During phentolamine, there was a small increase in heart rate and again no change in RR variation was observed (Table 2). This increase of heart rate probably resulted from a baroreceptor response to the decrease in mean blood pressure.[37] Thus, as has been suggested by others, it appeared that the SNS is important in determining heart rate but not RR variation.[35] However, during increased β-adrenergic stimulation with isoproterenol, there was both a decrease in RR interval (increase in heart rate) *and* a decrease in RR variation (Table 2). We hypothesize that during quiet nonstressed supine rest, there is very little cardiac SNS activity in normal man. Therefore, blockade of this low level of activity has very little effect on heart rate or RR variation. However, a large increase of adrenergic activity (such as during isopro-

FIGURE 3. Comparison of diabetics without and with clinical symptoms of autonomic neuropathy (Clin. A.N.) and age-matched normals. Both groups of these diabetics have both a faster heart rate and a smaller RR variation than the age-comparable normals, implying an impairment of the PNS activity to the heart. The P values are for the comparison of the diabetics to the normal subjects.



terenol or possibly with severe stress) can affect both heart rate and RR variation.

Previous studies have implied that the PNS is an important determinant of heart rate and RR variation.[35,38] In our studies, PNS blockade with atropine produced both a decrease in RR interval (faster heart rate) and a decrease in RR variation (Table 2). Thus, we have confirmed the findings of these previous studies. Furthermore, we have shown that the effects of PNS blockade could not be reversed or prevented with SNS blockade by propranolol (Table 2).

An increase in heart rate and a decrease in RR variation during either saline or propranolol was observed in diabetic patients compared with normal subjects of comparable age. The use of a comparable age control group is important because age has been shown to be an important determinant of RR variation and heart rate.[35] Although these studies are done in such a manner that it is unlikely that subjects have increased sympathetic tone due to medication, feeding, habits, or nonspecific stress, it cannot be assumed. The use of propranolol documents that the decrease in RR variation seen in diabetics is secondary to an impaired PNS and not due to an increase in SNS activity. Thus, although β-blockade with propranolol is not necessary to observe differences in normal and diabetic subjects when the studies are performed in a fasting, recumbent, nonstressful, standardized protocol, the use of propranolol confirms the fact that the difference was secondary to an impaired PNS.

Figure 3 demonstrates that diabetics with clinical symptoms of autonomic neuropathy have less RR variation during propranolol than normal controls. In asymptomatic diabetics, a similar abnormality was also observed. These findings suggest, as have others,[1,35] that autonomic neuropathy is present before clinical manifestations become apparent. In addition, in diabetic subjects whose measures of glycemia were stable, these methods were very reproducible from day to day. Therefore, these measurements may be useful for longitudinal studies in diabetics.

In summary, measurements of RR variation appear to be quantitative, sensitive, and reproducible procedures to evaluate the cardiac PNS integrity in man. However, appropriate control of patient position, respiratory rate, and metabolic state is needed before the studies can be interpreted. Furthermore, normal controls should be age- and probably sex-matched. Finally, if studies are done during β-adrenergic blockade, the observations between normal and diabetic subjects may be attributed to differences in the cardiac PNS. When performed in this manner, abnormalities of the PNS control of the heart can be identified in patients with diabetes, even when clinical symptoms of autonomic dysfunction are absent.

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REFERENCES

[1] Clarke, B. F., Ewing, D. J., and Campbell, I. W.: Diabetic autonomic neuropathy. Diabetologia *17*:195–212, 1979.

[2] Hosking, D. J., Bennett, T., Hampton, J. R., and Phil, D.: Diabetic autonomic neuropathy. Diabetes *27*:1043–54, 1978.

[3] Ewing, D. J., Campbell, I. W., and Clarke, B. F.: Mortality in diabetic autonomic neuropathy. Lancet *1*:601–603, 1976.

[4] Lloyd-Mostyn, R. H., and Watkins, P. J.: Total cardiac denervation in diabetic autonomic neuropathy. Diabetes *25*:748–51, 1976.

[5] Page, M. McB., and Watkins, P. J.: Cardiorespiratory arrest and diabetic autonomic neuropathy. Lancet *1*:14–16, 1978.

[6] Watkins, P. J., and Mackay, J. D.: Cardiac denervation diabetic neuropathy. Ann. Intern. Med. *92* (2):304–307, 1980.

[7] Faerman, I., Faccio, E., Milei, J., Nunez, R., Jadzinsky, M., Fox, D., and Rapaport, M.: Autonomic neuropathy and painless myocardial infarction in diabetic patients: histologic evidence of their relationship. Diabetes *26*:1147–58, 1977.

[8] Ewing, D. J., Campbell, I. W., and Clarke, B. F.: Assessment of cardiovascular effects in diabetic autonomic neuropathy and prognostic implications. Ann. Intern. Med. *92* (2):308–11, 1980.

[9] Helstad, D. D., Abbound, F. M., and Eckstein, J. W.: Vasoconstrictor response to simulated diving in man. J. Appl. Physiol. *25*:542–49, 1968.

[10] Gooden, B. A., Lehman, R. G., and Pym, J.: Role of the face in the cardiovascular responses to total immersion. Aust. J. Exp. Biol. Med. Sci. *48*:687–90, 1970.

[11] Hague, R., Scarpello, J., Sladen, G., and Cullen, D.: Autonomic function tests in diabetes mellitus. Diabete Metab. *4*:227–31, 1978.

[12] Hines, E. A.: The significance of vascular hyperreaction as measured by the cold pressor test. Am. Heart J. *19*:408–16, 1940.

[13] Ewing, D. J., Irving, J. B., Ken, F., Wildsmith, J. A. W., and Clarke, B. F.: Cardiovascular responses to sustained handgrip in normal subjects and in patients with diabetes mellitus: a test of autonomic function. Clin. Sci. Mol. Med. *46*:295–306, 1974.

[14] Griepp, R. B., Stinson, E. B., Dong, E., Jr., Clark, D. A., and Shumway, N. E.: Hemodynamic performance of the transplanted human heart. Surgery *70*:88–96, 1971.

[15] Hilsted, J., Galbo, H., and Christensen, N. J.: Impaired responses of catecholamines, growth hormone, and cortisol to graded exercise in diabetic autonomic neuropathy. Diabetes *29*:257–62, 1980.

[16] Hilsted, J., Galbo, H., and Christensen, N. J.: Impaired cardiovascular responses to graded exercise in diabetic autonomic neuropathy. Diabetes *28*:313–19, 1979.

[17] Taylor, S. H., and Meeran, M. K.: Different effects of adrenergic beta-receptor blockade on heart rate response to mental stress, catecholamines, and exercise. Br. Med. J. *4*:256–57, 1973.

[18] Eckberg, D. L., and Orshan, C. R.: Respiratory and baroreceptor reflex interactions in man. J. Clin. Invest. *59*:780–85, 1977.

[19] Low, P. A., Walsh, J. C., Huang, C. Y., and McLeod, J. G.: The sympathetic nervous system in diabetic neuropathy. A clinical and pathological study. Brain *98*:341–56, 1975.

[20] Lloyd-Mostyn, R. H., and Watkins, P. J.: Defective innervation of heart in diabetic autonomic neuropathy. Br. Med. J. *3*:15–17, 1975.

[21] Cryer, P. E., Silverberg, A. B., Santiago, J. V., and Shah, S. D.: Plasma catecholamines in diabetes: the syndromes of hypoadrenergic and hyperadrenergic postural hypotension. Am. J. Med. *64*:407–16, 1978.

[22] Ewing, D. J., Campbell, I. W., Murray, A., Neilson, J. M. M., and Clarke, B. F.: Immediate heart-rate response to standing: simple test for autonomic neuropathy in diabetes. Br. Med. J. *1*:145–47, 1978.

[23] Levin, A. B.: A simple test of cardiac function based upon the heart rate changes induced by the valsalva maneuver. Am. J. Cardiol. *18*:90–99, 1966.

[24] Nathanielsz, P. W., and Ross, E. J.: Abnormal response to valsalva maneuver in diabetics. Diabetes *16*:462–65, 1967.

[25] Baldwa, V. S., and Ewing, D. J.: Heart rate response to Valsalva maneuver: reproducibility in normals, and relation to variation in resting heart rate in diabetics. Br. Heart J. *39*:641–44, 1977.

[26] Hume, L., Ewing, D. J., Campell, I. W., Reuben, S. R., and Clarke, B. F.: Noninvasive assessment of left ventricular response to Valsalva manoeuvre in normal and diabetic subjects using praecordial accelerocardiography. Br. Heart J. *41*:199–230, 1979.

[27] Dyck, P. J., Sherman, W. R., Hallcher, L. M., Service, J., O'Brien, P. C., Grina, L. A., Palumbo, P. J., and Swanson, C. J.: Human diabetic endoneurial sorbitol, fructose, and myoinositol related to sural nerve morphometry. Ann. Neurol. *8*:590–96, 1980.

[28] Graf, R. J., Halter, J. B., and Porte, D., Jr.: Glycosylated hemoglobin in normal subjects and subjects with maturity-onset diabetes. Diabetes *27*:834–39, 1978.

[29] Pecoraro, R. A., Graf, R. J., Halter, J. B., Beiter, H., and Porte, D., Jr.: Comparison of a colorimetric assay for glycosylated hemoglobin with ion-exchange chromatography. Diabetes *28*:1120–25, 1980.

[30] Bennett, T., Farquhar, I. K., Hosking, D. J., and Hampton, J. R.: Assessment of methods for estimating autonomic nervous control of the heart in patients with diabetes mellitus. Diabetes *27*:1167–74, 1978.

[31] Bennett, T., Fentem, P. H., Fitton, D., Hampton, J. R., Hosking, D. J., and Riggott, P. A.: Assessment of vagal control of the heart in diabetes: measures of R-R interval variation under different conditions. Br. Heart J. *39*:25–28, 1977.

[32] Robertson, D., Johnson, G. A., Robertson, R. M., Nies, A. S., Shand, D. G., and Oates, J. A.: Comparative assessment of stimuli that release neuronal and adrenomedullary catecholamines in man. Circulation *59*:637–50, 1979.

[33] Gash, A., Karliner, J. S., Janowsky, D., and Lake, C. R.: Effects of smoking marihuana on left ventricular performance and plasma norepinephrine. Ann. Intern. Med. *89*:448–52, 1978.

[34] Mackay, J. D., Hayakawa, H., and Watkins, P. J.: Cardiovascular effects of insulin: plasma volume changes in diabetics. Diabetologia *15*:453–57, 1978.

[35] Wheeler, T., and Watkins, P. J.: Cardiac denervation in diabetes. Br. Med. J. *4*:584–86, 1973.

[36] Kollai, M., and Koizumi, K.: Reciprocal and non-reciprocal action of the vagal and sympathetic nerves innervating the heart. J. Auto. Nerv. Syst. *1*:33–52, 1979.

[37] Nickerson, M., and Collier, B.: Drugs inhibiting adrenergic nerves and structures innervated by them. *In* The Pharmacological Basis of Therapeutics. Goodman, L. S., and Gilman, A., Eds. New York, MacMillan Publishing Co., Inc., 1975, pp. 523–64.

[38] Ewing, D. J.: Cardiovascular reflexes and autonomic neuropathy. Clin. Sci. Mol. Med. *55*:321–27, 1978.