Sensitivity of R-R Variation and Valsalva Ratio in Assessment of Cardiovascular Diabetic Autonomic Neuropathy

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R-R variation and the Valsalva ratio are commonly used to quantitatively assess diabetic autonomic neuropathy (DAN). To assess the sensitivity of these two measures to parasympathetic ablation, 12 nondiabetic subjects were tested before and after graded doses (0.3–4.0 mg i.v.) of atropine. R-R variation was significantly reduced at 0.7 mg, whereas Valsalva ratio was not significantly smaller until the 2.0-mg dose of atropine. R-R variation continued to become progressively smaller during the 0.85-, 1.0-, and 2.0-mg doses. Valsalva ratio, but not R-R variation, was further reduced by the 4.0-mg dose. To further compare these two measures, two groups of diabetic subjects were compared with a group of nondiabetic subjects ($n = 22$). One group of diabetic subjects had symptoms of DAN ($n = 22$), and the other diabetic group had no symptoms of DAN ($n = 19$). In DAN subjects, both R-R variation (nondiabetic 33.2 ± 4.3 vs. DAN 9.8 ± 1.2, $P < .001$) and the Valsalva ratio (nondiabetic 1.98 ± 0.07 vs. DAN 1.55 ± 0.07, $P < .001$) were reduced. However, in asymptomatic subjects, R-R variation (23.2 ± 3.9, $P < .05$), but not Valsalva ratio (1.94 ± 0.13, NS), was less than nondiabetic subjects. Even after β-blockade, R-R variation was still less in both groups of diabetic subjects (nondiabetic 34.4 ± 4.2 vs. DAN 7.4 ± 1.3, $P < .001$; asymptomatic 21.8 ± 3.3, $P < .02$). Thus, the reduced R-R variation is more likely due to decreased parasympathetic rather than increased sympathetic activity. We conclude that R-R variation is more sensitive than Valsalva ratio in detecting parasympathetic ablation and is useful for early detection and follow-up of mild autonomic dysfunction. Valsalva ratio may be more useful for sequential long-term evaluation in subjects with severe diabetic autonomic dysfunction, in whom R-R variation is maximally suppressed. *Diabetes Care* 10:735–41, 1987

Clinical features of autonomic neuropathy comprise a myriad of problems, including postural hypotension, sweating abnormalities, disturbances of body temperature regulation, abnormalities with gastric emptying, intermittent nocturnal diarrhea, bladder problems, and impotence (1). Because these features generally occurred in patients with diabetes of long duration, diabetic autonomic neuropathy (DAN) was thought to be a late complication of diabetes (2). As newer techniques to test the autonomic nervous system have become available, it has become clear that various portions of the autonomic nervous system may be abnormal early in the course of diabetes (3–5). Reliance on clinical symptoms is an insensitive approach to detection of autonomic dysfunction. Morbidity associated with diabetic autonomic dysfunction is substantial and associated with high mortality (6). Sensitive, reliable, noninvasive, and quantitative means of detecting patients at high risk for developing symptomatic autonomic neuropathy should be established so that in the future, if any treatment modality is found to alter the progression of autonomic neuropathy, it can be applied as early as possible.

Techniques that assess the cardiovascular autonomic nervous system have proven useful in detecting the presence of DAN before the onset of clinical symptoms (3,4). Two techniques commonly used are R-R variation (the magnitude of respiratory sinus arrhythmia) and the Valsalva maneuver. The magnitude of R-R variation has been shown to be an index of a neurologic reflex arc including cardiac autonomic nervous system activity (4). When done with β-adrenergic blockage, R-R variation more specifically reflects cardiac para-

sympathetic activity (4). The Valsalva ratio is derived from the Valsalva maneuver (7). It is the maximum heart rate during the Valsalva maneuver divided by the slowest heart rate after the Valsalva maneuver. It serves as a more general cardiovascular autonomic nervous system test, incorporating cardiac parasympathetic and sympathetic nervous system activity as well as vascular sympathetic nervous system activity (8–11). In this study, we determine the sensitivity of these two measurements by first assessing the effect of parasympathetic withdrawal with graded doses of atropine in nondiabetic subjects and then comparing these two measures in a group of diabetic subjects with and without autonomic neuropathy.

METHODS

Subjects. Forty-one male diabetic and 28 male nondiabetic subjects participated in this study. Not all subjects participated in all studies. The nondiabetic subjects were obtained from advertisements and from a list of retired University of Washington faculty. The diabetics were recruited through referrals, clinics, and advertisements. All nondiabetic subjects had a normal medical history and physical examination, had no evidence or history of a chronic disease, and weren't on any medications. Twelve nondiabetic subjects were used in the first portion of this study (the sensitivity of R-R variation and Valsalva ratio to graded doses of atropine). Their average age was 28 ± 2 yr (mean ± SE). According to the 1959 Metropolitan Life Insurance Company tables, their ideal body weight was 103 ± 2%. Their fasting plasma glucose was 89 ± 1 mg/dl, and glycosylated hemoglobin was 7.7 ± 0.2%. Twenty-two nondiabetic subjects were used as a control group when these two techniques were applied to diabetic populations. The average age of these nondiabetic subjects was 41 ± 4 yr, ideal body weight 118 ± 5%, fasting plasma glucose 91 ± 1 mg/dl, and glycosylated hemoglobin 8.0 ± 0.1%. The neurological symptom score of these subjects based on the subjective neurological symptom scale described by Dyck et al. (12) was 0. The scale has a range of 0–17, with a score of 0 relating to no symptoms and a score of 17 indicating symptoms of motor, sensory, and autonomic nervous systems (Appendix).

The 41 diabetic subjects were divided into two groups: patients with (n = 22) and without (n = 19) clinical symptoms of DAN. These determinations were made on the basis of the Dyck scoring system (12). The average age of the patients with no symptoms of DAN was 48 ± 3 yr, ideal body weight 124 ± 7%, fasting plasma glucose 217 ± 23 mg/dl, glycosylated hemoglobin 12.0 ± 0.5%, Dyck score 0, and duration of diabetes 39 ± 12 mo. Six were treated with insulin, and 13 were treated with diet only or oral hypoglycemic agents to control blood sugar. The average age of the patients with symptoms was 49 ± 3 yr, ideal body weight 117 ± 5%, fasting plasma glucose 223 ± 25 mg/dl, glycosylated hemoglobin 11.6 ± 0.5%, duration of diabetes 114 ± 21 mo, and neuropathy score 6 ± 1 (range 1–13). Thirteen were treated with insulin and 9 with diet only or oral hypoglycemic agents to control blood sugar. Other than insulin or oral hypoglycemic agents, none of the diabetic subjects was using any medicines for at least 2 wk before these studies. All diabetic subjects were non–insulin dependent. This was determined by clinical criteria, e.g., no history of ketoacidosis on insulin withdrawal. No C-peptide test was performed nor was insulin stopped to prove they didn't develop ketoacidosis.

Study protocol and general procedures. Studies were performed in the Special Studies Unit of the Seattle Veterans Administration Medical Center. All patients fasted from midnight the night before, and studies were performed at ~0800 h the morning of the study. All subjects were instructed not to take any self-prescribed medications such as aspirin, vitamins, or antihistamines for 2 wk before the study. No medications (insulin or oral hypoglycemic agents) were allowed until completion of the study. Patients were not allowed to smoke cigarettes on the day of the study.

Autonomic studies. All studies were done in the recumbent position, because the upright position is known to stimulate neural input to the heart (13). A 19-gauge butterfly needle was introduced into a vein for infusion of drugs. Blood samples were obtained for fasting plasma glucose and glycosylated hemoglobin. The studies were started 30 min after starting the infusion. R-R intervals were measured for determination of R-R variation for 6 min. With the aid of an oscillating line on an oscilloscope, the subjects were instructed to breathe at a rate of 5 times/min for the entire 6 min. The last 5 min of this 6-min recording were used for analysis. After a 5-min rest period, the patient was asked to perform a Valsalva maneuver. The patient had previously practiced the Valsalva to acquaint himself with the procedure and equipment. The Valsalva was performed by having the patient blow into an open-ended spring manometer system fitted with a mouthpiece, maintaining a pressure of 40 mmHg for 20 s. The patient then resumed breathing at his normal rate. Three Valsalvas were performed, separated by 5-min rest periods, and analyzed separately. The mean of these studies was used to represent each individual. Heart rate response was determined with an ECG monitor and a microprocessor. The R-R intervals were measured to within 1 ms and recorded on magnetic tape for further analysis by a PDP-8 computer. Valsalva ratio was calculated as maximal R-R interval (ms) divided by minimal R-R interval (ms) (7). The magnitude of R-R variation was determined by a vector-analysis technique (14).

Atropine study. The study was performed in two groups of six nondiabetic subjects. In group 1, cardiac studies were performed before and after cumulative doses of 0.30, 0.40, 0.50, 0.60, 0.70, and 0.85 mg atropine (Table 1). Atropine was given every 30 min, and the actual doses given were 0.30, 0.10, 0.10, 0.10, 0.10, and 0.15 mg. In group 2, cardiac studies were performed before and after cumulative doses of 1.0, 2.0, and 4.0 mg atropine. Atropine was given every 30 min, and the actual doses given were 1.0, 1.0, and 2.0 mg. Studies were done 10 min after each dose of atropine was given.

TABLE 1
Results of atropine study

	R-R variation	P*	Valsalva ratio	P*
Group 1				
Saline (n = 6)	57.1 ± 10.4		2.49 ± 0.30	
0.30 mg atropine	56.0 ± 9.9	NS	2.63 ± 0.29	NS
0.40 mg atropine	51.2 ± 9.9	NS	2.71 ± 0.32	NS
0.50 mg atropine	50.6 ± 9.4	NS	2.69 ± 0.17	NS
0.60 mg atropine	53.5 ± 9.8	NS	2.85 ± 0.23	NS
0.70 mg atropine	43.4 ± 9.3	<.05	2.84 ± 0.17	NS
0.85 mg atropine	28.6 ± 4.8	<.02	2.77 ± 0.26	NS
Group 2				
Saline (n = 6)	53.9 ± 8.1		2.22 ± 0.17	
1.0 mg atropine	17.4 ± 4.5	<.02	2.28 ± 0.26	NS
2.0 mg atropine	6.5 ± 2.1	<.02	1.73 ± 0.14	<.05
4.0 mg atropine	6.1 ± 1.3	<.02	1.29 ± 0.05	<.02

Values are means ± SE.
*Value compared with saline by sign-rank test.

Group comparisons. To further compare these two measures, the autonomic studies were performed on diabetic subjects with and without symptoms of DAN and compared with nondiabetic subjects. Initially, R-R variation and three Valsalva maneuvers were completed during saline infusion. A second R-R variation was measured 30 min after β-adrenergic blockade with 10 mg propranolol i.v. push followed by an infusion at 0.1 mg/min (4).

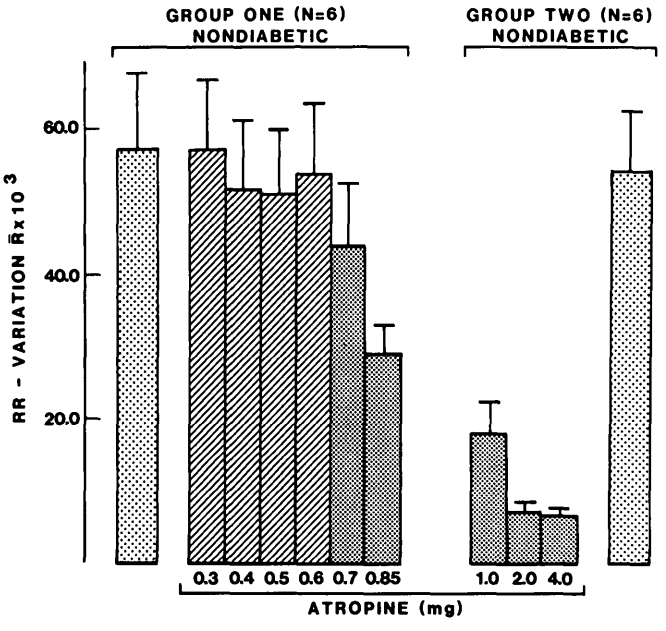

FIG. 1. Effects of graded doses of atropine on R-R variation in nondiabetic subjects. R-R variation is significantly reduced compared with that of saline after 0.7 mg atropine. Differences from saline and between doses remain quantitative and significant up to 2.0 mg. There is no difference between 2.0 and 4.0 mg atropine. Group 1 received low (0.30–0.85) and group 2 high (1.0–4.0) doses of atropine. *Lightly stippled bars* represent saline infusions. *Hatched bars* represent atropine infusions producing results that were not significantly different from saline. *Heavily stippled bars* represent infusions that produced results significantly different from saline. Values are means + SE.

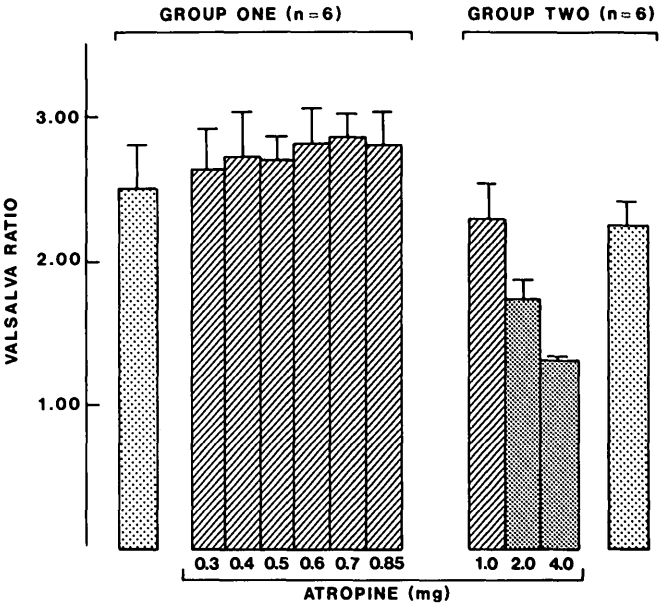

FIG. 2. Effects of graded doses of atropine on Valsalva ratio in nondiabetic subjects. In low-dose group, there is no significant reduction compared with saline after a cumulative dose of 0.85 mg atropine. In high-dose group, a significant decrease develops at 2.0 mg atropine compared with saline. Decrease at 4.0 mg remains quantitative and significant compared with 2.0 mg atropine and saline. *Lightly stippled bars* represent saline infusions. *Hatched bars* represent atropine infusions producing results that were not significantly different from saline. *Heavily stippled bars* represent infusions that produced results significantly different from saline. Values are means + SE.

Analytical methods. Plasma glucose was measured with the autoanalyzer glucose oxidase method (Technicon, Tarrytown, NY). Glycosylated hemoglobin was measured by the colorimetric method previously described (15). Measures for symptomatic and asymptomatic diabetic patients were compared with the two-tailed *t* test. Overall diminishment of the measures within individuals across graded doses of atropine was tested for each protocol with Page's distribution-free test for ordered alternatives, based on Friedman rank sums (16). When a significant dose response was detected, the minimal dose at which the measure differed from the response to saline was estimated with a nonparametric analogue of Fischer's least-significant difference method (17), where individual differences were tested with the sign-rank procedure.

RESULTS

Effects of graded doses of atropine on R-R variation and Valsalva ratio in nondiabetic subjects. Overall, a significant ($P < .001$) dose response was observed for R-R variation over the graded doses of atropine for both protocols (cumulative dose range

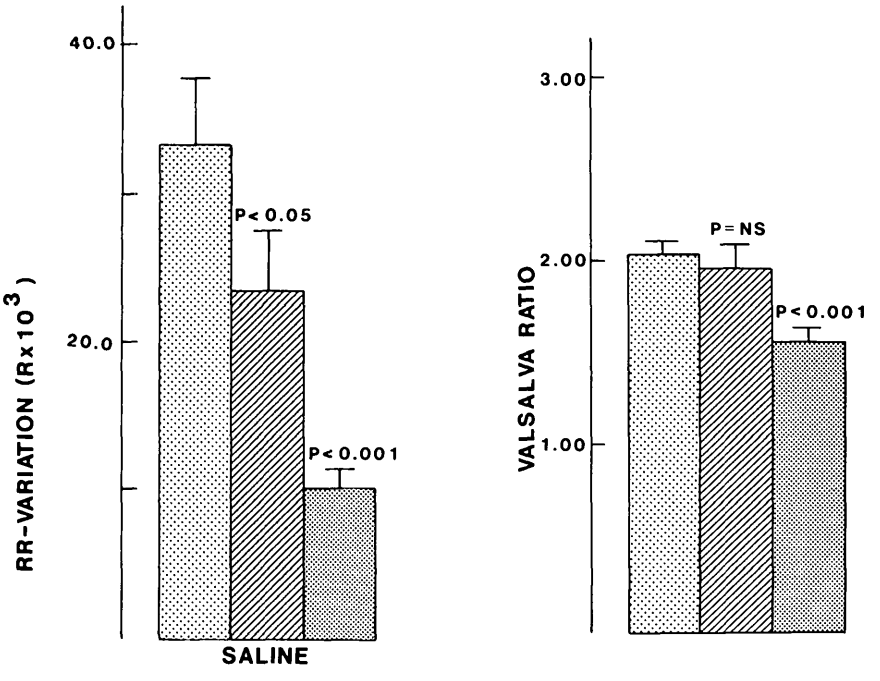

FIG. 3. Comparison of R-R variation and Valsalva ratio as measures of autonomic dysfunction in diabetic subjects. R-R variation and Valsalva ratio are both significantly decreased compared with nondiabetic subjects (*lightly stippled bars*, $n = 22$) in patients with symptomatic diabetic autonomic neuropathy (*heavily stippled bars*, $n = 22$). In asymptomatic group (*hatched bars*, $n = 19$), only R-R variation is significantly reduced compared with nondiabetic subjects. R-R variation was performed without β-blockade. P is for value compared with nondiabetic value by 2-sample *t* test. Values are means + SE.

0–0.85 and 0–4.0 mg; Table 1). The minimal dose producing a significant reduction in response compared with saline was 0.7 mg (Fig. 1). In contrast, the dose response for the Valsalva ratio over atropine doses ranging from 0 to 0.85 mg was not significant ($P > .05$). For doses ranging from 0 to 4 mg, there was overall significant evidence of effect ($P < .001$). The minimal dose producing a significant reduction in Valsalva ratio was 2 mg (Fig. 2). R-R variation continued to become progressively smaller during the 0.85-, 1.0-, and 2.0-mg doses. The Valsalva ratio, but not R-R variation, was further reduced by the 4.0-mg dose.

R-R variation and Valsalva ratio as measures of autonomic dysfunction in diabetic subjects. In patients with symptoms of DAN, both R-R variation (nondiabetic 33.2 ± 4.3 vs. DAN 9.8 ± 1.2, $P < .001$) and Valsalva ratio (nondiabetic 1.98 ± 0.07 vs. DAN 1.55 ± 0.07, $P < .001$) were reduced. However, in asymptomatic diabetic subjects, R-R variation (23.2 ± 3.9, $P < .05$), but not Valsalva ratio (1.94 ± 0.13, NS), was less than normal (Fig. 3). After β-blockade, R-R variation was still less in both groups of diabetic subjects (nondiabetic 34.4 ± 4.2 vs. DAN 7.4 ± 1.3, $P < .001$; asymptomatic 21.8 ± 3.3, $P < .02$; Fig. 4).

DISCUSSION

Diabetic autonomic neuropathy is a well-known complication of diabetes and contributes significantly to its morbidity and mortality. Research currently underway is looking at various treatment modalities and their ability to alter the progression of diabetic neuropathy. Because it appears that the onset of neural dysfunction occurs at a subclinical level as early as 1 yr after diagnosis in some patients with diabetes mellitus (3), it is imperative that a means of identifying patients at high risk of developing symptomatic diabetic neuropathy is available. In this study, we have found that R-R variation is a more sensitive measure of autonomic dysfunction than the Valsalva ratio.

The two measurements compared in this study are based on two complex cardiovascular reflex arcs. R-R variation is a measure of respiratory sinus arrhythmia. Modulation of heart rate associated with respiration appears to be due to an interaction of many smaller reflex arcs (18–23). This interaction involves central medullary respiratory and cardiomotor neurons, arterial baroreceptors in the carotid sinus and aortic arch, and stretch receptors in the cardiac chambers. It also involves thoracic stretch receptors in the lung parenchyma and chest wall (18,21,22). Afferent pathways are multiple but transverse mainly via the vagus. The efferent portion of this reflex arc involves vagal (parasympathetic) efferent fibers (19,24). It is known that a number of variables can influence R-R variation. Frequency of respiration, tidal volume, mechanism of lung inflation, position of the subject, baseline heart rate, and age have all been found to affect the assessment of this reflex (13,18,22,23,25). When performed under controlled conditions, as in this study, R-R variation is a quantitative, sensitive, and reproducible measure of cardiac parasympathetic activity (4).

The physiology of the heart rate and hemodynamic changes seen with the Valsalva maneuver were well described by Sharpey-Schafer (11) in 1965. This complex reflex involves baroreceptors inside and outside the chest and is dependent on vascular tone, intravascular volume, force of strain, duration of strain, and baseline heart rate (7,10,11,26). Performing the Valsalva maneuver and interpreting the data to produce a reliable and reproducible measure has proved to

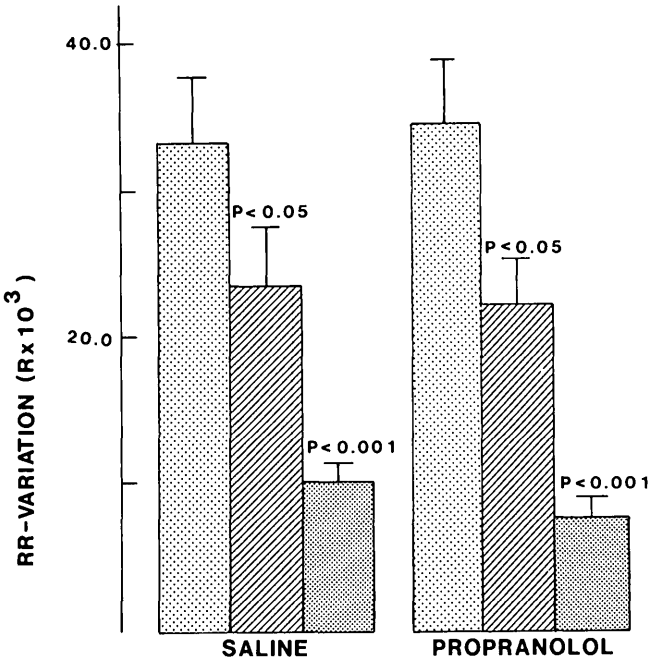

FIG. 4. Comparison of R-R variation as a measure of autonomic dysfunction with and without β-blockade. R-R variation is significantly decreased in both groups of diabetic subjects (asymptomatic, *hatched bars*, n = 19; autonomic neuropathy, *heavily stippled bars*, n = 22) before and after β-blockade. Decrease in magnitude of R-R variation is due to decreased parasympathetic tone and is unaffected by changes in sympathetic tone in supine resting state. P is for value compared with nondiabetic (*lightly stippled bars*, n = 22) value by 2-sample t test. Values are means + SE.

be difficult. To preclude the need for invasive hemodynamic monitoring, it was shown that monitoring heart rate response alone accurately reflected blood pressure changes in nondiabetic subjects (7). The decreasing pulse pressure seen during strain was accompanied by tachycardia, and the overshoot seen after release was accompanied by bradycardia. Several measurements of heart rate response have been used, but the Valsalva ratio has proved to be the simplest and most reproducible (7,11,13,26). Although technical variations still exist, a supine, rested, fasting patient straining at 40 mmHg for 20 s controls several variables and incorporates what produces the greatest response (1,7,9,10,26). Normality was considered to be a value >1.5 (7).

This study suggests that R-R variation is the more sensitive, reliable, and reproducible measure of the cardiovascular autonomic nervous system; R-R variation was decreased in both symptomatic and asymptomatic diabetic subjects, whereas the Valsalva ratio only became abnormal in symptomatic diabetic subjects (Fig. 3). In nondiabetic subjects, R-R variation decreased significantly after 0.7 mg atropine (Fig. 1), whereas the Valsalva ratio decreased significantly only after 2.0 mg atropine (Fig. 2). However, the Valsalva ratio did remain quantitative at higher (2.0 and 4.0 mg) doses of atropine (Fig. 2), whereas maximal suppression of R-R variation occurred at ≥2.0 mg atropine (Fig. 1).

Because we have previously shown that increased β-adrenergic stimulation can decrease R-R variation, R-R variation was repeated with β-blockade and compared with the results with saline (4). β-Blockade did not affect the results of R-R variation, indicating that the reduced R-R variation is more likely due to decreased parasympathetic rather than increased sympathetic activity (Fig. 4). This is consistent with the fact that R-R variation measured in the resting state is a reflection of parasympathetic tone on the heart. The Valsalva ratio seems to serve as more of a general cardiovascular autonomic nervous system test than a specific parasympathetic or sympathetic nervous system evaluator.

Previous studies have shown that R-R variation is a precise and reproducible measure of cardiac parasympathetic activity, with results showing a coefficient of variation of just 9.8% (4). Studies have also shown that R-R variation is sufficiently sensitive to detect cardiac parasympathetic dysfunction in recently diagnosed diabetic subjects before the onset of symptoms (3). This study demonstrates that R-R variation is more sensitive than the Valsalva ratio in detecting autonomic dysfunction in asymptomatic diabetic patients. The Valsalva ratio is not as sensitive as R-R variation but remains quantitative at levels of parasympathetic ablation that maximally suppress R-R variation. R-R variation is more sensitive and, therefore, useful as a screening test to detect early autonomic dysfunction. The Valsalva ratio is useful when R-R variation is maximally suppressed (patients with clinically evident severe DAN), because it remains quantitative with more severe dysfunction. Both tests may prove useful in clinical practice and trials designed to prevent and alter the course of diabetic neuropathy.

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APPENDIX

Name _____

Date _____

NEUROLOGICAL SYMPTOM SCORE
(Score 1 point for presence of a symptom)

I. Symptoms of muscle weakness		
A. Bulbar		
1. Extraocular	_____	
2. Facial	_____	
3. Tongue	_____	
4. Throat	_____	
B. Limbs		
5. Shoulder girdle and upper arm	_____	
6. Hand	_____	
7. Glutei and thigh	_____	
8. Legs	_____	MOTOR TOTAL _____
II. Sensory disturbances		
A. Negative symptoms		
9. Difficulty identifying objects in mouth	_____	
10. Difficulty identifying objects in hand	_____	
11. Unsteadiness in walking	_____	
B. Positive symptoms		
12. "Numbness," "asleep feeling," "like Novocain," "prickling,"—at any site	_____	
13. Pain-burning, deep aching, tenderness—at any location	_____	SENSORY TOTAL _____
III. Autonomic symptoms		
14. Postural faintingness, weakness	_____	
15. Impotence in male	_____	
16. Loss of urinary control	_____	
17. GI disturbance	_____	AUTONOMIC TOTAL _____
		COMBINED TOTALS _____

From DycK et al. (12).

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