Monotonicity of Nerve Tests in Diabetes

Subclinical nerve dysfunction precedes diagnosis of polyneuropathy

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OBJECTIVE — The objective of this study was to test whether monotone worsening of nerve function, attributable to diabetes, can be demonstrated before criteria for diabetic sensorimotor polyneuropathy (DSPN) have been met. Which nerve tests are best?

RESEARCH DESIGN AND METHODS — From a prevalence cohort of 504 individuals in the Rochester Diabetic Neuropathy study (RDNS), we identified 238 individuals (group 1) who at first examination were without polyneuropathy (DSPN) by a sum score of the normal deviates (from percentiles) of five attributes of nerve conduction of the legs (i.e., their five nerve conduction normal deviate values were <97.5th percentile) and were followed longitudinally two or more times. Of these 238, 90 (group 2) were followed six or more times at yearly or biyearly intervals. We compared different nerve tests for the ones most sensitive and reliable in showing latent nerve dysfunction and monotone (the extent to which a variable measured repeatedly over time reveals a significant trend of worsening or improvement).

RESULTS — In group 1 patients, the mean sum score of five attributes of nerve conduction (Σ 5 NC nds) at baseline was 1.08 and at the last examination (only patients with Σ 5 NC nds <97.5th percentile) was 3.63, markedly higher than that in healthy subjects (only of individuals with Σ 5 NC nds <97.5th percentile) (−0.12), indicating a subtle latent shift of nerve conduction tests toward abnormality. Serial evaluations of many individual and especially sum scores of nerve conduction tests in group 2 patients showed statistically significant worsening with time, even when nerve conduction tests were still well within normal limits. Neurologic signs also worsened but barely to significant levels; however, symptoms and quantitative sensation tests did not. Considering the composite score Σ 5 NC nds, 42 (of 90 group 2 patients) showed significant worsening, 22 were still without DSPN by nerve conduction test criteria, and some were even below the 50th percentile at the last evaluation.

CONCLUSIONS — Subtle and latent functional worsening of nerve conduction can be demonstrated even before nerve conduction test criteria for DSPN have been met. For demonstrating monotone worsening, the order (from best to worst) of tests was: some composite scores of nerve conduction and individual attributes of nerve conduction. We did not show monotone worsening of symptoms or of quantitative sensation test results. In multivariate analysis of risk factors and their association with worsening Σ 5 NC nds, 24-h microalbuminuria (a marker of microvessel disease) was found to be a significant covariate, an indication that the asymptomatic alterations of nerve conduction are meaningful.

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Recognizing the earliest alteration of nerves, eyes, kidneys, or blood vessels from diabetes may be important information useful for setting diagnostic criteria for diabetes (1,2), understanding pathophysiologic derangement of diabetes complications and adverse outcomes (3–5), and developing preventive treatments (6,7). Early indications of retinopathy are the retinal changes visualized directly or in photographs with or without use of a dye (8). For nephropathy, microalbuminuria and then decreased glomerular filtration rate develop (9,10). The early pathologic alteration of microvessels may be visualized in biopsied renal and nerve tissue (11). For neuropathies, associated with diabetes, the first manifestations varies with type of neuropathy (12). Focal and multifocal varieties of neuropathy associated with diabetes (cranial neuropathy, mononeuropathy [e.g., median neuropathy at the wrist], or multiple mononeuropathies and radiculoplexus neuropathies) tend to relate poorly to severity and duration of diabetes, and symptoms and signs are distinctive and prominent. By contrast, diabetic sensorimotor polyneuropathy (DSPN) often begins with silent dysfunction of nerves and abnormal signs but with few or no symptoms and relates more closely to severity and duration of diabetes (13,14). Nerve conduction tests are recommended because they are quantitative, objective, and reproducible (15,16), and abnormality relates to clinical impairment (16).

Here we explore early functional alteration of nerves, compare the tests and approaches that reveal it, and ask whether these early changes are meaningful. When hyperglycemia can be induced in groups of animals or in tissue culture, test results can be compared among test and control groups to recognize small, but significant, differences (e.g., of nerve conduction) (17–19). Nerve conduction tests have been compared among groups of patients recently diagnosed with diabetes with those of control subjects, but some of the patients may already have had DSPN so that differences in nerve conduction

tests may have been attributable to the polyneuropathy (DSPN) of some of them (20–22). A second approach, used by others and us to test for early functional alterations of nerves, is to test the sensitivity and specificity of tests as compared with a gold standard in cross-sectional cohort studies (23–26). Thomas and Tomlinson (27), reviewing the subject of diabetic polyneuropathy, write that abnormalities of NC "are the most consistent indicators of subclinical (perhaps asymptomatic) neuropathy" (27). The third approach and the one tested here is to assess for worsening function over time that is attributable to diabetes before the diagnosis of DSPN has been made. In this approach, one assesses for monotone worsening. Monotonicity may be defined as the extent to which the same variable measured on multiple occasions over time consistently indicates a trend of change of worsening or improvement.

In this study, we assess different end points of DSPN by the criterion of monotonicity in patients with diabetes who do not have DSPN when first tested but are expected to develop it. Because monotone worsening of nerve tests and clinical examinations might be due to diabetes but could also be from increasing age and weight, we do not use raw scores in our tests of monotonicity but use corrected (for age, sex, height, and weight) scores to isolate the effect of diabetes only.

In the present studies, we ask three questions: *1*) Can functional worsening of nerve tests be demonstrated even before the diagnosis of DSPN has been made? *2*) Among nerve tests, which ones provide the strongest evidence of monotone worsening with time and due to diabetes? *3*) Is this dysfunction, assuming that it is found, correlated with any of the known diabetes risk covariates?

RESEARCH DESIGN AND METHODS — For these studies we used cross-sectional and longitudinal data obtained from the Rochester Diabetic Neuropathy Study (RDNS) cohort, an epidemiologic study of 504 diabetic patients from Olmsted County (prevalence date 1 July 1986), who were Caucasian and mainly of northern European extraction (13,14,28). For the present study, a data audit was completed to the end of 2003.

The study population for the present study are the 238 (of 327 evaluated at least twice) patients with diabetes who at

first evaluation did not fulfill minimal nerve conduction test criteria for DSPN (i.e., their composite nerve conduction scores [Σ 5 NC nds], the derivation of which is given in the APPENDIX, were <97.5th percentile) (group 1) and a subgroup of 90 of these 238 patients that was evaluated at least six times (group 2). For the latter group, six or more visits was chosen as the smallest number of evaluations needed to test (with a reasonable degree of certainty) for worsening, improvement, or no change in linear regressions of nerve conduction tests (e.g., of Σ 5 NC nds) on time (years).

Normal values from the RDNS of healthy subjects cohort
Individual and sum scores of attributes of nerve conduction tests and modalities of quantitative sensation test (QST) thresholds (determined with CASE IV; WR Medical Electronics, Stillwater, MN) were expressed as measured units, percentiles, and normal deviates after correction for applicable biographic and demographic variables based on studies of a healthy subject cohort (RDNS of healthy subjects [RDNS-HS]) using precisely the same approaches and standards of testing and quality control as used for patients with diabetes (29,30).

Choice and derivation of Σ 5 NC nd used as the minimal criteria for DSPN
The Σ 5 NC nd was chosen as the minimal criteria for DSPN because nerve conduction abnormality is objective (results cannot be willed by the patient), provides a quantitative measure over a wide range, has been shown to be as (or more) sensitive and specific for sensorimotor polyneuropathy than other nerve conduction criteria, is more reproducible than most individual attributes of nerve conduction, and generally correlates well with neuropathic impairment, and its components include several nerves of the legs and representative attributes (conduction velocities, latencies, and amplitudes) (16). The denervation of the Σ 5 NC nds is provided in the APPENDIX. To set percentile (and normal deviate values) of sum scores such as Σ 5 NC nd, we plotted values of patients from the RDNS-HS cohort on age, fitted a common regression line using least-squares regression, and provided corresponding percentile lines empirically by increasing or decreasing the intercept. Considering its derivation, it follows that

if every one of the five nerve conduction values making up the composite score were at the 50th percentile, the composite score would add up to 0; if all were at the 1st percentile (−2.33 normal deviate), the composite score would be −11.65; and if all were at the 99th percentile (2.33 normal deviate), the composite score would be 11.65.

Performance of nerve tests
All clinical and nerve tests were prescheduled, standard, and independent (of information from previous or other examination test results) as part of a prospective cross-sectional and longitudinal study of diabetes and its complications and risk covariates (NS36797). Also, examination times were not linked to intercurrent health problems. The neurologic examinations were done by one of us (P.J.D.) without reviewing previous examination information or test results and without knowledge of symptoms (the examination done before inquiry regarding symptoms). All items of the standard Neuropathy Impairment Score (NIS) were graded by defined criteria. Then a 37-item symptom score (Neuropathy Symptoms and Change [NSC]) was completed. All of the clinical information was entered into a paper and electronic form (Clinical Neuropathy Assessment [CNA]). The CNA is a paper and electronic record of the study, biographic and demographic data, nerve test (NIS), neuropathy symptoms (NSC), and disability (Dyck modification of the Rankin score). The electronic record (CNA) provides a high level of quality control and accuracy and transfer of data, which is described in our previous article (31). Nerve conduction tests were performed by technicians following defined protocols of study under the supervision of one of us (C.M.H.), and QSTs were performed by technicians using verbal (from printed) instructions and programmed algorithms of testing and finding threshold. The stimulus-response pattern was quality controlled by visual inspection (by the technologists and P.J.D.), looking for inadequate patterns due to inadequate testing or inattention or drowsiness. When the record was inadequate, the test was repeated. For vibration and cooling thresholds, 4, 2, and 1 stepping with the null stimuli algorithm was used; for heat pain a nonrepeating ascending stepping algorithm with null stimuli was used (rev. in 16). As for clin-

Table 1—*Clinical features of patients*

	Group 1			Group 2		
	%	Mean ± SD	Median (range)	%	Mean ± SD	Median (range)
First evaluation						
Age (years)	—	57.2 ± 14.7	58.5 (16.0–86.0)	—	55.7 ± 13.3	58.0 (21.0–78.0)
Men (%)	51.3	—	—	55.6	—	—
Weight (kg)	—	84.1 ± 19.8	82.0 (46.0–181.8)	—	81.8 ± 17.1	81.0 (48.0–135.0)
BMI (kg/m^2)	—	29.9 ± 6.5	28.7 (20.0–52.1)	—	28.9 ± 6.0	28.2 (20.0–52.1)
Type of diabetes (type 1/type 2)	17.2/79.8	—	—	20.0/78.9	—	—
Duration of diabetes (years)	—	8.8 ± 7.6	7.1 (0.2–71.7)	—	8.1 ± 5.7	6.8 (0.2–24.8)
A1C (%)	—	7.9 ± 1.6	7.7 (4.8–13.4)	—	8.0 ± 1.7	7.7 (5.8–13.4)
FPG (mg/dl)	—	167.8 ± 64.0	155.5 (46.0–456.0)	—	161.4 ± 58.4	147.5 (46.0–328.0)
Last evaluation						
Age (years)	—	65.4 ± 14.9	68.0 (20.0–89.0)	—	69.6 ± 12.8	71.0 (32.0–89.0)
Average weight (kg)	—	84.5 ± 19.7	83.1 (45.1–183.1)	—	83.6 ± 17.4	82.9 (47.1–131.7)
Average BMI	—	30.2 ± 6.4	28.9 (20.4–51.8)	—	29.8 ± 6.0	28.7 (20.4–51.0)
Type of diabetes (type 1/type 2)	21.4/64.3	—	—	20.0/60.0	—	—
Duration of diabetes (years)	—	17.1 ± 8.8	16.2 (3.2–73.8)	—	22.1 ± 5.9	21.3 (12.0–40.8)
Duration of follow-up (years)	—	8.0 ± 5.1	7.0 (1.0–18.0)	—	13.8 ± 2.5	14.0 (9.0–18.0)
Average A1C (%)	—	7.8 ± 1.2	7.7 (5.1–12.5)	—	7.9 ± 1.0	7.7 (6.3–11.3)
Average FPG (mg/dl)	—	169.6 ± 37.5	163.8 (95.3–348.4)	—	168.9 ± 30.3	163.9 (118.2–259.5)

*Group 1 patients (n = 238) are from the RDNS cohort, have had diabetes evaluated yearly or biyearly two or more times, and at first examination did not have polyneuropathy (DSPN) by nerve conduction criteria (defined for this study as ≥97.5th percentile of Σ 5 NC nds). At last examination, <238 had Σ 5 NC nds <97.5th (see text). Group 2 patients (n = 90) are a subset of group 1 patients and were evaluated six or more times. Less than 90 had Σ 5 NC nds <97.5th at last examination. FPG, fasting plasma glucose.

ical evaluations review of earlier test results was not allowed for nerve tests. Nerve conductions were obtained using standard conditions and stimulating and recording electrode placements.

Analytical methods

In group 1 patients, we assessed whether the mean Σ 5 NC nd score of patients with diabetes but with values <97.5th percentile was higher at onset and at last examination than that of healthy subjects (also with values <97.5th percentile). If mean values are higher in diabetic subjects, and especially at the last examination, it would suggest that nerve conductions, even within normal limits, are gradually worsening in diabetes.

In the analysis of the 90 group 2 subjects (of 238) without DSPN at baseline, we regressed individual and sum scores of nerve conduction and other test results on time. This analysis was performed separately for each subject, resulting in a slope (b) and a correlation coefficient (r) for each subject. The mean slope ($\bar{b}$) and cor-

relation ($\bar{r}$) were calculated. One sample t test was used to test that the slopes and correlation coefficients differed from 0. The square of the mean slope was computed for each variable, indicating that the percent variance explained by the regression, which provided a measure of monotonic worsening ranging from 0.0 to 1.0, which can be compared among end points. Assessed as risk covariates against the slope of Σ 5 NC nds on time of group 2 patients were the risk covariates assessed in our previous article (14). Associations between the slope of worsening and risk factors were calculated quantitatively with Spearman rank correlation for continuous variables and rank sum tests for dichotomous variables. For multivariate analysis, stepwise regression was used, stepping up with $P < 0.05$ as the criterion.

RESULTS

Studies on group 1 patients (n = 238)
Of 327 patients in the RDNS who were evaluated two or more times, there were

238 subjects at the first evaluation and 196 at the last evaluation who were without DSPN by the criterion of Σ 5 NC nds <97.5th percentile. Their clinical features are summarized in Table 1. At baseline, the 238 patients had a mean Σ 5 NC nd score of 1.08, which was considerably higher than that in healthy subjects whose Σ 5 NC nds <97.5th percentile was −0.12. At the last examination the 196 patients had a normal deviate mean score of 3.63. These results suggest that in community diabetic subjects a shift of nerve conduction values toward abnormality has occurred even before they can be declared abnormal by the normal limits criterion of ≥97.5th percentile. It cannot be attributed to a change in age or weight during the follow-up period because these factors had already been corrected for.

To provide the differences in measured units of nerve conduction that a typical patient (a 45-year-old man, 1.78 m tall, and weighing 82 kg) would undergo if his Σ 5 NC nd value were to change from −0.12 nds (mean value of

Table 2—*Estimated measured nerve conduction values of a hypothetical man**

Patient	Σ 5 NC nd	Peroneal nerve CMAP (mV)	MNCV (m/s)	MNDL (ms)	Tibial nerve MNDL (ms)	Sural nerve SNAP (μV)
Healthy subject	−0.12	5.3	47.5	4.6	4.0	11.9
Diabetes baseline	1.08	4.9	47.0	4.7	4.0	10.7
Diabetes last examination	3.63	3.9	45.4	4.9	4.3	8.5

*Assumptions: the subject is 45 years old, 1.78 m tall, and weighs 82 kg. CMAP, compound muscle action potential; MNCV, motor nerve conduction velocity; MNDL, motor nerve distal latency; SNAP, sensory nerve action potential.

healthy subjects) to 1.08 nds (value at the first evaluation of RDNS patients) and 3.63 (value at the last evaluation of RDNS subjects), see Table 2. For these estimates, we make the assumption that each of the five attributes of nerve conduction in Σ 5 NC nds changed by an equal amount.

The same phenomenon (a shift toward abnormality while nerve conduction still remained within normal limits) can be seen by looking at the distribution of Σ 5 NC nds plotted against age among RDNS (diabetic patients) compared with healthy subjects (Fig. 1). In the RDNS-HS, there were 156 values above and 155 values falling below the 50th percentile line. For diabetic patients with Σ 5 NC nds <97.5th percentile at baseline, there were 147 values above the 50th percentile line and 90 below. At last examination, there were 154 above and 42 below the line.

Studies on group 2 patients
Table 3 shows information on which nerve tests provide the best measures of monotone worsening by the criteria of the number of individuals whose values significantly worsened, improved, or remained unchanged and on the magnitude of the mean change with time and the percentage of the variance explained ($\bar{r}^2$). Composite nerve conduction tests were markedly superior to individual attributes. Especially favorable indications of monotone worsening were Σ 5 NC nd and Σ Per. Tib. Ul. MNDL nds. By contrast, neurologic signs (e.g., NIS [lower limb]) barely showed a statistically significant change ($P = 0.032$), whereas symptoms (NSC) and QST did not.

The second question addressed was "Did patients show significant worsening over time even before minimal nerve conduction test criteria for DSPN had been met?" Statistically significant worsening of Σ 5 NC nds occurred in 42 of 90 patients (46.7% of patients); 22 of 90

(24.4%) had shown significant worsening but at the last examination had nerve conduction tests <97.5th percentile. It was of particular interest that of patients who at baseline had nerve conduction NC values <1st percentile, 13 (14.4%) had last values <50th percentile after 13.9 ± 2.25 years of follow-up.

Risk covariates relating to Σ 5 NC nds
Many univariate risk covariates were associated with rate of worsening of Σ 5 NC nds on time; but in multivariate analysis, only 24-h urine microalbuminuria (a measure of microvessel disease) remained as a significant risk covariate ($P < 0.001$) (Table 4).

CONCLUSIONS — To answer the question "Which nerve tests show the largest monotone worsening in a diabetic cohort expected to develop DSPN?", we compared the ability of nerve tests to demonstrate a statistically significant monotone worsening over time, assuming that such a change is taking place. Is the assumption of worsening correct? The studies by Pirart (32,33), the Diabetes Control and Complications Trial studies (34,35), our cross-sectional and longitudinal studies (36), and our present studies of group 1 and 2 patients unequivocally show that over time an increasing number of individuals with diabetes develop DSPN. It is therefore reasonable to compare different end point measures for their ability to recognize this worsening. Direct comparison among different nerve tests was optimized by expressing the abnormality of each test as a normal deviate value (from percentiles) with values corrected for applicable biographic and anthropomorphic characteristics (as obtained from the study of a healthy subject cohort). We did this for all our tests of individual and summed attributes of nerve conduction tests and for QSTs. For

clinical measures (i.e., NIS and NSC), corrections for the influence of age, sex, physical fitness, height, and weight were also attempted, but such corrections are based only on judgment, which presumably is at best only approximate. Although we have previously demonstrated that there can be a high level of agreement in NIS scores among investigators who are trained and agree on criteria for making judgments (28), it is unknown what degree of agreement would be achieved without this training and agreement.

The present studies did not address the closely related, but different, topic of the lowest level of cumulative glycemic exposure inducing polyneuropathy or the mechanisms that might be involved. Patients classified as having impaired glucose tolerance were not included in the present analyses.

By the criteria of monotone worsening in this cohort of subjects with diabetes but without DSPN at onset, nerve conduction tests were unequivocally superior to clinical impairment, symptoms, or QSTs. The best composite scores of nerve conduction test abnormality (Σ 5 NC nds) were a reliable indicator of functional worsening, as 42 subjects showed significant worsening and only 1 showed significant improvement, with the remainder showing no change. Neuropathic impairment (NIS and NIS [lower limb]) also showed a statistically significant mean slope of worsening, but it was not reliable for identification of worsening of individual patients, as five patients significantly worsened and three significantly improved. Symptoms (NSC severity) also worsened, but statistical significance was not quite achieved ($P = 0.053$). Here also symptoms were not reliable indicators of worsening because significant worsening was demonstrated in three subjects only, and in one there was significant improvement.
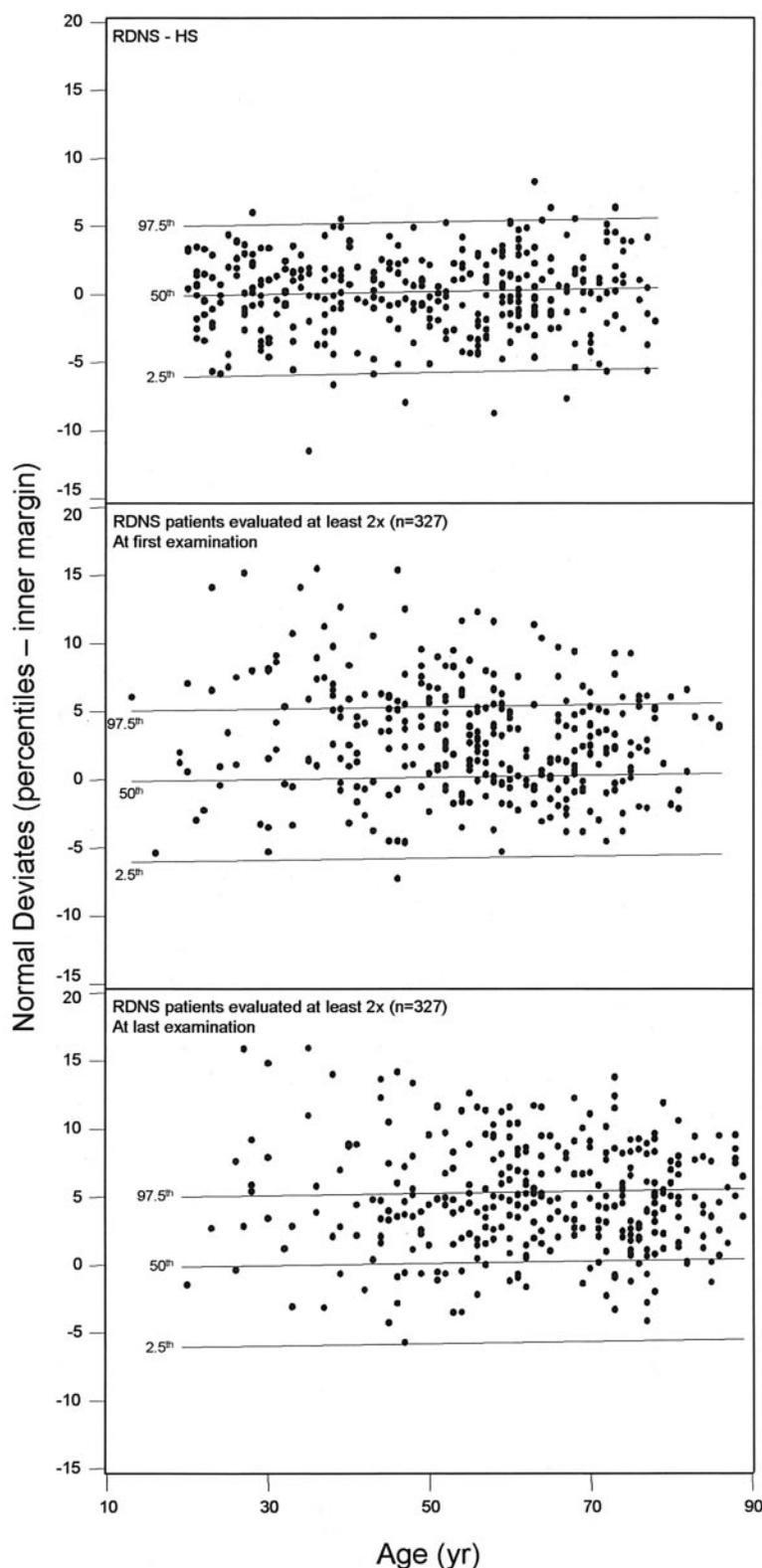
Next, which were the best indicators

Figure 1—*The composite nerve conduction scores Σ 5 NC nds of RDNS-HS and RDNS patients evaluated at least two times (n = 327) at baseline and at the last examination (defined in text) were plotted against age. The derivation of Σ 5 NC nds is provided in the APPENDIX. The estimated 2.5th, 50th, and 97.5th regression lines are superimposed. Observe that more diabetic patients fall between the 50th and 97.5th regression lines than between the 2.5th and 50th line and especially at the last evaluation, as described in the text. This and other evidence suggest that a subtle functional shift toward abnormality is occurring.*

of functional worsening of nerve conduction tests? Composite nerve conduction test scores performed better than individual nerve conduction test attributes. The two that performed best were Σ 5 NC nds and Σ Per. Tib. Ul. MNDL nds, but some individual attributes (e.g., Per. MNDL nds and Tib. MNDL) also performed well. We emphasize that this worsening can be attributed to actual worsening of nerve function due to diabetes, because corrections already had been made for the nondisease variables of change in age and weight.

Do the present results support the conclusion of the recently published evidence-based case definition of polyneuropathy (37)? These authors concluded that "The combination of neuropathic symptoms, signs, and electrodiagnostic findings provide the most accurate diagnosis of distal symmetric polyneuropathy." In addition to the evidence listed in the article, the conclusion these authors came to seems intuitively correct. The present study, however, extends the evidence on this question by showing that low levels of functional abnormalities of nerve conduction are earlier and more certain indicators of nerve dysfunction than are neurologic symptoms or signs. Alternatively or additionally, nerve conduction tests may provide reliable evidence of lesser involvement. This was recognized by the San Antonio consensus group who suggested a categorization of severity of DSPN, taking nerve conduction, QST, and neuropathic signs and symptoms into account (38). Our approach to staging severity of DSPN also recognizes that lesser degrees of functional abnormality are recognized by nerve conduction tests compared with clinical assessment. In our staging approach, abnormality of nerve conduction is the least degree of abnormality (stage 1A), followed by nerve conduction test abnormality and neurologic signs (stage 1B), and then by symptoms (stages 2A and 2B and 3) (12). In the present studies, direct comparison of nerve conduction test abnormality compared with neuropathic signs or symptoms by the criterion of monotone worsening over time showed that composite scores of nerve conduction tests are clearly superior. Although we argue here that nerve conduction tests at defined percentile levels are reliable minimal criteria for DSPN and are useful for epidemiologic surveys and

Table 3—*Summary statistics for longitudinal change of nerve tests in group 2 patients*

Nerve test	RDNS patients (of 90) who at baseline had Σ 5 NC nds <97.5th percentile			Significantly worsened vs. improved over time		
	Worse*	Better*	Unchanged*	$\bar{b}$†	P	$\bar{r}^2$‡
Ulnar CMAP nd	7	9	74	0.001	0.8815	0.000
Ulnar MNCV nd	22	0	68	−0.043	**<0.0001**	0.086
Ulnar MNDL nd	18	2	70	0.059	**<0.0001**	0.110
Ulnar F-wave nd	18	4	68	0.028	**<0.0001**	0.048
Peroneal CMAP nd	21	1	68	−0.033	**<0.0001**	0.053
Peroneal MNCV nd	23	6	61	−0.054	**<0.0001**	0.120
Peroneal MNDL nd	30	1	59	0.082	**<0.0001**	0.228
Tibial CMAP nd	16	12	62	−0.004	0.6130	0.000
Tibial MNCV nd	12	1	77	−0.026	**0.0003**	0.042
Tibial MNDL nd	25	1	64	0.085	**<0.0001**	0.226
Tibial F-wave nd	15	9	66	0.017	**0.0410**	0.023
Sural SNAP nd	18	5	67	−0.044	**<0.0001**	0.102
Σ 5 NC nds	42	1	47	0.296	**<0.0001**	0.354
Σ Per. Tib. Ul. CMAP nds	16	7	67	0.035	**0.0170**	0.017
Σ Per. Tib. CMAP & Sur. SNAP nds	28	1	61	0.082	**<0.0001**	0.082
Σ Per. Tib. Ul. MNCV nds	25	0	65	0.122	**<0.0001**	0.145
Σ Per. Tib. MNCV & Sur. SNCV nds	18	1	71	0.122	**<0.0001**	0.134
Σ Per. Tib. MNCV, Sur. SNCV & Tib. F-wave nds	21	2	67	0.143	**<0.0001**	0.119
Σ Per. Tib. Ul. MNCV & Per. Tib. F-wave nds	28	3	59	0.173	**<0.0001**	0.119
Σ Per. Tib. Ul. MNDL nds	35	1	54	0.223	**<0.0001**	0.290
NIS (total)	5	3	82	0.032	**0.0418**	0.011
NIS (lower leg)	5	4	81	0.032	**0.0383**	0.010
NSC severity (total)	3	1	86	−0.169	0.0523	0.042
Left foot VDT step nd	5	11	74	−0.020	0.0927	0.007
ln(Left foot CDT step) nd	11	2	77	0.024	0.0670	0.002
Left foot HP: 5 step nd	4	5	81	−0.005	0.8535	0.006
Left foot HP: 0.5 step nd	6	3	81	−0.013	0.6113	0.001
Left foot HP: 5–0.5 step nd	5	6	79	−0.016	0.5154	0.000

Data in bold are P values <0.05. *Statistically significant change ($P < 0.05$) of the nerve test over time of each patient using regression analysis. The mean ±SD number of neuropathic assessments was 7.8 ± 1.2. †Mean slope ($\bar{b}$) of regression lines of nerve tests on time. ‡Mean of the correlation of nerve tests on time. CDT, cooling detection threshold; CMAP, compound muscle action potential; HP, heat pain; MNCV, motor nerve conduction velocity; MNDL, motor nerve distal latency; Per., peroneal; SNAP, sensory nerve action potential; Sur. sural; Tib., tibial; Ul., ulnar; VDT, vibratory detection threshold.

therapeutic trials, there are compelling reasons (cost, complexity, expertise needed, and others) that for many clinical purposes they may not be needed.

The second question addressed here "Can latent dysfunction of nerves be demonstrated in patients with diabetes before nerve conduction test criteria for DSPN have been met?", has been unequivocally answered in the affirmative. In perhaps one-third of subjects who had not met minimal nerve conduction criteria for DSPN (i.e., their Σ 5 NC nds were <97.5th percentile), we were able to demonstrate subtle functional alterations by a shift of composite nerve conduction values within the range of normal values toward abnormality and more definitively by statistically significant worsening with time, even when all or most serial nerve

conduction test values fall within the normal range. This subtle functional alteration began anywhere within the range of normal test values (e.g., anywhere between the 1st and 97.5th percentile). Because these subtle alterations begin anywhere within the range of normal values, changing the minimal criteria to a lower level (e.g., to <95th or <90th percentile) would not have prevented the phenomenon from occurring. In fact, there were examples of nerve conduction test worsening within the ranges of the 1st to 50th percentile of the composite scores. We make the further observation that this worsening usually developed over years. In none of the subjects could this worsening be recognized by altered symptoms or impairments. Because serial measurements over time are needed to

show significant change, it would not be a useful minimal criterion for DSPN in clinical practice.

Is there an explanation for why the use of normal limits does not detect the latent functional abnormalities reported here? Perhaps the major reason is that normal NC values vary widely over a broad range (up to 100%) so that a change in nerve function is not readily recognized when values change within these normal limits. Following the course of nerve function, therefore, has clearly been shown to be more sensitive in recognizing such change.

The third question that our studies address is "Is there evidence that the functional worsening, mainly within the range of normal nerve conduction test values, which we have demonstrated, is mean-

Table 4—Risk covariates for monotone worsening of nerve conductions in group 2 patients

Continuous univariate risk factors

Characteristics	n	Spearman correlation coefficient	P*
Age at last visit	90	0.2465	**0.0192**
Average height	90	0.0047	0.9653
Average weight	90	0.0453	0.6717
Average body surface area	90	0.0358	0.7377
Average BMI	90	0.0562	0.5989
Average pulse	90	−0.1974	0.0621
Average systolic blood pressure	90	0.4385	**<0.0001**
Average diastolic blood pressure	90	0.0702	0.5110
Average energy expenditure (per person)	90	−0.1507	0.1563
Average 24-h proteinuria	90	0.4132	**0.0001**
Average 24-h microalbuminuria	87	0.4662	**<0.0001**
Duration of diabetes at last visit	90	−0.0587	0.5827
Average fasting plasma glucose	90	0.0918	0.3894
Average A1C	90	0.2919	**0.0052**
Average creatinine	90	0.2854	**0.0064**
Average cholesterol	90	0.1755	0.0980
Average triglycerides	90	0.1876	0.0767
Average HDL	90	−0.0764	0.4743
Average apolipoprotein A-I	90	−0.0761	0.4760
Average apolipoprotein A-II	90	0.0281	0.7924
Average apolipoprotein E	90	0.2421	**0.0215**
Average apolipoprotein B	90	0.1619	0.1274
Average lipoprotein(a)	90	−0.0626	0.5576
Smoking (pack-years) in last year	88	0.0936	0.3857
Alcohol drinking in last year	88	0.2589	**0.0149**
Smoking (pack-years) now	90	0.0316	0.7671
Alcohol drinking now	90	0.2088	**0.0483**
Average $A1C^{1/2}$ × duration of diabetes at last visit$^{1/8}$	90	0.2190	**0.0381**
Nephropathy stage	90	0.3568	**0.0006**
Retinopathy stage	90	0.1036	0.3314

Dichotomous univariate risk factors: characteristics	n		Median		Z statistic	P†
	1st	2nd	1st	2nd		
Last available type of diabetes by C-peptide (type 1/type 2)	26	64	0.1790	0.3030	−2.4170	0.0157
Sex (women/men)	40	50	0.2565	0.2870	−0.8364	0.4029

Multivariate risk factors: independent variable	Parameter estimates	SE	t statistic	P‡
Intercept	0.4443	0.0429		
$-1/(24\text{-h microalbuminuria}^{1/2})$	0.5781	0.1318	4.39	**0.001**

Change for variable periods up to 18 years. Data in bold are P values <0.05. *Two-sided Spearman rank correlation test. †Wilcoxon rank-sum test. ‡Least-squares multiple regression

ingful and relates to diabetes?" (39) The fact that the composite measure of nerve conductions (Σ 5 NC nds) significantly worsened in 42 of 90 patients and improved in only 1 patient and that this worsening was not explained by a change in age, height, or weight means that it is attributable to the diabetic condition. Furthermore, many univariate risk factors were related to this worsening. In multivariate analysis, 24-h microalbuminuria remained as the significant risk factor. In a previous study of the entire RDNS cohort, we showed that several markers of microvessel disease (retinopathy and 24-h proteinuria) and mean HbA_{1c} (A1C) assessed serially (and many times) over time were the major risk covariates for severity

of diabetic sensory polyneuropathy at last evaluation (14). (See also ref. 39 for a discussion of the role of vascular risk factors in DSPN.)

The present evidence of early asymptomatic worsening may be useful for conducting epidemiological and prospective controlled clinical trials. We advocate use of composite nerve conduction test scores for epidemiological and prospective controlled clinical trials because they have been shown to be sensitive and reproducible, to indicate monotone change with time, and to correlate with neuropathic impairment. The magnitude of change considered to be meaningful could be estimated by correlative studies of the degree of change of sum scores of nerve conduction tests equivalent to a clinical meaningful change (40).

APPENDIX

Derivations

CDT is the cooling detection threshold. In CASE IV testing, it is expressed as the just noticeable difference test level or step (from 1 to 25), Δ°C from 30°C, percentile or normal deviate considering modality, site, age, sex, and applicable physical variables.

DSPN indicates diabetic sensorimotor polyneuropathy; other causes of this pattern of polyneuropathy have been considered and excluded. The contribution of other diabetic neuropathies should also have been excluded (cranial, entrapment, or radiculoplexus neuropathies [cervical, thoracic or lumbosacral]).

The NIS consists of a summation of 37 items of the neuromuscular examination with muscle weakness graded from 0 = normal to 4 = paralyzed and tendon reflex and sensation loss graded 0 = normal, 1 = decreased, and 2 = absent. The NIS score may vary from 0 to 244 points. Abnormality is scored taking age, sex, height, weight, and physical fitness into account. Scores for the NIS of the lower limbs may vary from 0 to 88 points.

The NSC is a 38-item score of the number, severity, and change of neuropathic symptoms.

Σ 5 NC nds indicates summated normal deviates (nds) of peroneal motor nerve amplitude, velocity, and distal latency, tibial motor nerve distal latency, and sural nerve amplitude (point A). For each attribute of nerve conduction, the percentile value is estimated for age and applicable variables. Abnormality is expressed in the upper tail of the normal distribution. To illustrate, for amplitude and velocity a percentile of 5 is changed to 95 and so on. Each percentile is converted to a normal deviate value (e.g., the 50th percentile is 0 nd, the 5th is ~ -1.6, and the 95th is $\sim +1.6$. The nds of the five attributes are summed, divided by the number of measurable variables (when amplitude is 0 velocity and latency cannot be measured), and multiplied by the number of tests in the composite score (in this case five). Σ per, tib, ul, and MNDL are the peroneal, tibial, and ulner motor nerve distal latencies, respectively, normal deviate values from percentiles expressed in the upper tail of the normal distribution and then summated by the number of measureable values and multiplied by three.

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