Risk Factors for Severity of Diabetic Polyneuropathy

Intensive longitudinal assessment of the Rochester Diabetic Neuropathy Study cohort

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OBJECTIVE — Chronic hyperglycemia relates to the occurrence of diabetic polyneuropathy (DPN), but has not yet been shown to relate to its overall severity. In addition, the degree and duration of hyperglycemia, which measure of chronic hyperglycemia is most predictive of defined levels of severity of DPN, and which other putative risk factors are involved remain unknown.

RESEARCH DESIGN AND METHODS — In a longitudinal study of 264 diabetic individuals in Rochester, MN, risk factors and other diabetic complications assessed at regular intervals during an average of ~7 years were tested for their association with a composite score of severity of DPN at the last examination.

RESULTS — In multivariate analysis, diabetic retinopathy severity level (at last examination), mean ln(24-h proteinuria × duration of diabetes), and mean GHb were the main covariates for severity of DPN (R^2 = 0.33). Excluding markers of microvessel and macrovessel disease, the independent risk factors were mean ln(GHb × duration of diabetes), GHb, and type of diabetes (R^2 = 0.23).

CONCLUSIONS — We found that diabetic microvessel disease, chronic hyperglycemia exposure, and type of diabetes are associated with severity of DPN, and we believe these factors are implicated in its cause. Each of the five markers of microvessel disease was a strong covariate for severity of DPN. Mean GHb predicts severity of DPN better than duration of diabetes, and the latter predicts severity of DPN better than mean fasting plasma glucose. Knowing the severity of microvessel disease, the degree of chronic hyperglycemia exposure, and the type of diabetes provides useful information to evaluate whether a coexisting polyneuropathy and its severity is probably due to diabetes.

Diabetes Care 22:1479–1486, 1999

Prospective population-based studies like the one described herein are useful for ascertaining the frequency, severity, and risk factors for disease complications such as diabetic polyneuropathy (DPN) (1). However, the risk factors that are identified may provide important clues to etiology, or they may merely reflect epiphenomena or chance associations. The probability that a particular risk factor is implicated in the pathophysiology of a complication increases when the same risk factor is found consistently in different studies, when the factor relates not only to occurrence but also to severity of the outcome, and when there is a plausible mechanistic link between the factor and the disease complication. Ultimately, mechanistic studies or controlled clinical trials may be needed to establish that the risk factor is in fact implicated in causing the complication. Based on epidemiological studies and controlled clinical trials, total hyperglycemia exposure has been shown to be an important risk factor for the occurrence of DPN but has not yet been shown to relate to its severity. In addition, which marker of chronic hyperglycemia exposure is the best and the degree of near euglycemia needed to prevent the occurrence of DPN are known only approximately (2–5). Furthermore, the degree of severity of DPN (6) that can be prevented or improved by different levels of near euglycemia remains unknown.

Apart from chronic hyperglycemia (see CONCLUSIONS), there is only weak and inconsistent evidence implicating other risk factors such as older age, height, male sex, failure to lose weight, heart rate, decreased sinus arrhythmia, smoking, and lipoprotein concentration. These reported associations with DPN need reassessment because they are usually based only on univariate analysis because anthropometric characteristics were not adequately corrected for in reference values (7,8) and because other closely related complications were treated as covariates and may have masked recognition of causative factors (see CONCLUSIONS). In addition, inconsistencies in identified risk factors for DPN may have been due to methodological problems, mainly of four types: *1*) studies were generally not prospective and population based; *2*) risk factors were not assessed serially, over long periods, or at times unrelated to intercurrent illness; *3*) unrelated neurological disease or other diabetic neuropathies were not sufficiently ruled out; and *4*) severity of DPN was not assessed quantitatively and comprehensively (6). In most previous

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Received for publication 26 February 1999 and accepted in revised form 7 May 1999.

Abbreviations: apo, apolipoprotein; dBP, diastolic blood pressure; DPN, diabetic polyneuropathy; FPG, fasting plasma glucose; HS, healthy subject; Lp(b), lipoprotein(b); NIS(LL)+7 tests, Neuropathy Impairment Score of Lower Limbs plus 7 tests; RDNS, Rochester Diabetic Neuropathy Study; sBP, systolic blood pressure.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Calculating the NIS(LL)+7 tests score*

- Sum individual scores of the NIS(LL) (items 17–24, 28 and 29, and 34–37 of the NIS).
- In the NIS(LL), substitute transformed points for percentile abnormality* of vibration detection threshold for each great toe (obtained with CASE IV) for the clinical vibration sensation point score of great toes.
- Add transformed points for percentile abnormality* of heartbeat variation with deep breathing (one time only).
- Sum transformed points for percentile abnormality* of the five attributes of nerve conduction of lower limb (peroneal nerve [CMAP, MNCV, and MNDL], tibial nerve [MNDL], and sural nerve [sural sensory nerve action potential]) and divide by the number of attributes with obtainable values†, multiply by 5 (the number of attributes), and add this number to the global score.

*<95th = 0, ≥95th–99th = 1, ≥99th–99.9th = 2, and ≥99.9th = 3 (or >5th = 0 . . . ≤0.1th = 3, whichever end of the distribution is abnormal); †MNCV and MNDL cannot be estimated when CMAP is 0. CMAP, compound muscle action potential; MNCV, motor nerve conduction velocity; MNDL, motor nerve distal latency.

studies, therefore, risk factors were related to the presence or absence of DPN based on a limited evaluation without the use of standardized examinations or tests, without adequate reference values, and without quantitation of severity of DPN. As we have shown, estimation of the overall severity of DPN requires a composite and continuous score of severity that encompasses most or all of the major manifestations of DPN, with scoring of the abnormality based on reference values (percentiles) obtained from hundreds of healthy subjects without neurological disease drawn from the same population as the patients (6–10). In this study, we avoided many of the shortcomings of previous studies by assessing risk factors prospectively and on multiple occasions for up to 10 years and evaluated their association with a composite score of DPN, the Neuropathy Impairment Score of Lower Limbs plus 7 tests [NIS(LL)+7 tests] at the last evaluation. This composite score provides a robust measure of severity of DPN and is able to detect worsening of serial measurement in a cohort of diabetic patients (6).

RESEARCH DESIGN AND METHODS

The Rochester Diabetic Neuropathy Study (RDNS) cohort

This assessment of risk factors and severity of DPN is based on the longitudinal assessment of a representative cohort of patients with diabetes in Rochester, MN, many of whom were followed for more than 10 years. In previous reports, we have outlined the unique reasons why population-based studies are possible in this population (9,10). On 1 January 1986, all patients with diabetes (n = 870, by National Diabetes Data Group criteria [11]) who resided within the geographic confines of Rochester were invited to participate in a cross-sectional and longitudinal study of diabetic complications (neuropathies, retinopathies, and nephropathies and atherosclerotic complications and outcomes). Of those patients, 380 agreed to participate (9,10). Generally, patients were of northern European descent. We had evidence that comorbidity was not significantly different between the consenting and nonconsenting groups for patients aged <70 years. Generally, the diabetic cohort appeared to be representative of unselected diabetic patients from the community regarding complications (9,10,12).

Of the prevalence cohort of 380 patients at baseline, 264 were included in this longitudinal analysis. The composite score could not be calculated at the last examination in 116 patients because of confounding neurological disease (n = 28), missing test results (refusal or technical error [n = 17], disabling illness or death [n = 33], and moving out of the region or dropping out of the study [n = 38]). Although the nonparticipants (116) were different from the participants (264) regarding the criterion of confounding neurological disease or disabling illness or death, they were not significantly different in their metabolic control (mean GHb 10.3 and 10.2%, respectively, P = 0.88), diabetic complications (mean plasma creatinine 1.17 and 1.19 mg/dl, respectively, P = 0.51), or retinopathy severity level (R0–R3 18.1, 27.6, 15.5, and 7.8% and 31.1, 26.9, 26.5, and 11.0%; P = 0.25).

The healthy subject (HS) cohort of the RDNS (HS-RDNS)

The neuropathic symptoms, impairments, and test results used to determine the composite measure of severity of DPN [NIS(LL)+7 tests] had also been evaluated in a random sample of Rochester residents. Healthy subjects (15 men and 15 women for each hemidecade between ages 18 and 74 years who were without neurological disease or neuropathy or other diseases known to predispose to neuropathy) were recruited as an HS cohort (8). Percentile responses specific for age, sex, and other applicable anthropometric characteristics (e.g., height, weight, surface area, or BMI) were estimated for attributes of nerve conduction, heartbeat variation with deep breathing, vibration detection threshold, cooling detection threshold, and heat pain threshold (7,8). These estimated percentile responses provide a better measure of abnormality than values adjusted only for age because anthropometric features influence percentile estimates.

The NIS(LL)+7 tests for overall assessment of severity of DPN

Of the approaches tested, the composite score of the NIS(LL)+7 tests provided the best overall measure of severity of DPN (6). These tests combine the major sensory, autonomic, and motor weakness impairments and test abnormalities by transforming test percentile abnormalities into points so they can be added to the neuropathy impairment score. The version of the NIS(LL)+7 tests used here is shown in Table 1.

Risk factors assessed

The covariates assessed were evaluated on many occasions (Tables 2–6) at regular intervals for several years to obtain a representative measure of the variable uninfluenced by intercurrent disease. Nine standard photographs of the retina of each eye were graded for diabetic retinopathy severity level (R0–R3) by personnel in the Wisconsin Reading Center under the supervision of Dr. Ronald Klein. Standard foot X rays of RDNS patients and healthy subjects were randomly assigned and read by Dr. John Beabout at the Mayo Clinic with their disease status masked for calcification of foot arteries.

Data analysis

In testing for associations between most putative risk factors and DPN, we used averaged (calculating the average value for each year and then the mean of these annual values) longitudinal values of risk factors as the independent variable and

Table 2—*Characteristics of patients with diabetes in the RDNS

Characteristics	Type 1 diabetes		Type 2 diabetes			Duration of study (years)	Evaluations (*n*)
	n	Mean ± SD	*n*	Mean ± SD	*P*	(mean ± SD)	(mean ± SD)
Age (years)*	97	52.1 ± 16.6	149	69.7 ± 9.7	0.0001	—	—
Height (cm)	97	168.3 ± 10.4	149	166.2 ± 9.4	NS	5.7 ± 2.7	20.1 ± 11.2
Weight (kg)	97	77.1 ± 14.3	149	84.8 ± 16.9	0.0003	5.7 ± 2.7	19.9 ± 11.1
Body surface area (m^2)	97	1.9 ± 0.2	149	1.9 ± 0.2	0.0319	5.7 ± 2.7	6.3 ± 2.8
BMI (kg/m^2)	97	27.3 ± 5.1	149	30.7 ± 5.7	0.0001	5.7 ± 2.7	6.3 ± 2.8
Pulse (beats/min)	97	79.6 ± 7.1	148	77.6 ± 7.8	0.0100	5.7 ± 2.7	19.9 ± 11.0
sBP (mmHg)	97	127.2 ± 15.5	149	142.3 ± 14.3	0.0001	5.7 ± 2.7	20.0 ± 11.1
dBP (mmHg)	97	76.1 ± 7.4	149	80.1 ± 7.6	0.0001	5.7 ± 2.7	20.0 ± 11.1
Energy expended (kcal)‡	80	1,284 ± 219	100	1,162 ± 154	0.0001	6.9 ± 1.5	3.0 ± 1.3
Proteinuria (mg/24 h)	93	308 ± 636	135	278 ± 668	NS	6.2 ± 2.3	5.5 ± 2.5
Proteinuria concentration (mg/24 h)‡	93	18.5 ± 35.6	135	15.0 ± 31.2	NS	6.2 ± 2.3	5.5 ± 2.5
Microalbuminuria (mg/24 h)‡	90	146.3 ± 379.4	122	147.7 ± 442.4	NS	6.4 ± 2.1	3.8 ± 1.9
Duration of diabetes (years)*	97	20.8 ± 9.9	149	14.8 ± 7.7	0.0001	—	—
FPG (mg/dl)	97	178.9 ± 40.5	149	177.7 ± 42.2	NS	5.7 ± 2.7	20.3 ± 11.1
GHb (%)	97	10.8 ± 2.0	149	10.1 ± 2.0	0.0028	5.7 ± 2.7	20.3 ± 11.2
Creatinine (mg/dl)‡	97	1.1 ± 0.6	149	1.1 ± 0.3	0.0774 (NS)	5.7 ± 2.7	6.1 ± 2.9
Cholesterol (mg/dl)†	97	186.1 ± 36.2	149	199.8 ± 35.7	0.0022	5.7 ± 2.7	10.4 ± 5.4
Triglycerides (mg/dl)†	97	113.7 ± 70.8	149	192.7 ± 122.2	0.0001	5.7 ± 2.7	10.4 ± 5.4
HDL cholesterol (mg/dl)†	97	46.0 ± 14.4	149	36.4 ± 10.3	0.0001	5.7 ± 2.7	10.4 ± 5.4
apo(A-I) (mg/dl)†	97	136.5 ± 19.7	149	128.0 ± 16.0	0.0012	5.7 ± 2.7	10.4 ± 5.4
apo(A-II) (mg/dl)†	92	31.4 ± 5.1	148	31.3 ± 4.8	NS	3.9 ± 1.9	7.8 ± 3.3
apo(E) (mg/dl)†	97	4.9 ± 1.2	149	6.0 ± 1.4	0.0001	5.5 ± 2.5	9.9 ± 4.9
apo(B) (mg/dl)†	77	89.3 ± 22.6	102	104.2 ± 24.5	0.0001	7.0 ± 1.6	4.5 ± 2.4
Lp(a) (mg/dl)†	77	21.8 ± 18.0	102	20.8 ± 19.2	NS	6.6 ± 1.3	3.8 ± 1.8
Smoking (pack-years)*							
Now	96	3.5 ± 11.6	147	5.4 ± 17.5	NS	—	—
In last year	82	6.0 ± 16.2	124	2.8 ± 13.0	0.0189	—	—
Alcohol							
Drinking now	96	14.9 ± 35.6	148	26.3 ± 77.8	NS	5.7 ± 2.7	20.2 ± 11.1
Drinking within last year	82	15.9 ± 38.1	124	24.2 ± 74.0	NS	5.7 ± 2.7	20.2 ± 11.1

*Variables were averaged over 0–10 years of the study except as indicated. All characteristics were evaluated every 3 months, except for those marked with †, which were evaluated every 6 months, and those marked with ‡, which were evaluated every 12 months. For type 1 diabetes, men: 47.4%, insulin: 94.8%, and oral hypoglycemic drugs: 3.1%. For type 2 diabetes, men: 54.6%, insulin: 53.0%, and oral hypoglycemic drugs: 31.5%.

composite scores of neuropathic impairment [NIS(LL)+7 tests] at the last evaluation (as described above) as the dependent variable. Associations between severity and risk factors were evaluated univariately with Spearman rank correlations for quantitative risk factors and with rank-sum tests for dichotomous risk factors. For a multivariate assessment, stepwise regression was used, stepping up and down, as was the Kendall τ statistic for the correlation matrix as described in O'Gorman and Woolson (13). The criterion for inclusion of a variable in the model was $P < 0.05$. Variables identified by stepwise regression were then inspected for departures from a normal distribution, and appropriate transformations were made as necessary. A final model was then obtained using least-squares multiple regression. Because we anticipated that the effect of duration of diabetes and such markers of glycemic control might not be additive, we did not require main-effect terms in the model when considering the cross-product term.

RESULTS

Characteristics of the RDNS cohort

The 264 subjects ranged in age from 15 to 89 years (mean 62.8 years). There were approximately equal numbers of men (*n* = 138) and women (*n* = 126) (Table 2). Of the subjects, 149 patients had type 2 diabetes, 97 had type 1 diabetes, and 18 were not classified by their C-peptide response to glucagon. The cohort had a higher percentage of type 1 diabetes than was ascertained for the Rochester population (23%) (10). Their known duration of diabetes varied from 2 to 74 years (mean ± SD 17.1 ± 9.0 years). Diabetes treatment included insulin (*n* = 183 patients), oral hypoglycemic agents (*n* = 52), and no hypoglycemic agents (*n* = 29). The cohort was followed for up to 10 years (mean 6.9 years) and had an average of 5.7 assessments of NIS(LL)+7 tests to characterize the frequency and severity of DPN.

Statistically significant differences separated patients with type 1 and 2 diabetes (Table 2). On average, type 1 diabetic patients had diabetes for a longer period (20.8 years) than type 2 diabetic patients (14.8 years). Type 2 diabetic patients were significantly older (by 18 years on average), heavier (by ~7.7 kg), had a higher BMI, had higher blood pressure (sBP and dBP), had lower HDL cholesterol values, and had higher triglyceride values than type 1 diabetic patients. The percentage of type 2 diabetic patients using insulin was also lower than for type 1 diabetic patients

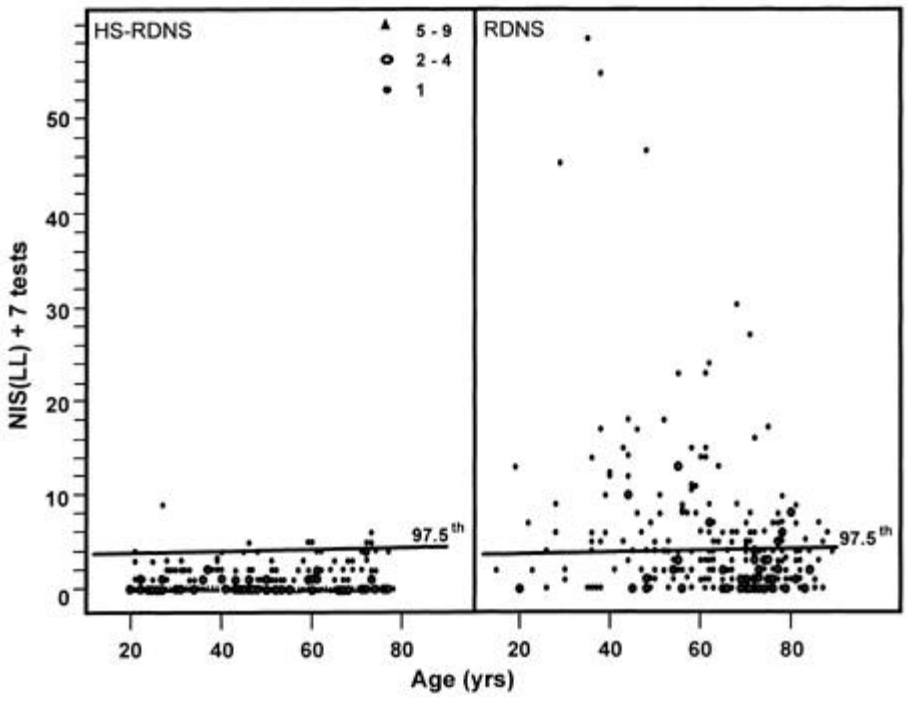

Figure 1—*The point values of NIS(LL)+7 tests plotted by age for each healthy subject in the HS-RDNS cohort* (left) *and for each patient with diabetes in the RDNS cohort* (right). *The composite score [NIS(LL)+7 tests] provides an overall measurement of severity of DPN based on an assessment of the sensory, autonomic, and motor impairments. In the RDNS-NS cohort, eight patients are above the 97.5th percentile line, and they are widely distributed over the age range, an indication that the algorithm used to estimate percentile lines has performed satisfactorily. In the RDNS cohort, about 36% of patients had values above the 97.5th percentile line. Note that the composite scores of patients with DPN vary widely in severity.*

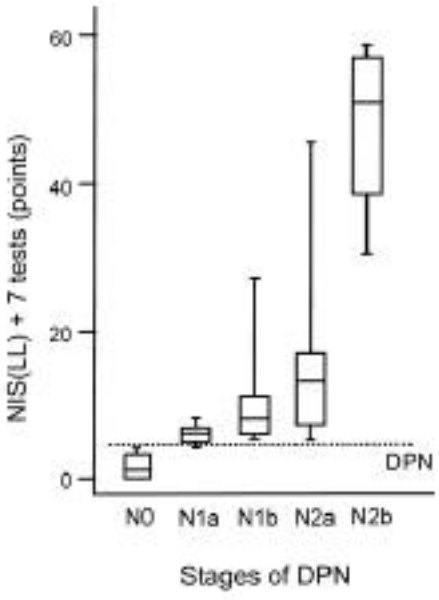

Figure 2—*The distribution of NIS(LL)+7 tests values for diabetic patients in the RDNS plotted by stage of severity of DPN. The stages are: N0 = no neuropathy and NIS(LL)+7 tests <97.5th,* n = *169; N1a = no neuropathic symptoms, NIS(LL)+7 tests ≥97.5th, and NIS(LL) <2 points,* n = *12; N1b = no neuropathic symptoms, NIS(LL)+7 tests ≥97.5th, and NIS(LL) <2 points,* n = *58; N2a = neuropathic symptoms are present, NIS(LL)+7 tests ≥97.5th, and dorsiflexion ankle muscles are <4 points,* n = *21; and N2b = neuropathic symptoms are present, NIS(LL)+7 tests ≥97.5th, and dorsiflexion muscles of leg are ≥4 points,* n = *4. The 25th, 50th, and 75th percentile values and the range are shown. Although there is an overlap of values, staged severity reflects severities of DPN.*

(53.0 vs. 94.8%, respectively, $P < 0.001$). Average FPG was not significantly different between the two diabetic disorders. On the other hand, mean GHb was significantly lower in type 2 patients. Diabetic retinopathy occurred in 58.7% of patients with diabetes: R1 (early nonproliferative) = 25.0%, R2 (late nonproliferative) = 22.4%, and R3 (proliferative) = 11.4%. Calcification of leg or foot vessels was observed in 10 of 45 individuals with type 1 diabetes and in 12 of 55 individuals with type 2 diabetes.

Severity of DPN

Figure 1 shows the distribution of NIS(LL)+7 tests values of the HS-RDNS cohort compared with RDNS patients at the last evaluation. Superimposed is the 97.5th percentile line as estimated from the HS-RDNS data. According to this criteria, 36% of the study cohort had DPN. By using the criterion of an abnormality (≥99th or ≤1st percentile, depending on which distribution tail is abnormal) of at least one nerve conduction attribute in two or more nerves, 36.4% of the cohort were abnormal. In contrast, only 16.7% were abnormal according to the criterion of decreased heartbeat variation with deep breathing (≤1st percentile).

Figure 2 shows the distribution of severity of DPN as measured by the NIS(LL)+7 tests by staged severity of DPN. In general, higher stages of DPN had increasingly greater NIS(LL)+7 tests scores. Although the frequency of DPN is high, severity of DPN was usually mild. In the study cohort, only 9.5% were symptomatic, 1.5% had stage 2B neuropathy (weakness of ankle dorsiflexion), and none had stage 3 neuropathy.

Risk factors for severity of DPN

Based on univariate analysis, >20 covariates were statistically associated with severity of DPN (Tables 3 and 4). To identify the most important risk factors for severity of DPN, we performed a series of multivariate analyses. In each analysis, we performed a stepwise multiple regression analysis by selecting risk factors from a list of candidate variables. The resulting best model associated with each list is reported. A measure of how closely each model correlated with severity is provided by the corresponding model R^2 value, where $R^2 \times 100$ is the percentage of variability in the NIS(LL)+7 tests explained by the model.

When all significantly associated univariate risk factors were included in multivariate analysis, only foot vessel calcification and retinopathy severity level (for type 1 diabetes), mean GHb (for type 2 diabetes) and retinopathy severity level, ln (duration of diabetes × mean 24-h proteinuria), and mean GHb (for all diabetes) were independent risk factors for severity of neuropathy (Table 5).

To test which of the five putative markers of microvessel disease (retinopathy severity level at last examination, mean 24-h proteinuria, mean 24-h microalbuminuria, or the product of the latter two markers and duration of diabetes) provided the strongest associations with severity of

Table 3—*Spearman rank correlation univariate analysis of continuous individual risk factors for composite neuropathy score [NIS(LL)+7 tests] at last evaluation*

	n	Spearman rank correlation coefficient	*P* value
Duration of diabetes	264	0.38	<0.001
FPG	264	0.17	0.006
GHb	264	0.42	<0.001
Duration of diabetes × FPG	264	0.43	<0.001
Duration of diabetes × GHb	264	0.50	<0.001
24-h proteinuria*	244	0.31	<0.001
24-h proteinuria (concentration)*	244	0.28	<0.001
24-h microalbuminuria*	228	0.29	<0.001
Pulse	263	0.24	<0.001
Age	264	–0.21	<0.001
Smoking (smoking within last year) (pack-years)	219	0.16	0.017
Stage of retinopathy*	232	0.49	<0.001
Stage of retinopathy × 24-h proteinuria	218	0.45	<0.001
Stage of retinopathy × 24-h microalbuminuria	203	0.43	<0.001
Creatinine × 24-h proteinuria	244	0.25	<0.001
Creatinine × 24-h microalbuminuria	228	0.24	<0.001
Duration of diabetes × 24-h proteinuria	244	0.43	<0.001
Duration of diabetes × 24-h microalbuminuria	228	0.38	<0.001

Analysis is for variable periods up to 10 years. NIS(LL)+7 tests include neurological examination of selective items of lower limbs plus vibration detection threshold of the toe, heartbeat variation with deep breathing, and five attributes of nerve conduction. *P* values are by two-sided rank correlation test. All characteristics were evaluated every 3 months, except for those marked with *, which were evaluated every 12 months. Tested but not significant were height, weight, cholesterol, triglycerides, creatinine, HDL cholesterol, apo(A1), apo(A2), apo(E), apo(B), Lp(a), sBP, dBP, energy expenditure, 24-h urine total protein, BMI, body surface area, smoking (smoking now) (pack-years), and alcohol (drinking now and drinking within last year).

DPN, we used only one (excluding the other four in each case) of the diabetic microvessel disease markers in the multivariate stepwise analysis and compared the correlation coefficients (R^2) in the final model. Of the markers, 24-h proteinuria, 24-h microalbuminuria, or the product of these two markers and duration of diabetes all had R^2 values of 0.48. Retinopathy severity level alone had a slightly lower value of 0.43.

On the assumption that markers of microvessel disease (e.g., retinopathy severity level, mean 24-h proteinuria, mean 24-h microalbuminuria, and the product of the latter two and duration of diabetes) and macrovessel disease (e.g., foot vessel calcification) are complications of diabetes themselves and are not directly the cause of DPN, we repeated the multivariate analysis excluding these markers (Table 6). For patients with type 1 diabetes, the independent covariate associated with severity of DPN was ln (GHb × duration of diabetes). For type 2 diabetes, the independent risk factor was mean GHb. For both type 1 and 2 diabetic patients combined, the covariates were mean ln (GHb × duration of diabetes), mean GHb, and type of diabetes.

To test which of the measures of total hyperglycemic exposure (mean FPG, mean GHb, duration of diabetes, mean FPG, or GHb × duration of diabetes) provided the highest correlation coefficient, we performed stepwise multivariate analysis testing on only one hyperglycemia marker and excluded the other four in the multivariate model. The highest to the lowest correlation coefficients were: mean GHb (R^2 = 0.19), mean GHb × duration of diabetes (R^2 = 0.16), duration of diabetes (R^2 = 0.15), mean FPG × duration of diabetes (R^2 = 0.13), and mean FPG (R^2 = 0.10).

CONCLUSIONS — The present study addresses four important questions in DPN research. *1*) What are the risk factors and other diabetic complications that are associated with severity of DPN? *2*) Which measure of diabetic microvessel disease best predicts severity of DPN? *3*) Which measure of total hyperglycemic exposure (duration of diabetes, mean GHb, mean FPG, mean GHb × duration of diabetes, or mean FPG × duration of diabetes) best predicts severity of DPN? *4*) Should other risk factors, in addition to the ones tested here, be evaluated in future assessments? This study was designed to answer these questions by analysis of data from a longitudinal study that minimized the sources of variability.

It is possible to group the independent covariates we studied into several broad mechanistic classes, such as microvessel disease (retinopathy severity level, mean 24-h proteinuria, mean 24-h microalbuminuria, and the product of mean 24-h proteinuria or mean 24-h microalbuminuria and duration of diabetes), total chronic hyperglycemic exposure (mean FPG, mean GHb, duration of diabetes and the product of mean FPG or mean GHb × duration of diabetes), kidney failure (mean creatinine), and type of diabetes. Our results show that all of these factors (except for kidney failure) are associated with severity of DPN. The order of importance appears to be diabetic microvessel disease, total hyperglycemic exposure, and type of diabetes.

Diabetic microvessel disease has been implicated in causing diabetic retinopathy, nephropathy, and polyneuropathy. The degree and duration of proteinuria or microalbuminuria are predictors of diabetic

Table 4—*Wilcoxon's rank-sum univariate analysis of dichotomous individual risk factors for composite neuropathy score [NIS(LL)+7 tests] at last evaluation for all patients with diabetes*

	n		Median NIS(LL)+7 tests		
	Present	Absent	Present	Absent	*P*
Type 1 and 2 diabetes					
Type 1 diabetes by C-peptide	97	149	5	2	<0.001
Insulin treatment	193	71	4	1	<0.001
Oral hypoglycemic agents	94	170	2	4	0.002
Calcification of vessels in foot*	22	82	7	1	<0.001

*Evaluated on one occasion. Analysis is for variable periods up to 10 years. NIS(LL)+7 tests include neurological examination of selective items of lower limbs plus vibration detection threshold of the toe, heartbeat variation with deep breathing, and five attributes of nerve conduction. *P* values are by two-sided rank-sum test. Sex was tested, but not significant.

Table 5—*Significant risk factors for NIS(LL)+7 tests in multivariate least-squares regression*

	Variables in model	Parameter estimates	Model R^2	*P* value
NIS(LL)+7 tests				
Type 1 and 2 diabetes (*n* = 218)	Intercept	−15.4724		—
	Retinopathy severity level	1.8954 ± 0.4333		<0.001
	ln(duration of diabetes × 24-h proteinuria)	1.4544 ± 0.3584		<0.001
	GHb	0.6675 ± 0.2042	0.33	0.001
Type 1 diabetes (*n* = 41)	Intercept	1.7453		—
	Calcification of vessels in foot	9.0719 ± 1.8916		<0.001
	Retinopathy severity level	1.5742 ± 0.7392	0.62	0.040
Type 2 diabetes (*n* = 149)	Intercept	−6.8369		—
	GHb	0.9583 ± 0.1326	0.26	<0.001

Data are means ± SEM. All significant univariate factors are included.

retinopathy (14,15) and nephropathy (16). In nondiabetic populations, microalbuminuria may be a predictor for vessel disease (17,18). We show here that proteinuria and microalbuminuria are strong predictors for severity of DPN, an association not previously emphasized.

Considerable earlier evidence indicates that diabetic microvessel disease is implicated in causing DPN (and diabetic retinopathy and nephropathy). A correlation exists between the occurrence of retinopathy and DPN (10,19). This study shows that graded severity of retinopathy is strongly associated with severity of DPN. Early studies of biopsied nerve reported occlusion (20) or closure of nerve microvessels (21) and basement membrane reduplication associated with pericyte degeneration (22). More recently, we were not able to confirm microvessel occlusion or closure (23,24). More importantly, we have shown that pericyte degeneration and reduplicated basement membranes (22) begin before development of DPN and increase in severity as DPN worsens (23,24). In the present study, retinopathy severity level, mean 24-h proteinuria, mean 24-h microalbuminuria, and the product of the latter two and duration of diabetes provide approximately similar correlates of DPN. Because they are all thought to be markers of microvessel disease, the concept that microvessel disease is implicated in causing DPN is strengthened by the finding that all of these markers are correlated with severity of DPN. However, we do not believe that retinopathy, proteinuria, or combinations per se cause DPN. We suggest that these are merely markers of microvessel disease that presumably also affect endoneurial microvessels. Hypoxia and ischemia may result from microvessel disease, or, alternatively, the damaged endoneurial microvessels may be more leaky and allow plasma constituents to enter the endoneurial microenvironment. There is evidence that entry of plasma constituents into the endoneurial microenvironment is harmful (25,26).

The second group of risk factors that correlate with severity of DPN relate to total hyperglycemia exposure. In this study, we found that all of the markers of this exposure were correlated with severity of DPN. In addition, we provide additional evidence that the degree of chronic hyperglycemia exposure relates to graded severity of DPN. Our study compares various measures of chronic hyperglycemia exposure as predictors of severity of DPN. Mean GHb × duration of diabetes, mean GHb, and even duration of diabetes appear to perform better than mean FPG or the product of mean FPG × duration of diabetes. Clearly GHb performs better than FPG when both are measured at 3-month intervals.

Although previous studies were not ideal in many respects, they have provided useful insights concerning the role of hyperglycemia and DPN. For example, based on cross-sectional data, Gregersen (27) found that ulnar and peroneal motor nerve conduction velocities were related to duration of diabetes and to poor glycemic control. Likewise, Pirart (28,29) attributed absent ankle reflexes (and later decreased vibration sensation of the feet) to poor

Table 6—*Significant risk factors for NIS(LL)+7 tests in multivariate least-squares regression*

	Variables in model	Parameter estimates	Model R^2	*P* value
NIS(LL)+7 tests				
Type 1 and 2 diabetes (*n* = 246)	Intercept	−16.0238		—
	ln(duration of diabetes × GHb)	3.4120 ± 0.9183		<0.001
	GHb	0.7993 ± 0.2318		<0.001
	Type of diabetes (by C-peptide)	−2.9341 ± 0.9441	0.23	0.002
Type 1 diabetes (*n* = 97)	Intercept	−41.5579		—
	ln(duration of diabetes × GHb)	9.3267 ± 1.9414	0.20	<0.001
Type 2 diabetes (*n* = 149)	Intercept	−6.8369		—
	GHb	0.9583 ± 0.1326	0.26	<0.001

Data are means ± SEM. All variables significant in the univariate analysis were used in stepwise linear regression except for retinopathy, calcification of vessels in foot, 24-h proteinuria, and microalbuminuria.

glycemic control, whereas Graf et al. (30) and Porte et al. (31) found that glycemic control, as measured by FPG or GHb, correlated with the degree of slowing of nerve conduction in type 2 diabetes. In a study of young patients with type 1 diabetes, Young et al. (32) related deterioration of motor, sensory, and autonomic nerve function; retinopathy; and microproteinuria to poor glycemic control. Hillson et al. (33) found that initial sensory threshold, age, sex, mean FPG, and failure to become thinner related to vibratory perception threshold in type 2 diabetes; however, corrections were not made for factors that influence this threshold in health (e.g., age, sex, height, and weight). Thus, it is unclear whether diabetes accounted for the effect. By studying vibratory perception threshold with biothesiometry in young type 1 diabetic patients, Sosenko et al. (34) found that this threshold was unrelated to GHb in prepubertal patients but was related in older patients. In a study of a type 1 diabetes, Maser et al. (35) identified the following risk factors for DPN: duration of diabetes, GHb, smoking, and LDL cholesterol. In the San Luis Valley Diabetic Study, a study of 277 type 2 diabetic patients that used a standard neurological history and examination, Franklin (36) found that increasing age, longer duration of diabetes, higher values of GHb, and insulin use were the independently related risk factors for DPN. Ziegler et al. (37), when testing somatosensory potentials, reported that abnormality related to stage of neuropathy rather than to glycemic control or duration of diabetes.

More direct evidence of the importance of total hyperglycemia exposure is found in controlled clinical trials. In a small but seminal early study, C-peptide–deficient type 1 diabetic patients with abnormal nerve conduction were randomly assigned to continue conventional therapy or to receive insulin administered continuously to achieve near euglycemia (2). At 4 and 8 months, mean glycemic control was strikingly better in the rigorous versus the conventional treatment group; however, only at 8 months were attributes of nerve conduction ($P = 0.03$) and vibration detection threshold ($P = 0.002$) significantly better in the rigorously treated group. In a second intervention trial, Amthor et al. (3) randomly assigned 45 type 1 diabetic patients to continuous infusion or multiple injections of insulin or to continuation of conventional therapy for 4 years. During the next 4 years, patients could choose to remain on the same schedule or to cross over. At 8 years, patients were divided into two groups: those whose mean GHb averaged <10 or $>10\%$. Peroneal motor nerve conduction velocity had worsened during the 8 years by an average of 2.2 ± 5.3 m/sec in the good control group and by 4.8 ± 4.9 m/sec in the poor control group ($P < 0.01$). A similar difference was found for other attributes of nerve conduction and for heart rate variability with deep breathing. However, the strongest evidence that chronic hyperglycemia is an important risk factor in the complications of diabetes was obtained in the Diabetes Control and Complications Trial (4,6).

Previous studies have not identified a consistent list of risk factors, other than total hyperglycemia exposure, for DPN. This study provides an explanation for some of this variability. Previous investigators who did not adequately correct their examination and test reference values for anthropometric characteristics subsequently found some of them to be predictive for neuropathy. This is not to say that height, weight, surface area, or BMI may not be risk factors for DPN, but corrections must first be made for these characteristics in reference values. In our data, however, anthropometric characteristics were not risk factors, as was found by Hillson et al. (33) for age, sex, and weight and by Franklin et al. (36) for age. We could not confirm that smoking, insulin use, lipids, lipoprotein, or apolipoprotein were risk factors. Maser et al. (35), in studying type 1 diabetic patients, found smoking and LDL cholesterol to be factors in DPN, and Franklin et al. (36) found insulin use to be a factor in DPN.

What are the likely reasons that our models account for at most ~50% of the variability in severity? We suggest that there may be four reasons why the correlation coefficients in the final models were not higher. The first reason may be that we are still not adequately assessing overall severity of DPN. We do not believe this is the main reason because we are measuring most impairments, using quantitative approaches, have developed robust reference values, and have demonstrated that we can measure subtle worsening over time (6). The second reason may be that we have not modeled all applicable risk factors. For example, genetic factors likely influencing the diabetic disorder or neuropathy were not modeled. The third reason may be that risk factors may not have been measured at the right time (e.g., early in the diabetes) or for a sufficiently long time. The fourth, and perhaps the most important reason, may be that most of our study subjects had relatively low levels of severity of DPN, so most of the cohort is relatively homogenous. With a higher proportion of patients having high NIS(LL)+7 tests abnormalities, one would expect a markedly higher correlation coefficient. Our goal, however, was to describe associations as they exist in a population-based cohort rather than an artificially constructed cohort. As a consequence of the low R^2, we still cannot predict the time of onset, rate of progression, or likely severity of DPN from the risk factors measured in this study. However, we can infer the approximate time of onset and severity from the state of diabetic microvessel disease, the degree of chronic hyperglycemia exposure, and the type of diabetes. In a future study, we will attempt to develop predictive models of DPN based on these known risk factors. We note here that, although still imperfect, knowing a patient's stage of diabetic retinopathy, total hyperglycemia exposure, and type of diabetes helps to determine whether a patient's sensorimotor polyneuropathy is due to diabetes and perhaps even to predict its severity depending on the level of chronic glycemic control achieved.

Acknowledgments — This work was supported in part from grants received from the National Institute of Neurological Disorders and Stroke (NS-36797).

We are grateful to the patients with diabetes from Rochester, MN, who participated in these studies and to Carol Overland and Mary Lou Hunziker for preparing the manuscript.

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