Glycemic Control Is Related to the Morphological Severity of Diabetic Sensorimotor Polyneuropathy

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OBJECTIVE — The aim of the current study was to determine the independent clinical risk factors for predicting morphological severity of distal diabetic sensorimotor polyneuropathy (DSP) as determined by fiber density (FD) on sural nerve biopsy.

RESEARCH DESIGN AND METHODS — A total of 89 patients with both type 1 and type 2 diabetes, ascertained from a large therapeutic randomized clinical trial, were included in this observational cohort study. Morphological severity of DSP was expressed as the myelinated FD in the sural nerve biopsy. General linear models were used to assess the relationship between the morphological severity of DSP and various clinical risk factors.

RESULTS — Glycated hemoglobin (GHb) was significantly related to FD in univariate and multivariate regression analyses. This relationship was present in models in which GHb was handled either as a continuous variable or as a categorical variable with the highest significance level, with a GHb cutoff level of 9%. After dividing patients into groups with optimal to moderate (GHb ≤9%) and suboptimal (GHb >9%) glycemic control, the difference in FD between the two groups ranged between 3,461 and 2,334 per mm^2. FD was also significantly related to duration of diabetes and age of the patient.

CONCLUSIONS — The severity of peripheral DSP expressed by morphological criteria was significantly related to glycemic control in type 1 and type 2 diabetic patients. Inconsistent with previously published electrophysiological data demonstrating a correlation between height and conduction velocity, increasing height is not associated with morphological severity. Based on the results of the present study, it might be hypothesized that improving glycemic control will lessen severity of DSP in terms of FD loss in subjects with diabetes.

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Diabetic sensorimotor polyneuropathy (DSP) is a frequent complication of both type 1 and type 2 diabetes. Clinical risk factors associated with an increased risk of presence and severity of neuropathy have been identified in cross-sectional cohorts and prospective cohort studies. Correlations exist between the electrophysiological presence of DSP and demographic parameters such as age and male sex, as well as with glycated hemoglobin (GHb) and duration of diabetes. In addition to these, increasing height is a consistently reported clinical risk factor for severity of DSP (1–6). A recent report of a large, longitudinal cohort study (7) identifies determinants of microvessel disease (retinopathy and the mean ln{24-h proteinuria × duration of diabetes}) and mean glycohemoglobin as the strongest independent risk factors for the severity of neuropathy. The authors imply the need to adjust electrophysiological data for anthropomorphic factors before interpretation. Although less consistently, cardiovascular risk factors such as hypertension, smoking, hypertriglyceridemia, and decreased HDL cholesterol levels have also correlated with the electrophysiological presence of DSP (1–3). Conclusions from the effect of glycemic control in randomized controlled trials vary, likely resulting from the use of different criterion standards. The Diabetes Control and Complications Trial Research Group demonstrated a 60% risk reduction in the onset of DSP with intensive glycemic control using nerve conduction studies (NCS) as the surrogate end point (8,9). In comparing an intensive glycemic control group with standard therapy in patients with type 2 diabetes, the U.K. Prospective Diabetes Study Group demonstrated a 40% relative risk reduction in the development of DSP by quantitative vibration perception thresholds with a biosthesiometer (10).

Recently, Tkac and Bril (11) investigated the risk factors associated with the electrophysiological severity, rather than simply the presence, of DSP in a cross-sectional examination of a cohort of subjects with type 1 and type 2 diabetes. Glycemic control, as estimated by GHb, was identified as the best correlated modifiable risk factor for severity of DSP by univariate and multivariate analyses. Similarly, age, duration of diabetes, and height were identified as nonmodifiable risk factors.

Most cohort and experimental studies have focused on identification of risk factors for the presence and severity of DSP measured by electrophysiological parameters. Studies evaluating the risk factors for DSP severity using morphological assessments of neuropathy have not been reported to date. The aim of the current study was to determine the independent clinical risk factors for predicting the

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Abbreviations: CV, conduction velocity; DSP, distal sensorimotor polyneuropathy; FD, fiber density; NCS, nerve conduction studies; SUMAMP, sum of distal amplitudes; SUMCV, sum of nerve conduction velocities; VPT, vibration perception threshold.

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

morphological severity of DSP as determined by sural nerve fiber density (FD).

RESEARCH DESIGN AND METHODS

— The present cross-sectional survey of a cohort study included 89 patients with DSP. These patients were initially enrolled in a double-blinded, placebo-controlled, randomized clinical trial of the effects of acetyl-L-carnitine in patients with mild to moderate DSP with normal renal function. This group represents an unselected cohort of patients with mild to moderate DSP. All data were collected before randomization and initiation of treatment with the active study drug or placebo. The study was approved by the Toronto General Hospital (University Health Network) Research Ethics Board. Criteria for selection into the randomized clinical trial included patients with type 1 or type 2 diabetes; GHb ≥ 5.9% by affinity chromatography at screening; peripheral neuropathy diagnosed by abnormalities in two of four major categories (symptoms, signs, NCS, and quantitative sensory testing); and the presence of bilateral sural potentials ≥1 μV. Patients with peripheral neuropathy with principal causes other than diabetes, such as alcohol abuse, liver or renal disease, toxic exposure, inflammatory diseases, monoclonal gammopathies, or endocrine, metabolic, or nutritional disorders, were excluded from the present study.

Standard NCS of the dominant peroneal motor and sural nerves were performed on all subjects using the Counterpoint instrument (Medtronic, Mississauga, Canada). Recordings were performed with temperature control (32–34°C), careful distance measurements, and recording of well-defined and artifact-free responses. Surface silver-silver chloride discs (standardized size 4 × 7 mm) were used to record all nerve responses. Three NCS were performed within 2–3 weeks. Latencies and amplitudes were determined automatically, distance values were entered into the Counterpoint, and conduction velocities (CVs) were calculated automatically. The mean values of repeat nerve CVs and amplitudes were calculated. The sum of nerve CVs (SUMCV) and sum of distal amplitudes (SUMAMP) are composite variables created from the three examined nerves.

Table 1 —*Demographic characteristics of 89 patients with DSP*

n	89
Sex (M/F)	65/24
Type 1 diabetes/type 2 diabetes	18/71
Mean age (years ± SD)	54.2 ± 10.2
Mean duration of diabetes (years ± SD)	11.2 ± 8.8
Mean duration of DSP (years ± SD)	2.9 ± 3.8
Mean GHb (%)	8.5 ± 1.7
Retinopathy	26
Nephropathy	2
Hypertension	44
Erectile dysfunction	58
Smokers	51
Coronary artery disease	26
VPT (finger and toe, vibration units)	1.2 ± 0.9, 4.7 ± 3.2
Mean myelinated FD	3,025.784 ± 1,912.132
Peroneal motor amplitude (mV)	3.7 ± 2.3
Peroneal motor CV (m/s)	37.7 ± 5.5
Sural amplitude (μV)	4.6 ± 3.2
Sural CV (m/s)	38.3 ± 5.8

Data are means ± SD or % unless otherwise indicated.

Full-thickness sural nerve biopsies were performed at an anatomical location posterior to the lateral malleolus by an experienced and protocol-trained surgeon. The biopsies were performed using local anesthetic (1% lidocaine without epinephrine). A 7-cm segment of nerve was obtained with care to avoid tension on the nerve, sectioned, and prepared for analysis. One portion was fixed with glutaraldehyde, and another portion was frozen with dry ice; these sections were shipped to a central laboratory where they were recoded for blinded analysis. The nerve segments were postfixed in 1% osmium (4% sucrose, 1.5% $K_3Fe[CN]_6$ in cacodylate buffer), dehydrated through ethanol (50–100%), and placed in propylene oxide before embedding in Epon 812 such that the cut faces of the nerve incised at the time of biopsy were oriented toward the face of the block. After curing, 1-μm sections were cut and stained with paraphenylenediamine to enhance the contrast of myelin for quantitative computer-assisted light microscopic morphometric analysis. The largest fascicle meeting criteria from cross-sectional area (≥100,000 μm^2), fixation, and mechanical distortion (≤6% endoneurial area) was selected for light microscopic morphometric analysis (12). The selected fascicle was digitally imaged at 400× and analyzed for total endoneurial area, the number of myelinated fibers, and the total axon areas of each myelinated fiber by a semiautomated image analysis system (12). The fiber count (all fibers in the fascicle) and fascicular area (in μm^2) were determined. Fascicular FD was obtained in the standard manner by dividing the total fiber count by total fascicular area and multiplying by 1,000,000 (13). The value is expressed in fibers per mm^2.

GHb, cholesterol, and triglyceride levels were measured using routine biochemical tests.

Statistical analysis

Statistical evaluation was performed with Super Analysis of Variance (version 1.11 for Macintosh, 1991) and Statview (version 4.5 for Macintosh, 1998) software (Abacus Concepts, Berkeley, CA). In univariate analyses, Pearson's coefficients of correlation between the FD and independent variables were calculated. General linear regression modeling using analysis of covariance was used in multivariate analyses to evaluate the effect of independent variables on FD and to adjust means for potential confounding variables. Additional analyses included correlations between FD and SUMCV and SUMAMP. Student's *t* test was used for comparison of two groups. Associations between clinical risk factors and NCS were not examined in this study.

RESULTS

— The study group included 65 men and 24 women; 71 subjects had type 2 diabetes and 18 subjects

Table 2 —*Pearson's correlation coefficients between the morphological severity of peripheral sensorimotor neuropathy and the investigated risk factors*

	FD	
	r	*P*
GHb	−0.207	0.0221
Duration of diabetes	−0.316	0.0027
Duration of neuropathy	−0.241	0.0241
Height	0.013	0.9074
Age	−0.145	0.1814

Severity of neuropathy is expressed as the FD.

had type 1 diabetes. Demographic characteristics of these patients are shown in Table 1. The mean age (±SD) of patients was 54.2 ± 10.2 years, the mean duration of diabetes was 11.2 ± 8.8 years, and the mean duration of symptoms of neuropathy was 2.9 ± 3.8 years. The mean GHb was 8.5 ± 1.7%.

The relationship between the severity of DSP and the examined risk factors was investigated by univariate correlation analysis. The results of correlation analysis are shown in Table 2. The FD significantly inversely correlated with GHb, duration of diabetes, and duration of clinical peripheral neuropathy. The FD demonstrated no correlation with height, weight, systolic or diastolic blood pressure, total cholesterol level, or triglyceride level.

Among binary or dichotomous variables, retinopathy, GHb at a threshold of 8, ataxia, erectile dysfunction, deep tendon reflexes, and position sense correlated with the FD (Fig. 1) (Table 3). Type of diabetes, sex, hypertension, pain, numbness, tingling, weakness, general autonomic symptoms, peripheral pulses, pinprick sensation, light-touch sensation, vibration sensation, and smoking history all failed to correlate with FD.

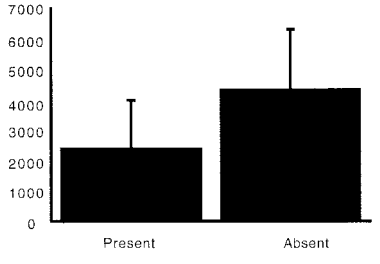

Figure 1—*Interaction bar plot for FD in men with DSP plus or minus erectile dysfunction (P < 0.0001).*

Table 3 —*Relationship between FD and clinical variables*

Variable	FD if variable present	FD if variable absent	*P*
Retinopathy	2,102	3,353	0.0063
Ataxia	1,754	3,153	0.0480
Erectile dysfunction	2,401	4,290	<0.0001
Abnormal position sense	1,549	3,153	0.0303
Abnormal DTR	2,827	4,172	0.0192

FD showed strong correlations with electrophysiological parameters as shown in Table 4. Vibration perception threshold (VPT) in the toe correlated significantly with FD, but this association was not observed with VPT in the finger.

In multivariate analysis, the variables that were significantly related to the severity of DSP in univariate analysis were studied: GHb, diabetes duration, retinopathy, ataxia, impotence, deep tendon reflexes, and position sense. Statistical significance of the individual risk factors related to FD in general linear models is displayed in Table 5. The duration of diabetes was a significant predictor of severity of neuropathy as shown by FD, even after correction for duration of polyneuropathy and after controlling for GHb as a categorical variable. After controlling for age, height, and weight, GHb remained a strong predictor of severity of neuropathy as shown by FD.

To identify which cutoff level of GHb would best differentiate between less and more severe forms of FD loss, GHb was investigated in different models predicting FD as a categorical variable (low versus high), with cutoff values of 8.0, 8.5, 9.0, 9.5, and 10.0%. Using the cutoff point of 9.0%, GHb as a categorical variable in a general linear model gave the highest level of statistical significance ($P = 0.0150$) (Table 6).

CONCLUSIONS— The present study has shown that diabetes control characterized by GHb level is related not only to the presence, but also to the morphological severity, of DSP. This relationship was shown in both univariate correlation analysis and in multivariate analysis using general linear modeling, thus controlling for potential confounding variables. The overall severity of DSP was expressed by using the sural nerve FD. In addition to GHb, duration of diabetes and the demographic variable of age were significant independent predictors of the severity of neuropathy. Surprisingly, height was not a predictor of DSP severity using FD. This result contrasts with previously published electrophysiological data demonstrating that increasing height is correlated with worsening nerve conduction velocity (1–6). Tkac and Bril (11) found that height is a predictor of severity of nerve conduction velocity change but not of nerve amplitude change. These earlier results and the current finding that height does not predict FD loss indicate that the association between height and conduction velocity is not determined by DSP but by other factors integral to conduction along peripheral nerves, such as nerve length, axon diameter, and thickness of the myelin sheath.

Table 4 —*Pearson's correlation coefficients between the morphologic severity of peripheral sensorimotor neuropathy and NCS parameters*

	FD			
	r	*P*	r*	*P*
SUMCV	0.717	<0.0001	0.624	<0.0001
SUMSURCV	0.074	<0.0001	0.635	<0.0001
SUMAMP	0.675	<0.0001		
VPT (toe)	−0.0437	<0.0001		
VPT (finger)	−0.069	0.5266		

Severity of neuropathy is expressed as the FD. SUMSURCV, sum of sural conduction velocities; r*, conduction velocity values corrected for anthropomorphic factors.

Table 5 —*Multiple linear regression model P values with FD as the dependent variable and risk factors for severity of diabetic sensorimotor neuropathy*

	FD
HbA_{1c}	0.0058
Diabetes duration	0.0259
Polyneuropathy duration	0.1451
Age	0.0195
Height	0.5869
Weight	0.7665
r^2	0.113

All relationships with continuous variables are inverse.

The lack of significant correlation between VPT in the finger with FD loss reflects the poor discrimination of neuropathy by VPT in the finger in patients with mild to moderate DSP. VPT in the toe reflects DSP severity better, as expected in this length-dependent pathophysiological process.

GHb as an index of long-term diabetes control has been shown to be related to the incidence and the prevalence of DSP in both cross-sectional and prospective epidemiological studies including mainly patients with type 1 diabetes, such as the Pittsburgh Epidemiology of Diabetic Complications Study (1,3), the European Diabetes Type I Study (2), the Seattle Prospective Diabetic Foot Study (4), the Diabetes Control and Complications Trial (8), and most recently, the Rochester Diabetic Neuropathy Study cohort (7). The study by Tkac and Bril (11) showed that GHb was a modifiable risk factor for electrophysiological severity of DSP. This finding can now be extended to demonstrate that GHb is also a risk factor for severity of FD loss in DSP. When GHb as a continuous variable was substituted in general linear models for a categorical variable, a cutoff point of 9% was identified to have the highest predictive value in those models, the same finding as with electrophysiological severity (11). Using this cutoff value, the difference in FD between patients with GHb ≤9% and GHb >9% ranged from 3,461 to 2,334 per mm^2 after adjustment for age, sex, height, and duration of diabetes.

Duration of diabetes, which was reported to be a risk factor for the presence of DSP (1,2,5,10), was shown in the present study to be related to the morphological severity of DSP. Height was not a significant predictor of FD loss, similar to the lack of prediction for electrophysiological amplitudes in the study by Tkac and Bril (11). Although some studies observed relationships between the presence of DSP and sex, hypertension, smoking, or triglyceride level (2,3), this study did not demonstrate a relationship between these factors and the morphological severity of DSP.

In summary, two major conclusions can be made from the present study. First, poor diabetes control is the most important factor related to the severity of DSP in patients with both type 1 and type 2 diabetes. Other nonmodifiable risk factors, specifically duration of diabetes and age, are correlated with the severity of DSP. Given that effective control of diabetes is beneficial for nerve CV in type 1 diabetic patients (9), it can be speculated that this type of intervention will have the same benefit on FD.

The second major conclusion from this study is that the relationship between increasing height and electrophysiological (CV) severity of DSP is spurious. Clearly, the influence of height on CV relates to factors such as the length of the nerve, the myelin thickness, and the measurement techniques, but not to nerve FD loss, the hallmark pathological finding of DSP. This conclusion emphasizes the need for caution when predicting nerve morphology from nerve function as measured by electrophysiological tests.

Table 6 —*FD in subjects with good to moderate (GHb ≤ 9%) and poor (GHb > 9%) glycemic control*

	GHb ≤ 9%	GHb > 9%	*P*
n	54	34	
FD	3,461 ± 1,988	2,334 ± 1,577	0.0150

The means are adjusted for age, sex, height, weight, and the duration of diabetes.

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References

1. Maser R, Steenkiste A, Dorman J, Nielsen V, Bass E, Manjoo Q, Drash AL, Becker DJ, Kuller LH, Greene DA: Epidemiological correlates of diabetic neuropathy: report from Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetes* 38: 1456–1461, 1989
2. Tesfaye S, Stevens L, Stephenson J, Fuller J, Plater M, Ionescu-Tirgoviste C, Nuber A, Pozz G, Ward JD: Prevalence of diabetic peripheral neuropathy and its relation to glycaemic control and potential risk factors: the EURODIAB Type I Complications Study. *Diabetologia* 39:1377–1384, 1996
3. Forrest K, Maser R, Pambianco G, Becker D, Orchard T: Hypertension as a risk factor for diabetic neuropathy: a prospective study. *Diabetes* 46:665–670, 1997
4. Adler A, Boyko E, Ahroni J, Stensel V, Forsberg R, Smith D: Risk factors for diabetic peripheral neuropathy: results of the Seattle Prospective Diabetic Foot Study. *Diabetes Care* 20: 1162–1167, 1997
5. Halonen J, Hamalainen H, Toikka T, Ronnemaa T: Population-based study on diabetic neuropathy with 7-year follow-up on 450 patients with type I and type II. Abstract presented at the 14th International Congress of EEG and Clinical Neurophysiology, Florence, Italy, 1997
6. Partanen J, Niskanen L, Lehtinen J, Mervaala E, Shtonen O, Uusitupa M: Natural history of peripheral neuropathy in patients with non-insulin-dependent diabetes mellitus. *N Engl J Med* 333:89–94, 1995
7. Dyck P, Davies J, Wilson D, Service F, Melton LR, O'Brien P: Risk factors for severity of diabetic polyneuropathy: intensive longitudinal assessment of the Rochester Diabetic Neuropathy Study cohort. *Diabetes Care* 22:1479–1486, 1999
8. The Diabetes Control and Complications Trial (DCCT) Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
9. The Diabetes Control and Complications Trial (DCCT) Research Group: Effect of intensive diabetes treatment on nerve conduction in the Diabetes Control and Complications Trial. *Ann Neurol* 38:869–880, 1995
10. UK Prospective Diabetes Study (UKPDS): Intensive blood-glucose control with sulfonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352:837–853, 1998
11. Tkac I, Bril V: Glycemic control is related to the electrophysiologic severity of diabetic peripheral sensorimotor polyneuropathy. *Diabetes Care* 21:1749–1752, 1998
12. Greene D, Brown M: Validation of sural

nerve fiber density and percent normal teased fibers as morphological endpoints in clinical trials of diabetic neuropathy. In *Diabetic Neuropathy: New Concepts and Insights*. Hotta N, et al., Eds. Amsterdam, Elsevier, 1995, p. 379–385

13. Dyck P: Diabetic neuropathy. In *Diabetic Neuropathy*. Dyck P, et al., Eds. Philadelphia, WB Saunders, 1987, p. 223–237