Lifestyle Intervention for Pre-Diabetic Neuropathy

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OBJECTIVE — The purpose of this study was to evaluate intraepidermal nerve fiber density (IENFD) as a sensitive measure of neuropathy change in patients with neuropathy associated with impaired glucose tolerance (IGT) receiving lifestyle intervention based on that used in the Diabetes Prevention Program.

RESEARCH DESIGN AND METHODS — We performed 3-mm skin biopsies with measurement of IENFD at the distal leg and proximal thigh at baseline and after 1 year in 32 subjects with IGT. Each received individualized diet and exercise counseling as a standard of care. Nerve conduction studies, quantitative sensory testing, quantitative sudomotor axon reflex testing, and the Michigan Diabetic Neuropathy score were performed, and a visual analog pain scale was completed. Two-hour oral glucose tolerance tests (OGTTs) following the American Diabetes Association guidelines were performed, and serum lipid levels were measured at baseline and 1 year later.

RESULTS — Baseline distal IENFD was 0.9 ± 1.2 fibers/mm and proximal IENFD was 4.8 ± 2.3 fibers/mm. Baseline distal IENFD correlated with fasting glucose ($P < 0.001$) and OGTT ($P < 0.01$). After 1 year of treatment, there was a 0.3 ± 1.1-fiber/mm improvement in distal IENFD and a 1.4 ± 2.3-fiber/mm improvement in proximal IENFD ($P < 0.004$). The change in proximal IENFD correlated with decreased neuropathic pain ($P < 0.05$) and a change in sural sensory amplitude ($P < 0.03$).

CONCLUSIONS — These findings indicate that diet and exercise counseling for IGT results in cutaneous reinnervation and improved pain. Skin biopsy was the most sensitive measure of neuropathy change over 1 year. IENFD should be included as an end point in future neuropathy trials.

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Idiopathic peripheral neuropathy is common. Population studies indicate that 5–14% of individuals >40 years old have neuropathy (1,2). Several groups have demonstrated a 40% prevalence of impaired glucose tolerance (IGT) among subjects with otherwise idiopathic neuropathy (3–5), compared with <15% in the age-matched general population (6). This observation supports the hypothesis that IGT-related neuropathy may represent the earliest stage of diabetic neuropathy. One practical test of this hypothesis is to determine whether treatment of IGT results in slowed progression of neuropathy. The Diabetes Prevention Program (DPP) demonstrated that intensive diet and exercise counseling slows progression of IGT to diabetes compared with placebo or metformin (7). We are studying patients with IGT and neuropathy in the Impaired Glucose Tolerance Neuropathy (IGTN) study, a National Institutes of Health–funded Clinical Pilot Project investigating the clinical features and progression of IGTN. As a standard of care, all IGTN study subjects receive diet and exercise intervention modeled on that used in the DPP.

The neuropathy associated with IGT and early diabetes is sensory predominant and painful and is characterized by prominent small-fiber injury. The most sensitive diagnostic measure is skin biopsy with measurement of intraepidermal nerve fiber density (IENFD) (5,8,9). A primary aim of the IGTN study is to pilot IENFD as a neuropathy progression measure.

RESEARCH DESIGN AND METHODS

— The IGTN study is a multicenter project led by the University of Utah with participation of the University of Michigan and Yale University. Institutional review board approval was obtained at each institution, and all subjects provided informed consent. All subjects had IGT and neuropathy confirmed by signs, symptoms, and an abnormality in results of nerve conduction studies (NCSs), quantitative sensory testing (QST), or quantitative sudomotor axon reflex testing (QSART). NCS values were compared with those for age-matched normal subjects. QST or QSART results were considered abnormal if they were greater than or less than the 95th percentile relative to a normal matched population. Oral glucose tolerance tests (OGTTs) were performed on two separate days using American Diabetes Association guidelines (10). All subjects were screened for other common causes for neuropathy including vitamin B_{12}, thyroid-stimulating hormone, and antinuclear antibody and serum protein electrophoresis and immunofixation.

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Abbreviations: DPP, Diabetes Prevention Program; IENFD, intraepidermal nerve fiber density; IGT, impaired glucose tolerance; IGTN, Impaired Glucose Tolerance Neuropathy; NCS, nerve conduction study; OGTT, oral glucose tolerance test; QST, quantitative sensory testing; QSART, quantitative sudomotor axon reflex testing; VAS, visual analog pain scale.

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

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Table 1—*Baseline characteristics and metabolic parameters of IGTN study participants*

	Baseline	1 year	Significance
Age (years)	60 ± 8.4		
Duration of neuropathy symptoms (months)	7 ± 31		
Exercise (min/week)	80 ± 73		
Sex			
Female	20		
Male	20		
Metabolic features			
BMI (kg/m^2)	32.1 ± 4.4	31.0 ± 4.4	$P < 0.001$
Fasting plasma glucose (mg/dl)	103 ± 13	104 ± 12	$P < 0.7$
2-h glucose (mg/dl)	160 ± 26	149 ± 41	$P < 0.05$
Cholesterol			
Total	200 ± 31	190 ± 33	$P < 0.01$
LDL	113 ± 27	97.9 ± 40	$P < 0.06$
HDL	49.6 ± 13	54.1 ± 15	$P < 0.06$
Triglycerides	237 ± 174	196 ± 156	$P < 0.7$
Systolic blood pressure (mmHg)	129 ± 13	125 ± 14	$P < 0.12$
Diastolic blood pressure (mmHg)	80.7 ± 8.6	77.7 ± 6.2	$P < 0.10$
n with other microvascular complications			
Retinopathy		0	
Microalbuminuria		4	

Data are means ± SD. Five subjects with IGT were included on the basis of isolated IFG (mean fasting plasma glucose 117 [range 112–125]). At 1 year, three of these five had IGT. Over the 1st year there was significant improvement in BMI, OGTT, and total cholesterol, with a trend toward improvement of LDL and HDL cholesterol. Retinal photography and assessment for microalbuminuria occurred at baseline only.

Those with a family history of neuropathy or a disease known to cause neuropathy were excluded.

Lifestyle intervention

Diet and exercise counseling was provided as a standard of care based on the results of the DPP (11). All subjects received individualized counseling with goals of reducing weight by 7% and increasing weekly exercise to 150 min. Dietary counseling occurred quarterly. Fitness was assessed using a 6-min walk test, a validated measure of exercise fitness (12). The distance walked at a brisk pace on a fixed course over 6 min was recorded. Subjects were taught to adjust their home exercise effort based on a ratings of perceived exertion value of 10–12 (sufficient exertion to limit casual conversation) (13). As a condition of study enrollment, all subjects identified a primary care provider who received laboratory results and was responsible for medical management of hyperlipidemia and hypertension.

Neuropathy evaluation

Before diet and exercise counseling, subjects received a neuropathy evaluation. A neurologic examination was performed. The Michigan Diabetic Neuropathy Score and Michigan Neuropathy Screening Inventory were performed (14). Gracely and 100-mm visual analog pain scales (VASs) were completed. Sural, median, ulnar, and radial sensory and peroneal, tibial, median, and ulnar motor NCSs were performed. QSTs for vibration and cold detection were performed on the foot using a CASE IV system (WR Medical, Stillwater, MN). QSART was performed at the foot, distal leg, proximal thigh, and forearm using a QSWEAT system (WR Medical). All studies were performed on the left side. All tests were repeated annually. An OGTT and serum lipid panel were performed at baseline and yearly. At baseline all subjects had 24-h urine collections to test for microalbuminuria and dilated 50° funduscopic digital photography examination. Images were analyzed by a blinded ophthalmologist.

Skin biopsy

Punch skin biopsies (3 mm) were performed at baseline and yearly at the distal leg and proximal thigh. The procedure was well tolerated, and there were no complications. Tissue was preserved in paraformaldehyde lysine phosphate for 24 h. Biopsies were cut into 50-μ sections and stained with protein gene product 9.5 using previously described methods (8). IENFD was calculated by a blinded observer who counted the number of nerve fibers crossing the dermal-epidermal junction using a magnification of ×40 in four randomly selected sections and divided this number by the epidermal length measured using a digital imaging system (Image Pro Plus). Qualitative abnormalities (e.g., axonal swellings, dystrophic changes, and abnormal branching patterns) were recorded (15,16). All biopsy slides were masked and mixed with a set of normal slides, and a single blinded observer (A.G.S.) determined normal versus abnormal.

Statistical analysis

Pearson's correlation coefficients were used to investigate correlation between neuropathy progression measures and metabolic parameters. Paired two-tailed Student's *t* tests were used to compare baseline and 1-year data. All data are presented as means ± SD.

RESULTS — Thirty-two subjects with IGT and with baseline and 1-year skin biopsies were evaluated. Baseline neuropathy and metabolic characteristics are summarized in Table 1. IGTN subjects had a sensory predominant neuropathy. All experienced some degree of neuropathic pain (mean VAS 37 ± 19 mm). As a group, subjects were obese, hyperlipidemic, and sedentary, with most performing no weekly exercise at baseline. Four subjects were taking an ACE inhibitor, four an angiotensive receptor blocker, three a statin, and one a fibrate. None started or stopped these medications during the follow-up period, and only one had a slight increase in their statin dose. Baseline distal IENFD was 0.8 ± 1.1 fibers/mm, and proximal IENFD was 4.8 ± 2.3 fibers/mm. Epidermal nerve fibers were absent distally in 24%. Baseline distal IENFD closely correlated with fasting plasma glucose ($P < 0.001$) and OGTT ($P < 0.01$), peroneal compound muscle action potential amplitude ($P < 0.05$), and vibration detection threshold ($P < 0.02$).

Baseline skin biopsy was abnormal in every subject. Distal IENFD was reduced in 83%. Each subject with normal IENFD had morphological abnormalities. Other measures were less sensitive and often demonstrated a lower dynamic range in which to measure change. This was true despite the fact that inclusion criteria required an abnormality of results of NCSs, QSTs, or QSARTs. Sural amplitude was normal in 36%, reduced in 29%, and absent in 36%. Peroneal motor amplitude

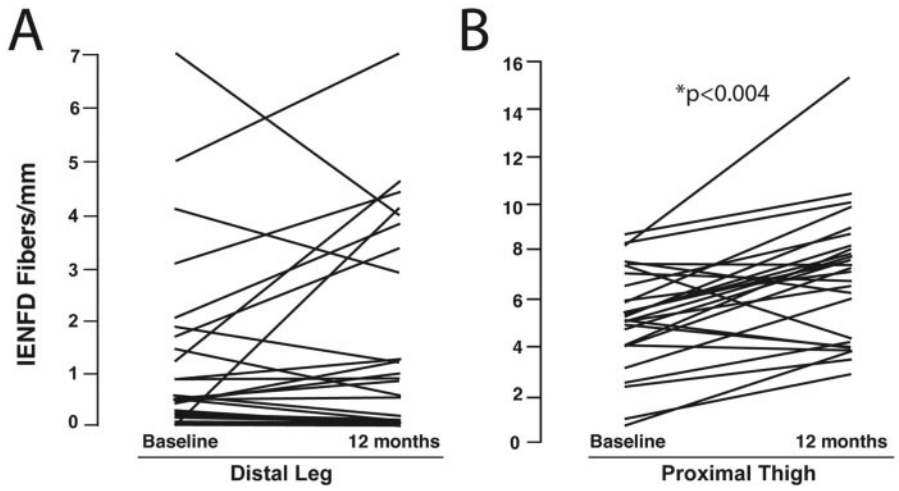

Figure 1—*The change in IENFD for each patient from the baseline visit to the 12-month visit is displayed for the distal leg* (A) *and the proximal thigh* (B). *Distal IENFD improved 0.3 ± 1.1 fibers/mm, and the proximal IENFD improved 1.3 ± 2.2 fibers/mm (*P < 0.004). *Improvement in proximal thigh IENFD was observed in 70% of subjects compared with 31% for the ankle.*

was reduced in 37%, and conduction velocity slowed in 39%. Overall, NCS were abnormal in 69%. Similarly, using the 95th percentile as the cutoff, the cold detection threshold was abnormal in 64% and the vibration detection threshold in 56%. Both cold and vibration thresholds were abnormal in 35%. Isolated abnormality of cold detection (i.e., abnormal cold and normal vibration) was observed in more than twice as many subjects as isolated abnormality of vibration detection (29 vs. 12%), consistent with relatively greater small-fiber injury. QSART was abnormal in 61% of subjects.

Diet and exercise counseling resulted in significant improvement in weight, glucose, and cholesterol (Table 1). After 1 year of therapy, the mean 2-h postload glucose during OGTT decreased 10.8 ± 30 mg/dl ($P < 0.05$) and the mean BMI decreased by 1.1 ± 1.4 ($P < 0.001$). There was a significant improvement in total serum cholesterol of 10.6 ± 20.1 mg/dl ($P < 0.01$).

Improvement in metabolic parameters was associated with significant improvement in measures of small-fiber function. There was a significant improvement in proximal IENFD of 1.3 ± 2.2 fibers/mm ($P < 0.004$). Distal IENFD improved 0.3 ± 1.1 fibers/mm ($P < 0.12$) (Fig. 1). There was no relationship between baseline IENFD, NCS, QST, QSART, or Michigan Diabetic Neuropathy Score and degree of subsequent reinnervation at the proximal thigh. However, subjects who had absent epidermal fibers and loss of the dermal nerve plexus were unlikely to experience significant reinnervation. However, some with absent epidermal but preserved dermal fibers did experience reinnervation (Fig. 2). Only 31% of subjects noted improvement in distal IENFD. In contrast, 70% experienced reinnervation at the proximal biopsy site. There was a significant improvement in foot sweat volume of 0.3 ± 0.8 ($P < 0.05$) measured using QSART. No significant change in sweat volumes was observed at the distal leg or thigh. Pain generally improved, although the change did not reach significance. The 100-mm VAS improved from 36.4 ± 19.4 to 32.8 ± 26.3 mm ($P < 0.4$) and the Gracely Pain Scale improved from 0.88 ± 0.4 to 0.74 ± 0.5 ($P < 0.1$). Other neuropathy measures did not change significantly.

The change in IENFD correlated with several other neuropathy measures. There was a significant correlation between change in proximal IENFD and improvement in pain assessed using the VAS ($P < 0.05$) (Fig. 3). The change in IENFD also correlated with a change in electrophysiologic measures: distal IENFD and peroneal motor conduction velocity ($P < 0.004$) and proximal IENFD and sural sensory amplitude ($P < 0.03$).

CONCLUSIONS — These results indicate that skin biopsy is the most sensitive measure of IGTN severity and suggest that treatment with diet and exercise counseling results in partial cutaneous reinnervation. IENFD change correlates with changes in electrophysiologic measures and pain severity, implying that it is

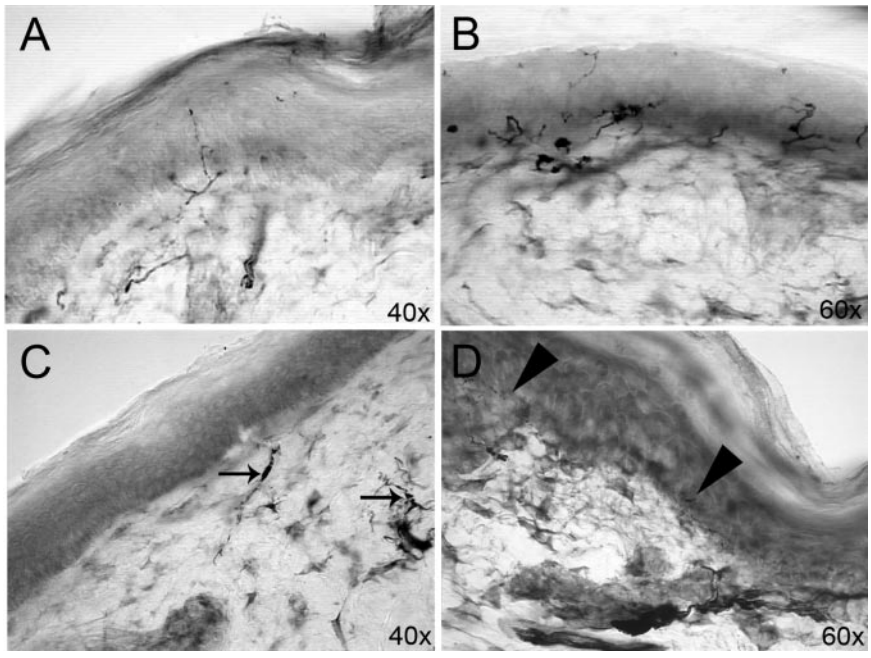

Figure 2—*Reinnervation was observed at the proximal site in most subjects. In the subject shown in* A, *at baseline the IENFD was 8.2 fibers/mm. At 12 months, IENFD at the same site had increased to 15.1 fibers/mm and frequent axonal swellings and dystrophic appearing axons were observed in both the dermis and the epidermis* (B). *A subject with absent epidermal fibers but preserved dermal nerve fibers at baseline* (C) *(arrows) did experience epidermal reinnervation after 12 months* (D) *(arrowheads, 4.4 fibers/mm). Subjects with absent dermal and epidermal fibers typically did not experience epidermal reinnervation.*

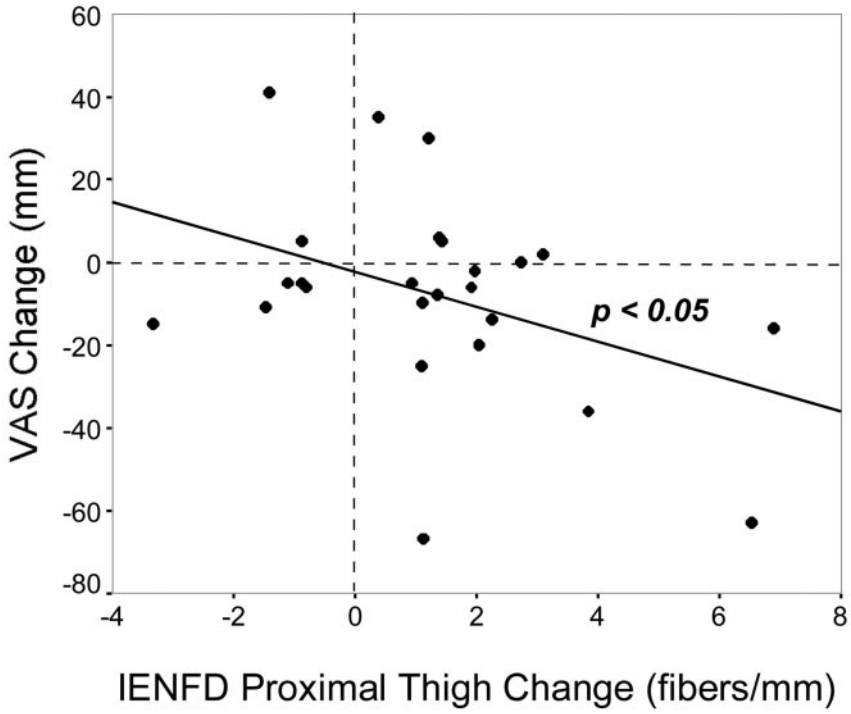

Figure 3—*There was a significant correlation between improvement in proximal IENFD and improvement in pain assessed by the 100-mm VAS (Pearson correlation coefficient −0.4,* $P < 0.05$*).*

a valid surrogate measure of neuropathy severity and progression.

IGT is the most frequent metabolic abnormality in subjects with idiopathic neuropathy (3–5,17). We hypothesize that IGTN represents the earliest stage of hyperglycemic neuropathy. The IGTN study was designed as an National Institutes of Health Clinical Pilot Project with the aim of validating measures of small-fiber injury and measuring rate of progression in preparation for a larger treatment study. The lifestyle intervention was included as a standard of care measure based on the DPP results. The study was not designed or powered to detect a treatment benefit. Nevertheless, during the 1st year of follow-up, there was significant improvement in BMI, OGTT, and serum cholesterol. This observation is not surprising, given the DPP, which demonstrated that a similar intervention reduced risk of progression of IGT to diabetes and resulted in significantly improved metabolic parameters (7). The significant improvement in IENFD and QSART was unexpected. That both IENFD and QSART improved supports the conclusion that improvement in metabolic parameters results in recovery of small-fiber function. The lack of correlation between the change in QSART and IENFD is probably a reflection of the large SD in sweat volumes and relatively low patient numbers. Change in IENFD correlated with improved pain, which is the most clinically relevant symptom in this population. The nerve fibers assessed by IENFD are thought to be nociceptive fibers, so this relationship is not unexpected.

These results are significant because the natural history of early diabetic neuropathy is of slow progression (18). No available therapy (including glycemic control) has previously been shown to result in diabetic neuropathy improvement. In the most dramatic example, "curing" type 1 diabetes with a pancreas transplant resulted in cessation of progression of neuropathy but with minimal improvement (19). The fact that axonal regeneration is observed after 1 year of therapy suggests that IGTN is more amenable to treatment than established diabetic neuropathy. Improvement after diet and exercise counseling also supports the overall hypothesis linking IGT and peripheral neuropathy, although a response to a more specific therapy for IGT (e.g., oral hypoglycemic therapy) must be demonstrated to more definitively support a causal relationship.

Interpretation of this study is limited because all subjects received diet and exercise counseling as a standard of care. Thus, there was no placebo or natural history group. Nevertheless, although the precise rate of IGTN progression over time is unknown, the observed improvement differs significantly from the natural history of early diabetic and IGT neuropathy. The improvement is also unlikely to represent a placebo effect. Whereas pain severity is prone to a placebo effect, IENFD is a quantitative histologic measurement performed by blinded observers. For the same reasons, it is unlikely that QSART would be susceptible to a placebo effect.

Data from animal models supports the sensitivity of skin biopsy for detection of reinnervation. A subset of streptozotocin-induced diabetic mice experienced spontaneous resolution of their diabetes. The mice that recovered had very dramatic epidermal reinnervation (20). There is also human data suggesting that therapy for diabetic neuropathy may result in small-fiber regeneration. Treatment with the aldose reductase inhibitor zenarestat improved nitric oxide–dependent nerve blood flow and increased the density of small-diameter sensory nerve fibers in sural nerve biopsies, although this medication was ultimately ineffective at slowing neuropathy progression (21,22). These data and ours suggest that small fibers may be particularly able to regenerate. Only measures of small-fiber function improved in the 1st year of follow-up in the IGTN cohort.

Efforts to evaluate treatment in early diabetic and IGT neuropathy are limited by a lack of validated measures of small-fiber function. Nerve conduction studies are useful and well-validated measures of progression for established diabetic neuropathy. Their utility in IGTN is limited, however, because they primarily measure large myelinated nerve fiber function. The most direct measure of sensory axonal function, the sural sensory response, varied widely among IGTN subjects. It was normal in 38% but was absent in a similar percentage. The high frequency of an absent response and poor test-retest reliability (23) limit the ability of the sural sensory amplitude to detect change, and it is therefore a poor surrogate measure. Peroneal motor conduction velocity assesses large myelinated motor fiber function and is the most commonly used end point measure in diabetic neuropathy studies. Although its reproducibility is good, it is relatively insensitive to the small-fiber neuropathy characteristic of

IGTN. Of IGTN study participants, 61% had normal peroneal motor conduction velocity. Cold detection threshold, a validated measure of small-fiber function, was abnormal in 69% of subjects but did not change over the follow-up period. Over 1 year of follow up there was no significant change in any NCS or QST parameter.

Skin biopsy was the most sensitive measure of neuropathy severity. IENFD was diminished in 83% of subjects, and morphologic changes were observed in the rest. Because IENFD can be measured at multiple sites, it has a very large dynamic range. Although 24% of subjects had absent epidermal nerve fibers distally, all had preserved fibers at the proximal site, making it possible to measure a change in IENFD in each subject. IENFD has the theoretical benefit of directly measuring small somatic fiber integrity, the same population of nerve fibers primarily involved in IGT. IENFD has excellent test-retest reliability with a variability of $<$10% and intraclass correlation coefficients of $>$90% (24).

Choice of biopsy site is important. Our data suggest that once there is a loss of the dermal plexus, the likelihood of epidermal reinnervation is remote. Subjects with IGT and with absent distal IENFD did not improve if there was loss of the dermal plexus as well (Fig. 2). Thus, whereas biopsy of the distal site provided correlative data with other neuropathy measures, it was less useful in measuring improvement. Future studies using skin biopsy should include a proximal site to increase dynamic range and optimize detection of a treatment effect over time. Our results support the logistical feasibility of using skin biopsy in multicentered studies. There has been concern that the utility of skin biopsy as an end point measure would be limited by poor patient tolerance and risk. However, the biopsy procedure was well tolerated without significant complications. Patient compliance with repeat biopsy was high. Biopsy samples were easily shipped to the central laboratory. This successful experience was shared by a previous multicenter HIV neuropathy treatment trial (25). Based on the results of the IGTN study, those conducting future treatment studies in early diabetic neuropathy and other small-fiber predominant neuropathies should consider inclusion of skin biopsy with use of IENFD as an end point measure.

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