

New Insights Into Diabetic Polyneuropathy

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DIABETES IS A SERIOUS, COSTLY disease that affects approximately 8% of adults in the United States.¹ Worldwide, the incidence of diabetes is increasing dramatically,² especially among children.³ The World Health Organization estimates that there will be 220 million people with diabetes by 2010. Diabetic peripheral neuropathy (DPN) is one of the most prevalent complications, yet it remains a challenging clinical problem. Diabetic polyneuropathy has been reported to affect nearly 50% of people with diabetes.⁴ While some studies suggest a further increased prevalence among people with type 2 diabetes,⁴⁻⁷ others suggest that symptoms are more severe in this population.⁴ Complications of DPN are a major cause for hospitalization among people with diabetes,⁸ and neuropathy ranks third in lifetime expenditures associated with the complications of diabetes, behind macrovascular disease and nephropathy.⁹ Diabetic polyneuropathy was once perceived as a late, inevitable complication of diabetes. There is now a growing impression that neuropathy may be associated with mild glucose dysmetabolism and may even be the presenting symptom of diabetes.¹⁰

CASE PRESENTATIONS

Patient 1

A 62-year-old woman presented with a 1-year history of burning pain in her feet. She described her symptoms as

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Patients with complaints of numbness, tingling, and dysesthesias in the toes and feet are frequently referred to neurologists. Often, the only objective evidence for peripheral nerve dysfunction in these patients is limited to small-caliber sensory nerve fibers. On examination these patients may have reduced distal pinprick sensation, and distal leg skin biopsies show loss of small-caliber nerve fibers. Studies focusing on small-caliber nerve fibers have led to a growing impression that neuropathy can be associated with early diabetes or impaired glucose tolerance (IGT). Often, neuropathy can be the presenting symptom of either diabetes or IGT. Furthermore, the oral glucose tolerance test appears to be a more sensitive measure of glucose dysmetabolism in these patients than levels of fasting blood glucose or glycated hemoglobin. Patients with IGT-associated neuropathy may represent an attractive target population for future regenerative studies given that their neuropathy is less severe and presumably more easily reversed than neuropathy occurring in patients with diabetes.

Historically, small-caliber fibers have not been extensively evaluated due to a lack of objective measures. Several measures to evaluate these fibers are emerging, including skin biopsy with visualization of epidermal nerve fibers. The accessibility of epidermal nerve fibers makes them an attractive target for nerve injury models, which have potential for development as novel outcome measures. Such approaches may address some of the challenges of past diabetic polyneuropathy trials.

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being worse at night and aggravated by standing. She experienced electric-like shocks, as well as hyperalgesia such that innocuous stimuli (eg, bed sheets rubbing against her feet) were painful. She reported that her feet often felt as if they were "on fire."

Her examination showed normal ankle reflexes and normal vibratory and proprioceptive sense. The only abnormality was a mild, stocking-pattern loss of pinprick sensibility. Diagnostic evaluation was unrevealing, with normal results of nerve conduction and electromyography testing. Results of a neuropathy screen were normal; this screen included a complete blood cell

count, chemistry and metabolic panel, serum protein electrophoresis with immunofixation, testing for rapid plasma reagin, and assessment of levels of thyroid-stimulating hormone, vitamin B₁₂, Sjögren syndrome antigens A and B, and

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glycosylated hemoglobin (HbA_{1c}). There was no history of familial neuropathy and no exposure to toxins. Results of the oral glucose tolerance test (OGTT) and of skin biopsies performed to assess epidermal nerve fiber (ENF) density were abnormal (2-hour OGTT value was 154 mg/dL [8.55 mmol/L]; results of skin biopsies revealed loss of ENFs at the distal leg site) (FIGURE 1).

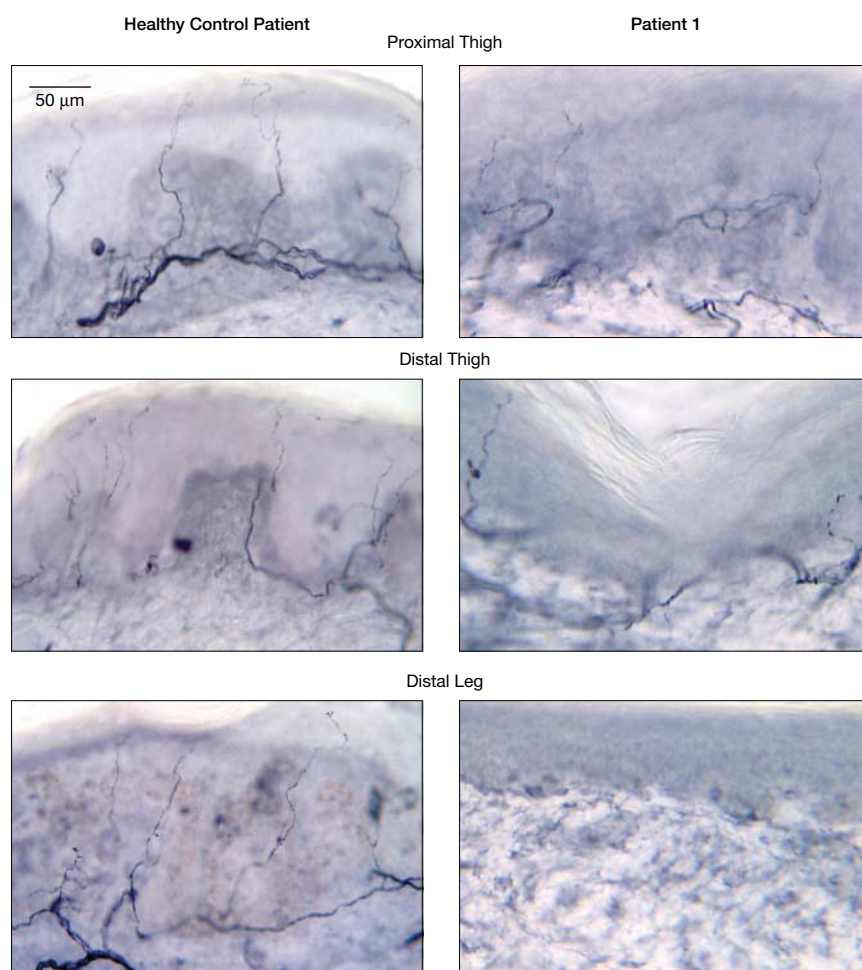
Patient 2

A 60-year-old woman presented with a 4-year history of sensory dysesthesias in

the feet. Symptoms initially consisted of a pins-and-needles sensation, tingling, burning, and sharp stabbing pains in the toes. Symptoms progressed proximally to involve the soles and distal legs and were accompanied by a sense of numbness. Past medical history was notable for carpal tunnel surgery 2 years earlier, which was not associated with any appreciable benefit. Five years ago, which was 1 year prior to symptom onset, a fasting blood glucose (FBG) level was normal (81 mg/dL [4.49 mmol/L]). One year later, at the time of symptom onset, an FBG level was 110 mg/dL (6.10 mmol/L).

One year after symptom onset, her HbA_{1c} level was 5.8%; 3 years after symptom onset, her HbA_{1c} level was 6.4%. She was diagnosed with diabetes 6 months later when her HbA_{1c} level was 7.8% and treatment was begun with an oral agent. A follow-up HbA_{1c} level 6 months later was 6.0%. Results of a nerve conduction/electromyography study at the time of her symptom onset were normal, results from a repeat study 2 years later were interpreted as borderline abnormal, and a third study nearly 4 years after onset of symptoms demonstrated a sensory neuropathy with reduced sural amplitudes. Examination revealed normal reflexes and power throughout. Sensory testing was notable for a stocking-pattern sensory loss with diminished pinprick sensitivity extending to the knees, and increased vibratory threshold and reduced proprioception at the toes. In addition, sensation was subjectively decreased in the distribution of the right median nerve.

Figure 1. Representative Regions of Skin Biopsy Samples From a Healthy Patient and Patient 1



Epidermal nerve fiber (ENF) density at all biopsy sites from the healthy control patient is normal. In patient 1, ENF density at proximal and distal thigh sites is normal, but there is complete denervation of the epidermis and subepidermal dermis at the distal leg site. Ubiquitin hydrolase expression in ENFs is indicated by blue staining and was immunolocalized using protein gene product polyclonal antibody and an avidin-biotin peroxidase complex method with Vector SG as the chromogen. The sections were counterstained with eosin Y (original magnification $\times 40$).

DISCUSSION

These patient histories underscore the growing impression that DPN can occur early in the course of glucose dysmetabolism and may even be the presenting symptom of diabetes. Considerable effort has been directed toward understanding the pathophysiology of DPN and has led to numerous well-conceived clinical trials. Despite these efforts, DPN remains a challenging clinical problem and there is currently no treatment approved by the US Food and Drug Administration aimed at halting or slowing disease progression. This article will review how the study of a subpopulation of nerve fibers—specifically, small-caliber sensory nerve fibers—has contributed to the understanding of DPN and may offer an opportunity to address some of the recognized challenges of past polyneuropathy trials.

The Skin Biopsy Technique

Patient 1 underwent skin biopsies for evaluation of cutaneous nerves. This technique has emerged over the past decade as a useful method to diagnose and study peripheral nerve disorders.¹¹⁻¹⁵ Pe-

peripheral nerve studies have preferentially focused on myelinated nerve fibers because methods available for assessment of the small, unmyelinated fibers were limited. Unmyelinated fibers remain “invisible” to standard nerve conduction studies, which assess large sensory and motor fibers. Assessment of unmyelinated fibers by sural nerve biopsy is problematic and requires electron microscopy. Nerve biopsy also only gives a window into 1 location along the nerve at a single time point. Finally, from a functional standpoint, cutaneous sensation is transduced by the nerve fibers that reach their targets in the skin, which are not identified by nerve biopsies. These limitations, and the observations that individuals with sensory neuropathies have neuropathic pain with marked allodynia, prompted studies of cutaneous innervation and led to development of methods to identify and quantify unmyelinated nerves in the skin.

Early studies of cutaneous nerves focused on the structure of myelinated nerves leading to Meissner corpuscles.^{16,17} Development of a monoclonal antibody against protein gene product 9.5, a panaxonal marker,¹⁸ has allowed small sensory nerve fibers to be visualized through immunohistochemical techniques. This approach has been used by a number of researchers to visualize the subpapillary plexus of small myelinated and unmyelinated nerve fibers. Epidermal nerve fibers have received the greatest scrutiny, mainly because they appear to be early indicators of neuropathy and because adequate samples can easily be obtained for quantitation.^{11,19-21} Robust normative data have been developed²² and demonstrate a distally dominant pattern of ENF loss in diabetic neuropathy²³ and other neuropathies.^{24,25} Comparison of unmyelinated nerve-fiber counts from sural nerve biopsies with ENF densities suggests that skin biopsy may be a more sensitive measure of small-caliber sensory nerve fibers.²⁶ Reduced epidermal innervation has been found in some individuals with normal tendon reflexes at the ankles, normal sural nerve action potential amplitudes, and normal quanti-

tative sensory tests, as well as in individuals with spontaneous allodynic pain.^{27,28} While the precise structural correlates of allodynia remain uncertain, it clearly can occur even with marked depletion of A delta and C fibers in the skin. Limitations of the skin biopsy technique relate to the high degree of variability in epidermal innervation among healthy controls. This will likely translate into a requirement for large sample sizes in clinical trials that use this measure. In addition, the absence of a reduction in density of ENFs with increasing age,²² as occurs with other measures, has led some investigators to question the technique.

IGT and Peripheral Neuropathy

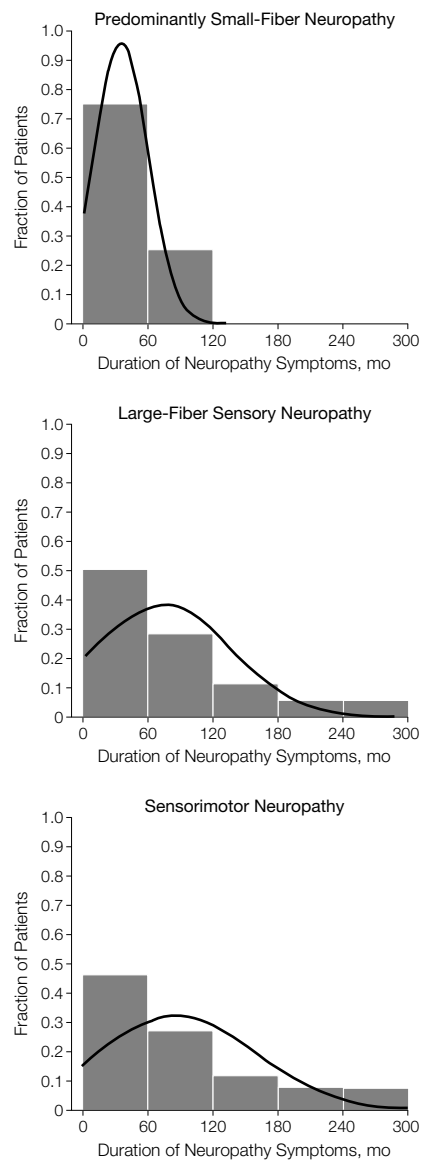
There is a growing consensus for future regenerative DPN trials to include patients with mild disease. Several recent studies have reported an association between impaired glucose tolerance (IGT) and peripheral neuropathy.²⁹⁻³¹ Evaluation for IGT requires a fasting 75-g 2-hour OGTT, with IGT being defined as a 2-hour serum glucose value between 140 and 200 mg/dL (7.77-11.10 mmol/L).³² Several large longitudinal studies have demonstrated that IGT precedes diabetes and confers an increased risk for subsequently developing diabetes.^{33,34} Modest weight loss and routine exercise and administration of metformin both reduce the risk of progression from IGT to diabetes.³³ Other studies have suggested that IGT confers an increased risk of heart disease and death.^{35,36}

Patient 1 had an FBG level of 104 mg/dL (5.77 mmol/L) and a 2-hour OGTT value of 154 mg/dL (8.55 mmol/L), consistent with a diagnosis of IGT. Large-fiber nerve function, measured by nerve conduction testing, was normal. Skin biopsies showed a loss of ENFs at the distal leg site, consistent with a diagnosis of small-fiber sensory neuropathy (Figure 1).

Several groups have reported an increased prevalence of IGT among patients with painful sensory neuropathy^{37,38} and randomly selected patients with sensory neuropathy and IGT were found to have reduced ENF densities.³⁹

Based on similar observations, we recently completed a study of patients with clinically confirmed, cryptogenic, predominantly sensory neuropathy.³¹ Of these patients, 56% had abnormal OGTT results, with 36% having IGT and 20% having frank diabetes. These percentages are 2- to 3-fold higher than those reported in the National Health and Nutrition Examination Survey (NHANES) III study, which showed an IGT prevalence of 15.8% among 2844 participants aged 40 to 74 years.¹ In our study, both the patients with IGT and those with diabetes had objective evidence of neuropathy, though the patients with IGT had less-severe neuropathy as measured by distal-leg skin biopsies, sural nerve amplitude, sural nerve velocity, and peroneal motor response when compared with their counterparts with diabetes. Similar to the relationship between hyperglycemia and cardiovascular disease,^{40,41} we found a direct, dose-response relationship between the degree of glucose dysmetabolism and the severity of neuropathy. When our patients' neuropathy was stratified by neuropathy type, those with predominantly small-fiber neuropathy had a shorter duration of neuropathy symptoms (FIGURE 2) and most had IGT. These results suggest that loss of small-caliber nerve fibers may be the earliest detectable sign of neuropathy in glucose dysmetabolism, and that the OGTT is a more sensitive measure of glucose dysmetabolism in patients with cryptogenic sensory neuropathy than levels of either FBG or HbA_{1c}.³¹

Our study was a cross-sectional study and therefore only demonstrates an association between IGT and peripheral neuropathy. Nevertheless, it provides indirect support for a causal relationship given the strength of the association, the dose-response relationship, the replication of findings in different populations, and biological plausibility.⁴² The findings also are consistent with the emerging theme that diabetic complications are found among patients with IGT. Clearly, longitudinal studies are needed to establish the temporal relationship between IGT-associated neuropathy and

Figure 2. Duration of Symptoms by Neuropathy Type (N=41)

Neuropathy type was classified using nerve conduction and skin biopsy results. Patients were classified as having predominantly small-fiber sensory neuropathy²⁵ if the only abnormality was their distal leg skin biopsy. Patients were classified as having large-fiber sensory neuropathy if they had abnormal sural responses and normal deep peroneal responses. Sensorimotor neuropathy was defined as abnormal sural and deep peroneal responses. Based on this classification, there appears to be a stepwise progression of involvement by fiber type, from small-caliber sensory axons to large myelinated sensory axons to motor-fiber involvement. Curves were generated from raw data. Adapted with permission.³¹

diabetic neuropathy. Additional studies will be needed to address the possibility of improving patients' neuropa-

thy through improved glycemic control.

Patient 2 had impaired levels of FBG (110 mg/dL [6.10 mmol/L]) at the time of her symptom onset. While an OGTT was not performed, we remain suspicious that she may have had IGT or diabetes at that time. If so, her neuropathy would have served as the presenting symptom and aggressive action directed toward improving glycemic control might have improved her neuropathy.

Challenges and New Approaches in DPN Trials

Clinical trials in DPN have focused on improving the neuropathic pain associated with DPN (symptomatic trials), preventing progression of existing disease (prevention trials), and reversing existing damage (regenerative trials). Significant advances have been made in the symptomatic treatment of DPN while progress in prevention and regenerative trials has been slower.

Several important lessons have been gleaned from the past several decades of regenerative DPN trials.⁴³ These lessons include the recognition that regenerative trials should include patients with milder disease, that trials need to have longer durations in order to detect a treatment effect, and that they should include larger sample sizes with more uniform patient populations.⁴⁴ Including patients with advanced DPN, such as those with absent sural responses in regenerative trials, may set too high a bar for potential neurotrophic or regenerative agents by expecting severely damaged tissue to regenerate.⁴⁴ Similarly, the chronic nature of DPN makes it difficult to detect differences among treatment groups in clinical trials lasting months or a few years, and a duration of 3 to 5 years is believed to be needed.^{43,45}

The patient histories presented earlier are instructive in highlighting potential solutions to these challenges. If IGT-associated neuropathy is a precursor to DPN, patients with IGT may represent an attractive study population given that their neuropathy is less severe and presumably more easily reversed than that in patients with diabetes.

In addition, a measure of small-fiber nerves would be a welcome addition to future DPN trials in light of evidence that these nerves can be preferentially affected early in glucose dysmetabolism. Existing measures of small-caliber sensory nerves include sural nerve biopsy, quantitative sensory testing, quantitative sudomotor autonomic reflex testing, and skin biopsy. Each measure has pros and cons, although the Peripheral Nerve Society has questioned the continued use of tandem sural nerve biopsies as part of future DPN trials due to the significant morbidity of the procedure.⁴⁶

The need for DPN trials to have durations up to 3 to 5 years in order to detect differences between treatment and control groups⁴⁷ is a daunting task, especially if large numbers of participants are involved. Over the past several years we have cultivated a novel approach to measure axonal regeneration that may compress this time frame. The ability to quantitate ENFs in a relatively noninvasive fashion through skin biopsy has allowed us to perform serial biopsies on human participants and measure regrowth of these nerves over time. Several standardized models of nerve injury have emerged. Two of the models involve transection of cutaneous nerve fibers, with subsequent denervation and reinnervation of the epidermis.⁴⁷ Both regenerative and collateral sprouting can be measured in such a fashion, with collateral growth occurring more slowly and incompletely than regenerative growth. Regenerative sprouting involves regrowth of axons along denervated Schwann cell bands, while collateral regeneration describes the branching of existing ENFs. A third model involves chemical denervation of the epidermal and subepidermal dermis achieved through the topical application of capsaicin cream. Capsaicin is the active ingredient in hot chili peppers and stimulates small nerve fibers through a nonselective cation channel receptor, VR1.⁴⁸ Almost all epidermal fibers are capsaicin-sensitive in humans, and capsaicin-mediated activation of VR1 produces an influx of calcium ions with consequent activation of calcium-sensitive

proteases⁴⁹ and axonal degeneration. Capsaicin-treated skin has reduced numbers of epidermal and subepidermal nerve fibers^{50,51} and the technique appears to be a powerful tool to experimentally denervate the epidermis and study subsequent regeneration.⁵² An example of a series of skin biopsies depicting capsaicin-induced denervation followed by reinnervation is shown in FIGURE 3.

Using such approaches, we have studied ENF regeneration as defined by recovery of ENF density in people with and without diabetes. Preliminary results suggest that people with diabetes have a reduced capacity to regenerate their axons and that changes in nerves begin early in the course of diabetes before any signs or symptoms of neuropathy are present.⁵³ These findings are consistent with previous animal studies⁵⁴ as well as with reports suggesting that persons with diabetes recover less well from peripheral nerve injury than people without diabetes.^{55,56} The implications for regenerative DPN trials are potentially far-reaching. Since recovery following ENF injury occurs over a matter of months this approach may compress trial duration significantly. Finally, because persons with nonneuropathic diabetes appear to have impaired regenerative capacity, an argument can be made for including individuals with diabetes and no evidence of neuropathy in regenerative neuropathy trials.

Lessons for the Clinic

The association of neuropathy with IGT and the finding of reduced axonal regeneration in people with diabetes have several important implications for patient care. We believe that patients presenting with sensory neuropathy should be evaluated for subtle abnormalities in glucose metabolism, such as IGT, and treated if such abnormalities are found. In addition, there is a rationale to treat nerve injuries in these patients more aggressively.

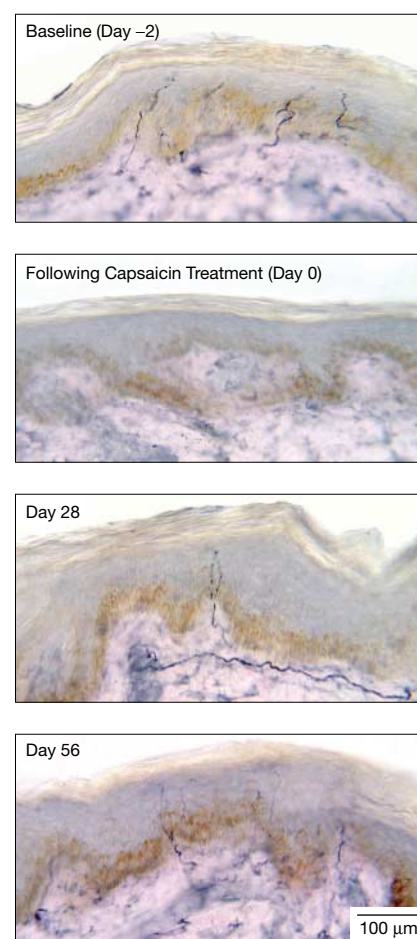
A 2-hour OGTT is required to identify patients with IGT. This differs from the routine practice of measuring FBG to screen patients for diabetes. There

is increasing evidence that glycemic control is not adequately reflected in HbA_{1c} values,⁵⁷ and the American Diabetes Association criteria for the diagnosis of diabetes do not include levels of HbA_{1c}.⁵⁸ A 2-hour postchallenge glucose value is a better predictor of all-cause and cardiovascular disease mortality than are levels of either HbA_{1c} or FBG.⁵⁹⁻⁶¹ In addition, IGT is an independent risk factor for cardiovascular disease^{35,36,62} and death.³⁵ Therefore, it is easy to rationalize performing an OGTT as part of an evaluation for peripheral neuropathy. If IGT is diagnosed, it should be treated. If IGT is diagnosed and treated, patients' peripheral neuropathy may improve or stabilize (M.P., personal observation) and their risk for developing diabetes and possibly cardiovascular disease will be reduced.³³

For patients with diabetes and nerve injuries, such as patient 2 with carpal tunnel syndrome, glycemic control is certainly important. There also may be a motivation to aggressively treat carpal tunnel syndrome in these patients. Conservative management with splinting or steroid injections may result in subsequent axon loss if they are not successful. Given that patients with diabetes have a reduced regenerative capacity, it appears preferable to treat such patients early with definitive surgical release. Similar arguments might be made for radicular injury due to disease of the spine, and potentially for patients with IGT.

In conclusion, DPN is a major medical concern with a high prevalence, a huge economic cost to society, and significant human suffering. Historically, prevention and regenerative trials in DPN have been challenging and have not yielded treatments approved by the Food and Drug Administration, yet DPN remains a dynamic field. The clinical observations that DPN can begin early in the course of glucose dysmetabolism and that axonal regenerative capacity may be reduced in patients with diabetes have important implications in how we approach these patients. In addition, the develop-

Figure 3. Representative Skin Biopsy Sections at Baseline, After Capsaicin Denervation, and at 2 Reinnervation Time Points



The baseline skin biopsy shows normal innervation of the epidermis. Following capsaicin treatment (day 0), there is complete denervation of the epidermis and subepidermal dermis. Biopsy samples at 28 and 56 days demonstrate reinnervation of the epidermis. Recovery of epidermal nerve fiber density was correlated with heat pain thresholds (data not shown). See Figure 1 legend for details of staining method (original magnification $\times 20$).

ment of novel measures of human axonal regeneration offers exciting potential for future DPN regenerative trials.

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