Longitudinal Assessment of Small Fiber Neuropathy
Evidence of a Non–Length-Dependent Distal Axonopathy

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**IMPORTANCE** Few data are available on the natural history of small fiber neuropathy (SFN). Peripheral neuropathy typically follows a length-dependent pattern, leading us to hypothesize that patients with SFN would lose intraepidermal nerve fibers at the distal leg more quickly than at more proximal thigh sites.

**OBJECTIVE** To compare the longitudinal rate and pattern of intraepidermal nerve fiber density (IENFD) change in idiopathic SFN (iSFN), impaired glucose tolerance–associated SFN (IGT-SFN), and diabetes mellitus–associated SFN (DM-SFN).

**DESIGN, SETTING, AND PARTICIPANTS** In this longitudinal, case-control study, patients diagnosed as having SFN from January 1, 2002, through December 31, 2010, and age- and sex-matched controls underwent additional evaluation at tertiary outpatient neurology clinics. Participants and healthy controls were evaluated twice separated by at least 2 years. Participants underwent standardized examinations, nerve conduction, and skin biopsy at 3 sites along the leg. A linear mixed-effects model was used to compare rates of IENFD decrease between cause and biopsy site.

**MAIN OUTCOMES AND MEASURES** We compared the rate of IENFD loss over time in subjects with iSFN, IGT-SFN and DM-SFN as well as the spatiotemporal pattern of IENF loss at different rostral-caudal sites along the leg.

**RESULTS** Fifty-two participants (25 with iSFN, 13 with IGT-SFN, and 14 with DM-SFN) and 10 healthy controls were evaluated. Mean (SD) ages were 50.9 (12.9), 63.1 (10.4), and 61.6 (11.6) years for the iSFN, IGT-SFN, and DM-SFN groups, respectively. There were 12, 7, and 8 female patients and 13, 6, and 6 male patients in the iSFN, IGT-SFN, and DM-SFN groups, respectively. The mean follow-up time was 24.2, 26.7, and 38.8 months for those with iSFN, IGT-SFN, and DM-SFN, respectively, and 32 months for healthy controls. At baseline, mean (SE) for distal leg IENFD (6.48 [1.06]) was lower than distal thigh (13.32 [1.08]) and proximal thigh IENFD (19.98 [1.07]) (P = .001). In addition, IENFD was significantly lower in patients with DM-SFN and IGT-SFN compared with iSFN at all biopsy sites (P = .001). All 3 neuropathy groups had significant IENFD decrease at follow-up at all 3 sites (P = .002), whereas there was no change in the control group. The mean yearly rates of IENFD change over time at the distal leg, distal thigh, and proximal thigh irrespective of cause were −1.42, −1.59, and −2.8 fibers per millimeter, respectively. The mean slopes of IENFD change over time by cause regardless of biopsy site are −0.179, −0.164, and −0.198 for iSFN, IGT-SFN, and DM-SFN, respectively. No difference was found between SFN groups in the rate of decrease. The rate of IENFD decrease was similar at all 3 biopsy sites.

**CONCLUSIONS AND RELEVANCE** Similar rates of IENFD decrease irrespective of cause were observed. Epidermal nerve fibers were lost at similar rates in proximal and distal sites, suggesting that SFN is a non–length-dependent terminal axonopathy.
mall fiber neuropathy (SFN), is a common distinguish axonal neuropathy that predominantly affects thinly myelinated and unmyelinated fibers and presents with a combination of neuropathic pain, hypoesthesia to pain and temperature sensation, and autonomic dysfunction. It can be a predominant or heralding feature of a generalized process that involves large and small fibers, or it can be a distinct disorder that predominantly affects small fibers. Clinical diagnosis of SFN can be challenging, and physical examination and functional assessments, such as nerve conduction studies (NCSs) or quantitative sensory tests, have limited diagnostic efficacy. Skin biopsy with intraepidermal nerve fiber (IENF) quantification is a useful diagnostic test to identify SFN with a diagnostic accuracy of approximately 88%. The cause of SFN is not always clear. Up to half of the cases can be idiopathic SFN (iSFN), with diabetes mellitus–associated SFN (DM-SFN) and impaired glucose tolerance–associated SFN (IGT-SFN) also common.

Typically, SFN is a distal, symmetric neuropathy with patients experiencing symptoms in a length-dependent pattern. A non-length-dependent type of SFN has also been reported in which the presentation of symptoms is patchy and IENF density (IENFD) is more prominently reduced in proximal sites relative to distal sites. In the length-dependent SFN, there is usually a gradient between the reduced density in the proximal and distal site that is not observed in the non-length-dependent cases, but nevertheless there is a reduction of nerve fiber densities in areas that are asymptomatic. This observation raises the possibility that even in cases that clinically present as a length-dependent SFN, the process might instead be a non-length-dependent terminal axonopathy.

There are limited longitudinal data regarding disease progression in patients with SFN. It is known that the correlation between IENFD and symptoms, such as neuropathic pain, is nonlinear and complex, and stability of clinical presentation cannot be interpreted as stability of the neuropathic process. In the current study, we longitudinally followed up patients with iSFN, IGT-SFN, and DM-SFN to explore progression over time. In addition, we were interested in the pattern of IENFD change at all 3 biopsy sites over time.

**Methods**

**Study Participants**

Patients with iSFN, DM-SFN, and IGT-SFN who were seen clinically from January 1, 2002, through December 31, 2010, were eligible. To qualify, individuals had to have physical examination, NCS, skin biopsy, and Neuropathy Impairment Score of the Lower Limb (NIS-LL) results available. Patients were diagnosed as having predominant SFN based on clinical presentation (hyperesthesia, allodynia, reduced sensation to pinprick and temperature but preserved vibratory and proprioceptive sensation, normal deep tendon reflexes) plus reduced IENFD on either set of skin biopsy specimens or the presence of large axon swelling and normal NCS results. These criteria are consistent with a previous study reporting that a combination of reduced IENFD and clinical findings can be used as diagnostic criteria for SFN and studies reporting that axon swellings can predict progressive reduction in IENFD. Qualified individuals (ie, electromyography technicians, laboratory technicians, examining physician) were invited back for masked repeat assessments, including examination (NIS-LL), skin biopsy, and NCS through a research protocol. Controls had normal peripheral nerve examination findings (vibration using a Rydell tuning fork, intact pin sensation at the toe, accurately detected 0.4-g monofilament at the foot) and no risk factors for peripheral neuropathy. Control subjects were selected from a database of over 150 controls based upon age and gender in order to achieve group matching with study subjects.

The Johns Hopkins Institutional Review Board approved this study; all participants signed consent forms. Data were de-identified.

Diabetes mellitus was defined as having a hemoglobin A1c level greater than 6.5% (to convert to proportion of hemoglobin, multiply by 0.01), a fasting plasma glucose level higher than 126 mg/dL (to convert to millimoles per liter, multiply by 0.0555), or a random plasma glucose level of 200 mg/dL or higher (to convert to millimoles per liter, multiply by 0.0555). Impaired glucose tolerance was defined as a plasma glucose level of 140 to 199 mg/dL 2 hours after a 75-g oral glucose load and a hemoglobin A1c level less than 6.5%. Other potential causes of SFN, such as human immunodeficiency virus (HIV), Sjögren syndrome, paraproteinemia, paraneoplastic syndromes, vitamin B12 deficiency, hypothyroidism, renal and hepatic dysfunction, alcohol abuse, and hereditary neuropathies, were excluded.

**Skin Biopsy**

Skin punch biopsies (3 mm) were performed with the patient under lidocaine local anesthesia, as previously described. Biopsy specimens were obtained from the distal leg (DL), distal thigh (DT), and proximal thigh (PT) at baseline and follow-up. Further details are in the eMethods in the Supplement.

**Statistical Analysis**

Group comparisons were made by multivariate general linear model (GLM) analysis of variance (ANOVA). A GLM with repetitive measures was used to compare NIS-LL and IENFD within groups between baseline and the follow-up from 3 locations and among the SFN groups. The change in IENFD was defined as IENFDfollow-up - IENFDbaseline. To correct for baseline IENFD differences and variable follow-up periods, the percentage of IENFD...
change from baseline per year was calculated as \([\text{IENFD}_{\text{follow-up}} - \text{IENFD}_{\text{baseline}}]/\text{IENFD}_{\text{baseline}}/(\text{Follow-up Duration}) \times 100\). A multivariate GLM ANOVA was used to compare the change, percentage of change from baseline, and these measures per year. In addition, linear regression was performed using IENFD as the dependent variable and time of biopsy, location of biopsy, and the cause of SFN as the independent variables to use the correlation coefficient (slope of the fitted line) as an independent unit-free measure to compare the rate of change in IENFD by biopsy location and cause. A negative slope indicates an IENFD decrease over time. Analyses were performed using SPSS statistical software, version 19 (SPSS Inc).

Although the underlying process of nerve degeneration is not fully understood, especially at the level of the individual nerve, we performed an additional analysis that assumed that IENFD loss over time is not linear but rather dependent on IENFD still being intact. To represent this process and better estimate the rate of decay, this can also be represented as the derivative

\[
\text{dN}_t / \text{d}t = \lambda N_0.
\]

To make results more translatable to clinical practice, we fit an equivalent model to estimate the percentage lost per year. This model took the form \(N_t = N_0 p^{1 - t} \), where \(p\) is the percentage of nerves lost per year. Results from the latter model are reported here. We fit these models to the data using a nonlinear regression using least squares to estimate the parameters \(\lambda\) and \(p\). Model fit was assessed using Akaike information criteria (AIC), and testing for statistical differences between groups and was done through pairwise t-tests. These analyses were performed in R version 3.2.2.

### Results

Fourteen individuals with DM-SFN, 25 with iSFN, and 13 with IGT-SFN were included in the study (Table 1). Follow-up duration was similar in the groups: mean (SD) 24.2 (17.9) months for iSFN, 26.7 (10.8) months for IGT-SFN, and 37.8 (22.7) months for DM-SFN (F\(_2\) = 1.845, \(P = .17\)). The most common reason for individuals not participating was the time requirement. Individuals who did not participate had similar demographics and baseline neuropathy measurements. Results of the baseline NCSS are summarized in Table 1. Those with iSFN had higher sural amplitudes than those with IGT-SFN or DM-SFN: mean (SD) 12.67 (1.68) vs. 11.56 (2.53) and 8.49 (1.46) (F\(_2\) = 4.207, \(P = .02\)). There was no difference in NIS-LL values at follow-up among those with DM-SFN and iSFN (F\(_2\) = 3.916, \(P = .052\)). Controls had similar ages and sex compositions (eTable 1 in the Supplement).

The IENFD values stratified by biopsy site and cause are summarized in Figure 1. At baseline, DL IENFD was significantly lower compared with DT and PT IENFD: mean (SD) 6.48 (1.06) vs. 13.32 (1.08) vs. 19.98 (1.07) (F\(_2\) = 54.296, \(P = .001\)). The IENFD was significantly lower in patients with DM-SFN and IGT-SFN compared with patients with iSFN at all sites (F\(_2\) = 8.030, \(P = .001\)). At follow-up, there was a significant IENFD decrease at all 3 sites (F\(_2\) = 10.903, \(P = .002\)). There was no difference among the SFN groups in the IENFD reduction over time (F\(_2\) = 0.035, \(P = .96\)). Furthermore, the IENFD decreases were similar irrespective of the biopsy site (F\(_2\) = 2.436, \(P = .10\)), suggesting that, although at baseline there was a distal, length-dependent pattern in IENFD reduction, over time there is a uniform reduction in IENFD at proximal and distal sites regardless of cause (Figure 1).

We further examined the hypothesis that IENFD decreases at a similar rate in both proximal and distal biopsy sites by comparing the rate of change in IENFD (Figure 2) and the

### Table 1. Demographic and Electrophysiologic Characteristics of Study Participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>iSFN</th>
<th>IGT-SFN</th>
<th>DM-SFN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>50.9 (12.9)</td>
<td>63.1 (10.4)</td>
<td>61.6 (11.6)</td>
</tr>
<tr>
<td>Sex, female/male (% female)</td>
<td>12/13 (48)</td>
<td>7/6 (54)</td>
<td>8/6 (57)</td>
</tr>
<tr>
<td>Fasting blood glucose, mg/dL</td>
<td>91.3 (2.1)</td>
<td>104.4 (1.8)</td>
<td>122.6 (0.8)</td>
</tr>
<tr>
<td>2-Hour postprandial glucose, mg/dL</td>
<td>83.37 (2.6)</td>
<td>162.142 (1.2)</td>
<td>220.33 (3.7)</td>
</tr>
<tr>
<td>Hemoglobin A1c, %</td>
<td>5.47 (0.17)</td>
<td>5.99 (0.093)</td>
<td>6.64 (0.087)</td>
</tr>
<tr>
<td>BMI</td>
<td>27.56 (4.8)</td>
<td>32.81 (2.1)</td>
<td>29.9 (2.75)</td>
</tr>
<tr>
<td>Duration of symptoms, mo</td>
<td>26.3 (9.4)</td>
<td>16.1 (7.4)</td>
<td>19.2 (9.1)</td>
</tr>
<tr>
<td>Sural SNAP, μV</td>
<td>24.2 (17.9)</td>
<td>26.7 (10.8)</td>
<td>37.8 (22.7)</td>
</tr>
<tr>
<td>Peroneal CMAP, mV</td>
<td>24.2 (17.9)</td>
<td>26.7 (10.8)</td>
<td>37.8 (22.7)</td>
</tr>
<tr>
<td>Tibial CMAP, mV</td>
<td>24.2 (17.9)</td>
<td>26.7 (10.8)</td>
<td>37.8 (22.7)</td>
</tr>
</tbody>
</table>

### Abbreviations:
- BMI: body mass index (calculated as weight in kilograms divided by height in meters squared); CMAP: compound motor action potential; DM-SFN: diabetes mellitus–associated small fiber neuropathy; IGT-SFN, impaired glucose tolerance–small fiber neuropathy; iSFN, idiopathic small fiber neuropathy; SNAP: sensory nerve action potential.
- SI conversion factors: to convert glucose to millimoles per liter, multiply by 0.0555; hemoglobin A1c to proportion of hemoglobin, multiply by 0.01.
- Data are presented as mean (SD) unless otherwise indicated.
- \(P < .05\).
Comparison of mean IENFD at baseline and follow-up across biopsy sites. IENFD was observed to decrease more in the distal leg compared with the proximal thigh and more in patients with diabetes mellitus–associated small fiber neuropathy (DM-SFN) and impaired glucose tolerance–associated small fiber neuropathy (IGT-SFN) than in patients with idiopathic small fiber neuropathy (iSFN). Overtime, there was a similar decrease in IENFD in the distal and proximal sites of biopsy with similar rates among patients with DM-SFN, IGT-SFN, and iSFN. Error bars indicate 95% CIs.

Discussion

Patients with predominant SFN of different causes were prospectively followed up to characterize the spatiotemporal pathologic progression of IENFD. Despite a relatively stable clinical course with respect to NIS-LL, IENFD decreased over time. The rate of IENF loss is similar among patients with iSFN, DM-SFN, and IGT-D. In addition, the data suggest that despite a length-dependent clinical presentation, epidermal nerve fibers are lost at similar rates along different rostral and caudal locations in the leg. This finding suggests that SFN follows a pattern of a non–length-dependent terminal axonopathy and not a length-dependent process based on slowly progressive distal to proximal axonal degeneration (dying back).

To our knowledge, relatively few longitudinal data regarding the natural course of SFN exist. In a retrospective study of 46 patients, more than half did not experience any worsening during a 2-year period. Similarly, in the Rochester diabetic neuropathy study, only approximately 3% of patients with diabetic neuropathy reported subjective worsening of symptoms associated with function of small sensory fibers, such as pain and paresthesia, after 2 years, and only approximately 8% experienced worsening in quantitative sensory testing results. In patients with symptoms of SFN with low-normal IENFD associated with prominent axonal swellings, IENFD decreased significantly at both sites similar rates during 19 months. The clinical findings of this study are in agreement with previous data that iSFN is a slowly progressive disease. In contrast, IENFD decreased over time in all patients at a similar rate. Our data also indicate that IENFD is lower in all sites at baseline in patients with DM-SFN and IGT-SFN compared with iSFN and are more likely to develop large fiber involvement.

It is well appreciated that there is a proximal to distal IENFD gradient among healthy controls, with PT having approximately

percentage change from baseline per year stratified by biopsy site and cause (Table 2). Multivariate ANOVA revealed no difference in these measures among the 3 leg sites ($F_2 = 0.1885$, $P = 0.47$), cause of neuropathy ($F_2 = 0.523$, $P = 0.72$), or interaction of these factors ($F_2 = 0.642$, $P = 0.74$; Figure 2). Similarly, the exponential decay model did not show statistically different rates of nerve loss across biopsy sites or across conditions. However, we did find quantitatively higher rates of nerve loss with increasing nerve length (eTable 2 in the Supplement). This finding was similarly observed within each condition. The rates of IENFD loss were greatest among individuals with iSFN-SFN and lowest in those with DM-SFN, although these findings were also not significant. All estimated rates of nerve loss were significantly greater than 0, with the exception of DL-IGT-SFN, which was likely attributable to low numbers (n = 13) and a high proportion with a count of 0 at the follow-up visit (9 [69%] of 13). The eFigure in the Supplement shows the predicted mean trajectory of IENFD decrease at each site according to this model.

The mean yearly rates of IENFD change over time at the DL, DT, and PT irrespective of cause are $\Delta 4.2$, $\Delta 1.59$, and $\Delta 2.8$ fibers per millimeter, respectively. The mean slopes of IENFD change over time by cause regardless of biopsy site are $\Delta 0.179$, $\Delta 0.164$, and $\Delta 0.198$ for iSFN, IGT-SFN, and DM-SFN, respectively. Together, these data indicate comparable rates of decrease across biopsy sites, suggesting that the pattern of unmyelinated fiber loss is more consistent with a non–length-dependent distal axonopathy than a length–dependent process (Figure 3). In contrast, healthy controls had stable IENFD at the 3 sites for a comparable period (eTable 3 in the Supplement).

Follow-up data from the NCSs revealed that 5 patients with DM-SFN (36%), 4 patients with IGT-SFN (31%), and 5 patients with iSFN (21%) developed a mild large fiber neuropathy and reduced ankle reflexes and vibratory sensation at the time of the follow-up visit. There was no progression to IGT-SFN or DM-SFN among 13 of the 25 patients with iSFN for whom there was follow-up hemoglobin A1c data.
Assessment of Small Fiber Neuropathy

Original Investigation Research

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Devigilietal6 reported that a mean DL IENFD in controls was 9.8, indicating a logic value, resulting in an apparent length-dependent pattern. A lowervalue and therefore will be the first site to reach a pathological stage of dis ease may simply reflect that the DL IENFD starts at one end. The reason that DL IENFD is more reduced at early stages of disease may simply reflect that the DL IENFD starts at a lower value and therefore will be the first site to reach a pathological value, resulting in an apparent length-dependent pattern. Devigili et al16 reported that a mean DL IENFD in controls was 9.8 fibers per millimeter compared with 21.7 in PT IENFD. In patients with SFN, these numbers were 4.4 and 12.9, respectively. Although these values are not from the same patients, they are consistent with a similar rate of decrease for proximal and distal sites (approximately 55% for DL and 41% for PT). In our study, we were able to compare IENFD from the same patients at 2 points. This finding revealed similar rates of IENF decrease after adjusting for follow-up duration of 17.6%, 10.4%, and 8.2% across all patients in the PT, DT, and DL sites, respectively.

Dying back is a common pattern seen in many degenerative and toxic conditions of the peripheral nervous systems.17 The pathophysiologic mechanism of distal axonal degeneration is incompletely understood, but the longest nerve fibers, presumably with higher metabolic demand, are postulated to be most susceptible to dying back. It has been suggested that distal axonal degeneration represents a lack of support to the most distal projections of the axon.18,19 We observed similar rates of IENF loss at proximal and distal sites, suggesting that axon length is less important in determining vulnerability. The pattern of disease progression in our patients with SFN is a non-length-dependent distal axonopathy and could be interpreted as evidence of the interaction between SFN terminals and its target (the skin) playing a critical role. Target-derived growth factors that are retrogradely transported to the neuronal cell body are critical during development and may continue to be vital into adulthood. Alternatively, fast retrograde transport, which is less affected by axon length, may contribute to SFN as reported with degenerative diseases,20 including Alzheimer disease,21 Parkinson disease,22 amyotrophic lateral sclerosis,23 and chemotherapy-induced peripheral neuropathy.24 Together, these observations are consistent with the increasing impression that distal portions of axons are selectively vulnerable in peripheral neuropathy. In DM, the peripheral nerve has metabolic derangements that are not present proximally in dorsal root ganglion.25,26 Similarly, in HIV, distal portions of nerve have higher rates of mitochondrial deletions compared with proximal portions or dorsal root ganglion.27 In chemotherapy-induced peripheral neuropathy, mitochondria are dysmorphic and reduced in number in axon terminals that correlate with the cumulative chemotherapy doses.28

These findings are consistent with evidence indicating involvement of distal axons in other organs of patients with neu-
ropathy. In patients with DM, with or without distal symmetric neuropathy, the density of corneal nerve fibers is reduced, and patients with non-length-dependent presentation of SFN also have a similar reduction in corneal nerve fiber density. Unmyelinated C fibers in the bladder mucosa of patients with DM have reduced responsiveness early in the disease that correlates with hypofunction of unmyelinated fibers in both upper and lower extremities. These observations are consistent with the longitudinal data presented here that SFN follows a non-length-dependent distal axonopathy, even in the cases where it clinically presents as a length-dependent neuropathy.

The present data can have important clinical implications. The diagnostic criteria and identification of a sensitive and specific cutoff for IENFD have focused on DL, based on the assumption that SFN is a length-dependent process. There is worldwide normative data available for IENFD at DL, and the current guidelines recommend a DL skin biopsy, whereas a proximal biopsy is considered a level C recommendation for a non-length-dependent process. Our data suggest that IENFD from proximal sites can be informative as well, including cases that clinically present as a classic distal symmetric SFN with a stocking-and-glove distribution. Furthermore, our data also suggest that follow-up skin biopsies and monitoring of the rate of reduction in IENFD could be a reliable marker for disease progression in treatment trials. This is of particular interest given the inherent challenges of available clinical scales as outcome measures in clinical trials and natural history studies. One limitation of the current study is that we simplified the analysis by only looking at the cause of SFN and the location of the skin biopsy as independent variables and did not include other variables that might affect progression of neuropathy (eg, medications such as angiotensin-converting enzyme inhibitors, comorbidities, and serum lipid levels) known to affect neuropathy progression. Another limitation is the relatively small sample size.

Conclusions

We observed that patients with SFN, irrespective of cause, experienced progressive axon loss during 2 to 3 years of follow-up, with many developing large fiber dysfunction. The rate of axon loss in IGT-SFN was similar to that in DM-SFN or iSFN. The spatiotemporal pattern of axon loss indicates that the distal terminals of axons are selectively vulnerable irrespective of axon length. This finding suggests that SFN is a non-length-dependent distal axonopathy and not a length-dependent process.
Assessment of Small Fiber Neuropathy

JAMA Neurology

Published online April 11, 2016
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ARTICLE INFORMATION

Accepted for Publication: January 5, 2015.
Published Online: April 11, 2016.


Author Contributions: Dr Buralgazi and Mr Truelove are equal contributors. Drs Khoshnooodi and Polydefkis had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Obtained funding: Polydefkis.

Administrative, technical, or material support: Polydefkis.

Study supervision: Polydefkis.

Conflict of Interest Disclosures: None reported.

Funding/Support: The study was supported by a philanthropic gift from Mark Rubenstein, MEng.

Role of the Funder/Sponsor: Obtained funding: Polydefkis.

REFERENCES


Supplementary Online Content


eMethods

eTable 1. Healthy Control Subject Demographics

eTable 2. Percent Decrease in IENFD Count per Year, as Estimated by an Exponential Decay Model

eTable 3. Intraepidermal Nerve Fiber Loss Over Time Stratified by Site Irrespective of Cause

eFigure. Predicted Mean Trajectory of IENFD Decline at Each Site, Starting at the Mean Values for This Population, According to the Exponential Decay Model

This supplementary material has been provided by the authors to give readers additional information about their work.
eMethods

Four 50 μM sections separated by regular intervals with a random start point were immunohistochemically stained using a free floating protocol with rabbit anti-human polyclonal PGP9.5 antibody (Serotec 1:10,000 dilution). The 5th percentile cutoffs for our laboratory are 5.2, 7.1 and 8.5 fibers/mm for DL, DT and PT, respectively. Slides were blinded using a random identifier and a single technician blinded to study details including biopsy location assessed IENFD values. Slides from different studies were mixed to further ensure blinding.
eTable 1. Healthy Control Subject Demographics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean (SD)</td>
<td>59.3 (3.5)</td>
</tr>
<tr>
<td>Gender (% female)</td>
<td>40</td>
</tr>
<tr>
<td>BMI (kg/m²) mean (SD)</td>
<td>-6.01 (4.15)</td>
</tr>
<tr>
<td>Follow up duration (years), mean (SD)</td>
<td>2.7 (0.4)</td>
</tr>
</tbody>
</table>
eTable 2. Percent Decrease in IENFD Count per Year, as Estimated by an Exponential Decay Model

<table>
<thead>
<tr>
<th>Condition</th>
<th>Distal Leg</th>
<th>Distal Thigh</th>
<th>Proximal Thigh</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>16.9 (6.0)</td>
<td>16.6 (3.8)</td>
<td>10.9 (2.4)</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>16.1 (8.0)</td>
<td>18.2 (5.9)</td>
<td>11.7 (3.6)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>16.2 (8.9)</td>
<td>12.1 (4.9)</td>
<td>6.6 (3.3)</td>
</tr>
<tr>
<td>IGT</td>
<td>49.0 (57.1)</td>
<td>23.6 (10.4)</td>
<td>16.7 (5.9)</td>
</tr>
</tbody>
</table>

*Pair-wise p-values ranged from 0.21-0.97 for comparisons across biopsy sites.*
eTable 3. Intraepidermal Nerve Fiber Loss Over Time Stratified by Site Irrespective of Cause

<table>
<thead>
<tr>
<th>Rate of change, mean (SD) fiber/mm</th>
<th>Distal Leg</th>
<th>Distal Thigh</th>
<th>Proximal Thigh</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.75 (0.52)</td>
<td>0.33 (1.6)</td>
<td>-1.74 (3.44)</td>
</tr>
<tr>
<td>Rate of change/year, mean (SD) (fiber/mm/year)</td>
<td>0.26 (0.20)</td>
<td>0.18 (0.64)</td>
<td>0.45 (1.21)</td>
</tr>
<tr>
<td>Percent change from baseline, mean (SD) (fiber/mm)*</td>
<td>-6.01 (4.15)</td>
<td>2.31 (16.03)</td>
<td>0.36 (10.02)</td>
</tr>
<tr>
<td>Percent change from baseline /year, mean (SD) (fiber/mm/year)**</td>
<td>-2.22 (1.7)</td>
<td>1.87 (5.95)</td>
<td>0.14 (4.23)</td>
</tr>
</tbody>
</table>

* (baseline value – follow up value) / baseline value × 100
** [(baseline value – follow up value) / baseline value] / duration of follow up × 100
eFigure. Predicted Mean Trajectory of IENFD Decline at Each Site, Starting at the Mean Values for This Population, According to the Exponential Decay Model

Percent decreases used were those of all conditions from Table 2. Transparent regions represent the 95% credible interval for IENFD values across time.