Small- and Large-Fiber Neuropathy After 40 Years of Type 1 Diabetes

Associations with glycemic control and advanced protein glycation: the Oslo Study

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OBJECTIVE—To study large- and small-nerve fiber function in type 1 diabetes of long duration and associations with HbA_{1c} and the advanced glycation end products (AGEs) N-ε-(carboxymethyl)lysine (CML) and methylglyoxal-derived hydroimidazolone.

RESEARCH DESIGN AND METHODS—In a long-term follow-up study, 27 persons with type 1 diabetes of 40 ± 3 years duration underwent large-nerve fiber examinations, with nerve conduction studies at baseline and years 8, 17, and 27. Small-fiber functions were assessed by quantitative sensory thresholds (QST) and intraepidermal nerve fiber density (IENFD) at year 27. HbA_{1c} was measured prospectively through 27 years. Serum CML was measured at year 17 by immunoassay. Serum hydroimidazolone was measured at year 27 with liquid chromatography–mass spectrometry.

RESULTS—Sixteen patients (59%) had large-fiber neuropathy. Twenty-two (81%) had small-fiber dysfunction by QST. Heat pain thresholds in the foot were associated with hydroimidazolone and HbA_{1c}. IENFD was abnormal in 19 (70%) and significantly lower in diabetic patients than in age-matched control subjects (4.3 ± 2.3 vs. 11.2 ± 3.5 mm, $P < 0.001$). IENFD correlated negatively with HbA_{1c} over 27 years ($r = -0.4$, $P = 0.04$) and CML ($r = -0.5$, $P = 0.01$). After adjustment for age, height, and BMI in a multiple linear regression model, CML was still independently associated with IENFD.

CONCLUSIONS—Small-fiber sensory neuropathy is a major manifestation in type 1 diabetes of 40 years duration and more prevalent than large-fiber neuropathy. HbA_{1c} and the AGEs CML and hydroimidazolone are important risk factors in the development of large- and small-fiber dysfunction in long-term type 1 diabetes.

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Peripheral neuropathy is one of many complications of type 1 diabetes, resulting in significant morbidity and mortality. Small-diameter nerve fibers represent 70–90% of all peripheral nerve fibers and are believed to be the earliest fibers to be damaged in diabetes (1). Small-fiber injury has also been associated with neuropathic pain (2,3), which is one of the most disabling symptoms in patients with diabetic neuropathy.

The severity of diabetic polyneuropathy increases with the duration of diabetes and degree of hyperglycemic exposure (4). In the Oslo Study, intensified insulin treatment with insulin pumps during 2 years improved large-fiber neuropathy at an early stage (5). After 8 years, near normoglycemia delayed progression of neuropathy (6), and after 18 years of fair glycemic control the peripheral nerve function was preserved for those with a mean HbA_{1c} <8.4% (68 mmol/mol) (7). In the Diabetes Control and Complications Trial (DCCT) and Epidemiology of Diabetes Interventions and Complications Study (EDIC) study, the benefits of previous intensive insulin treatment persisted for 13–14 years after DCCT closeout and provided further evidence of a durable effect of prior intensive treatment and that good glycemic control is important to prevent development of neuropathy (8). The EURODIAB study reported higher cumulative incidence of neuropathy related to higher HbA_{1c} value (9). Controversies exist regarding whether there is a definitive threshold of glycemic exposure for any diabetes complication to develop. Orchard et al. (10) did show that the average number of total glycemic exposure did not vary for the different microvascular complications to develop. They suggested an integrated measure of glycemic control called "A1c months" (both duration and degree) and that a value <1,000 A1c months was a minimal treatment goal (10).

How hyperglycemia may cause damage to the nervous system is not fully understood. One consequence of hyperglycemia is the generation of advanced glycation end products (AGEs) that can form nonenzymatically between glucose, lipids, and amino groups. It is believed that AGEs are involved in the pathophysiology of neuropathy. AGEs tend to affect cellular function by altering protein function (11). One of the AGEs, N-ε-(carboxymethyl)lysine (CML), has been found in excessive amounts in the human diabetic peripheral nerve (12). High levels of methylglyoxal in serum have been found to be associated with painful peripheral neuropathy (13).

In recent years, differentiation of affected nerves is possible by virtue of

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specific function tests to distinguish which fibers are damaged in diabetic polyneuropathy: large myelinated (Aα, Aβ), small thinly myelinated (Aδ), or small nonmyelinated (C) fibers. The DCCT/EDIC, EURODIAB, and other cohort studies have not focused especially on small-fiber damage. Therefore, little data are available on the prevalence and mechanisms of small-fiber dysfunction in long-term type 1 diabetes.

Our aims were to evaluate large- and small-nerve fiber function in long-term type 1 diabetes and to search for longitudinal associations with HbA_{1c} and the AGEs CML and methylglyoxal-derived hydroimidazolone.

RESEARCH DESIGN AND METHODS

—In the Oslo study from 1982, 45 patients with type 1 diabetes were included and randomized to either conventional treatment with two daily insulin injections or basal-bolus injection therapy with more than four injections daily or insulin pumps (14). Criteria for inclusion were age 18–45 years, diagnosis of type 1 diabetes at <30 years of age, disease duration >7 but <30 years, stimulated C-peptide <0.1 nmol/L, and no or minimal microvascular complications (5). After 4 years, intensified treatment retarded the progression of microvascular complications and all patients were offered intensified treatment and followed prospectively. After 27 years, 33 participants were still being followed. Two had died of causes not related to diabetes; one was "dead in bed," possibly associated with diabetes; eight were lost to follow-up, and one had to withdraw from the examinations because of newly diagnosed cancer.

A total of 27 of the patients from the Oslo study cohort were examined with neurophysiological investigations of large- and small-nerve fibers. The 27 examined did not differ significantly from the total group of participants regarding age, sex, or glycemic control. The patients were asked about symptoms of neuropathy and underwent a neurological examination of sensory and motor systems, including reflexes. Confirmed diabetic polyneuropathy was defined as suggested by the Toronto Consensus panels on diabetic polyneuropathy in 2009 (15). HbA_{1c} was measured prospectively every year for 27 years. The study was approved by the regional ethics committee and was carried out in accordance with the Declaration of Helsinki as revised in 2000.

Small-fiber investigations

Quantitative sensory testing (QST). Threshold temperatures for the sensation of warmth, cold, heat pain, and cold pain were determined using a computerized Thermo test (Somedic, Fastan, Sweden) as previously described (16,17). Warmth detection threshold (WD), cold detection threshold (CD), heat pain detection threshold (HPD), and cold pain detection threshold (CPD) were determined from a baseline temperature of 32°C, with a 1°C/s rate of change and with cutoff temperatures of 10°C and 50°C. WD, CD, HPD, and CPD were determined from the right thenar eminence, from the dorsum of both feet, and from the level of the knee bilaterally. Small-fiber dysfunction was defined as CD <25°C on the dorsum of the feet or WD >43°C. The actual values (for WD, CD, HPD, and CPD or cutoff temperatures when the patient lacked cold or warmth sensation) were used for statistical comparisons between patients and 27 age-matched control subjects. Skin temperature was measured at the sites of sensory testing and preceded each test session. An IR thermometer, SENSELAB Tempett (Somedic), was used.

Intraepidermal nerve fiber density. Two 3-mm skin punch biopsies were obtained from each patient after local anesthesia from the distal part of the right leg ~10 cm above the lateral malleolus. The method used has previously been described in detail (18,19). Fifty-micron frozen microtome sections were immunostained with PGP 9.5, and intraepidermal nerve fiber density (IENFD) in three sections from each biopsy was counted in addition to measurement of the total length of epidermis in order to record IENFD value per millimeter.

Autonomic testing

R-R interval in response to deep breathing. The R-R interval in response to 1 min deep breathing was assessed with the Keypoint apparatus (Keypoint, Sweden). Results were evaluated according to the Keypoint normal values.

Blood pressure in response to tilting. Mean blood pressure was measured every minute for 20 min before the patient was tilted to a 75° standing position. Mean blood pressure was again assessed every minute for 7 min before the patient regained a supine position. A ≥10% reduction of mean blood pressure was considered abnormal.

Galvanic skin response (GSR). The GSR from both hands and feet in response to one single electrical stimulus of 30 mA administered to one arm was recorded by the Keypoint apparatus. The result was considered normal when a response was present. Neither latency nor amplitude was evaluated.

Nerve conduction studies and electromyography. Large-fiber function was assessed by nerve conduction studies (NCSs) and electromyography (EMG) longitudinally (at baseline and years 8, 17, and 27). The initial examinations in this study have previously been described (6,19). Then, a Keypoint EMG apparatus was used, measuring motor nerve action potential amplitude (NAPA); distal latency; conduction velocity of the median, ulnar, peroneal, and posterior tibial nerves; and sensory amplitude and conduction velocity of the median, ulnar, sural, and superficial peroneal nerves bilaterally. EMG of the extensor digitorum and posterior tibial muscles of one leg was performed both assessing the properties of the motor unit potential and searching for possible signs of denervation. The results were evaluated according to the Keypoint normal values. Recordings were considered to be abnormal if the conduction motor or sensory velocities were <40 m/s (with normal or only slightly reduced motor or sensory amplitudes). Owing to the gradual disappearance of the nerve action potential amplitude in diabetic patients at this velocity level, nerve conduction velocities (NCVs) <30 m/s were displayed as 0 m/s. In such instances, NCV was scored as 15 m/s to avoid exaggeration of the glycemic effect.

Biochemical data including AGEs

All blood samples were taken after an overnight fast. Serum levels of CML were measured 10 years prior to the last neurological examinations with a competitive immunoassay developed in our laboratory (20). Serum levels of hydroimidazolone were determined at the time of skin biopsy by liquid chromatography–mass spectrometry (21).

HbA_{1c} was measured prospectively for 27 years. The different methods used for the first years of the cohort have previously been described (19). DCA 2000 (Bayer Diagnostics, Tarrytown, NY) has been used the last 10 years. The accuracy of the method has been secured by applying the same internal DCCT-adjusted standard. The interassay coefficient of variation was <3%. For estimation of the cumulative glycemic exposure (both degree and duration) A1c months were

calculated using the formula developed by Orchard et al. (10), which is determined by multiplying the number of HbA_{1c} units above the normal reference value by the number of months.

Statistical analyses

Comparisons between two independent groups were performed using Student *t* test and the nonparametric Mann-Whitney *U* test for skewed data. We used the Spearman rank correlation coefficient to estimate the association between continuous variables. Multiple linear regression models were applied to adjust the associations between CML and IENFD and between HbA_{1c} and IENFD for age, height, and BMI and to investigate the simultaneous associations among hydroimidazolone, HbA_{1c}, and heat-pain threshold in the leg. Linear mixed models were fitted to NCS variables to examine associations between A1c months and changes in NCS recordings over time. All models included fixed effects for A1c months dichotomized above and below 1,000 (group variable), time, and time × group interaction, a random intercept, and random-effects for time. An unstructured covariance matrix was used. The level of significance was set at $P < 0.05$. The statistical analyses were performed with SPSS, version 18.0 (SPSS, Chicago, IL) and Stata 12.1 (StataCorp, College Station, TX).

Table 1—*Clinical characteristics of the diabetic patients*

N	27
Patient age (years)	53 (7)
Female/male, *n*	12/15
Disease duration (years)	40 (35–58)
Total cholesterol (mmol/L)	5.1 (0.94)
HDL (mmol/L)	1.9 (0.46)
LDL (mmol/L)	2.8 (0.83)
Triglycerides (mmol/L)	0.9 (0.4–2.2)
Lipid-lowering treatment, yes/no (%)	9/18 (33)
Systolic BP (mmHg)	130.5 (13)
Diastolic BP (mmHg)	71.5 (8)
Antihypertensive treatment, yes/no (%)	14/13 (52)
Smokers/nonsmokers (%)	4/23 (17)
BMI (kg/m^2)	25 (19.7–33)
Vitamin B_{12} (pmol/L)	341 (263–453)
Hemoglobin (g/100 mL)	14.0 (1.15)
Creatinine (μmol/L)	69.4 (47–107)
HbA_{1c}27, %, mmol/mol	8.0 (0.59), 64 (4.1)
CML (units/mL)	3.35 (0.52)
Hydroimidazolone (MG-H1) (nmol/mg)*	427.4 (250)
Cardiovascular disease (yes/no, %)	7/20, 26
Albuminuria (yes/no, %)	4/23, 19
Retinopathy (yes/no, %)	20/7, 74

Data are means (SD), median (interquartile range), and *n* counts, %. HbA_{1c}27, mean HbA_{1c} over 27 years. *Serum protein.

RESULTS—Clinical and demographic characteristics of the study population are summarized in Table 1.

Symptoms and clinical findings

Fourteen patients (52%) reported sensory symptoms. Nine patients reported symptoms of a sensory neuropathy (reduced sensibility in feet or impaired balance), while three of these patients described pain. Five patients had symptoms compatible with carpal tunnel syndrome (pain or paresthesias within the innervation territory of the median nerve [data not shown]). An additional two had no symptoms but abnormal neurological tests with absent tendon reflexes and reduced sensibility. A total of 16 (59%) of the patients had symptoms or signs of neuropathy.

Small-fiber neuropathy

QST. Thermal thresholds in the feet and at the level of the knee were elevated in the patients compared with healthy control subjects (Supplementary Table 1). Twenty-two (81%) patients were diagnosed with small-fiber dysfunction: either a pure small-fiber neuropathy (with normal NCS) in seven patients or an additional large-fiber neuropathy ($n = 15$). The small-fiber dysfunction was most pronounced in the patients with large-fiber abnormalities. Only one patient with large-fiber neuropathy had normal thermal thresholds. No patient with symptoms of neuropathy had normal neurophysiological findings.

IENFD. IENFD was abnormal in 19 (70%) and significantly lower in diabetic patients than in age-matched control subjects (4.3 mm ± 2.3 vs. 11.2 mm ± 3.5, $P < 0.001$).

Results of autonomic testing (R-R interval, blood pressure after tilting, and GSR). Abnormal autonomic testing was observed in 7 (26%) of the patients and occurred together with neurophysiological signs of peripheral neuropathy. The abnormal tests were observed in most cases in patients with a combined large- and small-fiber neuropathy and in one patient with pure small-fiber neuropathy. Six patients had one abnormal test and one patient had two, but in none were all three tests abnormal (data not shown).

Large-fiber neuropathy (NCS and EMG)

NCS was in the normal range in 11 (41%) patients. The remaining 16 displayed abnormal findings compatible with large-fiber neuropathy. All patients with symptoms had abnormalities on NCS. When analyzing A1c months above or below 1,000 as a measure for a minimal treatment goal, there was significantly more progression to lower NCVs in the group with A1c months >1,000 for almost all nerves examined (Table 2).

Neuropathy and glycation

The presence of neuropathy correlated with mean HbA_{1c} for the first 17 years, A1c months over 17 years, and A1c months over 27 years (Table 3).

Small-nerve fibers and glycation and their associations with other recordings

IENFD correlated negatively with CML ($r = -0.50$ [95% CI −0.72 to −0.11]). For investigation of whether the association between IENFD and CML was independent of age, BMI, and height, a multiple regression analysis was performed, which gave an estimated reduction in IENFD of 1.24 (95% CI 0.08–2.39; $P = 0.037$) per unit increase of CML (Table 4).

IENFD was also negatively associated with HbA_{1c} over 27 years in simple regression analysis ($\beta = -1.47$ [−2.88 to −0.05], $P = 0.043$). After adjustment for age, BMI, and height in a multiple regression

Table 2—*Estimated changes in NCV divided into A1c months above or below 1,000 in type 1 diabetic patients*

	Estimated change from inclusion to 27 years, mean (95% CI)	Between-group difference in changes, mean (95% CI), *P*
Motor peroneal NCV		
A1 months <1,000	−6.66 (−10.9 to −2.42)	6.36 (−0.02 to 12.7), 0.051
A1 months ≥1,000	−13.0 (−17.8 to −8.26)	
Motor tibial NCV		
A1 months <1,000	−6.05 (−11.0 to −1.10)	10.4 (2.98–17.8), 0.006
A1 months ≥1,000	−16.5 (−22.0 to −10.9)	
Motor ulnar NCV		
A1 months <1,000	−5.16 (−13.7 to 3.35)	8.93 (−3.88 to 21.7), 0.17
A1 months ≥1,000	−14.1 (−23.7 to −4.51)	
Sensory ulnar NCV		
A1 months <1,000	−1.44 (−10.1 to 7.18)	17.3 (4.32–30.2), 0.009
A1 months ≥1,000	−18.7 (−28.4 to −9.04)	
Sensory sural NCV		
A1 months <1,000	−8.10 (−12.8 to −3.40)	7.16 (−0.12 to 14.4), 0.054
A1 months ≥1,000	−15.3 (−20.8 to −9.70)	

A1 months <1,000, n = 17; A1 months ≥1,000, n = 10 at year 27.

model, the association between HbA_{1c} and IENFD was no longer significant (P = 0.38).

IENFD was significantly associated with variables of QSTs: WD in the leg, r = −0.56 (95% CI −0.78 to −0.23), WD in the knee, r = −0.52 (95% CI −0.75 to −0.17), and HPD in the leg, r = −0.57 (95% CI −0.78 to −0.24).

Mean HbA_{1c} over 27 years correlated negatively with HPD, r = −0.45 (95% CI −0.71 to −0.08), and with CD in the hand, r = −0.60 (95% CI −0.80 to −0.29).

Positive correlations between methylglyoxal hydroimidazolone in serum and WD in the foot, r = 0.55 (95% CI 0.22–0.77), HPD in the foot, r = 0.47 (95% CI 0.11–0.72), and HPD in the knee, r = 0.43 (95% CI 0.06–0.70), were also observed.

In a multiple linear regression model, both HbA_{1c} (β = 1.42 [95% CI 0.41–2.43], P = 0.008) and hydroimidazolone (β = 0.005 [95% CI 0.001–0.008], P = 0.009) were independently associated with HPD in the foot.

Large-nerve fibers and glycation

The motor peroneal NCV correlated with all the markers of glycemic control over 17 and 27 years (Table 3). The motor tibial and sensory sural NCV all correlated significantly with A1c months over 27 years: r = −0.43 (95% CI −0.70 to −0.06) and r = −0.38 (−0.66 to 0.00), respectively. HbA_{1c} over the first 17 years of the cohort also correlated negatively with motor tibial NCV: r = −0.38 (−0.66 to 0.00).

Small- and large-nerve fibers and their association with traditional risk factors

No association was found between NCV and sex, duration of disease, cholesterol, smoking, creatinine, or systolic blood pressure. Diastolic blood pressure however, showed a positive correlation to motor tibial NAPA, peroneal NAPA, and NCV (data not shown). IENFD correlated negatively with sex and BMI but not with blood pressure, smoking, creatinine, or cholesterol. QST analysis did not correlate with any of these risk factors.

Table 3—*Correlations between markers of glycemic control at years 17 and 27 and tests of large- and small-fiber neuropathy*

	Presence of neuropathy	Large-fiber neuropathy: motor peroneal NCV	Small-fiber neuropathy: IENFD
N	27	27	27
HbA_{1c}17	0.44 (0.072–0.70)	−0.50 (−0.74 to −0.15)	−0.42 (−0.69 to −0.048)
A1c months 17	0.39 (0.012–0.67)	−0.38 (−0.67 to 0.00)	−0.23 (−0.56 to 0.16)
HbA_{1c}27	0.37 (−0.012 to 0.66)	−0.38 (−0.67 to 0.00)	−0.39 (−0.67 to −0.012)
A1c months 27	0.52 (0.17–0.75)	−0.51 (−0.75 to −0.16)	−0.22 (−0.55 to 0.10)

Spearman correlation. Set data are presented as r (95% CI). HbA_{1c}17, mean HbA_{1c} over 17 years; HbA_{1c}27, mean HbA_{1c} over 27 years.

CONCLUSIONS—Our study shows that small-fiber dysfunction is more prevalent than large-fiber dysfunction in diabetic neuropathy after long duration of type 1 diabetes. Although large-fiber abnormalities were less common than small-fiber abnormalities, almost 60% of the participants had their large nerves affected after 40 years with diabetes. Long-term blood glucose estimated by HbA_{1c} measured prospectively through 27 years and AGEs predict large- and small-nerve fiber function.

The relationship between C-fiber function and glycemic control was also shown previously by Vas, Green, and Rayman (22). Small-nerve fibers may be even more sensitive to metabolic changes than large fibers, making blood glucose control important for preserving small-fiber function in long-term type 1 diabetes.

We have demonstrated that the serum AGE CML measured 10 years prior to these examinations correlates with IENFD. This finding is consistent after adjustment for other known risk factors. This indicates that CML has a predictive value in the development of small-fiber dysfunction in this population and that AGEs are important in diabetes neuropathy.

A significant association between serum methylglyoxal-derived hydroimidazolone with the HPD in the foot was also demonstrated. This is consistent with a recent study from Bierhaus et al. (13), where significantly higher concentrations of plasma methylglyoxal were observed in type 2 diabetic patients with neuropathic pain compared with healthy control subjects and patients without pain. They also found that high level of methylglyoxal lowered the HPDs (13). Similarly, Monnier et al. (23) reported a positive association between skin levels of methylglyoxal hydroimidazolone and neuropathy in the DCCT.

Our data showed stronger correlations with HbA_{1c} for the first 17 years of the cohort than in the last 10 years (Table 3). This may be due to metabolic memory (24). Recently, Lind et al. (25) described in DCCT

Table 4—*Results of fitting linear regression models of IENFD on patient characteristics using simple (univariable) and multiple models*

	Simple regression models		Multiple regression model	
IENFD	Coefficient (95% CI)	*P*	Coefficient (95% CI)	*P*
CML	−1.63 (−3.17 to −0.08)	0.040	−1.24 (−2.39 to −0.08)	0.037
Age	−0.04 (−0.19 to 0.11)	0.61	−0.13 (−0.25 to −0.009)	0.036
BMI	−0.35 (−0.60 to −0.09)	0.010	−0.43 (−0.67 to −0.19)	0.001
Height	−0.11 (−0.18 to −0.03)	0.006	−0.09 (−0.15 to −0.03)	0.007

$N = 27$. $r^2 = 0.63$.

patients that HbA_{1c} values from 8 years earlier still had important impact on retinopathy development. There may also be a salutary effect of reducing HbA_{1c} that accelerates with time and becomes of clinical importance in neuropathy development in long-term type 1 diabetes. In the DCCT/EDIC study, it was argued that subjects with small-fiber neuropathy are more prone to the memory effect than are subjects with large-fiber neuropathy (24). We observed a significant predictive value of CML measured 10 years earlier on small-fiber neuropathy, supporting the DCCT/EDIC finding.

In our study, the strongest correlation between neuropathy and glycemic control was seen between A1c months over 27 years, which is a cumulative glycemic exposure measure that includes duration, HbA_{1c}, and NCS recordings. Significantly more progression to neuropathy was observed in the group with A1c months >1,000 (Table 2). Orchard et al. (10) did not observe that cumulative glycemic exposure predicted complications better than its components alone. However, that study had only 6 years of follow-up and a mean diabetes duration of 19 years, which is in contrast to our patients with mean disease duration of 40 years and nearly 30 years of follow-up. In the Rochester Diabetic Neuropathy study, a combination of HbA_{1c}, duration of diabetes, and age at onset of diabetes was found to better predict microvascular complications than single components alone (4).

We did not observe any association between large-fiber involvement and traditional risk factors, but this was partly found for small-fiber dysfunction. The reason for this might be the small sample size of our study. It might also be related to the selection criteria in 1982, excluding patients with clinical polyneuropathy at baseline.

We observed abnormal autonomic testing in 7 (26%) of the patients, which is in contrast to our other small-fiber recordings, where up to 80% had abnormal results. One possible confounder to these results is the comprehensive use of drugs among our diabetic patients. Both statins and ACE inhibitors may affect autonomic balance and can therefore underestimate these tests results (26).

Limitations

Our study comprises a relatively small number of participants. This makes negative results more difficult to interpret. However, we consider the accurate prospective long-term data on glycemic control and the use of comprehensive methods for quantification of neuropathy strengths of the study. The homogeneity of the group with minor variation in age and duration of disease does also to some degree compensate for the small number of participants. Many of our findings are in accordance with other large studies.

In summary, small-fiber dysfunction with reduced IENFD and dysfunction of A-Δ and C-fibers evaluated by QST is a major manifestation in type 1 diabetes of 40 years duration and is more prevalent than large-fiber neuropathy. HbA_{1c} and the AGEs CML and methylglyoxal-derived hydroimidazolone may be important risk factors in the development of small-fiber dysfunction. This study supports the importance of good glycemic control even in long-term type 1 diabetes for preserving small- and large-fiber function and that early good glycemic control may have an especially important impact on later neuropathy development. It also gives support to the knowledge that AGEs are important contributors in the development of neuropathy.

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K.A.S. designed the present follow-up study, performed non-neurological clinical assessments and skin biopsies, researched data, and wrote the manuscript. B.K. performed the neurological examinations and analyses. E.J. performed neurological analyses and contributed to writing the manuscript. S.I.M. contributed with IENFD recordings and to discussion and editing of the manuscript. M.W.F. performed statistical analysis and contributed to writing the manuscript. V.M.M. performed the cross-sectional AGE analysis and edited the manuscript. K.D.-J. and K.F.H. designed the study, followed the patients, and contributed to discussions and writing the manuscript. K.A.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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