Understanding Diabetic Neuropathy—From Subclinical Nerve Lesions to Severe Nerve Fiber Deficits: A Cross-Sectional Study in Patients With Type 2 Diabetes and Healthy Control Subjects

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Studies on magnetic resonance neurography (MRN) in diabetic polyneuropathy (DPN) have found proximal sciatic nerve lesions. The aim of this study was to evaluate the functional relevance of sciatic nerve lesions in DPN, with the expectation of correlations with the impairment of large-fiber function. Sixty-one patients with type 2 diabetes (48 with and 13 without DPN) and 12 control subjects were enrolled and underwent MRN, quantitative sensory testing, and electrophysiological examinations. There were differences in mechanical detection (Aβ fibers) and mechanical pain (Aδ fibers) but not in thermal pain and thermal detection clusters (C fibers) among the groups. Lesion load correlated with lower Aα-, Aβ-, and Aδ-fiber but not with C-fiber function in all participants. Patients with lower function showed a higher load of nerve lesions than patients with elevated function or no measurable deficit despite apparent DPN. Longer diabetes duration was associated with higher lesion load in patients with DPN, suggesting that nerve lesions in DPN may accumulate over time and become clinically relevant once a critical amount of nerve fascicles is affected. Moreover, MRN is an objective method for determining lower function mainly in medium and large fibers in DPN.

Distal symmetric diabetic polyneuropathy (DPN) is an important diabetes complication that significantly increases morbidity and mortality in affected patients (1). In some patients, DPN is painful, causing tingling, spontaneous pain or burning sensations, hyperalgesia, allodynia, or overt sensitivity to temperature changes, while other patients predominantly experience painless DPN often associated with numbness (2). For the central nervous system, several structural and functional differences between patients with painful and painless diabetic neuropathy have been described (3–6). However, no specific distinguishing features of the peripheral nervous system between painful and painless diabetic neuropathy have been determined so far (7). Moreover, the pathophysiology and natural course of DPN are poorly understood (8). It is assumed that both prediabetes and diabetes lead to microstructural alterations in affected nerves, which seem to begin in small unmyelinated C fibers (9), consequently leading to a loss of C fibers (10). Later findings over the course of the disease appear to be demyelination accompanied by axonal degeneration of myelinated fibers (Aβ, Aδ) (11). Studies of the underlying pathophysiological aspects of DPN in humans are limited because obtaining nerve tissue is both difficult and risky. Furthermore, nerve biopsies are restricted to distal nerves, which do not allow for an evaluation of proximal fibers. Therefore, it is of great importance to establish noninvasive, objective, in vivo methods that allow for the detection and exact localization

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of nerve damage of DPN at an early stage to gain better insight into the pathophysiology and evaluate potential therapeutic options. In recent studies, high-resolution magnetic resonance neurography (MRN) at 3 Tesla (3T) has proven effective for the detection and exact localization of peripheral nerve lesions in DPN (12,13). Nerve lesions that appear hyperintense in a T2-weighted (T2w), fat suppressed sequence have been shown to be negatively associated with parameters of nerve conduction (13). The exact clinical impact and relation to nerve fiber types of the lesions detected by MRN, however, have not been studied in detail, since to date lesions have only been correlated with basic clinical scores and electrophysiological testing (EPT) (12,13). Therefore, it is uncertain which types of nerve fibers are affected by T2w lesions. Moreover, detecting early stages of diabetic neuropathy would be beneficial for evaluating potential therapeutic methods in future clinical studies. The most sensitive clinical method for the characterization of neuronal impairment is complete quantitative sensory testing (QST), which includes mechanical testing for large-fiber function (2,14–17) and has not been used to evaluate the clinical relevance of MRN yet.

We therefore performed a cross-sectional study in patients with type 2 diabetes with and without DPN as well as in control subjects without diabetes or neuropathy by combining MRN, EPT, and QST as the potentially most sensitive and specific noninvasive clinical methods for evaluating both the exact clinical and neurophysiological status and the load of structural nerve lesions. We hypothesized that MRN mainly detects lesions in larger fibers because of its resolution. Therefore, we expected stronger correlations with clinical large- and medium-fiber functions than with small-fiber functions.

RESEARCH DESIGN AND METHODS

Participants

Patients aged between 18 and 85 years were recruited at the University Hospital Heidelberg (UHH) between 2016 and 2018. Clinical examinations were performed at the Clinical Study Center for Diabetes Research of the UHH. MRN studies were performed at the Department of Neuroradiology of the UHH. The study was approved by the Heidelberg University Medical Faculty ethics committee (numbers S-146/2015 and S-383/2016), and all participants gave written informed consent. Patients with type 2 diabetes with and without DPN as well as subjects without diabetes and neuropathy were included. To diagnose DPN, all participants were asked for symptoms associated with DPN for evaluating the Neuropathy Symptom Score (NSS) according to national German guidelines (18,19). Moreover, they underwent routine clinical examination, including TIP THERM (Tip Therm GmbH, Dorsten, Germany) vibration using a 64-Hz tuning fork and reflex testing as well as basic testing for pain sensations using a 512-mN von Frey filament (Optihair; MARSTOCK nerve test, Schriesheim, Germany) to calculate the Neuropathy Deficit Score (NDS) as previously described and recommended in national German guidelines (18,19). Patients with diabetes and an NSS and/or NDS of $\geq$3 were considered to be affected by DPN (13,18). For patients with diabetes without DPN as well as control subjects without diabetes, both NSS and NDS had to be 0. Individuals with scores of 1 and 2 were not included in the study to avoid inconclusive findings. Specifically trained personnel performed all clinical tests. Medical personnel performing the study strictly monitored for symptoms that were typical for distal symmetric DPN to rule out other causes for an elevated NSS, such as cramps because of magnesium deficit, neuropathic symptoms because of disc herniation, and others common causes. Patients with any contraindications for MRN examination and patients with other known potential causes for neuropathy, such as chronic alcoholism, end-stage renal disease, Parkinson disease, rheumatic autoimmune diseases, malignant tumors, or spinal lesions, were excluded from the study.

Fasting blood draw was performed for evaluation of HbA_{1c}, creatinine, and serum lipids, and urinary albumin/creatinine ratio (uACR) was determined. In control subjects without diabetes, oral glucose tolerance tests were performed to rule out disorders of glucose metabolism according to standard protocol (20). Blood and urine analyses were performed in the accredited Central Laboratory of the UHH according to standard protocols. Estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration equation (21).

QST

A full QST was performed on one dorsal foot, including cold detection threshold (CDT), warm detection threshold (WDT), cold pain threshold (CPT), heat pain threshold (HPT), thermal sensory limen (TSL), and paradoxical heat sensations, by use of a thermode (TSA-II; Medoc Ltd., Ramat Yishai, Israel) as previously described (14). Additionally, mechanical testing was performed using von Frey filaments (Optihair) for mechanical detection threshold (MDT) and a PinPrick stimulator set (MRC Systems GmbH, Heidelberg, Germany) for mechanical pain threshold (MPT), mechanical pain sensitivity (MPS), and wind-up ratio (WUR). For determining dynamic mechanic allodynia, a regular Q-tip, a cotton ball, and a brush (SENSELab Brush-05; Somedic SenseLab AB, Sösdala, Sweden) were used. Moreover, vibration detection threshold (VDT) was evaluated using a 64-Hz tuning fork and pressure pain threshold (PPT) by using a pressure algesiometer (FDN 200 with Rubber Tip 1 cm^2; Wagner Instruments, Greenwich, CT). CDT, WDT, and TSL represent thermosensory functions of small fibers (Aδ and C fibers), whereas CPT, HPT, PPT, and especially MPS and MPT reflect nociceptive functions of small fibers (Aδ and C fibers) (14). MDT and VDT represent tactile functions of larger Aβ fibers (14). All tests have previously been described in detail (14,16).

EPT

Nerve conduction velocities (NCVs), amplitudes of compound motor and sensory action potentials, and distal motor latencies were evaluated for tibial and peroneal nerves on the same leg as QST using the VikingQuest system (VIASYS Healthcare GmbH, Höchberg, Germany).

MRN Imaging Protocol

In all participants, high-resolution MRN of the right-side thigh was performed in a 3T magnetic resonance (MR) scanner (MAGNETOM Trio, A Tim System; Siemens Healthineers, Erlangen, Germany). A 15-channel transmit-receive extremity coil was used and an axial high-resolution T2w turbo spin echo 2-dimensional sequence with spectral fat saturation was applied. Sequence parameters were relaxation time 5,970 ms, echo time 55 ms, field of view 160×160 mm^2, matrix size 512×512, slice thickness 4 mm, interslice gap 0.35 mm, voxel size $0.5 \times 0.3 \times 4.0$ mm^3, and 24 slices, visualizing a total nerve length of ~10 cm. The sequence was centered on the sciatic nerve bifurcation in every patient. To avoid artificial alteration of the acquired T2w signal, we did not apply any prior image filters.

Image Postprocessing and Statistical Analysis

A total number of $73 \times 24 = 1{,}752$ images were recorded. All images were pseudonymized and analyzed with a semiautomatic approach using ImageJ and custom-written code in MATLAB v7.14.0.0739 (R2012a) (22,23). Anatomical segmentation of sciatic nerve fascicles was performed manually by two trained neuroradiologists (J.M.E.J., F.T.K.) blinded to clinical data. In accordance with previous studies (12), a T2w hyperintense lesion was defined as a nerve fascicle with an elevated T2w signal intensity of at least 25% above that of adjacent muscle tissue; a T2w hypointense lesion was defined as a nerve fascicle with a decreased T2w signal intensity of at least 25% below that of adjacent muscle tissue. Thresholds for lesions were adapted to the surrounding muscle signal on each of the 24 images per patient to avoid effects caused by image artifacts resulting from field and coil inhomogeneities as well as magic angle artifacts. The exact process of nerve segmentation has previously been described (12). Afterward, binarized images of lesions and healthy nerve tissue were analyzed in Matlab and the lesion load in percent of the full nerve volume was calculated. The process of image segmentation and binarization is illustrated in Fig. 1.

Statistical Analysis

Absolute values were used for analysis of EPT results (representing motor Aα-fiber functions). For QST results, each individual parameter was normalized to a published cohort of the same age, sex, and test region by subtracting the mean and dividing by the SD of the healthy control subject cohort (24). Resulting *z* scores have an expected zero mean and unity variance in healthy control subjects; *z* scores outside the ±1.96-SD range are outside the 95% CI and can be considered abnormal findings (14,16,24).

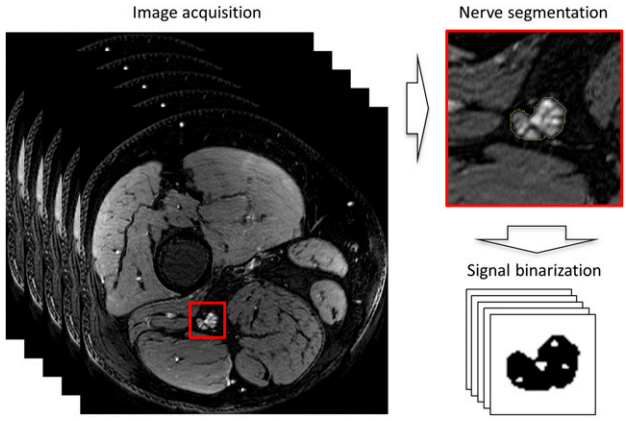

Figure 1— Nerve segmentation and binarization of lesions. Multislice segmentation of the sciatic nerve, binarization of nerve lesions, and statistical analysis of lesion load and vital nerve tissue.

Negative *z* scores (< −1.96 SDs) indicate lower thermoreceptive, nociceptive, or tactile functions. Positive *z* scores (>1.96 SDs) indicate elevated function (hyperalgesia or allodynia). The *z* scores of the single QST tests were clustered as follows to create compound *z* scores: thermal detection (average values of CDT, WDT, and TSL, representing sensory C fiber function), thermal pain (average values of CPT and HPT, representing sensory Aδ-fiber function), mechanical pain (MPT and MPS, representing sensory Aδ-fiber function), and mechanical detection (VDT and MDT, representing sensory Aβ-fiber function). For all absolute parameters, median and range are given. Both Kolmogorov-Smirnov and Shapiro-Wilk tests showed non-Gaussian distributions of T2w lesions and all absolute results for QST parameters except WDT. Therefore, nonparametric analyses were performed for absolute values (Kruskal-Wallis test and Mann-Whitney *U* test for group comparisons of absolute values, Spearman correlation coefficient [r_{Sp}] for correlation analyses), whereas ANOVA was used for group comparisons of compound *z* scores of QST parameters. Additionally, because of the wide distribution of lesion load in patients with diabetes and DPN, quartiles for the lesion load were calculated, and patients were divided into the following three groups to verify the relation between the severity of lesions in MRN and the clinical impairment observed: low lesion load <25th percentile (<4% of nerve tissue affected), moderate lesion load within the 25th–75th percentile (4–11% of nerve tissue affected), and high lesion load >75th percentile (>11% of nerve tissue affected).

For another statistical analysis, patients were categorized by their QST profiles into the following three groups: gain of function, loss of function, and no deficit. Gain of function was defined as pathological hyperalgesia (thermal or mechanical pain thresholds) with preserved nerve fiber function in detection thresholds. Loss of function was defined as pathological decrease of detection thresholds

in small, medium, and large fibers. Cutoff values for pathological test results of *z* scores have been set at 1.96 SD for gain of function and −1.96 SD for loss of function compared with a standardized control group as previously described (14,16).

Linear regression analysis was performed for evaluation of potential confounders. SPSS, version 23.0, software (IBM Deutschland, Ehningen, Germany) was used for all statistical analyses, whereas GraphPad Prism 7 (GraphPad Software, San Diego, CA) was used for all figures.

Data and Resource Availability

The data sets generated and/or analyzed in the current study are not publicly available because they contain patient data from UHH; data can be made available after anonymization from the corresponding author upon reasonable request for research purposes after approval by the local ethics committee.

RESULTS

In total, 73 participants were included: 48 patients with type 2 diabetes and an NSS and/or NDS of ≥3 (representing at least incipient DPN [18]), 13 patients with type 2 diabetes and an NSS and NDS of 0, and 12 control subjects without diabetes and an NSS and NDS of 0. All participants underwent MRN. The calculated T2w hyperintense lesion load ranged from 1 to 49%. Full patient characteristics are given in Table 1.

Age did not differ among the three groups ($P = 0.241$), but sex differed, with the control group mainly consisting of women, whereas there were more men than women in the diabetes groups (Table 1). No significant difference in lesion load in MRN among the three groups ($P = 0.238$) could be found, although the median percentage of lesions/healthy nerve tissue was 8.07% in patients with diabetes and DPN compared with 4.75% in control subjects (Table 1 and Fig. 2*A*). Moreover, HbA_{1c}, eGFR (by Chronic Kidney Disease Epidemiology Collaborative equation), creatinine, uACR, and diabetes duration did not differ between the two diabetes groups.

No correlations between clinical symptoms and T2w hyperintense lesion load in MRN could be found. However, tingling correlated significantly with both MDT ($r_{Sp} = 0.306$, $P = 0.049$) and VDT ($r_{Sp} = -0.327$, $P = 0.034$).

In a linear regression analysis that included all study participants, sex was the only significant parameter, with male sex being associated with a higher T2w hyperintense lesion load ($R^2 = 0.674$, $P = 0.041$), which remained when only patients with diabetes and DPN were included in the analysis ($R^2 = 0.259$, $P = 0.031$). Moreover, diabetes duration was a predictor for lesion load in patients with diabetes and DPN ($R^2 = 0.483$, $P = 0.022$). In both analyses, age, BMI, HbA_{1c}, uACR, insulin use, alcohol consumption, smoking, coronary heart disease, and peripheral artery disease were not associated with lesion load.

T2w Hyperintense Lesion Load and Large-Fiber Function

EPT was performed to evaluate function of large Aα motor fibers. In the comparison among control subjects, patients with diabetes without DPN, and patients with DPN, tibial NCV did not differ significantly ($P = 0.126$), while peroneal NCV was significantly different ($P = 0.02$) (Fig. 2*B* and *C*). In all participants, moderate negative correlations between lesion load and tibial NCV ($r_{Sp} = -0.362$, $P = 0.005$) and peroneal NCV ($r_{Sp} = -0.554$, $P < 0.001$) (Fig. 3*A* and *B*) as well as amplitudes of peroneal ($r_{Sp} = -0.317$, $P = 0.014$) and tibial ($r_{Sp} = -0.276$, $P = 0.035$) nerves and distal motor latency of the peroneal nerve ($r_{Sp} = 0.275$, $P = 0.037$) could be found. These correlations remained when only patients with diabetes and DPN were analyzed (peroneal NCV: $r_{Sp} = -0.497$, $P = 0.002$; tibial NCV: $r_{Sp} = -0.518$, $P = 0.002$; peroneal amplitude: $r_{Sp} = -0.337$, $P = 0.048$; tibial amplitude: $r_{Sp} = -0.518$, $P = 0.002$). In control subjects without diabetes, only the amplitude of the tibial nerve correlated with lesion load in MRN ($r_{Sp} = 0.654$, $P = 0.029$), whereas the other parameters did not show any correlations.

Patients with type 2 diabetes with DPN and high lesion load of >11% (>75th percentile) showed significant reductions in tibial ($P = 0.024$) and peroneal ($P = 0.032$) NCV (Fig. 4*A* and *B*) as well as in amplitudes of tibial ($P = 0.016$) and peroneal ($P = 0.048$) nerves compared with patients with moderate or low lesion load.

T2w Hyperintense Lesion Load and Nociceptive Medium- and Small-Fiber Function in All Participants

To evaluate the clinical relevance of MRN, associations between lesion load in MRN and clinical characteristics concerning sensory nerve functions evaluated by QST were studied. In the comparison between control subjects and patients with diabetes with and without DPN, significant differences could be found for the compound *z* scores for mechanical detection ($P < 0.001$, lower function in patients with DPN) and mechanical pain ($P = 0.02$, higher function in patients without DPN), whereas no significant differences for thermal pain or thermal detection could be found (ANOVA) (Table 2 and Fig. 2*D*–*G*).

In all study participants, irrespective of diabetes or DPN, moderate correlations between lesion load and composite *z* score clusters for mechanical detection ($r_{Sp} = -0.312$, $P = 0.007$) and mechanical pain ($r_{Sp} = -0.246$, $P = 0.036$) could be found, whereas thermal pain ($r_{Sp} = -0.152$, $P = 0.202$) and thermal detection ($r_{Sp} = -0.083$, $P = 0.488$) did not show correlations with lesion load (Fig. 3*C*–*F*).

T2w Hyperintense Lesion Load and Nociceptive Medium- and Small-Fiber Function in Patients With Type 2 Diabetes and DPN

In the group comparison of patients with type 2 diabetes and DPN with low, moderate, and high lesion load, patients with high lesion load showed the most severe clinical impairments in mechanical pain

Table 1—Patient characteristics

	Patients with type 2 diabetes with DPN	Patients with type 2 diabetes without DPN	Control subjects
Age (years)	65 (38–78)	65 (49–83)	61 (48–70)
Sex (*n*)			
Male	33	8	3
Female	15	5	9
Diabetes duration (years)	10 (0–30)	3 (1–20)	0
Patients with >10 years' diabetes duration	20	4	0
NSS (total score) (range 0–9)	6	0	0
NDS (total score) (range 0–10)	5	0	0
Retinopathy			
Yes	6	0	0
No	42	13	12
Nephropathy			
Yes	13	1	0
No	35	12	12
Stroke			
Yes	1	0	0
No	40	12	12
Unknown	7	1	0
Transient ischemic attack			
Yes	0	0	0
No	41	12	12
Unknown	7	1	0
Coronary heart disease			
Yes	7	0	0
No	36	12	12
Unknown	5	1	0
Myocardial infarction			
Yes	1	0	0
No	42	12	12
Unknown	5	1	0
Peripheral artery disease			
Yes	3	2	0
No	45	11	11
Unknown	0	0	1
Arterial hypertension			
Yes	40	4	2
No	8	9	10
Smoking			
Yes	6	1	0
No	42	12	12
Insulin therapy			
Yes	10	0	0
No	36	12	12
Unknown	2	1	0
BMI (kg/m^2)	29.7 (21.5–45.3)	30.5 (24.6–45.3)	27.2 (24.8–39.8)
n patients with BMI unknown	1	0	0
eGFR (mL/min)	94.6 (38.3–125.6)	71.1 (34–104.8)	85.1 (79.7–98.3)
n patients with eGFR <60	1	3	0
uACR (mg/mmol creatinine)	9.76 (2.42–653.94)	12.74 (4.07–129.33)	6.73 (3.77–67.5)
n patients with uACR 30–300	8	1	1
n patients with uACR >300	2	0	0
HbA_{1c} (%)	6.75 (5.4–10.8)	7.3 (5.2–7.9)	5.2 (4.7–5.8)
HbA_{1c} (IFCC) (mmol/mol)	50.5 (35.5–94.5)	56 (33–63)	33 (28–40)
Total cholesterol (mg/dL)	179 (113–280)	177 (151–223)	231 (158–300)

Continued on p. 441

Table 1—Continued

	Patients with type 2 diabetes with DPN	Patients with type 2 diabetes without DPN	Control subjects
Triglycerides (mg/dL)	181 (63–391)	108 (75–308)	120 (45–234)
HDL (mg/dL)	44 (32–111)	47 (36–70)	76 (46–93)
LDL (mg/dL)	95 (46–173)	100 (68–144)	140 (69–202)
Total body water (%)	43.9 (38.6–50.5)	45.7 (41.2–50.2)	51.2 (39.9–58.2)
T2w lesions/healthy nerve (%)	8.07 (1–49)	6.13 (3–14)	4.75 (2–12)
Tibial NCV (m/s)	39 (25–47)	41 (34–54)	44 (36–55)
Peroneal NCV (m/s)	38 (27–47)	40 (21–52)	45 (39–49)
Compound *z* score			
Mechanical detection	−1.74 (1.81)	0.89 (0.83)	0.69 (0.50)
Mechanical pain	0.63 (1.93)	2.08 (1.15)	1.39 (0.88)
Thermal pain	−0.51 (0.87)	−0.68 (0.90)	−0.34 (1.14)
Thermal detection	−1.15 (1.11)	−0.69 (1.05)	−0.33 (1.13)

Data are median (range) unless otherwise indicated. Data for compound *z* scores are mean (SD), since these scores are normally distributed. One patient only had known diabetes for 2 months; therefore, diabetes duration was defined as 0 years in this case. Distribution of NSS among patients with DPN was as follows: no symptoms (NSS 0), $n = 10$; mild symptoms (NSS 3–4), $n = 7$; moderate symptoms (NSS 5–6), $n = 13$; and severe symptoms (NSS 7–9), $n = 18$. Distribution of NDS among patients with DPN was as follows: no neuropathological deficits (NDS 0–2), $n = 12$; mild neuropathological deficits (NDS 3–5), $n = 16$; moderate neuropathological deficits (NDS 6–8), $n = 16$; and severe neuropathological deficits (NDS 9–10), $n = 4$. Patients with DPN and an NSS of 0 had a pathological NDS and vice versa, since inclusion criteria were NSS and/or NDS ≥3 points. Total body water was measured by body impedance analysis. IFCC, International Federation of Clinical Chemistry and Laboratory Medicine.

($P = 0.008$) and thermal pain clusters ($P = 0.009$), whereas no differences in mechanical detection or thermal detection clusters could be found (ANOVA) (Table 3 and Fig. 4*C–F*).

When categorizing patients with DPN according to QST results into three groups, namely, participants without any clinical deficits, with relative gain of function, and with relative loss of function, a significant difference in lesion load ($P = 0.033$) could be found between patients with loss of function and the two other patient groups without deficit or with gain of function in QST. These results are shown in Fig. 5.

T2w Hypointense Lesions

T2w hypointense lesions were also investigated in 70 participants (10 control subjects, 12 patients with type 2 diabetes without DPN, and 48 patients with type 2 diabetes and DPN). There was a mild positive correlation between T2w hyperintense and hypointense lesions ($r_{Sp} = 0.275$, $P = 0.021$). T2w hypointense lesions showed results similar to those of T2w hyperintense lesions, with no significant differences between the groups ($P = 0.77$) but with a moderate negative correlation between hypointense lesion load and the *z* scores of the mechanical detection cluster ($r_{Sp} = -0.343$, $P = 0.004$), whereas no correlations with the other QST clusters could be found. In EPT, there were moderate correlations between T2w hypointense lesion load and peroneal nerve function (NCV: $r_{Sp} = -0.378$, $P = 0.004$; amplitude: $r_{Sp} = -0.355$, $P = 0.007$; distal motor latency: $r_{Sp} = 0.318$, $P = 0.018$) but not with tibial nerve function (NCV: $r_{Sp} = -0.211$, $P = 0.122$; amplitude: $r_{Sp} = -0.118$, $P = 0.385$; distal motor latency: $r_{Sp} = 0.174$, $P = 0.199$).

DISCUSSION

This study confirmed our hypothesis that the load of T2w lesions in MRN is mainly associated with lower medium- and large-fiber function in patients with type 2 diabetes and DPN, since clinical parameters reflecting functions of sensory Aδ and Aβ fibers as well as Aα motor fibers were affected in patients with high lesion load. Since MRN itself does not allow for exact differentiation of nerve fiber types, these results lead to a better understanding of the lesions visualized, with the degree of clinical alterations in medium- and large-fiber function being related to the lesion load determined by MRN morphological alterations. This is of clinical importance because large-fiber dysfunction is more commonly associated with ulcerations, amputations, and cardiovascular mortality than small-fiber dysfunction (10,16). T2w hypointense lesions showed results similar to those of hyperintense lesions. Patients with higher hypointense lesion load showed lower function of Aβ fibers and peroneal motor fibers, whereas no associations with smaller sensory fiber functions could be found.

Every participant enrolled in this study, irrespective of diabetes or DPN, showed a certain amount of T2w hyperintense lesions in MRN in peripheral nerves. Therefore, one potential explanation might be that nerve lesions occur physiologically as part of the natural aging process. This idea is supported by findings from former studies on diffusion tensor imaging in peripheral nerve that demonstrated that a nerve's fractional anisotropy as a parameter

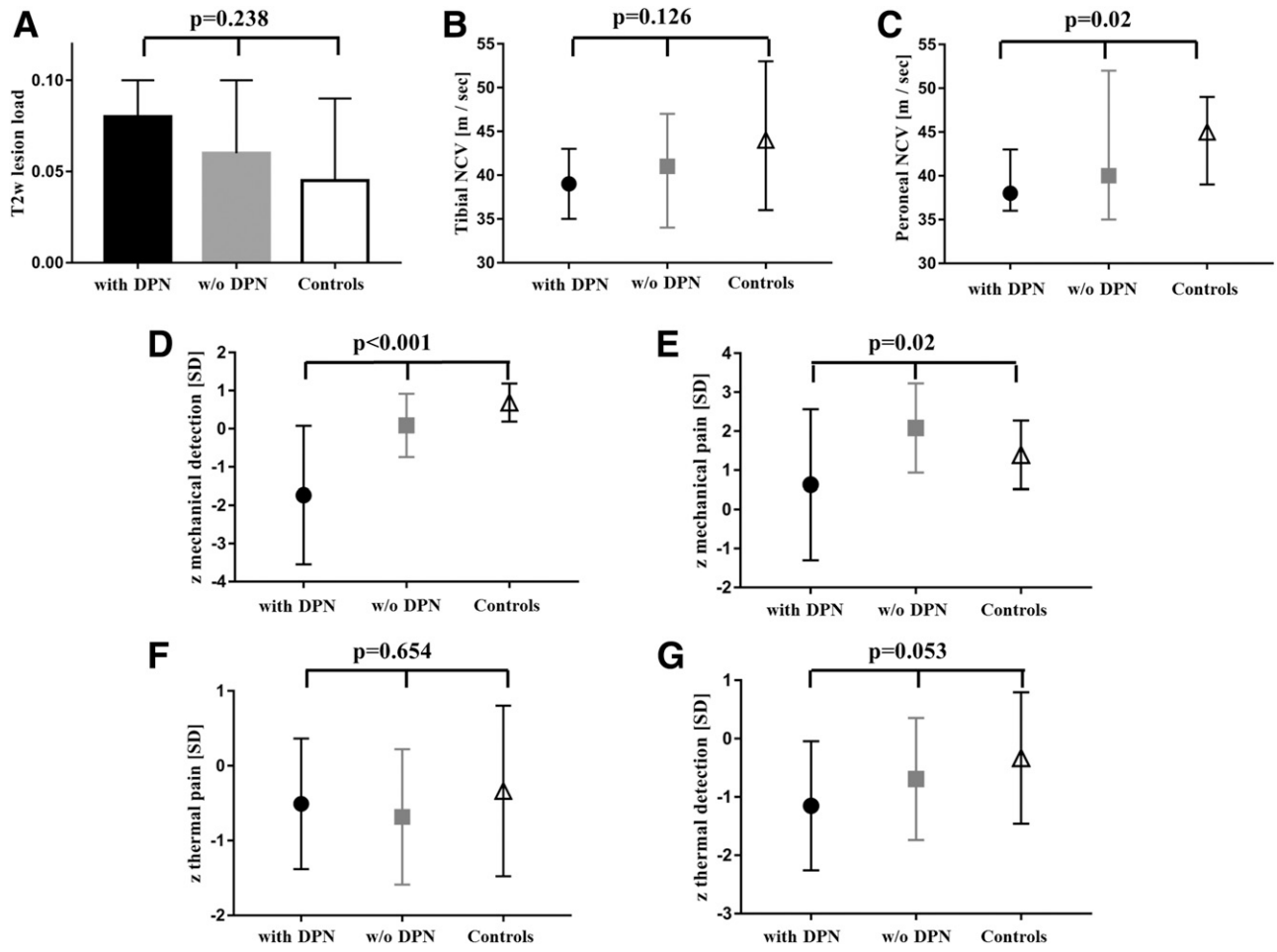

Figure 2—Comparison among groups of patients with type 2 diabetes with DPN, type 2 diabetes without DPN, and control subjects (as determined by NSS and NDS) with regard to T2w lesion load, EPT, and compound *z* scores. *A*: Group comparison of T2w lesion load in MRN. *B*: Group comparison of tibial NCV. *C*: Group comparison of peroneal NCV. *D*: Group comparison of compound *z* score for mechanical detection (comprising VDT and MDT) in QST. *E*: Group comparison of compound *z* score for mechanical pain (comprising MPT and MPS) in QST. *F*: Group comparison of compound *z* score for thermal pain (comprising CPT and HPT) in QST. *G*: Group comparison of compound *z* score for thermal detection (comprising CDT, WDT, and TSL) in QST. w/o, without.

for its structural integrity shows an age-dependent decline (25), indicating that nerve microstructure deteriorates with age. Because of the cross-sectional design of this study, however, this assumption remains hypothetical. Males showed a higher lesion load than females, since male sex was associated with higher lesion load in a linear regression analysis. This is in line with reference values for QST parameters, which are also age and sex dependent, with older people and men physiologically showing inferior results (14). This further renders possible that vascular damage has an impact on the development of T2w lesions, since vascular damage and atherosclerosis are usually more prominent and more frequent in males than in females (26). Also, changes in the relation of lipid material and water in the myelin microstructure may lead to significant changes in MR signal characteristics (27); however, the definitive pathophysiological mechanisms underlying the nerve lesions visualized by MRN remain unclear to this point. A critical aspect in the detection of T2w lesions is the echo time of the sequences applied. MRN sequences typically use strongly weighted T2 sequences with long echo times (28). Our sequence used an (optimized) echo time of 55 ms to generate an optimal contrast between healthy nerve and nerve lesions. While an alteration of echo time toward longer echo times may increase this contrast, lesion location will not change. Only lower echo times may reduce this contrast and, therefore, lesion detection (27). Other factors that may influence T2 contrast in nerve fibers are fiber orientation, myelin water ratio, neuronal g factors, or diffusion effects (27). Another critical aspect is the anatomical region of lesion detection, since it is known from previous studies that the number of nerve lesions differs between proximal and distal parts of the sciatic nerve (12). Therefore, scans were centered to the sciatic nerve's bifurcation to guarantee that the anatomical region examined was identical in all participants.

Lesions did not seem to be directly linked to hyperglycemia, since neither blood glucose nor HbA_{1c} was

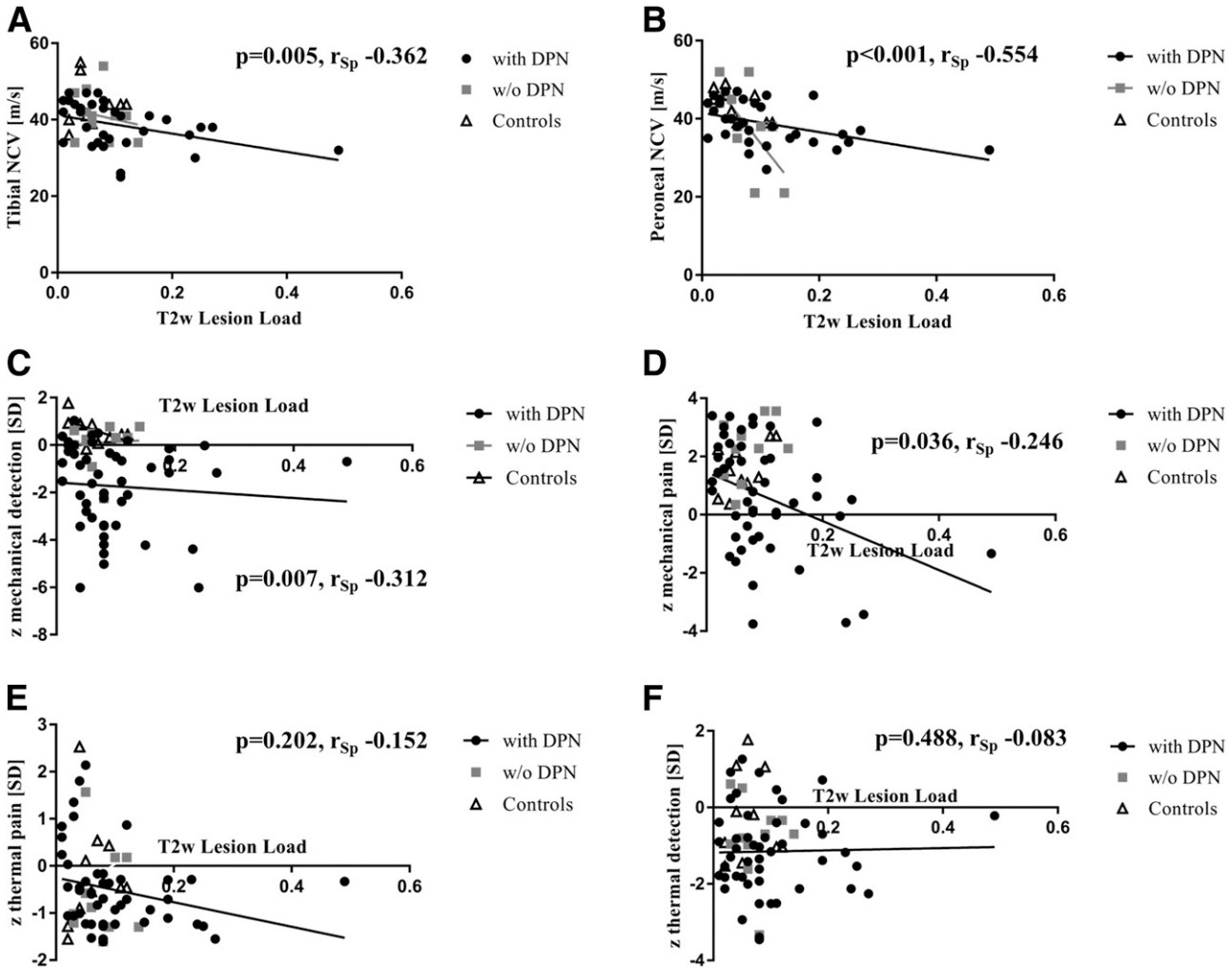

Figure 3— Correlation analysis of T2w lesion load with EPT and compound *z* scores in patients with type 2 diabetes with DPN, type 2 diabetes without DPN, and control subjects (as determined by NSS and NDS). *A*: Correlation between tibial NCV and T2w lesion load. *B*: Correlation between peroneal NCV and T2w lesion load. *C*: Correlation between compound *z* score for mechanical detection (comprising VDT and MDT) in QST and T2w lesion load. *D*: Correlation between compound *z* score for mechanical pain (comprising MPT and MPS) in QST and T2w lesion load. *E*: Correlation between compound *z* score for thermal pain (comprising CPT and HPT) in QST and T2w lesion load. *F*: Correlation between compound *z* score for thermal detection (comprising CDT, WDT, and TSL) in QST and T2w lesion load. w/o, without.

associated with lesion load. One has to consider, however, that the history of HbA_{1c} values was unknown, which is why HbA_{1c} values in this study only represented momentary glucose control. Moreover, several interventional studies only showed limited effects of glucose control on the course of DPN, indicating that other risk factors like dyslipidemia and microvascular damage might play an equal or even more important role (29). As for the progression of nerve damage, diabetes duration was independently associated with lesion load. Because of the cross-sectional nature of our data, further longitudinal studies on the effect of age, disease duration, and other risk factors for the formation of nerve lesions in patients with DPN are required.

Interestingly, patients without clinically apparent DPN according to NSS and NDS showed relative hyperalgesia compared with control subjects and patients with apparent DPN according to NSS and NDS, who showed relative hypoalgesia. Moreover, when only considering patients with type 2 diabetes and DPN, patients with lower function in QST showed significantly higher lesion load compared with participants without any deficit or with elevated function. These aspects could support the hypothesis that elevated function usually occurs at the early stages (whereas lower function signals later stages of DPN) and that hyperalgesia requires relatively intact fibers as visualized by low lesion load.

No significant differences in lesion load between patients with DPN and control subjects without diabetes could be found despite the median lesion load being almost twice as high in patients with diabetes and DPN compared with control subjects. The main reason for this finding

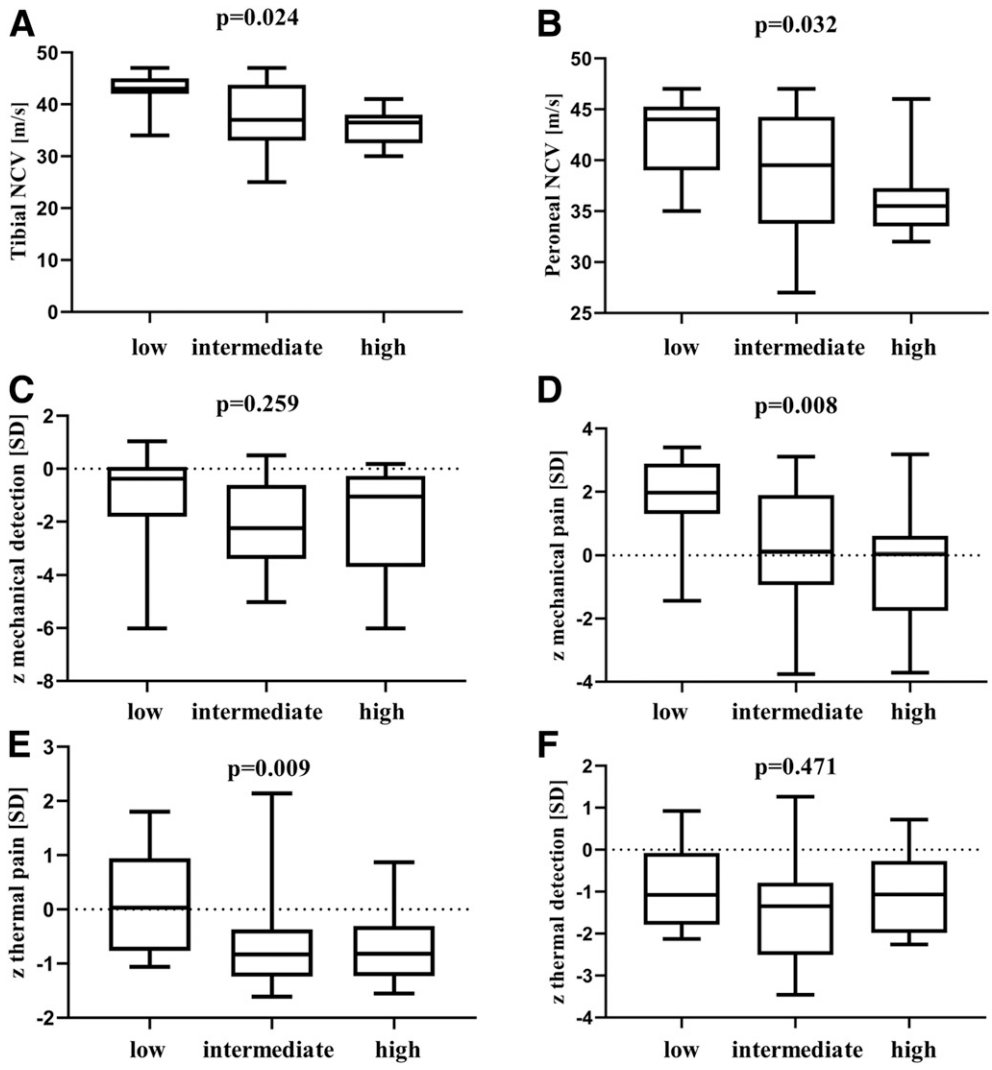

Figure 4—Comparison between groups of patients with type 2 diabetes and DPN divided by the lesion load in MRN. The low group consists of patients with a lesion load <25th percentile, which accounts for an absolute load of hyperintense lesions of <4%. The intermediate group consists of patients with moderate lesion load between the 25th and 75th percentiles, which accounts for an absolute load of hyperintense lesions of 4–11%. The high group consists of patients with a high lesion load in MRN >75th percentile, which accounts for an absolute load of hyperintense lesions of >11%. *A*: Comparison of tibial NCV. *B*: Comparison of peroneal NCV. *C*: Comparison of compound *z* score for mechanical detection (comprising VDT and MDT) in QST. *D*: Comparison of compound *z* score for mechanical pain (comprising MPT and MPS) in QST. *E*: Comparison of compound *z* score for thermal pain (comprising CPT and HPT) in QST. *F*: Comparison of compound *z* score for thermal detection (comprising CDT, WDT, and TSL) in QST.

could be the large range of lesion load in patients with DPN and the variability of patients with diabetes with regard to glucose control, phenotype, and other clinical parameters. Therefore, large cohorts would be required to achieve statistical significance. Also, one has to keep in mind that the diagnosis of DPN was based on the NSS and NDS, two scores that have been validated for the diagnosis of DPN in clinical settings but that do not allow for precise conclusions on axonal function.

Remarkably, EPT showed correlations between lesion load in MRN and both segmental demyelination and axonal damage in patients with type 2 diabetes and DPN. Both types of nerve damage have previously been described in DPN (30). In contrast, in control subjects without diabetes, only the tibial nerve amplitude correlated with the lesion load. This is supposedly due to the fact that only the lesion load of the tibial compartment of the sciatic nerve was evaluated in MRN, since the curving natural course of the peroneal compartment does not allow for precise binarization of nerve lesions. Moreover, it is possible that T2w lesions are associated with subclinical axonal damage, which might also occur with age in healthy subjects without neuropathy. Subclinical neuronal loss or axonal damage are well-established findings in the course of degenerative or inflammatory disorders of the central nervous system like Parkinson disease or multiple sclerosis (31,32). The findings of this study, therefore, indicate that subclinical axonal damage may also occur in the peripheral nervous system.

Table 2—ANOVA for comparison among overall groups

	Sum of squares	df	Mean square	*F*	Significance
z score mechanical detection * overall group					
Between groups (combined)	75.537	2	37.769	16.005	<0.001
Within groups	165.190	70	2.360		
Total	240.727	72			
z score mechanical pain * overall group					
Between groups (combined)	23.514	2	11.757	4.122	0.020
Within groups	199.649	70	2.852		
Total	223.163	72			
z score thermal pain * overall group					
Between groups (combined)	0.730	2	0.365	0.427	0.654
Within groups	58.988	69	0.855		
Total	59.717	71			
z score thermal detection * overall group					
Between groups (combined)	7.408	2	3.704	3.059	0.053
Within groups	84.759	70	1.211		
Total	92.167	72			

Comparison of QST parameters among control subjects, patients with type 2 diabetes without DPN, and patients with type 2 diabetes with DPN. Compound *z* scores were used.

The fact that no correlations between clinical symptoms and lesion load in MRN could be found in patients with DPN is not unusual because a discrepancy between clinical symptoms and functional impairment is a common finding in studies addressing DPN (33). However, to our knowledge, this study is the first to show a correlation between clinical symptoms and QST parameters, with VDT and MDT being associated with tingling. Potentially, longitudinal studies on larger cohorts might be necessary to detect further connections between symptoms and objective clinical impairment.

Since there was a strong correlation between the objectively detectable nerve lesions in MRN and most QST parameters, reflecting a broad range of sensory functions and nerve fibers, this study shows the validity and sensitivity of QST, even within physiological ranges, and likewise, a potential functional relevance of these lesions, even in subjects without diabetes or neuropathy. This finding is important because QST parameters are subjective and depend on a patient's cooperation during testing.

There are some limitations to this study. One may argue, for instance, that the in-plane resolution of MRN imaging was limited to 300 × 500 μm, which only allows for the visualization of fascicular structures but precludes imaging of different fiber types (e.g., Aα fibers with a diameter of 20 μm or C fibers with a diameter of ~5 μm) directly. It has to be acknowledged, however, that even preclinical MRI scanners at 9.4T or 14.4T do not allow for the visualization of single nerve fibers in rodents or in vitro

Table 3—ANOVA for comparison among patients with type 2 diabetes separated by lesion load

	Sum of squares	df	Mean square	*F*	Significance
z score mechanical detection * lesion load groups					
Between groups (combined)	8.981	2	4.491	1.392	0.259
Within groups	145.185	45	3.226		
Total	154.166	47			
z score mechanical pain * lesion load groups					
Between groups (combined)	33.587	2	16.794	5.330	0.008
Within groups	141.778	45	3.151		
Total	175.365	47			
z score thermal pain * lesion load groups					
Between groups (combined)	6.695	2	3.347	5.189	0.009
Within groups	29.030	45	0.645		
Total	35.725	47			
z score thermal detection * lesion load groups					
Between groups (combined)	1.900	2	0.950	0.766	0.471
Within groups	55.794	45	1.240		
Total	57.694	47			

Comparison of QST parameters in patients with type 2 diabetes and DPN with high, moderate, and low lesion load. Compound *z* scores were used.

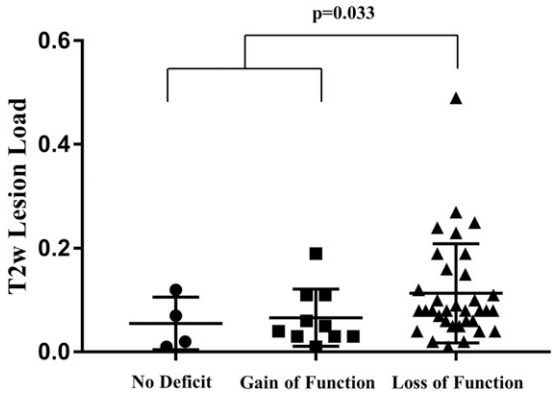

Figure 5—Comparison of lesion load among patients without deficits, with relative gain of function, and with relative loss of function in QST. Shown is the comparison of lesion load in patients with type 2 diabetes and DPN. In this case, because of the inherently low number of patients with DPN according to NSS but without deficit in QST (neuropathic symptoms without objective neural deficits in clinical testing), Kruskal-Wallis test was not significant. Therefore, Mann-Whitney *U* test was used to compare patients without deficit or with gain of function with patients with loss of function.

samples of human nerves. This is why in this study, in vivo MRN lesions at 3T were correlated with QST parameters as an established method for the differentiation of nerve fiber types. One could further argue that the control group mainly consisted of females, whereas patients with diabetes were predominantly male. For quantitative sensory testing, calculation of *z* scores accounted for any sex differences. Male sex was associated with higher lesion load. This effect remained when only patients with diabetes and DPN were considered. However, since the relevant results of the study were within the groups and the study was not powered for group comparison, a significant influence on the outcome is unlikely. Some of the correlations found in this study were only moderate, which is a common finding in studies that combined clinical and imaging data (34). Moreover, sample size was rather small, and the study was monocentric. The study was not powered to show differences in T2w lesion load among the groups; the primary outcome was to show the impact of T2w lesions on nerve function. Therefore, sample size seems valid because an association between T2w lesion load and nerve function in clinical testing could be shown. One may argue, of course, that the main limitation of our study is the cross-sectional design, which precludes any definite conclusions regarding the effect of age and disease duration, since no longitudinal MRI and QST data of the patients examined are available. While this is true, the finding of an association between type 2 diabetes duration and lesion load, indicating that neuropathy worsens with disease duration, is in line with the findings of several previous clinical studies. Long-term studies with larger sample sizes are needed to study the impact of these lesions on the natural course on diabetic neuropathy. For these studies, patients with subclinical DPN (low NSS and NDS) should be included as well.

This study exclusively included patients with diabetic neuropathy in type 2 diabetes, which precludes conclusions about the effects of MRN lesions in patients with type 1 diabetes. Since it has recently been shown that both hyperintense and hypointense nerve lesions also occur in type 1 diabetes but that the distribution of nerve lesions differs between diabetes types (12), it seems very likely that associations of lesions with QST parameters will also be found in type 1 diabetes. Therefore, further studies are required to investigate the impact of MRN lesions on QST parameters in type 1 diabetes.

In conclusion, this study suggests that lesions in T2w MRN are a physiological finding in peripheral nerves, potentially as a consequence of aging. They are mainly associated with lower medium- and large-fiber nerve function. Since the sciatic nerve's lesion load correlates with diabetes duration as well as with sensory and motor nerve function in patients with DPN and control subjects, it is very likely that lesions are of pathophysiological relevance. Therefore, future studies should focus on the composition of these lesions and their development or progression over time in a longitudinal design in subjects with and without diabetes.

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Author Contributions. J.B.G. was involved in the study concept, enrolled patients, performed clinical analyses, performed statistical analyses, wrote the manuscript, and designed figures and tables. J.M.E.J. was involved in the study concept, performed radiological imaging analyses, wrote the manuscript, and designed figures. F.T.K. performed radiological imaging analyses and reviewed the manuscript. Z.K. enrolled patients, performed clinical analyses, and reviewed the manuscript. R.-D.T. gave expertise in quantitative sensory testing, gave advice in statistical analyses, and contributed to the discussion. S.S.-H. gave expertise in quantitative sensory testing and contributed to the discussion. P.P.N. was involved in the study concept and reviewed the manuscript. M.B. gave expertise in radiological imaging analyses and reviewed the manuscript. S.K. was the head of the study, was involved in study concept, and reviewed and edited the manuscript. J.B.G. 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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