Diabetic Neuropathy Differs between Type 1 and Type 2 Diabetes: Insights from Magnetic Resonance Neurography

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Objective: To visualize and quantify differences of microstructural nerve damage in distal symmetric diabetic neuropathy (DPN) between type 1 diabetes (T1D) and type 2 diabetes (T2D), and to detect correlations between neuropathic symptoms and serological risk factors.
Methods: Three-tesla magnetic resonance neurography of the sciatic nerve was performed in 120 patients (T1D, n = 35; T2D, n = 85) with either DPN (n = 84) or no DPN (n = 36). Results were subsequently correlated with clinical, serological, and electrophysiological patient data.
Results: T2-weighted (T2w)-hyperintense lesions correlated negatively with tibial compound motor action potential ($r = -0.58$, $p < 0.0001$) and peroneal nerve conduction ($r = 0.51$, $p = 0.0002$), and positively with neuropathy disability score (NDS; $r = -0.54$, $p < 0.0001$), neuropathy symptom score (NSS; $r = 0.52$, $p < 0.0001$), and HbA1c level ($r = 0.23$, $p = 0.014$). T2w-hypointense lesions correlated positively with NDS ($r = 0.28$, $p = 0.002$), NSS ($r = 0.36$, $p < 0.0001$), and serum triglycerides ($r = 0.34$, $p = 0.0003$), and negatively with serum high-density lipoprotein (HDL; $r = -0.48$, $p < 0.0001$). For DPN in T1D, elevated values of T2w-hyperintense lesions ($19.67 \pm 4.13\%$ vs $12.49 \pm 1.23\%$, $p = 0.027$) and HbA1c ($8.74 \pm 0.29\%$ vs $7.11 \pm 0.16\%$, $p < 0.0001$) were found when compared to T2D. For DPN in T2D, elevated T2w-hypointense lesions ($23.41 \pm 2.69 mm^3$ vs $11.43 \pm 1.74 mm^3$, $p = 0.046$) and triglycerides (220.70 ± 23.70mg/dl vs 106.60 ± 14.51mg/dl, $p < 0.0001$), and lower serum HDL (51.29 ± 3.02mg/dl vs 70.79 ± 4.65mg/dl, $p < 0.0001$) were found when compared to T1D.
Interpretation: The predominant type of nerve lesion in DPN differs between T1D and T2D. Correlations found between lesion type and serological parameters indicate that predominant nerve lesions in T1D are associated with poor glycemic control and loss of nerve conduction, whereas predominant lesions in T2D are associated with changes in lipid metabolism. These findings may be helpful for future studies on the underlying pathophysiological pathways and possible treatments for DPN in T1D and T2D.

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Distal symmetric diabetic neuropathy (DPN) is one of the most frequent and yet most poorly understood complications of diabetes. About 30 to 50% of all adult diabetic patients are affected with high morbidity and health care costs.[1,2] It is generally accepted that poor glycemic control is a major risk factor for the development of DPN.[3,4] Elevated levels of blood glucose have been linked to complex inflammatory processes, endothelial dysfunction, and the deposition of advanced glycation end products (AGEs) in the extracellular matrix (ECM) of Schwann cells, causing the destruction of myelin and impairing axonal regeneration after damage.[5–7] Another risk factor for the development of DPN is dyslipidemia, especially low serum high-density lipoprotein (HDL) and elevated triglycerides, which have been linked to both increased neuropathic symptoms and

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microvascular angiopathy.[8] It is not yet clear whether dyslipidemia itself is an independent risk factor for the development of polyneuropathies or whether it just contributes to the effects caused by hyperglycemia in DPN.[1,8–10] There is ongoing controversy whether an impairment of kidney function contributes to the development of DPN.[11–13] Long duration of diabetes has also been linked to the development of DPN, suggesting that a long course of the disease is associated with an aggravation of effects caused mainly by elevated serum glucose level over time.[1,6]

Risk factors are not equally distributed among type 1 diabetes (T1D) and type 2 diabetes (T2D) patients; dyslipidemia and obesity, for example, occur more frequently in patients suffering from T2D.[14] Also, it has been shown that, for T1D patients, glycemic control is more beneficial for the course of DPN than in T2D.[2] This raises the question of whether damage to peripheral nerves in T1D and T2D originates from different risk factors and thus results in different patterns of nerve damage, which as a consequence would require different types of preventive treatment. It has been shown in histological studies of nerve tissue in vitro as well as in recent imaging studies in vivo that the earliest and most prominent nerve lesions in DPN occur at the level of the distal sciatic nerve.[15–19] This is crucial, because in vivo biopsies of the sciatic nerve are justified neither for clinical nor for scientific purposes. Postmortem studies of sciatic nerves mostly show very late stages of the disease and only allow limited retrospective correlation with serological and clinical data. Furthermore, the sciatic nerve is hard to access for proper electrophysiological examinations in vivo. Studies of peripheral nerves in rodents face the problem that especially results concerning the impact of dyslipidemia are limited because the effects of dyslipidemia in humans cannot be reproduced exactly due to differing fat and cholesterol metabolism.[20]

To our knowledge, so far, no study on DPN has shown a direct correlation of risk factors and peripheral nerve lesions of diabetes mellitus patients in vivo, which could be relevant for the understanding of the pathological mechanisms in DPN. The aim of this study was to correlate the load of lesions in the sciatic nerve with clinical findings and serological markers that have been identified as risk factors for the development of DPN in T1D and T2D patients in previous studies.

Patients and Methods

Study Design and Patients

This study was approved by the local ethics committee (HEIST-DiC, local ethics number S-383/2016, clinicaltrials.gov identifier NCT03022721), and all participants gave written informed consent. One hundred twenty patients (48 female, 72 male, mean age = 62.2 years, range = 25–78) with T1D (n = 36) and T2D (n = 84) took part in this prospective study between June 2015 and March 2017. Overall exclusion criteria were age < 18 years, pregnancy, any contraindications for magnetic resonance imaging (MRI), any history of lumbar surgery or disc protrusion, any history of anemia, vitamin deficiency, or chronic bowel diseases such as Crohn, coeliac, or inflammatory bowel disease, any other risk factors for neuropathy such as alcoholism, malignant or infectious diseases, any previous or ongoing exposure to neurotoxic agents, and any chronic neurological diseases such as Parkinson disease, restless legs syndrome, or multiple sclerosis. In addition, the total serum protein and albumin as well as the hematocrit (HKT), the mean corpuscular volume (MCV) of erythrocytes, and the red blood cell distribution width (RDW) were tested in every participant to rule out micro- or macrocytic anemia as a potential consequence of vitamin deficiency and to detect elevated total serum proteins as an indicator for monoclonal gammopathy.

Clinical and Electrophysiological Examination

A detailed medical history was documented for each patient, and an examination of neuropathic symptoms was performed, including evaluation of the neuropathy disability score (NDS) and the neuropathy symptom score (NSS). Whereas the NSS comprises a patient's subjective perception of neuropathic symptoms, including the qualities and localization of symptoms as well as their dependence on both time of day and patient posture, the NDS contains more objective clinical parameters such as the strength of tendon reflexes, the quality of vibration perception, pain perception, and the perception of temperature, all acquired during a clinical examination. Both scores range from 0 to 10 with 0 meaning no symptoms of any kind and 10 indicating a maximum of disability and neuropathic symptoms.[21]

The electrophysiological examination (VikingQuest; Viasys Healthcare, Höchberg, Germany) of the right leg included: distal motor latencies (DML) of the right tibial and peroneal nerve; motor (compound muscle action potential [CMAP]) and sensory (sensory nerve action potential [SNAP]) amplitudes of the tibial (CMAPtib), peroneal (SNAPper), and sural nerves; and nerve conduction velocities of the tibial (NCVtib), peroneal (NCVper), and sural nerves. It was ensured that skin temperature was at least 32 °C. Presence of DPN was determined according to the following criteria:

1. A score of ≥ 3 on NDS or NSS. If a discrepancy between NDS and NSS was found, the higher score was chosen in conformity with the guidelines issued by the German Society for Diabetology.[22]
2. Abnormal results of nerve conduction parameters mentioned above in at least 2 different nerves according to the guidelines issued by the German Society for Diabetology.[22]

Blood was drawn in fasting state and proceeded immediately under standardized conditions at the Central Laboratory of the University Hospital of Heidelberg. Albumin excretion in urine was detected in morning spot urine within all

TABLE 1. Magnetic Resonance Imaging, Demographic, Clinical, Electrophysiological, and Serological Data of Patients with NDPN and DPN

Parameter	NDPN	DPN	*p*
T2w-hypointense lesion volume, mm^3	11.43 ± 1.74	23.41 ± 2.69	0.002[a]
T2w-hyperintense lesion load, % of the full nerve	3.18 ± 0.004	13.93 ± 0.01	<0.0001[b]
Diabetes type 1, No.	17	18	n.a.
Diabetes type 2, No.	19	66	n.a.
Age, yr	62.55 ± 1.29	60.83 ± 1.61	0.180, ns
Disease duration, yr	17.40 ± 1.40	23.39 ± 2.59	0.048[c]
Body mass index, kg/m^2	28.89 ± 0.59	28.21 ± 0.83	0.631, ns
Neuropathy symptom score	0.06 ± 0.06	5.46 ± 0.26	<0.0001[b]
Neuropathy deficit score	1.44 ± 0.25	5.10 ± 0.30	<0.0001[b]
Tibial compound motor action potential, mV	15.99 ± 1.71	8.06 ± 1.01	0.0007[b]
Peroneal SNAP, mV	6.18 ± 0.71	4.00 ± 0.40	0.007[a]
Sural SNAP, mV	5.85 ± 0.96	3.73 ± 0.41	0.024[c]
NCV of tibial nerve, m/s	41.5 ± 1.59	36.77 ± 1.32	0.036[c]
NCV of peroneal nerve, m/s	44.09 ± 1.27	38.32 ± 0.99	0.005[a]
NCV of sural nerve, m/s	43.67 ± 1.05	38.76 ± 2.57	0.075, ns
DML of tibial nerve, ms	6.74 ± 1.40	5.767 ± 0.60	0.76, ns
DML of peroneal nerve, ms	5.97 ± 1.04	5.81 ± 0.53	0.49, ns
HbA1c, %	6.98 ± 1.22	7.46 ± 0.15	0.180, ns
Triglycerides, mg/dl	149.20 ± 18.81	211.30 ± 27.29	0.0007[b]
High-density lipoprotein, mg/dl	61.70 ± 2.70	55.64 ± 2.65	0.013[c]
Total cholesterol, mg/dl	181.00 ± 5.45	191.30 ± 6.87	0.507, ns
Low-density lipoprotein, mg/dl	98.25 ± 3.53	106.70 ± 6.18	0.209, ns
Creatinine, mmol/l	0.93 ± 0.10	0.86 ± 0.05	0.822, ns
Estimated glomerular filtration rate, $ml/min \times 1.73m^2$	85.15 ± 3.00	83.77 ± 3.59	0.937, ns
Albumin/creatinine ratio, mg/mmol	126.60 ± 62.49	23.89 ± 5.02	0.652, ns

All values are displayed as mean ± standard error. Level of significance: $p \geq 0.05$ ns.
[a]$p < 0.01$, [b]$p < 0.001$, [c]$p < 0.05$.
DML = distal motor latency; DPN = diabetic neuropathy; n.a., not applicable; NCV = nerve conduction velocity; NDPN = no DPN; ns = not significant; SNAP = sensory nerve action potential; T2w = T2-weighted.

participants. Estimated glomerular filtration rate were calculated with the CKD-EPI-formula.[23] Detailed clinical, electrophysiological, and serological data are presented in Tables 1 and 2.

Magnetic Resonance Neurographic Imaging Protocol

All participants underwent high-resolution MRN of the right leg in a 3.0T magnetic resonance scanner (Magnetom TIM-TRIO; Siemens Healthcare, Erlangen, Germany). A 15-channel transmit–receive extremity coil was used; we applied an axial high-resolution T2-weighted (T2w) turbo spin echo 2-dimensional (2D) sequence with spectral fat saturation of the right midthigh and mid lower leg and the following parameters: relaxation time (TR) 5,970 milliseconds, echo time (TE) = 55 milliseconds, field of view (FOV) = $160 \times 160mm^2$, matrix size = 512×512, slice thickness = 4mm, interslice gap = 0.35mm, voxel size = $0.5 \times 0.3 \times 4.0mm^3$, 24 slices. In addition, we recorded an axial T1w volumetric interpolated breath-hold examination 3D sequence of the right midthigh with TR = 3.3 milliseconds, TE = 1.11 milliseconds,

TABLE 2. Magnetic Resonance Imaging, Demographic, Clinical, and Serological Data of NDPN Patients with T1D and T2D

Parameter	NDPN T1D	NDPN T2D	*p*
T2w-hypointense lesion volume, mm^3	7.52 ± 0.97	16.83 ± 3.16	0.027[a]
T2w-hyperintense lesion load, % of the full nerve	2.80 ± 0.50	2.68 ± 0.43	0.838, ns
Age, yr	59.45 ± 2.22	67.04 ± 1.48	0.006[b]
Disease duration, yr	35.00 ± 2.64	13.2 ± 1.88	<0.0001[c]
Body mass index, kg/m^2	25.28 ± 1.00	31.43 ± 0.92	<0.0001[c]
Neuropathy symptom score	0.00 ± 0.00	0.013 ± 0.013	>0.999, ns
Neuropathy deficit score	1.73 ± 0.38	1.47 ± 0.41	0.640, ns
HbA1c, %	7.508 ± 0.30	6.87 ± 0.20	0.081, ns
Triglycerides, mg/dl	101.2 ± 17.65	149.2 ± 19.30	0.077, ns
High-density lipoprotein, mg/dl	63.00 ± 4.36	55.75 ± 4.36	0.257, ns
Low-density lipoprotein, mg/dl	104.3 ± 8.32	95.67 ± 7.65	0.460, ns
Total cholesterol, mg/dl	185.7 ± 8.83	179.4 ± 7.86	0.605, ns
Creatinine, mmol/l	0.844 ± 0.06	0.90 ± 0.10	0.639, ns
Estimated glomerular filtration rate, $ml/min \times 1.73m^2$	81.58 ± 7.49	83.24 ± 5.35	>0.999, ns
Albumin/creatinine ratio, mg/mmol	29.55 ± 11.36	15.35 ± 4.48	0.384, ns

All values are displayed as mean ± standard error. Level of significance: $p \geq 0.05$ ns.
[a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$.
NDPN = no diabetic neuropathy; ns = not significant; T1D = type 1 diabetes; T2D = type 2 diabetes; T2w = T2-weighted.

FOV = $160 \times 160mm^2$, matrix size = 128×128, slice thickness = 4mm, interslice gap = 0.35mm, voxel size = $0.5 \times 0.3 \times 4.0mm^3$, 24 slices.

Image Postprocessing and Statistical Analysis

All images generated were pseudonymized. To detect the location of the highest nerve lesion load in every patient and to test consistency with former studies,[19] the lesion load of the sciatic nerve at thigh level and of the tibial and common peroneal nerve at the level of the calves was compared visually by 2 trained neuroradiologists blinded to the clinical data. In all participants, the lesion load of the sciatic nerve was evaluated to be larger than the lesion load of the tibial and common peroneal nerve. Thus, the calculation of T2w-hyperintense and T2w-hypointense lesions was performed for the sciatic nerve at midthigh level.

Images at midthigh level were analyzed with a semiautomatic approach using ImageJ[24] and custom-written code in MATLAB (MathWorks, Natick, MA) v7.14.0.0739 (R2012a). A total number of 2,880 images were analyzed accordingly. Anatomical segmentation of the tibial and fibular fascicles of the sciatic nerve was performed manually by 2 trained neuroradiologists blinded to clinical data. Because the signal intensity of muscle and vital nerve is similar in a T2w fat-suppressed sequence,[18,19,25] a T2w-hyperintense lesion was defined as a nerve fascicle with an elevated T2w signal intensity of at least 25% above that of muscle tissue, whereas a T2w-hypointense lesion was defined as a fascicle showing a T2w signal decrease of at least 25% compared to that of muscle tissue. The threshold of 25% for both types was chosen to detect as many lesions as possible and to ensure that no vital nerve tissue was accidentally identified as a lesion, which would have happened if the threshold had been set at 2 standard deviations above or below the average muscle signal, respectively. To avoid effects caused by image artifacts due to field and coil inhomogeneities as well as magic angle artifacts,[26] thresholds for lesions were adapted to the surrounding muscle signal on each of the 24 image slices for each patient. No prior image filters were used, to avoid artificial alteration of the acquired T2w signal. Binarized images of lesions and healthy nerve tissue were then analyzed in MATLAB.[27] For the sciatic nerve, we calculated the lesion volume in cubic millimeters as well as lesion load in percentage of the full nerve volume. The determination of the 25% threshold and the process of lesion segmentation are illustrated in Figure 1.

Statistical data analysis was performed with Prism 6 (GraphPad, La Jolla, CA). All data were tested for Gaussian normal distribution using the D'Agostino–Pearson omnibus normality test. If a Gaussian normal distribution was given, *t* tests were used for comparisons of 2 groups, 1-way analyses of variance were applied for comparisons of >2 groups, and

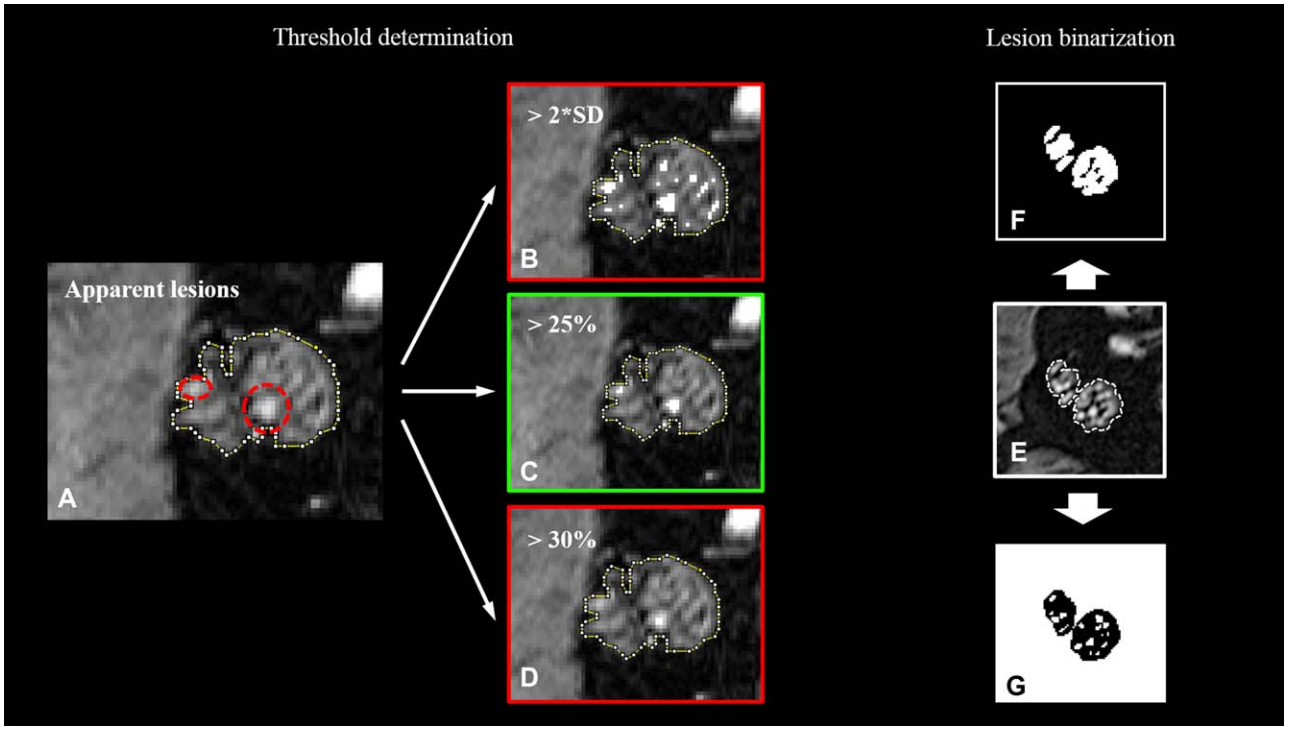

FIGURE 1: Lesion threshold and binarization of lesions. (A) Visible T2-weighted (T2w)-hyperintense lesions (encircled with dashed line) in a participant without diabetic neuropathy. (B) The automated segmentation of nerve lesions at a threshold of 2 standard deviations (SD) above the average muscle signal also identifies part of the vital nerve tissue as lesions. (C) Lesion segmentation at a threshold > 25% of the average muscle signal; lesions and vital tissue are differentiated correctly. (D) Insufficient detection of lesions at a threshold > 30% of the average muscle signal. (E) Sciatic nerve in a participant suffering from diabetic neuropathy. (F) Binarized map of T2w-hypointense lesions identified via thresholding. (F, G) Binarized maps of T2w-hypointense and T2w-hyperintense lesions, respectively, identified via thresholding. [Color figure can be viewed at www.annalsofneurology.org]

Pearson correlation coefficients were calculated for correlation analysis. If data were not Gaussian distributed, the Mann–Whitney test was used for comparisons of 2 groups, the Kruskal–Wallis test was used for multiple comparisons of >3 groups, and nonparametric Spearman correlation was applied for correlation analysis. For all tests, the level of significance was defined at $p < 0.05$. All results are presented as mean values ± standard error.

Results

MRI Results

Examples of T2w-hypointense and T2w-hyperintense lesions in comparison to a regular nerve signal are shown in Figure 2A–C. T2w-hypointense lesions appeared T1w-hyperintense (see Fig 2D, E), thus strongly suggesting that T2w-hypointense lesions contain a high level of lipids.

Patients with DPN showed an increased volume of T2w-hypointense lesions when compared to no DPN (NDPN; $23.41 \pm 2.69 mm^3$ vs $11.43 \pm 1.74 mm^3$, $p = 0.002$). Furthermore, T2w-hypointense lesion volume was higher in T2D compared to T1D in both DPN ($27.54 \pm 3.53 mm^3$ vs $19.74 \pm 5.57 mm^3$, $p = 0.046$) and NDPN ($16.83 \pm 3.16 mm^3$ vs $7.52 \pm 0.97 mm^3$, $p = 0.027$).

T2w-hyperintense lesion load was higher in DPN compared to NDPN ($13.93 \pm 0.01\%$ vs $3.18 \pm 0.004\%$, $p < 0.0001$) and higher in T1D compared to T2D for DPN ($19.67 \pm 4.13\%$ vs $12.49 \pm 1.23\%$, $p = 0.027$), but not for NDPN ($2.80 \pm 0.50\%$ vs $2.68 \pm 0.43\%$, $p = 0.838$).

Demographic, Clinical, Electrophysiological, and Serological Data

COMPARING DPN AND NDPN. Comparing all patients with and without diabetic neuropathy, we found no significant difference in patient age distribution or body mass index values. Also, no significant differences for total serum protein, serum albumin, HKT, MCV, and RDW were found. As expected, in DPN compared to NDPN, we found elevated scores for NSS (5.457 ± 0.26 vs 0.06 ± 0.06, $p < 0.0001$) and NDS (5.10 ± 0.3 vs 1.44 ± 0.25, $p < 0.0001$). In accordance with clinical symptoms, we found that nerve conduction qualities of all nerves examined were significantly reduced in DPN compared to NDPN (see Table 1).

With the additionally acquired serological data, we further found that in DPN compared to NDPN, triglycerides were elevated (211.30 ± 27.29mg/dl vs 149.20 ± 18.81mg/dl, $p = 0.0007$), whereas serum HDL was decreased (55.64 ± 2.65mg/dl vs 61.70 ± 2.70mg/dl,

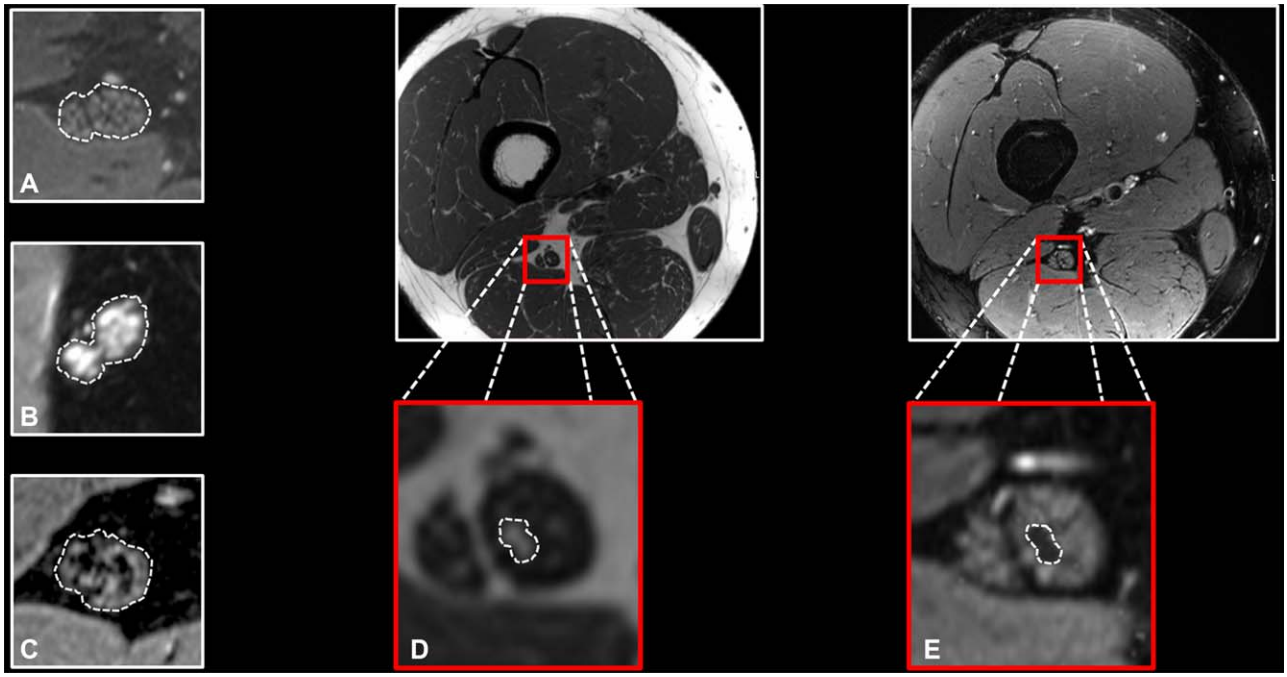

FIGURE 2: T2-weighted (T2w) sciatic nerve lesions in type 1 diabetes (T1D) and type 2 diabetes (T2D). (A) Regular nerve signal of a 35-year-old male T1D patient without signs of diabetic neuropathy (neuropathy symptom score [NSS] = 0, neuropathy disability score [NDS] = 2, HbA1c = 5.7%, high-density lipoprotein [HDL] = 95mg/dl, triglycerides = 41mg/dl). (B) T2w-hyperintense lesions in a 25-year-old female T1D patient with severe diabetic neuropathy (NSS = 6, NDS = 8, HbA1c = 8.7%, HDL = 81mg/dl, triglycerides = 116mg/dl). (C) T2w-hypointense lesions in a 56-year-old T2D patient with severe diabetic neuropathy (NSS = 7, NDS = 6, HbA1c = 6.7%, HDL = 30mg/dl, triglycerides = 492mg/dl). (D, E) T2w-hypointense lesions in the sciatic nerve are T1w hyperintense. [Color figure can be viewed at www.annalsofneurology.org]

$p = 0.013$). It should be noted that there was no significant difference in HbA1c level between DPN and NDPN. Also, no significant differences for parameters of renal function such as creatinine, glomerular filtration rate, and albumin/creatinine were found. A complete overview of all MRI findings and epidemiological, clinical, and serological parameters is given in Table 1.

COMPARING DPN AND NDPN IN T1D AND T2D. No differences for clinical scores were found between T1D and T2D (see Table 2). Although no difference was found for patients' age between T1D and T2D for DPN, T2D patients with NDPN were older than T1D patients (67.04 ± 1.48 years vs 59.45 ± 2.22 years), reflecting that patients suffering from T2D are generally older at disease onset than T1D patients. In contrast, disease duration was longer in T1D for both DPN (36.67 ± 2.44 years vs 11.64 ± 1.08 years, $p < 0.0001$) and NDPN (35.00 ± 2.64 years vs 13.2 ± 1.88 years, $p < 0.0001$). T2D patients showed higher body mass indices in both DPN (29.70 ± 0.92kg/m^2 vs 26.91 ± 2.01kg/m^2, $p = 0.044$) and NDPN (31.43 ± 0.92kg/m^2 vs 25.28 ± 1.00kg/m^2, $p < 0.0001$). When analyzing serological parameters associated with obesity and metabolic syndrome, we found that triglycerides were elevated in T2D for DPN (220.70 ± 23.70mg/dl vs 106.60 ± 14.51mg/dl, $p < 0.0001$) but not for NDPN. Also, for DPN, HDL was lower in T2D compared to T1D (51.29 ± 3.02mg/dl vs 70.79 ± 4.65mg/dl, $p < 0.0001$), whereas no such difference was found for NDPN, indicating that dyslipidemia is more present in T2D DPN than in T1D DPN. In contrast, we found that for T1D, HbA1c was higher in DPN ($8.74 \pm 0.29\%$ vs $7.11 \pm 0.16\%$, $p < 0.0001$), but not in NDPN. Furthermore, significantly elevated HbA1c level was only found in DPN compared to NDPN in T1D ($8.74 \pm 0.29\%$ vs $7.51 \pm 0.30\%$, $p = 0.006$), whereas in T2D no such difference could be detected. These results signify that elevated HbA1c level may bear a more important role in the development of DPN in T1D than in T2D. No differences for parameters of kidney function were found between T1D and T2D in DPN or NDPN. A detailed overview of demographic and clinical results of DPN and NDPN patients suffering from T1D or T2D is shown in Tables 2 and 3.

Correlation of MRI Results with Clinical, Serological, and Electrophysiological Data

T2W-HYPOINTENSE LESIONS. We found a positive correlation between T2w-hypointense lesion volume and NSS ($p < 0.0001$) as well as NDS ($p = 0.002$). In

TABLE 3. Magnetic Resonance Imaging, Demographic, Clinical, and Serological Data of DPN Patients with T1D and T2D

Parameter	DPN T1D	DPN T2D	*p*
T2w-hypointense lesion volume, mm^3	19.74 ± 5.57	27.54 ± 3.53	0.046[a]
T2w-hyperintense lesion load, % of the full nerve	19.67 ± 4.13	12.49 ± 1.23	0.027[a]
Age, yr	67.04 ± 1.48	63.72 ± 1.42	0.058, ns
Disease duration, yr	36.67 ± 2.44	11.64 ± 1.075	<0.0001[b]
Body mass index, kg/m^2	26.91 ± 0.92	29.7 ± 0.70	0.040[a]
Neuropathy symptom score	4.72 ± 0.64	5.67 ± 0.29	0.130, ns
Neuropathy deficit score	5.65 ± 0.51	6.19 ± 0.29	0.350, ns
HbA1c, %	8.74 ± 0.29	7.51 ± 0.30	<0.0001[b]
Triglycerides, mg/dl	106.6 ± 14.51	220.7 ± 23.70	<0.0001[b]
High-density lipoprotein, mg/dl	70.79 ± 4.65	51.29 ± 3.02	<0.0001[b]
Low-density lipoprotein, mg/dl	106.9 ± 6.33	95.39 ± 4.40	0.166, ns
Total cholesterol, mg/dl	187.90 ± 12.13	179.00 ± 6.413	0.168, ns
Creatinine, mmol/l	1.166 ± 0.40	0.87 ± 0.03	0.205, ns
Estimated glomerular filtration rate, $ml/min \times 1.73m^2$	87.95 ± 7.102	82.91 ± 3.13	0.387, ns
Albumin/creatinine ratio, mg/mmol	265.60 ± 247.10	88.56 ± 37.63	0.674, ns

All values are displayed as mean ± standard error. Level of significance: $p \geq 0.05$ ns.
[a]$p < 0.05$, [b]$p < 0.001$.
DPN = diabetic neuropathy; ns = not significant; T1D = type 1 diabetes; T2D = type 2 diabetes; T2w = T2-weighted.

addition, T2w-hypointense lesion load correlated negatively with serum HDL ($p < 0.0001$) and positively with triglycerides ($p = 0.0003$). Nonlinear regression analysis showed that an exponential increase of T2w-hypointense lesions was associated with a decrease in HDL ($r = 0.43$; Fig 3A) and with an increase in triglycerides ($r = 0.34$; see Fig 3C).

T2W-HYPERINTENSE LESIONS. With respect to NSS, NDS, and serum HbA1c level, we found that T2w-hyperintense lesion load was positively correlated (NSS, $p < 0.0001$; NDS, $p < 0.0001$; HbA1c, $p = 0.014$). A negative correlation was found for T2w-hyperintense lesions and CMAPtib ($p < 0.0001$), SNAPper ($p = 0.002$), NCVtib ($p = 0.0009$), and NCVper ($p = 0.0002$). Nonlinear regression analysis revealed that an increase in T2w-hyperintense lesion load is quadratically associated with HbA1c level ($r = 0.23$; see Fig 3B) and an exponential decrease of CMAPtib ($r = 0.58$; see Fig 3D). A detailed overview of correlations of T2w-hypointense and T2w-hyperintense lesions with all other parameters acquired is given in Table 4.

Discussion

To our knowledge, this is the first in vivo study showing that microstructural remodeling of the sciatic nerve in distal symmetric diabetic polyneuropathy differs between T1D and T2D, and to find associations between nerve lesions and serological parameters.

Specifically, we found the following. First, in our patient collective, both T2w-hyperintense and T2w-hypointense lesions were elevated in DPN. An increasing load of both lesion types was associated with an increasing severity of clinical symptoms. Furthermore, an increase of T2w-hyperintense lesions was positively correlated with an impairment of nerve conduction parameters. Second, T2w-hyperintense lesions were positively correlated with HbA1c level. Both T2w-hyperintense lesion load and HbA1c level were elevated in T1D DPN compared to T2D DPN. In addition, exclusively in T1D, T2w-hyperintense lesion load and HbA1c level were higher in DPN than in NDPN. Third, T2w-hypointense lesions were positively correlated with serum triglycerides and negatively with serum HDL. In T2D DPN, T2w-hypointense lesion load and serum

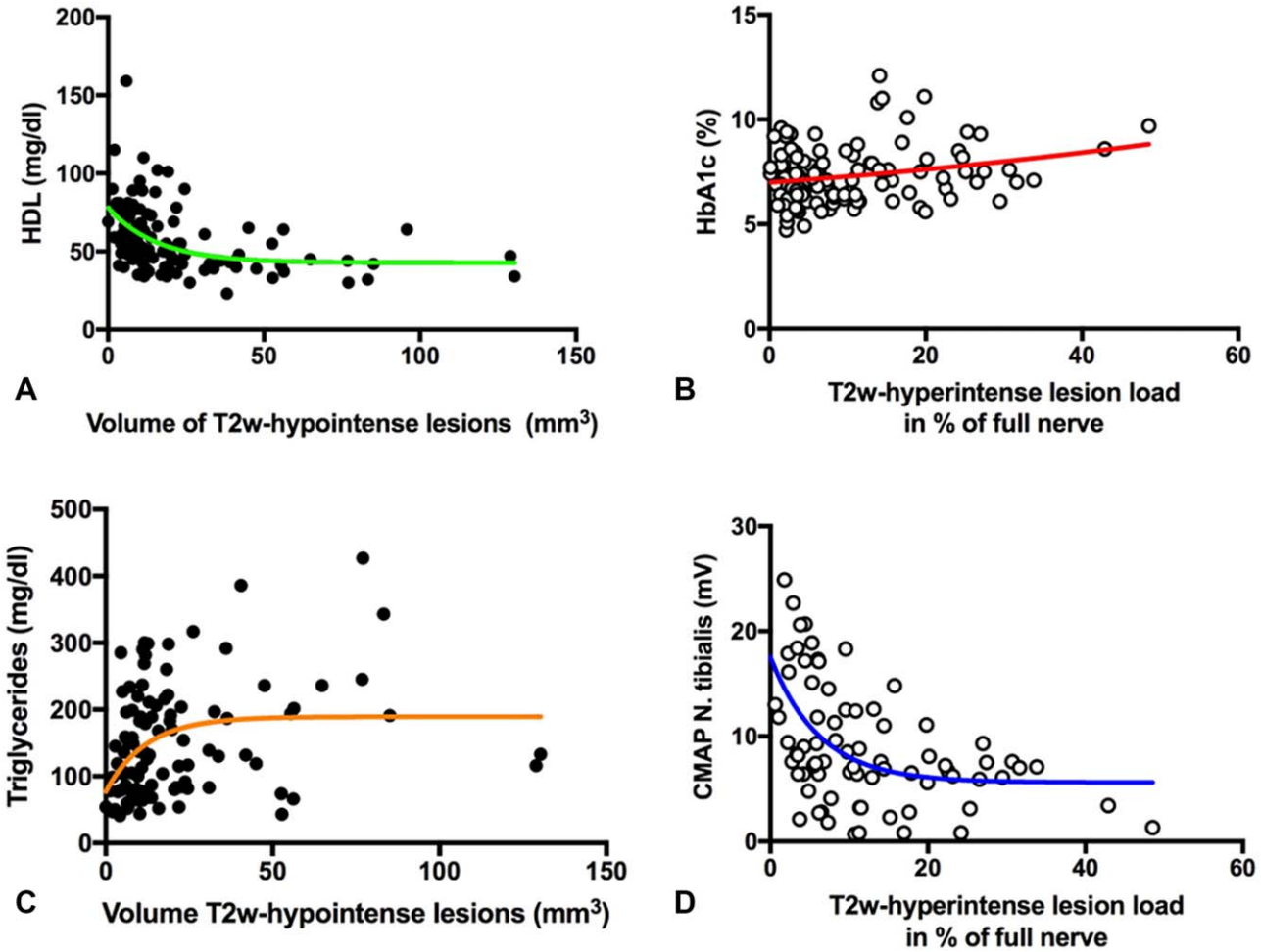

FIGURE 3: T2-weighted (T2w) sciatic nerve lesions in correlation with serological and electrophysiological data. (a) High-density lipoprotein (HDL) versus T2w-hypointense lesion volume. HDL (in mg/dl) decreases exponentially as a function of T2w-hypointense lesion volume (V; in mm^3) as $HDL(V) = 35.27 \cdot \exp(-0.064 \cdot V / mm^3) + 42.80$; $r = 0.43$. (B) HbA1c increases quadratically as a function of T2w-hyperintense lesion load (L; in % of full nerve) as $HbA1c(L) = 7 - 0.023 \cdot L + 0.0014 \cdot L^2$; $r = 0.23$. (C) Triglycerides (TGC; in mg/dl) increase with T2w-hypointense lesion volume (in mm^3) as $TGC(V) = -112.49 \cdot \exp(-0.083 \cdot V / mm^3) + 189.40$; $r = 0.34$. (D) Tibial compound muscle action potential (CMAPtib; in mV) decreases with T2w-hyperintense lesion load (in % of full nerve) as $CMAPtib(L) = 8.18 \cdot \exp(-0.167 \cdot L) + 2.58$; $r = 0.58$. [Color figure can be viewed at www.annalsofneurology.org]

triglycerides were elevated and HDL was decreased when compared to T1D DPN. Fourth, in NPDN, no differences of serological parameters or T2w-hyperintense lesion load were found between T1D and T2D. T2w-hypointense lesion load was elevated in T2D NDPN compared to T1D NDPN.

Considering the results of in vitro studies focusing on nerve damage due to glycation of ECMs as a result of aggregating AGEs, one may hypothesize that the positive correlations of T2w-hyperintense lesions with Hba1c level and nerve conduction impairment are suggestive of T2w-hyperintense lesions representing an imaging correlate of AGEs in the ECM of myelinating cells. AGEs have been identified both as a risk factor for the development of nerve damage due to inflammatory processes and endothelial dysfunction, and as a potential inhibitor of neural outgrowth after damage in in vitro studies.[5,28] Also, previous MRI studies have shown that T2w-hyperintense lesions in diabetic neuropathy occur due to an increase in proton spin density and not due to increased T2 relaxation time, which has also been shown for AGEs.[18,19] However, although our results support the hypothesis that T2w-hyperintense lesions in DPN represent AGEs, it should be noted that the small number of participants does not allow ruling out all possible confounders.

Similarly, one may hypothesize that the correlations of T2w-hypointense lesions with HDL and triglycerides and the predominance of T2w-hypointense lesions in T2D indicate dyslipidemia as a potential cause for nerve damage leading to neuropathic symptoms. This hypothesis is supported by in vivo studies showing that low

TABLE 4. Correlation of Lesions with Demographic, Clinical, Electrophysiological, and Serological Data

Parameters	Correlation with T2w-Hypointense Lesions		Correlation with T2w-Hyperintense Lesions	
	r	*p*	*r*	*p*
Age, yr	0.14	0.120, ns	0.07	0.478, ns
Disease duration, yr	−0.17	0.078, ns	−0.05	0.583, ns
Body mass index, kg/m^2	0.17	0.091, ns	0.03	0.753, ns
Neuropathy symptom score	0.36	<0.0001[a]	0.52	<0.0001[a]
Neuropathy deficit score	0.28	0.002[b]	0.54	<0.0001[a]
Tibial compound motor action potential, mV	−0.13	0.372, ns	−0.58	>0.0001[a]
Peroneal SNAP, mV	−0.15	0.300, ns	−0.44	0.002[b]
Sural SNAP, mV	−0.20	0.235, ns	−0.30	0.072, ns
NCV of tibial nerve, m/s	−0.06	0.677, ns	−0.47	0.0009[a]
NCV of peroneal nerve, m/s	−0.24	0.060, ns	−0.51	0.0002[a]
NCV of sural nerve, m/s	0.01	0.957, ns	−0.21	0.248, ns
DML of tibial nerve, ms	<0.01	0.994, ns	0.17	0.252, ns
DML of peroneal nerve, ms	−0.02	0.912, ns	0.21	0.162, ns
HbA1c, %	−0.13	0.179, ns	0.23	0.014[c]
Triglycerides, mg/dl	0.34	0.0003[a]	0.08	0.390, ns
High-density lipoprotein, mg/dl	−0.48	<0.0001[a]	0.12	0.225, ns
Total cholesterol, mg/dl	−0.09	0.369	−0.09	0.349, ns
Low-density lipoprotein, mg/dl	−0.04	0.714	−0.13	0.193, ns
Creatinine, mmol/l	0.13	0.167, ns	0.05	0.625, ns
Estimated glomerular filtration rate, ml/min × 1.73m^2	−0.04	0.690, ns	−0.03	0.751, ns
Albumin/creatinine ratio, mg/mmol	0.08	0.425, ns	0.09	0.387, ns

Correlation of T2w-hypointense lesion volume (in mm^3) and T2w-hyperintense lesions (in % of full nerve) with demographic, clinical, electrophysiological, and serological data. Level of significance: $p \geq 0.05$ ns.
[a]$p < 0.001$, [b]$p < 0.01$, [c]$p < 0.05$.
DML = distal motor latency; NCV = nerve conduction velocity; ns = not significant; SNAP = sensory nerve action potential; T2w = T2-weighted.

serum HDL itself is an independent risk factor for the development of peripheral neuropathy[9] and that decreased levels of HDL and elevated triglycerides increase the risk of microangiopathy independent of serum low-density lipoprotein level.[29] Because the T2w-sequence applied in this study is fat-suppressed, and T2w-hypointense lesions show a hyperintense signal in matching T1w images, our findings clearly indicate that T2w-hypointense lesions contain a high level of lipids. Put into context with previous in vitro studies on lipid deposits in nerves of patients suffering from DPN and dyslipidemia, T2w-hypointense lesions might represent an imaging correlate of intraneural aggregates of lipids or, more likely, of microvascular lipid deposits inside the wall of perineural blood vessels that occur as a result of elevated triglycerides and an impairment of reverse cholesterol transport, which is usually mediated by HDL.[8,30] This hypothesis is supported by the finding that T2w-hypointense lesions do not correlate with a decline of nerve conduction parameters but with the severity of clinical symptoms; vascular lipid deposits would not affect nerve conduction directly (in opposition to AGEs) by causing damage to axons or myelin. Instead, damage would be caused indirectly once a certain degree of

vascular occlusion in the affected vessel is reached.[31] In former studies, microangiopathy due to vascular lipid deposits has been identified as a potential risk factor for microangiopathy leading to hypoxic damage in peripheral nervous system neurons in vitro[8,17,32] and in central nervous system neurons in vivo.[31]

To comprehend the impact of T2w-hypointense lesion load on the development of DPN, one should acknowledge that disease duration in patients suffering from DPN due to T2D was much shorter than in DPN due to T1D. Considering the results mentioned above, one may hypothesize that the combination of dyslipidemia and elevated blood glucose causes nerve damage and neuropathic symptoms more rapidly than hyperglycemia alone. Apart from associations with metabolic parameters, in vivo visualization of structural nerve lesions containing lipids in DPN is of great importance, because DPN rodent models offer only very limited evidence for effects of pathologies in lipid metabolism.[20]

It should be noted that we found no correlation of nerve lesions and renal parameters, suggesting that, in our cohort, the development of the segmented lesions occurred independently from kidney function.

As mentioned above, our study is limited because the number of participants and different groups preclude multivariate analyses and all patients were only examined once, which does not allow longitudinal analysis of disease development. However, the correlation of lesions with serological parameters such as HbA1c and HDL, which are usually stationary for months, might indicate that lesions develop based on a prolonged exposure of nerve tissue to hyperglycemia and dyslipidemia. Also, concerning the correlation of clinical symptoms and serological parameters, our findings are consistent with previous longitudinal studies.[3]

One should note that no serological tests were performed to test for vitamins or their direct metabolites. However, because all participants with a history of digestive disorders or alcohol abuse were excluded, and because no cases of macro- or microcytic anemia were found, the accidental inclusion of patients with a vitamin B2, B6, or B12 deficiency is very unlikely in our cohort. Furthermore, total serum protein and albumin levels were normal in all participants, thus rendering monoclonal gammopathy an unlikely cause for the detected nerve lesions.

Another limitation may arise from different mean ages of T1D and T2D patients (57.5 years and 67 years, respectively). However, because no correlation of lesions and patient age could be found, it seems likely that the development of nerve lesions does not depend on a patient's age. Furthermore, the correlations found for serological risk factors and clinical data are consistent with recently published results of the SEARCH for Diabetes in Youth study, suggesting that the underlying pathological mechanisms in DPN do not differ between younger and older patients with diabetes mellitus.[33]

In summary, our results indicate that the predominant mechanisms of nerve damage in DPN differ between T1D and T2D. T2w-hyperintense lesions, correlated with serological markers for hyperglycemia, predominate in T1D DPN, whereas T2w-hypointense lesions, correlated with serological markers for dyslipidemia and an increasingly rapid progression of neuropathic symptoms, predominate in T2D DPN. Based on our results, one may hypothesize that an aggregation of AGEs in the nerve ECM due to hyperglycemia is a risk factor for developing diabetic neuropathy in T1D, whereas in T2D, microvascular or intraneural lipid deposition is an additional risk factor. Considering recent longitudinal clinical studies, our findings may offer an explanation for the poor effect of glycemic control on the course of DPN in T2D compared to T1D, and thereby indicate that future studies on the pathophysiology and the treatment of DPN should not only focus on glycemic control but also consider the role of dyslipidemia, especially in T2D.

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Author Contributions

J.M.E.J., F.T.K., S.H., M.P., J.B.G., S.K., P.N., and M.B. conceived and designed the study; J.M.E.J., F.T.K., J.B.G., S.K., D.O., and S.H. contributed to data acquisition and analysis; J.M.E.J., J.B.G., M.B., and F.T.K. contributed to drafting the text; J.M.E.J. and F.T.K. contributed to preparing the figures.

Potential Conflicts of Interest

Nothing to report.

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