ANNALS of Clinical and Translational Neurology
Open Access
AMERICAN NEUROLOGICAL ASSOCIATION

RESEARCH ARTICLE

Sciatic nerve microvascular permeability in type 2 diabetes decreased in patients with neuropathy

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Funding Information
The German Research Council (DFG, SFB 1158) provided financial support for personnel expenditure, MR imaging costs and costs for the technical equipment required for electrophysiological and serological analysis. The DFG had no influence on the study design, collection, and analysis of data or on the writing of the article. JJ further acknowledges support from the International Foundation for Research in paraplegia (IRP) and the Else-Kröner-Fresenius-Stiftung (EKFS), ZK received grants from the Deutsches Zentrum für Diabetesforschung (DZD) e.V., SH received a grant from the Dietmar Hopp Foundation and the German Research Council (DFG, SFB 1118), MB received grants and personal fees from Codman, Guerbet, Bayer, and Novartis, personal fees from Roche, Teva, Springer, Boehringer, Grifols, Braun and grants from the European Union, Siemens, the Dietmar Hopp Foundation, Stryker and the German Research Council (DFG, SFB 1118 and 1158), FK was supported by the German Research Foundation (KU 3555/1-1), the Hoffmann-Klose Foundation of Heidelberg University Hospital, and a research grant of Heidelberg University Hospital.

Received: 14 March 2022; Revised: 4 April 2022; Accepted: 8 April 2022

Annals of Clinical and Translational Neurology **2022; 9(6): 830–840**
doi: 10.1002/acn3.51563

Abstract

Objectives: Clinical and histological studies have found evidence that nerve ischemia is a major contributor to diabetic neuropathy (DN) in type 2 diabetes (T2D). The aim of this study was to investigate peripheral nerve microvascular permeability using dynamic contrast enhanced (DCE) magnetic resonance neurography (MRN) to analyze potential correlations with clinical, electrophysiological, and demographic data. **Methods**: Sixty-five patients (35/30 with/without DN) and 10 controls matched for age and body mass index (BMI) underwent DCE MRN of the distal sciatic nerve with an axial T1-weighted sequence. Microvascular permeability (K^{trans}), plasma volume fraction (v_p), and extravascular extracellular volume fraction (v_e) were determined with the extended Tofts model, and subsequently correlated with clinical data. **Results**: K^{trans} and v_e were lower in T2D patients with DN compared to patients without DN ($0.037\ min^{-1} \pm 0.010$ vs. $0.046\ min^{-1} \pm 0.014$; $p = 0.011$, and $2.35\% \pm 3.87$ vs. $5.11\% \pm 5.53$; $p = 0.003$, respectively). In individuals with T2D, K^{trans} correlated positively with tibial, peroneal, and sural NCVs ($r = 0.42$; 95%CI = 0.18 to 0.61, 0.50; 95%CI = 0.29 to 0.67, and 0.44; 95%CI = 0.19 to 0.63, respectively), with tibial and peroneal CMAPs ($r = 0.27$; 95%CI = 0.01 to 0.49 and $r = 0.32$; 95%CI = 0.07 to 0.53), and with the BMI ($r = 0.47$; 95%CI = 0.25 to 0.64). Negative correlations were found with the neuropathy deficit score ($r = -0.40$; 95%CI = −0.60 to −0.16) and age ($r = -0.51$; 95%CI = −0.67 to −0.31). No such correlations were found for v_p. **Conclusion**: This study is the first to find associations of MR nerve perfusion parameters with clinical and electrophysiological parameters related to DN in T2D. The results indicate that a decrease in microvascular permeability but not plasma volume may result in nerve ischemia that subsequently causes demyelination.

Introduction

Diabetic neuropathy (DN) is one of the most severe complications of diabetes mellitus with increasing prevalence.[1] Especially in type 2 diabetes (T2D), the pathophysiological mechanisms underlying the development of DN remain poorly understood.[1–4] Both clinical and histological studies have found evidence that microangiopathy resulting in nerve ischemia is a major contributor to demyelination and axonal damage in T2D.[5–7] In particular, post-mortem studies conducted on the nerves of the lower extremities by Dyck et al. found that the pattern of nerve damage in DN observed at the distal level of the sciatic nerve resembles changes that can be attributed to nerve ischemia.[8] To date, it has not been possible to assess perfusion-related structural changes of peripheral nerves in this particular region in the context of clinical studies. Magnetic resonance neurography (MRN) at 3 Tesla (3 T) is a noninvasive method that allows to assess peripheral nerves throughout the entire body.[9] Recent studies on MRN in patients with T2D have found that, in contrast to the progression of clinical symptoms, fascicular nerve damage in T2D predominates at thigh level and that structural nerve damage in this position is related to clinical and electrophysiological parameters.[10] It could further be demonstrated that fascicular nerve damage detected by MRN is associated with parameters of micro- and macroangiopathy such as intima-media-thickness or pulse wave velocity.[11] A pilot study on dynamic contrast enhanced (DCE) MRN in patients with mainly inflammatory neuropathies could show that DCE sequences allow to assess the perfusion of peripheral nerves.[12] The extended Tofts model allows calculating the constant of the examined nerve's capillary permeability (K^{trans}), the volume fraction of the plasma space (v_p), and the volume fraction of the extravascular extracellular space (v_e) from DCE MRN sequences and is widely used for DCE analysis of vascular permeability in MR imaging of the central nervous system.[13,14] These parameters provide an insight into the assessed nerve's microcirculation. The aim of this study was to combine DCE imaging of nerves at thigh level in patients with T2D with demographic, clinical, and electrophysiological data in order to assess potential associations between the microcirculation of nerves and clinical parameters related to DN in patients with T2D.

Methods

Study design and participants

This study was approved by the ethics committee of Heidelberg university hospital (HEIST-DiC, clinicaltrials.gov identifier NCT03022721, local ethics number S-383/2016) and all participants gave written informed consent. Overall exclusion criteria were age <18, pregnancy, an estimated glomerular filtration rate (eGFR) <60 mL/min, and any contraindications for MR imaging or administration of MRI contrast agents. Participants were also excluded in case of any history of spine surgery or disc extrusion, any risk factors for neuropathy other than diabetes such as malignant diseases, alcoholism, hypovitaminosis, any previous or ongoing exposure to neurotoxic agents, and any chronic neurological diseases such as Parkinson's disease, restless legs syndrome, or multiple sclerosis. The sample size was based on the results of previous MRN studies on DN.[11,15] Sixty-five patients with T2D (21 women, 44 men, mean age 63.77 years ± 9.71) and 10 controls (five women, five men, mean age 59.90 years ± 7.00) were enrolled in this prospective single-center study between January 2017 and March 2020 and underwent DCE 3-T MRN. The recruitment of participants is illustrated in Figure 1.

Clinical and electrophysiological examination

In every participant, a detailed medical history was documented. Blood was drawn in fasting state. Electrophysiological examinations (VikingQuest; Viasys Healthcare GmbH) included an assessment of the nerve conduction velocity (NCV) of the tibial, peroneal, and sural nerve, distal motor latency (DML) and compound muscle action potential (CMAP) of the tibial and peroneal nerve, and sensory nerve action potential (SNAP) of the sural nerve of the right leg. Skin temperature was kept at 32°C throughout the examination. Electrophysiological studies were conducted by two specially trained medical technical assistants with more than 6 years of experience. An examination of neuropathic symptoms was performed comprising the neuropathy disability score (NDS) and the neuropathy severity scale (NSS).[16] According to Gibbon's criteria, patients with an NDS ≥3 were assigned to the DN group.[17]

MRI imaging protocol

All participants underwent high-resolution MRN of the right thigh in a 3.0 Tesla MR-scanner (Magnetom SKYRA, Siemens Healthineers). A 15-channel transmit-receive extremity coil was used and the following sequences were applied:

1 Axial high resolution T2-weighted turbo spin echo (TSE) 2D sequence with spectral fat suppression. Repetition time (TR) = 5970 ms, echo time (TE) = 55 ms, field of view (FOV) = 160 × 160 mm^2, matrix size = 512 × 512, slice thickness = 4 mm, no interslice gap,

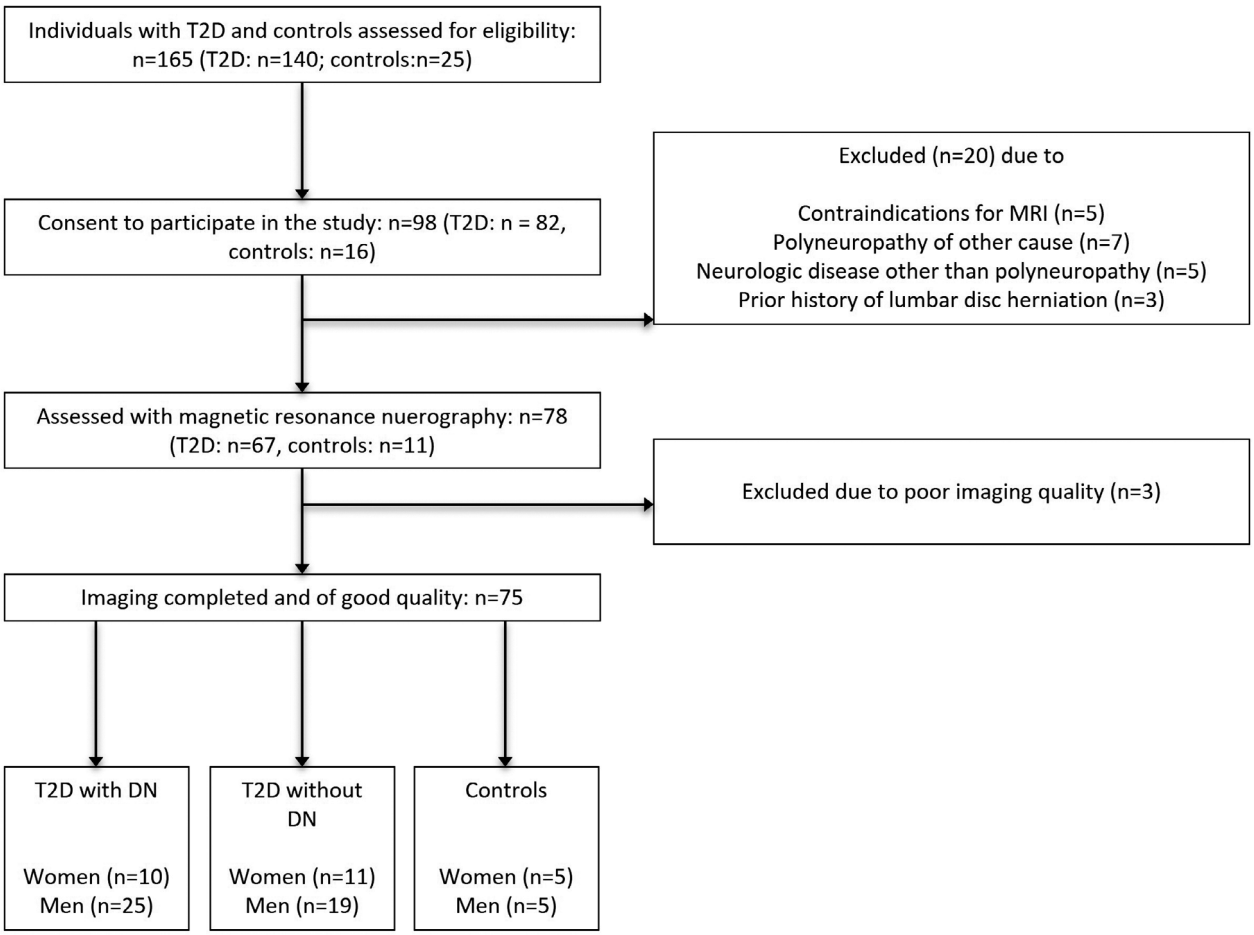

Figure 1. Flowchart on the recruitment of participants. DN, diabetic neuropathy.

voxel size = 0.3 × 0.3 × 4.0 mm^3, 24 slices, 24 acquired images, total acquisition time = 4:42 min.

2 Axial T1-weighted volume interpolated breath-hold examination (VIBE) sequence. Repetition time (TR) = 3.3 ms, echo time (TE) = 1.11 ms, field of view (FOV) = 160 × 160 mm^2, matrix size = 128 × 128, slice thickness = 4 mm, interslice gap = 0.8 mm, voxel size = 1.3 × 1.3 × 4.0 mm^3; single acquisition at a flip angle of 5°, 8°, 11°, 14°, 17° (24 slices = 144 acquired images), total acquisition time = 30s.

3 Axial T1-weighted volumetric interpolated breath-hold examination (VIBE) sequence. Repetition time (TR) = 3.3 ms, echo time (TE) = 1.11 ms, field of view (FOV) = 160 × 160 mm^2, matrix size = 128 × 128, slice thickness = 4 mm, interslice gap = 0.8 mm, voxel size = 1.3 × 1.3 × 4.0 mm^3, 50 repetitions (1200 acquired images) at a flip angle of 15°, contrast agent administration (Dotarem®, Guerbet, France, 0.1 mmol/kg, flow rate 3.5 mL/s) after completion of the sixth repetition, total acquisition time = 4:09 min.

The sequence was centered to the sciatic nerve bifurcation at distal thigh level in every participant.

MRI data analysis and statistical analysis

All images were pseudonymized. For each patient, the tibial compartment of the sciatic nerve was manually segmented on all slices of the T2-weighted sequence using ImageJ.[18] Segmented T2-weighted images were co-registered to the T1 VIBE sequence with custom-written code in Matlab (MathWorks, Natick, MA, R2020b), using affine transformations.[19] The process of image co-registration is illustrated in Figure 2. The arterial input function (AIF) was determined by manually segmenting a region of interest of the femoral artery on a representative imaging slice and using the average signal intensity of all artery voxels for consecutive imaging time points to obtain the signal intensity curve during contrast administration. The end of the signal intensity baseline (before signal intensity increase due to contrast administration)

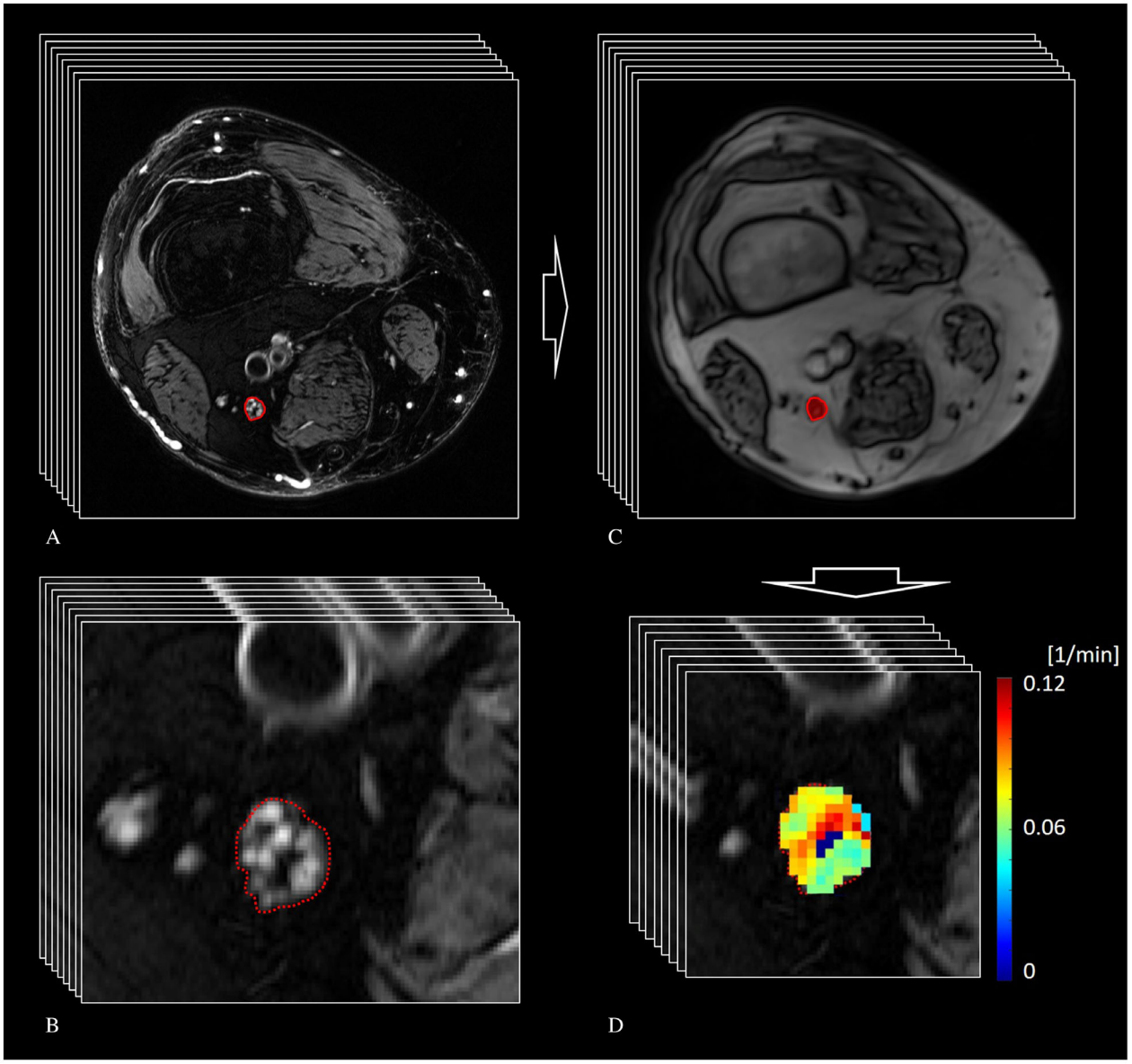

Figure 2. The process of image segmentation and co-registration. (A) Position of the distal sciatic nerve's tibial compartment (red circle). (B) Segmentation of the tibial compartment. (C) T1-weighted volumetric interpolated breath-hold examination (VIBE) sequence of the same position as in (A) with the co-registered region of interest for the sciatic nerve's tibial compartment. (D) Color-coded K^{trans} map of the sciatic nerve's tibial compartment calculated with the extended Tofts model.

was determined as the last imaging time point before signal intensity would increase by more than 25% above the averaged signal intensity of all preceding imaging time points. The resulting AIF was smoothed with a moving average filter of three images.

Relaxation time $T_{1,0}$ for the first imaging time point was subsequently determined for each voxel in dependence on flip angle α by assigning $T_{1,x}(\alpha) = \mathrm{SI}_0/\tan(\alpha)$ and $T_{1,y}(\alpha) = \mathrm{SI}_0/\sin(\alpha)$, where SI_0 corresponds to the initial signal intensity, and performing linear regression on $T_{1,x}$ and $T_{1,y}$ to obtain the slope m, see also.[20] The spin-lattice relaxation time $T_{1,0}$ then follows as $T_{1,0} = -\mathrm{TR}/\log(m)$, TR being the repetition time. With $\alpha_R = 15°$, we find at time t with signal intensity SI_t and relaxation time $T_{1,t}$ the following: $\frac{1}{\mathrm{T}_{1,t}} = -\log\left(\frac{[1-C_a]}{[1-\cos(\alpha_R)C_a]}\right)/\mathrm{TR}$, where $C_a = \frac{\frac{\mathrm{SI}_t}{\mathrm{SI}_0}[1-\exp[-\frac{\mathrm{TR}}{T_{1,0}}]]}{\left[1-\cos(\alpha_R)]\exp(-\frac{\mathrm{TR}}{T_{1,0}}\right)}$, see also Eq. (6) in Ref.[21] We eventually obtained the tissue concentration $C(t)$ at

Table 1. Demographic, clinical, electrophysiological, and MRN imaging data of all study participants.

	T2D	Controls
Total	65	10
Gender (w/m)	21 w/44 m	5 w/5 m
Age (years)	63.77 ± 9.71	59.90 ± 7.00
Disease duration	9.08 ± 8.55	n.a.
Body mass index (kg/m^2)	29.52 ± 4.73	27.96 ± 3.41
Sciatic nerve's K^{trans} (min^{-1})	0.041 ± 0.013	0.036 ± 0.011
Sciatic nerve's v_p (%)	4.59 ± 0.74	4.48 ± 0.40
Sciatic nerve's v_e (%)	3.63 ± 4.86	1.75 ± 1.77
Neuropathy disability score	3.48 ± 2.82	1.50 ± 1.51
Neuropathy severity scale	4.33 ± 3.39	2.20 ± 3.71
Sural nerve NCV (m/s)	36.92 ± 12.97	45.40 ± 5.17
Sural nerve SNAP (μV)	4.87 ± 3.07	8.91 ± 6.71
Peroneal nerve NCV (m/s)	39.90 ± 5.85	45.30 ± 4.32
Peroneal nerve CMAP (mV)	5.11 ± 3.64	10.35 ± 7.01
Peroneal DML (ms)	6.89 ± 10.62	3.94 ± 0.48
Tibial NCV (m/s)	40.19 ± 5.56	44.20 ± 3.43
Tibial CMAP (mV)	10.25 ± 6.70	18.06 ± 8.45
Tibial DML (ms)	5.75 ± 3.99	3.64 ± 0.56
HbA1c (%)	6.92 ± 1.16	4.71 ± 2.95
GFR (mL/min)	86.59 ± 15.82	87.95 ± 15.56

All values are displayed as mean ± standard deviation. K^{trans} = constant of capillary permeability; v_e = extravascular extracellular volume fraction; v_p = plasma volume fraction; NCV = nerve conduction velocity; CMAP = compound motor action potential; SNAP = sensory nerve action potential; m/s = meters per second; ms = milliseconds; mV = millivolts; μV = microvolts; GFR = glomerular filtration rate.

time t as $C(t) = \frac{1}{r_1}\left[\frac{1}{T_{1,t}} - \frac{1}{T_{1,0}}\right]$,[21] where $r_1 = 3.43$ L/mmol/s corresponds to the relaxivity of blood at 3 T.[22]

Within the extended Tofts model, that consists of a plasma and an extravascular extracellular compartment (see also Fig. S1), the following relation holds[23]:

$C_M(t) = K^{trans}\int_0^t \mathrm{AIF}(t')\exp\left(-\frac{K^{trans}[t-t']}{v_e}\right)dt' + v_p\mathrm{AIF}(t)$,

where K^{trans} represents the volume transfer constant between the plasma and the extravascular extracellular compartment, v_e corresponds to the volume fraction of extravascular extracellular space per unit volume of tissue, v_p corresponds to blood plasma volume per unit volume of tissue, and $C_M(t)$ represents the model tissue concentration at time t. Because of the relatively small molecular size of the employed contrast agent (559 Dalton), its capillary leakage into the extravascular space is reversible. The capillary leakage can therefore be assumed to be proportional to the difference in concentrations between the capillaries and the tissue. K^{trans} is the constant of this proportionality.[13,24] We used the Nelder–Mead simplex method to minimize the residual sum of least squares, $\sum_t [C_M(t) - C(t)]^2$, for model parameters K^{trans}, v_e, and v_p with starting values $K^{trans} = 0.007$/s, $v_e = 0.15$, $v_p = 0.025$.[12,25–27]

Statistical analysis

Statistical data analysis was carried out with MATLAB 7.14.0.0739 (R2012a) and GraphPad Prism 7. Gaussian normal distribution was tested with the D'Agostino-Pearson omnibus normality test. If a Gaussian normal distribution was given, t tests were used for comparisons of two groups, one-way ANOVAs were used for comparisons of more than two groups, and Pearson correlation coefficients were used for correlation analysis. If data were not Gaussian distributed, the Mann–Whitney test was used for comparisons of two groups, the Kruskal–Wallis test with post hoc Dunn correction was used for multiple comparisons of more than two groups, and nonparametric Bonferroni-corrected Spearman correlation was used for correlation analysis.

Results

Demographic and clinical data

Of 65 patients with T2D who took part in this study, 35 suffered from DN, while 30 showed no signs of DN according to Gibbon's criteria. A summary of demographic and clinical data of patients with T2D and controls is provided in Table 1, a summary of group comparisons of patients with and without DN, and controls is provided in Table 2.

MRN perfusion parameters

In patients with T2D and in controls K^{trans} was strongly correlated with v_e ($r = 0.86$; 95%CI = 0.77 to 0.91, and $r = 0.75$; $p = 0.013$, respectively). K^{trans} and v_e were lower in patients with DN compared to patients without DN (0.037(min^{-1}) ± 0.010 vs. 0.046(min^{-1}) ± 0.014; $p = 0.011$, and 2.35% ± 3.87 vs. 5.11% ± 5.53, $p = 0.003$, respectively). No such differences were found for v_p (4.62% ± 0.82 vs. 4.55 ± 0.65; $p > 0.999$). Compared to controls, v_e was higher in T2D patients without DN (5.11% ± 5.53 vs. 1.75% ± 1.77; $p = 0.038$), while K^{trans} was not (0.046(min^{-1}) ± 0.014 vs. 0.036(min^{-1}) ± 0.011; $p = 0.077$). Also, no differences for K^{trans}, v_e, and v_p were found between T2D patients with DN compared to controls. All group comparisons of perfusion parameters are found in Table 2.

Correlation of perfusion parameters with demographic data

In patients with T2D, K^{trans} and v_e correlated with age ($r = -0.51$; 95%CI −0.67 to −0.31, and $r = -0.34$; 95% CI = −0.55 to −0.10, respectively) and BMI ($r = 0.47$;

Table 2. Group comparisons of T2D patients with and without DN, and controls.

	T2D DN (n = 35)	T2D no DN (n = 30)	Controls (n = 10)	p-value	T2D DN vs. T2D no DN p-value	T2D DN vs. Controls p-value	T2D No DN vs. Controls p-value
K^{trans} (min^{-1})	0.037 ± 0.010	0.046 ± 0.014	0.036 ± 0.011	0.008^{A}	0.011	0.968	0.077
v_e	2.35 ± 3.87	5.11 ± 5.53	1.75 ± 1.77	0.002^{K}	0.003	>0.999	0.038
v_p	4.62 ± 0.82	4.55 ± 0.65	4.48 ± 0.40	0.981^{K}	>0.999	>0.999	>0.999
Age (years)	66.14 ± 8.62	61.00 ± 10.31	59.90 ± 7.00	0.042^{A}	0.116	0.106	>0.999
Disease duration (years)	10.65 ± 9.26	7.00 ± 7.64	n.a.	0.166^{T}	0.166	n.a.	n.a.
Gender (w/m)	10w/25 m	11w/19 m	5w/5 m	0.440^{K}	>0.999	0.637	>0.999
BMI (kg/m^2)	29.49 ± 4.55	29.55 ± 5.02	27.96 ± 3.41	0.613^{A}	0.998	0.625	0.619
NDS	5.49 ± 2.04	0.80 ± 0.92	1.50 ± 1.51	<0.001^{K}	<0.001	<0.001	>0.999
NSS	5.46 ± 2.96	2.73 ± 3.38	2.20 ± 3.71	0.001^{A}	0.003	0.018	>0.894
Sural NCV (m/s)	29.16 ± 11.96	45.24 ± 7.95	45.40 ± 5.17	<0.001^{A}	<0.001	0.007	>0.999
Sural SNAP (μV)	3.42 ± 1.53	6.53 ± 3.54	8.91 ± 6.71	<0.001^{A}	0.003	<0.001	0.161
Peroneal NCV (m/s)	37.71 ± 5.63	42.57 ± 5.01	45.30 ± 4.32	<0.001^{A}	0.002	<0.001	0.340
Peroneal CMAP (mV)	3.42 ± 2.53	7.16 ± 3.77	10.35 ± 7.01	<0.001^{K}	<0.001	<0.001	0.538
Peroneal DML (ms)	8.48 ± 14.10	4.96 ± 2.17	3.94 ± 0.48	0.010^{K}	0.155	0.014	0.476
Tibial NCV (m/s)	38.32 ± 5.67	42.25 ± 4.74	44.20 ± 3.43	0.002^{A}	0.011	0.006	0.548
Tibial CMAP (mV)	7.47 ± 6.33	13.41 ± 5.71	18.06 ± 8.45	<0.001^{A}	0.001	<0.001	0.054
Tibial DML (ms)	6.43 ± 4.52	4.98 ± 3.17	3.64 ± 0.56	0.004^{K}	0.150	0.005	0.257
HbA1c (%)	7.17 ± 1.09	6.73 ± 1.27	4.71 ± 2.95	<0.001^{A}	0.331	<0.001	0.015
GFR (mL/min)	82.61 ± 13.96	89.89 ± 17.89	87.95 ± 15.56	0.303 ^{A}	0.290	0.645	0.946

All values of participants are displayed as mean ± standard deviation. ^{A} = results obtained from One way ANOVA; K = Results obtained from Kruskal–Wallis test; ^{T} = Results obtained from t-Test; T2D DN = T2D patients with diabetic neuropathy; T2D no DN = T2D patients without diabetic neuropathy; K^{trans} = constant of capillary permeability; v_e = extravascular extracellular volume fraction v_p = plasma volume fraction; BMI = body mass index; NDS = neuropathy disability score; NSS = neuropathy severity scale; NCV = nerve conduction velocity; SNAP = sensory nerve action potential; CMAP = compound motor action potential; DML = distal motor latency; ms = milliseconds; mV = millivolts; μV = microvolts HbA1c = glycated hemoglobin; GFR = glomerular filtration rate.

95%CI = 0.25 to 0.64, and r = 0.41; 95%CI = 0.18 to 0.60), see also Figure 3A and B. In controls, no correlation with age was found for K^{trans} or v_e (r = 0.11; 95% CI = −0.56 to 0.69, and r = 0.39; 95%CI −0.31 to 0.82, respectively), while there was a positive correlation of K^{trans} with the BMI (r = 0.71; 95%CI 0.14 to 0.92). No correlations were found with gender in T2D patients or controls. Also, no correlation was found with diabetes duration, glomerular filtration rate, or HbA1c levels. Parameter v_p was not correlated with any of the acquired demographic parameters in T2D patients or controls.

Correlation of perfusion parameters with clinical and electrophysiological data

K^{trans} and v_e were positively correlated with NCVs of tibial (r = 0.42; 95%CI = 0.18 to 0.61, and r = 0.42; 95% CI = 0.17 to 0.61, respectively), peroneal (r = 0.50; 95% CI = 0.29 to 0.67, and r = 0.44; 95%CI = 0.21 to 0.63, respectively), and sural (r = 0.44; 95%CI = 0.19 to 0.63, and r = 0.40; 95%CI = 0.15 to 0.60) nerves, see also Figure 3C and D. Further positive correlations were found for K^{trans} and v_e with CMAPs of tibial (r = 0.27; 95% CI = 0.01 to 0.49, and r = 0.34; 95%CI = 0.08 to 0.55, respectively) and peroneal (r = 0.32; 95%CI = 0.07 to 0.53, and r = 0.33; 95%CI = 0.08 to 0.54, respectively) nerves. Negative correlations were found for K^{trans} and v_e with the NDS (r = −0.40; 95%CI = 0.60 to −0.16, and r = −0.48; 95%CI = −0.66 to −0.26, respectively). No such correlations were found for the NSS, and no correlations between perfusion parameters and electrophysiological data were found in the control group. A detailed summary of all correlations of MRN perfusion parameters with clinical and electrophysiological data in T2D patients is provided in Table 3.

In a partial correlation analysis controlled for age and BMI, correlations of peroneal NCVs with K^{trans} and v_e remained significant (r = 0.34; p = 0.044, and r = 0.34; p = 0.045). For tibial NCVs, correlations remained significant for v_e (r = 0.33; p = 0.042). In addition, partial

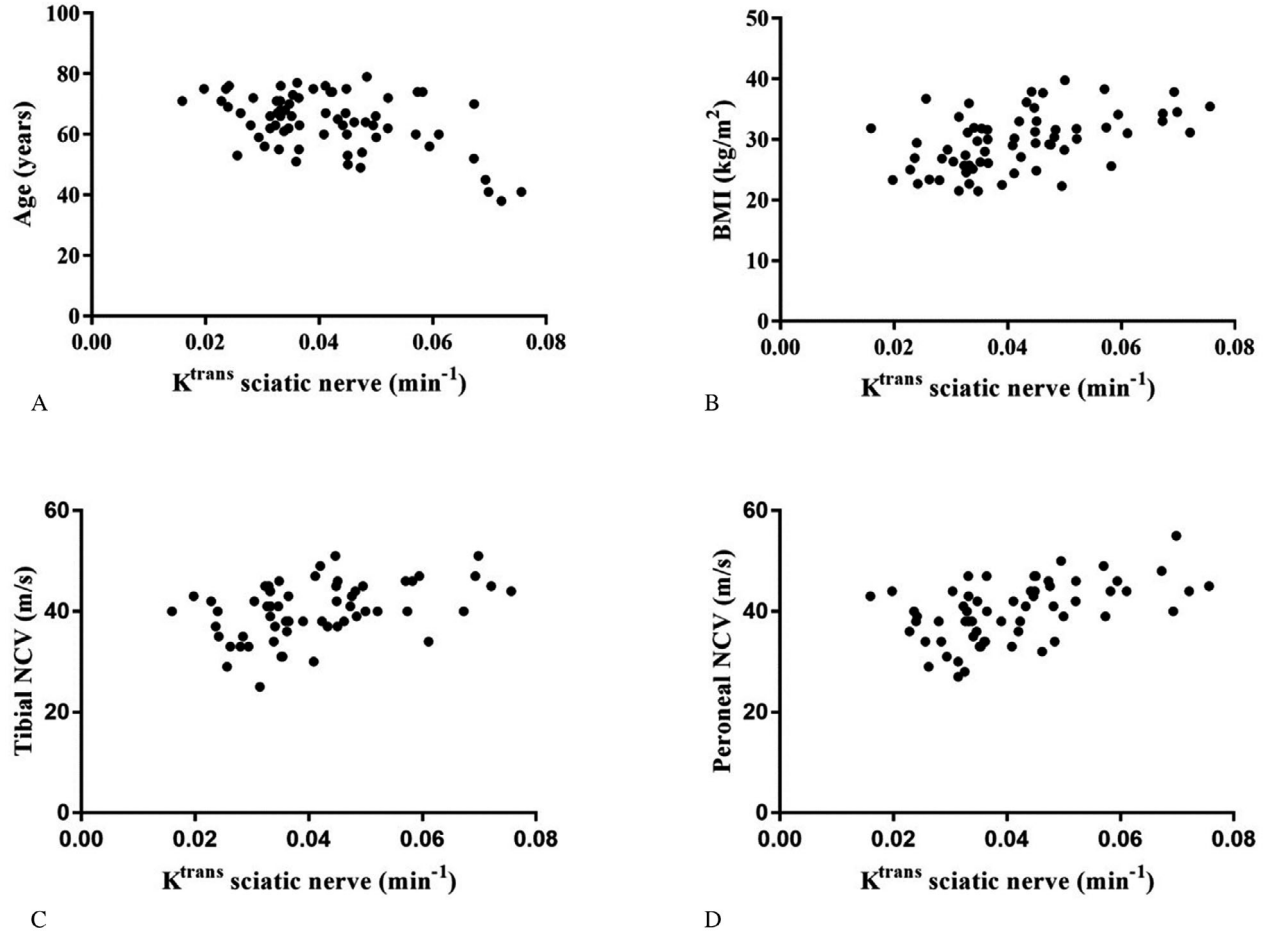

Figure 3. Correlations of the sciatic nerve's K^{trans} with demographic and electrophysiological parameters. (A) Correlation of K^{trans} with age ($r = -0.46$; $p < 0.001$). (B) Correlation of K^{trans} with body mass index (BMI; $r = 0.48$; $p < 0.001$). (C) Correlation of K^{trans} and tibial nerve conduction velocity (NCV; $r = 0.42$; $p = 0.001$). (D) Correlation of K^{trans} and peroneal NCV ($r = 0.50$; $p < 0.001$).

correlation analysis revealed a significant correlation between v_e and sural SNAPs ($r = 0.41$; $p = 0.019$). A detailed summary of partial correlation analyses of electrophysiological parameters is provided in Table S1.

Discussion

This study investigated peripheral nerve perfusion in patients with T2D using DCE 3 T MRN. The main findings were (i) DCE MRN at sciatic nerve level detected lower values of K^{trans} and v_e in T2D patients with DN compared to T2D patients without DN while no such difference was found between T2D patients with DN and controls; (ii) K^{trans} and v_e were correlated with the NDS score and electrophysiological parameters in T2D patients while no such correlation was found for v_p; (iii) K^{trans} and v_e correlated with age and BMI.

The range of perfusion parameter values of this study is in line with a reference study on DCE MRN, which underlines that DCE-MRN is feasible in patients with T2D.[12] The positive association of K^{trans} with v_e shows that an increase in the sciatic nerve's microvascular permeability is correlated with an increase of the extravascular extracellular volume (EEV) fraction, and, therefore, with a better supply of nerve structures via the blood stream. In turn, this correlation suggests that a decrease in nerve permeability results in a reduction of EEV and, ultimately, in nerve ischemia. The finding that both K^{trans} and v_e were positively correlated with the assessed nerve conduction velocities and compound motor action potentials further suggests that a decrease in nerve permeability and the associated decrease in EEV fraction contribute to nerve ischemia causing demyelination and axonal damage. This hypothesis is further supported by the finding that K^{trans} and v_e were lower in DN patients compared to patients without DN. This is in line with the results of histological studies conducted by Dyck et al. that

Table 3. Correlations of MRN perfusion parameters with demographic, clinical, and electrophysiological data in patients with T2D.

	K^{trans} (min^{-1})		v_p (%)		v_e (%)	
	r	95%CI	*r*	95% CI	*r*	95% CI
K^{trans} (min^{-1})			−0.15[s]	−0.39 to 0.11	0.86[s]	0.77 to 0.91
v_p	−0.15[s]	−0.39 to 0.11			0.15[s]	−0.11 to 0.38
v_e	0.86[s]	0.77 to 0.91	0.15[s]	−0.11 to 0.38		
Age (years)	−0.51[p]	−0.67 to −0.31	<0.01[s]	−0.25 to 0.25	−0.34[s]	−0.55 to −0.10
Diabetes duration (years)	0.01[p]	−0.24 to 0.24	0.15[s]	−0.37 to 0.14	−0.01[s]	−0.25 to 0.24
Gender	0.23[s]	−0.02 to 0.46	−0.17[s]	−0.40 to 0.09	0.21[s]	−0.05 to 0.44
BMI	0.47[p]	0.25 to 0.64	0.12[s]	−0.14 to 0.36	0.41[s]	0.18 to 0.60
NDS	−0.40[s]	−0.60 to −0.16	<0.01[s]	−0.25 to 0.26	−0.48[s]	−0.66 to −0.26
NSS	−0.31[s]	−0.52 to −0.06	0.06[s]	−0.19 to 0.31	−0.29[s]	−0.51 to −0.04
Sural NCV (m/s)	0.44[s]	0.19 to 0.63	−0.17[s]	−0.42 to 0.10	0.40[s]	0.15 to 0.60
Sural SNAP (μV)	0.34[s]	0.09 to 0.56	−0.11[s]	−0.36 to 0.16	0.38[s]	0.13 to 0.58
Peroneal NCV (m/s)	0.50[p]	0.29 to 0.67	−0.15[s]	−0.39 to 0.12	0.44[s]	0.21 to 0.63
Peroneal CMAP (mV)	0.32[s]	0.07 to 0.53	−0.03[s]	−0.28 to 0.23	0.33[s]	0.08 to 0.54
Peroneal DML (ms)	−0.48[s]	−0.65 to −0.25	−0.03[s]	−0.29 to 0.22	−0.52[s]	−0.69 to −0.30
Tibial NCV (m/s)	0.42[p]	0.18 to 0.61	−0.17[s]	−0.41 to 0.10	0.42[s]	0.17 to 0.61
Tibial CMAP (mV)	0.27[s]	0.01 to 0.49	<0.01[s]	−0.26 to 0.26	0.34[s]	0.08 to 0.55
Tibial DML (ms)	−0.27[s]	−0.50 to −0.01	0.01[s]	−0.25 to 0.27	−0.33[s]	−0.55 to −0.08
HbA1c (%)	−0.06[s]	−0.32 to 0.20	−0.02[s]	−0.27 to 0.24	<0.01[s]	−0.26 to 0.26
GFR (mL/min)	−0.08[p]	−0.37 to 0.22	−0.06[s]	−0.36 to 0.25	−0.07[s]	−0.37 to 0.23

[s]Spearman correlation coefficient; [p]Pearson correlation coefficient; K^{trans} = constant of capillary permeability; v_e = extravascular extracellular volume fraction v_p = plasma volume fraction; BMI = body mass index; NDS = neuropathy disability score; NSS = neuropathy severity scale; NCV = nerve conduction velocity; SNAP = sensory nerve action potential; CMAP = compound motor action potential; DML = distal motor latency; m/s = meters per second; ms = milliseconds; mV = millivolts; μV = microvolts HbA1c = glycated hemoglobin; GFR = glomerular filtration rate.

identified nerve ischemia as a potential contributor to demyelination at distal sciatic nerve level.[6,8] In addition to those findings, the results of this study suggest that the main contributor to nerve ischemia is a decrease in microvascular permeability but not in miscrovascular plasma volume, since no correlations were found for the plasma volume fraction v_p.

One should note that this study only found differences in perfusion parameters K^{trans} and v_e between T2D patients with and without neuropathy, and differences of v_e between T2D patients without DN and controls, while no such difference was found between controls and T2D patients with DN. Given the positive correlations of K^{trans} and v_e with parameters of nerve conduction, and the negative correlations with the NDS in patients with T2D described above, however, one would assume both K^{trans} and v_e to be lower in T2D patients with DN compared to controls. Instead, it appears that K^{trans} and v_e are at nearly the same level in T2D DN patients and controls, while both K^{trans} and v_e are higher in T2D patients without DN. One possible explanation for this finding might be that, in the setting of T2D, the metabolic demand of myelinating cells requires an increase of capillary permeability to keep the nerve in a vital condition. This is supported by previous studies on animal models of T2D that have found capillary permeability of peripheral nerves to be increased due to leakiness of the blood–nerve barrier.[28] This in mind, the results of our study suggest that, compared to controls without diabetes, capillary permeability of peripheral nerves is increased in T2D patients due to the nerves' metabolic demand.[29] During the course of T2D, various processes known to be associated with the disease such as perivascular fibrosis and endothelial damage, which can be aggravated by smoking,[30] cause a decrease in nerve permeability, which ultimately results in nerve ischemia leading to DN.

The finding that K^{trans} and v_e were negatively correlated with age suggests that the microvasculature of the sciatic nerve becomes less permeable during the process of aging. This is in line with numerous clinical studies which have found that higher age is an important risk factor for DN.[28] The positive correlations of K^{trans} and v_e with the BMI suggest that a higher BMI is associated with an increasing permeability of the sciatic nerve's microcirculation. The latter is surprising since the majority of clinical studies has found obesity to be a risk factor for DN.[29] One possible explanation might be that metabolic changes associated with obesity such as dyslipidemia may have a more severe impact on demyelination than microvascular changes alone.[2] It remains to be determined, however, in how far metabolic changes associated with obesity also contribute to an increase in microvascular permeability in T2D patients and controls.

One may of course argue that the correlations between MRN perfusion parameters and parameters of nerve conduction simply reflect the combined effects of age and obesity on nerve structure in patients with T2D. It should be taken into account, however, that correlations of tibial and peroneal NCVs with v_e and of peroneal~~tibial~~ NCVs with K^{trans} remained significant after partial correlation analysis double controlled for age and BMI. Thus, it is justified to assume that both nerve permeability and EEV fraction have a relevant impact on demyelination in T2D.

The focus of this study was to investigate possible associations between in vivo parameters of microvascular nerve perfusion with parameters of nerve conduction and basic clinical neuropathy scores in T2D patients. This study did not investigate pain in the context of DN. Since pain is arguably related to nerve ischemia in T2D, future studies on MRN perfusion should investigate the potential association between pain and parameters of microvascular permeability in DN, using specific scales and methods such as quantitative sensory testing.[31]

An important technical limitation of this study is that the resolution of the MR perfusion sequence does not allow distinguishing endoneurial blood flow from perineural blood flow. We therefore cannot tell whether the observed changes in K^{trans} apply to both compartments at the same extent. This study is further limited by the fact that the cross-sectional design does not allow to draw conclusions on the impact of nerve perfusion on the progression of DN. It should be kept in mind, however, that the primary aim of this study was to assess the feasibility of DCE MRN in patients with T2D and to investigate potential correlations with clinical and demographic parameters. Another limitation is the fact that our cohort was not equally balanced for women and men. It should be considered, however, that no correlation was found between gender and any of the acquired parameters, indicating that gender does not have a relevant impact on nerve perfusion. Another limitation is the fact that the number of patients and controls was not equally balanced. It should be considered, however, that the primary aim of this study was to detect the impact of nerve perfusion on nerve function and structural integrity in patients with T2D and not to detect differences to healthy controls. The age-matched control group was included in this study in order to assess whether the perfusion parameters of T2D patients strongly differed from those of individuals without T2D, and, therewith, to determine whether the analysis of nerve perfusion on MRN DCE sequences is a viable method in patients with T2D, since there are no reference values for nerve perfusion determined by the extended Tofts model in the literature so far.

In summary, this study found that the constant of permeability, K^{trans} and the extravascular extracellular volume fraction, v_e, but not the plasma volume fraction v_p were correlated with clinical and electrophysiological parameters of the tibial and peroneal nerve in patients with T2D. The results indicate that a decrease in microvascular permeability but not in microvascular blood volume contribute to nerve ischemia resulting in demyelination and axonal damage. It was further found that nerve permeability declines with age whereas an increase in BMI is correlated with an increase in nerve permeability. Further longitudinal studies on the impact of nerve perfusion on the progression of DN and on the impact of obesity on nerve microcirculation are warranted.

Acknowledgments

The German Research Council (DFG, SFB 1158) provided financial support for personnel expenditure, MR imaging costs and costs for the technical equipment required for electrophysiological and serological analysis. The DFG had no influence on the study design, collection, and analysis of data or on the writing of the article. JJ further acknowledges support from the International Foundation for Research in paraplegia (IRP) and the Else-Kröner-Fresenius-Stiftung (EKFS), ZK received grants from the Deutsches Zentrum für Diabetesforschung (DZD) e.V., SH received a grant from the Dietmar Hopp Foundation and the German Research Council (DFG, SFB 1118), MB received grants and personal fees from Codman, Guerbet, Bayer, and Novartis, personal fees from Roche, Teva, Springer, Boehringer, Grifols, Braun and grants from the European Union, Siemens, the Dietmar Hopp Foundation, Stryker and the German Research Council (DFG, SFB 1118 and 1158), FK was supported by the German Research Foundation (KU 3555/1-1), the Hoffmann-Klose Foundation of Heidelberg University Hospital, and a research grant of Heidelberg University Hospital. Open Access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

JJ: Conception of study, organization of participants, collection of MR data, image segmentation, data analysis and interpretation, literature search, writing of manuscript, and arrangement of Figures. CM: Collection of MR data, data analysis and interpretation, literature search, writing of manuscript, and arrangement of Figures. ZK, MD, PhD: Collection of clinical, electrophysiological and serological

data, and organization of participants. LS: Collection of clinical, electrophysiological and serological data, and organization of participants. AJ: organization of participants, collection of MR data, and data analysis. SH: Conception of MRN sequence protocol. PN: Study design and coordination. MB: Study design and coordination, development of MR sequence protocol, and writing of manuscript. SK: Development of clinical and electrophysiological study protocol, collection of clinical, electrophysiological, and serological data. FK: Conception of study, programming of image analysis tools, image segmentation, data analysis and interpretation, literature search, writing of manuscript, and arrangement of figures.

Data Availability Statement

Anonymized data will be shared by request from any qualified investigator.

References

1. Alleman CJM, Westerhout KY, Hensen M, et al. Humanistic and economic burden of painful diabetic peripheral neuropathy in Europe: a review of the literature. Diabetes Res Clin Pract. 2015;109:215-225.
2. Feldman EL, Nave K-A, Jensen TS, Bennett DLH. New horizons in diabetic neuropathy: mechanisms, bioenergetics, and pain. Neuron. 2017;93:1296-1313.
3. Toth PP, Simko RJ, Palli S, Koselleck D, Quimbo RA, Cziraky MJ. The impact of serum lipids on risk for microangiopathy in patients with type 2 diabetes mellitus. Cardiovasc Diabetol. 2012;11:109-109.
4. Shillo P, Sloan G, Greig M, et al. Painful and painless diabetic neuropathies: what is the difference? Curr Diab Rep. 2019;19:32-32.
5. Tesfaye S, Chaturvedi N, Eaton SEM, et al. Vascular risk factors and diabetic neuropathy. New Engl J Med. 2005;352:341-350.
6. Dyck PJ, Karnes J, O'Brien P, Nukada H, Lais A, Low P. Spatial pattern of nerve fiber abnormality indicative of pathologic mechanism. Am J Pathol. 1984;117:225-238.
7. Dyck PJ, Lais A, Karnes JL, Obrien P, Rizza R. Fiber loss is primary and multifocal in sural nerves in diabetic polyneuropathy. Ann Neurol. 1986;19:425-439.
8. Dyck PJ, Karnes JL, O'Brien P, Okazaki H, Lais A, Engelstad JN. The spatial distribution of fiber loss in diabetic polyneuropathy suggests ischemia. Ann Neurol. 1986;19:440-449.
9. Jende JME, Groener JB, Oikonomou D, et al. Diabetic neuropathy differs between type 1 and type 2 diabetes: insights from magnetic resonance neurography. Ann Neurol. 2018;83:588-598.
10. Jende JME, Groener JB, Kender Z, et al. Structural nerve remodeling on 3-T MR neurography differs between painful and painless diabetic polyneuropathy in either type 1 or type 2 diabetes. Radiology. 2020;294:405-414.
11. Jende JME, Groener JB, Kender Z, et al. Troponin T parallels structural nerve damage in type 2 diabetes: a cross-sectional study using magnetic resonance neurography. Diabetes. 2020;69:713-723.
12. Bäumer P, Reimann M, Decker C, et al. Peripheral nerve perfusion by dynamic contrast-enhanced magnetic resonance imaging: demonstration of feasibility. Invest Radiol. 2014;49:518-523.
13. Sourbron SP, Buckley DL. On the scope and interpretation of the Tofts models for DCE-MRI. Magn Reson Med. 2011;66:735-745.
14. Xu Z, Zeng W, Sun J, et al. The quantification of blood-brain barrier disruption using dynamic contrast-enhanced magnetic resonance imaging in aging rhesus monkeys with spontaneous type 2 diabetes mellitus. Neuroimage. 2017;158:480-487.
15. Vaeggemose M, Haakma W, Pham M, et al. Diffusion tensor imaging MR neurography detects polyneuropathy in type 2 diabetes. J Diabetes Complicat. 2020;34:107439.
16. Young MJ, Boulton AJM, Macleod AF, Williams DRR, Onksen PHS. A multicentre study of the prevalence of diabetic peripheral neuropathy in the United Kingdom hospital clinic population. Diabetologia. 1993;36:150-154.
17. Gibbons CH, Freeman R, Veves A. Diabetic neuropathy. Diabetes Care. 2010;33:2629-2634.
18. Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an open-source platform for biological-image analysis. Nat Methods. 2012;9:676-682.
19. Mattes D, Haynor DR, Vesselle H, Lewellen TK, Eubank W. PET-CT image registration in the chest using free-form deformations. IEEE Trans Med Imaging. 2003;22:120-128.
20. Cheng H-LM, Wright GA. Rapid high-resolutionT1 mapping by variable flip angles: accurate and precise measurements in the presence of radiofrequency field inhomogeneity. Magn Reson Med. 2006;55:566-574.
21. Chikui T, Obara M, Simonetti AW, et al. The principal of dynamic contrast enhanced MRI, the method of pharmacokinetic analysis, and its application in the head and neck region. Int J Dent. 2012;2012:1-10.
22. Shen Y, Goerner FL, Snyder C, et al. T1 relaxivities of gadolinium-based magnetic resonance contrast agents in human whole blood at 1.5, 3, and 7 T. Invest Radiol. 2015;50:330-338.
23. Tofts PS, Brix G, Buckley DL, et al. Estimating kinetic parameters from dynamic contrast-enhanced T(1)-weighted MRI of a diffusable tracer: standardized quantities and symbols. J Magn Reson Imaging. 1999;10:223-232.
24. Cuenod CA, Balvay D. Perfusion and vascular permeability: basic concepts and measurement in DCE-CT and DCE-MRI. Diagn Interv Imaging. 2013;94:1187-1204.

25. Lagarias JC, Reeds JA, Wright MH, Wright PE. Convergence properties of the Nelder--Mead simplex method in low dimensions. SIAM J Optim. 1998;9:112-147.
26. Padhani AR, Hayes C, Landau S, Leach MO. Reproducibility of quantitative dynamic MRI of normal human tissues. NMR Biomed. 2002;15:143-153.
27. Faranesh AZ, Kraitchman DL, McVeigh ER. Measurement of kinetic parameters in skeletal muscle by magnetic resonance imaging with an intravascular agent. Magn Reson Med. 2006;55:1114-1123.
28. Seneviratne KN. Permeability of blood nerve barriers in the diabetic rat1. J Neurol Neurosurg Psychiatry. 1972;35:156-162.
29. Shami SK, Chittenden SJ. Microangiopathy in diabetes mellitus: II. Features, complications and investigation. Diabetes Res. 1991;17:157-168.
30. Jende JME, Mooshage C, Kender Z, et al. Magnetic resonance neurography reveals smoking-associated decrease in sciatic nerve structural integrity in type 2 diabetes. Front Neurosci. 2022;15:811085.
31. Groener JB, Jende JME, Kurz FT, et al. 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. Diabetes. 2020;69:436-447.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Illustration of the two compartment exchange model. Plasma flows into and out of the capillary (white cylinder) with the flow F_p. The exchange of contrast agent between the plasma inside the capillary and the extravascular extracellular space (grey rectangle) between the capillary and the nerve fascicles (yellow tubes) is assumed to be symmetric and is defined by the permeability surface product, represented by K^{trans}. v_p represents the volume fraction of capillary plasma whereas v_e represents the volume fraction of the extravascular extracellular space.
Table S1. Correlations of MRN perfusion parameters with electrophysiological data in a partial correlation analysis double controlled for age and BMI.

