Supplementary materials

Supplementary methods

The following neurological assessments were performed:

1) Toronto Clinical Neuropathy Score (TCNS) to assess neuropathy symptoms and signs.(1)

2) Doleur Neuropathique en 4 (DN4) questionnaire to assess neuropathic pain symptoms and signs(2) and Neuropathic Pain Symptom Inventory (NPSI) to evaluate different symptoms of neuropathic pain.(3)

3) Nerve conduction studies performed at a stable skin temperature of 31°C and a room temperature of 24°C, using an electrophysiological system (Medelec; Synergy Oxford Instruments, Oxford, UK). The following peripheral nerve attributes were measured: a) sural sensory nerve action potential; b) common peroneal motor nerve distal latency, compound muscle action potential, and conduction velocity; and c) tibial motor nerve distal latency.

4) German Research Network of Neuropathic Pain (DFNS) quantitative sensory testing (4) where the following sensory sub-modalities were tested: cold (CDT) and warm detection thresholds (WDT; TSA-II, MEDOC, Israel), cold (CPT) and heat pain (HPT), mechanical detection (MDT, von Frey hairs, Optihair2-set, Marstock Nervtest Germany) and pain (MPT, PinPrick stimulator set, Medizintechnische Systeme, Germany) thresholds, mechanical pain sensitivity (MPS), dynamic mechanical allodynia (DMA; Standardized brush, Somedic, Sweden; cotton wool tip, cotton wisp), pressure pain thresholds (PPT; Pressure gauge device, Wagner Instruments, USA), and VDT (Rydel-Seiffer tuning fork, 64 Hz, 8/8 scale). As per the DFNS protocol, all modalities were tested using the same technique at the dorsum of the foot, except for vibration detection thresholds in which the tuning fork is placed on the medial malleolus and pressure pain threshold where the pressure algometer is placed on the abductor hallucis muscle. The QST data was z-transformed for each parameter and compared to site, gender, and age specific reference data. A positive z-score denotes a gain of function whereas a loss of function is denoted by a negative z-score. Formal training for the protocol was obtained at Bochum Hospital, Germany.

We graded diabetic retinopathy (DR) severity (0, no DR; 1, mild non-proliferative DR; 2, moderate/severe non-proliferative and proliferative DR) using two-field mydriatic digital photography images acquired as part of the National Diabetic Retinopathy Screening Program for England. Diabetic nephropathy was defined as a urinary albumin/creatinine ratio of ≥30 mg/mmol on more than two urine samples greater than three months apart.

Analysis was performed using the statistical package Statistical Product and Service Solutions Version 26 (SPSS, IBM Corporation, New York, USA). Data was tested for normality using the Shapiro-Wilk test. Normally distributed characteristics are presented as means and standard deviations and those with a non-parametric distribution are presented as medians and inter-quartile ranges. Categorical and dichotomous variables are presented as number

of cases and group percentages. Normally distributed continuous data was analysed using the independent-samples t-test. The Chi-squared test was used to compare categorical and dichotomous variables. The Pearson's correlation test was used to measure the association between ^{31}P-MRS and clinical, demographic, and neurological variables. Additionally, participants with painful-DPN were stratified according to their pain score during the MRI scan into moderate/severe pain (NRS ≥3) and low pain (NRS <3). The Mann-Whitney U test was used to compare pain scores and 31P-MRS variables between these groups as the data was non-parametric.

Supplementary display items

	Painless-DSPN (*n*=12)	Painful-DSPN (*n*=20)	*p* value
CDT	-2.3 ± 1.2	-2.7 ± 0.8	0.341
WDT	-1.9 ± 0.9	-2.0 ± 0.52	0.727
TSL	-2.4 ± 1.3	-2.4 ± 0.7	0.922
CPT	-0.9 ± 0.4	-0.9 ± 0.3	0.936
HPT	-1.2 ± 1.3	-1.4 ± 0.6	0.456
PPT	0.9 ± 2.0	0.5 ± 2.0	0.549
MPT	-2.4 ± 1.3	-1.8 ± 1.7	0.353
MPS	-1.8 ± 0.5	-1.6 ± 0.5	0.486
MDT	-2.6 ± 1.7	-2.3 ± 1.5	0.673
VDT	-2.4 ± 2.3	-2.6 ± 1.9	0.865

Supplementary Table 1. DFNS QST *z*-score results in study participants. CDT, cold detection threshold; CPT, cold pain threshold; HPT, heat pain threshold; MDT, mechanical detection threshold; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; PPT, pressure pain threshold; TSL, thermal sensory limen; VDT, vibration detection threshold; WDT, warm detection threshold. All tests were t-test.

	Low pain (*n*=6)	Moderate/severe pain (*n*=14)	*P* value
Age, years	65.2 ± 6.6	60.1 ± 9.0	0.23
Sex, *n* female (%)	2 (33%)	10 (71%)	0.11
Duration DM, years	12.8 ± 8.6	12.4 ± 8.5	0.92
Pack Years Smoking	15.8 ± 24.5	14.9 ± 16.6	0.93
Body mass index (kg/m^2)	34.3 ± 6.2	30.0 ± 4.3	0.08
HbA1c (mmol/mol)	63.8 ± 25.6	65.5 ± 21.1	0.88
Creatinine (µmol/L)	70.7 ± 14.0	71.4 ± 17.3	0.93
Total Cholesterol (mmol/l)	4.4 ± 0.9	4.3 ± 1.0	0.82
Blood glucose During MR (mmol/l)	9.1 ± 3.8	10.0 ± 6.0	0.76
TCNS	13.0 ± 2.6	14.3 ± 4.6	0.53
Peroneal CMAP (mV)	2.3 ± 1.5	37.7 ± 5.1	0.62
Peroneal MNCV (m/s)	37.7 ± 5.1	38.5 ± 5.6	0.78
Peroneal MNDL (msec)	5.4 ± 1.1	6.6 ± 3.5	0.44
Tibial MNDL (msec)	7.2 ± 2.2	6.3 ± 2.1	0.45
Sural SNAP (mV)	2.8 ± 4.1	6.3 ± 7.4	0.32
NPSI (Total score)	14.4 ± 3.6	25.8 ± 9.9	0.01
DN4	6.5 ± 2.0	7.0 ± 1.4	0.52
Depression, *n* (%)	0 (0%)	3 (21%)	0.22*
Anxiety, *n* (%)	0 (0%)	4 (29%)	0.14*
Medications, *n* (%)			N/A
α2-δ ligands	1 (16%)	7 (50%)	0.16*
Duloxetine	1 (16%)	3 (21%)	0.81*
Amitriptyline	2 (33%)	4 (28%)	0.83*
Opiates	0 (0%)	3 (21%)	0.22*
Topical agents	1 (16%)	0 (0%)	0.12*
Other	0 (0%)	2 (14%)	0.33*

Supplementary table 2. Demographic details, clinical, biochemical and neurophysiological assessments of study participants with low pain (NRS <3) and moderate/severe pain (NRS ≥3). One pack year smoking is defined as: 20 cigarettes x number of years of smoking. CMAP, compound muscle action potential; DM, diabetes mellitus; DN4, doleur neuropathique en 4 questions;, motor nerve conduction velocity; MNDL, motor nerve distal latency; MR, magnetic resonance scan; NIS-LL+7, Neuropathy Impairment Score of the Lower Limbs plus 7 neurophysiological tests; NPSI, neuropathic pain symptom inventory; SNAP, sensory nerve action potential; TCNS, Toronto Clinical Neuropathy Score. All tests were t-test unless stated, * Chi-squared test. Boldface type used to denote group comparisons with a *p*-value <0.05.

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