ORIGINAL ARTICLE

Hypertension Contributes to Neuropathy in Patients With Type 1 Diabetes

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BACKGROUND
Diabetic peripheral neuropathy (DPN) can lead to foot ulceration and amputation. There are currently no disease-modifying therapies for DPN. The aim of this study was to determine if hypertension contributes to DPN in patients with type 1 diabetes mellitus (T1DM).

METHODS
Subjects with T1DM ($n = 70$) and controls ($n = 78$) underwent a comprehensive assessment of DPN.

RESULTS
Hypertension was present in 40 of 70 T1DM subjects and 20 of 78 controls. Hypertension was associated with abnormal nerve conduction parameters ($P = 0.03$ to <0.001), increased vibration perception threshold ($P = 0.01$) and reduced corneal nerve fiber density and length ($P = 0.02$) in subjects with T1DM. However, after adjusting for confounding factors only tibial compound motor action potential and nerve conduction velocity were associated with hypertension ($P = 0.03$) and systolic blood pressure ($P < 0.01$ to <0.0001). Hypertension had no effect on neuropathy in subjects without diabetes.

CONCLUSIONS
This study shows that hypertension is associated with impaired nerve conduction in T1DM. It supports previous small trials showing that angiotensin-converting enzyme inhibitors improve nerve conduction and advocates the need for larger clinical trials with blood pressure lowering agents in DPN.

Keywords: blood pressure; corneal confocal microscopy; diabetic peripheral neuropathy; hypertension; nerve conduction, quantitative sensory testing.

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There are currently no US Food and Drug Administration (FDA)-approved treatments for diabetic peripheral neuropathy (DPN).[1] Although tight glycemic control is advocated for the treatment of DPN, it has only been shown to limit progression of neuropathy in patients with type 1 diabetes mellitus (T1DM) and has shown no benefit in patients with type 2 diabetes mellitus (T2DM).[2] However, clinical and experimental studies suggest that hypertension is an independent risk factor for DPN in patients with T1DM[3–8] and T2DM.[9–12] In relation to the underlying pathophysiology, we have previously demonstrated loss of myogenic tone and vascular hypertrophy in resistance vessels of hypertensive patients with T2DM,[13] with partial amelioration of these abnormalities after improved glycemic control[14] or treatment with the angiotensin-receptor blocker candesartan.[15]

Detailed experimental studies suggest that hypertension predominantly affects the myelinated fibers. Hypertensive streptozotocin rats with diabetes show myelinated fiber

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abnormalities.[7] Spontaneously hypertensive rats with diabetes show a reduction in sciatic nerve blood flow with a reduction in motor and sensory nerve conduction velocity and myelinated fiber density, but no loss of intraepidermal nerve fibers.[8] In a hypertensive T2DM model, there was a reduction in sensory nerve conduction velocity and increased expression of matrix metalloproteinase at sites of myelin thinning[10] In nondiabetic hypertensive rats impaired epineurial arteriolar function was shown to contribute to reduced endoneurial perfusion and neuropathy[16] as well as axonal atrophy and myelin splitting with endoneurial microangiopathy.[17] However, treatment with fosinopril prevented the development and maintenance of tactile allodynia[18] and a combination of enalapril, α-lipoic acid and menhaden oil improved thermal hypoalgesia, intraepidermal nerve fiber profiles, and corneal subbasal nerve fiber length in a normotensive T2DM model.[19] These improvements were related to improved vascular relaxation to acetylcholine and calcitonin gene-related peptide in sciatic nerve epineurial arterioles. Recently, sacubitril/valsartan, a combination drug containing a neprilysin inhibitor and angiotensin II receptor blocker has been shown to prevent and reverse nerve conduction and intraepidermal and corneal nerve abnormalities in type 2 diabetic rats.[20]

We have shown that treatment of diabetic patients with the angiotensin-converting enzyme inhibitor trandolapril, improved nerve conduction, but had no impact on neuropathic symptoms/deficits, vibration perception, or autonomic function.[21] Other studies have reported a significant improvement in nerve conduction, neuropathic symptoms, and thermal thresholds in hypertensive patients with diabetes treated with an angiotensin-converting enzyme inhibitor.[22,23] Treatment of normotensive patients with DPN with the angiotensin-receptor blocker losartan for 12 weeks did not show an improvement in nerve conduction studies (NCS).[24] In the neurological assessment of thioctic acid in diabetic neuropathy 1 trial, patients treated with α-lipoic acid on angiotensin-converting enzyme inhibitors showed improved heart rate variability (deep breathing heart rate variability, DB-HRV).[25]

We have undertaken a detailed study to identify the exact impact of hypertension on both large and small fiber measures of DPN in patients with T1DM. We believe this may explain the disparate results of previous studies assessing the benefits of blood pressure (BP) lowering agents on DPN. It will also help to identify the neuropathy end points that should be used to determine the efficacy of BP lowering therapies in DPN.

METHODS

Participants with T1DM and controls without diabetes were recruited from the Manchester Diabetes Centre, Manchester Royal Infirmary and the NIHR Wellcome Trust Clinical Research Facility. The study was performed at the NIHR Wellcome Trust Clinical Research Facility.

Exclusion criteria included corneal trauma/dystrophy, corneal surgery in the last 6 months, vitamin B12 deficiency, hypothyroidism, neuropathy from nondiabetic causes and diabetes or impaired glucose tolerance in the control group. This study was approved by the local research ethics committee and all participants gave informed consent to take part in the study. The research adhered to the tenets of the Declaration of Helsinki.

BP measurement

BP was assessed in all participants on the nondominant arm, assuring correct cuff size, with an automated device DINAMAP PRO 400 (Critikon, FL) in the sitting position after 5 minutes rest on 2 occasions. Hypertension was defined according to either an average systolic blood pressure (SBP) ≥ 140 mm Hg from 2 sets of measurement as described in the WHO/ISH Guidelines or if subjects were on antihypertensive treatment.

Clinical measures

All participants underwent assessment of body mass index (BMI), glycated haemoglobin (HbA1c), cholesterol, and triglycerides.

Neuropathy and neuropathic pain assessment

DPN was diagnosed according to the criteria established by the Toronto Diabetic Neuropathy Expert Group[26] These criteria include neuropathy symptoms or neuropathy signs and an abnormality of NCS or a validated measure of small fiber neuropathy (corneal nerve fiber length, CNFL).[27,28] The assessments were performed by different researchers who were blinded to subject group and the researchers were acting independently, with no exchange of results during the study.

Neuropathic symptoms were assessed using the DNS score,[29] a 4-item validated symptom score for symptoms of unsteadiness in walking, neuropathic pain, paresthesia, and numbness, giving a maximum score of 4 points, with a score of ≥ 1 defining the presence of neuropathic symptoms. Neuropathy signs were defined using the neuropathy disability score (NDS)[30] that includes examination of vibration perception using a 128-Hz tuning fork, pinprick on the tip of the large toe, temperature perceptions in the dorsum of the feet, and the presence or absence of ankle reflexes. Subjects scoring > 2 of 10 were considered to have signs of neuropathy.

Neuropathic pain was defined by a combination of deficits with an NDS score > 2 and the presence of painful symptoms using the McGill Pain Questionnaire to assess the type of pain using descriptors such as throbbing, shooting, distressing, excruciating etc.[31]

Corneal confocal microscopy

Participants underwent examination with the Heidelberg Retina Tomograph (HRT III RCM) *in vivo* corneal confocal microscope (Heidelberg Engineering GmbH, Heidelberg, Germany) using our established methodology.[32] Three corneal confocal microscopy images from the subbasal nerve plexus in the central cornea were captured per eye. Corneal nerve fiber density (CNFD), number of main nerve fibers

per mm^2 (no./mm^2), corneal nerve branch density, number of nerve branches per mm^2 (no./mm^2), and CNFL, length of nerve fibers per mm^2 (mm/mm^2) were quantified manually using CCMetrics, a validated image analysis software.[32] The cutoff values of CNFD (≥19 no./mm^2), corneal nerve branch density (≥42 no./mm^2), and CNFL (≥16 mm/mm^2) were based on the study by Petropoulos et al.[33] that assessed the validity of corneal confocal microscopy in diagnosing DPN.

Intraepidermal nerve fiber density

A 3-mm punch skin biopsy was taken from the dorsum of the foot under 1% lidocaine local anesthesia. Skin samples were immediately fixed in 4% (wt/vol) paraformaldehyde for 24 hours and then cryoprotected in sucrose, frozen and cut into 50 m sections. Immunohistochemistry was performed as previously described.[34] A Zeiss Axio Imager M2 microscope (Carl Zeiss, Jena, Germany) was used to quantify intraepidermal nerve fiber density, which is the total number of nerve fibers per millimeter length of epidermis (no./mm), in accordance with established criteria.[35]

Autonomic neuropathy

Cardiac autonomic neuropathy was evaluated using the ANX 3.0 autonomic nervous system monitoring device (ANSAR Medical Technologies, Philadelphia, PA).[36] Deep breathing heart rate variability DB-HRV was assessed by R-R interval variation *via* surface electrodes over 1 minute at a frequency of 6 breaths/minute.

Peripheral autonomic dysfunction was assessed using the Neuropad (miro Verbandstoffe, Wiehl-Drabenderhöhe, Germany) applied to the plantar aspect of the 1st metatarsal head for 10 minutes, followed by quantification of the percentage color change of the Neuropad.

Quantitative sensory testing

Quantitative sensory testing included measurement of vibration perception threshold (VPT) on the tip of the large toe using the Neurothesiometer (Horwell, Scientific Laboratory Supplies, Nottingham, UK) and warm and cold perception thresholds on the dorsum of the left foot using the method of limits with the MEDOC *TSA II* (Medoc, Ramat Yishai, Israel).

Nerve conduction

Electrodiagnostic studies were undertaken using a Dantec "Keypoint" system (Dantec Dynamics , Bristol, UK) equipped with a DISA temperature regulator to keep lower limb temperature constantly between 32 and 35 °C. Sural sensory nerve action potential (SNAP), sural nerve conduction velocity (SNCV), tibial compound motor action potential (TCMAP), tibial motor nerve conduction velocity (TMNCV), peroneal compound motor action potential (PCMAP), and peroneal motor nerve conduction velocity (PMNCV) were assessed in the right lower limb by a consultant neurophysiologist. Sural sensory responses were measured using a bipolar bar electrode (interelectrode distance 3cm) attached over the sural nerve at the lateral malleolus. Stimulation was performed 140 mm proximal to the active recording electrode in the calf. Abnormal nerve conduction was defined based on 2 abnormal nerve conduction velocities of either SNCV, TMNCV, or PMNCV. The cutoff values of the nerve conduction velocities were defined on the - 2 SD from the mean based on our control population.

Statistical analysis

The sample size needed to detect significant differences in corneal confocal microscopy and NCS between the groups was calculated from our previously published data.[28] Given a reported difference in population means of 8 no./mm^2 for CNFD and 5 m/s for PMNCV, estimated SD for within group differences of 7 for CNFD and 3 for PMNCV, and aiming for a study power of 80% and an alpha of 0.05, we estimated that ~17 participants for each group would be needed to conduct this study.

Differences between normotensive and hypertensive groups in continuous variables were compared using independent *t*-test. Categorical variables were compared using chi-square or Fisher's exact test (when sizes were less than 5). Data are expressed, based on the scale of measurements, as mean (SD) or frequency distribution. This analysis was done separately for the control group and the diabetic group. The analysis was performed using StatsDirect, version 3.0.

The aforementioned analysis was repeated while adjusting for baseline imbalances between the 2 groups (normotensive and hypertensive) using multiple linear regression analysis for continuous variables and multiple logistic regression analysis for categorical variables. Assumptions of linear regression were satisfied for normality, collinearity, and outliers. In addition, residual plots were used to determine whether the models fit the assumptions. Finally, a multiple linear regression model was created to test the association between SPB and neuropathy measures adjusting for potential confounders. The analysis was performed using SPSS (version 23; SPSS, Chicago, IL).

A 2-tailed *P* value of ≤0.05 was considered significant.

RESULTS

Clinical data

The demographic and clinical characteristics are summarized in Table 1. Fifty-eight normotensive controls, 20 hypertensive controls, 30 normotensive, and 40 hypertensive T1DM participants were studied. All 4 groups had comparable age and gender. The duration of diabetes was comparable between hypertensive and normotensive T1DM participants. Both SBP and diastolic blood pressure were significantly higher in the hypertensive compared to normotensive groups (142.58–151.35 mm Hg vs. 117.89–121.58 mm Hg and 74.08–82.15 vs. 67.68–70.54 mm Hg, respectively, $P < 0.01$ to <0.0001). Hypertensive controls had significantly higher cholesterol levels compared to normotensive controls (5.54 [SD 0.75] vs. 4.98 [SD 0.79] mmol/l, $P = 0.01$), but HbA1c, triglycerides, and BMI were comparable. Hypertensive T1DM participants had significantly

Table 1. Demographic characteristics of the study population

	Control					T1DM				
	Normotensive		Hypertensive		*P* value	Normotensive		Hypertensive		*P* value
n	58		20			30		40		
Age, years	47.84	(11.91)	53.35	(13.40)	NS	44.19	(11.11)	49.52	(12.19)	NS
Gender (F, M), *n*	29	29	10	10	NS	16	14	13	27	NS
SBP, mm Hg	121.58	(12.63)	151.35	(12.17)	<0.0001	117.89	(10.19)	142.58	(17.74)	<0.0001
DBP, mm Hg	70.54	(8.19)	82.15	(9.75)	<0.0001	67.68	(8.10)	74.08	(9.83)	<0.01
Diabetes duration, years	N/A		N/A			27.23	(12.89)	31.63	(15.95)	NS
HbA1c, %	5.63	(0.34)	5.58	(0.33)	NS	7.89	(1.86)	8.30	(1.40)	NS
HbA1c, mmol/l	38.06	(3.72)	37.31	(3.57)		66.53	(14.86)	67.24	(15.35)	
Chol. mmol/l	4.98	(0.79)	5.54	(0.75)	0.01	4.40	(0.88)	4.24	(0.90)	NS
Trig. mmol/l	1.42	(0.74)	1.70	(0.73)	NS	0.95	(0.53)	1.39	(0.73)	<0.01
BMI, kg/m^2	26.72	(4.84)	29.01	(4.46)	NS	25.55	(4.12)	27.71	(3.70)	<0.05

Comparing the characteristics between normotensive vs. hypertensive control subjects, and normotensive vs. hypertensive T1DM subjects. Values presented as mean (SD) unless otherwise stated. Unpaired *t*-test was applied to assess for parametric data. Abbreviations: BMI, body mass index; Chol., cholesterol; DBP, diastolic blood pressure; SBP, systolic blood pressure; T1DM, type 1 diabetes mellitus; Trig., triglycerides.

higher triglycerides (1.39 [SD 0.73] vs. 0.95 [SD 0.53] mmol/l, $P < 0.01$) and BMI (27.71 [SD 3.70] vs. 25.55 [SD 4.12] kg/m^2, $P < 0.05$) compared to normotensive T1DM participants, but HbA1c and cholesterol were comparable.

Neuropathy and neuropathic pain

The neuropathy findings between normotensive and hypertensive subjects in the T1DM and control group are summarized in Table 2. The prevalence of DPN (53.8% vs. 51.7%) and painful DPN (38.5% vs. 23.3%) were comparable between patients with T1DM with and without hypertension, respectively. There were no difference in the prevalence of DPN (10.0% vs. 7.0%) and painful DPN (5.3% vs. 1.8%) between the hypertensive and normotensive controls.

Corneal and intraepidermal nerve fiber morphology

The T1DM group with hypertension had a significantly lower CNFD (22.04 [SD 10.33] vs. 27.61 [SD 7.60] no./mm^2, $P = 0.02$) and CNFL (16.40 [SD 6.83] vs. 20.28 [SD 5.58] mm/mm^2, $P = 0.02$) compared to the normotensive group. However, the significant difference was lost after adjusting for age, gender, triglycerides, and BMI. There was no difference in corneal nerve branch density (46.83 [SD 31.86] vs. 60.80 [SD 30.55] no./mm^2) and intraepidermal nerve fiber density (5.12 [SD 3.77] vs. 6.89 [SD 4.43] no./mm^2) between the normotensive and hypertensive T1DM groups (Table 2, Figures 1 and 2). CNFD, corneal nerve branch density, CNFL, and intraepidermal nerve fiber density were comparable between the normotensive and hypertensive control groups.

Autonomic neuropathy

There were no differences in deep breathing heart rate variability (DB-HRV) and Neuropad response between the T1DM and control participants with and without hypertension.

Quantitative sensory testing

VPT was significantly higher in hypertensive (15.37 [SD 11.38] compared to normotensive (9.40 [SD 7.04] V, $P = 0.01$) patients with T1DM, but the difference was no longer significant after adjusting for age, gender, triglycerides, and BMI. The cold and warm perception thresholds were comparable. However, after adjusting for baseline imbalances the cold perception threshold was significantly higher in the hypertensive group ($P = 0.02$). There were no differences in VPT, cold perception threshold, or warm perception threshold between the normotensive and hypertensive control groups.

Nerve conduction studies

T1DM patients with hypertension had a significantly lower SNAP (6.95 [SD 6.75] vs. 11.33 [SD 7.31] μV, $P = 0.01$), TCMAP (6.38 [SD 4.62] vs. 10.87 [SD 4.10] mV, $P < 0.001$), TMNCV (39.39 [SD 5.82] vs. 44.92 [SD 4.08] m/s, $P < 0.001$) and PCMAP (2.56 [SD 2.06] vs. 3.76 [SD 2.20] mV, $P = 0.03$). However, after adjusting for age, gender, triglycerides, and BMI the differences were no longer significant apart from TCMAP and TMNCV. SNCV (39.63 [SD 7.84] vs. 41.98 [SD 10.31] m/s) and PMNCV (39.06 [SD 6.52] vs. 41.87 [SD 6.93] m/s) were comparable between the 2 subgroups. In the control group, only SNAP (14.87 [SD 6.92] vs. 21.82 [SD 10.43] μV, $P = 0.01$) was lower in the hypertensive compared to the normotensive group but the difference was no longer significant after adjusting for age, gender, and cholesterol and SNCV, TCMAP, TMNCV, PCMAP, and PMNCV were comparable.

Association between neuropathy and SBP

Simple linear regression analysis shows that all measures of DPN including CNFD, CNFL, HRV, SNAP, SNCV, TCMAP, TMNCV, PCMAP, PMNCV, and VPT were associated with SBP in patients with T1DM. However, after

Table 2. Neuropathy measures in the study population

	Control			T1DM		
	Normotensive	Hypertensive	*P* value/*P* value*	Normotensive	Hypertensive	*P* value/*P* value*
n	58	20		30	40	
Neuropathy, *n* (%)	4 (7.0)	2 (10.0)	NS/NS	15 (51.7)	21 (53.8)	NS/NS
Neuropathic pain, *n* (%)	1 (1.8)	1 (5.3)	NS/NS	7 (23.3)	15 (38.5)	NS/NS
Nerve fiber morphology						
CNFD, no./mm^2	36.99 (6.39)	35.42 (6.69)	NS/NS	27.61 (7.60)	22.04 (10.33)	0.02/NS
CNBD, no./mm^2	90.95 (40.35)	84.07 (28.65)	NS/NS	60.80 (30.55)	46.83 (31.86)	NS/NS
CNFL, mm/mm^2	25.99 (5.50)	25.26 (5.10)	NS/NS	20.28 (5.58)	16.40 (6.83)	0.02/NS
IENFD, no./mm	9.49 (4.21)	10.17 (1.76)	NS/NS	6.89 (4.43)	5.12 (3.77)	NS/NS
Autonomic neuropathy						
HRV-DB, beats/min	28.88 (12.60)	27.89 (10.97)	NS/NS	25.49 (10.68)	20.11(10.58)	NS/NS
Neuropad, %	84.33 (23.16)	89.25 (14.38)	NS/NS	76.46 (28.71)	70.92 (34.31)	NS/NS
Quantitative sensory testings						
VPT, V	6.24 (5.11)	7.27 (5.40)	NS/NS	9.40 (7.04)	15.37 (11.38)	0.01*/NS
CPT, °C	28.43 (2.06)	27.49 (2.13)	NS/NS	24.51 (6.66)	25.37 (4.50)	NS/0.02
WPT, °C	37.34 (3.32)	36.63 (2.13)	NS/NS	39.62 (4.06)	40.59 (4.37)	NS/NS
Nerve conduction						
SNAP, μV	20.82 (10.43)	14.87 (6.92)	0.01/NS	11.33 (7.31)	6.95 (6.75)	0.01/NS
SNCV, m/s	51.08 (4.81)	49.49 (4.07)	NS/NS	41.98 (10.31)	39.63 (7.84)	NS/NS
TCMAP, mV	12.69 (4.18)	10.92 (4.19)	NS/NS	10.87 (4.10)	6.38 (4.62)	<0.001/0.03
TMNCV, m/s	48.96 (3.20)	48.57 (3.95)	NS/NS	44.92 (4.08)	39.39 (5.82)	<0.001/0.03
PCMAP, mV	5.12 (2.04)	4.66 (2.22)	NS/NS	3.76 (2.20)	2.56 (2.06)	0.03/NS
PMNCV, m/s	49.03 (3.63)	47.00 (4.02)	NS/NS	41.87 (6.93)	39.06 (6.52)	NS/NS

Characteristics of normotensive vs. hypertensive control subjects, and normotensive vs. hypertensive T1DM subjects. Values presented as mean (SD) unless otherwise stated. Unpaired *t*-test was applied to assess parametric data. * Mann–Whitney test was applied to assess nonparametric data. *P* value* were adjusted for baseline imbalances in each group according to Table 1. Abbreviations: CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; CPT, cold perception threshold; HRV-DB, heart rate variability with deep breathing; IENFD, intraepidermal nerve fiber density; PCMAP, peroneal compound motor action potential; PMNCV, peroneal motor nerve conduction velocity; SNAP, sural nerve action potential; SNCV, sural nerve conduction velocity; T1DM, type 1 diabetes mellitus; TCMAP, tibial compound motor action potential; TMNCV, tibial motor nerve conduction velocity; VPT, vibration perception threshold; WPT, warm perception threshold.

adjusting for confounding factors including age, gender, duration of diabetes, HbA1c, cholesterol, triglyceride, and BMI, multiple linear regression analysis showed that only TCMAP ($\beta = -1.12$, $P < 0.0001$) and TMNCV ($\beta = -0.10$, $P < 0.01$) were independently associated with SBP (Table 3).

In the control group, simple linear regression analysis showed that all nerve conduction parameters apart from PCMAP were associated with SBP. However, after adjusting for confounding factors, only SNAP ($\beta = -0.16$, $P = 0.01$) was independently associated with SBP.

DISCUSSION

This study shows that DPN is associated with hypertension and raised SBP in T1DM. It also shows that hypertension has varying effects on small and large fibers, providing an explanation as to why previous studies of BP lowering therapy have shown an improvement in some but not other measures of diabetic neuropathy. We show that hypertension worsens deficits in NCS and vibration perception in subjects with T1DM, indicating an abnormality of large nerve fibers, but is also associated with loss of corneal nerve fibers using corneal confocal microscopy. This is clinically relevant as small nerve fibers are the earliest to be damaged and underlie the pathogenesis of foot ulceration[37–39] and painful DPN.[40] However, after adjusting for baseline imbalances including age, gender, triglyceride, and BMI, only TCMAP and TMNCV were affected by hypertension. Similarly, after adjusting for confounding factors including age, gender, duration of diabetes, HbA1c, cholesterol, triglyceride, and BMI, multiple linear regression analysis showed that only TCMAP and TMNCV remained independently associated with SBP.

Given that there are no disease-modifying therapies for DPN, this encourages the need for clinical trials of BP lowering agents in DPN and provides direction for the end points which should be used in these trials. Both clinical

and experimental studies have shown that treatment with an angiotensin-converting enzyme inhibitor leads to an improvement in NCS,[19,21–23] but has no impact on symptoms, deficits, VPT, or autonomic function. Indeed, we show that hypertension does not influence neuropathic symptoms or thermal thresholds, and therefore may not change. Istenes et al.[41] reported an association between hypertension and cardiac autonomic neuropathy in T2DM, which is associated with silent myocardial ischemia, cardiac arrhythmias, and cardiorespiratory instability.[42,43] In a study of T1DM and T2DM patients with cardiac autonomic neuropathy, 12 months of treatment with quinapril, losartan, or a combination of both showed an improvement in cardiac

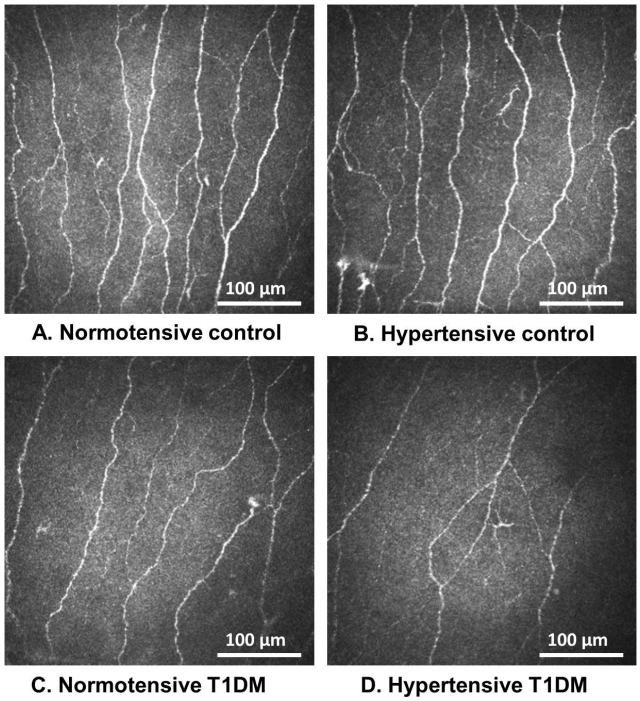

Figure 1. Corneal confocal microscopy (CCM) images of the subbasal nerve plexus in a normotensive control (**a**), hypertensive control (**b**) showing normal corneal nerve morphology and a normotensive T1DM patient (**c**), and hypertensive T1DM patient (**d**) showing a reduction in corneal nerve fiber density, branch density, and length. Abbreviation: TIDM, type 1 diabetes mellitus.

Table 3. Multiple linear regression analysis showing the association between measures of neuropathy and systolic blood pressure in subjects with T1DM after adjusting for confounding factors

	Coefficient	95% confidence interval	*P* value
Corneal nerve morphology			
CNFD	−0.09	−0.20 to 0.02	NS
CNFL	−0.08	−0.16 to 0.003	NS
Cardiac autonomic neuropathy			
HRV	−0.02	−0.15 to 0.11	NS
Quantitative sensory testing (QST)			
VPT	0.08	−0.03 to 0.19	NS
Nerve conduction (NC)			
SNAP	−0.05	−0.13 to 0.03	NS
SNCV	−0.1	−0.21 to 0.02	NS
TCMAP	−0.12	−1.17 to −0.07	<0.0001
TMNCV	−0.10	−0.16 to −0.03	<0.01
PCMAP	−0.01	−0.04 to 0.01	NS
PMNCV	0.003	−0.08 to 0.08	NS

Variables affecting diabetic neuropathy were considered in the fitted model with a *P* value ≤0.05. Abbreviations: CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; HRV, heart rate variability; PCMAP, peroneal compound motor action potential; PMNCV, peroneal motor nerve conduction velocity; SNAP, sural sensory nerve action potential; SNCV, sural nerve conduction velocity; TCMAP, tibial compound motor action potential; TMNCV, tibial motor nerve conduction velocity; VPT, vibration perception threshold.

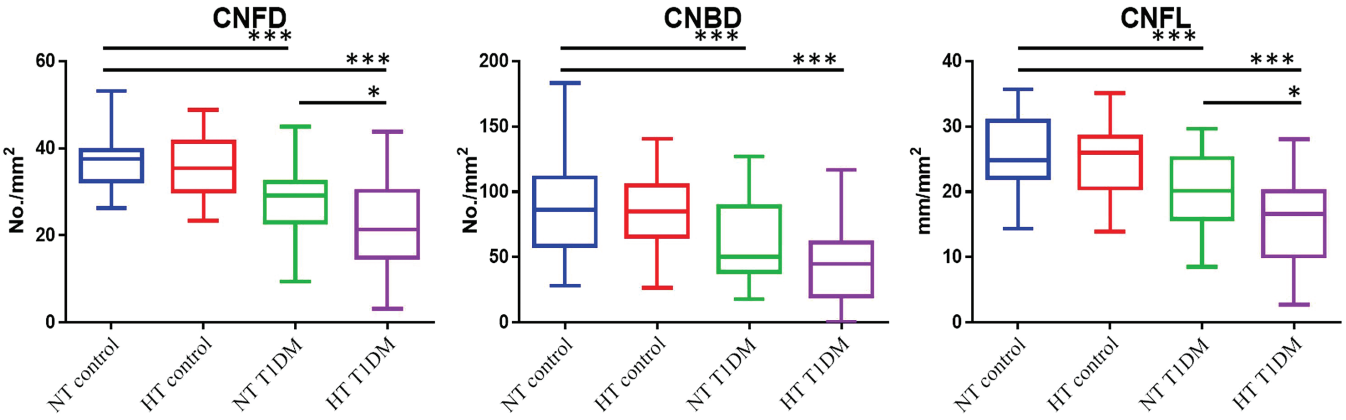

Figure 2. Corneal nerve morphology in normotensive controls (blue), hypertensive controls (red), normotensive T1DM participants (green), and hypertensive T1DM participants (purple). Box plots of corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD), and corneal nerve fiber length (CNFL). The line in the middle of the boxes represents the median and the boxes extend from the 25th to 75th percentiles. The whiskers extend from the highest to the lowest value. Significant differences between the groups were expressed as $*P \leq 0.01$ and $***P < 0.0001$. Abbreviation: TIDM, type 1 diabetes mellitus, NT, normotensive, HT, hypertensive.

autonomic neuropathy.[44] However, in this study we show a limited association between deep breathing heart rate variability (DB-HRV) and SBP, which was lost after adjusting for age, gender, duration of diabetes, triglycerides, and BMI. In addition, there was no effect of hypertension on sudomotor dysfunction.

Limitations of this study include the use of a single as opposed to cumulative burden of BP and glucose control on DPN and the relatively small numbers of subjects studied. We acknowledge that a cross-sectional study showing an association between hypertension and nerve conduction cannot imply cause and effect. However, a major strength of this study is the homogeneity of age, gender, and duration of diabetes as well as the detailed neuropathy assessments, which have enabled us to identify the exact associations between hypertension and specific measures of neuropathy. It provides an explanation as to why some studies assessing the effect of BP treatment have been positive, whereas others have been negative, depending on the measures chosen to assess DPN.

This study shows that hypertension is associated with nerve conduction abnormalities in T1DM but has no impact in subjects without diabetes. It also shows that the detrimental impact of T1DM on DPN may be mediated by hypertension on the myelinated fibers and by a number of metabolic risk factors including hyperglycemia, high triglycerides and obesity affecting the small fibers. These data suggest that nerve conduction studies should be used as the primary end points in clinical trials assessing the benefits of BP lowering therapy on diabetic neuropathy.

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DISCLOSURE

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship and are not listed. We confirm that the order of authors listed in the manuscript has been approved by all authors. None of the authors have received or anticipate receiving income, goods, or benefit from a company that will influence the design, conduct, or reporting of the study.

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