Painful diabetic neuropathy is associated with increased nerve regeneration in patients with type 2 diabetes undergoing intensive glycemic control

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ABSTRACT
Aims/Introduction: Painful diabetic peripheral neuropathy (pDPN) is associated with small nerve fiber degeneration and regeneration. This study investigated whether the presence of pDPN might influence nerve regeneration in patients with type 2 diabetes undergoing intensive glycemic control.

Materials and Methods: This exploratory substudy of an open-label randomized controlled trial undertook the Douleur Neuropathique en 4 questionnaire and assessment of electrochemical skin conductance, vibration perception threshold and corneal nerve morphology using corneal confocal microscopy in participants with and without pDPN treated with exenatide and pioglitazone or basal–bolus insulin at baseline and 1-year follow up, and 18 controls at baseline only.

Results: Participants with type 2 diabetes, with (n = 13) and without (n = 28) pDPN had comparable corneal nerve fiber measures, electrochemical skin conductance and vibration perception threshold at baseline, and pDPN was not associated with the severity of DPN. There was a significant glycated hemoglobin reduction (P < 0.0001) and weight gain (P < 0.005), irrespective of therapy. Participants with pDPN showed a significant increase in corneal nerve fiber density (P < 0.05), length (P < 0.0001) and branch density (P < 0.0005), and a decrease in the Douleur Neuropathique en 4 score (P < 0.01), but no change in electrochemical skin conductance or vibration perception threshold. Participants without pDPN showed a significant increase in corneal nerve branch density (P < 0.01) and no change in any other neuropathy measures. A change in the severity of painful symptoms was not associated with corneal nerve regeneration and medication for pain.

Conclusions: This study showed that intensive glycemic control is associated with greater corneal nerve regeneration and an improvement in the severity of pain in patients with painful diabetic neuropathy.

INTRODUCTION
In patients with type 1 diabetes, 6.5 years of intensive glycemic control reduced the incidence of diabetic peripheral neuropathy (DPN) by 60%, prevented peroneal nerve conduction velocity...
slowing1 and continued to benefit patients 8 years after completion of the DCCT2. However, in patients with type 2 diabetes, the United Kingdom Prospective Diabetes Study (UKPDS)3 and Veterans Affairs Co-operative Study in Type 2 Diabetes Mellitus (VA-CSDM) trial4 reported no impact on the incidence of DPN, and the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial showed no effect on vibration perception over a period of 6 years5. Furthermore, multiple phase III clinical trials failed to show an improvement in diabetic neuropathy, and there are currently no US Food and Drug Administration approved therapies for DPN. It is unclear whether this failure is a consequence of inadequate translation of experimental therapies, inadequate end-points or the enrollment of patients with a widely varying severity of DPN7.

The prevalence of painful diabetic peripheral neuropathy (pDPN) and DPN increases with age and duration of diabetes8. Hyperglycemia, hyperlipidemia and hypertension are associated with DPN; whereas obesity, physical inactivity and smoking cigarettes are associated with pDPN8,10. Neuropathic pain might be present at any stage of DPN11, and has been linked to a complex interplay between ongoing small nerve fiber degeneration and regeneration12,13. Indeed, skin biopsy studies have shown comparable intra-epidermal nerve fiber density in patients with and without painful neuropathy14 and painful diabetic neuropathy15,16. However, more detailed immunohistological studies have shown an increase in regenerating intra-epidermal17,18 and dermal nerve fibers containing substance P and calcitonin gene-related peptide in patients with painful compared with painless diabetic neuropathy19. Recently, Bönhof et al.20 showed comparable intra-epidermal nerve fiber density, but increased growth-associated protein–43 staining indicative of regenerating dermal nerves in patients with painful diabetic neuropathy. We also utilized corneal confocal microscopy (CCM) to show significantly greater corneal sub-basal nerve plexus degeneration in patients with painful compared with painless diabetic neuropathy15,21,22. These studies suggest that patients with painful diabetic neuropathy might have greater small fiber degeneration, but also an increased capacity for nerve regeneration.

CCM has been used to identify early small fiber regeneration in several clinical trials23. Indeed, early corneal nerve regeneration occurred 6 months24 after pancreas and kidney transplantation, and was followed by an improvement in nerve conduction and neuropathic symptoms after 24 months24,25. We also recently reported that enexatide and pioglitazone or basal–bolus insulin effectively reduce glycated hemoglobin (HbA1c)26 and induce corneal nerve regeneration27.

The present substudy of the Qatar study26 assessed whether the presence of pDPN might influence nerve regeneration in patients with type 2 diabetes undergoing intensive glycemic control with exenatide and pioglitazone or insulin.

MATERIALS AND METHODS
This was an exploratory substudy of an open-label, randomized controlled trial (clinicaltrials.gov ID: NCT02887625)26, that examined the efficacy of exenatide and pioglitazone versus basal–bolus insulin in patients with poorly controlled type 2 diabetes. This substudy has not been registered in a public clinical trial database. Participants with type 2 diabetes were enrolled from the National Diabetes Center in Hamad General Hospital, Doha, Qatar, and studied at baseline and 1-year follow-up, and control participants without diabetes were enrolled from Rumalithe Hospital, Doha, Qatar, and studied at baseline only from October 2016 to November 2018.

The present study received ethical approval from the Hamad Medical Corporation IRB (IRB# 13-00076), and all participants consented to participate in the study. The study followed the tenets of the declaration of Helsinki.

Study cohort
Individuals aged 18–75 years with HbA1c >7.5% (>58 mmol/mol) on near maximum dose of metformin (>1,500 mg/day) and sulfonylurea (>3 mg glimepiride or >60 mg gliclazide); with normal liver and kidney function, and electrocardiogram; and stable bodyweight (±1 kg) in the past year were recruited. The exclusion criteria are described in detail in our previous report27, but included any cause of neuropathy apart from diabetes, corneal dystrophies, corneal surgery or trauma in the past year, antidiabetic treatment other than metformin and sulfonylureas, diabetic proliferative retinopathy, and abnormally high albumin excretion.

Interventions
Participants were randomized to receive exenatide and pioglitazone or glargine and aspart insulin treatment to achieve and maintain an HbA1c <7.0% (<53 mmol/mol)27.

Demographic and metabolic measures
Age, sex, diabetes duration, bodyweight, body mass index (BMI), blood pressure, HbA1c, total cholesterol, triglyceride, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were recorded from the electronic health record.

DPN assessment
pDPN was defined on a Douleur Neuropathique en 4 (DN4) questionnaire score ≥4, as previously described28. The DN4 questionnaire has been validated for distinguishing neuropathic pain from non-neuropathic pain29 in Arabic30 and for pDPN28. It consists of 10 questions relating to symptoms and signs, and each question is equally weighted. A score ≥4 has 80% sensitivity and 92% specificity for pDPN28. The questionnaire was administered by the investigator in English or Arabic. Medications for pDPN were recorded.

CCM was carried out using the HRT-3-RCM device (Heidelberg Engineering GmbH, Heidelberg, Germany), as described in our previously published protocol31. Corneal nerve fiber density (CNFD; fibers/mm²), length (CNFL) (mm/mm²) and branch density (CNBD) (branches/mm²) were quantified manually using CCMetrics32.
Sudomotor function was measured by electrochemical skin conductance (ESC) using Sudoscan (Impeto Medical SAS, Paris, France), as described previously. Sudoscan evaluates sympathetic innervation based on sweat chloride concentrations generated by the sweat gland in response to the voltage applied, and is reported as ESC in microSiemens (µS).

Vibration perception threshold (VPT) was measured using a Neurothesiometer (Horwell Scientific Laboratory Supplies, London, UK) on the pulp of the large toe on both feet, and the average value of three measurements was recorded as a VPT in Volts (V) ranging from 0 to 50 V.

**Statistical analysis**

Continuous variables between controls, participants with type 2 diabetes, with and without pDPN were compared using one-way ANOVA. Continuous variables were compared between controls and participants with type 2 diabetes with and without painful diabetic peripheral neuropathy were compared using one-way ANOVA, and significant differences between them are denoted as $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$. BMI, body mass index; BP, blood pressure; DN4, Douleur Neuropathique en 4; CNFD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; ESC, electrochemical skin conductance; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VPT, vibration perception threshold. Bold values are indicates statistically significant.

**RESULTS**

**Difference between participants with and without pDPN and healthy controls**

A total of 41 participants with type 2 diabetes, with $(n = 13)$ and without $(n = 28)$ pDPN, and 18 control participants were studied (Table 1). The proportion of those treated with basal–bolus insulin $(P = 0.84)$ or a combination of exenatide and pioglitazone $(P = 0.84)$ were comparable between the two groups. Three out of 13 participants with pDPN (23%) were taking medication to

**Table 1 | Comparison of baseline characteristics between patients with type 2 diabetes with and without painful diabetic peripheral neuropathy and healthy controls**

|                      | Controls $(n = 18)$ | Patients without painful diabetic neuropathy $(n = 28)$ | Patients with painful diabetic neuropathy $(n = 13)$ | $P$-value |
|----------------------|--------------------|--------------------------------------------------------|----------------------------------------------------|-----------|
| Age (years)          | 53.0 ± 11.0        | 50.7 ± 9.4                                             | 57.6 ± 5.1                                          | <0.01     |
| Diabetes duration (years) | 12.0 ± 8.0     | 9.3 ± 6.3                                             | 9.3 ± 6.3                                           | 0.27      |
| Male, n (%)          | 16/28 (69.6)       | 7/12 (30.4)                                           | 7/12 (30.4)                                         | 0.94      |
| Basal–bolus insulin, n (%) | 12/28(42.9) | 6/13(46.2)                                           | 6/13(46.2)                                          | 0.84      |
| Exenatide plus pioglitazone, n (%) | 16/28(57.1) | 7/13(53.8)                                           | 7/13(53.8)                                         |           |
| Physical activity    | 11/27 (49.7)       | 1/12(8.3)                                             | 1/12(8.3)                                          | <0.05     |
| HbA1c (mmol/mol)     | 9.01 ± 21.1        | 8.70 ± 20.7                                           | 8.70 ± 20.7                                         | 0.66      |
| HbA1c (%)            | 10.4 ± 1.9         | 10.1 ± 1.9                                            | 10.1 ± 1.9                                         |           |
| Total cholesterol (mmol/L) | 4.9 ± 0.9   | 4.9 ± 1.2                                             | 4.9 ± 1.2                                          | 1.00      |
| Triglyceride (mmol/L) | 1.9 ± 1.2          | 2.1 ± 1.1                                             | 2.1 ± 1.1                                          | 0.72      |
| HDL (mmol/L)         | 1.2 ± 0.5          | 1.1 ± 0.3                                             | 1.1 ± 0.3                                          | 0.28      |
| LDL (mmol/L)         | 2.9 ± 0.9          | 2.6 ± 0.7                                             | 2.6 ± 0.7                                          | 0.45      |
| Systolic BP (mmHg)   | 129.1 ± 15.9       | 127.5 ± 25.4                                          | 127.5 ± 25.4                                       | 0.83      |
| Diastolic BP (mmHg)  | 78.1 ± 11.4        | 77.6 ± 14.2                                           | 77.6 ± 14.2                                        | 0.91      |
| Bodyweight (kg)      | 85.0 ± 13.4        | 89.5 ± 22.2                                           | 89.5 ± 22.2                                        | 0.51      |
| BMI (kg/m²)          | 29.9 ± 4.7         | 33.7 ± 7.6                                            | 33.7 ± 7.6                                         | 0.12      |
| DN4 score            | 0 ± 0              | 1.1 ± 1.0***                                          | 5.5 ± 1.4***                                       | <0.0001   |
| CNFD (fibers/mm²)    | 33.7 ± 5.7         | 27.4 ± 8.0†                                           | 26.0 ± 8.7†                                        | 0.64      |
| CNBD (branches/mm²)  | 1104 ± 45.0        | 673 ± 32.1†                                           | 540 ± 27.6†                                        | 0.20      |
| CNFL (mm/mm²)        | 25.1 ± 4.3         | 18.8 ± 48***                                          | 174 ± 56***                                        | 0.46      |
| VPT (V)              | 7.2 ± 4.1          | 7.8 ± 4.4†                                           | 14.1 ± 8.0†                                        | 0.07      |
| ESC (µS)             | 66.9 ± 18.4        | 66.5 ± 17.4                                           | 64.2 ± 24.1                                        | 0.79      |

Numeric variables and frequency distribution for categorical variables are summarized as the mean ± standard deviation or n (%), and were compared between patients with and without painful diabetic peripheral neuropathy using the unpaired t-test and $\chi^2$-test, respectively. Variables between controls and patients with type 2 diabetes with and without painful diabetic peripheral neuropathy were compared using one-way ANOVA, and significant differences between them are denoted as $^{t}P \leq 0.05$, $^{t}P \leq 0.01$, $^{t+}P \leq 0.001$, $^{t++}P \leq 0.0001$. BMI, body mass index; BP, blood pressure; DN4, Douleur Neuropathique en 4; CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; ESC, electrochemical skin conductance; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VPT, vibration perception threshold. Bold values are indicates statistically significant.
relieve pain. Participants with type 2 diabetes were age-matched with control participants. Participants with pDPN were older (P < 0.01), had a higher DN4 score (P < 0.0001) and a lower percentage undertook physical activity (P < 0.05) compared with participants without pDPN. Sex (P = 0.94), duration of diabetes (P = 0.27), HbA1c (P = 0.66), total cholesterol (P = 1.00), triglyceride (P = 0.72), HDL (P = 0.28), LDL (P = 0.45), systolic blood pressure (P = 0.83), diastolic blood pressure (P = 0.91), bodyweight (P = 0.51) and BMI (P = 0.12) were comparable between participants with and without pDPN.

Participants with type 2 diabetes had a significantly higher DN4 score (P < 0.001) and VPT (P < 0.01), and lower corneal nerve fiber measures (P < 0.05), but comparable ESC compared with healthy controls. Corneal nerve fiber measures, ESC and VPT were comparable between participants with and without pDPN.

**Table 2 | Baseline and 1-year follow-up clinical and neuropathy measures of patients with type 2 diabetes with and without painful diabetic neuropathy**

|                  | Patients without painful diabetic neuropathy (n = 28) | P-value     | Patients with painful diabetic neuropathy (n = 13) | P-value     |
|------------------|-----------------------------------------------------|-------------|---------------------------------------------------|-------------|
| **Baseline**     |                                                     |             |                                                   |             |
| HbA1c (mmol/mol) | 90.1 ± 21.1                                         | <0.0001     | 87.0 ± 20.7                                       | <0.0001     |
| HbA1c (%)        | 10.4 ± 1.9                                          | <0.0001     | 10.1 ± 1.9                                        | <0.0001     |
| Total cholesterol (mmol/L) | 4.9 ± 0.9                                   | <0.01       | 4.9 ± 1.2                                         | 0.06        |
| Triglyceride (mmol/L) | 1.9 ± 1.2                                      | <0.01       | 2.1 ± 1.1                                         | 0.26        |
| HDL (mmol/L)     | 1.2 ± 0.5                                          | 0.30        | 1.1 ± 0.3                                         | 0.72        |
| LDL (mmol/L)     | 2.8 ± 0.9                                          | <0.01       | 2.6 ± 0.7                                         | 0.19        |
| Systolic BP (mmHg) | 129 ± 15.9                                     | 0.12        | 127.5 ± 25.4                                      | 0.16        |
| Diastolic BP (mmHg) | 78.1 ± 11.4                                     | <0.0001     | 77.6 ± 14.2                                       | 0.39        |
| Bodyweight (kg)  | 85.0 ± 13.4                                        | <0.0001     | 89.5 ± 22.2                                       | <0.01       |
| BMI (kg/m²)      | 29.9 ± 4.7                                         | 0.19        | 33.7 ± 7.6                                        | 0.18        |
| DN4 score        | 1.1 ± 1.0                                          | 0.66        | 5.5 ± 1.4                                         | <0.01       |
| CNFD (fibers/mm²) | 27.4 ± 8.0                                        | 0.91        | 26.6 ± 8.7                                        | <0.05       |
| CNBD (branches/mm²) | 67.3 ± 32.1                                     | <0.01       | 54.0 ± 27.6                                       | <0.01       |
| CNFL (mm²/mm²)   | 188 ± 4.8                                          | 0.13        | 17.4 ± 5.6                                        | <0.0001     |
| VPT (µV)         | 78 ± 4.8                                           | 0.77        | 14.1 ± 8.0                                        | 0.32        |
| ESC (µS)         | 666 ± 17.4                                         | 0.54        | 642 ± 24.1                                        | 0.96        |

Numeric variables are summarized as the mean ± standard deviation. Variables were compared using the paired t-test. BMI, body mass index; BP, blood pressure; DN4, Douleur Neuropathique en 4; CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; ESC, electrochemical skin conductance; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VPT, vibration perception threshold. Bold values are indicates statistically significant.
Association of change in CCM measures and painful symptoms with clinical characteristics

The change in CCM measures was not associated with the type of treatment ($P = 0.47$) and decrease in HbA1c ($P = 0.61$). However, it was negatively associated with bodyweight gain, with every 2-kg increase in bodyweight, CNFD decreased by 1 fiber/mm (95% confidence interval $-1.0$–$-0.0$), but this association just missed statistical significance ($P = 0.0501$).

The change in DN4 score had no association with medications for neuropathic pain at baseline ($P = 0.21$), decrease in HbA1c ($P = 0.81$) or gain in bodyweight ($P = 0.67$).

There was no association between the change in DN4 score and change in CNFD ($P = 0.93$), CNBD ($P = 0.25$) or CNFL ($P = 0.28$).

DISCUSSION

The present study shows that treatment of patients with type 2 diabetes and poor glycemic control with exenatide and pioglitazone or basal–bolus insulin markedly improves glycemic control, and is associated with an improvement in painful diabetic neuropathy and corneal nerve regeneration.

Painful symptoms in DPN have been associated with active nerve degeneration and regeneration$^{12}$. Indeed, although there are no differences in intra-epidermal nerve fiber density between those with and without pDPN$^{34,35}$, there was a significantly lower CNFL in patients with pDPN compared with those with painless DPN$^{15,21}$; and in another study, CNFD was significantly lower in patients with pDPN$^{22}$. Quantitative sensory testing has also shown increased thermal thresholds in patients with pDPN compared with those with painless DPN$^{36,37}$. More recently, we showed lower intra-epidermal nerve fiber density and corneal nerve fibers in a large group of patients with painful compared with painless diabetic neuropathy$^{38}$. In this study, CCM measures, sudomotor function and vibration perception threshold were comparable between patients with and without pDPN, although the number of patients with painful diabetic neuropathy was much smaller than in previous studies$^{15,21,22,38}$.

A large improvement in HbA1c (>2–3%) has been reported to be associated with treatment-induced neuropathic pain and autonomic neuropathy$^{39}$. However, the present study showed that despite a mean reduction in HbA1c of 3.4% among those without pDPN, and 2.5% among those with pDPN, there was no increase in the DN4 score, consistent with our previous findings$^{27}$. Furthermore, of the 27 patients without pDPN, only one developed pDPN after 1 year of intensive glycemic control.
There is a need for better neuropathy phenotyping to enable trial enrichment of participants who are more likely to respond to therapies, whether to reduce the severity of pain with therapies targeting pain or nerve regeneration in clinical trials of disease-modifying therapies for DPN. Thus, there has been a resurgence of interest in identifying biomarkers of specific pain mechanisms that might allow more effective targeted use of existing therapies. Quantitative sensory testing has been used in a phenotype-stratified randomized, double-blind, placebo-controlled study to show that oxcarbazepine had a significantly greater effect in patients with an irritable nociceptor phenotype. Similarly, the conditioned pain modulation test has been used to identify altered descending spinal pathways to predict greater efficacy of duloxetine. We also showed altered rate-dependent depression of the H-reflex, indicative of abnormal descending inhibitory pathways in patients with pDPN. However, a deep phenotyping approach to identify outcomes of disease-modifying therapies has not been undertaken to date. Indeed, despite multiple trials of disease-modifying therapies, there are currently no US Food and Drug Administration-approved therapies for DPN. Several studies showed that subclinical small nerve fiber injury precedes large fiber damage in DPN. Furthermore, early small fiber repair has been shown in several small clinical intervention trials, and after pancreas and kidney transplantation, normalization of glycemia was associated with corneal nerve regeneration after 6 months, followed by an improvement in neuropathic symptoms and nerve conduction after 24 months. More recently, we showed that both exenatide and pioglitazone or basal–bolus insulin effectively reduce HbA1c and induce corneal nerve regeneration, independent of changes in HbA1c, bodyweight and lipids. Preclinical studies have reported that glucagon-like peptide-1 receptor agonists have a neuroprotective effect and suppresses pain hypersensitivity in diabetes, and although earlier clinical trials showed no benefit, we recently showed corneal nerve regeneration with exenatide. Thiazolidinediones have been reported to alleviate neuropathic pain by attenuating proinflammatory cytokine expression, and preclinical studies show a prevention of nerve conduction slowing. Indeed, the Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) trial reported that rosiglitazone significantly reduced the 4-year cumulative incidence of clinical DPN compared with insulin. Insulin treatment has also been shown to have a neurotrophic effect and reduce tactile allodynia, and intensive insulin treatment might prevent nerve conduction slowing and loss of ankle reflexes.

We acknowledge this is a small exploratory study with potential confounders in relation to the small cohort size and effect of different treatments. Nevertheless, we showed that patients with pDPN have optimal nerve regeneration in
Variables were compared using unpaired t-test. BMI, body mass index; BP, blood pressure; DN4, Douleur Neuropathique en 4; CNBD, corneal nerve branch density; CNFL, corneal nerve fiber density; CNFD, corneal nerve fiber density; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

**Table 3** Comparison of changes in clinical and neuropathy measures over a 1-year period between patients with and without painful diabetic neuropathy

| Variables                        | Patients with painful diabetic neuropathy | Patients with painful diabetic neuropathy | P-value |
|----------------------------------|------------------------------------------|------------------------------------------|---------|
| ΔHbA1c (mmol/mol)                | −36.6 ± 16.2                             | −26.8 ± 20.2                             | 0.14    |
| ΔHbA1c (%)                       | −3.4 ± 1.5                               | −2.5 ± 1.9                               | 0.14    |
| ΔTotal cholesterol (mmol/L)      | −0.6 ± 0.9                               | −0.7 ± 1.2                               | 0.79    |
| ΔTriglyceride (mmol/L)           | −0.4 ± 0.8                               | −0.3 ± 0.9                               | 0.72    |
| ΔHDL (mmol/L)                    | −0.1 ± 0.4                               | 0.3 ± 0.3                                | 0.59    |
| ΔLDL (mmol/L)                    | −0.4 ± 0.8                               | −0.3 ± 0.8                               | 0.87    |
| ΔSystolic BP (mmHg)              | −49 ± 16.1                               | 7.7 ± 18.7                               | <0.05   |
| ΔDiastolic BP (mmHg)             | −76 ± 9.6                                | −3.2 ± 13.0                              | 0.30    |
| ΔBodyweight (kg)                 | 4.0 ± 4.7                                | 6.1 ± 6.3                                | 0.28    |
| ΔBMI (kg/m²)                     | 0.1 ± 1.7                                | 0.8 ± 1.9                                | 0.24    |
| ΔDN4 score                       | 0.1 ± 1.1                                | −0.6 ± 2.7                               | 0.40    |
| ΔCNFD (fibers/mm²)               | 0.2 ± 8.0                                | 5.5 ± 7.4                                | 0.06    |
| ΔCNBD (branches/mm²)             | 14.5 ± 25.4                              | 48.2 ± 46.0                              | <0.05   |
| ΔCNFL (mm/mm²)                   | 1.2 ± 3.9                                | 6.1 ± 3.8                                | 0.001   |
| ΔVPT (V)                         | −0.2 ± 3.8                               | −1.1 ± 2.8                               | 0.53    |
| ΔESC feet (µS)                   | −20 ± 14.1                               | −0.2 ± 14.4                              | 0.77    |

Numeric variables are summarized as the mean ± standard deviation. Variables were compared using unpaired t-test. BMI, body mass index; BP, blood pressure; DN4, Douleur Neuropathique en 4; CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; ESC, electrochemical skin conductance; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VPT, vibration perception threshold. Bold values are indicated statistically significant.

response to improved glycemic control. We also showed that nerve regeneration might be limited due to weight gain, and, of course, recently we showed that weight loss with bariatric surgery is associated with corneal nerve regeneration. Disease-modifying treatments are also more likely to be of benefit in early or mild neuropathy where there is predominantly small fiber damage. These findings highlight the complex pathogenesis and risk factors determining outcomes in clinical trials of diabetic neuropathy and argue strongly for pre-trial enrichment of participants.

We conclude that pDPN is associated with greater corneal nerve regeneration and improvement in painful neuropathic symptoms in patients with type 2 diabetes after intensive glycemic control. The underlying mechanism is not clear and merits further study.

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