




# Progressive loss of corneal nerve fibers is associated with physical inactivity and glucose lowering medication associated with weight gain in type 2 diabetes

Georgios Ponirakis<sup>1</sup> , Ibrahim Al-Janahi<sup>2</sup>, Einas Elgassim<sup>1</sup>, Hoda Gad<sup>1</sup>, Ioannis N Petropoulos<sup>1</sup>, Adnan Khan<sup>1</sup> , Hamda Ali<sup>2</sup>, Mashhood A Siddique<sup>2</sup>, Wajiha Gul<sup>2</sup>, Maryam Ferdousi<sup>3</sup>, Alise Kalteniece<sup>3</sup>, Fatima FS Mohamed<sup>1</sup>, Lina HM Ahmed<sup>1</sup>, Youssra Dakroury<sup>1</sup>, Abeer MM El Shewehy<sup>1</sup>, Abdulrahman Al-Mohamedi<sup>1</sup>, Fatema AlMarri<sup>1</sup>, Moayad Homssi<sup>1</sup>, Murtaza Qazi<sup>1</sup>, Nebras H Hadid<sup>1</sup>, Fatima Al-Khayat<sup>1</sup>, Ziyad R Mahfoud<sup>1</sup>, Shazli Azmi<sup>3,4</sup>, Uazman Alam<sup>5</sup>, Mahmoud A Zirie<sup>2</sup>, Yousuf Al-Ansari<sup>2</sup>, Amin Jajyousi<sup>2</sup>, Alan S Rigby<sup>4,6</sup>, Eric S Kilpatrick<sup>3,4,6</sup>, Stephen L Atkin<sup>7</sup>, Rayaz A Malik<sup>1,3,8\*</sup> 

<sup>1</sup>Department of Medicine, Weill Cornell Medicine-Qatar, Qatar Foundation, Doha, Qatar, <sup>2</sup>National Diabetes Center, Hamad General Hospital, Hamad Medical Corporation, Doha, Qatar, <sup>3</sup>Division of Cardiovascular Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK, <sup>4</sup>Centre for Diabetes, Endocrinology and Metabolism, Manchester University NHS Foundation Trust, Manchester, UK, <sup>5</sup>Department of Cardiovascular & Metabolic Medicine and the Pain Research Institute, Institute of Life Course and Medical Sciences, Liverpool University Hospital NHS Foundation Trust, University of Liverpool, Liverpool, UK, <sup>6</sup>Hull York Medical School, University of Hull, Kingston Upon Hull, UK, <sup>7</sup>Royal College of Surgeons in Ireland Bahrain, Adliya, Bahrain, and <sup>8</sup>Faculty of Science and Engineering, Manchester Metropolitan University, Manchester, UK

## Keywords

Corneal confocal microscopy, Diabetic neuropathy, Physical inactivity

## \*Correspondence

Rayaz A Malik  
Tel.: +974 44928256  
Fax: +974 44928422  
E-mail address:  
ram2045@qatar-med.cornell.edu

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## ABSTRACT

**Aims/Introduction:** Limited studies have identified risk factors linked to the progression of diabetic peripheral neuropathy (DPN) in type 2 diabetes. This study examined the association of risk factors with change in neuropathy measures over 2 years.

**Materials and Methods:** Participants with type 2 diabetes ( $n = 78$ ) and controls ( $n = 26$ ) underwent assessment of clinical and metabolic parameters and neuropathy using corneal confocal microscopy (CCM), vibration perception threshold (VPT), and the DN4 questionnaire at baseline and 2 year follow-up.

**Results:** Participants with type 2 diabetes had a lower corneal nerve fiber density (CNFD), branch density (CNBD), and fiber length (CNFL) ( $P \leq 0.0001$ ) and a higher VPT ( $P \leq 0.01$ ) compared with controls. Over 2 years, despite a modest reduction in HbA1c ( $P \leq 0.001$ ), body weight ( $P \leq 0.05$ ), and LDL ( $P \leq 0.05$ ) the prevalence of DPN ( $P = 0.28$ ) and painful DPN ( $P = 0.21$ ) did not change, but there was a significant further reduction in CNBD ( $P \leq 0.0001$ ) and CNFL ( $P \leq 0.05$ ). CNFD, CNBD, and CNFL decreased significantly in physically inactive subjects ( $P < 0.05$ – $0.0001$ ), whilst there was no change in CNFD ( $P = 0.07$ ) or CNFL ( $P = 0.85$ ) in physically active subjects. Furthermore, there was no change in CNFD ( $P = 0.82$ ), CNBD ( $P = 0.08$ ), or CNFL ( $P = 0.66$ ) in patients treated with glucose lowering medication associated with weight loss, whilst CNBD ( $P = 0.001$ ) decreased in patients on glucose lowering medication associated with weight gain.

**Conclusions:** In participants with type 2 diabetes, despite a modest improvement in HbA1c, body weight, and LDL there was a progressive loss of corneal nerve fibers; except in those who were physically active or on glucose lowering medication associated with weight loss.

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## INTRODUCTION

Diabetic peripheral neuropathy (DPN) is associated with painful neuropathy, erectile dysfunction, and foot ulceration, imposing a significant burden on patient morbidity and mortality<sup>1</sup>. The pathogenesis of DPN is complex and multifactorial and there are currently no FDA approved modifying therapies for DPN.

Poor glycemic control is a major risk factor for DPN<sup>2,3</sup> and intensive glycemic control can prevent or delay DPN progression in type 1 diabetes<sup>4</sup> but not in type 2 diabetes<sup>5</sup>. Obesity and hyperlipidemia are also driving DPN in type 2 diabetes<sup>2,6–8</sup> and we have shown previously that obesity is associated with painful DPN and hyperlipidemia is associated with DPN, independent of poor glycemic control<sup>2</sup>. In a recent cross-sectional study, Ferdousi *et al.*<sup>9</sup> reported that age, HbA1c and body weight are associated independently with corneal nerve fiber loss in patients with type 2 diabetes. Corneal confocal microscopy (CCM) is able to detect corneal nerve degeneration<sup>10–12</sup> or regeneration<sup>13,14</sup> 6–12 months earlier compared with quantitative sensory testing and nerve conduction studies (NCS), which show changes 24–36 months<sup>14,15</sup> after intervention. Longitudinal studies have shown that weight loss<sup>16,17</sup> and physical activity or exercise<sup>15,18–21</sup> are associated with an improvement in neuropathic symptoms and small nerve fiber regeneration.

The objective of this prospective study was to assess whether physical activity, medication use, and change in HbA1c, BMI, lipid profile, or blood pressure was associated with a change in neuropathic symptoms, vibration perception, and corneal nerve morphology over 2 years.

## MATERIALS AND METHODS

In this prospective study, subjects with type 2 diabetes were enrolled from the National Diabetes Center in Hamad General Hospital in Qatar between January 2017 and December 2020 and control subjects without diabetes were enrolled from the University of Manchester in the UK between October 2016 and November 2019 and studied at baseline and after 2 years. This study obtained ethics approval by the WCM-Q IRB (#14-00058), HMC IRB (#15103/15), and NRES Committee North West – Greater Manchester North (#08/H1004/1). All participants consented before taking part in the study. The research adhered to the principles of the declaration of Helsinki.

### Study cohort

Subjects with type 2 diabetes eligible for the study were aged 18–70 years old and were not participating in any other interventional study. Healthy controls were aged 18–70 years old and had a HbA1c <42 mmol/mol (<6%).

Exclusion criteria were anemia (as a Hb <11 and <10 g/dL for males and females, respectively, can affect the validity of HbA1c measurement), renal failure (CKD Stage 4 and 5), medication leading to insulin resistance (e.g. corticosteroids), pregnancy, active retinopathy, any cause of neuropathy other than

diabetes, including Sjogren's syndrome, systemic lupus erythematosus, HIV, hepatitis B and C, inherited neuropathies, tumors, alcoholism, and factors that may affect the corneal nerves, including severe dry eyes and corneal dystrophies, injury, and surgery in the preceding 12 months as determined by physical examination and medical history.

### Clinical and metabolic measures

Age, sex, diabetes duration, body weight, BMI, systolic (SBP), and diastolic blood pressure (DBP), HbA1c, lipid profile were recorded from the electronic medical register (Cerner). Poor glycemic control was defined as HbA1c  $\geq 7\%$  based on the ADA standards of medical care<sup>22</sup>. Hypertension was defined as SBP  $\geq 140$  mmHg and/or the use of blood pressure medication based on the WHO/ISH Guidelines<sup>23</sup>. Hyperlipidemia was defined as a total cholesterol  $\geq 6.2$  mmol/L and/or triglyceride  $\geq 2.3$  mmol/L, or the use of a statin. Obesity was defined as BMI  $\geq 30$  kg/m<sup>2</sup> based on the WHO criteria<sup>24</sup>. Renal impairment was defined as creatinine  $\geq 110$   $\mu$ mol/L and/or eGFR  $\leq 60$  mL/min/L. Physical inactivity and activity were recorded using the Global Physical Activity Questionnaire version 2 (GPAQv2), defined as complete lack of physical activity and regular activity at work, travel to and from places and/or recreational activities, respectively, at baseline and 2 year follow-up visit. Glucose lowering medications were classified into two groups: those associated with weight gain (insulin, sulfonylureas, and thiazolidinediones) and weight loss (metformin, glucagon-like peptide-1 (GLP-1), and sodium-glucose cotransporter-2 (SGLT2) inhibitors).

### Diabetic neuropathy assessment

Corneal confocal microscopy (CCM) assessments were performed using the HRT-3 RCM device (Heidelberg Engineering GmbH). In brief, the preparation included topical corneal anesthesia using 0.4% oxybuprocaine hydrochloride (Bausch & Lomb UK Ltd, Kingston upon Thames, London, UK) and lubrication using 0.2% carbomer transparent gel (Blumont Healthcare Ltd). Participants were instructed to fixate on a target light during the assessment. Multiple images of the sub basal corneal nerves in the central cornea were captured from both eyes. Image selection and extraction were performed at a separate time by one investigator blinded to the diagnosis and medical history. Three high clarity images per eye were selected based on depth, focus, position, and contrast<sup>25</sup>. Corneal nerve fiber density (CNFD, fibers/mm<sup>2</sup>), length (CNFL, mm/mm<sup>2</sup>), and branch density (CNBD, branches/mm<sup>2</sup>) were manually measured using CCMetrics, a validated image analysis software<sup>26</sup>.

Vibration perception threshold (VPT) on the pulp of the large toe on both feet was measured three times using a Neurothesiometer (Horwell Scientific Laboratory Supplies). The mean VPT value was recorded. VPT is measured in units of volts (V) and ranges from 0 to 50 V.

Painful diabetic peripheral neuropathy (pDPN) was assessed using the DN4 questionnaire validated in English<sup>27</sup> and Arabic<sup>28</sup>. It distinguishes neuropathic from non-neuropathic pain. It consists of seven questions for painful symptoms, including burning, painful cold, electric shocks, tingling, pins and needles, numbness, and itching and three questions for abnormal signs, including hypoesthesia to touch and pin prick and allodynia to brushing in the painful area. Each question was scored with either 1 point for yes or 0 point for no. The score of each question is equally weighted.

Diabetic peripheral neuropathy was defined as one or more neuropathic symptoms using the DN4 questionnaire and a VPT  $\geq 15$  V<sup>29</sup>. Painful DPN (pDPN) was defined based on a DN4 questionnaire score of  $\geq 4$ , which has 80% sensitivity and 92% specificity for pDPN<sup>30</sup> and/or the use of medication for pDPN.

### Statistical analysis

This exploratory 2 year prospective study was not adjusted for multiple comparisons<sup>31</sup>. Baseline continuous and categorical variables between subjects with type 2 diabetes and controls were compared using an unpaired *t*-test and  $\chi^2$ , respectively. Changes in continuous and categorical variables between baseline and 2 year follow-up within the group were compared using a paired *t*-test and  $\chi^2$ , respectively.

Bivariate linear regression analysis was performed with CNFD, CNBD, CNFL, VPT, DN4 score,  $\Delta$ CNFD,  $\Delta$ CNBD,  $\Delta$ CNFL,  $\Delta$ VPT, and  $\Delta$ DN4 score as dependent variables, and poor glycemic control, obesity, hyperlipidemia, hypertension, physical inactivity, microalbuminuria at baseline or change in HbA1c, body weight, waist circumference, BMI, lipid profile, and blood pressure as independent variables. All dependent variables were normally distributed as assessed by Q-Q plots and histograms. Dependent variables that were significant on the bivariate level were included in the multiple linear regression analysis. The regression coefficient (95% CI) is presented. All statistical calculations were performed using IBM-SPSS version 26 (SPSS Inc, Armonk, NY, USA). A two-tailed *P* value of  $\leq 0.05$  was considered significant.

## RESULTS

### Demographic and clinical characteristics (Table 1)

Subjects with  $12.9 \pm 6.6$  years of type 2 diabetes ( $n = 78$ ) and controls ( $n = 26$ ) of comparable age ( $50.1 \pm 10.6$  years vs  $54.5 \pm 8.6$  years,  $P = 0.06$ ), and sex (female: 56% vs 42%,  $P = 0.21$ ) were studied. In the type 2 diabetes cohort, 92% had poor glycemic control, 65% had obesity, 58% were physically inactive at baseline and 2 year follow-up visit, 22% had hyperlipidemia, 21% had hypertension, 28% had microalbuminuria, and 5.1% had renal impairment. The prevalence of type 2 diabetes duration  $<10$ ,  $10$ – $19$ , and  $\geq 20$  years was 29, 45, and 26%, respectively. The prevalence of DPN and pDPN was 18% and 26%, respectively. The percentage of patients on glucose

lowering drugs associated with weight gain was 75% and with weight loss it was 25% at baseline and 2 year follow-up visit.

Compared with the controls, subjects with type 2 diabetes had comparable SBP and DBP ( $P = 0.22$ ), higher BMI ( $P \leq 0.0001$ ), HbA1c ( $P \leq 0.0001$ ), and triglycerides ( $P \leq 0.0001$ ), and lower total cholesterol, LDL, and HDL ( $P \leq 0.0001$ ). Subjects with type 2 diabetes had higher VPT ( $P \leq 0.01$ ) and lower corneal nerve fiber density (CNFD) ( $P \leq 0.0001$ ), length (CNFL) ( $P \leq 0.0001$ ), and branch density (CNBD) ( $P \leq 0.0001$ ) compared with the controls.

### Change in risk factors and neuropathy measures over 2 years (Table 1)

Subjects with type 2 diabetes showed a small but significant reduction in HbA1c (64.9 to 59.5 mmol/mol or 8.1 to 7.6%;  $P \leq 0.001$ ), body weight (84.4 to 82.9 kg;  $P \leq 0.05$ ) and LDL (2.3 to 2.0 mmol/L;  $P \leq 0.05$ ) and an increase in HDL (1.0 to 1.1 mmol/L;  $P \leq 0.05$ ) and SBP (112.7 to 129.8 mmHg;  $P \leq 0.01$ ) with no change in BMI, DBP, total cholesterol, or triglycerides. The percentage of patients on glucose lowering medication associated with weight gain or both weight gain and loss remained the same (75% vs 65%,  $P = 0.19$ ).

The prevalence of DPN (18 to 12%;  $P = 0.28$ ), pDPN (26 to 18%;  $P = 0.21$ ), or VPT (9.2 to 9.5 V;  $P = 0.57$ ) did not change, although there was a significant decrease in the DN4 score (2.6 to 1.8;  $P \leq 0.001$ ). There was no significant change in CNFD (27.0 to 26.1 fibers/mm<sup>2</sup>;  $P = 0.28$ ), but there was a significant reduction in CNBD (62.4 to 42.9 branches/mm<sup>2</sup>;  $P \leq 0.0001$ ) and CNFL (17.8 to 16.4 mm/mm<sup>2</sup>;  $P \leq 0.05$ ). In controls there was an increase in VPT (6.1 to 7.2 V;  $P \leq 0.005$ ), but no change in CNFD (37.0 to 36.3 fibers/mm<sup>2</sup>;  $P = 0.44$ ), CNBD (97.2 to 89.5 branches/mm<sup>2</sup>;  $P = 0.26$ ), or CNFL (26.6 to 25.0 mm/mm<sup>2</sup>;  $P = 0.20$ ).

### Associations of risk factors with baseline and follow-up changes in neuropathy measures (Table 2, Figure 1)

Bivariate analysis showed that poor glycemic control, obesity, hyperlipidemia, hypertension, microalbuminuria, and renal impairment were not associated with VPT, DN4 score, or CCM measures at baseline. Multiple linear regression analysis showed that the duration of diabetes ( $P < 0.01$ ) and physical inactivity ( $P < 0.05$ ) were associated with a deterioration of VPT ( $P < 0.05$ ). Age ( $P < 0.05$ ) and glucose lowering medication promoting weight gain ( $P < 0.01$ ) were associated with neuropathic symptoms and signs.

There was no association between  $\Delta$ HbA1c,  $\Delta$ body weight,  $\Delta$ waist circumference,  $\Delta$ BMI,  $\Delta$ lipid profile, or  $\Delta$ blood pressure with  $\Delta$ VPT,  $\Delta$ DN4 or  $\Delta$ CCM measures. The duration of diabetes was negatively associated with  $\Delta$ CNBD ( $P < 0.05$ ) and physical inactivity was negatively associated with  $\Delta$ CNFD ( $P < 0.01$ ) and  $\Delta$ CNFL ( $P < 0.01$ ). Glucose lowering medication associated with weight gain ( $P < 0.01$ ) was negatively associated with  $\Delta$ CNFL ( $P = 0.054$ ).

**Table 1** | Clinical, metabolic and neuropathy measures at baseline and their change over 2 years in patients with type 2 diabetes and control subjects

|                                | Baseline             |                             |          | Change over 2-years  |                             |           |
|--------------------------------|----------------------|-----------------------------|----------|----------------------|-----------------------------|-----------|
|                                | Controls<br>(n = 26) | Type 2 diabetes<br>(n = 78) | P value* | Controls<br>(n = 26) | Type 2 diabetes<br>(n = 78) | P value** |
| HbA1c, mmol/mol                | 38.9 ± 2.5           | 64.9 ± 9.7                  | ≤0.0001  | −3.0 <sup>†††</sup>  | −5.4 <sup>††</sup>          | 0.15      |
| Hb1Ac, %                       | 5.7 ± 0.2            | 8.1 ± 0.9                   | ≤0.0001  | −0.3 <sup>†††</sup>  | −0.5 <sup>††</sup>          | 0.15      |
| Total cholesterol, mmol/L      | 5.2 ± 0.7            | 4.2 ± 1.0                   | ≤0.0001  | −0.25                | −0.28                       | 0.86      |
| Triglyceride, mmol/L           | 1.6 ± 0.8            | 1.8 ± 1.1                   | ≤0.0001  | 0.02                 | −0.10                       | 0.48      |
| HDL, mmol/L                    | 1.4 ± 0.3            | 1.0 ± 0.3                   | ≤0.0001  | 0.01                 | 0.09 <sup>‡</sup>           | 0.10      |
| LDL, mmol/L                    | 3.1 ± 0.6            | 2.3 ± 0.8                   | ≤0.0001  | −0.32 <sup>†</sup>   | −0.31 <sup>‡</sup>          | 0.95      |
| Systolic BP, mmHg              | 128.1 ± 14.5         | 122.7 ± 14.0                | 0.22     | −4.7                 | 7.1 <sup>†</sup>            | ≤0.01     |
| Diastolic BP, mmHg             | 73.2 ± 7.8           | 74.3 ± 8.6                  | 0.22     | −1.3                 | 1.0                         | 0.41      |
| Body weight, kg                | 76.5 ± 13.5          | 84.4 ± 12.2                 | ≤0.05    | 1.7                  | −1.5 <sup>‡</sup>           | 0.23      |
| BMI, kg/m <sup>2</sup>         | 27.0 ± 5.0           | 31.5 ± 3.4                  | ≤0.0001  | 0.0                  | −0.6                        | 0.26      |
| CNFD, fibers/mm <sup>2</sup>   | 37.0 ± 5.6           | 27.0 ± 8.8                  | ≤0.0001  | −0.8                 | −0.9                        | 0.93      |
| CNBD, branches/mm <sup>2</sup> | 97.2 ± 30.0          | 62.4 ± 36.4                 | ≤0.0001  | −7.7                 | −19.5 <sup>†††</sup>        | 0.14      |
| CNFL, mm/mm <sup>2</sup>       | 26.6 ± 4.4           | 17.8 ± 6.2                  | ≤0.0001  | −1.6                 | −1.3 <sup>‡</sup>           | 0.85      |
| VPT, V                         | 6.1 ± 4.8            | 9.2 ± 5.9                   | ≤0.01    | 1.1 <sup>‡</sup>     | 0.3                         | 0.31      |
| DN4, score                     | –                    | 2.6 ± 2.5                   | N/A      | –                    | −0.8 <sup>††</sup>          | N/A       |

Numeric variables are summarized as mean ± standard deviation. Baseline data and changes during a 2 year period between controls and subjects with type 2 diabetes were compared using unpaired *t*-test. Changes between baseline and 2 year follow-up were compared using paired *t*-test:

<sup>‡</sup>*P* ≤ 0.05, <sup>†</sup>*P* ≤ 0.01, <sup>††</sup>*P* ≤ 0.001, <sup>†††</sup>*P* ≤ 0.0001. \**P* value for comparison of baseline data between controls and subjects with type 2 diabetes. \*\**P* value for comparison of changes during a 2 year period between controls and subjects with type 2 diabetes. BP, blood pressure; CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; VPT, vibration perception threshold.

**Table 2** | Associations of risk factors at baseline and change at follow-up with change in neuropathy measures

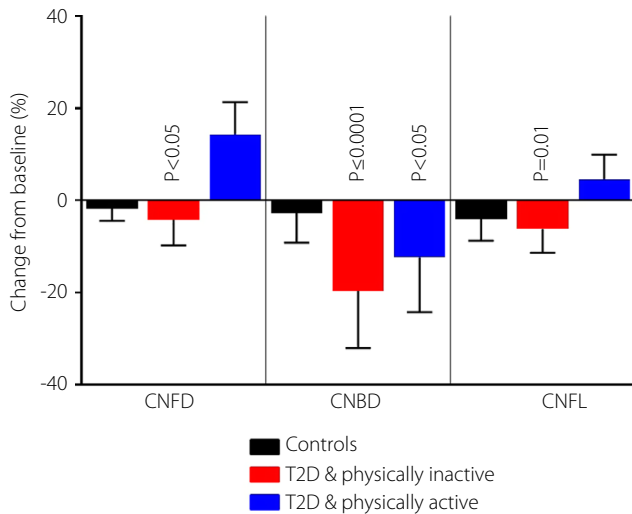
| Dependent variable                                | Independent variable                                    | Adjusted mean difference | 95% CI       | P value |
|---|---|--------------------------|--------------|---------|
| Associations with neuropathy measures at baseline |   |                          |              |         |
| CNFD, fibers/mm <sup>2</sup>                      | No associations   |                          |              | NS      |
| CNBD, branches/mm <sup>2</sup>                    | No associations   |                          |              | NS      |
| CNFL, mm/mm <sup>2</sup>                          | No associations   |                          |              | NS      |
| VPT, V  | Duration of diabetes <sup>†</sup>                       | 2.0                      | 0.07, 3.82   | <0.01   |
|   | Physical inactivity                                     | 2.8                      | 0.01, 5.6    | <0.05   |
| DN4, score  | Age <sup>†</sup>  | 0.9                      | 0.17, 1.67   | <0.05   |
|   | Glucose lowering medication associated with weight gain | 1.8                      | 0.47, 3.03   | <0.01   |
| Associations with changes in neuropathy measures  |   |                          |              |         |
| ΔCNFD, fibers/mm <sup>2</sup>                     | Physical inactivity                                     | −4.2                     | −7.23, −1.09 | <0.01   |
| ΔCNBD, branches/mm <sup>2</sup>                   | Duration of diabetes                                    | −11.3                    | −21.9, −0.6  | <0.05   |
| ΔCNFL, mm/mm <sup>2</sup>                         | Physical inactivity                                     | −3.2                     | −5.33, −1.05 | <0.01   |
|   | Glucose lowering medication associated with weight gain | −2.3                     | −4.68, 0.04  | 0.054   |
| ΔVPT, V   | No associations   |                          |              | NS      |
| ΔDN4, score                                       | pDPN  | −2.0                     | −2.8, −1.1   | ≤0.0001 |

All the variables considered in the fitted model had *P* ≤ 0.05. <sup>†</sup><10, 10–19, ≥20 years. CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; CNBD, corneal nerve branch density; VPT, vibration perception threshold; pDPN, painful diabetic peripheral neuropathy; Δ, differences.

### Change in CCM measures in relation to physical activity (Table 3)

In physically inactive subjects with type 2 diabetes, CNFD (26.9 to 24.1 fibers/mm<sup>2</sup>, *P* < 0.05), CNBD (61.1 to 38.9 branches/

mm<sup>2</sup>, *P* ≤ 0.0001), and CNFL (17.4 to 15.3 mm/mm<sup>2</sup>, *P* = 0.01) decreased significantly. Whereas in physically active subjects with type 2 diabetes there was no change in CNFD (27.1 to 29.0 fibers/mm<sup>2</sup>, *P* = 0.07), or CNFL (18.3 to



**Figure 1** | The effect of physical activity and inactivity on corneal nerve fiber measures over 2 years.

18.1 mm/mm<sup>2</sup>, *P* = 0.85) with a decrease only in CNBD (64.3 to 48.7 branches/mm<sup>2</sup>, *P* < 0.05). There was no change in CNFD (37.0 to 36.3 fibers/mm<sup>2</sup>, *P* = 0.44), CNBD (97.2 to 89.5 branches/mm<sup>2</sup>, *P* = 0.26), or CNFL (26.6 to 25.0 mm/mm<sup>2</sup>, *P* = 0.20) in controls. Diabetes duration was comparable

between physically inactive and physically active subjects (12.9 ± 6.7 vs 12.9 ± 6.6, *P* = 0.99).

**Change in CCM measures in relation to type of glucose lowering medication affecting weight (Table 3)**

In subjects with type 2 diabetes on glucose lowering medication associated with weight loss there was no change in CNFD (27.1 to 28.5 fibers/mm<sup>2</sup>, *P* = 0.82), CNBD (55.7 to 47.8 branches/mm<sup>2</sup>, *P* = 0.08), and CNFL (17.3 to 17.8 mm/mm<sup>2</sup>, *P* = 0.66). However, in subjects on glucose lowering medication associated with weight gain or a combination of medication associated with both weight gain and loss, CNBD (64.9 to 39.1 branches/mm<sup>2</sup>, *P* = 0.001) decreased significantly with no change in CNFD (27.1 to 25.2 fibers/mm<sup>2</sup>, *P* = 0.27) or CNFL (17.9 to 15.8, *P* = 0.12).

**DISCUSSION**

In this cohort of patients with type 2 diabetes, a modest improvement in HbA1c, body weight, and LDL cholesterol was not associated with a change in the prevalence of DPN, pDPN, or VPT. However, there was evidence of progressive corneal nerve loss in patients who were physically inactive or were being treated with anti-diabetic medications associated with weight gain.

Poor glycemic control is associated with DPN in type 2 diabetes<sup>2,3</sup>. We and others have shown that a higher HbA1c is

**Table 3** | Change in CCM measures in relation to physical activity and glucose lowering medication associated with weight gain or both weight gain and loss

|   | Baseline    | 2 year follow-up | Mean difference | Rate (%)      | <i>P</i> value |
|---|-------------|------------------|-----------------|---------------|----------------|
| <b>CNFD, fibers/mm<sup>2</sup></b>          |             |                  |                 |               |                |
| Controls                                    | 37.0 ± 5.6  | 36.3 ± 7.0       | -0.8 ± 5.0      | -1.8 ± 13.6   | 0.44           |
| Type 2 diabetes & physically active         | 27.1 ± 8.0  | 29.0 ± 7.3       | 1.9 ± 5.6       | 14.3 ± 39.9   | 0.07           |
| Type 2 diabetes & physically inactive       | 26.9 ± 9.5  | 24.1 ± 8.4       | -2.3 ± 7.3      | -2.9 ± 37.9↓  | <0.05          |
| <b>CNBD, branches/mm<sup>2</sup></b>        |             |                  |                 |               |                |
| Controls                                    | 97.2 ± 30.0 | 89.5 ± 28.0      | -7.7 ± 34.0     | -2.8 ± 32.7   | 0.26           |
| Type 2 diabetes & physically active         | 64.3 ± 31.5 | 48.7 ± 33.0      | -15.6 ± 36.6    | -12.4 ± 65.0↓ | <0.05          |
| Type 2 diabetes & physically inactive       | 61.1 ± 39.7 | 38.9 ± 30.9      | -18.3 ± 33.7    | -19.7 ± 81.3↓ | ≤0.0001        |
| <b>CNFL, fiber length mm/mm<sup>2</sup></b> |             |                  |                 |               |                |
| Controls                                    | 26.6 ± 4.4  | 25.0 ± 5.5       | -1.6 ± 6.1      | -4.0 ± 24.3   | 0.20           |
| Type 2 diabetes & physically active         | 18.3 ± 5.3  | 18.1 ± 5.2       | -0.1 ± 4.2      | 4.5 ± 30.9    | 0.85           |
| Type 2 diabetes & physically inactive       | 17.4 ± 6.8  | 15.3 ± 5.9       | -1.7 ± 5.2      | -4.6 ± 34.7↓  | 0.01           |
| <b>CNFD, fibers/mm<sup>2</sup></b>          |             |                  |                 |               |                |
| Not on weight gain therapy                  | 27.1 ± 8.4  | 28.5 ± 8.4       | 1.4 ± 7.1       | 13.1 ± 36.2   | 0.82           |
| On weight gain therapy                      | 27.1 ± 9.2  | 25.2 ± 8.3       | -1.9 ± 7.0      | -0.6 ± 40.7   | 0.27           |
| <b>CNBD, branches/mm<sup>2</sup></b>        |             |                  |                 |               |                |
| Not on weight gain therapy                  | 55.7 ± 28.8 | 47.8 ± 31.7      | -7.9 ± 28.5     | -4.1 ± 61.3   | 0.08           |
| On weight gain therapy                      | 64.9 ± 39.1 | 39.1 ± 29.0      | -25.8 ± 36.1    | -25.3 ± 75.5↓ | 0.001          |
| <b>CNFL, fiber length mm/mm<sup>2</sup></b> |             |                  |                 |               |                |
| Not on weight gain therapy                  | 17.3 ± 5.6  | 17.8 ± 5.7       | 0.5 ± 3.5       | 7.2 ± 25.7    | 0.66           |
| On weight gain therapy                      | 17.9 ± 6.5  | 15.8 ± 5.7       | -2.2 ± 5.2      | -6.1 ± 35.1   | 0.12           |

Variables are summarized as mean ± standard deviation. The mean difference of CCM measures between baseline and 2 year follow-up were compared using a paired *t*-test. CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length.

associated with corneal nerve fiber loss in type 2 diabetes<sup>9,32</sup>. However, evidence for the efficacy of intensive glycemic control on DPN in type 2 diabetes is conflicting. The Kumamoto trial<sup>33</sup>, ACCORD<sup>34</sup>, and BARI 2D<sup>35</sup> trials showed that intensive glycemic control delayed DPN progression, whilst the UKPDS<sup>36</sup>, VA-CSDM<sup>37</sup>, and Steno-2 trial<sup>38</sup> showed no benefit, which was confirmed in a Cochrane review<sup>5</sup>. In this study, a small improvement in HbA1c showed no improvement in neuropathy. A recent longitudinal study reported that despite a modest improvement in HbA1c and LDL cholesterol, there was a progressive worsening of thermal thresholds, nerve conduction, and corneal nerve degeneration<sup>39</sup>. However, a >3% reduction in HbA1c and weight loss of about 7 kg in type 2 diabetes over 4 years was associated with corneal nerve regeneration<sup>40,41</sup>. Furthermore, we showed previously that treatment with exenatide and pioglitazone or basal-bolus insulin over 12 months resulted in about a 3% reduction in HbA1c, corneal nerve regeneration, and reduction in pain, particularly in those with painful DPN<sup>42,43</sup>.

In type 2 diabetes, obesity, hyperlipidemia, and hypertension are independently associated with DPN development<sup>2,6-8</sup>. Our previous study in patients with type 2 diabetes showed that hyperlipidemia was independently associated with DPN and obesity was associated with painful DPN<sup>2</sup>. The prevalence of DPN in normoglycemic individuals with obesity was higher compared with lean normoglycemic individuals, suggesting that obesity may be an independent risk factor for DPN<sup>6</sup>. In the KORA F4/FF4 cohort followed over 6.5 years, BMI and waist circumference were associated with incident DPN<sup>7</sup>. Similarly, in a longitudinal study of 1,256 patients with type 2 diabetes followed over 13 years, Andersen *et al.*<sup>8</sup> showed that weight, BMI, waist circumference, HDL, and LDL were associated with incident DPN. We<sup>44</sup> and others<sup>3</sup> have shown that systolic blood pressure was independently associated with DPN. However, in the current study, obesity, hyperlipidemia, and blood pressure were not associated with DPN progression. This might be attributed to the small sample size and short follow up time.

Weight loss may have a beneficial effect on DPN<sup>19</sup>. Weight loss after bariatric surgery was associated with an improvement in neuropathic symptoms and deficits, and corneal nerve regeneration in normoglycemic individuals with morbid obesity<sup>16</sup> and individuals with type 2 diabetes and obesity<sup>17</sup>. In the current study, a modest improvement in body weight or treatment with anti-diabetic medication which promotes weight loss was not associated with an improvement in DPN. However, there was a progressive reduction in corneal nerve branches in patients on anti-diabetic medication associated with weight gain. This association was lost after adjusting for confounders which may be attributed to the small sample size.

Physical activity may have a beneficial effect on DPN<sup>15,18-21</sup> and previously we have shown that physical inactivity is associated with painful DPN<sup>2</sup>. In a longitudinal study, diet and exercise, with a modest reduction in BMI and total cholesterol, was associated with an improvement in neuropathic pain, sural

nerve amplitude, and cutaneous reinnervation over 12 months in patients with impaired glucose tolerance (IGT)<sup>18</sup>. In a 4 year study, aerobic exercise training was associated with a reduced incidence of impaired vibration perception and abnormal NCS in patients without DPN<sup>15</sup>. A 10 week aerobic and strengthening exercise program showed an improvement in neuropathic pain and intraepidermal nerve branching in patients with DPN<sup>19</sup>. Aerobic exercise training in patients with type 2 diabetes and DPN over 12 weeks was associated with improved sural nerve conduction velocity<sup>20</sup>. Physical activity was also associated with increased corneal nerve migration<sup>21</sup>. Interestingly, in the current study progressive corneal nerve degeneration occurred in physically inactive patients with type 2 diabetes.

We acknowledge that the small cohort size and relatively short duration of follow up may have limited the associations between risk factors and their change in relation to baseline severity and progression of DPN. We have not assessed nerve conduction or IENFD<sup>45</sup>, however, both previously failed to identify improvement after simultaneous pancreas and kidney transplantation<sup>14</sup>. Renal impairment was not associated with neuropathy measures, however, the overall severity of impairment was moderate and it was only present in 5.1% (4/78) of participants.

In conclusion, a modest improvement in HbA1c, weight, and lipids does not impact on the overall prevalence of diabetic peripheral neuropathy or painful neuropathy and cannot prevent progressive small nerve fiber loss. Furthermore, physical inactivity and the use of glucose lowering medication associated with weight gain may be associated with increased small nerve fiber loss quantified using corneal confocal microscopy.

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## DISCLOSURE

The authors declare no conflict of interest.

Approval of the research protocol: The research protocol was approved by the WCM-Q IRB (#14-00058), HMC IRB (#15103/15) and NRES Committee North West - Greater Manchester North (#08/H1004/1).

Informed consent: All subjects consented to take part in the study. The study acted in accordance to the tenets of the declaration of Helsinki.

Registry and the registration no. of the study/trial: N/A.

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## REFERENCES

- Pop-Busui R, Boulton AJ, Feldman EL, *et al.* Diabetic neuropathy: a position statement by the American Diabetes Association. *Diabetes Care* 2017; 40: 136–154.
- Ponirakis G, Elhadd T, Chinnaiyan S, *et al.* Prevalence and risk factors for diabetic neuropathy and painful diabetic neuropathy in primary and secondary healthcare in Qatar. *J Diabetes Investig* 2020; 12: 592–600.
- Callaghan BC, Xia R, Banerjee M, *et al.* Metabolic syndrome components are associated with symptomatic polyneuropathy independent of glycemic status. *Diabetes Care* 2016; 39: 801–807.
- Diabetes C, Complications Trial Research G, Nathan DM, *et al.* The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993;329:977–986.
- Callaghan BC, Little AA, Feldman EL, *et al.* Enhanced glucose control for preventing and treating diabetic neuropathy. *Cochrane Database Syst Rev* 2012; (6): CD007543.
- Callaghan BC, Xia R, Reynolds E, *et al.* Association between metabolic syndrome components and polyneuropathy in an obese population. *JAMA Neurol* 2016; 73: 1468–1476.
- Schlesinger S, Herder C, Kannenberg JM, *et al.* General and abdominal obesity and incident distal sensorimotor polyneuropathy: insights into inflammatory biomarkers as potential mediators in the KORA F4/FF4 cohort. *Diabetes Care* 2019; 42: 240–247.
- Andersen ST, Witte DR, Dalsgaard EM, *et al.* Risk factors for incident diabetic polyneuropathy in a cohort with screen-detected type 2 diabetes followed for 13 years: ADDITION-Denmark. *Diabetes Care* 2018; 41: 1068–1075.
- Ferdousi M, Kalteniece A, Azmi S, *et al.* Diagnosis of neuropathy and risk factors for corneal nerve loss in type 1 and type 2 diabetes: a corneal confocal microscopy study. *Diabetes Care* 2021; 44: 150–156.
- Pritchard N, Edwards K, Russell AW, *et al.* Corneal confocal microscopy predicts 4-year incident peripheral neuropathy in type 1 diabetes. *Diabetes Care* 2015; 38: 671–675.
- Lovblom LE, Halpern EM, Wu T, *et al.* In vivo corneal confocal microscopy and prediction of future-incident neuropathy in type 1 diabetes: a preliminary longitudinal analysis. *Can J Diabetes* 2015; 39: 390–397.
- Edwards K, Pritchard N, Dehghani C, *et al.* Corneal confocal microscopy best identifies the development and progression of neuropathy in patients with type 1 diabetes. *J Diabetes Complications* 2017; 31: 1325–1327.
- Tavakoli M, Mitu-Pretorian M, Petropoulos IN, *et al.* Corneal confocal microscopy detects early nerve regeneration in diabetic neuropathy after simultaneous pancreas and kidney transplantation. *Diabetes* 2013; 62: 254–260.
- Azmi S, Jeziorska M, Ferdousi M, *et al.* Early nerve fibre regeneration in individuals with type 1 diabetes after simultaneous pancreas and kidney transplantation. *Diabetologia* 2019; 62: 1478–1487.
- Balducci S, Iacobellis G, Parisi L, *et al.* Exercise training can modify the natural history of diabetic peripheral neuropathy. *J Diabetes Complications* 2006; 20: 216–223.
- Azmi S, Ferdousi M, Liu Y, *et al.* Bariatric surgery leads to an improvement in small nerve fibre damage in subjects with obesity. *Int J Obes (Lond)* 2021; 45: 631–638.
- Adam S, Azmi S, Ho JH, *et al.* Improvements in diabetic neuropathy and nephropathy after bariatric surgery: a prospective cohort study. *Obes Surg* 2021; 31: 554–563.
- Smith AG, Russell J, Feldman EL, *et al.* Lifestyle intervention for pre-diabetic neuropathy. *Diabetes Care* 2006; 29: 1294–1299.
- Kluding PM, Pasnoor M, Singh R, *et al.* The effect of exercise on neuropathic symptoms, nerve function, and cutaneous innervation in people with diabetic peripheral neuropathy. *J Diabetes Complications* 2012; 26: 424–429.
- Gholami F, Nikookheslat S, Salekzamani Y, *et al.* Effect of aerobic training on nerve conduction in men with type 2 diabetes and peripheral neuropathy: a randomized controlled trial. *Neurophysiol Clin* 2018; 48: 195–202.
- Al Rashah K, Pritchard N, Dehghani C, *et al.* Corneal nerve migration rate in a healthy control population. *Optom Vis Sci* 2018; 95: 672–677.
- American Diabetes A. 6. Glycemic targets: standards of medical care in diabetes-2020. *Diabetes Care* 2020; 43(Suppl 1): S66–S76.
- Moser M. World Health Organization-International Society of Hypertension Guidelines for the management of hypertension-do these differ from the U.S. recommendations? Which guidelines should the practicing physician follow? *J Clin Hypertens (Greenwich)* 1999; 1: 48–54.
- Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser 2000;894:i-xii, 1–253.
- Kalteniece A, Ferdousi M, Adam S, *et al.* Corneal confocal microscopy is a rapid reproducible ophthalmic technique for quantifying corneal nerve abnormalities. *PLoS One* 2017; 12: e0183040.
- Dabbah MA, Graham J, Petropoulos IN, *et al.* Automatic analysis of diabetic peripheral neuropathy using multi-scale quantitative morphology of nerve fibres in corneal confocal microscopy imaging. *Med Image Anal* 2011; 15: 738–747.
- Bouhassira D, Attal N, Alchaar H, *et al.* Comparison of pain syndromes associated with nervous or somatic lesions and development of a new neuropathic pain diagnostic questionnaire (DN4). *Pain* 2005; 114: 29–36.
- Terkawi AS, Abolkhair A, Didier B, *et al.* Development and validation of Arabic version of the douleur neuropathique 4 questionnaire. *Saudi J Anaesth* 2017; 11(Suppl 1): S31–S39.
- Wiles PG, Pearce SM, Rice PJ, *et al.* Vibration perception threshold: influence of age, height, sex, and smoking, and

- calculation of accurate centile values. *Diabet Med* 1991; 8: 157–161.
30. Spallone V, Morganti R, D'Amato C, *et al.* Validation of DN4 as a screening tool for neuropathic pain in painful diabetic polyneuropathy. *Diabet Med* 2012; 29: 578–585.
  31. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology* 1990; 1: 43–46.
  32. Andersen ST, Grosen K, Tankisi H, *et al.* Corneal confocal microscopy as a tool for detecting diabetic polyneuropathy in a cohort with screen-detected type 2 diabetes: ADDITION-Denmark. *J Diabetes Complications* 2018; 32: 1153–1159.
  33. Ohkubo Y, Kishikawa H, Araki E, *et al.* Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. *Diabetes Res Clin Pract* 1995; 28: 103–117.
  34. Ismail-Beigi F, Craven T, Banerji MA, *et al.* Effect of intensive treatment of hyperglycaemia on microvascular outcomes in type 2 diabetes: an analysis of the ACCORD randomised trial. *Lancet* 2010; 376: 419–430.
  35. Pop-Busui R, Lu J, Brooks MM, *et al.* Impact of glycemic control strategies on the progression of diabetic peripheral neuropathy in the Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) Cohort. *Diabetes Care* 2013; 36: 3208–3215.
  36. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 1998; 352: 837–853.
  37. Azad N, Emanuele NV, Abaira C, *et al.* The effects of intensive glycemic control on neuropathy in the VA cooperative study on type II diabetes mellitus (VA CSDM). *J Diabetes Complications* 1999; 13: 307–313.
  38. Gaede P, Vedel P, Larsen N, *et al.* Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Engl J Med* 2003; 348: 383–393.
  39. Dhage S, Ferdousi M, Adam S, *et al.* Corneal confocal microscopy identifies small fibre damage and progression of diabetic neuropathy. *Sci Rep* 2021; 11: 1859.
  40. Jia X, Wang X, Wang X, *et al.* In vivo corneal confocal microscopy detects improvement of corneal nerve parameters following glycemic control in patients with type 2 diabetes. *J Diabetes Res* 2018; 2018: 8516276.
  41. Ishibashi F, Taniguchi M, Kosaka A, *et al.* Improvement in neuropathy outcomes with normalizing HbA1c in patients with type 2 diabetes. *Diabetes Care* 2019; 42: 110–118.
  42. Ponirakis G, Abdul-Ghani MA, Jayyousi A, *et al.* Effect of treatment with exenatide and pioglitazone or basal-bolus insulin on diabetic neuropathy: a substudy of the Qatar Study. *BMJ Open Diabetes Res Care* 2020; 8: e001420.
  43. Ponirakis G, Abdul-Ghani MA, Jayyousi A, *et al.* Painful diabetic neuropathy is associated with increased nerve regeneration in patients with type 2 diabetes undergoing intensive glycemic control. *J Diabetes Investig* 2021; 12: 1642–1650.
  44. Ponirakis G, Petropoulos IN, Alam U, *et al.* Hypertension contributes to neuropathy in patients with type 1 diabetes. *Am J Hypertens* 2019; 32: 796–803.
  45. Devigili G, Rinaldo S, Lombardi R, *et al.* Diagnostic criteria for small fibre neuropathy in clinical practice and research. *Brain* 2019; 142: 3728–3736.