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CLINICAL TRIAL

Insulin resistance limits corneal nerve regeneration in patients with type 2 diabetes undergoing intensive glycemic control

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ABSTRACT

Aims/Introduction: This study aimed to investigate whether insulin resistance (IR) in individuals with type 2 diabetes undergoing intensive glycemic control determines the extent of improvement in neuropathy.

Materials and Methods: This was an exploratory substudy of an open-label, randomized controlled trial of individuals with poorly controlled type 2 diabetes treated with exenatide and pioglitazone or insulin to achieve a glycated hemoglobin <7.0% (<53 mmol/mol). Baseline IR was defined using homeostasis model assessment of IR, and change in neuropathy was assessed using corneal confocal microscopy.

Results: A total of 38 individuals with type 2 diabetes aged 50.2 ± 8.5 years with (n = 25, 66%) and without (n = 13, 34%) IR were studied. There was a significant decrease in glycated hemoglobin ($P < 0.0001$), diastolic blood pressure ($P < 0.0001$), total cholesterol ($P < 0.01$) and low-density lipoprotein ($P = 0.05$), and an increase in bodyweight ($P < 0.0001$) with treatment. Individuals with homeostasis model assessment of IR <1.9 showed a significant increase in corneal nerve fiber density ($P \leq 0.01$), length ($P \leq 0.01$) and branch density ($P \leq 0.01$), whereas individuals with homeostasis model assessment of IR ≥1.9 showed no change. IR was negatively associated with change in corneal nerve fiber density after adjusting for change in bodyweight ($P < 0.05$).

Conclusions: Nerve regeneration might be limited in individuals with type 2 diabetes and IR undergoing treatment with pioglitazone plus exenatide or insulin to improve glycemic control.

INTRODUCTION

Diabetic peripheral neuropathy (DPN) occurs in approximately 50% of patients with diabetes, and is associated with neuropathic pain, erectile dysfunction and foot ulcers[1]. Currently there are no Food and Drug Administration approved treatments for DPN. Intensive glycemic control can delay DPN progression in type 1 diabetes[2], but has a limited effect in type 2 diabetes[3-6].

Insulin resistance (IR) is characterized by decreased responsiveness of the liver fat and muscle to circulating insulin[7]. IR is an important risk factor for type 2 diabetes[8] and atherosclerosis[9], and is associated with hypertension[10], obesity[11] and dyslipidemia[12], many of the risk factors for DPN[13]. Neurons also show IR[14], and it might attenuate the neurotrophic effect of insulin[15]. IR has been associated with DPN in individuals with type 2 diabetes[16,17] and type 1 diabetes[13]. Furthermore, in the BARI-2D study, the incidence of DPN was lower in patients receiving insulin-sensitizing treatment compared with insulin providing treatment over a 4-year period, even after adjusting for the change in glycated hemoglobin (HbA1c)[5].

Previously, we have reported that both exenatide plus pioglitazone or basal–bolus insulin improve corneal nerve branch

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density and length, independent of change in HbA1c, bodyweight and lipid profile[18]. However, after simultaneous pancreas and kidney transplantation in patients with type 1 diabetes, corneal nerve fiber density increased significantly at 6 months followed by an increase in corneal nerve fiber length and corneal nerve branch density[19,20]. Treatment with the erythropoietic peptide, cibinetide, showed a significant increase in corneal nerve fiber density in patients with type 2 diabetes[21], and an increase in corneal nerve fiber area in patients with sarcoidosis and small fiber neuropathy[22]. A detailed analysis of the change in different corneal nerve parameters showed that an initial increase in the corneal nerve branch density, length and area was indicative of repair to the terminal parts of the basal plexus followed by an increase in corneal nerve fiber density, indicative of more proximal nerve repair[23]. The objective of the present study was to investigate whether the presence of IR affects corneal nerve regeneration in individuals with type 2 diabetes undergoing intensive glycemic control with exenatide and pioglitazone or basal–bolus insulin over a 12-month period.

MATERIALS AND METHODS

This was an exploratory substudy of an open-label, randomized controlled trial (clinicaltrials.gov ID: NCT02887625)[24] 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 were enrolled from the National Diabetes Center in Doha, Qatar, at baseline and 1-year follow up from October 2016 to November 2018. This study received ethical approval from the Hamad Medical Corporation institutional review board (IRB approval number 13-00076) on 9 May 2016. All subjects consented to participate in the study. The study followed the tenets of the declaration of Helsinki.

Inclusion and exclusion criteria

Individuals aged 18–75 years and with HbA1c >7.5% (>58 mmol/mol) on near maximum dose of metformin (>1,500 mg/day) and sulfonylurea (>4 mg glimepiride or >60 mg gliclazide), normal liver and kidney function, electrocardiogram, and stable bodyweight (±1 kg) in the past year were recruited.

Exclusion criteria are described in detail in our previous report[18], 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 exenatide and pioglitazone or glargine and aspart treatment to achieve and maintain an HbA1c <7.0% (<53 mmol/mol)[24]. There was no limit on the upper value of HbA1c for enrolment. Participants randomized to combination treatment were started on weekly subcutaneous extended release exenatide (2 mg/week Bydureon) and pioglitazone (30 mg/day). Participants receiving insulin were started on glargine before breakfast. The Treat-to-Target Trial (4T) algorithm was used to calculate the starting glargine dose, and the dose was adjusted weekly to achieve a fasting plasma glucose of <6.11 mmol/L. After the fasting plasma glucose goal was achieved, if the HbA1c was >53 mmol/mol (>7.0%), 4–6 units of insulin aspart was started before each meal, and the dose was adjusted to achieve a post-prandial plasma glucose concentration of <7.78 mmol/L, 2 h after meals. Patients were seen monthly during the first 4 months or as required, based on the results of the plasma glucose concentration, and bimonthly thereafter.

Demographic and metabolic measures

Age, sex, diabetes duration, body weight, 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.

Insulin secretion and resistance

The oral glucose tolerance test was administered in the morning after an overnight fast. Blood samples were collected at −30, −15, 0, 30, 60, 90 and 120 min through a small polyethylene catheter inserted into an antecubital vein to measure plasma glucose and insulin concentrations. Plasma glucose and insulin concentration were measured by the glucose oxidase reaction (Glucose Oxidase Analyzer, Fullerton, CA, USA) and radioimmunoassay (Coat A Coat; Diagnostic Products, Los Angeles, CA, USA), respectively. The insulinogenic index was calculated by the incremental area under the curve of insulin divided by the incremental area under the curve of glucose during the 0- to 120-min (total) time period of the oral glucose tolerance test. Homeostasis model assessment of insulin resistance (HOMA-IR), which reflects hepatic IR[25], was used as a surrogate measure of IR[26,27]. There is considerable variability in the threshold levels that define IR and it does not exclusively reflect insulin sensitivity in patients with type 2 diabetes. However, in two large population-based cohorts, the upper 95th percentiles of HOMA-IR were 1.9 and 2.0[28]. Therefore, in the current study, participants were categorized as having IR if the HOMA-IR was ≥1.9.

Neuropathy assessment

Corneal confocal microscopy (CCM) was undertaken using the HRT-III-RCM device (Heidelberg Engineering GmbH, Heidelberg, Germany), as described previously[29]. Corneal nerve fiber density (CNFD; fibers/mm^2), length (CNFL) (mm/mm^2) and branch density (CNBD; branches/mm^2) were quantified using CCMetrics[30].

Statistical analysis

This was an exploratory study, no power calculation was determined, and the results were not adjusted for multiple

comparisons. Participants were randomly assigned to either treatment[31]. Variables between baseline and 1-year follow up were compared using a paired *t*-test. Changes in clinical, metabolic and CCM measures from baseline to 1-year follow up between participants were compared using an unpaired *t*-test.

Univariate linear regression analysis was carried out with the corneal nerve measures at baseline and change in the corneal nerve measures as the dependent variables, and HOMA-IR, insulin secretion, blood pressure, bodyweight, BMI, HbA1c, cholesterol, triglyceride, HDL and LDL as independent variables. All significant variables were included in the multiple linear regression analysis. The regression coefficient and corresponding 95% confidence interval are presented. All analyses were calculated using IBM SPSS v. 26 (SPSS Inc., Armonk, NY, USA). A two-tailed *P*-value ≤0.05 was considered significant.

RESULTS

Baseline and 1-year follow-up clinical and metabolic characteristics

The clinical and metabolic characteristics at baseline and 1-year follow up are summarized in Table 1. The study cohort was comprised of 15 (40%) women and 23 (60%) men aged 50.2 ± 8.5 years, with type 2 diabetes for 10.2 ± 5.2 years. The mean fasting plasma glucose at baseline was 243.5 ± 54.4 mg/dL. There was a significant reduction in HbA1c (10.7% [93 mmol/mol] vs 7.9% [62 mmol/mol], $P < 0.0001$), diastolic blood pressure (DBP; mmHg) (79.8 vs 73.5, $P < 0.0001$), total cholesterol (mmol/L; 5.0 vs 4.5, $P < 0.01$) and LDL (mmol/L; 2.9 vs 2.6, $P = 0.05$), but no change in systolic blood pressure (SBP; mmHg) (128.8 vs 127.1, $P = 0.58$), BMI (kg/m^2; 31.6 vs 32.0, $P = 0.19$), triglycerides (mmol/L; 1.9 vs 1.8, $P = 0.49$) and HDL (mmol/L; 1.2 vs 1.1, $P = 0.28$). The mean bodyweight increased significantly (kg; 86.5 to 91.5, $P < 0.0001$).

Table 1 | Clinical and metabolic characteristics of patients with type 2 diabetes at baseline and 1-year follow up

	Baseline	1-year follow up	*P*-value
HbA1c (mmol/mol)	93.3 ± 19.2	62.4 ± 22.4	<0.0001
HbA1c (%)	10.7 ± 1.8	7.9 ± 2.1	<0.0001
Total cholesterol (mmol/L)	5.0 ± 1.2	4.5 ± 1.1	<0.01
Triglyceride (mmol/L)	1.9 ± 1.2	1.8 ± 1.5	0.44
HDL (mmol/L)	1.2 ± 0.5	1.1 ± 0.3	0.26
LDL (mmol/L)	2.9 ± 1.1	2.6 ± 1.0	<0.05
Systolic BP (mmHg)	128.8 ± 18.0	127.1 ± 17.0	0.65
Diastolic BP (mmHg)	79.8 ± 11.7	73.5 ± 9.8	<0.0001
Bodyweight (kg)	86.5 ± 16.8	91.5 ± 19.1	<0.0001
BMI (kg/m^2)	31.6 ± 6.3	32.0 ± 6.5	0.50
CNFD (fibers/mm^2)	27.6 ± 9.1	29.3 ± 8.4	0.28
CNBD (branches/mm^2)	67.8 ± 37.0	87.5 ± 47.1	<0.01
CNFL (mm/mm^2)	19.2 ± 5.6	21.0 ± 6.0	<0.05

Numeric variables are summarized as the mean ± standard deviation. Changes between baseline and 1-year follow up were compared using paired *t*-test. BMI, body mass index; BP, blood pressure; CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Shades denote the content of each column.

There was a significant increase in CNBD (branches/mm^2; 67.8 vs 87.5, $P < 0.01$) and CNFL (mm/mm^2; 19.2 vs 21.0, $P < 0.05$), and a non-significant increase in CNFD (fibers/mm^2; 27.6 vs 29.3, $P = 0.26$).

Association of IR with clinical characteristics and corneal nerve fiber measures

Of the 38 individuals studied, 25 (65.8%) had HOMA-IR ≥1.9 and 13 (34.2%) had HOMA-IR <1.9. Comparison of the plasma glucose and insulin concentrations, and index of insulin secretion and resistance, and the change in clinical characteristics and corneal nerve fiber measures between those with HOMA-IR <1.9 and ≥1.9 are summarized in Table 2. Participants with IR (HOMA-IR ≥1.9) had a significantly lower mean age (45.1 ± 8.4 vs 54.4 ± 7.2, $P < 0.05$), but comparable duration of diabetes (11.2 ± 5.3 vs 9.6 ± 5.2, $P = 0.40$) and proportion of women (46.2% vs 36.0%, $P = 0.54$) compared with those without IR. In both groups, HbA1c decreased ($P \leq 0.001$) and bodyweight increased ($P \leq 0.001$), with comparable changes in HbA1c (−2.5% [−28 mmol/mol] vs −3.0% [−32.6 mmol/mol], $P = 0.49$) and bodyweight (kg; 6.6 vs 4.2, $P = 0.19$). Participants with HOMA-IR ≥1.9 showed a significantly greater decrease in DBP ($P \leq 0.01$) and total cholesterol ($P \leq 0.01$), but the change in DBP (mmHg; −6.8 vs −5.9, $P = 0.81$) and total cholesterol (mmol/L; −0.5 vs −0.5, $P = 0.97$) was comparable between both interventions. The change in triglycerides (mmol/L; −0.1 vs −0.2, $P = 0.79$), HDL (mmol/L; −0.1 vs −0.1, $P = 0.78$), LDL (mmol/L; −0.3 vs −0.4, $P = 0.88$), SBP (mmHg; 0.8 vs −3.1, $P = 0.53$) and BMI (kg/m^2; −0.1 vs 0.3, $P = 0.61$) was comparable between patients with and without IR.

Participants with HOMA-IR <1.9 showed a significant increase in CNFD ($P \leq 0.01$), CNBD ($P \leq 0.01$) and CNFL ($P \leq 0.01$), whereas participants with IR ≥1.9 showed no change (Figure 1). There was a significant difference in the change in CNFD (fibers/mm^2; 7.5 vs −1.3, $P \leq 0.01$) and CNFL (mm/mm^2; 4.7 vs 0.3, $P < 0.05$), but not in CNBD (branches/mm^2; 33.7 vs 12.4, $P = 0.16$) between patients with and without IR.

Association of insulin secretion, HOMA-IR and clinical characteristics with corneal nerve fiber measures

Linear regression analysis showed a negative association between baseline HOMA-IR as a continuous variable with change (Δ) in CNFD ($P < 0.05$), but no association with ΔCNBD ($P = 0.66$) or ΔCNFL ($P = 0.75$; Table 3). HOMA-IR ranged from 0.2 – 9.1 and for every 1-unit increase, the CNFD decreased by 1.38 fibers/mm^2 after adjusting for change in bodyweight. There was no association between baseline

Table 2 | Comparison of demographic and clinical characteristics at baseline and change over a 1-year period between participants with type 2 diabetes with and without insulin resistance

	HOMA-IR <1.9 (n = 13)	HOMA-IR ≥1.9 (n = 25)	P-value
Age (years)	54.4 ± 7.2	45.1 ± 8.4	<0.05
Sex (female), n (%)	6 (46.2)	9 (36.0)	0.54
Duration of diabetes (years)	11.2 ± 5.3	9.6 ± 5.2	0.40
Fasting glucose (mg/dL)	227.2 ± 54.7	257.5 ± 52.6	0.11
2-h glucose (mg/dL)	397.9 ± 46.0	416.7 ± 69.9	0.31
Fasting insulin (μU/mL)	1.8 ± 1.2	6.7 ± 2.6	<0.0001
2-h insulin (μU/mL)	5.9 ± 7.2	10.4 ± 5.3	0.06
Insulinogenic index (mU/L/mg/dL)	2.7 ± 3.5	3.2 ± 3.2	0.66
HOMA-IR	1.0 ± 0.6	4.2 ± 1.8	<0.0001
Matsuda insulin sensitivity index	24.9 ± 25.6	4.2 ± 1.4	0.01
ΔHbA1c (mmol/mol)	−27.8 ± 19.3†††	−32.6 ± 20.5†††	0.49
ΔHbA1c (%)	−2.5 ± 1.8†††	−3.0 ± 1.9†††	0.49
ΔTotal cholesterol (mmol/L)	−0.5 ± 1.1	−0.5 ± 1.1†	0.97
ΔTriglyceride (mmol/L)	−0.1 ± 0.8	−0.2 ± 1.3	0.79
ΔHDL (mmol/L)	−0.1 ± 0.3	−0.1 ± 0.4	0.78
ΔLDL, mmol/L	−0.3 ± 0.9	−0.4 ± 1.0	0.88
ΔSystolic BP (mmHg)	0.8 ± 17.6	−3.1 ± 18.4	0.53
ΔDiastolic BP (mmHg)	−6.8 ± 11.9	−5.9 ± 8.3†	0.81
ΔBodyweight (kg)	6.6 ± 5.2††	4.2 ± 5.1†††	0.19
ΔBMI (kg/m^2)	−0.1 ± 2.1	0.3 ± 1.4	0.61
ΔCNFD (fibers/mm^2)	7.5 ± 9.6‡	−1.3 ± 7.6	≤0.01
ΔCNBD (branches/mm^2)	33.7 ± 41.8‡	12.4 ± 44.5	0.16
ΔCNFL (mm/mm^2)	4.7 ± 5.0†	0.3 ± 4.9	<0.05

Numeric variables and frequency distribution for categorical variables are summarized as the mean ± standard deviation and n (%), and were compared between participants with type 2 diabetes with homeostasis model assessment of insulin resistance (HOMA-IR) <1.9 and ≥1.9 using unpaired t-test and χ^2-test, respectively. Changes between baseline and 1-year follow up were compared using paired t-test: $^{‡}P \leq 0.05$, $^{†}P \leq 0.01$, $^{††}P \leq 0.001$, $^{†††}P \leq 0.0001$. BMI, body mass index; BP, blood pressure; CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

HOMA-IR and baseline CNFD (P = 0.17), CNBD (P = 0.85) or CNFL (P = 0.82), and between baseline insulin secretion and baseline CNFD (P = 0.80), CNBD (P = 0.82), CNFL (P = 0.89) and ΔCNFD (P = 0.43), ΔCNBD (P = 0.77) or ΔCNFL (P = 0.65). There was no association between type of treatment and ΔCNFD (P = 0.13), ΔCNBD (P = 0.35) or ΔCNFL (P = 0.30).

The change in CCM measures was not associated with age (P = 0.14–0.63), duration of diabetes (P = 0.11–0.23), ΔSBP (P = 0.15–0.95), ΔDBP (P = 0.57–079), ΔHbA1c (P = 0.37–0.83), Δtriglyceride (P = 0.12–0.61) or ΔLDL (P = 0.47–0.80). There was a negative association between Δbodyweight and ΔCNFD ($P < 0.01$), but not with ΔCNBD (P = 0.53) or ΔCNFL (P = 0.77), and between Δtotal cholesterol and ΔCNBD ($P < 0.05$), but not with ΔCNFD (P = 0.36) or ΔCNFL (P = 0.81).

Effect of treatment on neuropathy progression with HOMA-IR <1.9 and ≥1.9

Of 38 participants, 26 (68%) received pioglitazone and exenatide, and 12 (32%) received basal–bolus insulin. Of 25 participants with HOMA-IR ≥1.9, 18 (72%) received pioglitazone and exenatide, and seven (28%) received insulin. There was no significant difference in CNFD (fibers/mm^2; 3.2 vs −3.0, P = 0.13), CNBD (branches/mm^2; 36.8 vs 2.9, P = 0.12) and CNFL (mm/mm^2; 2.1 vs −0.4, P = 0.15) between participants receiving insulin or combination treatment.

Of the 13 participants with HOMA-IR <1.9, eight (61.5%) received pioglitazone and exenatide, and five (38.5%) received insulin. The change in CNFD (fibers/mm^2; 5.8 vs 8.5, P = 0.67), CNBD (branches/mm^2; 36.6 vs 31.9, P = 0.84) and CNFL (mm/mm^2; 4.4 vs 4.8, P = 0.86) was comparable between participants receiving combination compared to insulin treatment.

DISCUSSION

The present study found that the presence of IR might impact on the capacity for corneal nerve regeneration after an improvement in glycemic control, irrespective of treatment with pioglitazone and exenatide or basal–bolus insulin. Participants with IR showed no change in corneal nerve measures, whereas participants without IR showed a significant increase in corneal

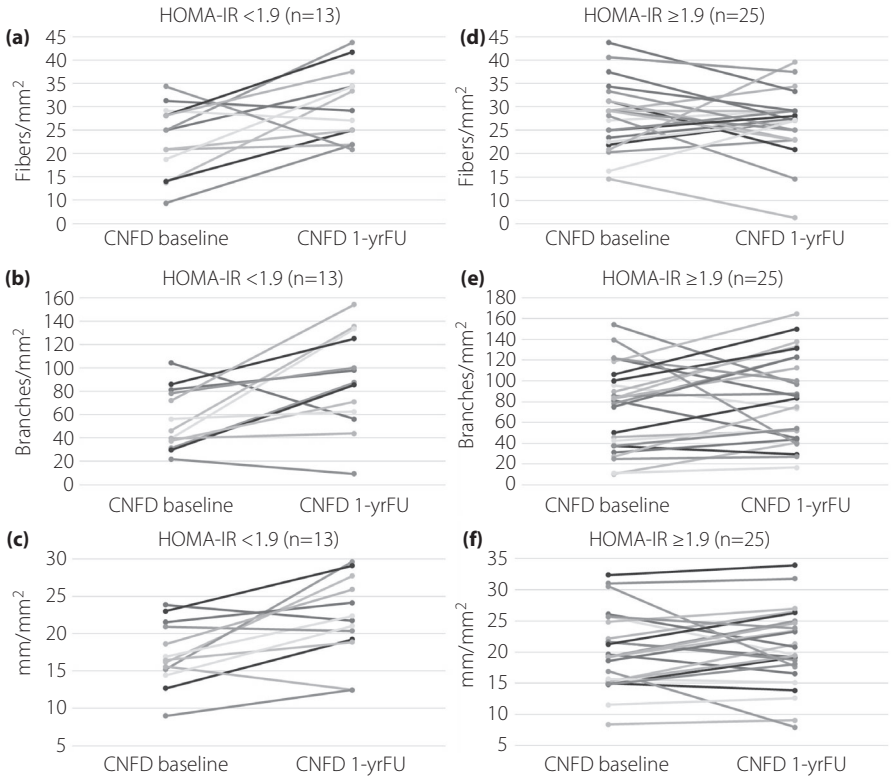

Figure 1 | Change in corneal nerve fiber density, branch density and fiber length in participants with (a–c) homeostasis model assessment of insulin resistance (HOMA-IR) <1.9 and (d–f) HOMA-IR ≥1.9 between baseline and 1-year follow up. CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, Corneal nerve fiber length; FU, follow up.

Table 3 | Linear regression analyses to determine the association of corneal nerve fiber measures with insulin resistance as a continuous variable

	Beta coefficient	95% Confidence interval	*P*-value
Baseline CNFD (fibers/mm^2)			
HOMA-IR	0.94	−0.41, 2.29	0.17
Change in CNFD (fibers/mm^2)			
HOMA-IR	−1.38	−2.64, −0.11	<0.05
Change bodyweight (kg)	−0.82	−1.36, −0.28	<0.01
Baseline CNBD (branches/mm^2)			
HOMA-IR	0.53	−5.11, 6.18	0.85
Change in CNBD (branches/mm^2)			
HOMA-IR	2.55	−3.65, 8.76	0.41
Change in total cholesterol	−16.19	−29.27, −3.11	<0.05
Baseline CNFL (mm/mm^2)			
HOMA-IR	0.10	−0.77, 0.96	0.82
Change in CNFL (mm/mm^2)			
HOMA-IR	−0.13	−0.94, 0.68	0.75

All the variables considered in the fitted model had $P < 0.05$. CNBD, corneal nerve branch density; CNFD, corneal nerve fiber density; CNFL, corneal nerve fiber length; HOMA-IR, homeostasis model assessment of insulin resistance. Shades denote the content of each column.

nerve fiber density, branch density and length indicative of both proximal and distal nerve regeneration. Baseline insulin secretion was not associated with change in corneal nerve measures. Consistent with our recent study[18], the improvement in HbA1c and other risk factors associated with IR including diastolic blood pressure, total cholesterol and LDL were not associated

with change in corneal nerve measures, whereas weight gain in both treatment groups was inversely associated with the change in corneal nerve fiber density.

There is a considerable literature linking IR and insulin action to neuronal integrity in the brain of patients with Alzheimer's disease[32]; and the effect of pioglitazone[33] and glucagon-like peptide-1 therapy[34] on dementia is currently being evaluated in ongoing trials. A few studies have investigated the impact of IR on DPN in individuals with type 2 diabetes[16,17] and type 1 diabetes[13]. A cross-sectional study[16] of 86 individuals with type 2 diabetes in Korea reported a significant association between IR and DPN assessed using a neurological examination and nerve conduction studies. In the current study, we found no association between IR and corneal nerve measures, which might be attributed to differences in BMI, HbA1c and duration of diabetes in our cohort compared with the Korean study. Both obesity[35] and higher HbA1c[36,37] are associated with reduced corneal nerves and DPN[38], as well as painful DPN[39]. In relation to the impact of IR on incident DPN, a prospective study[17] showed an association between IR and a reduction in sensory nerve action potential over a period of 6 years. The European Diabetes (EURODIAB) Prospective Complications Study[13] of 1,172 individuals with type 1 diabetes reported that lower estimated glucose disposal rate (higher IR) at baseline was associated with the development of DPN after adjusting for diabetes duration and HbA1c. The current study shows that IR at baseline reduced the impact of improved glycemic control with pioglitazone and exenatide or basal–bolus insulin on small nerve fiber regeneration. Lifestyle interventions through increased physical activity and weight loss reduce IR, and lower the risk for type 2 diabetes in individuals with high IR[40], reduce the prevalence of cardiovascular risk factors[41], and improve painful neuropathic symptoms and intraepidermal nerve fiber density in individuals with impaired glucose tolerance[42].

In individuals with insulin resistance, basal–bolus insulin treatment showed a trend for greater corneal nerve regeneration compared with pioglitazone plus exenatide treatment. This might be attributed to the direct neurotrophic effect of insulin, independent of underlying IR[15,18,43]. We have previously reported greater corneal nerve fiber regeneration, and an improvement in vibration perception with insulin treatment[18]. Furthermore, Azmi *et al.*[43] showed greater corneal nerve regeneration in patients treated with continuous subcutaneous insulin infusion compared with multiple daily insulin injection despite comparable HbA1c, suggesting that continuous subcutaneous insulin infusion might provide more stable blood glucose control and a direct neurotrophic benefit of continuous insulin on nerves[44].

The increase in corneal nerve fiber density, length and branch density in patients without insulin resistance indicates both proximal and distal nerve regeneration. Our previous studies after simultaneous pancreas and kidney transplantation in type 1 diabetes patients[20], and after bariatric surgery in obese patietns[45] have also shown an increase in corneal nerve fiber density, length and branch density. Cibinetide showed an increase in corneal nerve fiber density in patients with type 2 diabetes[21] and an increase in corneal nerve fiber area in patients with sarcoidosis[22]. Treatment of patients with type 1 diabetes with Omega-3 resulted in an increase in corneal nerve fiber length and branch density with no change in nerve fiber density[46]. These differences in the extent and type of nerve regeneration highlight the need to take into account underlying patient characteristics when determining outcomes in clinical trials.

A significant study limitation of the present study was the small sample size, and as such, subgroup analysis based on categorization of IR was not powered to adequately assess the response to different treatment arms. The classification of patients into those with and without significant IR based on the HOMA-IR cut-off of 1.9 was useful, but should be interpreted with caution, as it depends on insulin secretion, the insulin assay and HOMA-IR calculation[47]. CCM is an objective biomarker of small nerve fiber degeneration and regeneration, and can predict the development of DPN[48-50]. However, the current study was not powered to assess whether the presence of insulin resistance can prevent or delay incident DPN. We also acknowledge a lack of blinding for patients and investigators due to weekly injections of exenatide and multiple daily insulin injections. However, the investigator who measured the corneal nerve morphology was masked to the treatment group.

In conclusion, the present study found that patients with type 2 diabetes and IR show blunted small nerve fiber regeneration despite a substantial improvement in glycemic control. Therefore, lifestyle interventions, such as physical activity and weight reduction to improve IR might benefit DPN. Furthermore, IR should be considered as a potential confounder when assessing the benefits of disease-modifying therapies in clinical trials for DPN.

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DISCLOSURE

The authors declare no conflict of interest.

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