Distal Corneal Small Nerve Fibre Damage in Subjects With Obesity

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Research Article

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Abstract

Background: We have previously shown that subjects with obesity have elevated vibration and thermal perception thresholds and central corneal nerve loss and patients with diabetic neuropathy have greater corneal nerve loss at the inferior whorl compared to the central cornea. In the current study, we assessed whether there is evidence for a dying-back neuropathy in subjects with obesity with and without diabetes.

Methods: 57 obese subjects, with and without diabetes (DM^+, n=30; DM^-, n=27 respectively) and age- and sex-matched controls (n=21) underwent venous blood sampling and assessment of the neuropathy symptom profile (NSP), neuropathy disability score (NDS), vibration, cold and warm threshold testing, cardiac autonomic function, and corneal confocal microscopy (CCM).

Results: NSP and NDS were significantly elevated in obese DM^+ ($p<0.0001$; $p=0.001$) and DM^- ($p<0.0001$; $p=0.001$) subjects compared to controls. Vibration perception threshold was significantly higher in DM^+ ($p=0.001$), but not in DM^- ($p=0.06$), compared to controls, whilst cold ($p = 0.87$) and warm ($p = 0.52$) perception thresholds did not differ between groups. Deep breathing heart rate variability was significantly lower in DM^+ ($p=0.01$), but not DM^- ($p=0.9$) subjects compared to controls. Corneal nerve fibre density [26.8 ±6.22 vs 26.8 ±6.01 vs 35.3 ±7.41, $p<0.0001$], branch density [55.4 ±28.2 vs 58.4 ±28.5 vs 88.2 ±31.1, $p<0.001$], fibre length (CNFL) [17.6 ±4.43 vs 19.9 ±5.43 vs 26.7 ±5.31, $p <0.0001$], inferior whorl length (IWL) [17.9 ±6.10 vs 18.6 ±7.42 vs 35.3 ±9.70, $p<0.0001$] and total nerve fibre length (TNFL) [35.5 ±9.58 vs 38.5 ±11.0 vs 62.0 ±12.3, $p<0.0001$] were significantly lower in obese subjects without and with diabetes compared to controls. In comparison to controls, there was a greater relative reduction in IWL compared to CNFL in DM^+ (47.3% vs 25.5%) and DM^- (49.3% vs 34.1%).

Conclusion: We demonstrate evidence of peripheral neuropathy characterised by neuropathic symptoms, neurological deficits, elevated vibration perception and autonomic dysfunction with a dying-back neuropathy affecting the corneal nerves in obese subjects with and without type 2 diabetes.

Introduction

Small fibre neuropathy (SFN) affects unmyelinated C fibres or myelinated A- delta fibres [1], and presents with symptoms including numbness, burning, cold-like pain or pins and needles [2, 3]. Corneal confocal microscopy (CCM) is a rapid ophthalmic imaging tool which has identified small fibre damage in a range of peripheral neuropathies [4, 5] and has been utilised to show nerve repair in clinical trials [6]. Corneal nerves function chiefly as somatic and autonomic sensory nerves, extending from the peripheral to the central cornea, radiating to a vortex-like structure called the inferior whorl (IW) and terminate as intraepithelial nerves [7]. Comparison of corneal nerve length at the more distal inferior whorl with more proximal central corneal nerves enables an assessment for a dying-back neuropathy. Zhang et al. showed that a reduction in inferior whorl length occurred in in patients with sub-clinical familial amyloid polyneuropathy [8]. In a cross-sectional study, we have previously reported greater corneal nerve loss at

the IW compared to the central cornea in patients with diabetic neuropathy [9], and there was greater corneal nerve loss at the IW compared to the central cornea in a longitudinal study [10].

Obesity is associated with a small fibre neuropathy which is often more insidious in onset [11], and less severe than diabetic neuropathy [11, 12]. We have previously shown that obesity-related small fibre neuropathy can be identified using CCM and that it improves 12 months after bariatric surgery [13]. In a murine model of peripheral neuropathy, Davidson et al. showed greater and earlier loss of nerve fibres in the inferior whorl in both obese and hyperglycaemic rats [14]. In this study we have undertaken detailed neuropathy phenotyping and compared corneal nerve loss at the IW compared to the central cornea to assess for evidence of a dying-back neuropathy in subjects with obesity with and without diabetes.

Research Design And Methods

Selection of patients

Fifty-seven obese subjects without (DM^-, n=27) and with (DM^+, n=30) type 2 diabetes were recruited from a specialised and complex obesity service in Salford Royal Hospital, prior to undergoing bariatric surgery. Control subjects (n=21) were recruited from patients' family members and University of Manchester staff. Patients with a history of or any other cause of neuropathy, current or active diabetic foot ulceration, current contact lens wear, corneal trauma or surgery, ocular disease or systemic disease that may affect the cornea were excluded from the study. Approval was granted by the Central Manchester Research and Ethics Committee. Informed consent was obtained from all subjects prior to their participation. This research adhered to the tenets of the declaration of Helsinki.

Blood pressure, anthropometric and laboratory assessments

All study participants underwent assessment of height, weight, body mass index (BMI), blood pressure, glycosylated haemoglobin (HbA1c), fasting total cholesterol, high density lipoprotein cholesterol (HDL-C), calculated low density lipoprotein cholesterol (LDL-C), and triglycerides. Total cholesterol and triglycerides were measured using cholesterol oxidase phenol 4-aminoantipyrine peroxidase method and glycerol phosphate oxidase phenol 4-aminoantipyrine peroxidase method respectively. HDL-C was assayed using a second-generation homogenous direct method (Roche Diagnostics, Burgess Hill, UK). All tests were performed on a Randox daytona+ analyser (Randox Laboratories, Crumlin, UK). LDL-C was estimated using the Friedewald formula [15]. HbA1c was measured using standard laboratory methods in the Department of Biochemistry, Manchester University Foundation Trust (taking part in the United Kingdom National External Quality Assessment Service).

Assessment of Neuropathy

Each subject underwent assessment of the neuropathy symptoms profile (NSP) which evaluates sensory, motor and autonomic symptoms giving a score out of 38 [16], and the neuropathy disability score (NDS) which assesses vibration perception, pin prick, temperature sensation and presence or absence of ankle reflexes to give a score out of 10 [17]. Vibration perception threshold (VPT) was quantified on both toes using a neurothesiometer (Horwell, Scientific Laboratory Supplies, Wilford, Nottingham, UK). Cold thermal (CT) and warm thermal (WT) thresholds were assessed on the dorsolateral aspect of the left foot (S1) using the TSA-II NeuroSensory Analyser (Medoc Ltd., Ramat-Yishai, Israel). Deep breathing heart rate variability (DB-HRV) was assessed using the ANX 3.0 autonomic nervous system monitoring device (ANSAR Medical Technologies Inc., Philadelphia, PA, USA) by measuring the heart rate response to deep breathing over two 8-cycle breathing series separated by a 5-min period of normal breathing [18].

Ophthalmic assessment

Patients underwent CCM in both eyes using the Heidelberg Retinal Tomograph III Rostock Cornea Module (Heidelberg Engineering GmbH, Heidelberg, Germany) as per our previously established protocol [19]. Six non-overlapping images (3 per eye) from the central cornea and four (2 per eye) images from the IW region were selected and manually quantified in a masked fashion following our previously established protocols [20, 21] using purpose designed software called “CCMetrics” (University of Manchester, UK) [22].

Six corneal nerve fibre parameters were quantified: corneal nerve fibre density (CNFD) — the total number of major nerves per square millimetre (No./mm^2), corneal nerve branch density (CNBD) — the number of branches emanating from the major nerves per square millimetre (No./mm^2), corneal nerve fibre length (CNFL) — the total length of all nerve fibres and branches per square millimetre (mm/mm^2), inferior whorl length (IWL) — the total length of the nerve structures per square millimetre in the inferior whorl region (mm/mm^2), average nerve fibre length (ANFL= CNFL+IWL/2)(mm/ mm^2), and total nerve fibre length (TNFL=CNFL+IWL)(mm/ mm^2).

Statistical analysis

Statistical analysis was carried out on SPSS (Version 19.0, IBM Corporation, New York, USA) and Prism (Version 9.0 for macOS). Data normality was determined using the Shapiro-Wilk Normality test and by direct visualisation of the histogram and normal Q-Q plots. Data were expressed as mean ± standard deviation (SD) for parametric variables or median (interquartile range, IQR) for non-parametric variables. To test for differences among groups, we used a one-way analysis of variance (post hoc – Tukey) for normally distributed data and Friedman’s test for non-normally distributed data. Pearson’s correlation coefficient (Spearman’s for non-parametric) was calculated to assess the correlations between different variables. Receiver operating characteristic (ROC) curves were used to compare the diagnostic utility of different CCM parameters. To define the optimum cut-off points with highest sensitivity and specificity

for the diagnosis of diabetic peripheral neuropathy (DPN), Youden's index (J=Sensitivity+specificity-1) was measured. The relative difference between the IW and central corneal nerves was evaluated by determining the percentage difference between controls and patients by the controls. A significant *p* value was <0.05.

Results

Clinical demographic and biochemical parameters

Clinical and demographic data are presented in Table 1.Age in years was comparable (p=0.2) between controls (48 ± 14) and patients without (46 ± 11) and with (52 ±11) diabetes. There were no significant differences in sex (p=0.5) and systolic (p=0.2) and diastolic blood pressure (p=0.3) between groups. BMI was significantly higher in both groups: without (46.6±11.3) (p < 0.0001) and with (47.3±11.9) (p < 0.001) diabetes compared to controls with no difference between the two groups (p = 0.9). HbA1c was significantly higher (p<0.0001) in obese subjects with (53.9±11.1) compared to without (39.7±4.38) diabetes and controls (32.6±4.62). Although below the diabetes threshold, the HbA1c was higher in obese subjects without diabetes compared to controls (p<0.0001). There were no significant differences in total cholesterol (p = 0.2) or triglycerides (p = 0.9) between groups. LDL-C was significantly lower in patients with compared to without diabetes (2.21±1.19 vs. 3.05±0.96, p = 0.001) and HDL-C was significantly lower in the patient without (1.21±0.35, p = 0.006) and with diabetes (1.22±0.37, p = 0.007) compared to controls (1.58±0.43).

Neuropathy assessments

The results of neuropathy assessments are presented in Table 2. The neuropathy symptom profile (NSP) was significantly higher in patients with (p<0.0001) and without diabetes (p<0.0001) compared to controls. The NDS was significantly higher in patients without (p =0.001) and with diabetes (p = 0.001) compared to controls. VPT was significantly higher in with patients with diabetes [8.00 (5.00-16.5), p=0.001] compared to controls [4.50 (3.00-5.50). CT (p = 0.8) and WT (p = 0.5) did not differ among subjects and controls. DB-HRV was significantly lower in patients with diabetes [15.0 (10.0-21.5)] compared to controls (26.8 (17.8-33.5, p= 0.01) with no difference compared to those without diabetes [20.5 (11.8-33.0), p=0.9].

Corneal Confocal Microscopy

The results of CCM are presented in Table 3 and depicted graphically in figure 1. Example images from the central and peripheral cornea from recruited patients are shown in figure 2. Patients with and without diabetes had significantly lower CNFD (P<0.0001, P<0.0001), CNBD (P=0.001, P=0.002), CNFL (P<0.0001, P<0.0001), IWL (P<0.0001, P<0.0001), ANFL (P<0.0001, P<0.0001) and TNFL (P<0.0001, P<0.0001) compared to controls. There was no significant difference in CNFD (P=0.9), CNBD (P=0.9), CNFL (P=0.1),

IWL (P=0.9), ANFL (P=0.3) and TNFL (P=0.5) between obese patients with and without diabetes. In subjects without and with diabetes, the percentage reduction in IWL (49.3%, 47.3%) was greater compared to CNFL (34.1%, 25.5%) compared to controls, respectively.

Correlations

NDS correlated significantly with CNBD ($r = -0.35$, $p < 0.05$), IWL ($r = -0.27$, $p < 0.05$), ANFL ($r = -0.28$, $p < 0.05$) and TNFL ($r = -0.28$, $p < 0.05$) and ANFL correlated with WT ($r = -0.46$, $p = 0.008$) amongst subjects. There were no correlations between corneal nerve parameters, lipid parameters, HbA1c or BMI.

Diagnostic utility of CCM parameters.

The diagnostic ability of CCM parameters to identify neuropathy (NDS greater than or equal to 3) in obese subjects was determined by construction of receiver operator curves (Figure 3). Outliers, defined as values greater than the 90^{th} percentile of the data were excluded. The area under the curve (AUC) for CNFD was 0.62, CNBD was 0.70, CNFL was 0.67, IWL was 0.72 and TNFL was 0.75 (Table 4)

Discussion

The underlying pathophysiology of obesity-related peripheral neuropathy is poorly understood [12]. This study shows a significant reduction in central corneal nerve fibre parameters in obese subjects with and without diabetes, in agreement with our previously published studies in subjects with obesity [13, 23] and diabetes [5, 24]. We now further report greater corneal nerve damage in the more distal inferior whorl compared to the central region providing support for a dying-back neuropathy, consistent with studies in adults [25, 26] and children [27] with diabetes.

Small fibre damage is known to precede large fibre damage in diabetes [28], although the diagnosis of small fibre neuropathy (SFN) is not made easily given that the current diagnostic gold standard — skin biopsy — is not widely available in routine clinical practice [29]. Our cohort of patients had mild clinical neuropathy based on the NSP and ND and the thermal thresholds were normal, which contrasts with our previous study in patients with obesity with more severe neuropathy [13, 23]. Despite a mild neuropathy, there was significant distal and proximal corneal nerve loss in subjects with and without diabetes. There was also evidence of cardiac autonomic neuropathy only in obese subjects with diabetes, consistent with previous studies [23]. CCM has been used to show both progression of neuropathy in patients with type 2 diabetes over 6.5 year [5] and nerve regeneration in patients with type 1 diabetes after simultaneous pancreas-kidney transplantation [30] and in subjects with obesity with [23] and without diabetes [31] after bariatric surgery. This suggests that it has utility to capture change in neuropathy over time.

NDS is a widely used neurological assessment for the diagnosis of diabetic neuropathy [32] and other neuropathies [33] and has been related to the severity of sural nerve pathology [34] and deficits in nerve

conduction velocity and amplitude [35]. Despite being a measure of established neuropathy, we found significant correlations between NDS and CNBD, IWL, ANFL and TNFL.

In 2008, Devigilli et al. proposed diagnostic criteria for SFN based on at least 2 abnormalities from the distal sensory exam, thermal thresholds and IENFD [36]. However, Botez et al. [29] have challenged the inclusion of IENFD as it may be normal in patients with a high suspicion of SFN [37]. Previously we found that CCM has comparable diagnostic accuracy to IENFD for the diagnosis of diabetic neuropathy [38] [39]. More recently, our metanalysis which included over 4000 patients with diabetes showed that CCM detects corneal nerve loss in both sub-clinical and clinical DPN [40]. Furthermore, in patients with SFN we have recently shown that abnormal distal IENFD and neurological examination most frequently reflected small fibre disease. However, CCM further enhanced diagnosis, whilst QST, QSART, and proximal IENFD were of lower impact [41].

Conclusion

Small fibre damage occurs in patients with obesity both with and without type 2 diabetes and can be detected using CCM. Here we have demonstrated evidence of peripheral neuropathy characterised by neuropathic symptoms, neurological deficits, elevated vibration perception and autonomic dysfunction with a dying-back neuropathy affecting the most distal corneal nerves in obese subjects with and without type 2 diabetes.

Declarations

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Author Contributions

H.S & R.A.M conducted literature searches and conceived the idea for this study. Z.I, J.H.H, S.A recruited patients to the study, collected data, interpreted, and analysed data, M.F & A.K. analysed and interpreted data. Z.I wrote the first draft of the manuscript. Y.L, R.D, A.S, B.A & B.B. contributed to data analysis and interpretation. All authors have critically reviewed the final version of the manuscript. H.S funded the study

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Tables

Table 1. Demographic, anthropometric and biochemical assessment in controls and subjects without (DM^-) and with (DM^+) diabetes. * represents significant difference compared to controls; †represents significant difference compared to subjects without diabetes. BMI, body mass index. BP, blood pressure. LDL, low density lipoprotein. HDL, high density lipoprotein. Data presented as Mean±SD or Median (Interquartile range).

	Controls	Without Diabetes (DM^-)	With Diabetes (DM^+)	P value
Number	21	27	30	
Age (years)	48 ± 14	46 ± 11	52 ± 11	0.22
Sex (Female/ Male)	12/9	22/5	24/6	0.5
Anthropometric				
BMI (kg/m^2)	26.2 ± 5.4	46.6 ± 11.3*	47.3 ± 11.9*	**< 0.0001**
Systolic BP (mmHg)	121 ± 19.7	129 ± 18	129 ± 17	0.24
Diastolic BP (mmHg)	67.0 ± 9.97	72.9 ± 15.2	70.9 ± 11.1	0.30
Biochemical				
HbA1c (mmol/mol)	32.6 ± 4.62	39.7 ± 4.38*	53.9 ± 11.1*†	**<0.0001**
Total cholesterol (mmol/L)	4.97 ± 0.89	4.97 ± 1.19	4.55 ± 1.04	0.24
Triglycerides (mmol/L)	1.5 (0.9 – 1.95)	1.55 (1.23 – 2.04)	1.60 (0.9- 1.89)	0.93
LDL cholesterol (mmol/L)	2.7 ± 0.71	3.05 ± 0.96	2.21 ± 1.19†	0.012
HDL Cholesterol (mmol/L)	1.58 ± 0.43	1.21 ± 0.35*	1.22 ± 0.37*	**0.01**

Table 2. Neuropathy assessment in controls and subjects without (DM^-) and with (DM^+) diabetes. * represents significant difference compared to controls. NSP, neuropathy symptom profile. NDS, neuropathy disability score. VPT, vibration perception threshold. CT, cold perception threshold. WT, warm perception threshold. DB-HRV, deep breathing heart rate variability. All data presented as Median (Interquartile range).

	Controls	Without Diabetes (DM^-)	With Diabetes (DM^+)	P value
NSP (0-38)	0.0 (0 – 0)	4 (2 – 8)*	3 (2 – 7)*	**< 0.0001**
NDS (0-10)	0.0 (0 – 0)	2 (0 – 4)*	2 (0 – 4)*	**0.0004**
VPT (V)	4.50 (3.00 – 5.50)	4.58 (3.21 – 8.28)	8.00 (5.00 – 16.5)*	**0.001**
WT (°C)	36.5 (35.2 – 39.5)	39.0 (36.8 – 40.0)	37.0 (36.4 – 41.2)	0.52
CT (°C)	28.7 (26.4 – 29.1)	27.8 (26 – 29.1)	28.5 (26.3 – 30.0)	0.87
DB-HRV (beats /min)	26.8 (17.8 – 33.5)	20.5 (11.8 – 33)	15.0 (10.0 – 21.5)*	**0.01**

Table 3. Corneal nerve parameters in controls and subjects without (DM^-) and with (DM^+) diabetes. * represents significant difference compared to controls. CNFD, corneal nerve fibre density. CNBD, corneal nerve branch density. CNFL, corneal nerve fibre length. IWL, inferior whorl length. ANFL, average nerve fibre length. TNFL, total nerve fibre length. All data presented as Mean±SD

	Controls	Without Diabetes (DM^-)	With Diabetes (DM^+)	P value
CNFD (no./mm^2)	35.3 ± 7.41	26.8 ± 6.22*	26.8 ± 6.01*	**< 0.0001**
CNBD (no./mm^2)	88.2 ± 31.1	55.4 ± 28.2*	58.4 ± 28.5*	**0.0006**
CNFL (mm/mm^2)	26.7 ± 5.31	17.6 ± 4.43*	19.9 ± 5.43*	**< 0.0001**
IWL (mm/mm^2)	35.3 ± 9.70	17.9 ± 6.10*	18.6 ± 7.42*	**< 0.0001**
ANFL (mm/mm^2)	31.0 ± 6.10	17.4 ± 4.81*	19.3 ± 5.50*	**< 0.0001**
TNFL (mm/mm^2)	62.0 ± 12.3	35.5 ± 9.58*	38.5 ± 11.0*	**< 0.0001**

Table 4. AUC with optimal cut-off and sensitivity and specificity for the diagnosis of peripheral neuropathy in obese subjects.

	AUC	p value	Optimal cut off	Sensitivity (%)	Specificity (%)
CNFD	0.62	0.144	28.64 no./mm^2	78	53
CNBD	0.70	**0.017**	36.01 no./mm^2	50	88
CNFL	0.67	**0.028**	16.65 mm/mm^2	67	68
IWL	0.72	**0.009**	17.69 mm/mm^2	72	74
TNFL	0.75	**0.003**	33.86 mm/mm^2	77	74

Figures

Figure 1

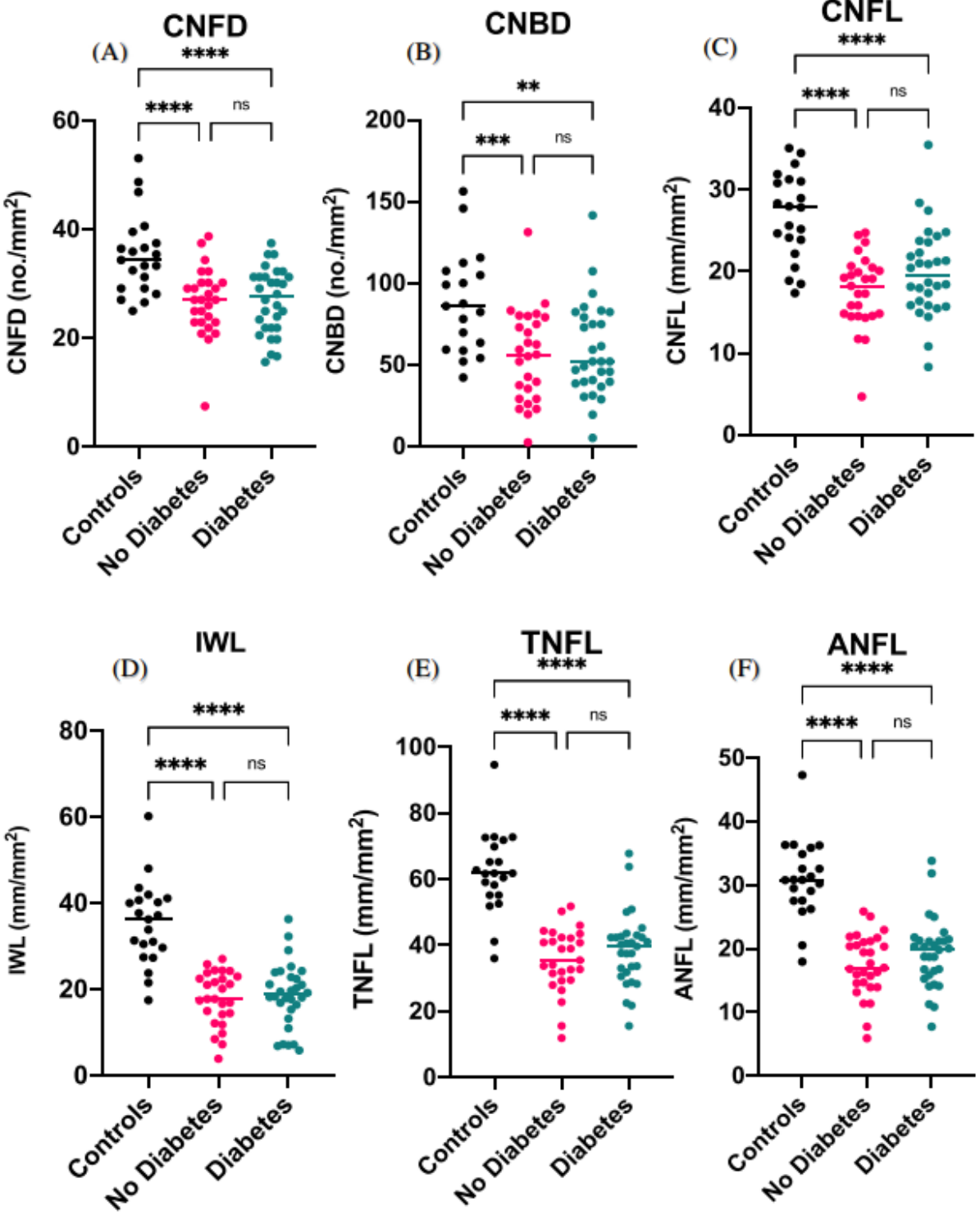

Figure 1

Corneal nerve parameters in controls and obese subjects without and with diabetes. Corneal nerve fibre density (CNFD) (A), Corneal nerve branch density (CNBD) (B), Corneal nerve fibre length (CNFL) (C), Inferior whorl length (IWL) (D) , Total nerve fibre length (TNFL) (E), Average nerve fibre length (F).

Figure 2

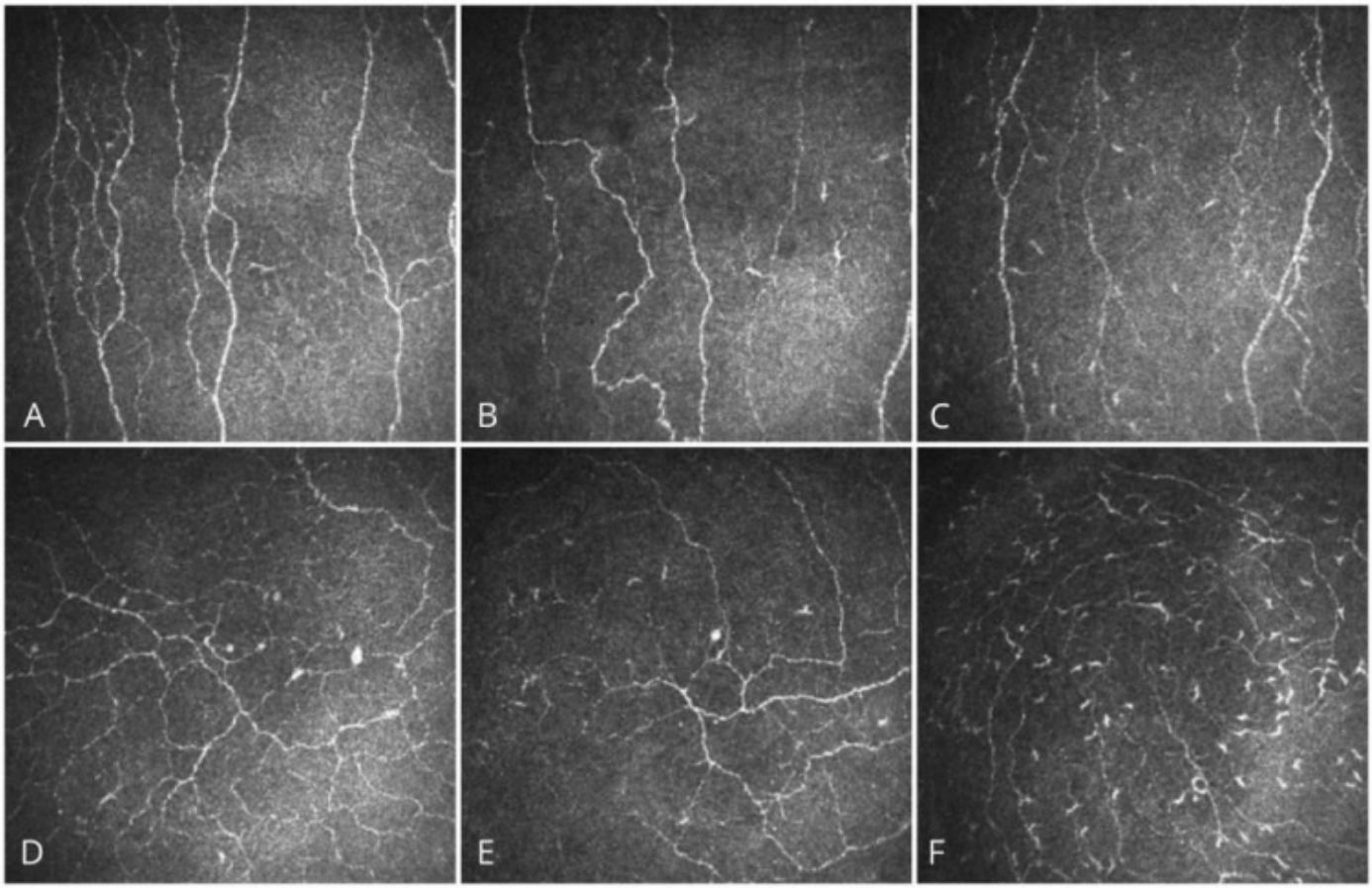

Figure 2

CCM images of the sub-basal nerve plexus from the central cornea in a healthy subject (A), obese subjects without (B) and with (C) diabetes and inferior whorl in a healthy subject (D), obese subjects without (E) and with (F) diabetes.

Figure 3

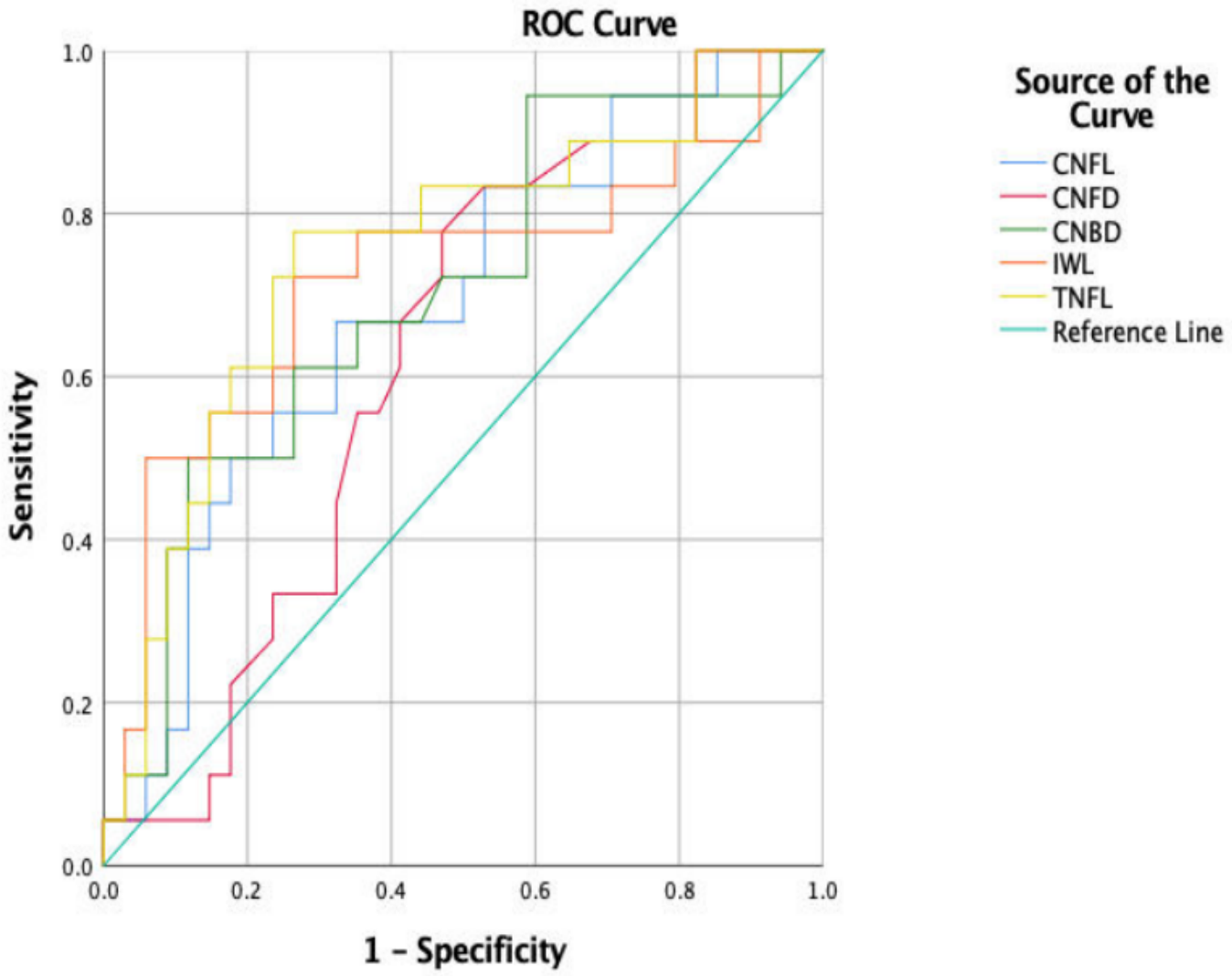

Figure 3

ROC curves for CNFD – corneal nerve fibre density, CNBD – corneal nerve branch density, CNFL – corneal nerve fibre length, IWL – inferior whorl length and TNFL – Total nerve fibre length.