# Corneal Confocal Microscopy Detects Neuropathy in Subjects With Impaired Glucose Tolerance

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## OBJECTIVE

**Impaired glucose tolerance (IGT) represents one of the earliest stages of glucose dysregulation and is associated with macrovascular disease, retinopathy, and microalbuminuria, but whether IGT causes neuropathy is unclear.**

## RESEARCH DESIGN AND METHODS

**Thirty-seven subjects with IGT and 20 age-matched control subjects underwent a comprehensive evaluation of neuropathy by assessing symptoms, neurological deficits, nerve conduction studies, quantitative sensory testing, heart rate variability deep breathing (HRVdb), skin biopsy, and corneal confocal microscopy (CCM).**

## RESULTS

**Subjects with IGT had a significantly increased neuropathy symptom profile ($P < 0.001$), McGill pain index ($P < 0.001$), neuropathy disability score ($P = 0.001$), vibration perception threshold ($P = 0.002$), warm threshold ($P = 0.006$), and cool threshold ($P = 0.03$), with a reduction in intraepidermal nerve fiber density ($P = 0.03$), corneal nerve fiber density ($P < 0.001$), corneal nerve branch density ($P = 0.002$), and corneal nerve fiber length ($P = 0.05$). No significant difference was found in sensory and motor nerve amplitude and conduction velocity or HRVdb.**

## CONCLUSIONS

**Subjects with IGT have evidence of neuropathy, particularly small-fiber damage, which can be detected using skin biopsy and CCM.**

The association between impaired glucose tolerance (IGT) and peripheral neuropathy was first highlighted when subjects with idiopathic small-fiber neuropathy were found to have an unexpectedly high prevalence of IGT (1). Subsequently in the population-based San Luis Valley (2) and Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA)/Cooperative Health Research in the Region Augsburg (KORA) (3) studies, neuropathy occurred in 26–28% of patients with diabetes, in 11–13% of those with IGT, and in 4–8% of control subjects. In contrast, Dyck et al. (4) did not find an increased prevalence of neuropathy among subjects with impaired glycemia.

Establishing neuropathy in IGT is important because it may provide insights into the early pathogenetic components of diabetic neuropathy and highlights that neuropathy may occur with minimal metabolic derangement. The detection of peripheral neuropathy in IGT remains challenging, especially because most of the studies have used a combination of symptoms and neurologic signs that are large-fiber weighted. An increasing body of evidence suggests a predominantly small-fiber

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neuropathy, with a significant reduction in intraepidermal nerve fiber density (IENFD) and minimal large-fiber involvement in subjects with IGT (5).

Corneal confocal microscopy (CCM), a novel surrogate measure of small-fiber neuropathy, has been shown to detect early small-fiber damage in diabetic patients (6,7). The purpose of this study was to undertake a comprehensive assessment of neuropathy in subjects with IGT using symptoms and neurological deficits, neurophysiology, quantitative sensory testing, and in particular, skin biopsy and CCM, as sensitive measures of small-fiber neuropathy.

## RESEARCH DESIGN AND METHODS

### Study Subjects

The study comprised 37 subjects aged 30–75 years with IGT (oral glucose tolerance test: 2-h glucose = 7.8–11.1 mmol/L) and 20 age-matched control subjects with glucose tolerance within normal limits. Subjects with any other cause of peripheral neuropathy, active corneal disease, surgery, or chronic contact lens use were excluded. This research adhered to the tenets of the declaration of Helsinki and was approved by the North Manchester Research Ethics committee. Informed written consent was obtained from all subjects before participation.

### Clinical and Peripheral Neuropathy Assessment

All subjects underwent assessment of systolic and diastolic blood pressure, BMI, $HbA_{1c}$, lipid fractions (total cholesterol, LDL, HDL, and triglycerides), and estimated glomerular filtration rate. Signs and symptoms of neuropathy were assessed using the neuropathy symptom profile, neuropathy disability score, vibration perception threshold (VPT; Horwell Scientific Laboratory Supplies, Wilford, Nottingham, U.K.), and cool (CT) and warm (WT) thresholds (Medoc Ltd., Ramat-Yishai, Israel). Sural sensory nerve amplitude and conduction velocity, and peroneal motor nerve amplitude and conduction velocity were assessed. Heart rate variability deep breathing (HRVdb) was assessed with an ANX 3.0 autonomic nervous system monitoring device (ANSAR Medical Technologies Inc., Philadelphia, PA). Sudomotor function was assessed using Neuropad (miro Verbandstoffe, Wiehl-Drabenderhole, Germany).

### Skin Biopsy

A 3-mm punch skin biopsy specimen was obtained from the dorsum of the foot ~2 cm above the second metatarsal head after local anesthesia (1% lidocaine), and IENFD was quantified in accord with established criteria (8).

### CCM

Patients underwent examination with CCM (Heidelberg Engineering GmbH, Heidelberg, Germany), and three established corneal nerve parameters—corneal nerve fiber density (CNFD), corneal nerve branch density (CNBD), and corneal nerve fiber length (CNFL)—were quantified in a masked fashion, as previously described (9).

### Statistical Analysis

SPSS for Mac version 19.0 software (IBM Corp., Armonk, NY) was used for descriptive and frequency statistics. An unpaired *t* test (or nonparametric Mann-Whitney *U* test) was used to study differences between means. All data are expressed as mean ± SEM, and a $P < 0.05$ was considered significant.

## RESULTS

### Clinical Assessment

Subjects with IGT and control subjects were matched for age. Subjects with IGT had a significantly greater 2-h glucose level for the oral glucose tolerance test (9.2 ± 1.0 vs. 6.5 ± 0.6 mmol/L, $P = 0.001$), and they had a higher BMI (31.7 ± 1.0 vs. 27.9 ± 1.2 kg/m$^2$, $P = 0.02$) and $HbA_{1c}$ (6.0 ± 0.2/43.9 ± 1.0 vs. 5.4 ± 0.1/36.0 ± 0.3 mmol/mol, $P < 0.001$), with a lower total (4.8 ± 0.2 vs. 5.5 ± 0.2 mmol/L, $P = 0.02$) and HDL (1.2 ± 0.1 vs. 1.7 ± 0.1 mmol/L, $P < 0.001$) cholesterol compared with control subjects. There was no difference in systolic/diastolic blood pressure (132/78 vs. 137/79 mmHg, $P = 0.5$), LDL cholesterol (2.1 ± 1.1 vs. 3.2 ± 0.6 mmol/L, $P = 0.1$), and triglycerides (2.8 ± 1.0 vs. 1.7 ± 0.9 mmol/L, $P = 0.2$).

### Neuropathy Assessment

The neuropathy symptom profile (4.1 ± 1.0 vs. 0.5 ± 0.2, $P < 0.001$), the McGill pain index (2.8 ± 0.3 vs. 0.2 ± 0.1, $P < 0.001$), the neuropathy disability score (2.9 ± 0.5 vs. 0.6 ± 0.2, $P = 0.001$), VPT (15.9 ± 2.3 vs. 6.5 ± 1.1, $P = 0.002$), and WT (40.6 ± 0.8 vs. 37.6 ± 0.6, $P = 0.006$) were significantly increased, whereas CT (24.9 ± 1.3 vs. 27.5 ± 0.6, $P = 0.03$), neuropad response (71.0 ± 2.8% vs. 93.0 ± 5.6%, $P = 0.05$), IENFD (6.3 ± 0.6 vs. 9.1 ± 0.7, $P = 0.03$), CNFD (27.6 ± 1.2 vs. 37.4 ± 1.6, $P < 0.001$), CNBD (55.8 ± 6.0 vs. 89.2 ± 8.4, $P = 0.02$), and CNFL (22.1 ± 1.2 vs. 25.7 ± 1.2, $P = 0.05$) were significantly decreased in the IGT group compared with the control group (Fig. 1). There was no significant difference in sural sensory nerve amplitude (14.0 ± 1.4 vs. 16.6 ± 1.9, $P = 0.2$) and conduction velocity (49.9 ± 0.9 vs. 49.9 ± 1.0, $P = 0.8$), in peroneal motor nerve amplitude (4.6 ± 0.4 vs. 5.3 ± 0.5, $P = 0.1$) and conduction velocity (45.6 ± 0.7 vs. 47.5 ± 0.7, $P = 0.1$), or in HRVdb (9.5 ± 6.9 vs. 11.9 ± 6.9, $P = 0.09$).

Under the assumption that CNFD is normally distributed in controls and IGT (Shapiro-Wilk W test, $P > 0.05$) and based on a cutoff point of 2 SD from the control average (CNFD = 24.0 no./mm$^2$), subjects with IGT were restratified into two groups: 22 without IGT neuropathy (IGTN; CNFD >24.0 no./mm$^2$) and 15 (40.5%) with IGTN (CNFD <24.0 no./mm$^2$). There was significantly greater self-reported pain intensity (McGill Pain Index, $P = 0.04$) and reduction in CNBD ($P = 0.02$) and CNFL ($P < 0.001$) in subjects with IGTN compared with IGT (Fig. 1*E*).

## CONCLUSIONS

A recent study has shown a significant reduction in IENFD and abnormal corneal nerve morphology in patients with type 2 diabetes of short duration, suggesting that neuropathy may be an early complication (10), and of course, longitudinal data from the Rochester cohort (11) suggest that duration and severity of exposure to hyperglycemia are related to the severity of neuropathy. In the current study, we show a significant increase in neuropathic symptoms, consistent with the MONICA/KORA Augsburg Surveys (3), which also found a threefold increase in neuropathic pain in subjects with IGT. We also show a significant alteration in sudomotor function, whereas cardiac autonomic function and electrophysiology were normal, similar to a previous study in subjects with IGT demonstrating an abnormal sympathetic skin response but normal results on electrophysiology and standard autonomic function tests (12).

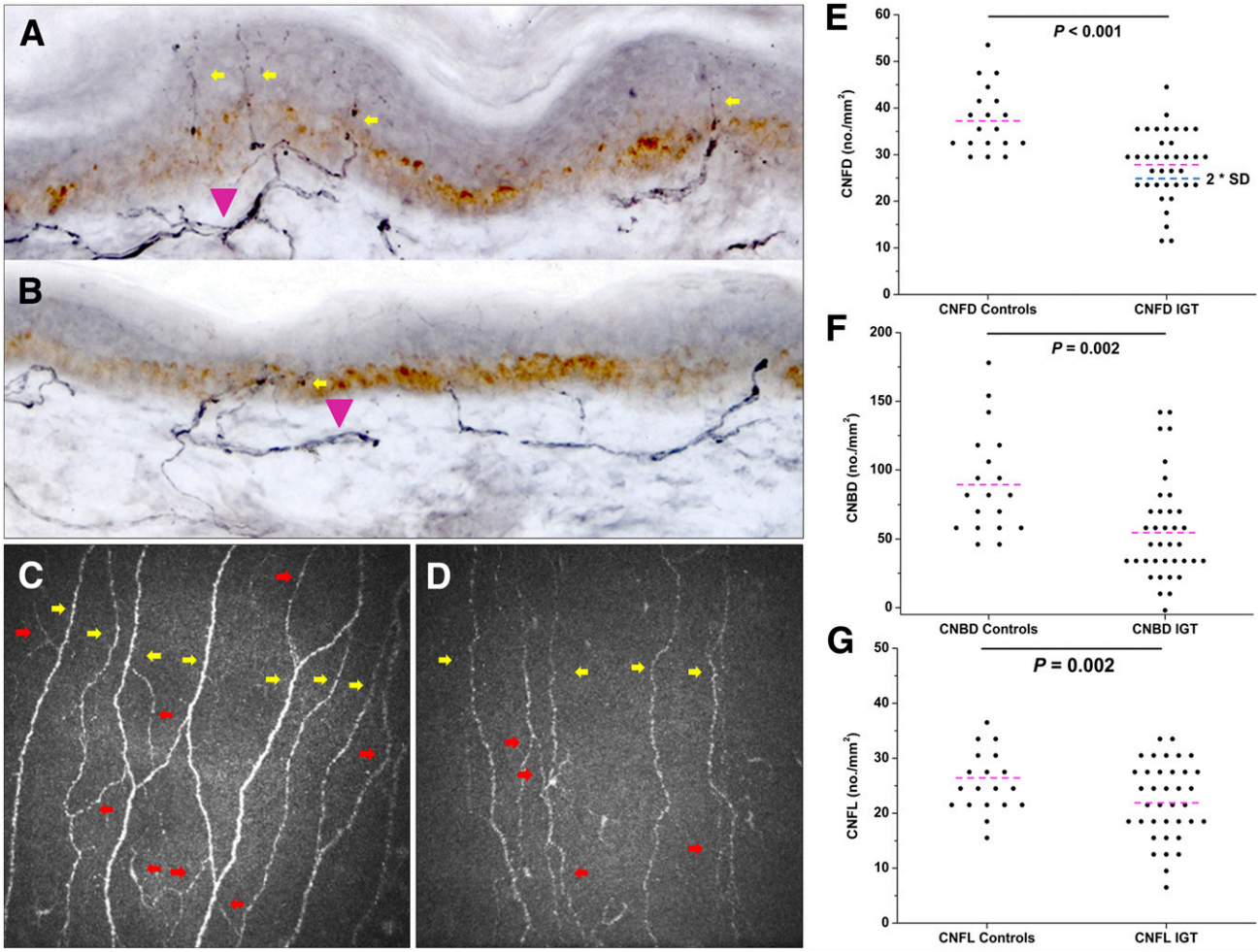


**Figure 1**—Skin punch biopsy specimens immunostained for PGP9.5 (*A* and *B*) and CCM images (*C* and *D*) from a healthy control subject vs. a subject with IGT. The graphs show the distribution of CNFD (*E*), CNBD (*F*), and CNFL (*G*) in control subjects vs. IGT subjects. In *C* compared with *D*, a significant reduction in corneal nerve fibers (yellow arrows) and nerve branches (red arrows) is observed, which mirrors the reduction in the same subject in intraepidermal nerve fibers (yellow arrows) reaching the upper levels of epidermis in *B* compared with *A*. The subepidermal nerve plexus is also visible (purple arrowhead). Data points in *E*, *F*, and *G* represent actual corneal subbasal nerve parameters in control subjects ($n$ = 20) vs. IGT subjects (n = 37). The purple dashed lines represent group averages, and the blue dashed line in *E* represents a cutoff for "risk of neuropathy" (IGTN).

These latter findings are similar to a large population-based study that also showed no electrodiagnostic abnormalities in subjects with impaired fasting glucose or IGT (4). However, we demonstrate a significant abnormality in VPT, WT, and CT, similar to the San Luis Valley study (2), which also reported impaired VPT in 11.2% of subjects with IGT compared with 3.5% in control subjects.

Previous studies have demonstrated a reduction in IENFD in subjects with IGT, which improved after lifestyle intervention (5), suggesting that this early abnormality may be amenable to treatment. We now confirm a significant reduction in IENFD in subjects with IGT. In addition, we also demonstrate a significant abnormality in corneal nerve morphology using the noninvasive technique of CCM and indeed show that 40.5% of subjects with IGT have significant small-fiber damage based on CNFD reduction. CCM provides a unique opportunity to noninvasively and rapidly assess unmyelinated C fibers in vivo, which has important diagnostic (6) and prognostic (8) implications. In conclusion, this study shows evidence of neuropathy in subjects with IGT, as evidenced by abnormal symptoms, signs, quantitative sensory testing, skin biopsy, and CCM, but not neurophysiology (13).

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