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No Difference in Small or Large Nerve Fiber Function Between Individuals With Normal Glucose Tolerance and Impaired Glucose Tolerance.

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Running title: Neuropathy and glucose metabolism

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**Objective**

To assess small and large nerve fiber function in persons with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and type 2 diabetes (T2D).

**Research Design and Methods**

Participants were recruited consecutively from a population-based cohort; NGT (n=39), IGT (n=29) and T2D (n=51). Electrophysiological measures included nerve conduction studies and thermal thresholds. Intraepidermal nerve fiber density (IENFD) in skin biopsies was calculated.

**Results**

There was no difference between IGT and NGT in sural nerve conduction, IENFD and thermal thresholds. IENFD was significantly lower in T2D (median 2.8 fibers/mm, IQR 1.1–4.7) than NGT individuals (median 4.5 fibers/mm, IQR 3.4–6.1, p<0.05). Type 2 diabetes participants had poorer nerve conduction and higher heat thresholds than NGT and IGT.

**Conclusions**

Large and small nerve function in people with IGT did not differ from those with NGT. Our finding does not support the existence of neuropathy in a pre-diabetic stage.

**Abbreviations**

<table>
<thead>
<tr>
<th>Term</th>
<th>Abbreviation</th>
</tr>
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<tbody>
<tr>
<td>Amplitude</td>
<td>AMP</td>
</tr>
<tr>
<td>Conduction velocity</td>
<td>CV</td>
</tr>
<tr>
<td>Impaired glucose tolerance</td>
<td>IGT</td>
</tr>
<tr>
<td>Intraepidermal nerve fiber density</td>
<td>IENFD</td>
</tr>
<tr>
<td>Normal glucose tolerance</td>
<td>NGT</td>
</tr>
</tbody>
</table>
A high prevalence of IGT in individuals with idiopathic neuropathy has been reported (1), but whether neuropathy already exists in the pre-diabetic stage, i.e. impaired glucose tolerance (IGT) is unknown (2, 3). In a population-based study, neuropathy was marginally more common in IGT than in normoglycemic controls (4), but others reported no difference in measures of neuropathy between IGT and normal glucose tolerance (NGT) (5, 6).

When addressing the question of whether ‘IGT neuropathy’ truly exists, objective measures of nerve dysfunction are frequently crude and focused on large nerve fibers, while small nerve fiber dysfunction is often overlooked (1, 4, 6).

Thus, our aim was to study measures of both small and large nerve function in well-characterized normoglycemic, IGT, and type 2 diabetes individuals.

**Research Design and Methods**

**Study population**

The study population, their glycemic status verification and other possible causes of neuropathy were considered and have been described earlier (7). All individuals gave informed consent to participation. The regional ethical review board of Umeå University, Umeå, Sweden, approved the study.

**Measurements**

Blood samples were drawn and measured for cholesterols, triglycerides, creatinine, fasting plasma glucose and HbA1c. Anthropometry and other measurements have been described elsewhere (7).
Neurophysiological assessment

Nerve conduction
Standardized motor and sensory nerve conduction studies were performed on the right peroneal and sural nerve by a neurophysiologist blinded to the individuals’ group identity.

Thermal threshold testing
Thermal threshold tests were performed with Thermotest® equipment (Somedic AB, Hörby, Sweden) by using the method of limits (8).

Skin biopsy
Thin skin biopsies (5 µm) were taken for microscopical assessment. Procedures were developed (10) and modified (11) from published guidelines (9). The intraepidermal nerve fiber density (IENFD) denotes number of fibers/mm of epidermal length (mean counts in three sections). Intra- and inter-observer reliabilities were $r_i = 0.98$ and 0.84, respectively.

Statistical analyses
Data are presented as numbers (n), proportions (%) and distribution as mean and standard deviation (SD) or median and interquartile range (IQR). Differences between groups were tested by ANOVA and subsequent Student’s t-test for normally distributed variables. For non-normally distributed variables, the Kruskal-Wallis test was applied with subsequent Mann-Whitney U-testing. A p-value < 0.05 was considered statistically significant. Statistical analyses were performed with SPSS 19 (SPSS Inc., Chicago, IL).
Results

Baseline characteristics
Clinical characteristics of the 119 participants are presented in Table 1. Ages were similar in all three groups. People with IGT showed no significant metabolic differences compared to NGT, while patients with diabetes had metabolic perturbations compared to both NGT and IGT (Table 1).

Nerve conduction
Sural nerve conduction did not differ between IGT and NGT. No difference was seen in sural amplitude between the groups (Table 1). People with IGT had a lower conduction velocity of the peroneal nerve than those with NGT. The conduction velocity of the peroneal and sural nerve was lower in type 2 diabetics compared with NGT individuals (Table 1).

Thermal thresholds
There were no differences in heat or cold thresholds between IGT and NGT (Table 1). The proportion of abnormal heat thresholds was significantly higher in individuals with type 2 diabetes than NGT and IGT.

IENFD
IENFD did not differ significantly between IGT and NGT (Table 1), but was significantly lower in T2D compared with NGT. Women had higher IENFD than men (median 4.8 fibers/mm, IQR 3.2–6.4 vs. median 2.7 fibers/mm, IQR 1.6–4.7, p<0.001). However, there was no interaction between gender and small or large nerve fiber function (data not shown).
Conclusions

IGT individuals did not show different large and small nerve fiber function compared with NGT. As expected, patients with T2D had poorer small and large nerve fiber function than NGT and IGT.

Impaired glucose tolerance and nerve dysfunction

It is not clear if neuropathy is found in the pre-diabetic individuals with IGT (2-4, 6). A high prevalence of IGT in individuals with idiopathic neuropathy has been reported (1, 12), but these were individuals with existing neuropathy and in whom glycemic status was subsequently assessed. The retrospective study design is less appropriate for ascribing IGT as a potential cause of neuropathy. A reduction in IENFD has been reported in individuals with diabetes without clinical or electrophysiological indications of nerve dysfunction (13). In addition, it has been reported that there is a loss of IENFD in individuals with IGT (14, 15) suggestive of small nerve fiber dysfunction being present in a pre-diabetic stage. In a population-based study neuropathy was marginally increased in IGT, but the measure of neuropathy was rather crude and mainly on large fiber (4). One recent similar study showed no difference between IGT and NGT (6); however, no detailed measures of small nerve fiber function, particularly IENFD, were assessed.

Limitations and strengths

Our study is limited by a relatively small group size, which probably reduced the power to detect differences in IENFD between groups. However, our study provides detailed assessment of nerve function in individuals with IGT and NGT without any trend in results suggesting differences between the two groups. Secondly, the cross-sectional design did not enable us to study cause and effect. Moreover, when assessing IENFD, we used thin sections
of 5 µm as compared to thick 50 µm sections suggested by published guidelines. However, it still allows for group comparison between NGT, IGT and type 2 diabetes within our study, but hampers comparisons to studies using thicker sections.

Strengths of our study are that all individuals were recruited consecutively from a population-based sample, were well-defined in terms of glycemic status with a strict definition of IGT based on two oral glucose tolerance tests, and the participants were all of the same age. To avoid bias, neurophysiologic measurements were performed by personnel blinded for glucose status of participants.

In conclusion, we found no significant differences in large and small nerve function between IGT and NGT. Our finding questions the existence of neuropathy in a pre-diabetic stage.

**Acknowledgments**

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No potential conflicts of interest relevant to this article were reported.

K.P. analyzed data, evaluated the skin biopsies, and wrote the manuscript. L.B.D. contributed to the discussion and reviewed and edited the manuscript. E.E. evaluated the skin biopsies, contributed to the discussion, and reviewed and edited the manuscript. O.R. helped design the study, analyzed data, contributed to the discussion, and reviewed and edited the manuscript. O.R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

The authors would like to thank the late Göran Sundqvist (University Hospital Malmö, Lund University, Sweden.), who was one of the initiators of the study. The authors are also grateful to Dr. Rolf Libelius and Dr. Erik Nordh (Umeå University, Sweden) for examining the
neurophysiologic tests and invaluable comments. The authors acknowledge biomedical technician Kerstin Sturesson (Lund University, Sweden) for the handling of specimens and logistics and laboratory researcher Annette Persson (Lund University, Sweden) for providing optimal conditions for the immunohistochemical staining. The authors also thank Dr. Sigbritt Rasmark (Umeå University, Sweden) for taking the skin biopsies.
References

**Table 1** Clinical characteristics of the study population by glycemic status.

<table>
<thead>
<tr>
<th></th>
<th>NGT</th>
<th>IGT</th>
<th>Type 2 diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n (m/f)</strong></td>
<td>39 (19/20)</td>
<td>29 (15/14)</td>
<td>51 (30/21)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>61 ± 0.6</td>
<td>61 ± 0.8</td>
<td>61 ± 1.3</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>–</td>
<td>–</td>
<td>7.2 ± 0.9</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.72 ± 0.11</td>
<td>1.72 ± 0.10</td>
<td>1.71 ± 0.09</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.4 ± 16.0</td>
<td>81.1 ± 24.0</td>
<td>85.6 ± 15.2*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.0 ± 3.6</td>
<td>26.9 ± 5.4</td>
<td>29.4 ± 4.6*</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.4 (5.3–5.4)</td>
<td>5.5 (5.4–5.6)</td>
<td>7.3 (7.0–7.7)*†</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>36 (34–36)</td>
<td>37 (36–38)</td>
<td>56 (53–61)*†</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>5.1 (4.7–5.4)</td>
<td>5.2 (4.9–5.8)</td>
<td>8.2 (6.8–9.7)*†</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>128 ± 17</td>
<td>128 ± 16</td>
<td>131 ± 14</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>76 ± 7</td>
<td>75 ± 11</td>
<td>76 ± 7</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>74 (69–79)</td>
<td>72 (67–78)</td>
<td>73 (67–79)</td>
</tr>
<tr>
<td>Statin treatment n (%)</td>
<td>3 (7.7)</td>
<td>4 (13.8)</td>
<td>28 (54.9)*†</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.8 ± 0.8</td>
<td>5.4 ± 0.9</td>
<td>4.7 ± 0.7*†</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.8 ± 0.9</td>
<td>3.4 ± 0.7</td>
<td>2.8 ± 0.6*†</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.4 ± 0.3</td>
<td>1.3 ± 0.4</td>
<td>1.2 ± 0.3*</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.1 (0.9–1.5)</td>
<td>1.4 (1.0–1.7)</td>
<td>1.5 (1.1–2.0)*</td>
</tr>
</tbody>
</table>

**Nerve conduction**

|                          |                        |                         |                          |
| CV peroneal nerve (m/s)  | 50.0 (45.0–52.0)       | 48.0 (43.0–50.0)*       | 45.0 (41.0–50.0)*        |
| CV sural nerve (m/s)     | 49.0 (44.0–53.0)       | 47.0 (43.0–51.5)        | 45.0 (42.0–49.8)*        |
| AMP sural nerve (µV)     | 10.0 (6.0–14.5)        | 9.5 (5.3–15.0)          | 8.0 (3.3–14.0)           |
Thermal threshold tests

<table>
<thead>
<tr>
<th></th>
<th>Abnormal cold threshold, n (%)</th>
<th>Abnormal heat threshold, n (%)</th>
<th>Intraepidermal nerve fiber density</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9 (24)</td>
<td>5 (19)</td>
<td>4.5 (3.4–6.1)</td>
</tr>
<tr>
<td></td>
<td>11 (29)</td>
<td>9 (35)</td>
<td>3.8 (2.2–6.0)</td>
</tr>
<tr>
<td></td>
<td>15 (31)</td>
<td>28 (58)*†</td>
<td>2.8 (1.1–4.7)*</td>
</tr>
</tbody>
</table>

IENFD tertiles, n (%):  
1st  6 (16)  10 (35)  25 (49)*  
2nd  16 (42)  7 (24)  16 (31)  
3rd  16 (42)  12 (41)  10 (20)*

Data are given as mean ± SD or median (interquartile range Q1–Q3) and proportions (%). NGT, normal glucose tolerance; IGT, impaired glucose tolerance; BMI, body mass index; IENFD, intraepidermal nerve fiber density; CV, conduction velocity; AMP, amplitude.

HbA1c values are shown in both the Diabetes Control and Complications Trial (DCCT) (%) standard values and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (mmol/mol) units. The range of IENFD was 0–10.4 fibers/mm and the distributions (%) of individuals in the IENFD tertiles are given.* p<0.05 vs. NGT and † p<0.05 vs. IGT by Mann-Whitney and Student’s t-test, where appropriate.