

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

KAVEH POURHAMIDI, MD<sup>1</sup>  
LARS B. DAHLIN, MD, PHD<sup>2</sup>

ELISABET ENGLUND, MD, PHD<sup>3</sup>  
OLOV ROLANDSSON, MD, PHD<sup>1</sup>

Anthropometry and other measurements have been described elsewhere (7).

**OBJECTIVE**—To assess small and large nerve fiber function in people 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 [interquartile range 1.1–4.7 fibers/mm]) than NGT individuals (4.5 fibers/mm [3.4–6.1 fibers/mm]);  $P < 0.05$ . T2D 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 prediabetic stage.

*Diabetes Care* 36:962–964, 2013

A high prevalence of impaired glucose tolerance (IGT) in individuals with idiopathic neuropathy has been reported (1), but whether neuropathy already exists in the prediabetic stage, i.e., 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, and 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 diabetic (T2D) 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 approved the study.

### Measurements

Blood samples were drawn and measured for cholesterol, triglycerides, creatinine, fasting plasma glucose, and HbA<sub>1c</sub>.

## 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 Thermostest equipment (Somedic AB, Hörby, Sweden) by using the method of limits (8).

## Skin biopsy

Thin skin biopsies (5  $\mu$ m) were taken for microscopical assessment. Procedures were developed (9) and modified (10) from published guidelines (11). The intraepidermal nerve fiber density (IENFD) denotes the number of fibers per millimeter of epidermal length (mean counts in three sections). Intra- and interobserver reliabilities were  $r_s = 0.98$  and  $0.84$ , respectively.

## Statistical analyses

Data are presented as numbers ( $n$ ) and proportions (%), and distribution as mean and SD or median and interquartile range (IQR). Differences between groups were tested by ANOVA and subsequent Student  $t$  test for normally distributed variables. For nonnormally 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 with NGT, whereas patients with

From the <sup>1</sup>Department of Public Health and Clinical Medicine, Family Medicine, Umeå University, Umeå, Sweden; the <sup>2</sup>Department of Clinical Sciences Malmö, Hand Surgery, Skåne University Hospital, Lund University, Malmö, Sweden; and the <sup>3</sup>Division of Neuropathology, Department of Pathology, Lund University, Lund, Sweden.

Corresponding author: Kaveh Pourhamidi, kaveh.pourhamidi@fammed.umu.se.

Received 6 July 2012 and accepted 28 September 2012.

DOI: 10.2337/dc12-1331

© 2013 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See <http://creativecommons.org/licenses/by-nc-nd/3.0/> for details.

Table 1—Clinical characteristics of the study population by glycemic status

	NGT	IGT	T2D
n (male/female)	39 (19/20)	29 (15/14)	51 (30/21)
Age (years)	61 ± 0.6	61 ± 0.8	61 ± 1.3
Duration of diabetes (years)	—	—	7.2 ± 0.9
Height (m)	1.72 ± 0.11	1.72 ± 0.10	1.71 ± 0.09
Weight (kg)	77.4 ± 16.0	81.1 ± 24.0	85.6 ± 15.2*
BMI (kg/m <sup>2</sup> )	26.0 ± 3.6	26.9 ± 5.4	29.4 ± 4.6*
HbA <sub>1c</sub> (%)	5.4 (5.3–5.4)	5.5 (5.4–5.6)	7.3 (7.0–7.7)*†
HbA <sub>1c</sub> (mmol/mol)	36 (34–36)	37 (36–38)	56 (53–61)*†
Fasting plasma glucose (mmol/L)	5.1 (4.7–5.4)	5.2 (4.9–5.8)	8.2 (6.8–9.7)*†
Systolic blood pressure (mmHg)	128 ± 17	128 ± 16	131 ± 14
Diastolic blood pressure (mmHg)	76 ± 7	75 ± 11	76 ± 7
Creatinine (μmol/L)	74 (69–79)	72 (67–78)	73 (67–79)
Statin treatment, n (%)	3 (7.7)	4 (13.8)	28 (54.9)*†
Total cholesterol (mmol/L)	5.8 ± 0.8	5.4 ± 0.9	4.7 ± 0.7*†
LDL cholesterol (mmol/L)	3.8 ± 0.9	3.4 ± 0.7	2.8 ± 0.6*†
HDL cholesterol (mmol/L)	1.4 ± 0.3	1.3 ± 0.4	1.2 ± 0.3*
Triglycerides (mmol/L)	1.1 (0.9–1.5)	1.4 (1.0–1.7)	1.5 (1.1–2.0)*
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			
Abnormal cold threshold, n (%)	9 (24)	5 (19)	15 (31)
Abnormal heat threshold, n (%)	11 (29)	9 (35)	28 (58)*†
IENFD (nerve fibers/mm)	4.5 (3.4–6.1)	3.8 (2.2–6.0)	2.8 (1.1–4.7)*
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 (IQR = Q1–Q3) and proportions (%). AMP, amplitude. HbA<sub>1c</sub> 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 by Mann-Whitney *U* test and Student *t* test, where appropriate. †*P* < 0.05 vs. IGT by Mann-Whitney *U* test and Student *t* test, where appropriate.

diabetes had metabolic perturbations compared with 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 (CV) of the peroneal nerve than those with NGT. The CV of the peroneal and sural nerve was lower in T2D patients 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 T2D 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 fibers/mm] vs. 2.7 fibers/mm [1.6–4.7 fibers/mm]; *P* < 0.001). However, there was no interaction between sex 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.

### IGT and nerve dysfunction

It is not clear if neuropathy is found in the prediabetic 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 prediabetic stage. In a population-based study, neuropathy was marginally increased in IGT, but the measure of neuropathy was rather crude and mainly on large fibers (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

First, 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. Second, 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 with thick 50-μm sections suggested by published guidelines. However, it still allows for group comparison between NGT, IGT, and T2D within our study, but hampers comparisons to studies using thicker sections.

Our study has the following strengths: 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 were all of the same age. To avoid bias, neurophysiological measurements were performed by personnel blinded for the 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 prediabetic stage.

**Acknowledgments**—The study was funded by the County Council of Västerbotten, Sweden.

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 Rasmak (Umeå University, Sweden) for taking the skin biopsies.

## References

1. Singleton JR, Smith AG, Bromberg MB. Increased prevalence of impaired glucose tolerance in patients with painful sensory neuropathy. *Diabetes Care* 2001;24:1448–1453
2. Papanas N, Vinik AI, Ziegler D. Neuropathy in prediabetes: does the clock start ticking early? *Nat Rev Endocrinol* 2011;7:682–690
3. Dyck PJ, Dyck PJ, Klein CJ, Weigand SD. Does impaired glucose metabolism cause polyneuropathy? Review of previous studies and design of a prospective controlled population-based study. *Muscle Nerve* 2007;36:536–541
4. Ziegler D, Rathmann W, Dickhaus T, Meisinger C, Mielck A; KORA Study Group. Prevalence of polyneuropathy in pre-diabetes and diabetes is associated with abdominal obesity and macroangiopathy: the MONICA/KORA Augsburg Surveys S2 and S3. *Diabetes Care* 2008;31:464–469
5. Eriksson KF, Nilsson H, Lindgärde F, et al. Diabetes mellitus but not impaired glucose tolerance is associated with dysfunction in peripheral nerves. *Diabet Med* 1994;11:279–285
6. Dyck PJ, Clark VM, Overland CJ, et al. Impaired glycemia and diabetic polyneuropathy: the OC IG Survey. *Diabetes Care* 2012;35:584–591
7. Pourhamidi K, Dahlin LB, Boman K, Rolandsson O. Heat shock protein 27 is associated with better nerve function and fewer signs of neuropathy. *Diabetologia* 2011;54:3143–3149
8. Heldestad V, Linder J, Sellersjö L, Nordh E. Reproducibility and influence of test modality order on thermal perception and thermal pain thresholds in quantitative sensory testing. *Clin Neurophysiol* 2010;121:1878–1885
9. Thrainsdottir S. *Peripheral polyneuropathy in type 2 diabetes mellitus and impaired glucose tolerance. Correlations between morphology, neurophysiology and clinical findings*. Lund, Sweden, Lund University, 2009
10. Thomsen NO, Englund E, Thrainsdottir S, Rosén I, Dahlin LB. Intraepidermal nerve fibre density at wrist level in diabetic and non-diabetic patients. *Diabet Med* 2009;26:1120–1126
11. Lauria G, Hsieh ST, Johansson O, et al.; European Federation of Neurological Societies; Peripheral Nerve Society. European Federation of Neurological Societies/Peripheral Nerve Society Guideline on the use of skin biopsy in the diagnosis of small fiber neuropathy. Report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society. *Eur J Neurol* 2010;17:903–912, e44–e49
12. Novella SP, Inzucchi SE, Goldstein JM. The frequency of undiagnosed diabetes and impaired glucose tolerance in patients with idiopathic sensory neuropathy. *Muscle Nerve* 2001;24:1229–1231
13. Umapathi T, Tan WL, Loke SC, Soon PC, Tavintharan S, Chan YH. Intraepidermal nerve fiber density as a marker of early diabetic neuropathy. *Muscle Nerve* 2007;35:591–598
14. Sumner CJ, Sheth S, Griffin JW, Cornblath DR, Polydefkis M. The spectrum of neuropathy in diabetes and impaired glucose tolerance. *Neurology* 2003;60:108–111
15. Smith AG, Ramachandran P, Tripp S, Singleton JR. Epidermal nerve innervation in impaired glucose tolerance and diabetes-associated neuropathy. *Neurology* 2001;57:1701–1704