Peripheral Somatic Nerve Function in Relation to Glucose Tolerance in an Elderly Caucasian Population: the Hoorn Study

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Only sparse and contradictory data are available on peripheral somatic nerve function in relation to the total range of glucose tolerance. A random sample ($n = 708$) of people, stratified by age, sex, and glucose tolerance, from a Caucasian population aged 50 to 74 years was invited to undergo an examination including measures of large-fibre nerve function (ankle and knee reflexes, vibration sense, vibratory perception threshold (VPT) at the foot) and one measure of small-fibre function (thermal discrimination threshold (TDT) at the foot). A total of 267 subjects with a normal glucose tolerance (NGT), 167 with impaired glucose tolerance (IGT), 90 with newly diagnosed diabetes mellitus (NDM), and 73 with previously known diabetes (KDM) were included. KDM was associated with the highest prevalence of large-fibre nerve dysfunction. Within the range from NGT to NDM, most large-fibre function measures showed a decline with decreasing glucose tolerance. The TDT showed a decrease with an increase in fasting and post-load insulin levels ($p < 0.05$). We conclude that glucose intolerance is associated with impaired peripheral large-fibre nerve function, an association which seems to apply even in the non-diabetic range. Higher insulin levels were associated with a better small-fibre nerve function.

KEY WORDS **Glucose tolerance Specific insulin Ankle tendon reflexes Quantitative sensory testing Population-based survey Caucasians**

Introduction

Diabetic neuropathy is most commonly a distal and symmetrical polyneuropathy, involving a variable set of somatic and autonomic nerve fibres.[1,2] Its somatic component may be manifested by pain, paresthesia, cramps or muscle weakness, predominantly in the lower limbs. It may also progress barely recognized by the patient, playing nevertheless an important role in the development of diabetic foot lesions, via loss of sensory function and small muscle wasting.[3] Although distal symmetrical polyneuropathy typically develops slowly over many years, it may be the presenting feature of diabetes in older patients[4,5] and a number of studies have shown that measurable deficits in peripheral somatic nerve function may be present in subjects with newly diagnosed non-insulin-dependent diabetes.[6–10] It is not known whether this early nerve dysfunction reflects a period of undiagnosed diabetes, or arises gradually during a state of impaired glucose tolerance (IGT), prior to the onset of diabetes. With respect to the risk of other diabetes-related complications, such as cardiovascular disease[11–14] and macro- and microproteinuria,[15] evidence is accumulating for IGT to take an intermediate position between normal glucose tolerance (NGT) and diabetes. With respect to distal symmetrical polyneuropathy, however, the few available studies have reported contradictory results.[16–18] Therefore we took the opportunity to study, as part of an epidemiologic survey in a 50–74-year-old general Caucasian population, the relation between glucose tolerance and peripheral somatic nerve function, across the total range of glucose tolerance, from NGT to known diabetes. Peripheral nerve function was assessed by examining the deep tendon reflexes of the lower limbs and the vibration sensation at toes and ankles. In addition, quantitative vibratory and thermal sensory thresholds, indexing large and small-diameter nerve fibre function, respectively, were measured at the foot. Age, sex, body height,[18–20] alcohol intake,[21] and smoking[22] were considered as potentially confounding factors. In the analyses with the sensory threshold data, the skin temperature[23] and the use of neuroleptic drugs or benzodiazepines were also taken into account, the

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Abbreviations: NGT normal glucose tolerance, IGT impaired glucose tolerance, NDM newly-diagnosed diabetes mellitus, KDM known diabetes mellitus, OGTT oral glucose tolerance test, VPT vibratory perception threshold, TDT temperature discrimination threshold, CI confidence interval, OR odds ratio, IQD interquartile difference.

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latter because of the possible adverse influence on the concentration of the study subjects and, thus, on the sensory threshold levels.

Subjects and Methods

Subjects

All study participants were members of the cohort of the Hoorn Study, an epidemiologic survey on glucose metabolism and related disorders in a general Caucasian population. For this study, a random sample of 50–74-year-old subjects was taken from the population register of the town of Hoorn in the Netherlands.[24] All participants not on oral hypoglycaemic agents or insulin treatment underwent a 75 g oral glucose tolerance test (OGTT).[25] Those with a 2-h post-load venous plasma glucose level $\geq$11.1 mmol l^{-1} (n = 122), an 81 % (n = 254) sample from the subjects with a 2-h post-load glucose $\geq$7.5 mmol l^{-1} and <11.1 mmol l^{-1}, and a 13 % (n = 256) age and sex-stratified random sample from those with a post-load glucose <7.5 mmol l^{-1} were invited to undergo a second OGTT within 3 to 5 weeks. They were also asked to participate in an examination concerning diabetes-related disorders, as were the members of the Hoorn Study cohort who were on oral hypoglycaemic agents or insulin treatment for diagnosed diabetes mellitus (n = 76). Thus, 708 people in total were invited to take part in the diabetes-related disorder study, which included an assessment of peripheral somatic nerve function. Further details on the sampling procedure are provided elsewhere.[26]

The study protocol was approved by the Ethics Committee of the Vrije Universiteit Academic Hospital. Informed consent was given by all participants.

Physical Examination

Knee and ankle reflexes and vibration sensation at big toes and medial malleoli (128 Hz tuning fork) were classifed as present or absent in each participant by a single observer, who was unaware of the glucose tolerance status of the subjects. Bilateral absence was considered to be a sign of distal symmetrical nerve dysfunction.

Sensory Threshold Tests

Vibratory perception and thermal discrimination thresholds were assessed, as described elsewhere.[27] Briefly, the vibratory perception threshold (VPT) was assessed at the dorsum of the right big toe by means of a Vibrameter (Somedic, Stockholm, type IV) as the mean of 6 out of 10 ascending thresholds (in μm vibratory amplitude), excluding the two highest and the two lowest values. The temperature discrimination threshold (TDT) was assessed at the dorsum of the right foot, by means of a two-alternative forced-choice testing procedure. Two thermostimulators were applied, one after the other, on the dorsum of the right foot, the temperature of the second being set at random either higher or lower than that of the first one. The subject was asked whether the second stimulator was warmer or colder than the first one, and the magnitude of the temperature difference between the two was doubled or halved, depending on the responses of the participant. The test was continued until the course of the temperature difference showed eight 'reversal points', and the TDT was then computed as the mean temperature difference (in °C) at the last six reversal points. All sensory thresholds were measured by one of two observers, who used the same instruments and were unaware of the glucose tolerance status of the participants.

Parameters of Glucose Metabolism

Fasting and 2-h post-load venous plasma glucose levels were determined with a glucose dehydrogenase method (Merck, Darmstadt, Germany). Mean values of two OGTTs, if available, were used in the data analysis. HbA_{1c} and serum fructosamine were assessed once, by ion-exchange high performance liquid chromatography using a Modular Diabetes Monitoring System (Bio-Rad, Veenendaal, NL, normal range 4.3–6.1 %), and by a second generation serum fructosamine assay (Roche Diagnostics, Basel, Switzerland), respectively.

Fasting and 2-h post-load specific serum insulin levels were quantified (in duplicate for each OGTT) with an insulin-specific double-antibody radioimmunoassay (antibody: Linco SP21, St Louis, MO, USA). No cross-reactivity with proinsulin and split proinsulin was found. The intra-assay and inter-assay coefficient of variation was 5–8 % and 7–11 %, respectively. Mean insulin values were used in the data analysis.

In subjects treated with oral hypoglycaemic agents or insulin, fasting plasma glucose and fasting serum insulin (in duplicate) were measured on one occasion only. These subjects were classified as having previously known diabetes mellitus (KDM). All other participants were classified, according to the World Health Organization criteria[25,26] applied to the mean fasting and post-load glucose values of two OGTTs, as having normal glucose tolerance (NGT), impaired glucose tolerance (IGT), or diabetes, either newly-diagnosed (NDM), or KDM (the latter if they were previously diagnosed as having diabetes, receiving dietary advice as their only treatment).

Other Measurements

The medical history was obtained by means of a self-administered questionnaire.[26] On their visit to the study centre, the participants brought with them the completed questionnaire and any drugs they were using. The names of the drugs and the dosages prescribed were registered from the labels on the packages. Subjects using phenytoin,

or having a neurological disorder which could influence peripheral somatic nerve function, were excluded from the analysis. Questions on alcohol use and smoking habits were incorporated in questionnaires designed specifically for the study population at hand. A number of variables representing different categorizations of alcohol intake and smoking history were examined for a possible confounding effect in the data analysis.

Analysis

The sensory threshold distributions were skewed and a logarithmic transformation ($\log_{10}$) was carried out to obtain distributions which were close to normal, conditionally on age, sex, and body height. Logistic regression analysis was used to estimate odds ratio (with 95 % confidence intervals (CIs)) contrasting the (odds of the) prevalences of signs of peripheral somatic dysfunction at different levels of the independent variables. The associations between parameters of glucose metabolism (e.g. HbA_{1c}) and sensory threshold levels (e.g. VPT) were assessed by means of least squares regression analysis. Regression models were fitted as follows:

$$\log_{10}(\text{VPT}) = a + b_1 \bullet \text{HbA}_{1c} + b_2 \bullet \text{age} + b_3 \bullet \text{sex} + b_4 \bullet \text{height} + \text{etc.}$$

With these equations fitted to the data, the regression coefficients can be interpreted (provided that the residuals are normally distributed, which was checked in all analyses), as the logarithms of the ratio of median values or, briefly, the logarithms of median ratios, corresponding to unit contrasts in the independent variables.[28] All parameters of glucose metabolism were expressed in units which were equal to the difference between their 75th and 25th centile (the interquartile difference, IQD), in order to be able to compare the magnitude of the regression coefficients. Thus, the antilog of the coefficient b_1 (10^{b1}), for example, is equal to the median VPT in subjects with $\text{HbA}_{1c} = x_i + 1 \bullet \text{IQD}$, divided by the median VPT in those with $\text{HbA}_{1c} = x_i$, adjusted for age and other characteristics represented in the regression model. The standard errors of the regression coefficients were used to obtain the 95 % CIs of the median ratios.

In all regression analyses, age, sex, height, alcohol-use, and smoking were considered as being characteristics which could confound the relations at issue. The skin temperature and the use of neuroleptic drugs or benzodiazepines were regarded as potential confounders only in the analyses with sensory threshold data.

Double-sided p values <0.05 were considered to be statistically significant.

Results

Of the subjects invited to take part in the peripheral nerve function assessment, 89 % ($n = 631$) participated. The mean time which elapsed between the second OGTT and the nerve function assessment was 54 days (95th centile: 93 days). Thirty-three participants were excluded because of histories of lumbar disc disease, Parkinson's disease, cerebrovascular events, systemic lupus erythematosus or spinal cord lesion. Also excluded was one subject using phenytoin. Of the remaining 597 subjects, 267 had NGT, 167 had IGT, 90 had NDM, and 73 had KDM, of whom 11 were treated by dietary advice only (Table 1).

All subjects with KDM were older than 30 years of age when diabetes was diagnosed, except for one woman using insulin who was aged 29 at the time of diagnosis. The median known duration of their diabetes was 6.6 years (5th and 95th centile: 1.0 and 25.9 years). Physical examination data were missing for one subject, the VPT for two, and the TDT for five subjects. For two persons, the signs of polyneuropathy were scored as present on those parts of the lower extremities which had been amputated because of diabetic foot problems. With the exception of the TDT, all measures of peripheral somatic nerve function indicated a poorer function with reduced glucose tolerance (Table 2).

Logistic regression analysis showed that, adjusting for age, sex, and height, KDM was associated with the highest prevalence of signs of peripheral nerve dysfunction (Table 3). The IGT and NDM categories showed a smaller increase relative to NGT, which was statistically significant only for the absence of ankle reflexes. Excluding the subjects with KDM from the analysis, the bilateral absence of vibration sensation at the big toes and the absence of ankle and knee reflexes were associated statistically significantly with various measures of glycaemic level (Table 3). There were no significant relations with fasting or post-load insulin levels (Table 3). The odds ratios for the associations with the parameters of glycaemia remained in the same order of magnitude when only the subjects with NGT and IGT were included in the analysis (data not shown). In this reduced population, however, only the relation between post-load glucose and absence of ankle reflexes was statistically significant (OR = 2.4 (95 % CI: 1.4–4.2), $p = 0.001$). All odds ratio estimates were influenced only marginally by including in the regression model variables for smoking and alcohol-intake (data not shown).

Adjusting for age, sex, and height by regression analysis, the median VPT showed a statistically significant increase relative to NGT in KDM, but not in IGT and NDM (Table 4). The TDT was not significantly increased in any of the glucose intolerance categories. In the population without KDM, an increase in the parameters of glycaemia equal to the interquartile difference was accompanied by an increase in the medians of both thresholds of about 5 %. However, only the association between serum fructosamine and VPT was statistically significant. Unlike the VPT, the median TDT showed a 10 % decrease with an IQD increase in fasting and post-load insulin levels. The associations between fasting glucose and insulin levels, on the one hand, and median TDT on the other, did not change when glucose

Table 1. Characteristics of study subjects according to categories of glucose tolerance

	Normal glucose tolerance ($n = 267$)	Impaired glucose tolerance ($n = 167$)	Newly-diagnosed diabetes mellitus ($n = 90$)	Known diabetes mellitus ($n = 73$)
Sex (% men)	50	47	47	41
Age (yr)	63.3 ± 7.4	65.2 ± 6.9	65.9 ± 6.6	65.9 ± 6.8
Body height (cm)	168.8 ± 9.0	167.6 ± 8.9	166.7 ± 9.0	167.9 ± 10.1
Fasting glucose (mmol l^{-1})	5.4 ± 0.5	6.1 ± 0.7	8.4 ± 3.1	10.5 ± 3.7
Post-load glucose (mmol l^{-1})	5.6 ± 1.3	9.1 ± 0.9	15.5 ± 5.7	–[a]
HbA_{1c} (%)	5.4 ± 0.5	5.6 ± 0.5	6.7 ± 1.9	7.7 ± 1.6
Serum fructosamine (μmol l^{-1})	240 ± 17	246 ± 19	284 ± 72	328 ± 66
Fasting insulin (pmol l^{-1})	73 (56, 93)	90 (68, 135)	116 (84, 157)	100 (73, 136)
Post-load insulin (pmol l^{-1})	302 (183, 420)	605 (377, 1050)	535 (277, 891)	–[a]
Drinking ≥20 g alcohol day^{-1}	17	12	18	17
Current cigarette smoking	30	24	21	25

Data expressed as percentage, mean ± SD, or median (25th, 75th centile).
[a]Not assessed in subjects treated with oral hypoglycaemic agents or insulin.

Table 2. Measures of peripheral somatic nerve function according to categories of glucose tolerance

	Normal glucose tolerance ($n = 267$)	Impaired glucose tolerance ($n = 167$)	Newly diagnosed diabetes mellitus ($n = 90$)	Known diabetes mellitus ($n = 73$)
Vibration sensation (128 Hz) bilaterally absent at				
big toes	28	27	39	50
medial malleoli	5	5	7	8
Bilateral absence of				
ankle reflexes	16	26	29	49
knee reflexes	2	3	3	7
Vibratory perception threshold (μm)	7.6 (3.8, 19.7)	8.5 (4.0, 17.6)	11.1 (4.1, 24.6)	16.3 (7.2, 44.3)
Thermal discrimination threshold (° C)	0.4 (0.2, 0.7)	0.3 (0.2, 0.7)	0.4 (0.2, 0.7)	0.5 (0.3, 0.8)

Data expressed as percentage or median (25th, 75th centiles).

Table 3. Prevalence of signs of peripheral somatic dysfunction in relation to parameters of glucose metabolism: odds ratios (with 95 % CIs) estimated by logistic regression analysis, adjusted for age, sex, and body height

		Odds ratio for bilateral absence of			
Independent variable	Interquartile difference	Vibration sensation (128 Hz) at big toes	medial malleoli	Ankle reflexes	Knee reflexes
Glucose tolerance					
IGT vs NGT		0.8 (0.5–1.3)	0.9 (0.4–2.2)	1.7[b] (1.1–2.8)	1.2 (0.4–4.1)
NDM vs NGT		1.5 (0.9–2.7)	1.1 (0.4–3.0)	2.0[b] (1.1–3.6)	1.3 (0.3–5.5)
KDM vs NGT		2.3[c] (1.3–4.2)	1.3 (0.5–3.8)	4.8[d] (2.7–8.6)	2.7 (0.8–9.3)
Fasting glucose[a]	1.1 mmol l^{-1}	1.1[b] (1.0–1.3)	1.1 (1.0–1.3)	1.1[b] (1.0–1.3)	1.2[c] (1.1–1.5)
Post-load glucose[a]	4.2 mmol l^{-1}	1.2 (1.0–1.4)	1.1 (0.8–1.5)	1.4[d] (1.1–1.7)	1.4[b] (1.1–2.0)
HbA_{1c}[a]	0.8 %	1.1 (1.0–1.4)	1.1 (0.9–1.4)	1.2[c] (1.1–1.4)	1.2 (0.9–1.5)
Serum fructosamine[a]	27 μmol l^{-1}	1.2[b] (1.0–1.4)	1.1 (0.9–1.4)	1.1 (1.0–1.3)	1.2[b] (1.0–1.5)
Fasting insulin[a]	52 pmol l^{-1}	1.1 (0.9–1.4)	1.2 (0.8–1.8)	1.2 (1.0–1.5)	1.2 (0.7–1.9)
Post-load insulin[a]	423 pmol l^{-1}	0.9 (0.7–1.1)	0.7 (0.4–1.1)	1.1 (0.9–1.3)	1.0 (0.7–1.5)

NGT, normal glucose tolerance; IGT, impaired glucose tolerance; NDM, newly-diagnosed diabetes mellitus; KDM, known diabetes mellitus.
[a]Subjects with KDM excluded; odds ratios correspond to an increase in the independent variable equal to the interquartile difference; [b]$p < 0.05$; [c]$p < 0.01$; [d]$p < 0.001$.

Table 4. Sensory thresholds in relation to parameters of glucose metabolism: median ratios (with 95 % CIs) estimated by regression analysis, adjusted for age, sex, and body height

Independent variable	Interquartile difference	Median ratio	
		Vibratory perception threshold	Thermal discrimination threshold
Glucose tolerance			
IGT vs NGT		0.97 (0.81–1.16)	0.90 (0.73–1.11)
NDM vs NGT		1.12 (0.89–1.41)	1.00 (0.77–1.30)
KDM vs NGT		1.63[c] (1.27–2.08)	1.17 (0.89–1.56)
Fasting glucose[a]	1.1 mmol l^{-1}	1.05 (0.99–1.11)	1.06 (1.00–1.12)
Post-load glucose[a]	4.2 mmol l^{-1}	1.06 (0.98–1.15)	1.02 (0.94–1.12)
HbA_{1c}[a]	0.8 %	1.03 (0.97–1.10)	1.06 (0.98–1.14)
Serum fructosamine[a]	27 μmol l^{-1}	1.07[b] (1.01–1.14)	1.06 (0.99–1.14)
Fasting insulin[a]	52 pmol l^{-1}	1.07 (0.98–1.16)	0.90[b] (0.82–1.00)
Post-load insulin[a]	423 pmol l^{-1}	0.99 (0.92–1.06)	0.92[b] (0.85–0.99)

NGT, normal glucose tolerance; IGT, impaired glucose tolerance; NDM, newly diagnosed diabetes mellitus; KDM, known diabetes mellitus. [a]Subjects with KDM excluded; median ratios correspond to an increase in the independent variable equal to the interquartile difference; [b]$p < 0.05$; [c]$p < 0.001$.

and insulin were entered into the regression model simultaneously (Table 5). There was no indication of the association between insulin levels and TDT being dependent on the glucose level. When the subjects with NDM were excluded from the analysis, the relation between serum fructosamine and VPT was the only one which remained statistically significant (median ratio = 1.16 (95 % CI: 1.01–1.32), $p = 0.03$; other data not shown). None of these analyses was more than marginally influenced when variables were included for smoking, alcohol-intake, skin temperature or use of neuroleptic drugs or benzodiazepines (data not shown).

Discussion

Most measures of peripheral somatic dysfunction used in our study clearly reflected the generally acknowledged fact that subjects with non-insulin-dependent diabetes mellitus are at an increased risk of a distal symmetrical polyneuropathy. The most pronounced difference between subjects with NGT and those with KDM was found by means of a simple clinical test: the examination of the Achilles tendon reflexes. Bilateral loss of ankle jerks was also the only test which could detect statistically significant differences between subjects with IGT or NDM, and those with NGT. The absence of the 128 Hz vibration sensation at the big toe and the loss of knee reflexes, though not clearly more prevalent in the IGT and NDM categories, nevertheless did show a decline of nerve function with increasing glycaemic level, measured on a continuous scale, within the range from NGT to NDM. The same applied to the VPT at the big toe. The relation between fructosamine and VPT in the population including subjects with NGT and IGT only, suggests that large-fibre sensory function starts to decline in the range of non-diabetic glucose intolerance.

Table 5. Temperature discrimination threshold in relation to fasting glucose and insulin levels, entered into the regression model simultaneously: median ratios (with 95 % CIs), adjusted for age, sex, and body height

Independent variable	Interquartile difference	Median ratio thermal discrimination threshold
Model 1		
Fasting glucose[a]	1.1 mmol l^{-1}	1.07[b] (1.01–1.14)
Fasting insulin[a]	52 pmol l^{-1}	0.88[b] (0.80–0.98)
Model 2		
Fasting glucose[a]	1.1 mmol l^{-1}	1.06 (1.00–1.12)
Post-load insulin[a]	423 pmol l^{-1}	0.92[b] (0.85–0.99)

[a]Subjects with known diabetes excluded; median ratios correspond to an increase in the independent variable equal to the interquartile difference; [b]$p < 0.05$.

Our findings confirm other reports on peripheral somatic nerve dysfunction in newly diagnosed non-insulin-dependent diabetes.[6–10] Contrary to another study,[18] our data suggest that some degree of nerve dysfunction can be demonstrated already when diabetes is detected by screening, rather than by clinical diagnosis. The increased prevalence of the absence of ankle jerks in subjects with IGT is in accordance with the results of a previous study, based on a two-stage household sampling procedure, in which, mainly on the basis of bilateral abnormality of ankle reflexes, an odds ratio of 3.5 (95 % CI: 1.5–7.9) was found for the prevalence of distal symmetrical polyneuropathy comparing IGT with NGT.[17,29] Other investigators, however, found IGT subjects to be equal or even superior to NGT subjects with respect to nerve conduction[16] and sensory function.[18] All nerve function measures which were included in this study, are indices of large-fibre function, except for the TDT. The TDT was also the only measure which was associated with both fasting and post-load insulin levels:

lower insulin levels were accompanied by a higher TDT. This finding is consistent with experimental evidence suggesting that C-peptide[30] or insulin—which shares several molecular, structural, and physiological properties with nerve growth factor[31]—may have direct beneficial effects on nerve-tissue metabolism or function. In agreement with this, a recent study showed that in subjects with non-insulin-dependent diabetes mellitus, low serum insulin concentrations were associated with the development of polyneuropathy, regardless of the degree of glycaemia.[32] The association between insulin and TDT in our study suggests that in a population without known diabetes, insulin or C-peptide may also have a subtle beneficial effect on peripheral nerve function, which is detectable in small nerve fibres only.

In conclusion, the results of this population-based study show that glucose intolerance is associated with an impaired peripheral somatic large-fibre nerve function, an association which seems to apply even in the non-diabetic range. As far as small nerve fibres are concerned, the findings are consistent with the hypothesis that C-peptide or insulin may have a beneficial effect on nerve-tissue function.

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