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Large fibre dysfunction in diabetic peripheral neuropathy is predicted by cardiovascular risk factors

Running title: J Elliott et al.: Risk factors for large fibre dysfunction

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Abstract

Objective: Diabetic large nerve fibre dysfunction, as measured by vibration perception threshold (VPT) predicts foot ulceration, amputation and mortality. Thus, determination of modifiable risk factors is of great clinical importance.

Research Design and Methods: We assessed 1407 patients with type 1 diabetes and normal VPT participating in the EURODIAB Prospective Complications Study, at baseline mean age 32.7±10.2 years; mean diabetes duration 14.7±9.3 years; and mean follow-up of 7.3±0.6 years. VPT was measured using biothesiometry on the right big toe and medial malleolus. An abnormal result was defined as >2SD from the predicted mean for the patient's age.

Results: An abnormal VPT was associated with an increased incidence of gangrene, amputation, foot ulceration, leg bypass or angioplasty and mortality ($p \leq 0.02$). The incidence of abnormal VPT was 24% over the 7.3 year follow-up. Duration of diabetes and HbA_{1c} significantly influenced the incidence of abnormal VPT ($p<0.0001$). After correction for these, established risk factors for cardiovascular disease including, male sex ($p=0.0004$), hypertension ($p<0.0001$), total cholesterol ($p=0.002$), LDL cholesterol ($p=0.01$), smoking ($p<0.0001$), weight ($p<0.0001$); and diabetic complications (retinopathy ($p=0.0001$), nephropathy ($p=0.01$) and autonomic neuropathy ($p=0.001$)) were all found to be significant risk factors. A previous history of cardiovascular disease doubled the incidence of abnormal VPT.

Conclusions: This prospective study indicates that cardiovascular risk factors predict development of large fibre dysfunction. This may account for the high mortality rate in

patients with abnormal VPT, and emphasises the importance of an early determination of VPT to detect sub-clinical neuropathy, and to address cardiovascular risk factors.

Keywords Diabetic neuropathy, Cardiovascular risk factors, Type 1 Diabetes Mellitus, Epidemiology

Abbreviations VPT: vibration perception threshold, AER: albumin excretion rate, CAN: cardiac autonomic neuropathy, CVD: cardiovascular disease

Chronic diabetic peripheral neuropathy (DPN) is a slowly progressive process, the pathogenesis of which is poorly understood. However, we do know that large fibre dysfunction, as measured by vibration perception threshold (VPT) predicts foot ulceration, lower-limb amputation and mortality (1-3). The consequences of these clinical complications results in a massive economic burden, estimated in 2001 to be $10.9 billion in the US (4, 5). Early in the natural history of the disease patients are usually asymptomatic. Thus reliable identification of individuals in the early stages of the neuropathic process is required so that more rigorous modification of risk factors and foot-care education can be implemented. The best method to identify such patients is still a matter of some debate (6-9).

In patients with type 2 diabetes the prevalence of abnormal VPT has been shown to be 11.4% (10) and the incidence of an abnormal VPT was 19.9% over a 12 year period (11). Some investigators have used absolute values of VPT as predictors of foot ulceration. In one study a VPT >25V was associated with a seven-fold increase in ulcer risk, compared to individuals with a VPT <15V (12). Another study found an incidence of 7.2% of first foot ulceration within 1 year in patients with a VPT $\geq$25 (13). In addition, a cut-off of 25V has been shown to be a more sensitive way of detecting patients at risk of foot ulceration than the 10g monofilament (14). Small fibre dysfunction may be determined by measuring thermal thresholds, but in terms of discriminating between patients with and without ulceration it does not appear to provide any additional value above measuring VPT (15).

Thus ample evidence exists to highlight the clinical importance of an abnormally high VPT, but as yet there is little evidence examining the risk factors involved in its development. Poor glycaemic control is associated with an abnormal VPT even at diagnosis in patients with type 2 diabetes (16). Evidence also exists to suggest that height is a determinant of VPT (10, 17).

Importantly, VPT is known to increase with age (18, 19). The majority of research regarding large fibre dysfunction, as measured by VPT, has been conducted in patients with type 2 diabetes. In this study we have examined the incidence of abnormal VPT in a large cohort of patients with type 1 diabetes, in order to identify possible modifiable risk factors.

Research Design and Methods

Study design The EURODIAB Prospective Complications Study recruited 3250 patients (1668 men and 1582 women; mean [±SD] age, 32.7±10.2 years; mean duration of diabetes, 14.7±9.3 years). Subjects with type 1 diabetes were randomly selected from 31 diabetes clinics across Europe. The selection criteria and methods have been described in detail previously (20, 21). The study was approved by the ethics committee at each centre and written informed consent was gained from all subjects. All measurements were obtained by trained physicians following standardised procedures. The baseline examinations were conducted from 1989 to 1991, and a subsequent follow-up visit occurred between 1997 and 1999.

Assessment of vibration perception threshold (VPT) was measured by centrally calibrated biothesiometers (Bio-Medical Instrument Company, Newbury, Ohio, USA). Three readings on the right big toe and right medial malleolus were obtained and averaged. Results were classified according to age-related criteria (19).

Neuropathy was defined as the presence of two or more of the following criteria: the presence of one or more symptoms, the absence of two or more reflexes of the ankle or knee tendons (with reinforcement if necessary), an abnormal VPT and the presence of CAN, as described

previously (21). Symptoms of neuropathy were present if the subject had experienced any of the following in the preceding six months: "asleep" numbness or "dead feeling" in the feet, a prickling sensation in the feet, deep aching or burning pains in the legs, unusual difficulty in climbing stairs, difficulty with bladder control, and nocturnal diarrhoea. Symptoms were assessed carefully to exclude non-diabetic causes.

Blood samples, taken after an overnight fast, if possible, were sent to central laboratories. In order to compare the results with those of the DCCT, measured glycosylated haemoglobin values were converted as previously reported (22). Lipid measurements included total cholesterol, HDL cholesterol and triglycerides. Levels of LDL cholesterol were calculated.

Urinary albumin excretion rate (AER) was obtained from a single 24-h urine collection. An AER between 20 and 200 μg/min was defined as microalbuminuria, while >200 μg/min was defined as macroalbuminuria. Diabetic retinopathy was determined from centrally graded retinal photographs and was either classified as non-proliferative (background) or proliferative.

Cardiovascular disease (CVD) was defined as either a history of physician-diagnosed cardiovascular disease (e.g., previous myocardial infarction, angina, coronary-artery bypass grafting, or stroke) or ischaemic changes on a 12-lead electrocardiogram (classified by two observers according to the Minnesota Code).

Of the 3250 patients examined at baseline, 722 had abnormal VPT, whereas 1407 with normal VPT were assessed at follow-up (Figure 1). Baseline investigations and determination of VPT were repeated after a mean period of 7.3±0.6 years of follow-up.

Statistical analysis The data were analysed using the statistical package SAS (version 8). A p-value less than 0.05 was considered statistically significant. Where variables were continuous and normally distributed the t-test was used to compare group means. If, however, the variable had a skewed distribution the Mann-Whitney U test was used to compare group medians and the variable was log transformed for any subsequent analysis. To compare group percentages the chi-square test was used.

In order to assess which risk factors were associated with the development of VPT multiple logistic regression was used to calculate odd ratios adjusted for HbA_{1c} and duration. So that the odds ratios could be compared, for each risk factor standardised odds ratios were calculated. For a continuous variable this was the odds ratio associated with an increase of 1 standard deviation (SD), and for a dichotomous variable the reference group was those patients without the respective risk factor.

Similarly, multiple logistic regression was used to assess which variables were associated with the incidence of VPT while adjusting for all other key risk factors.

In order to assess the effect of glycaemic exposure on the incidence of abnormal VPT linear regression was performed by the centre to compare the results of HbA_{1c} measured locally and centrally at the same time, both at baseline. This provided a formula to convert locally measured HbA_{1c} to the centralized assay at each centre. An average of all local HbA_{1c} which had been measured for each patient, prior to the baseline visit, was calculated and converted to the central measure.

A breakpoint or threshold effect for the relation between HbA_{1c} and progression to abnormal VPT was tested by a two phase segmented weighted regression, where two straight lines are fitted through a series of defined points. These points were calculated by logistic regression adjusted for diabetes duration. This segmented regression was compared to the line of best fit using weighted linear regression. Logistic regression was also used to test for a threshold.

Results

Baseline characteristics according to whether VPT was assessed at follow-up

The baseline characteristics of the 1407 patients examined in this study were compared to those that qualified for follow-up (n=660), but did not have a follow-up assessment. Those that died or did not have VPT assessed at follow-up had higher baseline glycosylated haemoglobin (8.5 vs 8.1%, p<0.0001), waist-to-hip ratios (0.85 vs 0.84, p=0.04), systolic blood pressure (118 vs 116mmHg, p=0.05), total cholesterol (5.35 vs 5.21mmol/l, p=0.005), LDL-cholesterol (3.37 vs 3.27 mmol/l, p=0.05) and triglyceride levels (0.96 vs 0.87mmol/l, p<0.0001). In addition, they were more likely to have a history of smoking (51 vs 46%, p=0.03), proliferative retinopathy (8 vs 5%, p=0.02) and had lower Von Willebrand factor levels (1.09 vs 1.14U/ml, p=0.02).

Incidence of clinical outcomes

An abnormal VPT at baseline predicted the incidence of several lower-limb complications, i.e., foot ulcers 7.7 vs 1.7% (p<0.0001), leg bypass or angioplasty 1.6 vs 0.4% (p=0.02), gangrene 2.2 vs 0.6% (p=0.004), amputation 2.4 vs 0.4% (p=0.0004) and indeed mortality 8.0 vs 2.1% (p<0.0001).

Risk factors for the development of an abnormal VPT

Of the 1407 patients with normal VPT at baseline, an abnormal VPT developed in 333 at follow-up, an incidence of 23.7%. Table 1 shows baseline characteristics of the study group divided into those that had an abnormal versus normal VPT at follow-up. Patients that developed abnormal VPT were significantly older (2.5 years), with longer duration of diabetes (2.2 years), poorer blood glucose control (HbA_{1c} 0.6% higher) and were taller (2 cm) at baseline than those patients who maintained a normal VPT. Furthermore, patients that developed an abnormal VPT had significantly higher levels of traditional cardiovascular risk factors, i.e., male sex, history of smoking, weight, body-mass index, waist-to-hip ratio, hypertension, total cholesterol, LDL-cholesterol, triglycerides, history of cardiovascular disease and lower HDL-cholesterol levels. They were also more likely to have established complications of diabetes, i.e., micro- or microalbuminuria, higher albumin excretion rates, any retinopathy, proliferative retinopathy, and cardiac autonomic neuropathy.

By adjusting for the established confounding factors of duration of diabetes and HbA_{1c}, risk factors for the development of an abnormal VPT were determined using regression analysis, Table 2. Virtually all the traditional risk factors of cardiovascular disease and established complications of diabetes remained significantly associated with the development of abnormal VPT, the only exception being HDL-cholesterol. The risk factors with the highest odds ratios were macroalbuminuria and proliferative retinopathy, i.e., 2.48 and 2.17 respectively. Furthermore, as expected taller patients were more likely to develop an abnormal VPT, and this association remained statistically significant after also adjusting for male sex, OR = 1.38 (1.16, 1.64), p=0.003.

Multivariate Models

In order to examine independent associations between key risk factors and the incidence of abnormal VPT multiple logistic regression analysis was performed, Table 3. In Model 1 the analysis mutually adjusted for the presence of all other risk factors. It was found that duration of diabetes, glycosylated haemoglobin value, triglyceride, body-mass index, history of smoking and presence of hypertension at baseline remained significantly associated with the incidence of abnormal VPT. The relation between total cholesterol level and the incidence of abnormal VPT did not reach statistical significance, and neither did albumin excretion rate. The strongest relation was with history of smoking, which carried an odds ratio of 1.71, and the second strongest relation was with hypertension with an odds ratio of 1.65.

When complications of diabetes were added to the model (Table 3, Model 2) duration of diabetes as expected was not statistically significant. Glycosylated haemoglobin value, triglyceride, body-mass index, history of smoking and presence of hypertension at baseline remained significantly related to the incidence of abnormal VPT. In addition, the presence of cardiovascular disease at baseline was independently related to the incidence of abnormal VPT, with an odds ratio of 2.13. The relation between any retinopathy and abnormal VPT was not significant, $p = 0.1$.

As expected chronic glycaemic exposure was higher in the group who developed an abnormal VPT (HbA_{1c} 0.3% higher, $p=0.001$). When the above analyses were repeated using the adjusted mean HbA_{1c} the findings were virtually identical. Furthermore, when the relationship between HbA_{1c} and progression to abnormal VPT was tested there was no threshold effect.

Conclusions

Determination of vibration perception threshold using a biothesiometer is a quick and easy way of detecting large fibre dysfunction, and therefore identifies patients at risk of foot ulceration, lower-limb amputation and mortality (1, 2). During a period of 12 years, the incidence of abnormal VPT in patients with type 2 diabetes is estimated to be 19.9% (11). In this larger prospective study of patients with type 1 diabetes we observed an incidence of 23.7% over 7.3 years of follow-up. Apart from glycaemic control and duration of diabetes we have demonstrated that cardiovascular risk factors such as hypertension, smoking, obesity, elevated triglyceride levels and the presence of cardiovascular disease at baseline appear to predict the development of abnormal VPT. The patients who qualified for follow-up, but were not assessed had worse glycaemic and cardiovascular profiles and hence our results may, if anything, have underestimated the associations. The same independent risk factors have previously been shown to be related to newly diagnosed DPN (21). Both hypertension and a history of smoking were strong risk factors for development of neuropathy, and abnormal VPT. As expected a higher mean HbA_{1c} predicted the development of abnormal VPT, but no threshold effect was identified. A similar outcome was found in a previous study of a mixed cohort of type 1 and type 2 diabetes patients (9).

An abnormal VPT at baseline predicted the development of foot ulcers, leg bypass or angioplasty, gangrene, amputation and mortality. This finding is consistent with previous studies (12, 13). In a general diabetes clinic it is probably more important to routinely use a test which is highly sensitive, as opposed to highly specific, as the potential risk of DPN being under diagnosed is greater than the risk of over preventative measures being implemented in patients with false-positive results. Such measures include foot-care education, and more frequent follow-up, for instance in podiatry-led neuropathy clinics (23). Studies have shown

that measurement of VPT is more sensitive than the 10g monofilament in assessing foot ulcer risk (14). Despite this evidence, at present monofilaments are routinely used in outpatient clinics. Whilst the loss of ability to feel a 10g monofilament is known to predict ulceration (13), it usually occurs at a late stage in the disease process. Conversely, an abnormal VPT is often evident earlier in the natural history of the disease (24). Another advantage of the VPT method is that it provides a quantitative result, and for each unit increase, the risk of foot ulceration increases (13).

It is well recognised that patients with diabetes are at increased cardiovascular disease risk. Within the EURODIAB cohort, in cases where the cause of mortality is known, CVD was the cause of death in ~40% of subjects (25). This present study shows that even in a young cohort of patients with type 1 diabetes (average age 32.7 years at baseline) an abnormal VPT identifies a subset of patients with adverse cardiovascular risk parameters. This knowledge enables identification of those patients in need of more aggressive treatment of their CVD risk factors. The all-cause mortality rate of patients with an abnormal VPT at baseline was four times higher than those with a normal value, 8 versus 2% over 7.3 years follow-up.

In conclusion, determination of an abnormal VPT is important for two reasons. Firstly, it identifies a cohort of patients with large fibre dysfunction and its inherent clinical risks, and secondly, it identifies a population at increased cardiovascular risk. Therefore, intensive management of cardiovascular risk factors could not only reduce cardiovascular events, but also the clinical sequelae of neuropathy.

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Duality of interest None of the authors have declared a duality of interest

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Table 1. Baseline characteristics of 1407 patients according to the incidence of abnormal VPT.*

Variable	Progression to abnormal VPT	Maintenance of normal VPT	p-value
Number of patients	333	1074	
Age (yr)	33.4±10.2	30.9±8.8	<0.0001
Duration of diabetes (yr)	14.8±9.0	12.6±8.1	<0.0001
Male sex (%)	56	46	0.001
History of smoking (%)	56	42	<0.0001
Height (cm)	170±9	168±9	0.0004
Weight (kg)	70.1±10.8	65.5±10.1	<0.0001
Body-mass index	24.3±3.0	23.2±2.6	<0.0001
Waist-to-hip ratio	0.85±0.11	0.83±0.10	0.0053
HbA_{1c} (%)	8.6±2.0	8.0±1.8	<0.0001
Insulin dose / kg body weight (IU)	0.67±0.21	0.69±0.22	0.1
Total cholesterol (mmol/l)	5.45±1.17	5.14±1.02	<0.0001
LDL-cholesterol (mmol/l)	3.48±1.05	3.20±0.91	0.0005
HDL-cholesterol (mmol/l)	1.46±0.44	1.52±0.42	0.04
Triglyceride (mmol/l)	0.95 (0.54, 2.55)	0.84 (0.48, 2.06)	<0.0001
Fibrinogen (g/l)	3.26±0.95	3.15±0.87	0.1
Von Willebrand factor (U/ml)	1.25 (0.64, 2.32)	1.10 (0.54, 2.11)	0.0005
Systolic Blood pressure (mm Hg)▼	117 (98, 152)	116 (96,140)	0.06
Diastolic Blood pressure (mm Hg)▼	75 (57, 93)	73 (57, 92)	0.09
Hypertension (%)	27	15	<0.0001
History of cardiovascular disease (%)	11	6	0.005
Albumin excretion rate (μg/min)	12.6 (3.7, 447.4)	9.5 (3.2, 122.5)	<0.0001
Macroalbuminuria (%)	9.5	3.3	<0.0001
Micro- or Macroalbuminuria (%)	33	22	<0.0001
Any retinopathy (%)	52	33	<0.0001
Proliferative retinopathy (%)	9	4	0.0001
Cardiac autonomic neuropathy (%)	39	28	0.0001

* Plus-minus values are means ±SD. Alternatively, in cases of skewed distributions, data are medians (5^{th} percentile, 95^{th} percentile). P values are derived from Student's t-test or, in cases of skewed distributions from a Mann-Whitney U test and in cases of percentages from a chi-squared test.

▼ The blood pressure data exclude patients who were undergoing antihypertensive therapy

Table 2. Risk factors for the incidence of abnormal VPT after adjustment for HbA_{1c} and duration of diabetes.*

Variable	Odds ratio (95% CI)	p-value
Male sex (%)	1.58 (1.23 - 2.04)	0.0004
History of smoking (%)	1.69 (1.31 - 2.18)	<0.0001
Height (cm)	1.40 (1.22 - 1.59)	<0.0001
Weight (kg)	1.64 (1.44 - 1.86)	<0.0001
Body-mass index	1.43 (1.26 - 1.62)	<0.0001
Waist-to-hip ratio	1.17 (1.04 - 1.32)	0.009
Insulin dose / kg body weight (IU)	0.88 (0.77 - 0.99)	0.05
Total cholesterol (mmol/l)	1.22 (1.08 - 1.39)	0.002
LDL-cholesterol (mmol/l)	1.22 (1.04 - 1.42)	0.01
HDL-cholesterol (mmol/l)	0.90 (0.79 - 1.02)	0.10
Triglyceride (mmol/l)	1.35 (1.15 - 1.57)	0.002
Von Willebrand Factor (U/ml)	1.27 (1.08 - 1.51)	0.005
Hypertension (%)	1.88 (1.38 - 2.56)	<0.0001
History of cardiovascular disease (%)	1.62 (1.04 - 2.52)	0.03
Albumin excretion rate (μg/min)▼	1.29 (1.14 - 1.45)	<0.0001
Macroalbuminuria (%)	2.48 (1.47 - 4.19)	0.0006
Micro- or Macroalbuminuria (%)	1.44 (1.08 - 1.93)	0.01
Any retinopathy (%)	1.88 (1.36 - 2.61)	0.0001
Proliferative retinopathy (%)	2.17 (1.22 - 3.86)	0.008
Cardiac autonomic neuropathy (%)	1.58 (1.20 - 2.07)	0.001

* Standardized odds ratios are expressed per SD increase in each continuous risk factor. Odds ratios for dichotomous variables have as a reference group those patients without the respective risk factor. CI denotes confidence interval.

▼ Log transformation was used

Table 3. Odds Ratios for Associations between Key Risk Factors and the Incidence of Abnormal VPT with the Use of Two Logistic-Regression Models.*

	◆Model 1		◆◆Model 2	
Variable	**Odds ratio (95% CI)**	**p-value**	**Odds ratio (95% CI)**	**p-value**
Duration of diabetes (yr)	1.21 (1.03 - 1.43)	0.02	1.12 (0.90 - 1.39)	0.3
HbA_{1c} (%)	1.26 (1.06 - 1.49)	0.008	1.30 (1.07 - 1.68)	0.009
History of smoking (%)	1.71 (1.23 - 2.37)	0.001	1.85 (1.27 - 2.70)	0.01
Hypertension (%)	1.65 (1.08 - 2.50)	0.02	1.82 (1.12 - 2.94)	0.01
Body-mass Index	1.32 (1.12 - 1.56)	0.001	1.26 (1.04 - 1.53)	0.002
Total cholesterol (mmol/l)	1.06 (0.88 - 1.27)	0.6	0.94 (0.76 - 1.17)	0.6
Triglyceride (mmol/l)	1.21 (1.01 - 1.45)	0.04	1.27 (1.03 - 1.58)	0.03
Albumin excretion rate (μg/min)▼	1.15 (0.98 - 1.35)	0.09	1.20 (1.00 - 1.44)	0.05
History of cardiovascular disease (%)			2.13 (1.10 - 4.12)	0.03
Any retinopathy (%)			1.44 (0.92 - 2.26)	0.1

* Standardized odds ratios are expressed per SD increase in each continuous risk factor. Odds ratios for dichotomous variables have as a reference group those patients without the respective risk factor. CI denotes confidence interval.

▼ Log transformation was used

◆ Model 1 – mutually adjusted for all other risk factors

◆◆ Model 2 – mutually adjusted for all other risk factors and complications of diabetes