Peripheral vascular and nerve function associated with lower limb amputation in people with and without diabetes

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ABSTRACT

Multiple factors, including peripheral vascular disease and neuropathy, contribute to the development and perpetuation of complications of the lower extremities in diabetes. The main aim of the present study was to assess the peripheral vascular and nerve status of diabetic and non-diabetic subjects that had undergone lower limb amputation. Various non-invasive tests of peripheral vascular and nerve function were carried out on subjects who had undergone unilateral lower limb amputation and were now attending a Rehabilitation Centre. The control group ($n=23$), the diabetic amputee group ($n=64$) and the non-diabetic amputee group ($n=32$) were age-matched. Only the diabetic amputee group had evidence of medial arterial calcification. Transcutaneous oxygen levels were significantly lower in the diabetic amputee group (median 43 mmHg; interquartile range 33–49 mmHg) than in the control (59; 56–74 mmHg) and non-diabetic amputee (57; 43–65 mmHg) groups (control compared with diabetic amputee group, $P<0.001$; diabetic amputee compared with non-diabetic amputee group, $P>0.01$). The same trend was found for carbon dioxide levels in the skin [mmHg: diabetic amputees, 25 (21–37); controls, 38 (32–42); non-diabetic amputee, 34 (31–39)] (control compared with diabetic amputee, $P<0.01$; diabetic amputee compared with non-diabetic amputee, $P<0.05$). Vibration and pressure perception measurements (which assess Aβ nerve fibre function) showed that both the diabetic amputee and non-diabetic amputee subjects had significantly greater impairment than the controls. However, measures of Aα and C nerve fibre function were abnormal only in the diabetic amputee group. Thus the peripheral vascular and nerve functions of age-matched diabetic and non-diabetic subjects having undergone lower limb amputation show specific differences, with non-diabetic amputees exhibiting signs of neuropathy. This indicates that factors characteristic of diabetes (such as hyperglycaemia and non-enzymic glycation) are associated with calcification, lower oxygen and carbon dioxide levels in the skin, and abnormal Aα and C nerve fibre function.

Key words: amputee, diabetes mellitus, nerve, oxygen, peripheral vascular.

Abbreviations: ABPI, ankle brachial pressure index; HbA1c, glycated haemoglobin; MNCV, motor nerve conduction velocity; NDS, neurological disability score; PPT, pressure perception threshold; $TcPCO_2$, transcutaneous partial pressure of carbon dioxide; $TcPO_2$, transcutaneous partial pressure of oxygen; TPT, temperature perception threshold.

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INTRODUCTION

It is clear from the numerous prospective and cross-sectional studies examining risk factors for foot ulceration and amputation in diabetes that a multitude of physiological and sociological factors contribute to the development and perpetuation of lower extremity complications [1–4]. These include peripheral vascular disease [5,6], peripheral neuropathy [2,7,8], limited joint mobility [9], and trauma, infection and poor wound healing [10,11], among others.

Distal symmetrical polyneuropathy affects the sensory, motor and autonomic sections of the peripheral nervous system [1,8,12–15] and is a primary cause of foot ulceration. Peripheral vascular disease has also been implicated in foot ulceration in diabetes, but may play a greater role in problems ultimately necessitating lower limb amputation [4,5]. In addition, hypoxia resulting from factors unrelated to diabetes can also result in altered neuronal function [16,17].

Prospective studies are useful in determining which subjects are likely to require lower limb amputation in the future, but it is also important to determine lower limb physiology at the time of amputation in cross-sectional studies, to obtain information regarding the active processes involved. One of the problems associated with prospective studies in diabetes is that patients may change their glycaemic control, and/or their drug regimens may be altered, during the course of the study, all of which may affect outcome.

The main aim of the present cross-sectional study was to assess the peripheral nerve and vascular status of diabetic and non-diabetic subjects (with peripheral vascular disease) that had undergone lower limb amputation, in comparison with age-matched controls. The non-diabetic amputees (with peripheral vascular disease) were studied in order to assess the effects of peripheral vascular disease alone on neurological function, and to determine whether there is a diabetes-specific neurovascular disorder associated with problems leading to lower limb amputation.

METHODS

Subjects

It is ethically unacceptable to carry out neurovascular testing on the limb that is about to be amputated. In addition, many of the patients were in pain and/or had gangrene/infection at the time of amputation. Consequently, subjects attending a Sub-Regional Rehabilitation Centre at Withington Hospital Disablement Services Centre (post-amputation), and who agreed to participate, were recruited for the study and assigned to the following groups: (1) control non-diabetic subjects (staff, relatives and friends); (2) diabetic amputee group, comprising diabetic subjects having undergone unilateral lower limb amputation; (3) non-diabetic amputee group, comprising non-diabetic subjects having undergone unilateral lower limb amputation (due to peripheral vascular disease alone). All other causes of neuropathy, such as toxic (e.g. alcohol-induced), malignant (e.g. bronchogenic carcinoma) and iatrogenic (e.g. isoniazid-induced) neuropathy were excluded.

The study was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association, and was approved by the South Manchester Research Ethics Group. The patients gave informed consent.

The various tests were carried out on the remaining limb (peripheral neuropathy and vascular disease follow a symmetrical distribution, so the test results are representative of the systemic disease). All assessments were made at a skin temperature of $\geq 30 \, ^\circ\text{C}$, measured with a Mikron Thermometer (model M806-OC; Mikron, Wyckoff, NJ, U.S.A.) and maintained using a controllable heating pad, within a leg trough.

Peripheral vascular assessment

The ankle brachial pressure index (ABPI) was determined using a Doppler ultrasound machine (Sonicaid, Oxford, U.K.) and a portable sphygmomanometer (Accoson). A value of $< 0.8$ indicated vascular insufficiency. Values of $\geq 0.8$ were subclassified as normal or calcified (if the dorsalis pedis pressure was $> 280 \, \text{mmHg}$).

The transcutaneous partial pressures of oxygen ($\text{TcPO}_2$) and carbon dioxide ($\text{TcCO}_2$) were measured at the foot dorsum, just proximal to the second and third metatarsal heads, using a Transcutaneous $\text{PO}_2/\text{PCO}_2$ monitoring system (Radiometer, Crawley, W. Sussex, U.K.). The electrode combines a heating element, two temperature sensors, a Clark-type oxygen electrode and Severinghaus-type carbon dioxide electrode in a single unit. Prior to each measurement, the system was calibrated. After calibration, the electrode was fixed at the skin surface and generated heat was transferred to the skin surface to heat the skin to $43 \, ^\circ\text{C}$. This produced local vasodilation to increase the permeability of the skin to oxygen and carbon dioxide, rendering a measurement at the skin surface possible. When the oxygen values had stabilized (20 min), the values were recorded at 1 min intervals for a period of 5 min, and the mean value taken as the partial pressure. $\text{PO}_2$ values of $< 30 \, \text{mmHg}$ were considered abnormal; no abnormal values for $\text{PCO}_2$ have been established.

Peripheral neurological assessment

A modified neurological disability score (NDS) [15] was assessed for each patient. This is a composite score derived from the assessment of pain, temperature, vibration sense and Achilles reflex (no./5 for one leg).
The vibration perception threshold (VPT) was measured using a Neurothesiometer (Horwell Scientific Laboratory Supplies, Wilford, Nottingham, U.K.) at the hallux and medial malleolus.

The cutaneous pressure perception threshold (PPT) was determined using Semmes Weinstein Monofilaments (1 g, 10 g and 75 g; Gillis W. Long Hansen's Disease Center, Carville, LA, U.S.A.) at one mid-dorsal site and three plantar sites (first metatarsal head, fifth metatarsal head and heel) on the foot. The dorsal site was scored separately from the plantar sites. If the patient felt the 1 g filament at the dorsal site or at all three sites on the plantar surface, a score of 6 was given for each surface of the foot. If the patient could not feel the 1 g filament at any site, the 10 g filament was used; if it was felt at all sites, a score of 5 was given for that surface of the foot. If the 10 g filament was not felt at any site, the 75 g filament was used; if it was felt at all sites, a score of 6 was given. If it was not felt at any site a score of 7 was given. The filaments were tested three times at each site.

Motor nerve conduction velocity (MNCV) in the common peroneal nerve was measured using an MS92a EMG machine (Medelec Ltd, Old Woking, Surrey, U.K.). Action potentials were recorded using surface electrodes placed at the extensor digitorum brevis muscle, and the common peroneal nerve was stimulated (300 V intensity; 0.1 ms duration) to obtain a supramaximal stimulus. Stimulation was carried out at the head of the fibula and mid-way between the malleoli on the anterior surface of the limb. Skin temperature was recorded, the length of the nerve was measured, and proximal and distal latencies were recorded.

The temperature perception threshold (TPT) (for heat) was determined using a forced-choice procedure, with a Therm-aesthesiometer (model AZVU; Medical Instruments Department, VU Hospital, Amsterdam, The Netherlands) at the foot dorsum.

Statistical analysis

Data from the three groups of subjects that followed a non-parametric distribution were ranked and compared using the Kruskal–Wallis and Dunn’s multiple comparison tests (Graph Pad Prism 3.0). This method compares the differences in the sums of the ranked data between groups with an expected average difference. In addition, the chi-square test (Excel; Microsoft Office 2000) was employed where appropriate.

RESULTS

The control group (n = 23; 10 male/13 female), the diabetic amputee group (n = 64; 41 male/23 female) and the non-diabetic amputee group (n = 32; 18 male/14 female) were age-matched [medians and interquartile ranges: 61 (57–66), 67 (58–72) and 69 (63–73) years respectively; no significant difference by the Kruskal–Wallis test]. The duration of confirmed diabetes in the diabetic amputee group was 15.6 ± 10.4 years (mean ± S.D.). The absence of diabetes in the control group and in the non-diabetic amputee group was confirmed by random assessment of blood glucose in these two groups (5.92 ± 1.58 and 4.76 ± 1.84 mmol/l respectively; means ± S.D.).

The median follow-up time between amputation and neurovascular assessment was 109 days (interquartile range 68–153 days). The primary causes of amputation in the diabetic group recorded in the notes were sub-classified as follows: general peripheral vascular disease/ischaemia, 2; sudden blockage/embolism, 4; failed angioplasty/grafting, 13; severe symptoms of peripheral vascular disease, 1; gangrene, 13; ulcers/infection, 21; failed minor amputation, 10. For the non-diabetic group, the primary causes of the amputations were: general peripheral vascular disease/ischaemia, 13; sudden blockage/embolism, 4; failed angioplasty/grafting, 10; severe symptoms of peripheral vascular disease, 2; gangrene, 3. In addition, smoking status for the three groups was defined as never smoked, past smoker and smoker. The distribution of the control group for these three categories was 83%, 17% and 0% respectively, that for the diabetic amputees was 26%, 45% and 29% respectively, and that for the non-diabetic amputees was 9%, 56% and 35% respectively.

The results from the ABPI assessment were compared in various ways, as shown in Table 1. Comparisons using ranked data and the Kruskal–Wallis test showed that both the diabetic and non-diabetic amputee groups had a significantly lower ABPI than the age-matched controls. A greater proportion of non-diabetic amputees had an ABPI of < 0.8 (indicating vascular insufficiency) compared with the diabetic amputee and control groups, and the only group that had any significant calcification of the dorsalis pedis artery was the diabetic amputee group. Both TcPO₂ and TcPCO₂ were significantly lower in the diabetic amputee group than in the control and the non-diabetic amputee groups (Table 1).

The neurological status of the subjects is given in Table 2. The NDS was highest in the diabetic amputee group, but the non-diabetic amputee subjects also demonstrated some neurological impairment that was significantly different from that in both the control and the diabetic amputee groups.

Both VPT and PPT measurements (which assess Aβ nerve fibre function) showed that both diabetic amputees and non-diabetic amputees had significantly greater dysfunction than control subjects. However, MNCV (which assesses Aα nerve fibre function) and TPT (which assesses C nerve fibre function) values were significantly abnormal only in the diabetic amputee group, and not in the non-diabetic amputee group, when compared with controls.
Table 1  Peripheral vascular assessments
Values are given as median (interquartile range), or as number of subjects (%).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (C)</th>
<th>Diabetic amputees (DA)</th>
<th>Non-diabetic amputees (A)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABPI</td>
<td>1.07 (1.03–1.29)</td>
<td>0.83 (0.62–1.00)</td>
<td>0.87 (0.56–1.00)</td>
<td>C vs DA, P &lt; 0.01; C vs A, P &lt; 0.01</td>
</tr>
<tr>
<td>ABPI unobtainable</td>
<td>0/23 (0%)</td>
<td>8/63 (12.7%)</td>
<td>6/32 (18.8%)</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>ABPI &lt; 0.8</td>
<td>0/23 (0%)</td>
<td>19/55 (34.5%)</td>
<td>11/26 (42.3%)</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>ABPI indicating calcification</td>
<td>0/23 (0%)</td>
<td>10/55 (18.1%)</td>
<td>0/26 (0%)</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>TcPO2 (mmHg)</td>
<td>59 (56–74)</td>
<td>43 (33–49)</td>
<td>57 (43–65)</td>
<td>C vs DA, P &lt; 0.001; DA vs A, P &lt; 0.01</td>
</tr>
<tr>
<td>TcPO2 &lt; 30 mmHg</td>
<td>0/22 (0%)</td>
<td>10/52 (19.2%)</td>
<td>3/30 (10%)</td>
<td></td>
</tr>
<tr>
<td>TcPCO2 (mmHg)</td>
<td>38 (32–42)</td>
<td>25 (21–37)</td>
<td>34 (31–39)</td>
<td>C vs DA, P &lt; 0.01; DA vs A, P &lt; 0.05</td>
</tr>
</tbody>
</table>

Table 2  Peripheral neurological assessments
Values are given as median (interquartile range). See the Methods section for a description of the scoring system used for PPT.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (C)</th>
<th>Diabetic amputees (DA)</th>
<th>Non-diabetic amputees (A)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDS (no./5)</td>
<td>0.0 (0.0–1.5)</td>
<td>3.0 (2.5–4.0)</td>
<td>2.0 (1.0–3.0)</td>
<td>C vs DA, P &lt; 0.001; C vs A, P &lt; 0.05; DA vs A, P &lt; 0.01</td>
</tr>
<tr>
<td>VPT, hallux (V)</td>
<td>7.0 (5.5–11.0)</td>
<td>25.0 (16.5–35.0)</td>
<td>16.5 (13.5–25.5)</td>
<td>C vs DA, P &lt; 0.001; C vs A, P &lt; 0.001</td>
</tr>
<tr>
<td>VPT, medial malleolus (V)</td>
<td>10.0 (5.5–11.5)</td>
<td>25.0 (18.0–35.0)</td>
<td>18.5 (13.0–28.5)</td>
<td>C vs DA, P &lt; 0.001; C vs A, P &lt; 0.05</td>
</tr>
<tr>
<td>PPT, dorsum</td>
<td>4.0 (4.0–4.0)</td>
<td>4.0 (4.0–5.0)</td>
<td>4.0 (4.0–5.0)</td>
<td>C vs DA, P &lt; 0.01</td>
</tr>
<tr>
<td>PPT, plantar</td>
<td>4.0 (4.0–4.5)</td>
<td>5.0 (5.0–6.0)</td>
<td>5.0 (4.0–6.0)</td>
<td>C vs DA, P &lt; 0.001; C vs A, P &lt; 0.05</td>
</tr>
<tr>
<td>MNCV (m/s)</td>
<td>47.9 (46.2–49.2)</td>
<td>36.3 (32.0–39.5)</td>
<td>43.3 (41.3–46.7)</td>
<td>C vs DA, P &lt; 0.001; DA vs A, P &lt; 0.001</td>
</tr>
<tr>
<td>TPT, dorsum (°C)</td>
<td>0.5 (0.5–0.5)</td>
<td>2.75 (0.5–11.0)</td>
<td>0.5 (0.5–1.0)</td>
<td>C vs DA, P &lt; 0.01; DA vs A, P &lt; 0.01</td>
</tr>
</tbody>
</table>

DISCUSSION

This study shows that peripheral vascular function not only was different in the two groups of amputees compared with age-matched controls, but also differed between the diabetic and non-diabetic groups. The study highlights the problems associated with the interpretation of ABPI data. Primarily, in some patients it is not possible to determine the ABPI at all, due to the loss of pulsatile flow in the dorsalis pedis artery. In addition, measurement of the median value did not discriminate between the two amputee groups. However, it was clear that calcification (as assessed by the methods described here) is specific to diabetes. Calcification is often seen as a problem when measuring ABPI; however, it may play an important physiological role, and its presence should be examined carefully rather than being regarded as an impairment to data collection. Medial arterial calcification has been suggested to result from the binding of calcium to elastin in the arterial wall, and it has been reported that the glycation of arterial elastin is correlated with increased calcium deposition in diabetic rats [18]. The interaction of various sugars with elastin has been shown to alter its biochemical and physical properties, and this is dependent on pH [19]. As assessment of TcPO2 is an indirect measure of pH, this may indicate a role for such assessment in relation to arterial stiffening and calcification. It is possible that the measurement of calcification at annual review may be a simple, useful indicator for a physiological role of non-enzymic glycation, in a similar way that HbA1c (glycated haemoglobin) measures the biochemical role of glycation for the subject with diabetes.

TcPO2 is an assessment of the oxygen availability in the skin and therefore depends upon both macrovascular and microvascular delivery of oxygen. It is assumed that the non-diabetic amputees have a decreased oxygen supply to the skin that is mainly due to macrovascular impairment. Surprisingly, there was no significant decrease in TcPO2 in the non-diabetic amputee group compared with the control subjects, whereas TcPO2 values were significantly lower in the diabetic amputee group. This may be related to a different distribution of peripheral vascular disease in diabetic subjects compared with that seen in non-diabetic subjects, i.e. macrovascular disease is the major finding in non-diabetic subjects, whereas microvascular disease is more often associated with diabetes.

The problems of accurately assessing peripheral vascular function are numerous, especially with regard to TcPO2 measurement [20]. However, TcPO2 may be a better
predictor of ulcer healing than toe blood pressure [21]. In addition, a TcPO₂ value of < 30 mmHg is an independent predictor of foot ulceration in diabetes [6]. Clearly, improved simple methods for the assessment of peripheral vascular function are required in the clinical setting.

The finding of a significant difference in TcPO₂ values between the non-diabetic and diabetic amputee groups studied here requires further investigation. There is evidence in the literature that alterations in acid/base balance can lead to altered vascular and nerve function [22,23]. In their experiments with isolated peripheral nerves, Strupp et al. [22] showed that high glucose availability and low PO₂ in combination with decreased buffering power and/or inhibition of HCO₃⁻-dependent pH regulatory mechanisms may damage peripheral mammalian nerves due to pronounced intracellular acidosis.

Many different peripheral vascular abnormalities have been associated with the diabetic foot, including arteriovenous shunting [24–26] and an impaired neurogenic vascular response [27]. Numerous studies have examined the impaired endothelium-dependent and -independent vasodilation that occurs in diabetic patients by various methods [28–32], but there is still no consensus as to which method is preferable for diagnosis in the clinical rather than the research setting.

Other factors, such as dyslipidaemia, are important in both the development of peripheral vascular disease and peripheral neuropathy [33], and consequently lower limb amputation in diabetes. High low-density lipoprotein cholesterol, high HbA1c and smoking are predictive of lower extremity arterial disease in Type I diabetes [34]. In addition, low high-density lipoprotein is a predictor of foot lesions in Type II diabetes [7]. Finally, serum lipoprotein(a) is also associated with peripheral vascular disease in diabetes [35].

Our study showed that the non-diabetic amputees also had a significant degree of nerve dysfunction that is not attributable to raised blood glucose levels. The impairment of VPT (Aβ fibres) was not as severe in the non-diabetic amputees as in the diabetic amputees. Diabetic patients exhibit hyperglycaemia and hypoxia to some degree; it is likely that the metabolic nerve dysfunction results from both of these conditions, and that the effects are additive [36]. Even though non-diabetic amputees are not hyperglycaemic, they do exhibit degrees of hypoxia due to the occlusive nature of the disease. It is interesting that the impairment seen in the non-diabetic amputees with peripheral vascular disease was only present in the Aβ nerve fibres, which are involved in both vibration and pressure perception. The impairments of both temperature perception (small unmyelinated C fibres) and motor nerve conduction velocity (large myelinated Aβ fibres) were specific to the diabetic amputees, and presumably to the environment brought on by the combination of increased blood glucose and hypoxia (non-enzymic glycation, oxidative stress and increased aldose reductase activity, among others).

In conclusion, the present study has shown that vascular dysfunction associated with amputation differs between subjects with and without diabetes. Calcification was specific for the diabetic amputees, and skin oxygen levels (at least as assessed by the techniques described here) were decreased only in the diabetic subjects. Skin carbon dioxide levels were also decreased in the diabetic amputees, which is a novel finding requiring more investigation. The neurological findings in the non-diabetic amputees indicate that peripheral vascular disease per se is associated with impaired vibration and pressure perception, and that hyperglycaemia is not involved. However, diabetes-associated hyperglycaemia is important in the development of impaired MNCV and temperature sensation.

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A. L. Carrington and others


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