Diabetes mellitus affects about one in 25 Australians. Neuropathic foot ulceration is a frequent complication in persons with diabetes. This study evaluates the importance of different risk factors for the development of this condition. The role of nonenzymatic glycosylation and pressure beneath the sole of the foot in the pathogenesis of neuropathic foot ulcers was investigated. Twenty-seven subjects with diabetes with a recent history of neuropathic foot ulceration were matched by age and sex with a group of 50 control subjects without neuropathy or history of foot ulceration. The degree of nonenzymatic glycosylation was assessed by analyzing the average level of glycosylated hemoglobin in the 3 years prior to the development of the foot ulcer and a goniometer assessment of peripheral joint (hand and ankle) flexibility. Dynamic pressure of the plantar aspect of the foot was recorded using a Musgrave Footprint System® pedobarograph during a normal gait cycle. There was no significant difference in age, sex, body mass index, and duration or type of diabetes between the ulcer and control groups. The pressure of the plantar aspect of the foot was significantly elevated \((p < 0.01)\). Ankle joint flexibility was reduced \((p < 0.01)\) in cases with neuropathic foot ulceration compared with the control group. There was a trend toward elevation of glycosylated hemoglobin (HbA1c fraction) or HbA1c in the ulcer group \((p = 0.06)\). The results suggested that nonenzymatic glycosylation occurs at a more significant level in patients with diabetes with a history of neuropathic foot ulceration.

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Diabetes mellitus is a common condition with an estimated prevalence in western societies of 3\% to 4\% using the strict criteria of elevated blood glucose of greater than 10 mmol/L in a glucose tolerance test.\(^1\)

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\(^1\) W.M. Automation, Newtonabbey, Northern Ireland, United Kingdom.

About 400,000 Australians have diabetes mellitus. It has been estimated that 50\% of nontraumatic lower extremity amputations (approximately 2,000 per year) are performed on diabetic patients and 20\% to 25\% of all hospital admissions of diabetic patients are for foot-related problems. At any one time, 2\% of hospital beds are occupied with diabetic foot complications and the annual cost of treatment exceeds $30 million.\(^2\) The lifetime risk of lower extremity amputation for diabetic individuals is 5\% to 15\%.\(^3\) The molecular and mechanical events leading to foot ulceration in diabetes have yet to be fully elucidated. Reports in the literature have shown that pa-
tients with diabetes with peripheral neuropathy and elevated foot pressure are at risk of developing ulcers on the sole of the foot. Recent research has also suggested that nonenzymatic glycosylation of connective tissue may contribute to foot ulceration in diabetic patients. Nonenzymatic glycosylation occurs when the ambient blood glucose level is elevated. Free glucose molecules attach to proteins in the blood and tissues, including collagen. Glycosylation of collagen results in cross-linking of the collagen fibers. The resulting decrease in the flexibility of collagen may reduce the shock absorption capacity of the foot and compromise joint mobility.

This study was to investigate whether nonenzymatic glycosylation was associated with increased foot pressure and increased risk of neuropathic ulceration on the plantar aspect of the foot in patients with diabetes mellitus. The hypothesis was that elevated levels of HbA1c and increased foot pressure indicate a significantly increased risk of developing foot ulcers in diabetic patients with peripheral sensory neuropathy. The study assessed the strength of association of risk factors rather than tested a specific causal hypothesis.

The public health benefits of this research may be realized in the accurate and early detection of persons with diabetes at greatest risk of developing foot ulceration, so that resources may be appropriately directed to preventative health programs.

**Methodology**

A case control study was selected to investigate the relationship of nonenzymatic glycosylation of collagen and foot pressure of the plantar aspect of feet in patients with a history of neuropathic foot ulceration. Seventy-seven subjects were recruited from a register of patients attending the diabetic units at the Princess Alexandra Hospital in Brisbane, Queensland, and the Royal Hobart Hospital in Hobart, Tasmania. The patients had to have at least one episode of neuropathic foot ulceration on the plantar aspect in the 12 months preceding the study, but to be free of ulceration at the time of the study.

Control subjects were matched by age and sex and were selected on the basis that they had attended the hospital diabetic unit at least once during the previous 12 months and did not have a history of foot ulceration. A standard questionnaire obtaining information on the subjects' medical history, history of foot ulceration, and previous treatment was administered to all participants.

Peripheral nerve function was assessed using two tests. One was of the subjects' sensitivity to vibration using a biothesiometer. A probe, vibrating at varying amplitudes proportional to the voltage applied, was placed on the hallux and plantar aspect of the foot. The patients indicated the threshold at which they were first able to perceive vibrations. Patients with a vibratory perception threshold of greater than 30 volts were classified as having clinically severe peripheral neuropathy.

A second test using Semmes-Weinstein monofilaments confirmed each subject's level of sensitivity to pressure. Small filaments were placed on the hallux and forefoot and a range of pressures applied. The various monofilaments represented the common logarithm of 10 times the monofilament buckling force. Subjects incapable of perceiving a force less than 5.07 units were classified as having peripheral sensory neuropathy.

Peripheral joint flexibility was measured as an indicator of the extent to which connective tissue had been affected by nonenzymatic glycosylation and the proportion of HbA1c in the blood was assessed as an indicator of the rate of nonenzymatic glycosylation over an extended time.

The ankle joint dorsiflexion range of motion from the perpendicular was measured with a goniometer as described by Mueller et al. The flexibility of the first metacarophalangeal joint of the left hand was also measured using the technique described by Delbridge.

The levels of HbA1c during the 36 months prior to the study were assessed by averaging the results of tests recorded in the subjects' medical records. At least three test results recorded at greater than 6 monthly intervals were used for each subject.

Pressure of the plantar aspect of the foot was measured using a Musgrave Footprint System which relays information to a computer for automatic analysis using Musgrave software. Dynamic pressure recordings were made of six foot prints with the highest and lowest pressure prints discarded and the average foot pressure obtained from the remaining four prints. The metatarsal heads, hallux and heel, were selected for pressure analysis because these are the most frequent sites of plantar foot ulceration. The maximum peak pressure (kg/cm²) was recorded for statistical analysis.

**Data Analysis**

All statistical analysis was done using the Statistical Analysis System, except for the conditional logistic regression for which Egret was used. All data were assessed for consistency by checking frequency and range and scatter plotting all variables.

Mean and standard deviation for each test were calculated and the normality of distribution was tested by using the Wilcoxon signed rank test. The Student's t-test was used to assess the significance
of differences between case and control subjects in all tests that showed a normal distribution of data as tested by the Shapiro-Wilk test. In cases where the distribution of data was not normal, a nonparametric test (the Wilcoxon signed rank test) was used to assess the differences.

Conditional logistic regression (appropriate for use in matched case-control studies) was used to assess the strength of association between the factors assessed and the risk of diabetic foot ulceration. The thresholds of foot pressure, joint flexibility and levels of HbAlc below which foot ulceration does not occur were assessed using the likelihood ratio statistic. This test compares odds ratios at several designated levels of foot pressure, joint flexibility, and HbAlc.

**Results**

There was no significant difference between the case group (patients with diabetes with a history of neuropathic plantar foot ulcers) and the control group (patients with diabetes without a history of ulceration) in age, sex, body mass index, and duration or type of diabetes.

Case subjects had significant levels of neuropathy as assessed by their vibratory perception threshold and confirmed by pressure perception threshold. Their peripheral nerve function was significantly worse than the control group in both tests. Vibratory perception threshold: 40.1 ± 12.3 versus 24.7 ± 14.2, p < 0.01.

Pressure of the plantar aspect of the foot was significantly elevated in case subjects compared with the control group (8.7 ± 2.3 versus 6.0 ± 2.1 kg/cm², odds ratios 4.8, 95% confidence intervals 1.44 - 16.3, p < 0.01). The case subjects also had higher HbA1c levels than the control group, but this did not achieve statistical significance (10.4% ± 1.9 versus 8.9% ± 1.3, odds ratios 1.69, 95% confidence intervals 0.96 - 2.99, p = 0.06).

Ankle joint flexibility was significantly reduced in case subjects compared to the control subjects (5.1° ± 4.0 versus 11.0° ± 5.3, odds ratio 0.89, 95% confidence intervals 0.82 - 0.96, p < 0.01).

The peripheral nerve function of all subjects in this study was assessed. Those subjects with a history of foot ulceration were shown to have significant peripheral neuropathy, as assessed by subject perception of vibration and pressure on the foot. This result is consistent with previous studies that showed significant differences in peripheral nerve function between subjects with neuropathic ulcers and controls. It has been suggested that the pathogenesis of neuropathy involves prolonged hyperglycemia and glycosylation of nerve protein.

Influences from other potential risk factors for foot ulceration in patients with diabetes, including age, sex, body mass index, and type and duration of

### Table 1. Comparison of Variables Associated With Foot Ulcer Group and Controls (Mean ± Standard Deviation)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases</th>
<th>Controls</th>
<th>P-Value</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>27</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>55 ± 13</td>
<td>58 ± 13</td>
<td>0.26</td>
<td>t</td>
</tr>
<tr>
<td>Sex ratio (male/female)</td>
<td>18/9</td>
<td>36/13</td>
<td>0.6</td>
<td>X</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>17.5 ±12.0</td>
<td>14.5 ±10.2</td>
<td>0.24</td>
<td>t</td>
</tr>
<tr>
<td>Type of diabetes</td>
<td>15/12</td>
<td>27/23</td>
<td>0.89</td>
<td>X</td>
</tr>
<tr>
<td>Body mass index (%)</td>
<td>28.4 ± 5.5</td>
<td>27.1 ± 5.0</td>
<td>0.35</td>
<td>t</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>10.4 ± 1.9</td>
<td>8.9 ± 1.3</td>
<td>0.01*</td>
<td>t</td>
</tr>
<tr>
<td>Vibratory perception threshold (volts)</td>
<td>40.1 ±12.3</td>
<td>24.7 ±14.2</td>
<td>0.01*</td>
<td>t</td>
</tr>
<tr>
<td>Hand flexibility (°)</td>
<td>15.4 ± 5.6</td>
<td>20.1 ± 8.5</td>
<td>0.01*</td>
<td>Φ</td>
</tr>
<tr>
<td>Ankle flexibility (°)</td>
<td>5.1 ± 4.0</td>
<td>11.0 ± 5.3</td>
<td>0.01*</td>
<td>Φ</td>
</tr>
<tr>
<td>Maximum foot pressure (kg/cm²)</td>
<td>8.7 ± 2.3</td>
<td>6.0 ± 2.1</td>
<td>0.01*</td>
<td>t</td>
</tr>
</tbody>
</table>

*Statistically significant difference at a p < 0.01, t = Student’s t-test, Χ = chi-square test, Φ = Wilcoxon test.

### Table 2. Odds Ratios, 95% Confidence Limits and Probability Values Estimated by Conditional Logistic Regression Analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>95% Confidence Intervals</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.87</td>
<td>0.71 - 1.0</td>
<td>0.17</td>
</tr>
<tr>
<td>Body mass index</td>
<td>1.0</td>
<td>0.93 - 1.13</td>
<td>0.60</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>1.0</td>
<td>0.97 - 1.06</td>
<td>0.37</td>
</tr>
<tr>
<td>Vibratory perception threshold</td>
<td>4.9</td>
<td>1.0 - 24.0</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Glycosylated hemoglobin</td>
<td>1.69</td>
<td>0.96 - 2.99</td>
<td>0.06</td>
</tr>
<tr>
<td>Hand flexibility</td>
<td>0.89</td>
<td>0.82 - 0.96</td>
<td>0.02*</td>
</tr>
<tr>
<td>Ankle flexibility</td>
<td>0.78</td>
<td>0.67 - 0.91</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Maximum foot pressure</td>
<td>4.8</td>
<td>1.44 - 16.3</td>
<td>0.01*</td>
</tr>
</tbody>
</table>

*Significant at p-value < 0.01.

The peripheral nerve function of all subjects in this study was assessed. Those subjects with a history of foot ulceration were shown to have significant peripheral neuropathy, as assessed by subject perception of vibration and pressure on the foot. This result is consistent with previous studies that showed significant differences in peripheral nerve function between subjects with neuropathic ulcers and controls. It has been suggested that the pathogenesis of neuropathy involves prolonged hyperglycemia and glycosylation of nerve protein.

Influences from other potential risk factors for foot ulceration in patients with diabetes, including age, sex, body mass index, and type and duration of
diabetes, were minimized in this study by matching case and control subjects for these variables.

This study showed that patients with diabetes with a history of neuropathic foot ulceration have significantly higher rates of nonenzymatic glycosylation than do patients with diabetes without a history of ulceration. This was shown by a trend toward higher average levels of glycosylated hemoglobin during 36 months in subjects with a history of foot ulceration than in control subjects.

Patients with diabetes with a history of foot ulceration were also shown to have significantly compromised joint mobility in their ankles and hands, indicating that glycosylation of collagen had reduced the flexibility of their connective tissue. Mobility was reduced by an average of 10% to 30% in patients with diabetes with a history of foot ulceration compared with controls. This finding supports studies by other researchers showing an association between limited joint mobility and the risk of foot ulceration.11

It was hypothesized that nonenzymatic glycosylation of collagen would reduce the capacity of the connective tissue in the foot to absorb shock, thus leading to increased pressure on the foot. This study showed that mean pressure of the plantar aspect of the foot, regardless of its location, was significantly higher in subjects with a history of foot ulceration (8.7 ± 2.3 kg/cm²) than in control subjects (6.0 ± 2.1 kg/cm²). Increased foot pressure should therefore be considered a significant risk factor for ulceration (odds ratio 4.8; confidence intervals 1.4 - 16.3).

Reduced ankle joint mobility may disrupt the biomechanical function of the foot and compromise the distribution of pressure under the foot. A previous study has, in fact, shown a strong association between limited joint mobility and high foot pressure.12

Areas of high pressure on the plantar surface of the foot are most susceptible to tissue breakdown, possibly progressing to neuropathic ulceration.

Previous research has shown an association between prolonged hyperglycemia and foot ulcers in patients with diabetes.3 It has been demonstrated that cross-linking of glucose caused by nonenzymatic glycosylation occurs at or near physiologic concentrations of glucose and that it is a time-dependent reaction.13 The results of this study support the suggestion that glycosylation of collagen is one of the links between hyperglycemia and foot ulceration.

A suggested sequence of events is that prolonged hyperglycemia leads to significant nonenzymatic glycosylation of collagen and contributes to neuropathy. Glycosylation of collagen causes cross-linking of the fibers and reduces the flexibility of collagen, compromising joint mobility and the capacity of the foot to absorb pressure. Both of these factors contribute to increased pressure on the sole of the foot. Sites of maximum pressure are then susceptible to tissue breakdown and ulceration.

In this study, a strong association has been demonstrated between foot ulceration in diabetic patients with peripheral neuropathy and nonenzymatic glycosylation and foot pressure. The authors therefore recommend that physicians use these risk factors to assess diabetic patients for their risk of developing foot ulcers according to the following protocol:

1) examine the patient for evidence of peripheral neuropathy;
2) if neuropathy is present, then evaluate the patient’s HbA1c level;
3) if the HbA1c level is shown to be chronically elevated over a period of months, then refer for analysis of pressure of the plantar aspect of the foot;
4) if the plantar foot pressure at any place exceeds 8.0 kg/cm², then prescribe pressure-relieving foot insoles. This can reduce pressure on the plantar aspect by approximately 30%.

Conclusion

Diabetes mellitus is a complex disease with significant long-term vascular and neurologic complications. Treatment is expensive and time-consuming, and the morbidity associated with foot problems threatens mobility and independence, which can have a profound effect on life-style.

This study showed that patients with diabetes with peripheral neuropathy and protracted hyperglycemia have a significant risk of ulceration of the plantar aspect of the foot. Peripheral joint flexibility was shown to be reduced in subjects with a history of plantar foot ulceration, indicating that nonenzymatic glycosylation of connective tissue is a contributory risk factor. Pressure of the plantar aspect of the foot was also significantly elevated in patients with diabetes with a history of neuropathic foot ulceration. Increased foot pressure was calculated to increase the risk of ulceration approximately five times.

Preventative programs must rely on patient education, podiatric physician and professional staff awareness, regular foot examinations, proper footwear, and good diabetic control. Investigating pressures of the foot’s plantar aspect using a Musgrave Footprint System may yield vital information about the level of risk of foot ulceration in patients with diabetes.

References

1. BRAUNWALD E, ISSELBACHER KJ, PETERSDORF RG, ET AL. “Diabetes Mellitus,” in Harrison’s Principles of Internal