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Post-occlusive reactive hyperaemia of skin microvasculature and foot complications in type 2 diabetes

S. M. Lanting¹, A. L. Barwick², S. M. Twigg^{2,3}, N. A. Johnson^{2,4}, M. K. Baker⁵, S. K. Chiu¹, I. D. Caterson^{2,3,5} and V. H. Chuter^{1,7}

1 – School of Health Sciences, University of Newcastle, Australia

2 – Charles Perkins Centre, University of Sydney, Australia

3 – Sydney Medical School, University of Sydney, Australia

4 - Discipline of Exercise and Sport Science, University of Sydney, Australia

5 – School of Exercise Science, Australian Catholic University, Australia

6- Boden Institute of Obesity, Nutrition, Exercise and Eating Disorders, University of Sydney, Australia

7- Priority Research Centre for Physical activity and Nutrition, University of Newcastle

Corresponding author: Sean Lanting

Sean.Lanting@newcastle.edu.au

University of Newcastle

PO Box 127 Ourimbah

NSW, 2258, Australia

Abstract

Aims: Diabetes-related microvascular disease has been implicated in the development of foot ulceration and amputation. Assessment of microvascular function may be effective in identifying those at risk of diabetic foot complications. We investigated the relationship between active or previous foot complication and post-occlusive reactive hyperaemia (PORH) measured by laser-Doppler fluxmetry (LDF) in people with type 2 diabetes.

Methods: PORH measures were obtained from the hallux apex in 105 people with type 2 diabetes. Associations were investigated between active or previous foot complication and PORH measures: time to peak (TtPeak) and peak as a percentage of baseline (P%BL). Multinomial logistic regression was used to determine the association of PORH with the likelihood of active foot ulcer or previous foot complication.

Results: For each second increase in TtPeak, the likelihood of a participant having a history of foot complication is increased by 2% (OR=1.019, $p=0.01$). This association was not reflected in people with an active foot ulcer (OR=1.003, $p=0.832$). P%BL was not found to be significantly different between those with a current or previous foot complication and those without ($p=0.404$).

Conclusions: This investigation in a cohort with type 2 diabetes has demonstrated that longer TtPeak is associated with history of diabetic foot complications.

Key words: post-occlusive reactive hyperaemia; diabetes; foot complication

1. Introduction

Microvascular disease in people with diabetes is associated with longer duration of diabetes and chronic hyperglycaemia and is widely recognised to cause kidney, retinal and neurological pathology (Chao & Cheing, 2009). In the lower limb microvascular dysfunction has been implicated in the development of foot complications and poor wound healing (Chao & Cheing, 2009; Lowry, Saeed, Narendran, & Tiwari, 2016). Endothelial dysfunction related to the synthesis and function of nitric oxide is also considered characteristic of diabetes, even in its early stages (Khan, Elhadd, Greene, & Belch, 2000; Veves et al., 1998) and is central to the development of neuropathy. In addition, both sensory and autonomic neuropathies are proposed to contribute to a reduction in functional capacity of the microvasculature. This includes the presence of Capillary Steal Syndrome which results in reduced flow in the capillary beds through dysfunction of arteriovenous shunts (Krishnan & Rayman, 2006), and structurally and neurologically driven impairment to microvascular reactivity (Barwick, Tessier, de Jonge, Ivers, & Chuter, 2016) and capacity for tissue response to injury (Guo & DiPietro, 2010; Tooke, 1995). Recent research has also highlighted histological changes to capillary structure in the foot occurring in association with neuro-ischaemic and neuropathic foot ulcers in an older type 2 diabetes cohort (Fiordaliso et al., 2016). There is a reduction in capillary lumen diameter, basement membrane thickening and a reduction in capillary density. The increasing evidence of the contribution of microvascular dysfunction to foot complications supports a role for assessment of microvascular function in clinical practice to assist in determining those patients at risk.

Post occlusive reactive hyperaemia (PORH) is a measure of microvascular function performed by a period of proximal arterial occlusion with subsequent release and quantification of the following rapid rise in blood flow (Cracowski, Minson, Salvat-Melis, & Halliwill, 2006). It is proposed that shear stress associated with vessel occlusion stimulates release of vasodilators from the endothelium, although other factors such as those of myogenic, sensory nervous and metabolic origin also appear to be involved (Cracowski et al., 2006; Lorenzo & Minson, 2007). Overall, the measurement is said to provide a combined assessment of endothelial dependent and independent function (Cracowski et al., 2006) which may be of advantage when assessing global changes to microvascular function in those with diabetes.

PORH has been shown to be diminished in people with peripheral arterial disease (PAD), most likely as a result of maldistribution of blood flow (Bongard & Fagrell, 1989), and has been associated with increased cardiovascular risk in healthy females (Vuilleumier, Decosterd, Maillard, Burnier, & Hayoz, 2002) and with presence of coronary artery disease (Shamim-Uzzaman et al., 2002). In a study involving 305 adults (aged 40-65), Strain et al., determined that a rapid peak flux post-occlusion was associated with increased albumin excretion rate and cardiovascular risk (Strain, Chaturvedi, Bulpitt, Rajkumar, & Shore, 2005). In type 2 diabetes, microvascular dysfunction determined by PORH index has been demonstrated to be an independent predictor of cardiovascular disease (CVD) (Yamamoto-Suganuma & Aso, 2009) and associated with the presence of overt peripheral neuropathy (Barwick et al., 2016). When compared to a healthy control group and a non-neuropathic diabetes group, the reactive hyperaemia in response to heat and acetylcholine (ACh) iontophoresis has also been shown to be reduced (Arora et al., 1998). However, the relationship between the PORH response and active, or previous, history of diabetic foot complications including foot ulceration and amputation, has not been established.

The aim of this study was to investigate the relationship between presence of active or previous diabetic foot complications, including foot ulceration and amputation, and PORH performed using laser-Doppler Fluxmetry (LDF) in people with type 2 diabetes.

2. Subjects

Participants were recruited from two community Podiatry clinics in New South Wales with informed written consent obtained prior to their involvement in the study. Ethical approval for the study was granted from the University of Newcastle Human Research Ethics Committee. To be included in the study participants required a diagnosis of type 2 diabetes confirmed by medical records. Patients were excluded (n=5) where there existed contraindications to PORH and toe pressure measurement including injury, ulceration or amputation of both halluces, the inability to remain in a supine position for 20 minutes or history of vasospastic disorder. No exclusions in relation to current pharmacotherapy were applied. At the commencement of the testing session demographic data was documented including age, sex, height, weight and smoking history. Pertinent medical history (duration of diabetes, measures of

glucose control including most recent fasting plasma glucose and haemoglobin A1c [HbA1c], as well as history of hypertension, CVD, cerebrovascular disease and microvascular disease [nephropathy and retinopathy]) were confirmed from medical records. Diagnosis of nephropathy was made with an estimated glomerular filtration rate $<60\text{mL/min/1.73m}^2$, and a urinary albumin-to-creatinine ratio $>25\text{ mg/mmol}$ for men and $>35\text{ mg/mmol}$ for women (American Diabetes Association, 2014). Retinopathy was considered to be present if proliferative or non-proliferative changes were recognized at screening (American Diabetes Association, 2014). Medical records and clinical examination were used to establish presence of active or previous foot ulceration or amputation and the specific location. Amputations of the lower limb were classified as per Nather and Wong (Nather & Wong, 2013). Significant PAD was defined by a systolic toe pressure of less than 55mmHg (Schaper et al., 2012).

3. Materials and methods

3.1 Equipment

A biothesiometer (Briggate Medical Company) and 10g 5.07 Semmes-Weinstein monofilament (North Coast Medical, California) were used to determine peripheral neuropathy status. LDF was used to determine PORH parameters. Specifically, a MoorVMS-LDF2 laser Doppler in combination with a VHP2 skin heater probe (Moor Instruments Ltd, Axminster, United Kingdom) were used to measure cutaneous flux at the distal plantar aspect of the right hallux. A MoorVMS-PRES automated pressure unit with toe cuff (Moor Instruments Ltd) was used to allow for calculation of toe pressure and PORH. MoorVMS analysis software Version 3.1 (Moor Instruments Ltd) was used for PORH data analysis.

3.2 Procedure

Eligible participants were advised to refrain from caffeine, alcohol, smoking, and strenuous exercise for two hours prior to the testing session. Demographic data collection and lower limb measurements were performed by one of two Podiatrists in a temperature controlled room (22-24°C). Following a 10-minute period of horizontal supine rest and acclimatisation, participants underwent screening for peripheral neuropathy using VPT and loss of protective sensation (LOPS) to determine neuropathy status. Loss of perception of VPT less than 25mV and inability to identify the 10g monofilament at four of four sites were considered a deficit. Neuropathic status was classified as abnormal if deficits were

observed in both neurological tests (Boulton et al., 2008). For LDF, the laser probe was embedded in the heat probe with the temperature set at 33°C to maintain consistent thermo-neutral skin temperature at the site being measured. The toe cuff was applied over the proximal phalanx of the hallux avoiding contact with the laser/heat probe unit. The automated recorded sequence adhered to was: baseline flux for three minutes, cuff inflation and occlusion to 220mmHg for three minutes followed by four minutes of post-occlusive flux, where flux is an arbitrary perfusion unit.

PORH measures used for analysis included peak flux as a percentage of baseline flux (P%BL) and the time to peak (TtPeak), measured in seconds' post-occlusion. These variables were chosen due to their ability to depict the extent of response and response over time in the context of their previously demonstrated acceptable reliability by the authors (Barwick, Lanting, & Chuter, 2015). A typical PORH response is depicted in Figure 1.

3.3 Statistical analysis

Statistical analysis was undertaken using R version 3.1.1. Data from one limb per person were used in the analysis to satisfy the assumption of independence of data (Menz, 2004). For participants with an active foot ulceration or previous foot complication, data from the affected limb were used. Where amputation was present, data for that limb were used if possible. Where both limbs had been affected by foot ulceration (n=6), only one leg was randomly selected and the data for that leg used. Where one limb had an active foot ulceration and the other had a previous foot complication, the limb with the active ulceration was used (n=1). For those without history of foot complication, data from the right lower limb were used.

Between group comparisons (history of foot complication, no history of foot complication/no current foot ulcer, active foot ulcer) for demographic, relevant diabetes and PORH variables were determined using an ANOVA to compare the means between the comparison groups for continuous variables. The Fischer exact test was used to compare proportions for categorical data due to the low numbers of participants with active ulcers. For the purposes of this analysis, retinopathy and nephropathy were collapsed into a single variable (microvascular complications [MC]). From these results, variables

demonstrating significant differences to the reference group (no history of ulcer) were identified (TtPeak, diabetes duration, HbA1c, MC and neuropathy). Multinomial logistic regression (MLR) was used to compare the associations between significantly different variables and outcome variables between those with a history of foot complication versus an active ulcer compared to the reference category of no foot complication history/no current ulcer.

Binomial logistic regression was also used as part of the sensitivity analysis, where the categories were collapsed by considering "history of foot complication" and "active foot ulcer" as the same category and included each of the variables identified as significant in the MLR. This was done to measure the difference in the change in odds ratio (OR) and compare the results to the MLR results. Additionally, the larger sample means that the event per variable ratio would support an adjusted multivariate model.

Finally, a multivariate binomial logistic regression model was then used to determine the level of association of diabetes specific risk factors (HbA1c, diabetes duration) and TtPeak on the likelihood of the participants having an active foot ulcer or previous foot complication. Neuropathy was not included in this analysis due to a significant moderate correlation with TtPeak and the potential for this to distort the model (Tu, Kellett, Clerehugh, & Gilthorpe, 2005).

4. Results

4.1 Between group comparisons

The between group participant characteristics are detailed in Table 1. Notably, comparisons showed that HbA1c differed within groups ($p=0.046$) with those with active ulcers having the highest HbA1c ($p=0.018$). These results also indicate that, although not statistically significant ($p=0.334$), the participants with a history of foot complication had a high HbA1c. There was also a significant ($p=0.005$) difference between groups and their respective TtPeak. This difference was primarily driven by a mean TtPeak in the participants with a history of foot complication.

There was a significant ($p=0.036$) difference in the presence of MC between groups, with those with a history of foot complication much more likely to have either retinopathy and/or nephropathy than those

with no history of foot complication or those with active ulcer. The Fischer test for neuropathy showed that there was a significant ($p<0.001$) difference in the distribution, with more observed participants with neuropathy with both a history of foot complication and active ulcers compared to those with no history.

4.2 Multinomial Logistic Regression

Multinomial logistic regression analysis results for TtPeak, HbA1c, diabetes duration, MC and neuropathy are detailed in Table 2. The TtPeak model showed that one unit increase in TtPeak (seconds) significantly ($p=0.007$) increased the odds of a participant having a history of foot complication by approximately 2% ($OR=1.019$). Conversely, the data failed to show that the same unit increase in TtPeak significantly increased the odds of a participant having an active ulcer ($p=0.832$).

The HbA1c model showed that a one unit increase in HbA1c increased the odds of a participant having a history of foot complication compared to no history by 20%, however the data failed to support that this difference was significantly different ($p=0.315$). Conversely, a one-unit increase in HbA1c significantly ($p=0.024$) increased the odds of a participant having an active ulcer by a factor of 1.6. Although there appeared to be a significant effect between the reference category (no ulcer) and active ulcer, the global p-value for this variable suggested that there is no significant ($p=0.069$) gain of information by including the HbA1c variable in the model.

The MC model showed that a presence of a history of MC significantly increased the odds of the participant having a history of foot complication by a factor of 4.08 ($p=0.025$). Conversely, the same change in the MC decreased the odds of an active ulcer by 51%, though this was not significant ($p=0.406$).

The neuropathy model showed that there is strong evidence to suggest that the presence of neuropathy increased the odds of a history of foot complication by a factor of 25.6 ($p<0.001$) and of active ulcer by a factor of 5.48 ($p=0.045$).

4.3 Logistic regression

Table 3 shows the results for the univariate logistic regression for the variables of interest, when the categories “history of foot complication” and “active ulceration” are merged into a single category. The results show that for every one unit increase in TtPeak, the odds of a history of foot complication or active ulcer increased by 1.5% ($p=0.02$). Additionally, the inclusion of TtPeak significantly ($p=0.018$) increased the model fit compared to the null model. The model containing the variable HbA1c shows that for every one unit increase in HbA1c the odds of the participant having a history of foot complication or active ulcer increases by 34% ($p=0.048$). The global p-value indicates that this variable significantly describes the variation in the observed data compared to the null. The last significant variable found was the categorical variable, neuropathy. A participant with neuropathy was 12.18 times more likely to have either a history of foot complication or active ulcer. This result was highly significant ($p<0.001$) and additionally, the likelihood ratio (LR) test comparing the model to the null shows that the inclusion of neuropathy significantly ($p<0.001$) improved the model fit.

4.4 Multivariate logistic regression

Lastly, the multivariate binomial logistic regression model determined a significant association between the grouped “history of foot complication” and “active ulceration” and TtPeak ($p=0.027$) with every unit (second) increase associated with an increased risk of 1.5%, while the association of HbA1c (OR = 1.315) was approaching significance ($p=0.072$) (Table 4).

5 Discussion

This study found that the TtPeak variable of PORH was independently associated with both active and previous foot complication in a population of older people with type 2 diabetes in a community based setting. In addition, consistent with previous research (Adler, Boyko, Ahroni, & Smith, 1999; Reiber et al., 1999), peripheral neuropathy was found to significantly increase the likelihood of active and previous foot complication. These findings demonstrate that aspects of microvascular and neurological function are strongly associated with foot complications and can identify those individuals even when the incidence of complications is quite low (22% in this study). Elevated HbA1c had a strong association with active ulceration indicating a likely increased ulcer risk with poorly controlled blood glucose

levels. The association between HbA1c and history of foot complications was weaker though this may be as it is unlikely that recent measures of glycaemic control accurately reflect historical data for individuals, particularly HbA1c levels prior to, and during periods of foot ulceration. Our findings of elevated HbA1C in those with active foot ulceration although conducted in a small sample are consistent with previous results from a larger cohort study involving 250 people with diabetes of which 36% had active foot ulceration. In this study, 58% of those with ulceration had poor glycaemic control (HbA1c > 8.5% in this study) compared with 32% in the non-ulcer group ($p = 0.0001$) (Yekta, Pourali, & Ghasemi-Rad, 2011).

Peripheral neuropathy is well established as a major risk factor for foot ulcer development (Reiber et al., 1999) and a significant risk factor for lower limb amputation in people with diabetes (Adler et al., 1999). Our finding that presence of neuropathy increased the likelihood of a previous foot complication (OR 25.59) and current foot ulcer (OR 5.48) is consistent with this. The diagnosis of peripheral neuropathy in participants in this study was based on abnormal 10g monofilament and VPT test results. In people with diabetes, peripheral neuropathy diagnosed with these testing methods has previously been shown to be predictive of a 7 to 12-fold increase in risk of foot ulceration (Frykberg et al., 1998; Perkins, Olaleye, Zinman, & Bril, 2001; Young, Breddy, Veves, & Boulton, 1994). These findings support the utility of clinically based measures of assessing neuropathic status in people with diabetes to identify those at risk of foot ulceration.

In relation to measures of microvascular function, only one PORH variable (TtPeak) was found to be associated with active and previous foot complication. TtPeak has previously been reported as the most predictive PORH measure for CVD in an older population (Strain et al., 2005) and has also been shown to correlate with coronary microvascular dynamics (Larkin & Williams, 1993). These existing data indicate that TtPeak may identify microvascular dysfunction in relation to CVD however, little is known about the clinical usefulness of this measure in determining risk of foot ulceration in people with diabetes. Our present study suggests TtPeak may also be indicative of microvascular dysfunction associated with foot complications, though by contrast P%BL demonstrated no significant association.

Several studies have also identified a role for sensory nerves in the PORH response (Larkin & Williams, 1993; Lorenzo & Minson, 2007). Neurogenic vasodilator release mediated by small nerve fibres is required for the increased blood flow necessary for healing following an injury (Hile & Veves, 2003). Increasing TtPeak has been shown to be associated with clinically diagnosed peripheral neuropathy, with neuropathy predicting 22% of the variance in the measurement (Barwick et al., 2016). Interestingly in this present study the MLR demonstrated the significant relationship with TtPeak occurred in those with a history of foot complication rather than those with active ulcer and this was supported by a reduction in the OR (1.19 to 1.15) when the “active ulceration” and “previous foot complication” categories were collapsed. It is possible that this difference may be an influencing factor in potential for ulcer healing as all those participants with previous amputation due to non-healing were in the “previous foot complication” group. In addition, due to the high number of participants with previous foot complications and neuropathy, it is very likely that the presence of neuropathy contributed to change in TtPeak in these participants. However, this would have also been expected to occur in those with neuropathy with and without active or previous foot complication.

Although these findings support a relationship between altered microvascular function and previous foot complication it is possible that the clinical methods of assessment of neurological status may have affected these results. Early stage neurological dysfunction may have affected TtPeak yet it may not have been detected by neurological testing used in this study. In addition, three of the participants in this study had an active or previous ulceration without overt neuropathy. Although it is likely that these ulcers were ischaemic in nature, it is possible that neuropathy was present but not diagnosed.

The lack of association between P%BL and active or previous foot complication is noteworthy. Earlier research has demonstrated that a reduction in nerve conduction speed is associated with a drop in PORH peak (Yamamoto-Suganuma & Aso, 2009). Given that 47% of participants in this study had neuropathy our data suggests that while neurological dysfunction may impact this variable it does not differentiate between those with active or previous foot complication and those without. It has also previously been demonstrated that increased myogenic activity may compensate for the impaired neural responsiveness resulting in the peak of the measurement being maintained in early stage peripheral neuropathy (Sun et

al., 2008). As neuropathy testing in the study was performed using basic clinical tests it is likely that some participants had neuropathy that was not detected, meaning that these participants may also have had some degree of change in P%BL making it less likely a difference in the sub-group with history of foot complications could be identified. Based on these initial findings, further assessment of the relationship between microvascular function and diabetic neuropathy and foot complications using methods to assess early stage peripheral neuropathy is warranted.

5.1 Limitations

This study investigated the relationship between active ulceration or history of diabetic foot complications and cutaneous microvascular reactivity. We did not perform any prospective assessments of these outcomes and are unable to comment on the predictive capacity of these measures for future foot complications. This study was not designed to solely relate to people with existing foot complications of diabetes but rather to investigate the potential associations between cutaneous microvascular reactivity and foot complications for people with diabetes in the community. Although the percentage of people with foot complications was quite low (22%), this is reflective of the community based population and is consistent with the study aim.

The mechanism of PORH is not comprehensively understood and there is currently no consensus as to the extent to which endothelium-dependent and -independent vasodilation are involved. Our study therefore does not explain the exact mechanisms underlying the reactivity. Although we have previously demonstrated the PORH variables used in this study to be reliable, this form of measurement is susceptible to several external and internal factors which may influence the results. This includes measurement technique and pharmacological agents including antihypertensives and drugs for dyslipidemia which were not excluded from this study as they are in widespread use in the target population. In addition, the likelihood participants in the study had co-existing morbidities that affect microvascular function such as PAD, is high and these were not accounted for. Similarly, hypoglycaemia may influence endothelial function. Although no hypoglycaemic events were observed in our study, this was based on participant self-report only, and the results of our study need to be considered in this light.

There are a range of variables that may be assessed within the PORH measurement. In order to limit the number of variables in the analysis we selected only two (TtPeak and P%BL). These two measurements have previously demonstrated acceptable reliability (Barwick et al., 2015) and together give an indication of the temporality and magnitude of the PORH response. TtPeak has historically been used as a measure of endothelial function (Yamamoto-Suganuma & Aso, 2009) and has demonstrated discriminative capabilities between people with PAD and healthy control patients (Morales et al., 2005). It is possible that other PORH variables may provide additional information relating to microvascular function and diabetes-related foot complications. Assessment for example of area under the curve pre- and post-occlusion or magnitude of the peak could be more physiologically relevant and may help to explain the contrasting results of our study with regards to P%BL and TtPeak. Similarly, there is evidence that the ratio of area under the curve 1 min after the release of the cuff relative to the AUC of a 1-min period before cuff inflation is more strongly associated with markers of microvascular and macrovascular disease in a diabetes cohort than the more widely used TtPeak measure used in our present study (Yamamoto-Suganuma & Aso, 2009), and therefore may be better able to identify those at risk of diabetes related foot complications.

5.2 Conclusions

This investigation has demonstrated in a cohort with type 2 diabetes that every one-second increase in TtPeak – a variable of PORH (as measured by LDF) is associated with a 1.5% increased likelihood of active or previous diabetic foot complication and a 2% increase likelihood of previous foot complication only. These findings support the need for further investigation into the relationship between measures of microvascular function and development, prospectively, and outcomes of, diabetic foot complications. In addition, measures of microvascular function may be a useful indicator for more precisely determining future risk of diabetic foot complications and healing potential.

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Author contributions: SL collected data, inputted data, drafted the manuscript and contributed to statistical analysis. AB collected data, inputted data and contributed to drafting of the manuscript. ST contributed to statistical analysis and drafting of the manuscript. NJ and MB contributed to study design and drafting of the manuscript. SC contributed to statistical analysis and drafting of the manuscript. IC contributed to drafting of the manuscript and data interpretation. VC conceived the study, collected data, contributed to statistical analysis and drafting of the manuscript. All authors approved the final version of this manuscript.

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8. Conflicts of interest

All authors declare that there are no conflicts of interests.

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Table legends

Table 1: Between group participant characteristics

Table 2: Multinomial regression analysis

Table 3: Univariate binomial regression analysis

Table 4: Multivariate binomial regression analysis

Figure 1: Typical PORH response

Tables

Table 1: Between group participant characteristics.

	No history (n=82)	History of ulcer/amputation (n=15) [#]	Active ulceration (n=8) ^{##}	p-value
Age	69.97	67.07	70.63	0.559
Male (%)	63.41	66.67	75.00	0.873
BMI	34.09	32.73	29.63	0.228
Diabetes duration	11.75	12.77	15.75	0.513
HbA1c (%)	7.21	7.62	8.56*	0.046
TtPeak	17.11	47.49**	19.17	0.005
P%BL	321.16	404.47	258.67	0.404
CVD (%)	40.24	33.33	62.50	0.371
Neuropathy (%)	35.37	93.33	75.00	<0.001
Smoking history (%)	52.44	66.67	75.00	0.873
MC (%)	40.24	73.33	25.00	0.036

CVD, cardiovascular disease; MC, microvascular complication (retinopathy and/or nephropathy).

* significant at $p < 0.05$

** significant at $p \leq 0.005$

[#] Previous amputation n=4 (3 digits only, 1 ray [metatarsal and digit]), previous ulceration n=11, with neuropathy n=10

^{##} Current ulceration with neuropathy n=7, plantar forefoot n=5, lesser digit n=1, lateral forefoot n=1

Table 2: Multinomial regression analysis

	History of ulcer					Active ulcer					LR test
	OR	LCL	UCL	SE	p	OR	LCL	UCL	SE	p	
Intercept	0.107	0.051	0.222	0.373	<0.001	0.093	0.039	0.222	0.444	<0.001	0.0214
TtPeak	1.019	1.005	1.033	0.007	0.007	1.003	0.978	1.029	0.013	0.832	
Intercept	0.048	0.003	0.72	1.378	0.028	0.002	0	0.08	1.781	0.001	0.0688
HbA1c	1.197	0.843	1.7	0.179	0.315	1.605	1.064	2.42	0.21	0.024	
Intercept	0.082	0.029	0.226	0.52	<0.001	0.122	0.052	0.286	0.432	<0.001	0.0299
MC	4.083	0.029	0.226	0.626	0.025	0.495	0.052	0.286	0.847	0.406	
Intercept	0.019	0.003	0.136	1.009	<0.001	0.038	0.009	0.155	0.72	<0.001	<0.001
Neuropathy	25.586	0.003	0.136	1.061	0.002	5.483	0.009	0.155	0.849	0.045	

MC, microvascular complication; OR, odds ratio; LCL, lower control limit; UCL, upper control limit; SE, standard error; LR, likelihood ratio

Table 3: Univariate binomial regression analysis

	OR	LCL	UCL	SE	p	LR test
Intercept	0.193	0.103	0.337	0.299	<0.001	0.018
TtPeak	1.015	1.003	1.029	0.006	0.02	
Intercept	0.031	0.003	0.289	1.154	0.003	0.048
HbA1c	1.336	1.003	1.794	0.146	0.048	
Intercept	0.204	0.097	0.386	0.347	<0.001	0.166
MC	1.93	0.761	5.025	0.477	0.168	
(Intercept)	0.057	0.014	0.153	0.593	<0.001	<0.001
Neuropathy	12.184	3.784	54.87	0.661	<0.001	

MC, microvascular complication; OR, odds ratio; LCL, lower control limit; UCL, upper control limit; SE, standard error; LR, likelihood ratio

Table 4: Multivariate binomial regression analysis

	Odds ratio	95% CI		p
		Lower	Upper	
Diabetes duration (per year)	1.018	0.971	1.068	0.454
HbA1c (per 1.0 % increase in NGSP units)	1.315	0.976	1.771	0.072
PORH Time to Peak (per second)	1.015	1.002	1.028	0.027*

* statistically significant at $p < 0.05$

Figure 1: Typical PORH response