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Microangiopathy in diabetic polyneuropathy revisited

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Abstract. – OBJECTIVE: Microangiopathy is a major cause in diabetic polyneuropathy (DPN). This review examines evidence from both human and animal studies to elucidate the important microvascular factors in DPN.

MATERIALS AND METHODS: This is a literature review of articles published on PubMed in English.

RESULTS: There is an abundance of evidence linking endoneurial microvascular abnormalities to peripheral nerve dysfunction and pathology in patients with diabetes. These structural changes result in an abnormal diffusion barrier leading to endoneurial hypoxia. Furthermore, the functional changes of endoneurial microvessels characterized by reduced vasodilation and potentiated vasoconstriction also exacerbate the endoneurial hypoxia. Although reduced endoneurial blood flow has also been widely reported in established DPN, there is some evidence that blood flow may be elevated early in the course of the disease. Capillary dysfunction in DPN, which reduces the amount of oxygen and glucose that can be extracted by the tissue for a given blood flow, may explain that the tissue may be hypoxic even in the context of normal or elevated nerve blood flow. The pathogenesis of painful DPN also remains unclear although neural hemodynamic changes have been demonstrated both in the peripheral and central nervous system, offering potential new insights for the treatments of this distressing condition.

CONCLUSIONS: Compelling experimental and human work has highlighted the close association connection between endoneurial microangiopathy and diabetic polyneuropathy. Future investigations will need to investigate the role of microvascular factors both in the periphery and the central nervous system in the pathogenesis of painful DPN.

Key Words:
Diabetic polyneuropathy, Microangiopathy, Endoneurial hypoxia, Painful diabetic polyneuropathy, Magnetic resonance imaging.

Abbreviations

DPN = diabetic polyneuropathy, IGT = impaired glucose tolerance, NGT = normal glucose tolerance, NO = nitric oxide, STZ = streptozotocin.

Introduction

Microangiopathy, or dysfunction of small blood vessels, is considered relevant to the pathogenesis of several forms of peripheral nerve diseases, in particular diabetic polyneuropathy (DPN). It is probably incorrect to conclude that microangiopathy is the primary trigger of neuropathic complications, an assumption that ignores the insults from metabolic disorders. However, it might be more accurate to indicate that both microangiopathy and metabolic disturbances lead to a vicious interacting cycle of nerve damage. This paper will review the structural and functional changes of microvessels in DPN, the reports of alterations in nerve blood flow both from experimental and human studies, and last but not least, will examine the reported hemodynamic changes in painful DPN.

Structural Changes of Microvessels in Diabetic Polyneuropathy

Peripheral nerve axons are anatomically unique; they are extremely long relative to their diameter and a great distance from the parent cell body. These axons are exquisitely dependent on the nerve microenvironment for the blood supply, oxygenation, and nutrition. Peripheral nerve trunks have a double blood supply: the epineurial vascular plexus and the intrinsic endoneurial blood supply. Because of this rich blood supply, nerve fibers suffer functional or structural changes only when there is severe and long-lasting ischemia due to widespread vascular damage. Long-term hyperglycemia induces the thickening of the capillary basement membrane, which leads to occlusive angiopathy and to tissue hypoxia and damage. These changes are the structural hallmark of diabetic microangiopathy. Capillary basement membrane thickening is widespread in diabetic neuropathy. The early work done by Fagerberg (Acta Med Scand 1957; 159: 59-62) showed the

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thickening and hyalinization of intraneural vessel wall by a material staining PAS positive, together with a reduction in vessel caliber, in diabetic neuropathy. These changes were later found to be due to reduplication of capillary basal lamina, although found in other neuropathies, the phenomenon is more common in diabetes. Malik et al[1] found endoneurial capillary basement membrane, endothelial cell and total diffusion barrier were significantly increased in DPN which resulting in a reduction in luminal size of transperineurial capillaries (Figure 1), in some cases leading to complete occlusion of small vessels. Furthermore, the ultrastructural studies of Dyck et al[2] have demonstrated that the increase in basement membrane area was associated with the severity of polyneuropathy[2]. In fact, changes in endoneurial capillary density in the sural nerve precede the development of diabetes in subjects with Impaired Glucose Tolerance (IGT); and decreased capillary luminal area in the sural nerve precedes deterioration in glucose tolerance in both IGT and NGT[3]. These results would suggest that diabetic polyneuropathy might also be associated with other factors besides hyperglycemia.

Functional Changes of Microvessels in Diabetic Polyneuropathy

Studies by the Eurodiab group on type 1 diabetes complications showed that in addition to glycemic control and duration of diabetes, conventional markers of macro- and micro-vascular disease are strongly associated with DPN[4]. Similar correlations have been observed for DPN in type 2 diabetes[5]. A potential link between macro-vascular risk factors and microvascular complications including neuropathy is their association with endothelial dysfunction[6]. Very recently work done by Roustit et al[7] showed flow-mediated dilation (FMD), a marker of endothelial dysfunction, was strongly associated with Neuropathy Disability Score. This study suggested that endothelial dysfunction mediates the deleterious effects of diabetes on macrovascular risk factors and DPN. Endothelial dysfunction is characterized by an imbalance between endothelium-derived vasodilator and vasoconstrictor substances. Reduced endothelium-dependent vasodialation in peripheral arteries has been reported in patients with type 1 and type 2 diabetes[8]. Nitric oxide (NO), a potent endothelium-derived vasodilator, plays an important role in the endothelial dysfunction associated with diabetic polyneuropathy. Other vasodilators, such as prostacyclin, have also been found to decrease in diabetes[9]. Increased circulating levels of potent vasoconstrictor peptides such as endothelin-1 in patients with type 2 diabetes[8] and increased sensitivity to angiotensin II in diabetic rats[10] have also been demonstrated.

Hemodynamic Disturbance in Diabetic Polyneuropathy

Evidence from Experimental Studies

Tuck et al[11] initially reported that streptozotocin (STZ)-treated rat was associated with a decline of sciatic nerve blood flow using the hydrogen clearance technique. The perfusion deficit causes endoneurial hypoxia sufficient to compromise nerve function and initiate neurodegenerative processes. Findings from initial research in the STZ-induced rat model of type 1 diabetes have been extended to other models, using the similar hydrogen clearance technique, including the BB-Wor type 1 model[12], the type 2 models Zucker diabetic fatty rat[13], and Otsuka Long-Evans Tokushima fatty rats[14]. The reduced blood flow is not restricted to peripheral nerve trunks, but is also observed in autonomic ganglia[15], dorsal root ganglia[16], and even centrally in some brain structures such as the hippocampus[17]. On the basis of above studies, there are substantial interventions, which have been reported to correct both nerve blood flow and diabetic electrophysiological abnormalities. Although those studies have undoubtedly provided evidence of a linkage, cause and effect have not been proven. The idea that microvascular changes cause diabetic polyneuropathy has been questioned. Some researchers[18] have failed to identify declines in nerve blood flow in DPN. On the contrary, they have reported that endoneurial blood flow may be elevated early following the induction of experimental diabetes in rats[19]. The elevated nerve blood flow has also been demonstrated in early human diabetic polyneuropathy as described below.

Evidence from Human Investigations

There have been relatively fewer human studies to measure peripheral nerve-trunk blood flow because of technical limitations. Using techniques of nerve photography and fluorescein angiography, Tesfaye et al[20] demonstrated delayed fluorescein transit time in patients with advanced diabetic polyneuropathy. Fluorescein transit time was also prolonged in patients with mild to moderate DPN[21]. However, it is uncertain whether such reductions fully account for polyneuropa-

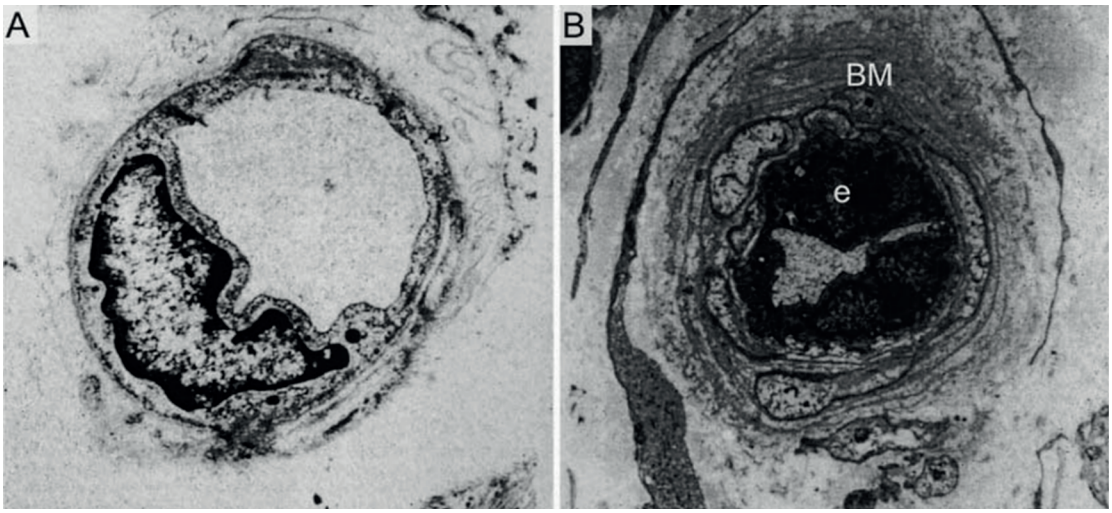

Figure 1. Photomicrographs of endoneurial capillaries from sural nerve biopsies showing ***(A)*** a normal capillary from a diabetic patient without neuropathy and ***(B)*** a closed capillary from a subject with diabetic neuropahty, with basement membrane (BM) thickening and endothelial cell (e) proliferation. Photomicrographs courtesy of Dr. R. A. Malik, University of Manchester, Manchester UK.

thy. Theriault et al[22] measured human sural nerve blood flow using multiple epineurial laser Doppler flowmetry. They found in patients with mild diabetic polyneuropathy sural nerve blood flow was slightly higher compared to those with other polyneuropathies, except those with vasculitic neuropathy. Such observations remained constant over a 1-year time period during which nerve fibre density decreased. Blood flow may be affected by a number of factors. According to hydrodynamics, blood velocity is influenced by local arterial pressure, blood viscosity, temperature and the vessel morphology including the vessel caliber, vessel winding and inner-surface contour (roughness). Besides structural factors, changes in blood flow may also be due to methodological factors[23], including failure to strictly maintain near nerve temperature; the use of single, uncontrolled laser Doppler flowmetry measurements; incorrectly used hydrogen microelectrodes; and the use of excessively large microelectrodes. The arteriovenous shunting, which is well described in DPN[24] may further complicate the measurement of nerve blood flow. Endoneurial hypoxia has been the more consistent findings in patients with DPN. Abundant proofs, both from animal models and human investigations have identified endoneurial hypoxia in diabetic polyneuropathy. Generally, the higher tissue blood flow is, the higher the tissue oxygenation will be, according to the classic flow-diffusion equation. This classic equation assumes all tissue capillaries are equally perfused, however, the blood velocities vary considerably among capillaries especially those with endothelial dysfunction. Hyperglycemia, oxidative stress and oxidized lipoproteins interfere with the capillary micro-environment, and tissue blood flow must be adjusted to ensure sufficient oxygen extraction. When capillary dysfunction becomes more severe, as a result, tissue blood flow increases lead to little or no longer improvements in tissue oxygenation. That tissue may be hypoxic in the absence of demonstrable signs of ischaemia[25]. The proposed hypothesis may give potential physical linkage between endothelial dysfunction, which is well-established pathological state in macrovascular complications, with diabetic polyneuropathy. It is also consistent to the results of clinical investigation from Eurodiab group[4].

Microvascular Changes in Painful Diabetic Polyneuropathy

Painful diabetic polyneuropathy is the most distressing complication of diabetes. However, the cause of painful DPN is unclear. While there is now strong evidence for the importance of nerve microvascular disease in the pathogenesis of DPN, the evidence for its detail mechanism in painful DPN is less clear. The fluctuant nature of painful neuropathic symptoms might suggest a more dynamic underling cause, such as metabolic or hemodynamic factors, rather than structural lesions. Studies using sural nerve epineurial vessel photography and fluorescein angiography

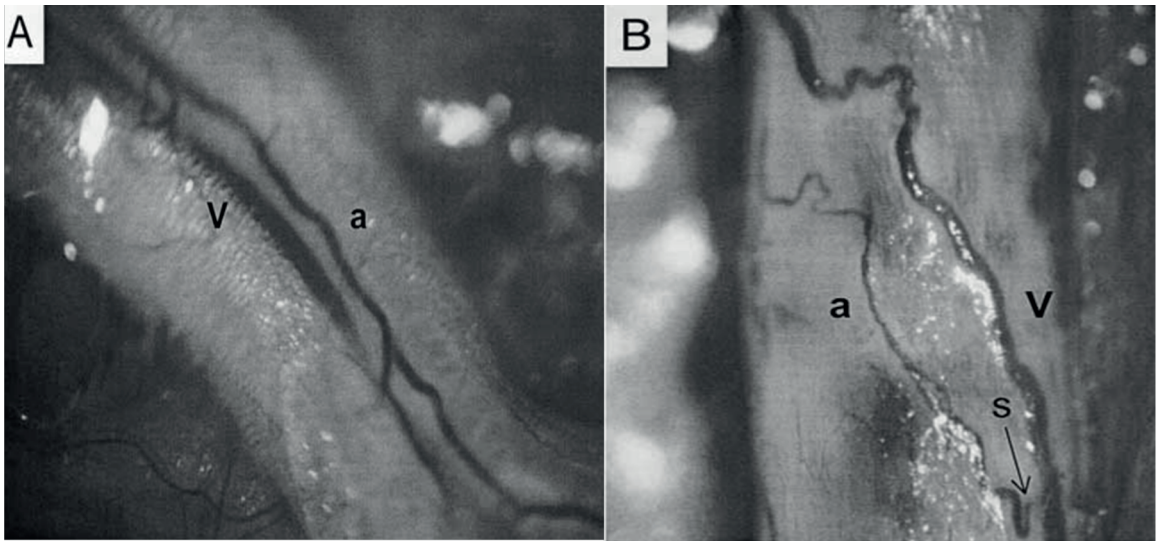

Figure 2. Surface view of the sural nerve from a non-diabetic subject ***(A)*** showing normal arterial (a) and venous (V) anatomy. This contrasts with findings for a patient with chronic diabetic neuropahty ***(B)***, where there was arterial (a) attenuation, venous (V) tortuosity and arterio-venous shunting (s). Reproduced with permission.

in vivo showed increased epineurial shunt flow in patients with severe pain due to "insulin neuritis" (acute painful neuropathy following rapid glycemic control), which might be the consequence of epineurial arteriovenous shunting[24]. Such hemodynamic disturbance has also been found in diabetic polyneuropathy (Figure 2). Tesfaye et al[24] used the techniques of microlight guide spectrophotometry and fluorescein angiography to measure sural nerve epineurial intravascular oxygen saturation and blood flow respectively. Epineurial oxygen saturation was higher and epineurial blood flow was faster in the group with painful neuropathic symptoms compared to those without (Figure 3)[26]. Such epineurial findings are not only confined to the peripheral nerve but are also found in other vascular beds, such as cutaneous microcirculation of the foot, using a noninvasive laser Doppler technique[27]. These results indicate that there may be distinct differences in haemodynamics within the epineurium of the sural nerve in

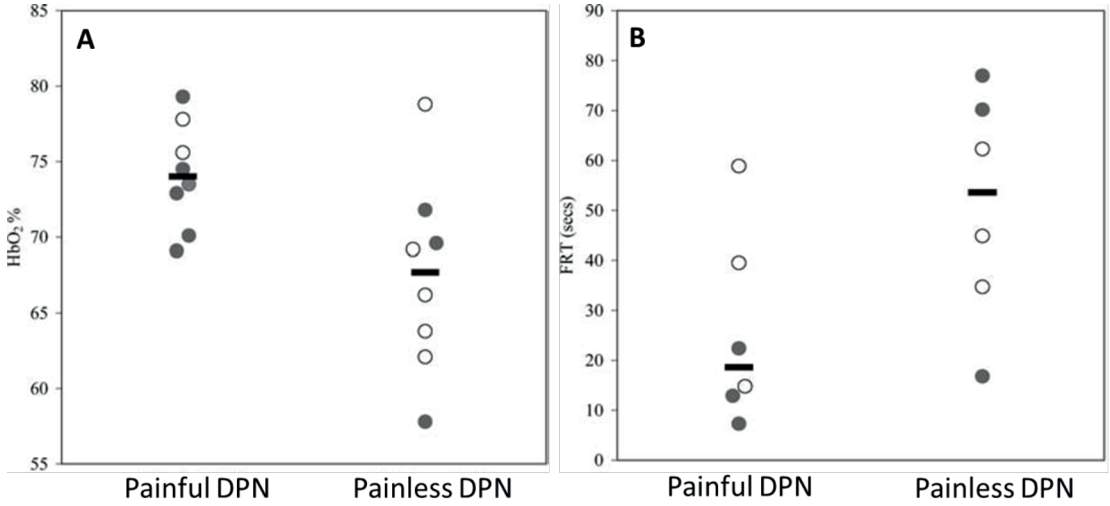

Figure 3. Sural nerve intravascular oxygen saturation (HbO_2%) (A) and fluorescein rise time (FRT) (B) in subjects with painful and painless DPN. Bars represent median values. Empty circles represent patients with T1DM, filled circles T2DM. Reproduced with permission..

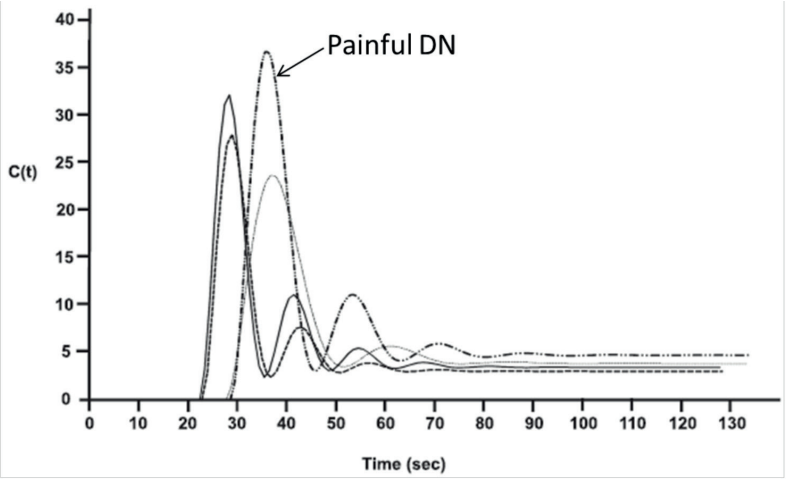

Figure 4. Composite concentration time profiles of the bolus passage of exogenous contrast agent (Gd-DTPA) through the thalamus in each subgroup: health volunteer, Diabetes without polyneuropathy, painless DN, and painful DN. Reproduced with permission.

subjects with painful and painless neuropathy. Haemodynamics disturbance may contribute to the pathogenesis of neuropathic pain, by inducing endoneurial hypoxia[28].

In previous studies, Archer et al[29] demonstrated the blood flow in the feet of patients with diabetic neuropathy was five times higher than in normal controls. Reduction of this high flow by sympathetic arousal stimuli was associated with reduction in neuropathic pain, similar to the pain relief reported by some patients when cooling the feet (which would be expected to cause local vasoconstriction). Also they demonstrated painful DPN still retained the ability to constrict their peripheral blood vessels in response to arousal and reduce peripheral flow whereas patients with painless DPN did not. Later, Doupis et al[30] found nerve axon reflex-related vasodilation induced by stimulating c-nociceptive fibers in skin were lower in the painless neuropathy, when compared to painful neuropahy. On the other hand, Quattrini et al[27] found not significantly different results in foot skin vasodilator responses to acetylcholine and sodium nitroprusside but significantly impaired vasoconstrictor responses to sympathetic (deepest possible gasp) stimulation in patients with painful DPN compared those with painless DPN[27], suggesting a role of sympathetic denervation in the development of cutaneous shunting and consequent reduction in dermal nutrition blood flow. Tack et al[31] showed neurochemical and positron emission tomography (PET) scanning evidence for regionally selective sympathetic denervation in painful polyneuropathy. These findings seemed inconsistent; however, it might suggest the hypothesis that microvascular hemodynamic disturbance might caused by abnormal innervations associated with the pathogenesis of painful diabetic polyneuropathy. Hemodynamic factors may also affect central nerve systems. In order to accommodate the diverse metabolic needs in different part of cerebrum, the microvascular perfusion characteristics that are critical to the biophysics of oxygen extraction has to be adjusted[32]. Magnetic resonance based brain imaging technologies provide a suite of merits that can be used to test hypotheses about central nervous system mechanisms underlying pain perception[33], among which magnetic resonance perfusion imaging and blood oxygen level-dependent (BOLD) signals were correlated with regional cerebral blood flow. Tseng et al[34] found enhanced BOLD signals in limbic and striatal circuits using functional magnetic resonance imaging, which contributed to the development and maintenance of burning pain and thermal hyperalgesia in diabetes. A study from Sheffield[35] assessed the microvascular perfusion characteristics of thalamus and caudate nucleus using the magnetic resonance perfusion imaging. The caudate nucleus was chosen to serve as an *in vivo* control region. The study demonstrated increased thalamic vascularity with sluggish flow in painful DPN but not in painless DPN (Figure 4). There was no significant difference in markers of caudate nucleus perfusion. The thalamus plays a central role in modulating/processing somatosensory information that is relayed to the cerebral cortex. Hemodynamic

changes in thalamus may have an important role in the pathogenesis of neuropathic pain[35]. The latest electrophysiology findings in experimental animals further prove the thalamic hyperactivity can substantially transform ascending sensory input in diabetic neuropathy[36]. Further work is required to clarify the haemodynamic changes in the pain processing areas of the brain in order to elucidate their relevance to the mechanisms of pain in diabetic neuropathy.

Conclusions

Compelling experimental and human work has highlighted the close association connection between endoneurial microangiopathy and diabetic polyneuropathy. Endoneurial hypoxia caused by structural and functional microvascular changes is also well-recognized in diabetic polyneuropathy. However, the early metabolic changes triggering these microvascular hemodynamic changes are yet to be fully elucidated. Future work will also need to investigate the role of microvascular factors both in the periphery and the central nervous system in the pathogenesis of painful DPN.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Malik RA, Tesfaye S, Thompson SD, Veves A, Hunter A, Sharma AK, Ward JD, Boulton AJ. Transperineurial capillary abnormalities in the sural nerve of patients with diabetic neuropathy. Microvasc Res 1994; 48: 236-245.
2) Giannini C, Dyck PJ. Ultrastructural morphometric abnormalities of sural nerve endoneurial microvessels in diabetes mellitus. Ann Neurol 1994; 36: 408-415.
3) Thrainsdottir S, Malik RA, Dahlin LB, Wiksell P, Eriksson KF, Rosen I, Petersson J, Greene DA, Sundkvist G. Endoneurial capillary abnormalities presage deterioration of glucose tolerance and accompany peripheral neuropathy in man. Diabetes 2003; 52: 2615-2622.
4) Tesfaye S, Chaturvedi N, Eaton SE, Ward JD, Manes C, Ionescu-Tirgoviste C, Witte DR, Fuller JH. Vascular risk factors and diabetic neuropathy. N Engl J Med 2005; 352: 341-350.
5) Ziegler D, Rathmann W, Dickhaus T, Meisinger C, Mielck A. Prevalence of polyneuropathy in pre-diabetes and diabetes is associated with abdominal obesity and macroangiopathy: the MONICA/KORA Augsburg Surveys S2 and S3. Diabetes Care 2008; 31: 464-469.
6) Bilir B, Ekiz Bilir B, Yilmaz I, Soysal Atile N, Yildirim T, Kara SP, Gumustas SA, Orhan AE, Aydin M. Association of apelin, endoglin and endocan with diabetic peripheral neuropathy in type 2 diabetic patients. Eur Rev Med Pharmacol Sci 2016; 20: 892-898.
7) Roustit M, Loader J, Deusenbery C, Baltzis D, Veves A. Endothelial dysfunction as a link between cardiovascular risk factors and peripheral neuropathy in diabetes. J Clin Endocrinol Metab 2016; 101: 3401-3408.
8) Tabit CE, Chung WB, Hamburg NM, Vita JA. Endothelial dysfunction in diabetes mellitus: molecular mechanisms and clinical implications. Rev Endocr Metab Disord 2010; 11: 61-74.
9) Ward KK, Low PA, Schmelzer JD, Zochodne DW. Prostacyclin and noradrenaline in peripheral nerve of chronic experimental diabetes in rats. Brain 1989; 112: 197-208.
10) Kihara M, Mitsui MK, Mitsui Y, Okuda K, Nakasaka Y, Takahashi M, Schmelzer JD. Altered vasoreactivity to angiotensin II in experimental diabetic neuropathy: role of nitric oxide. Muscle Nerve 1999; 22: 920-925.
11) Tuck RR, Schmelzer JD, Low PA. Endoneurial blood flow and oxygen tension in the sciatic nerves of rats with experimental diabetic neuropathy. Brain 1984; 107: 935-950.
12) Stevens MJ, Zhang W, Li F, Sima AA. C-peptide corrects endoneurial blood flow but not oxidative stress in type 1 BB/Wor rats. Am J Physiol Endocrinol Metab 2004; 287: E497-505.
13) Oltman CL, Davidson EP, Coppey LJ, Kleinschmidt TL, Yorek MA. Treatment of Zucker diabetic fatty rats with AVE7688 improves vascular and neural dysfunction. Diabetes Obes Metab 2009; 11: 223-233.
14) Nakamura J, Hamada Y, Sakakibara F, Hara T, Wakao T, Mori K, Nakashima E, Naruse K, Kamijo M, Koh N, Hotta N. Physiological and morphometric analyses of neuropathy in sucrose-fed OLETF rats. Diabetes Res Clin Pract 2001; 51: 9-20.
15) Cameron NE, Cotter MA. Diabetes causes an early reduction in autonomic ganglion blood flow in rats. J Diabetes Complications 2001; 15: 198-202.
16) Sasaki H, Schmelzer JD, Zollman PJ, Low PA. Neuropathology and blood flow of nerve, spinal roots and dorsal root ganglia in longstanding diabetic rats. Acta Neuropathol 1997; 93: 118-128.
17) Manschot SM, Biessels GJ, Cameron NE, Cotter MA, Kamal A, Kappelle LJ, Gispen WH. Angiotensin converting enzyme inhibition partially prevents deficits in water maze performance, hippocampal synaptic plasticity and cerebral blood flow in streptozotocin-diabetic rats. Brain Res 2003; 966: 274-282.
18) Zochodne DW, Ho LT. Normal blood flow but lower oxygen tension in diabetes of young rats:

microenvironment and the influence of sympathectomy. Can J Physiol Pharmacol 1992; 70: 651-659.

19) Pugliese G, Tilton RG, Speedy A, Chang K, Santarelli E, Province MA, Eades D, Sherman WR, Williamson JR. Effects of very mild versus overt diabetes on vascular haemodynamics and barrier function in rats. Diabetologia 1989; 32: 845-857.

20) Tesfaye S, Harris N, Jakubowski JJ, Mody C, Wilson RM, Rennie IG, Ward JD. Impaired blood flow and arterio-venous shunting in human diabetic neuropathy: a novel technique of nerve photography and fluorescein angiography. Diabetologia 1993; 36: 1266-1274.

21) Ibrahim S, Harris ND, Radatz M, Selmi F, Rajbhandari S, Brady L, Jakubowski J, Ward JD. A new minimally invasive technique to show nerve ischaemia in diabetic neuropathy. Diabetologia 1999; 42: 737-742.

22) Theriault M, Dort J, Sutherland G, Zochodne DW. Local human sural nerve blood flow in diabetic and other polyneuropathies. Brain 1997; 120: 1131-1138.

23) Zochodne DW. Nerve and ganglion blood flow in diabetes: an appraisal. Int Rev Neurobiol 2002; 50: 161-202.

24) Tesfaye S, Malik R, Harris N, Jakubowski JJ, Mody C, Rennie IG, Ward JD. Arterio-venous shunting and proliferating new vessels in acute painful neuropathy of rapid glycaemic control (insulin neuritis). Diabetologia 1996; 39: 329-335.

25) Ostergaard L, Finnerup NB, Terkelsen AJ, Olesen RA, Drasbek KR, Knudsen L, Jespersen SN, Frystyk J, Charles M, Thomsen RW, Christiansen JS, Beck-Nielsen H, Jensen TS, Andersen H. The effects of capillary dysfunction on oxygen and glucose extraction in diabetic neuropathy. Diabetologia 2015; 58: 666-677.

26) Eaton SE, Harris ND, Ibrahim S, Patel KA, Selmi F, Radatz M, Ward JD, Tesfaye S. Increased sural nerve epineurial blood flow in human subjects with painful diabetic neuropathy. Diabetologia 2003; 46: 934-939.

27) Quattrini C, Harris ND, Malik RA, Tesfaye S. Impaired skin microvascular reactivity in painful diabetic neuropathy. Diabetes Care 2007; 30: 655-659.

28) Lim TK, Shi XQ, Johnson JM, Rone MB, Antel JP, David S, Zhang J. Peripheral nerve injury induces persistent vascular dysfunction and endoneurial hypoxia, contributing to the genesis of neuropathic pain. J Neurosci 2015; 35: 3346-3359.

29) Archer AG, Roberts VC, Watkins PJ. Blood flow patterns in painful diabetic neuropathy. Diabetologia 1984; 27: 563-567.

30) Doupis J, Lyons TE, Wu S, Gnardellis C, Dinh T, Veves A. Microvascular reactivity and inflammatory cytokines in painful and painless peripheral diabetic neuropathy. J Clin Endocrinol Metab 2009; 94: 2157-2163.

31) Tack CJ, van Gurp PJ, Holmes C, Goldstein DS. Local sympathetic denervation in painful diabetic neuropathy. Diabetes 2002; 51: 3545-3553.

32) Merkle CW, Srinivasan VJ. Laminar microvascular transit time distribution in the mouse somatosensory cortex revealed by dynamic contrast optical coherence tomography. Neuroimage 2016; 125: 350-362.

33) Davis KD, Moayedi M. Central mechanisms of pain revealed through functional and structural MRI. J Neuroimmune Pharmacol 2013; 8: 518-534.

34) Tseng MT, Chiang MC, Chao CC, Tseng WY, Hsieh ST. fMRI evidence of degeneration-induced neuropathic pain in diabetes: enhanced limbic and striatal activations. Hum Brain Mapp 2013; 34: 2733-2746.

35) Selvarajah D, Wilkinson ID, Gandhi R, Griffiths PD, Tesfaye S. Microvascular perfusion abnormalities of the thalamus in painful but not painless diabetic polyneuropathy: a clue to the pathogenesis of pain in type 1 diabetes. Diabetes Care 2011; 34: 718-720.

36) Freeman OJ, Evans MH, Cooper GJ, Petersen RS, Gardiner NJ. Thalamic amplification of sensory input in experimental diabetes. Eur J Neurosci 2016; 44: 1779-1786