Microvascular Changes in the Diabetic Foot

Thanh Dinh, DPM and Aristidis Veves, MD, DSc

INTRODUCTION

It has been nearly half a century since the concept of “small vessel disease” was introduced as a unique entity in the microvasculature of the patient with diabetes. This misconception was arrived at through a retrospective histological study demonstrating the presence of periodic acid Schiff-positive material occluding the arterioles in amputated limb specimens of patients with diabetes (1). From these observations, Goldenberg and his colleagues deduced that the deposits in the small and medium-sized arterioles were the hallmark of vascular disease in the patient with diabetes. Perpetuation of this erroneous idea led to the belief that preferential occlusion of the small vessels in the patient with diabetes produced a poorer prognosis with limited revascularization options.

Since then, numerous studies have successfully refuted the notion of “small vessel disease.” In a blinded, prospective analysis of amputated limbs, periodic acid Schiff staining showed a similar meager pattern of occlusive disease in both diabetic and nondiabetic limbs at the arteriole level (2). Using a sophisticated casting technique, Conrad also demonstrated a lack of significant occlusive disease at the arteriole level in both the patients with and without diabetes (3). Furthermore, vascular reactivity in the vessels of patients with diabetes has been shown to be comparable to those of patients without diabetes based on physiological studies involving the administration of a papaverine (a vasodilator) into femoro-popliteal bypass grafts (4). These data, coupled with a vast clinical experience of nearly three decades of successful arterial reconstruction in patients with diabetes, have thoroughly dispelled the notion of diabetic “small vessel disease” (5).

However, recent work suggests that although an occlusive disease of the microcirculation does not exist, the microcirculation (predominantly capillaries and arterioles) is impaired in the patient with diabetes. In simplest terms, microvascular dysfunction in diabetes may be described by an increased vascular permeability and impaired autoregulation of blood flow and vascular tone. It is postulated that metabolic derangements as a result of hyperglycemia and insulin resistance work synergistically to cause microvascular dysfunction. Consequently, these metabolic alterations produce functional and structural changes at multiple levels within the arteriolar and capillary level.
STRUCTURAL CHANGES IN THE MICROCIRCULATION

Basement Membrane Thickening

Structurally, the most notable changes in the microcirculation involve thickening of the basement membrane and an observed reduction in the capillary size (6,7). However, the density of the skin capillaries does not differ from healthy subjects (8). These structural changes are more pronounced in the legs, likely the result of increased hydrostatic pressures in that part of the body (9). The extent of basement membrane thickening has also been observed to be related to the level of glycemic control, with increased basement thickening in poorly controlled patients with diabetes (10).

In the diabetic foot, basement membrane thickening has been demonstrated in the muscle capillaries (11). The sequence of events leading to basement membrane thickening begins with the increased hydrostatic pressure and shear force in the microcirculation. This is thought to evoke an injury response on the part of the microvascular endothelium with subsequent release of extravascular matrix proteins. Subsequently, thickening of the basement membrane with arteriolar hyalinosis occurs (12).

Because changes in the basement membrane can affect numerous cellular functions, such as vascular permeability, cellular adhesion, proliferation, differentiation, and gene expression, alterations in its components may cause vascular dysfunctions. Thickening of the basement membrane impairs the normal exchange of nutrients and activated leukocyte migration between the capillary and interstitium. Furthermore, the elastic properties of the capillary vessel walls are diminished, limiting their ability to vasodilate (13). As a result, the normal hyperemic response to injury is impaired, limiting the compensatory arteriolar dilatation in response to local injury, resulting in a reduced hyperemic response (14). It is important to note that basement membrane thickening does not appear to lead to narrowing of the capillary lumen, instead arteriolar blood flow is observed at normal levels or even increased despite these changes (15).

FUNCTIONAL CHANGES IN THE MICROCIRCULATION

The observed failure of the microcirculation to vasodilate in response to injury has been described as a functional ischemia and has been demonstrated to be a result of a number of factors at play in the microcirculation of the patient with diabetes. Alteration in the microcirculation of the foot has been postulated to be an important factor in the poor wound healing associated with chronic diabetic foot ulcerations. Recent work from our unit has addressed these changes, with specific emphasis placed on the changes in the diabetic foot microcirculation, nerve function, and muscle metabolism.

DIABETIC FOOT MICROCIRCULATION

The resting total skin microcirculation in the diabetic foot is comparable with that of the nondiabetic foot, when peripheral neuropathy is absent. However, when neuropathy is present, the capillary blood flow has been shown to be reduced (16,17). This may indicate a maldistribution of blood flow to the skin, with a resultant functional ischemia. As previously mentioned, the hyperemic response is impaired in the diabetic microcirculation, thereby failing to achieve maximal blood flow following injury. Development of new techniques to evaluate the microcirculation has expanded our understanding of
these functional changes. Therefore, review of these techniques may be of particular importance.

**Methods of Evaluating the Microcirculation of the Feet**

Recent technological advances over the past decade have enabled us to evaluate the functional microcirculation of the feet. Methods such as laser Doppler flowmetry, flow-video microscopy, cannulation measurements of capillary pressure, and transcutaneous oxygen tension measurements have all been used. The most commonly used technique, and the one used in our lab, for evaluating blood flow in the skin remains laser Doppler flowmetry.

**Laser Doppler Flowmetry**

This method is considered the most widely accepted technique for evaluating capillary blood flow in the skin microcirculation based on its ease of use and reproducibility. Presently employed in our unit, this method uses a red laser light that is transmitted to the skin through a fiberoptic cable. The frequency shift of light back-scattered from the moving red blood cells beneath the probe tip is used to give a measure of the superficial microvascular perfusion (17).

There are two types of laser probes available for use with this method, a single-point laser probe or a real-time laser scanner. The single-point laser probe measures the microvascular blood flow at a single point in the skin, and has been used for evaluating the hyperemic response to a heat stimulus, or for evaluating the nerve–axon-related hyperemic response. Measurement of the hyperemic response to a heat stimulus is performed by first taking baseline blood flow measurements. Next, the skin is heated to 44°C for 20 minutes using a small brass heater, or in our experience, maintaining the ambient room temperature at this level. Following this, the maximum blood flow is determined by the magnitude of blood flow change in response to heat.

In conjunction with the technique of iontophoresis and the addition of a second probe, the single-point laser probe method can be used to assess the integrity of the nerve–axon reflex. The technique of iontophoresis involves the delivery of a vasoactive substance (acetylcholine chloride or sodium nitroprusside [SNP]) transdermally with a low-voltage current (200 μA) in order to effect vasodilation. In the two single-point laser probe technique, the first probe is exposed to acetylcholine, in order to measure the blood flow to a specified area of skin. The second probe is situated in close proximity (5 mm) to the first probe, and consequently measures the indirect effect of the iontophoresed acetylcholine. The indirect effect of the acetylcholine results from stimulation of the C-nociceptive nerve fibers and therefore, the nerve–axon reflex hyperemic response.

The method of laser scanning also uses the technique of iontophoresis to evaluate the endothelium-dependent microvascular reactivity. More specifically, a device consisting of two chambers that accommodate two single-point laser probes are applied to the skin. A small quantity (<1 mL) of the vasoactive substance is placed in the chamber whereas a second nonactive electrode is placed 10–15 cm away from the chamber. A constant current of 200 μA is applied, creating movement of the solution toward the skin, causing vasodilation. Iontophoresis of acetylcholine chloride measures the endothelium-dependent vasodilation, whereas SNP measures the endothelium-independent vasodilation.
Following iontophoresis of the vasoactive substance, the adhesive device is removed and the area of skin is scanned with a laser Doppler scanner. The laser Doppler perfusion imager uses 1-mW helium–neon laser beam of 633-nm wavelength to sequentially scan the area of skin. Increased blood flow at the skin level is recorded by the scanner and expressed in volts. This technique has been validated against direct measurements of the capillary flow velocity with consistent measurements achieved.

**Flow-Video Microscopy**

Flow-video microscopy enables measurements of capillary blood flow along with such parameters as average flow velocity, peak postocclusive hyperemic flow velocity, and the response to other physiological maneuvers. With the use of an image-shearing monitor, the capillary red cell column width is calculated. This is performed by lighting mercury vapor onto skin that has previously been brushed with a thin film of oil or varnish in order to limit the scattering of light. The image of the moving blood elements can then be recorded with a low-light sensitive video system (18). More recently, a digitized system has been developed that is capable of recording continuous capillary blood flow. Measurements are then calculated through an integrated software program. However, this method may under-estimate the true capillary lumen resulting from the unvisualized marginal plasma layer.

**Capillary Pressure Measurements**

The measurement of capillary pressure involves direct cannulation of a single vessel. Following cannulation of the vessel, the transmitted pressure can be measured manometrically or through use of an electronic device. This invasive technique is capable of detecting small changes in the capillary pressure as low as 1–2 mmHg. Additionally, it has the added benefit of being able to measure the capillary pressure continuously. However, the procedure can be complex and may require significant expertise.

**Transcutaneous Oxygen Tension Measurements**

The measurement of oxygen transcutaneously can be performed based on the fact that oxygen is capable of diffusing throughout the body tissue and skin. Although the rate of diffusion is very low at normal surface body temperature, application of heat to a localized area can sufficiently enhance the flow of oxygen through the dermis to allow for noninvasive measurement of the capillary oxygen level. However, these measurements can be inaccurate as they appear to fluctuate with the skin temperature and room temperature.

**MICROVASCULAR REACTIVITY IN THE DIABETIC FOOT**

Functional changes in the microcirculation appear to impact the ability of the microcirculation to vasodilate in periods of stress or injury. Clinical examination of the neuropathic diabetic foot with an ulcer may demonstrate a warm foot with palpable pulses and distended veins. Although there appears to be no reduction in blood flow to the foot, the blood flow to the skin microcirculation may be reduced (19,20) through shunting of blood from the nutritional capillaries to the subpapillary arteriovenous shunts of a much lower resistance (21).

As these shunts are innervated by sympathetic nerves, the existence of diabetic autonomic neuropathy with sympathetic denervation may lead to opening of these shunts with a resultant augmentation of the maldistribution of blood between the nutritional
capillaries and subpapillary vessels (8,22). Ultimately, arteriovenous shunting further aggravates the functionally ischemic foot, as evidenced by studies using venous occlusion plethysmography, Doppler sonography, and venous oxygen tension measurements (23).

Much work has been done in the past decade to investigate why the microvasculature in diabetes fails to respond appropriately to stress and injury. The recent development of noninvasive techniques that can reliably quantify blood flow in the skin microcirculation and evaluate endothelial function has made it possible to study changes in microvascular function in patients with diabetes. Those findings will be discussed in detail.

**Endothelial Dysfunction**

The vascular endothelium plays an important role in controlling the microvascular tone by synthesizing and releasing substances such as prostacyclin, endothelin, prostaglandins, and nitric oxide that modulate the vasomotor tone and prevent thrombosis. Nitric oxide is the most important vasodilator substance responsible for endothelium-dependent vasodilation. After its secretion from the endothelium it diffuses to the adjacent smooth muscle cells and stimulates the guanylate cyclase enzyme which leads to smooth muscle relaxation and vasodilation (24).

There is substantial evidence that endothelial function is abnormal in patients with both type 1 (insulin-dependent) and type 2 (noninsulin-dependent) diabetes mellitus (25,26). As a result, the causes of endothelial function have been postulated to include both hyperglycemia and hyperinsulinemia as possible mediators of abnormal endothelium-dependent responses. The significance of endothelial dysfunction on the micro- and macrocirculation and the variety of proposed mechanisms affecting normal function will be discussed in further detail.

**Endothelium-Dependent Vasodilation**

The majority of studies agree that the endothelium-dependent vasodilation in the large vessels is impaired in diabetes, irrespective of the presence or absence of long-term complications (25–29). Initial studies of endothelium-dependent vasodilation used venous occlusion plethysmography whereas subsequent studies employed flow-mediated vasodilation, a noninvasive technique. Through these techniques, endothelium-dependent vasodilation has been shown to be impaired in adolescents with type 1 diabetes, a population that is generally spared from the micro and macrovascular complications of diabetes (30). This finding suggests that endothelial dysfunction is present before the development of these vascular complications and may play an important role in their development. Finally, endothelial function in type 1 diabetes has been shown to be associated with total cholesterol, red cell folate, blood glucose levels, and duration of diabetes (31–33).

In the past decade, extensive research effort has focused on the relationship of type 2 diabetes and vascular disease. Thus, it is currently well established that the endothelium-dependent vasodilation are impaired in both the micro- and macrocirculation in type 2 diabetes. Furthermore, there is almost universal agreement that changes in the endothelial function precedes the development of diabetes and is present in the prediabetic stage. It is also of interest that endothelial dysfunction is associated with insulin resistance in subjects without diabetes, suggesting a cause–effect relationship of these two conditions.
Findings from our unit further corroborate impairment of endothelium-dependent vasodilation in patients with diabetes. We have evaluated the effect of neuropathy and hypoxia on foot circulation on five groups of patients, patients with diabetes and neuropathy, patients with neuropathy and clinical signs of vascular disease, patients with diabetes and Charcot neuroarthropathy, nonneuropathic patients with diabetes, and healthy controls. (27) (Fig. 1). The endothelial-dependent vasodilation was studied by using laser Doppler imaging to measure the vasodilatory response to iontophoresis of
acetylcholine. Administration of acetylcholine directly stimulates the production of nitric oxide, resulting in vasodilation. We found that the vasodilatory response to acetylcholine was reduced in patients with neuropathy, neuropathy and vascular disease, and Charcot neuroarthropathy, whereas no difference was found between nonneuropathic subjects and the healthy controls. Additionally, the vasodilatory response was not diminished in subjects with neuropathy and vascular disease in comparison with subjects with neuropathy alone.

Interestingly enough, impairment in the microcirculation was found to be present in the absence of large vessel disease. These findings implied that the main reason for reduced microvascular reactivity was the presence of neuropathy, as indicated by the fact that no abnormalities were found in the nonneuropathic patients with diabetes. Further support for this claim is provided by the findings that the coexistence of neuropathy and vascular disease did not result in a greater decrease in endothelium-dependent vasodilation than that resulting from neuropathy alone.

**Endothelium-Independent Vasodilation**

Regarding the endothelium-independent vasodilation, which evaluates the function of the vascular smooth muscle cell, the majority of published studies have been conflicting. However, it should be emphasized that most of the early studies that demonstrated no impairment of endothelium-independent vasodilation in type 1 diabetes composed of a small number of subjects whereas the power analysis was mainly based on the results from the endothelial function. Thus, the possibility of not detecting a difference because of methodological problems cannot be excluded. Despite contrary initial reports, there is mounting evidence that endothelium-independent vasodilation is impaired in type 1 diabetes, even in diabetes without secondary complications (34,35).

In the presence of secondary complications such as microalbuminuria in type 1 diabetes, there exists conflicting data on the function of endothelium-independent vasodilation. An early study found that endothelium-independent vasodilation is normal in the presence of microalbuminuria (36), whereas subsequent work has revealed that endothelium-independent vasodilation dysfunction was present in the absence of microalbuminuria, potentially predicting the development of microalbuminuria in type 1 diabetes and other cardiovascular complications (37–40).

Endothelium-independent vasodilation in type 2 diabetes has also met with contradictory early results. However, most studies agree that endothelium-independent vasodilation in type 2 diabetes is unchanged in the macrocirculation, and diminished, along with impairment of the vascular smooth muscle function, in the microcirculation (41,42). Of note, these changes were also found in patients with type 2 diabetes without secondary complications of the disease (42).

In our unit, endothelium-independent vasodilation was also found to be decreased in patients with diabetes (17). (Fig. 1) The endothelium-independent vasodilation was studied using laser Doppler imaging to measure the vasodilatory response to iontophoresis of SNP. This response was most severely reduced in diabetic patients with vascular disease, suggesting that the endothelium-independent response may be spared. Because acetylcholine stimulates the production of nitric oxide, it was surmised that an impaired nitric oxide production was responsible for the impaired vasodilatory response observed.
Mechanisms of Endothelial Dysfunction

In 1980, Furchgott and Zawadzki (43) discovered that arterial vasodilation was dependent on an intact endothelium and its release of a substance they called endothelium-derived relaxing factor, which causes arterial smooth muscle relaxation in response to acetylcholine and other vasodilators. Later identified as endothelial-derived nitric oxide (EDNO), its roles include activation of vascular smooth muscle guanylate cyclase, elevation of cGMP levels, and may increase Na⁺, K⁺-ATPase activity (44). In addition to acetylcholine, there appear to be a number of substances that produce EDNO-mediated vasodilation. These other substances appear to cause vasodilatory effects through the nitric oxide pathway, with insulin mediating the vasodilation through modulating the synthesis and release of EDNO (45, 46).

A variety of mechanisms have been proposed for endothelial dysfunction, principally abnormalities in the EDNO pathway. The main mechanisms that are involved include the activation of protein kinase C (PKC), the increased vasoconstrictor prostanoids, reduction in Na⁺, K⁺-ATPase activity, poly (adenosine diphosphate [ADP]-ribose) polymerase (PARP) activation, alterations in oxidative stress, and advanced glycosylated end products (AGEs).

Increased vascular permeability is another characteristic abnormality observed as a result of endothelial dysfunction in diabetes. This can occur as early as 4–6 weeks after the onset of diabetes and is probably caused by a loss of integrity in the tight junctions between the endothelial cells (47). Increased activation of PKC, a key player in intercellular signal transduction for hormone and cytokines, may be the result of hyperglycemia. PKC inhibitors have also been used to restore impaired vascular reactivity, lending support to the role of increased PKC activation in endothelial dysfunction (41). Finally, PKC activation can also regulate vascular permeability and neovascularization via the expression of growth factors, such as vascular endothelial growth factor (VEGF)/vascular permeability factor.

Experimental studies in diabetic animals have also indicated that abnormal endothelial production of vasoconstrictor prostanoids may be a cause of endothelial cell dysfunction. Increased levels of thromboxane (TXA₂) and prostaglandin (PGH₂) have been isolated from segments of diabetic vascular tissue. In human studies, however, the role of vasoconstrictor prostanoids is less clear. Flow-dependent vasodilation in healthy subjects, which may be used as an index of endothelial function is unaffected by aspirin, thus demonstrating that it is entirely mediated by EDNO and independent of vasoactive prostanoids (48).

Na⁺, K⁺-ATPase is an integral component of the sodium pump and is involved in the maintenance of cellular integrity and functions of contractility, growth, and differentiation. Therefore, impairment of this mechanism can lead to vascular dysfunction. It is well established that activity of the Na⁺, K⁺-ATPase is generally decreased in the vascular tissues of patients with diabetes.

Recent work has also shed light on the role of PARP in endothelial function (49). PARP is a nuclear enzyme that responds to oxidative DNA damage by activating an inefficient cellular metabolic cycle, often leading to cell necrosis. Interestingly, PARP activation has been observed in patients with diabetes as well as those healthy patients at risk for developing diabetes. This finding suggests that changes in the microcirculation resulting from PARP activation may begin in the prediabetic state.

In a study performed in our unit (45), nondiabetic controls were compared with three groups: those with type 2 diabetes, those with glucose intolerance only, and those with
a family history of type 2 diabetes but no intolerance themselves. PARP activation was higher in all three diabetes-associated groups than in the healthy controls. The activation of PARP was associated with changes in the vascular reactivity of the skin microcirculation in from biopsies taken from these subjects, supporting the hypothesis that PARP activation contributes to changes in microvascular reactivity. Further study is required to prove this association and possible benefits from inhibiting this activation.

It has recently been proposed that oxidative stress contributes to the development of diabetic vascular complications, through an increased production of oxygen-derived free radicals. This increased production in diabetes directly inactivates endothelium-derived nitric oxide, thereby reducing the bioavailability of EDNO (50). In animal models, endothelium-derived free radicals impaired EDNO-mediated vasodilation. In human studies, administration of vitamin E (400 IU/day), a potent free radical scavenger, had no apparent effect on cardiovascular outcomes in patients with diabetes with complications (51). However, early studies showed that high-dose vitamin E (1800 IU/day) normalized hemodynamic abnormalities, suggesting that administration of an antioxidant may reduce the risks of diabetic vascular complications (52). Later studies involving long-term high-dose vitamin E found no beneficial effects on endothelial function or left ventricular function in patients with type 1 and patients with type 2 diabetes (53). Furthermore, high-dosage vitamin E was also associated with worsening in some vascular reactivity measurements when compared with control subjects.

AGEs result from a nonenzymatic reaction when proteins are exposed to hyperglycemic environments. The resultant Schiff bases can be rearranged to form Amadori products, AGEs, and reactive oxygen species. Increased AGE levels have been found in patients with diabetes and may contribute to the increased vascular permeability of diabetes, because blockade of a specific receptor for AGE reverses diabetes-mediated vascular hyperpermeability (54). Furthermore, the generated reactive oxygen species have been shown to cause severe disturbances in the regulation of coronary flow and cellular hemostasis, leading to the severe macrovascular lesions typically observed in diabetic patients after more than 10 years of disease (55). Interestingly, inhibition of reactive oxygen species also prevents the generation of AGE products, suggesting that the autoxidative process plays an important role in the complex reaction cascade leading to AGE.

**Biochemical Markers of Endothelial Dysfunction**

When the endothelium has been injured, a number of vasoactive substances are produced in response. As a result, these biochemical markers, such as von Willebrand factor (vWF) and cellular adhesion molecules, have been employed to evaluate endothelial dysfunction. vWF, a multimeric glycoprotein mainly synthesized by endothelial cells, is involved in platelet adhesion and aggregation and acts as the carrier of coagulation factor VIII in plasma. Increased levels of vWF, reflecting activation of or damage to endothelial cells, have been described in association with atherosclerosis and diabetes. Initial studies in patients with diabetes have demonstrated increased plasma levels of vWF (56). Furthermore, these elevations preceded the development of albuminuria and peripheral nerve dysfunction. Therefore, it has been suggested that vWF could be used as a predictive indicator of vascular complications.

Cellular adhesion molecules are expressed on endothelial cells in response to inflammation and facilitate the adhesion of circulating leukocytes to their surface. Increased
levels of soluble intercellular adhesion molecule (sICAM) in healthy individuals have been linked with a higher risk of future cardiovascular complications (57). Furthermore, both sICAM and soluble vascular cell adhesion molecule levels have been reported to be higher in patients with diabetes and in some instances, individuals with impaired glucose tolerance (58,59).

In vitro studies of sICAM and soluble vascular cell adhesion molecule demonstrated that these biochemical markers were expressed by endothelial cells following a short period of incubation in high glucose conditions (60), lending support that hyperglycemia plays a role in activation of these molecules. Furthermore, a direct correlation has been detected between vascular cell adhesion molecule (VCAM)-1 and VEGF, suggesting that cellular adhesion and neovascularization may be linked processes (61).

**Nerve–Axon Reflex**

Nerve dysfunction contributes to the diminished vasodilatory response observed in diabetes. Under normal conditions, the ability to increase blood flow to the skin depends on the existence of an intact neurogenic vascular response. This response is referred to as Lewis’ triple flare response and begins with stimulation of C-nociceptive nerve fibers, leading to antidromic stimulation of the adjacent C fibers (Fig. 2). These fibers then secrete substance P, calcitonin gene-related peptide, and histamine, causing vasodilation and increased blood flow to the injured tissues. Typically, this response is equal to one-third of the maximal vasodilatory capacity.

Measurements in the diabetic neuropathic foot have shown that this neurovascular response is impaired, leading to a significant reduction in blood flow under conditions of stress. Thus, the diabetic neuropathic foot fails to respond to injury or infection in the usual manner, providing a plausible explanation for the clinically observed lack of hyperemia in the infected or injured diabetic foot. It has been postulated that the observed reduction in the nerve–axon reflex-related vasodilation in diabetic neuropathy is related to both impaired C-nociceptive fiber function and impaired ability of the microvasculature to respond to vasomodulators secreted by these fibers (62).
Evidence for this vasodilatory impairment related to the presence of diabetic neuropathy is provided by studies in our lab that used the previously described single-point laser probe technique to evaluate the nerve–axon-related vasodilatory response. The indirect response to the iontophoresis of acetylcholine was significantly reduced in diabetic patients with neuropathy, diabetic patients with neuropathy and peripheral vascular disease, and diabetic patients with Charcot arthropathy, compared with patients with diabetes without complications or healthy subjects (45). The abnormality in axon-related vascular reactivity is believed to further aggravate the abnormalities in the microcirculation, and a vicious cycle ensues (17). Subsequently, involvement of the C-nociceptive fibers in diabetes not only leads to impaired pain perception, but also to impaired vasodilation under conditions of stress.

The contribution of the nerve–axon reflex-related vasodilation response to the total endothelium-dependent and endothelium-independent vasodilation was also studied in a group of patients with diabetes and a group of healthy control subjects (63). The nerve–axon-related response in healthy subjects was found to be 35% of the total response at the forearm level and 29% at the foot level. By contrast, response to SNP, a substance that does not specifically excite the C-nociceptive fibers was 13% at the level of the forearm and 12% at the level of the foot. This indicates that the presence of a non-specific galvanic response may also be responsible.

In the presence of diabetic neuropathy, the total response was reduced to 8%, a significant reduction in comparison with the healthy controls (Fig. 3). These findings indicate that although the neurovascular response is an important factor in the skin microcirculation, it is not the sole nor dominant pathway through which vasodilation is
achieved (64). However, the presence of neuropathy appears to render the diabetic foot functionally ischemic, as blood flow fails to increase under periods of stress.

**CONCLUSION**

In conclusion, although an occlusive disease of the microcirculation does not exist, functional impairment of the microcirculation in diabetes may contribute to secondary complications such as foot infections and ulcerations. Microcirculation to the diabetic foot suffers both structural and functional derangements. Nerve–axon-related microvascular reactivity is clearly impaired in the diabetic population and there is a growing belief that both the failure of the vessels to dilate and the impairment of the nerve-axon reflex are major causes for impaired wound healing in patients with diabetes. Further studies are necessary to clarify the precise etiology of observed endothelial dysfunction in diabetic and neuropathic patients and to identify the possible potential therapeutic interventions to prevent or to retard its progression.

**REFERENCES**


