Cutaneous microcirculation in the neuropathic diabetic foot improves significantly but not completely after successful lower extremity revascularization

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Objective: **The purpose of this study was the examination of the effect of successful large vessel revascularization on the microcirculation of the neuroischemic diabetic foot.**
Research design and methods: **We measured the cutaneous microvascular reactivity in the foot in 13 patients with diabetes with peripheral arterial disease and neuropathy (group DI) before and 4 to 6 weeks after successful lower extremity arterial revascularization. We also compared them with age-matched and sex-matched groups of 15 patients with diabetes and neuropathy, seven patients without neuropathy, and 12 healthy patients for control. We used single-point and laser Doppler scan imaging for the measurement of the foot skin vasodilatation in response to heating to 44°C and to iontophoresis of 1% acetylcholine (endothelial-dependent response) and 1% sodium nitroprusside (endothelial-independent response).**
Results: **The group DI response to heat increased from 289% ± 90% before surgery (percent increase over baseline measured in volts) to 427% ± 61% ($P < .05$) after surgery but was still comparable with the response of the patients with diabetes and neuropathy (318% ± 51%) and lower than the responses of the patients without neuropathy (766% ± 220%) and the healthy patients for control (891% ± 121%; $P < .0001$). The group DI acetylcholine response also improved from 6% ± 4% before surgery to 26% ± 8% after surgery ($P < .05$) and was similar to the responses of patients with diabetes and neuropathy (18% ± 3%) and patients without neuropathy (38% ± 8%) but still lower when compared with the response of the patients for control (48% ± 9%; $P < .001$). The sodium nitroprusside response for group DI improved from 10% ± 4% to 29% ± 9% ($P < .05$) and was similar to the responses of the neuropathic (25% ± 9%), nonneuropathic (32% ± 6%), and control (40% ± 5%) groups. The group DI neurovascular response, which depends on the healthy function of the C-fiber nociceptors, was similar at baseline (5% ± 9%) and after surgery (14% ± %10) and in the neuropathic group (33% ± 21%), but it was dramatically reduced when compared with the nonneuropathic (110% ± 40%) and control (198% ± 54%) groups ($P < .001$).**
Conclusion: **Impaired vasodilation in the diabetic neuropathic lower extremity leads to functional ischemia, which improves considerably but is not completely corrected with successful bypass grafting surgery. Therefore, patients with diabetes and neuropathy may still be at high risk for the development of foot ulceration or the failure to have an existing ulcer heal despite adequate correction of large vessel blood flow.**
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Peripheral arterial disease is four to six times more prevalent in patients with diabetes between the ages of 45 and 75 years than in those without diabetes, and the male to female ratio approaches one.[1,2] The pattern of occlusive peripheral arterial disease with diabetes is such that medium-sized arteries, mainly at the popliteal trifurcation, are affected. However, there is sparing of the distal pedal vessels from occlusive disease with diabetes, and the so-called "small vessel disease" that referred to occlusive disease of the small pedal arteries in the past has been shown to be incorrect.[3] Distal arterial bypass grafting surgery to the pedal arteries is common practice today in patients with diabetes, and long-term follow-up studies have shown encouraging results.[4]

Despite the lack of occlusive disease in the small pedal arteries with diabetes, it is currently realized that there are functional abnormalities in the microcirculation (ie, the capillaries and arterioles), which seem to exist even in the prediabetic stage at both the forearm and foot level.[5,6] In addition, diabetic neuropathy impairs the nerve axon reflex that depends on healthy C-fiber nociceptor function and causes local vasodilation in response to a painful stimulus. This condition further compromises the vasodilatory response that is present in conditions of stress, such as injury or inflammation, in the diabetic neuropathic foot.[7] This impairment may be one of the reasons that some ulcers in the diabetic neuropathic foot fail or are slow to heal despite successful lower extremity revascularization.

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Clinical characteristics of study groups

	DI group	*DN group*	*D group*	*C group*
No. of patients	13	15	7	12
Male:female ratio	11:2	13:2	5:2	8:4
Age (years)	62 ± 3	60 ± 2	55 ± 6	58 ± 3
No. of patients with diabetes mellitus type 1 or type 2*	1:12	5:8	5:2	–
Diabetes mellitus duration (y)	20 ± 3	22 ± 3	15 ± 4	–
Transcutaneous oxygen pressure†	40 ± 6	61 ± 4	70 ± 4	76 ± 2
Neuropathy Disability Score‡	12 ± 4	18 ± 2	0.4 ± 0.3	0.7 ± 0.4
Vibration Perception Threshold‡	43 ± 3	43 ± 4	18 ± 3	13 ± 2
Semmes-Weinstein filaments‡	5.51 ± 0.34	6.59 ± 0.15	4.00 ± 0.23	4.07 ± 0.12

Data expressed as mean ± standard error of mean.
*DI group versus DN group and D group.
†DI group versus DN, D, and C groups and DN group versus C group.
‡DI group and DN group versus D group and C group.
DI, Patients with diabetes with neuropathy and vascular disease; *DN,* patients with diabetes with neuropathy; *D,* patients with diabetes; *C,* patients for control.

The effect of successful lower extremity arterial revascularization (LEAR) on the impaired foot microcirculation with diabetes is not known. In this study, we have evaluated the effect of successful lower extremity arterial reconstruction on the microcirculation of the ischemic and neuropathic diabetic foot.

SUBJECTS AND METHODS

Subjects. Four groups of subjects who were matched for age and gender were studied. The first group (group DI) consisted of 13 patients with diabetic neuropathy and lower extremity ischemia that needed lower extremity revascularization for limb salvage. The second group consisted of 15 patients with diabetic neuropathy but no evidence of ischemia (palpable pedal pulses and no history of claudication). The third group consisted of seven subjects with no neuropathy or ischemia, and the fourth group consisted of 12 healthy patients for control. The first group was studied before surgery and 4 to 6 weeks after successful lower extremity revascularization. The main reason for the allowance of 4 to 6 weeks after the operation was to minimize the effects of postoperative edema that is present immediately after the bypass grafting procedure. The remaining groups were examined once. The demographics of the four groups of subjects studied are shown in the Table.

All the subjects studied were nonsmokers or had not smoked in the previous 6 months, had normotensive conditions or conditions that were well controlled with no more than two drugs, had serum creatinine levels of less than 2 mg/dL with no microalbuminuria, and had no severe dyslipidemia (triglyceride levels, >600 mg/dL; cholesterol levels, >250 mg/dL). The protocol was approved by the Ethics Committee/Institutional Review Board of the medical center. Fully informed consent was obtained from the volunteers who participated in the study.

Methods. The subjects were studied after an overnight fast and abstinence from caffeine-containing beverages for 24 hours. A general physical examination was performed by a physician and included blood pressure measurement. Neuropathy was diagnosed according to previously described criteria.[8] More specifically, the Neuropathy Symptom Score, the Neuropathy Disability Score, the Vibration Perception Threshold, and the Semmes-Weinstein monofilaments were used as previously described.[9] The systolic and diastolic blood pressures were recorded to the nearest 2 mm Hg as the mean of two measurements with the subjects seated. Height and weight were also recorded, and the body mass index was calculated.

Assessment of microvascular reactivity. All the vascular reactivity tests were performed on the dorsum of the foot immediately after the clinical examination with fasting conditions according to methods that have been previously described.[7] In summary, the subjects were seated comfortably in a reclining chair in a quiet warm environment (room temperature, 21°C to 22°C). The cutaneous microcirculation on the dorsum of the foot was evaluated with laser Doppler scan perfusion imaging (LISCA Development AB, Linkoping, Sweden) before and after iontophoresis of 1% acetylcholine and 1% sodium nitroprusside (MICI iontophoresis system, Moor Instruments LTD, Millwey, Devon, England). The term iontophoresis denotes the introduction of soluble ions into the human skin with the application of a small electric current (200 μA). Acetylcholine was used for the evaluation of endothelium-dependent vasodilatation (nitric oxide [NO]–mediated response), and sodium nitroprusside was used to evaluate endothelium-independent vasodilatation (direct smooth muscle cell response). The cutaneous hyperemic response to heat (44°C) also was evaluated at a site away from the iontophoresis site with single-point laser Doppler scanning.

The data analysis was performed with the Minitab statistical package (Minitab, State College, Pa). The Kruskal-Wallis test was used for nonparametric data, and analysis of variance was used for parametric data for comparisons among the various groups. The Fisher exact test was used for the identification of differences between individual groups. For the comparison of measurements before and

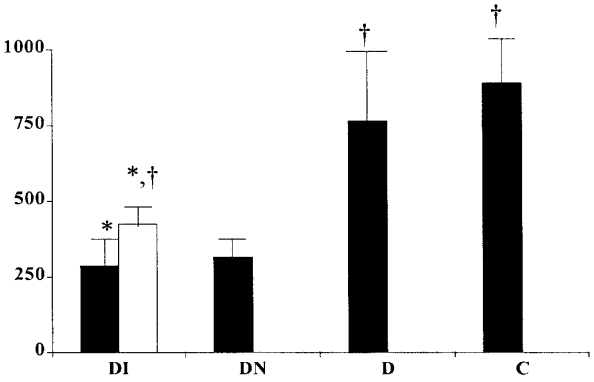

Fig 1. Maximal hyperemic response to heat improved in patients with neuropathy and peripheral arterial disease (*DI* group) after successful bypass grafting operation *(white bar)* when compared with baseline measurements before operation *(black bar)*. However, postoperative response was similar to that of patients with neuropathy (*DN* group) and lower when compared with patients with nonneuropathic diabetes (*D* group) and healthy patients for control (*C* group). Results are on basis of single-point laser probe measurements and are expressed as percent of increase over baseline. *Before versus after surgery in DI group, $P < .05$. †After surgery, DI group versus D and C groups, $P < .0001$.

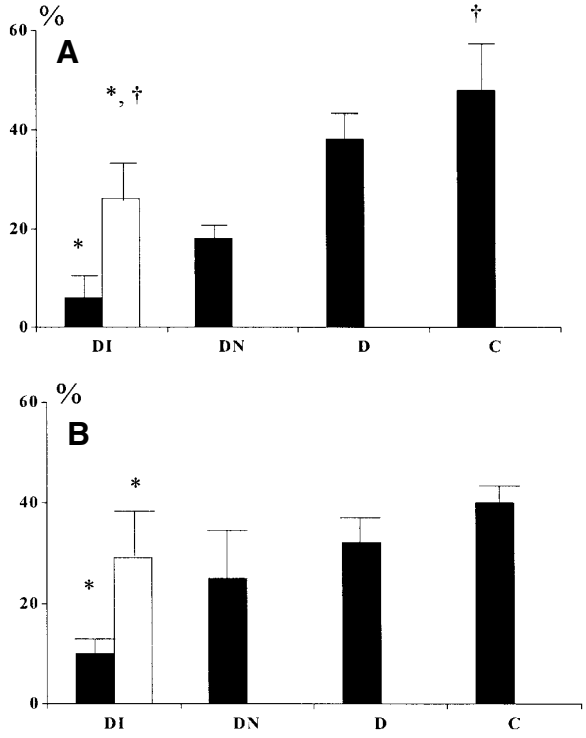

Fig 2. A, Response to iontophoresis of acetylcholine (endothelium dependent) improved in patients with neuropathy and peripheral arterial disease (*DI* group) after successful bypass grafting operation *(white bar)* when compared with baseline measurements before operation *(black bar)*. However, postoperative response of DI group was similar to that of patients with neuropathy (*DN* group) and patients with nonneuropathic diabetes (*D* group) and was lower when compared with healthy patients for control (*C* group). **B,** Response to iontophoresis of sodium nitroprusside (endothelium independent) improved in patients with neuropathy and peripheral arterial disease (*DI* group) after successful bypass grafting operation *(white bar)* when compared with baseline measurements before operation *(black bar)*. Postoperative response was similar to that of patients with neuropathy (*DN* group), patients with nonneuropathic diabetes (*D* group), and healthy patients for control (*C* group). Results are on basis of laser Doppler scan imaging measurements and are expressed as percent of increase over baseline. *Before versus after surgery in DI group, $P < .05$. †After surgery, DI group versus C group, $P < .001$.

after revascularization in the first group, the paired *t* test was used. The results were expressed as the mean ± standard error of the mean.

RESULTS

The patient demographics are depicted in the Table. All the groups were matched for age, sex, comorbid conditions, and, in the case of the patients with diabetes, the duration of diabetes. All the patients in the DI group had undergone successful distal bypass grafting surgery for limb salvage. A total of 13 distal bypass grafting procedures were performed. Of these, eight were from the femoral to the dorsalis pedis artery and five were from the femoral to the posterior tibial artery. There were eight patients with tissue loss. Four patients had nonhealing ulcers that healed 4 to 6 weeks after surgery. Two patients had ulcers that were clean and healing. Two patients underwent amputations of dry gangrenous toes soon after the bypass grafting procedures, and their amputation sites were healing satisfactorily 4 to 6 weeks after surgery. The outcome of the operation was defined on clinical criteria, such as healthy foot pulses (n = 7) and palpable graft pulses (n = 13), nonobstructive continuous wave Doppler scan signal over the graft, satisfactory wound healing for ulcers, and amelioration of rest pain.

The cutaneous vasodilatory response to heating to 44°C (percent increase over baseline measured in volts) was significantly improved after LEAR in the DI group when compared with the preoperative measurement (289% ± 90% versus 427% ± 61%; $P < .05$; Fig 1). However, the postoperative response was significantly lower when compared with the responses in the patients who were diabetic without neuropathy and in the healthy patients for control (766% ± 220% and 891% ± 121%, respectively; $P < .001$), and the response was comparable with that in the neuropathic group (318% ± 51%).

The acetylcholine response also improved from 6% ± 4% before surgery to 26% ± 8% after surgery ($P < .05$, measured in volts) in the DI group (Fig 2). The postoperative response was similar to that of the patients with neuropathic diabetes (18% ± 3%) and the patients with

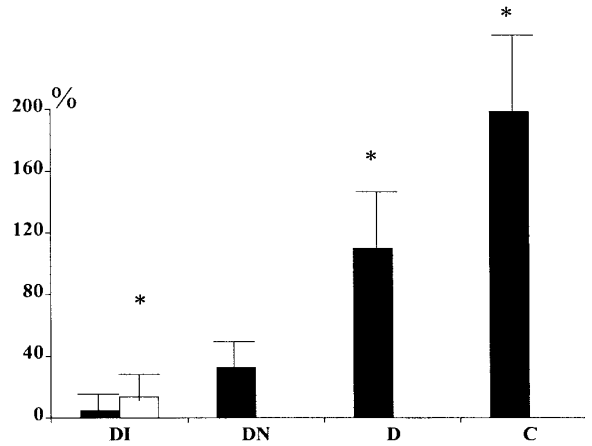

Fig 3. Nerve axon reflex–related response remained unchanged in patients with neuropathy and peripheral arterial disease (*DI* group) after successful bypass grafting operation *(white bar)* when compared with baseline measurements before operation *(black bar)*. Both preoperative and postoperative responses were similar to those of patients with neuropathy (*DN* group) and lower when compared with patients with nonneuropathic diabetes (*D* group) and healthy patients for control (*C* group). Results are on the basis of single-point laser probe measurements and are expressed as percent of increase over baseline. *Before and after surgery, DI group versus D and C groups, $P < .001$.

nonneuropathic diabetes (38% ± 8%) but was still lower when compared with the response of the healthy patients for control (48% ± 9%; $P < .001$). The sodium nitroprusside response for the DI group improved from 10% ± 4% to 29% ± 9% ($P < .05$, measured in volts) after surgery. The postoperative response was similar to that of the patients with neuropathic diabetes (25% ± 9%), the patients with nonneuropathic diabetes (32% ± 6%), and the healthy patients for control (40% ± 5%).

The group DI neurovascular response, which depends on the healthy function of the C-fiber nociceptors, was similar at baseline (5% ± 9%) and after surgery (14% ± 10%, measured in volts; Fig 3). Both these responses were similar to those of the patients with neuropathic diabetes (33% ± 21) but were dramatically reduced when compared with the responses of the patients with nonneuropathic diabetes (110% ± 40%; $P < .001$) and the healthy patients for control (198% ± 54%; $P < .001$).

DISCUSSION

In this study, we have shown that successful LEAR has a favorable effect on the skin microcirculation. However, this improvement does not reach the levels seen in healthy subjects, and the postoperative vascular reactivity was similar to the levels that were observed in patients with neuropathic diabetes without any clinical peripheral arterial disease. Finally, the nerve axon reflex–related response that depends on the function of healthy C-fiber nociceptors failed to show any improvement after LEAR.

Atherosclerotic arterial occlusive disease is common in patients with diabetes and has a characteristic peritibial distribution with relative sparing of the foot vessels. The pattern of atherosclerotic occlusive disease seen with diabetes often presents several options with regard to arterial reconstruction. Peripheral arterial disease is the most common indication for surgery in patients with diabetes. Successful LEAR for the treatment of diabetic foot ulceration in the presence of ischemia and neuropathy plays a major role in the healing of ulcers.[2,4] However, some ulcers do not heal or are slow to heal or do recur despite the restoration of maximum perfusion to the foot via bypass grafting surgery. This occurs even in the absence of known factors that prevent or delay wound healing, such as wound infection, poor wound care, and inadequate weight offloading.

The cutaneous microcirculation of the diabetic and neuropathic foot is known to be functionally impaired.[5,7] Our study results have confirmed four abnormalities in the physiology of the microcirculation in the diabetic foot in the presence of ischemia and neuropathy. Thus, the studied patients with neuropathy and peripheral arterial disease showed diminished cutaneous vasodilatory response to heat, acetylcholine (endothelium-dependent vasodilatation), sodium nitroprusside (smooth muscle cell response), and the neurovascular response. The maximal hyperemic response to heat and the endothelium-dependent vasodilatation improved after LEAR but were still less than healthy levels. The same applies to the endothelium-independent vasodilatation, but in this case, the response approached the levels that were seen in patients with nonneuropathic diabetes and did not differ statistically from the response of the healthy patients for control. These results indicate that the restoration of maximum blood flow in the large vessels results in an increased blood flow in the microcirculation. However, a successful bypass grafting procedure cannot reverse the existing endothelial dysfunction that is associated with diabetes, and therefore, the postoperative endothelium-dependent vasodilatation should not be expected to reach healthy levels. It is also of interest that the improvement was more pronounced in the endothelium-independent vasodilatation (response to sodium nitroprusside), which indicated that the smooth muscle cell was still able to vasodilate satisfactorily despite the existence of neuropathy and endothelial dysfunction.

The foot skin microvascular reactivity is reduced when compared with the forearm, not only in patients with diabetes but also in healthy subjects, and this may be a result of the high capillary pressures related to the erect posture.[10,11] In addition, neuropathy also has a detrimental effect on the skin microcirculation.[12-14] Reversal of hypoxia has been shown to halt the progression of diabetic neuropathy but not reverse the neuropathy that is already present.[15,16] Therefore, the results of this study with regards to the nerve axon–related vasodilatation, which did not change after LEAR, are not surprising. Recent study results from our unit have shown that the nerve axon reflex–related vasodilatation can account for as much as one third of the total skin vasodilatation.[17] Inability to restore this vasodilatory response after LEAR may be

responsible for the failure of an existing ulcer to heal that is observed in some patients with neuropathic diabetes.

Although the exact cause of neuropathy with diabetes is not known, the microvascular endothelial and smooth muscle cell vasodilatory response is known to be impaired the most in patients with diabetes with ischemia and neuropathy.[7] Skin biopsy results from the dorsum of the foot of patients with diabetes have also shown a significant decrease in endothelial NO synthase expression (eNOS).[7,18] Although the exact contribution of diabetes and neuropathy to this reduced expression of eNOS is not known, accumulating evidence suggests that this abnormality may play a pivotal role in the impairment of wound healing with diabetes.[19,20] Therefore, it is not surprising that methods that increase NO production or eNOS expression have shown promising results in wound healing in experimental diabetes.[21,22] We believe that the methods we have used in this study can be helpful in the evaluation of potential new treatments in human clinical trials for the assessment of healing of diabetic foot ulcers.

Finally, a word of caution should be added regarding the interpretation of these data. This study was mainly statistically powered for the examination of differences in the vascular reactivity before and after LEAR and for the comparison of the levels that were achieved after surgery with the healthy levels. Therefore, the study lacks the power to compare possible differences between patients with nonischemic and nonneuropathic diabetes and healthy patients for control. We believe that this lack of power does not affect the conclusions regarding the effect of LEAR on microvascular reactivity. In addition, this study was not designed to evaluate whether the inability to vasodilate is related to reduced NO production, increased NO destruction, or inability of the smooth muscle cell to respond to NO. Other investigators have attempted to study these issues further.[5-7,14,17] Because the subjects in the DI group had baseline impairment in both endothelial-dependent and endothelial-independent vasodilatation, by definition the impaired sodium nitroprusside response may have blunted the acetylcholine response. The interpretation of the acetylcholine response in the presence of a defect in the endothelial-independent vasodilatation is potentially difficult. This is one of the weaknesses of the study. However, after revascularization, both responses improved significantly but did not reach levels seen in healthy control subjects, which is of clinical significance.

In summary, in this study, we have shown that impaired vasodilation in the diabetic neuropathic lower extremity leads to functional ischemia, which improves but is not fully corrected with successful lower extremity bypass grafting surgery. Therefore, patients with neuropathic diabetes may still be at high risk for the development of foot ulceration or for failure of an existing ulcer to heal despite adequate correction of large vessel blood flow.

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