Use of Laser Doppler Imaging to Assess Microvascular Response to Thermal Stress

Dixie R. Aragaki, MD, Min-Ning Huang, MD, PhD, Peggy Chen, MD, Peter Yeung, MD, Charles Kunkel, MD, Dorene Opava-Rutter, MD, A.M. Erika Scremin, MD, and Oscar U. Scremin, MD, PhD

Assessment of cutaneous microvascular perfusion can provide important clues about wound and amputation healing in patients with diabetes. These investigators examined the practicality of laser Doppler imaging in this setting.

Diabetes is a major public health problem, especially in the VA, whose patient population has a substantially higher prevalence of the disease (11.4%) compared with the general U.S. population (7.2%).[1] Among the many complications of diabetes, lower extremity amputation is particularly distressing. Diabetes is currently the leading cause of nontraumatic amputation in the United States, and the disease has resulted in about 80,000 lower extremity amputations per year since 1997.[2] Three years after a diabetes-related lower extremity amputation, the mortality rate is between 35% and 50%.[3] Furthermore, since proximal amputation levels demand higher energy expenditure to ambulate with a prosthesis,[4] patients who've had such amputations face limited mobility and independence. This high cost to individual health and functioning is sadly matched by the immense financial cost of associated health care.[5] In 2003, diabetes-related lower extremity amputations accounted for approximately 810,000 hospital days.[2]

Given the scope of this problem, there is an urgent need to further our understanding of the pathophysiology leading to amputations in the diabetic population.[6–8] Microvascular function of the skin—which is important to wound healing, hemostasis, temperature regulation, and immune system modulation—has emerged as a physiologic variable of interest.[9]

The cutaneous vasculature is a fine network of arterioles, capillaries, and venules that is not readily studied by routine ultrasonic Doppler imaging or ankle-brachial index calculations. In the past, methods of estimating skin perfusion included radioisotope studies to record skin perfusion pressure[10] or tissue tracer uptake,[11] segmental systolic blood pressure,[10] arteriography,[12] and capillary microscopy.[13] These methods are time consuming, however, and have varying degrees of reproducibility.[14] In 2000, the Transatlantic Inter-Society Consensus identified transcutaneous PO_2 ($TcPO_2$) measurement, radionuclide scans, laser Doppler methods, and capillary microscopy as useful adjuncts in assessing critical limb ischemia.[15] In recent years, $TcPO_2$ and laser Doppler methods have become widely used to assess skin microperfusion and oxygenation.[16]

Laser Doppler flowmetry (LDF), which can monitor skin blood flow in real time over a small area, has been used in many areas of medicine, including plastic surgery,[17] vascular medicine,[18] burn management,[19] and dermatology.[20] Additionally, some investigators have reported its utility in assessing amputation level and

Dr. Aragaki is the assistant program director for the physical medicine and rehabilitation (PM&R) residency program at the Greater Los Angeles VA Healthcare System (GLAVAHS) and an assistant clinical professor in the department of medicine at the University of California, Los Angeles (UCLA) David Geffen School of Medicine, both in Los Angeles, CA. **Dr. Huang**, **Dr. Chen**, and **Dr. Yeung** are all practicing physiatrists who, at the time of this study, were PM&R residents at the GLAVAHS. **Dr. Kunkel** is the assistant chief of PM&R services at the GLAVAHS and an associate professor in the department of medicine at UCLA David Geffen School of Medicine**. Dr. Opava-Rutter** is the director of PM&R polytrauma at the GLAVAHS. **Dr. A. Scremin** is the chief of PM&R services at the GLAVAHS and a professor and the chair of the PM&R division of the department of medicine at UCLA David Geffen School of Medicine. **Dr. O. Scremin** is a rehabilitation research and development senior research career scientist in the department of research at the GLAVAHS and a professor emeritus in the department of physiology at the UCLA David Geffen School of Medicine.

wound healing in diabetic and nondiabetic patients.[18,21] LDF used in conjunction with thermal stressing (application of controlled heat) has revealed significantly lower perfusion in amputees than in control participants,[22] and it has resulted in better clinical correlation with ischemia severity than either $TcPO_2$ or Doppler ankle pressure measurement.[23]

Scanning laser Doppler imaging (LDI) is a more recently developed technique that provides advantages over the LDF device—primarily the ability to produce a relatively large, two-dimensional image with superior spatial resolution[24] and temporal reproducibility[25,26] without direct skin contact. LDI achieves this by using a continuous raster scanning laser beam across the skin surface. Doppler-shifted light from moving blood is reflected and processed to provide a flux measurement expressed in arbitrary perfusion units (PU) proportional to tissue blood flow.

At the West Los Angeles VA Medical Center (WLAVAMC), the Functional Perfusion Laboratory team is called upon to evaluate patients who are referred from various clinical services, such as the limb preservation and amputee clinics, for diagnosis of microcirculation abnormalities and for guidance regarding wound care, treatment of peripheral vascular disease, and surgical planning. Traditionally, the laboratory team relied upon $TcPO_2$ to assess cutaneous microperfusion after thermal stress in such patients. Recently, however, the staff wondered whether scanning LDI technology, applied in a similar manner, could add valuable information to these assessments.

As a first step in evaluating the practicality and reproducibility of LDI technology in this setting, we undertook a study to increase our understanding of the expected normal and abnormal LDI values and to characterize the physiologic nature of the thermal response. To that end, we analyzed the microvascular response to thermal stress, as measured by scanning LDI, on three cutaneous calf sites of healthy individuals and individuals with peripheral vascular disease and diabetes mellitus (PVD-DM). Specific aims were to identify useful microperfusion parameters that can distinguish between people with and without PVD-DM and to characterize the spatial and temporal course of heat-induced microvascular hyperemic response in normal skin.

METHODS

Participants

For the study, we enrolled 12 healthy individuals (11 men and one woman) aged 28 to 63 years (median, 35 years) and 11 men with PVD-DM aged 50 to 79 years (median, 62 years). Healthy volunteers were recruited for the study through flyers posted at the WLAVAMC. Among volunteers, individuals were excluded if they had known peripheral vascular disease, diabetes, hypercholesterolemia, Raynaud disease, coronary artery disease, cerebral vascular disease, prior amputation, skin diseases, or acute illness; had used tobacco in the previous six months; or were taking medications known to alter vessel reactivity (nitrates).

The participants with PVD-DM had been referred to the Functional Perfusion Laboratory for vascular studies and were considered candidates for lower extremity amputation. Of these 11 participants, 10 underwent an amputation four to 98 days after the LDI study (eight below the knee, one through the knee, and one transmetatarsal).

All participants gave informed consent to participate in the study, which was approved by the VA Greater Los Angeles Institutional Review Board.

Procedure

Participants were placed prone on a hospital bed. Pillows were placed strategically to offer comfort and minimize movement artifacts. Skin temperature was measured with an infrared thermometer at each test site before skin heating. Circular probes that were 19 mm in diameter and contained at their centers a heating element 8 mm in diameter (Transcutaneous CO_2/O_2 Monitor, Novametrix Medical Systems Inc., Wallingford, CT) were applied with a 7-mm wide adhesive tape ribbon, concentric and external to the probe border.

Probes were placed posteriorly at approximately 10 cm (proximal), 15 cm (middle), and 20 cm (distal) below the knee joint line. In order to raise skin temperature to a nonpainful 44°C, the probes were left in place for 15 minutes and then removed. Within one minute of removal, a laser Doppler imager (Moor Instruments, Devon, UK) scanned the three heated sites and adjacent area. In healthy participants only, LDI scanning was repeated at five, 10, 15, 20, 25, and 30 minutes after removal of the heating probes. LDI measurements were not repeated in participants with PVD-DM due to concerns about participants' discomfort.

Data analysis

Regions of interest (ROIs) were mapped onto the LDI photo images by tracing the outer edge of the heating probes. When the probes were removed, these ROIs were superimposed on the serial LDI images to ensure accurate localization of the thermally challenged skin areas.

Mean and peak flux levels were recorded, using the Moor LDI software, within ROIs at the heated sites

and in equivalent, adjacent, nonheated areas. Group averages, differences, and ratios of peak and mean flux were calculated for both heated and nonheated areas. Significance between group mean differences was assessed by analysis of variance, followed by multiple contrasts with the Fisher least significant difference procedure when three means were contrasted (comparisons between the three sites in healthy participants) or by Student *t* tests when means of healthy and PVD-DM populations were compared.

The dependence of flux on distance from the center of the heated area (thermal hyperemia profile) was assessed using an originally developed spatial modeling (SM) program. It involved measuring flux on all pixels within a 1,600-mm^2 region containing the heated area drawn on the LDI flux image. This process was automated by exporting the imager data as a text file and processing it with a script written in MATLAB (MathWorks, Inc.). The values of all points equidistant to the center of the heated area were averaged and displayed as a function of distance from the center at 1-mm intervals. The dissipation of the heat hyperemia with distance was quantified by fitting the values of flux beyond the border of the heating element (F_D) and their distance (D) to the first order rate equation: $F_D = A \bullet e^{-B \bullet D} + C$. In this model, the parameter A represents the difference between flux at the border of the heating element and at the distance farthest away from it, B is the reciprocal of the space constant for flux decay, and C is the asymptotic value of flux away from the heated area. These parameters were calculated by nonlinear regression with a Newton-Gauss algorithm.

RESULTS

The microvascular response: Normal versus ischemic limbs

A sharp increase in blood flow was observed within the skin areas covered by the heating probes in both healthy and PVD-DM groups. This response was more blunted, however, in the PVD-DM subjects. Since there was no significant difference between flux values at the three posterior calf sites in either group, these values were averaged. The postheat mean flux in healthy participants was more than twice that of the participants with PVD-DM (Table). Even in nonheated skin areas, the mean flux was 34% greater in the healthy group than in the PVD-DM group. Flux ratios were calculated by dividing the mean and peak flux values for heated skin regions by values of neighboring nonheated skin areas. These ratios also were greater in the healthy group than the PVD-DM group, which reflects a more vigorous microvascular hyperemic response to heat among the healthy participants.

SM analysis

Images from individual heated sites indicated a significant spatial variability that precluded a simple direct evaluation of flux (Figure 1). In order to provide average flux values while preserving the spatial information, the data were analyzed using the SM approach described earlier. With this technique, we created plots of average flux of all pixels within the image over their Euclidian distance away from the center of the heated area. Within each group of participants, we found no statistically significant differences between the three calf positions studied. Thus, an average of the three decay slopes was used to calculate a single set of equation parameters for each participant. These values were used for comparison between healthy participants and the participants with PVD-DM. Average flux was found to decay exponentially with distance away from the heating element in both healthy and PVD-DM participants, with a space constant of 2.6 mm (Figure 2).

Temporal response in healthy participants

The time course of the blood flow response to topical heat was studied in the healthy participants (Figure 3). Flux levels recorded at one and five minutes after removal of the heating probe were similar, but values beyond five minutes showed slow temporal decay. No significant differences be-

Table. Comparison of heated and nonheated flux values and flux ratios

Group	Mean flux (PU[a])		Ratio of heated to nonheated[b]	
	Heated	Nonheated	Mean flux	Peak flux
Healthy group (SE)	403.2 (49.4)	113.7 (8.6)	4.09 (0.47)	5.08 (0.47)
PVD-DM[c] group (SE)	192.7 (25.4)	84.8 (7.8)	2.24 (0.16)	3.09 (0.26)
P value	< .001	.02	< .001	< .001

[a]PU = perfusion units. [b]A higher ratio reflects a more vigorous microvascular response to heat. [c]PVD-DM = peripheral vascular disease and diabetes mellitus.

tween the three calf positions were found. When the averaged values were analyzed, heated and nonheated areas showed statistically significant differences in peak and mean flux at all time points. Average (SE) mean flux recorded 30 minutes after probe removal was still much higher in the heated areas than in the nonheated areas: 188 (17.8) PU versus 100 (5.9) PU, repectively ($P < .001$). The same was true for average (SE) peak flux, which was 694 (93.6) PU in the heated areas and 230 (17.8) PU in the nonheated areas ($P < .001$).

Investigating possible confounders

Since the two groups of participants had substantially different median ages (35 years for the healthy participants versus 62 for those with PVD-DM), we investigated any possible dependence of flux on age using linear regression analysis. Only the PVD-DM population exhibited a significant trend of decreasing flux with age, and this phenomenon was observed only for the nonheated areas.

Baseline skin surface temperature did not differ between the three calf positions used in healthy participants. The pooled average (SE) of the three sites was 32.7°C (0.16°C) for the healthy participants and 33.1°C (0.27°C) for those with PVD-DM—a nonsignificant difference.

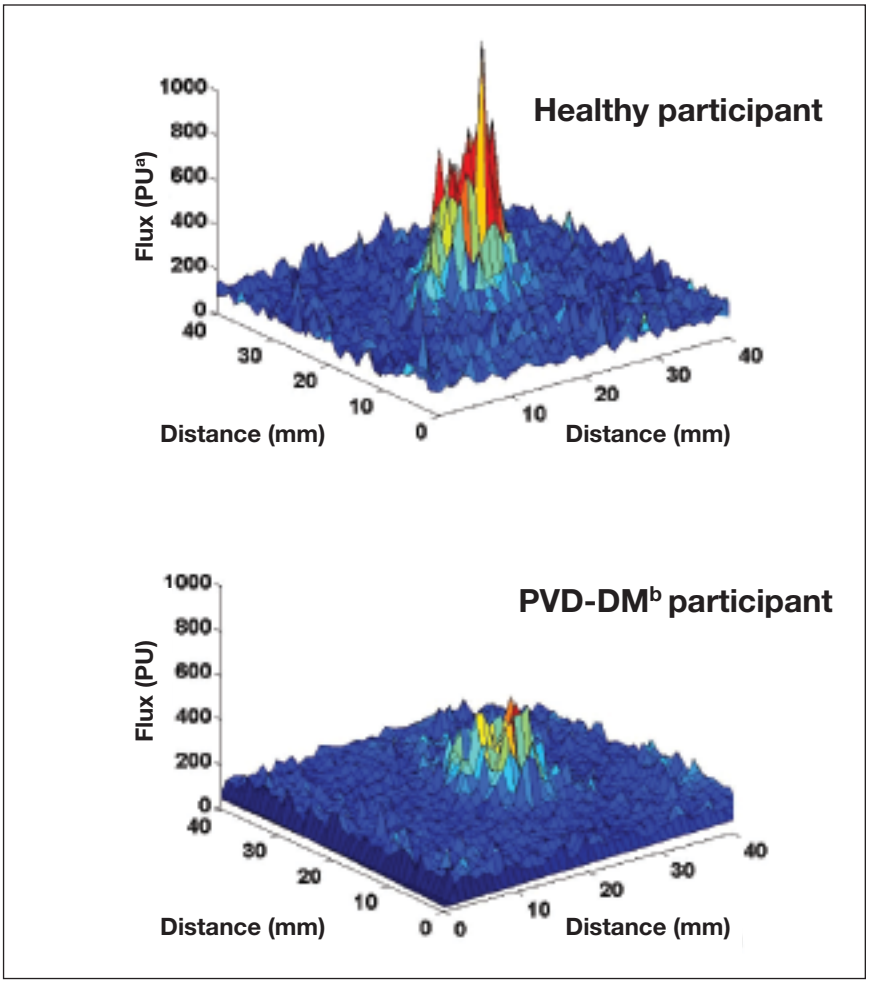

Figure 1. Laser Doppler images of one sample heated region in a sample healthy participant (top) and a sample participant with PVD-DM (bottom). Scanning laser Doppler images from individual heated sites displayed spatial variability and increased flux measured in PU in both healthy participants and those with PVD-DM. Images were analyzed using a spatial modeling technique to calculate a space constant to reflect the decay of hyperemic response over distance from the center of the heating probe. [a]PU = perfusion units. [b]PVD-DM = peripheral vascular disease and diabetes mellitus.

DISCUSSION

Considerations for the use of LDI in diabetic patients

In this study, scanning LDI was able to differentiate skin microperfusion function between groups of healthy participants and participants with PVD-DM. The participants with PVD-DM had significantly lower peak and mean flux values in both heated and nonheated test sites. The greater disparity between the two groups occurred in the heated condition. This finding can be attributed, in part, to the direct effect of diabetes-associated impairment of microvascular perfusion reserve capacity. Such decreases have been reported in diabetic patients without ischemia.[9,27,28] There is also, however, a likely contribution from macrovascular dysfunction, as Arora and colleagues have shown that such deficits improve after successful revascularization.[29]

The unique advantages of scanning LDI allowed us to preserve spatial information and characterize the flux decay with distance from the heated center using a simple method that would be implausible with other known microvascular techniques. The distance over which the hyperemic response tapered was very short

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(with a space constant of 2.6 mm) in both the healthy participants and those with PVD-DM. In practical terms, this means that neighboring test probes could be placed within a centimeter of one another without interfering significantly with true regional microperfusion variability. This may be helpful when assessing patients with multiple skin defects or variable sites of interest in the course of planning amputations, wound care, or other treatment interventions.

Temporal decay of the heat-induced hyperemia was very slow, with greater than 55% of the maximal flux increase still present 30 minutes after probe removal. This finding suggests that LDI is not feasible for making serial images over a short period of time—but would be more suitable for discrete flux measurements.

This phenomenon also suggests that the flux increase is independent of an axonal reflex. The response of skin blood flow to local heat is mediated by different mechanisms than those of thermoregulatory responses to enhanced core temperature.[30] Nociceptive C fibers appear to be involved in the initial phase of heat-induced vasodilation, and it has been proposed that an axonal reflex is responsible for the propagation of the response at a distance from the site of thermal challenge.[31] The sustained hyperemic level of greater than 15 minutes we observed, however, suggests other mechanisms.[32,33] Locally released nitric oxide may play a role, as nitric oxide synthase inhibitors have been shown to attenuate late hyperemia.[32] Other vasoactive mechanisms probably are involved as well,[34] since antidromic vasodilation is known to decay with a half-life of one to three minutes once stimulation is discontinued.[35]

At present, the ability to base clinical recommendations upon LDI findings is hampered by a lack of data. To date, wound healing and amputation outcomes have been better correlated with $TcPO_2$ cut-off values than LDI results, and the 2000 report of the Transatlantic Inter-Society Consensus included diagnostic guidelines only for $TcPO_2$ measurements. For this reason, while our Functional Perfusion Laboratory currently uses both $TcPO_2$ and scanning LDI to assess cutaneous microvascular perfusion after thermal stress, we report diagnostic impressions and predictions based on $TcPO_2$ findings. LDI data, which are collected after the $TcPO_2$ measurements at the same limb sites of interest, are included in the report with less emphasis on the predictive value. Given the advantages LDI offers, however, we hope that future research will provide clinical correlations with specific flux values to serve as additional guidelines in our reports.

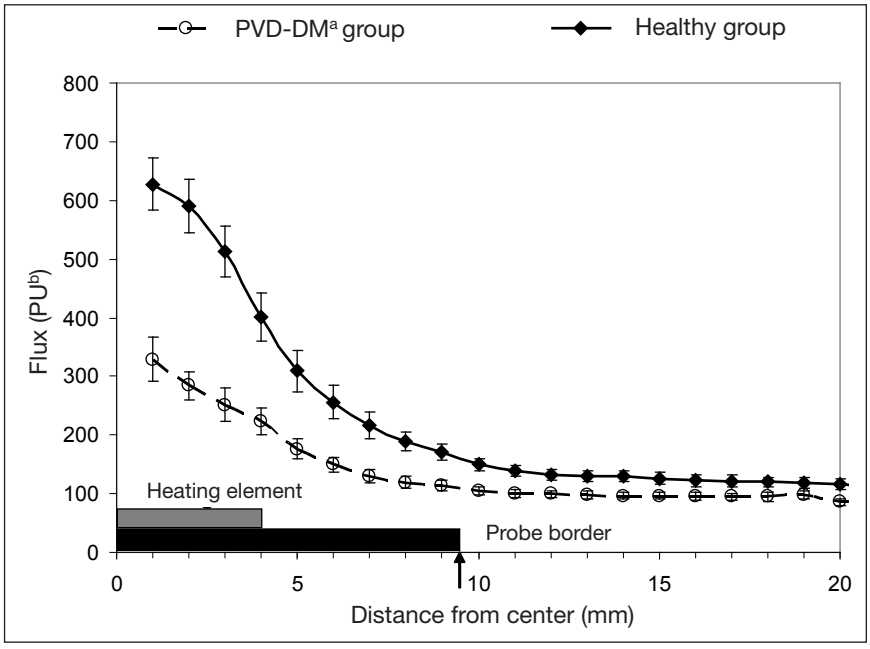

Figure 2. Spatial decay of heat-induced flux response for healthy and PVD-DM groups recorded one minute after removal of heat probes. Average flux values were significantly greater in healthy participants than in those with PVD-DM at all points measured. [a]PVD-DM = peripheral vascular disease and diabetes mellitus. [b]PU = perfusion units.

Is age a factor?

The dependence of heat-induced hyperemia on age has been studied by several authors.[36–38] Evans and colleagues found that hyperemia decreased with age in certain skin areas when comparing a group of young individuals (mean age, 21 years) with an older group (mean age, 76 years).[37] Minson and colleagues found that the response in a group of participants aged 69 to 84 years was about 10% lower than that of a group aged 18 to 24 years.[38] The lack of age-related effects observed in our study could be due to the limited age range (27 to 63 years) and the small number of study participants.

IN SUMMARY

Scanning LDI is a simple, noninvasive tool that can be used to detect abnormal microvascular function in diabetic patients with macrovascu-

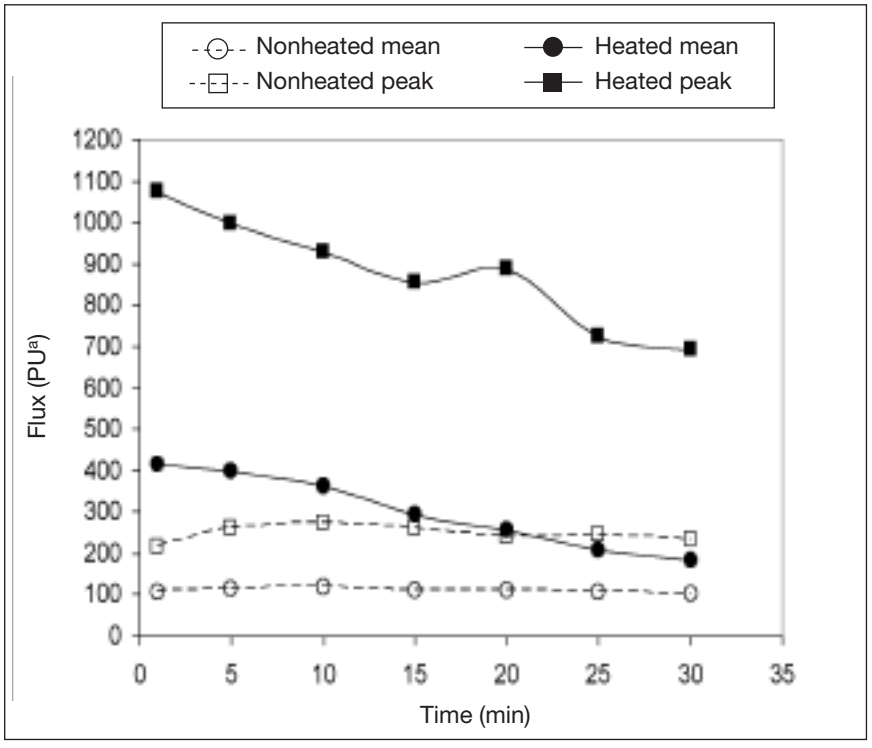

Figure 3. Temporal decay of flux in healthy participants. More than 55% of the maximal heat-induced flux increase was still present at 30 minutes. Differences between heated and nonheated areas were statistically significant at all points in time. [a]PU = perfusion units.

lar disease who are at high risk for amputation. The lower peak and mean flux in the PVD-DM group was evident in both the thermally stressed and baseline skin regions in comparison to healthy control participants. We found mean flux values and heated/nonheated flux ratios to be meaningful parameters for distinguishing normal from ischemic lower limb microperfusion. The sharp spatial decay of heat-induced hyperemia justifies close placement of test probes without risk of interference when attempting to quantify regional skin perfusion deficits. Temporal dissipation of the normal hyperemic response is too slow to be explained solely by an axonal reflex and makes the technique better suited for distinct "snapshot" flux assessments, rather than serial measurements within a short timeframe. Further study of the correlation between maximal microvascular reserve capacity (in terms of flux ratios) and clinical outcomes of diabetes-related lower extremity amputations may help establish better guidelines for treatment intervention and prediction of wound healing for specific amputation levels.

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Author disclosures

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REFERENCES

1. Miller DR, Safford MM, Pogach LM. Who has diabetes? Best estimates of diabetes prevalence in the Department of Veterans Affairs based on computerized patient data. *Diabetes Care*. 2004;27(suppl 2): B10–B21.
2. Diabetes data and trends: Hospitalization. CDC web site. http://www.cdc.gov/diabetes/statistics/hospitalization_national.htm. Accessed May 21, 2008.
3. Reiber GE, Boyko EJ, Smith DG. Chapter 18. Lower extremity foot ulcers and amputations in diabetes. In: *Diabetes in America*. 2nd ed. Bethesda, MD: National Institutes of Health; 1995:409–428. http://diabetes.niddk.nih.gov/dm/pubs/america/pdf/chapter18.pdf. Accessed May 21, 2008.
4. Gonzalez EG, Corcoran PJ, Reyes RL. Energy expenditure in below-knee amputees: Correlation with stump length. *Arch Phys Med Rehab*. 1974;55(3):111–119.
5. Harrington C, Zagari MJ, Corea J, Klitenic J. A cost analysis of diabetic lower-extremity ulcers. *Diabetes Care*. 2000;23(9):1333–1338.
6. Pecoraro RE, Reiber GE, Burgess EM. Pathways to diabetic limb amputation. Basis for prevention. *Diabetes Care*. 1990;13(5):513–521.
7. Humphrey LL, Palumbo PJ, Butters MA, et al. The contribution of non–insulin-dependent diabetes to lower-extremity amputation in the community. *Arch Intern Med*. 1994;154(8):885–892.
8. Williams DT, Harding KG, Price P. An evaluation of the efficacy of methods used in screening for lower-limb arterial disease in diabetes. *Diabetes Care*. 2005;28(9):2206–2210.
9. Mayrovitz HN, Larsen PB. Functional microcirculatory impairment: A possible source of reduced skin oxygen tension in human diabetes mellitus. *Microvasc Res*. 1996;52(2):115–126.
10. Lassen NA, Holstein P. Use of radioisotopes in assessment of distal blood flow and distal blood pressure in arterial insufficiency. *Surg Clin North Am*. 1974;54(1):39–55.
11. Ohta T. Noninvasive technique using thallium-201 for predicting ischaemic ulcer healing of the foot. *Br J Surg*. 1985;72(11):892–895.
12. Beard JD, Scott DJ, Evans JM, Skidmore R, Horrocks M. Pulse-generated runoff: A new method of determining calf vessel patency. *Br J Surg*. 1988;75(4):361–363.
13. Gschwandtner ME, Ambrózy E, Schneider B, Fasching S, Willfort A, Ehringer H. Laser Doppler imaging and capillary microscopy in ischemic ulcers.

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Atherosclerosis. 1999;142(1):225–232.
14. Ubbink DT, Jacobs MJ, Tangelder GJ, Slaaf DW, Reneman RS. The usefulness of capillary microscopy, transcutaneous oximetry and laser Doppler fluxmetry in the assessment of the severity of lower limb ischaemia. *Int J Microcirc Clin Exp*. 1994;14(1-2):34–44
15. Management of peripheral arterial disease (PAD). TransAtlantic Inter-Society Consensus (TASC). Section D: Chronic critical limb ischaemia. *Eur J Vasc Endovasc Surg*. 2000;19(suppl A):S144–S243.
16. Krishnan ST, Baker NR, Carrington AL, Rayman G. Comparative roles of microvascular and nerve function in foot ulceration in type 2 diabetes. *Diabetes Care*. 2004;27(12):1343–1348.
17. Heller L, Levin LS, Klitzman B. Laser Doppler flowmeter monitoring of free-tissue transfers: Blood flow in normal and complicated cases. *Plast Reconstr Surg*. 2001;107(7):1739–1745.
18. Gebuhr P, Jørgensen JP, Vollmer-Larsen B, Nielsen SL, Alsbjørn B. Estimation of amputation level with a laser Doppler flowmeter. *J Bone Joint Surg Br*. 1989;71(3):514–517.
19. Barachini P, Vezzoni GM, Palombo C, Franzoni F, Bigalli G. Skin blood flow pattern in burns outcomes. *Burns*. 2004;30(4):312–316.
20. Choi CM, Bennett RG. Laser Dopplers to determine cutaneous blood flow. *Dermatol Surg*. 2003;29(3):272–280.
21. Karanfilian RG, Lynch TG, Zirul VT, Padberg FT, Jamil Z, Hobson RW II. The value of laser Doppler velocimetry and transcutaneous oxygen tension determination in predicting healing of ischemic forefoot ulcerations and amputations in diabetic and nondiabetic patients. *J Vasc Surg*. 1986;4(5):511–516.
22. Fairs SL, Ham RO, Conway BA, Roberts VC. Limb perfusion in the lower limb amputee—A comparative study using a laser Doppler flowmeter and a transcutaneous oxygen electrode. *Prosthet Orthot Int*. 1987;11(2):80–84.
23. Lantsberg L, Goldman M. Laser Doppler flowmetry, transcutaneous oxygen tension measurements and Doppler pressure compared in patients undergoing amputation. *Eur J Vasc Surg*. 1991;5(2):195–197.
24. Essex TJ, Byrne PO. A laser Doppler scanner for imaging blood flow in skin. *J Biomed Eng*. 1991;13(3):189–194.
25. Stücker M, Heese A, Hoffmann K, Röchling A, Altmeyer P. Precision of laser Doppler scanning in clinical use. *Clin Exp Dermatol*. 1995;20(5):371–376.
26. Kubli S, Waeber B, Dalle-Ave A, Feihl F. Reproducibility of laser Doppler imaging of skin blood flow as a tool to assess endothelial function. *J Cardiovasc Pharmacol*. 2000;36(5):640–648.
27. Khan F, Elhadd TA, Greene SA, Belch JJ. Impaired skin microvascular function in children, adolescents, and young adults with type 1 diabetes. *Diabetes Care*. 2000;23(2):215–220.
28. Jaap AJ, Shore AC, Tooke JE. Relationship of insulin resistance to microvascular dysfunction in subjects with fasting hyperglycaemia. *Diabetologia*. 1997;40(2):238–243.
29. Arora S, Pomposelli F, LoGerfo FW, Veves A. Cutaneous microcirculation in the neuropathic diabetic foot improves significantly but not completely after successful lower extremity revascularization. *J Vasc Surg*. 2002;35(3):501–505.
30. Kellogg DL, Pérgola PE, Piest KL, et al. Cutaneous active vasodilation in humans is mediated by cholinergic nerve cotransmission. *Circ Res*. 1995;77(6):1222–1228.
31. Magerl W, Treede RD. Heat-evoked vasodilatation in human hairy skin: Axon reflexes due to low-level activity of nociceptive afferents. *J Physiol*. 1996;497(pt 3):837–848.
32. Minson CT, Berry LT, Joyner MJ. Nitric oxide and neurally mediated regulation of skin blood flow during local heating. *J Appl Physiol*. 2001;91(4):1619–1626.
33. Golay S, Haeberli C, Delachaux A, et al. Local heating of human skin causes hyperemia without mediation by muscarinic cholinergic receptors or prostanoids. *J Appl Physiol*. 2004;97(5):1781–1786.
34. Vinik AI, Erbas T, Park TS, Stansberry KB, Scanelli JA, Pittenger GL. Dermal neurovascular dysfunction in type 2 diabetes. *Diabetes Care*. 2001;24(8):1468–1475.
35. Häbler HJ, Wasner G, Jänig W. Interaction of sympathetic vasoconstriction and antidromic vasodilatation in the control of skin blood flow. *Exp Brain Res*. 1997;113(3):402–410.
36. Algotsson A, Nordberg A, Winblad B. Influence of age and gender on skin vessel reactivity to endothelium-dependent and endothelium-independent vasodilators tested with iontophoresis and a laser Doppler perfusion imager. *J Gerontol Series A Biol Sci Med Sci*. 1995;50(2):M121–M127.
37. Evans E, Rendell M, Bartek J, et al. Thermally-induced cutaneous vasodilatation in aging. *J Gerontol*. 1993;48(2):M53–M57.
38. Minson CT, Holowatz LA, Wong BJ, Kenney WL, Wilkins BW. Decreased nitric oxide- and axon reflex-mediated cutaneous vasodilation with age during local heating. *J Appl Physiol*. 2002; 93(5):1644–1649.