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Standard and Near-Surface Laser-Doppler Perfusion in Foot Dorsum Skin of Diabetic and Nondiabetic Subjects with and without Coexisting Peripheral Arterial Disease

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Appropriate assessment of microvascular function is now recognized as an important adjunct to the diagnostic workup and medical follow-up for a variety of conditions. Laser Doppler fluxmetry (LDF)-derived rbc perfusion (Q) and the volume (V) and velocity (U) components are useful in this regard but the fact that the sampled volume includes both nutritional and nonnutritional components may limit its specificity and range of usefulness. It was reasoned that if the depth of penetration could be reduced without significantly altering essential optical transmission features, then the detected signal would better represent the nutritional component. A 0.68-mm-thick Delrin spacer was fabricated and used to compare LDF values with (WITH) and without (WITHOUT) its use on the foot dorsum of 71 limbs of 44 diabetic (DM) and nondiabetic (NO-DM) subjects with lower extremity arterial disease (LEAD, $n = 39$) and without disease (NORM, $n = 32$). Overall LDF values WITH as compared to WITHOUT had a slightly greater U (1.01 vs 0.89 mm/sec, $P < 0.01$) and much lower V (0.06 vs 0.63%, $P < 0.001$) and Q (0.25 vs 1.88 ml/min/100 g, $P < 0.001$). In NO-DM subjects, WITH detected a lower Q in limbs with LEAD (0.14 vs 0.27, $P < 0.05$) but WITHOUT did not (1.48 vs 1.47, ns). In DM subjects, WITH measured a significantly lower U in LEAD limbs (1.05 vs 1.22 mm/sec, $P < 0.05$), which was not detected WITHOUT. Without the spacer, NORM limb LDF values were all greater in DM vs NO-DM subjects. With spacer use, only the DM velocity component was significantly greater. Use of a modified LDF procedure has shown both utility and promise as a method for evaluation of skin microcirculation and appears to offer some potential benefits as compared with the currently used standard method. Previously undocumented differences between LEAD and NORM limbs in DM and NO-DM patients as herein reported represent initial findings using a 0.68-mm spacer.

INTRODUCTION

Increased recognition that alterations in the microvasculature can importantly impact the etiology and outcome of vascular disease processes and complications has led to intensified efforts to deal with these at the clinical level. Laser Doppler fluxmetry (LDF) is a method for the evaluation of skin red blood cell perfusion (Bonner and Nossal, 1980; Nilsson and Tenland, 1980), which can be a useful complement to other diagnostic procedures in the evaluation of certain features in patients (Fronek, 1993). These include those with diabetes (Stevens *et al.*, 1991; Belcaro *et al.*, 1989; Belcaro and Nicolaides, 1991; Rendell *et al.*, 1989; Tooke

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et al., 1987), lower extremity arterial disease (LEAD) (Ubbink *et al.*, 1992, 1993; Schmidt *et al.*, 1993; Hoffmann *et al.*, 1993; Ranft *et al.*, 1986; Van den Brande, 1988), and skin ulcers (Kristensen *et al.*, 1986; Mayrovitz and Larsen, 1994). It can also be used as an aid to assess effects on skin perfusion (Mayrovitz *et al.*, 1993; Mayrovitz and Larsen, 1992) and ulcer healing likelihood (Holloway and Burgess, 1983; Karanfilian *et al.*, 1986; Belcaro and Nicolaides, 1993; Gebuhr *et al.*, 1989).

Clinical uses of laser Doppler are in the process of evolving, with ongoing efforts attempting to pinpoint which parameters and test procedures best discriminate between normal and abnormal, which can stratify among levels of abnormality, which can be used singly or in combination diagnostically, which can best monitor the effectiveness of therapeutic interventions, and which can best aid in the choice of medical management. One of the drawbacks of standard LDF when applied in the clinical setting is that the signal represents the composite of photon scattering events of an admixture of skin nutritional and nonnutritional red blood cell (rbc) movements. When it is the quality of the nutrient rbc perfusion (Q) which carries the necessary clinically important diagnostic information, then the standard basal skin measurement is useful only when the standardly measured perfusion (QQ) is directly related to Q. Unfortunately, there is usually no a priori knowledge that this is true in any specific patient or disease class.

It was reasoned that if the laser probe was elevated from the skin surface without altering the essential optical transmission features, then the resultant detected signal would be more representative of the nutritional component. Such a "spacer" was fabricated and used between the probe and the skin to compare the LDF values obtained with and without its use on the foot dorsum of patients with and without lower extremity arterial disease. The present report documents the initial findings as measured in a total of 71 limbs of 44 subjects.

METHODS

Spacer

The premise for spacer use is that if the penetration depth of the laser could be sufficiently diminished so as to substantially exclude the deeper vasculature, then the resultant perfusion would more closely reflect events in the nutritional capillary network. To this end a cup-like adapter to accommodate the laser probe was machined to have a bottom surface thickness of 0.68 mm. The material used was a plastic, white Delrin, which at the wavelength of the laser has optical transmission, absorption, and reflectance properties similar to skin. Thus, the bottom surface simulates skin devoid of circulation and effectively diminishes the depth of penetration without alternating the laser Doppler operational features. This same material is used to obtain zero calibration levels by the manufacturer of the laser Doppler system. The spacer weight is only 3.4 g, and the combined probe–spacer weight is less than the routinely used thermal probe system.

Laser Doppler

With the subject resting in a supine position, a 19-mm-diameter laser probe alone (Model P-430, Vasamedics) and the probe affixed to the spacer were se-

quentially placed at a standardized site on the distal medial dorsum with double-sided electrode tape on 71 legs of 44 subjects. The choice of which to apply first was made on a random basis. When measurements were taken on both limbs (27 subjects) the order for the second limb was reversed. Once the probe or probe plus spacer was affixed to the skin, the LDF perfusion was monitored, and when any transient effects due to probe placement had normalized (typically 1–2 min and never more than 3 min), data recording was initated for an interval of 6 min. At the end of this interval the remaining probe combination replaced the initial one and the cycle was repeated.

The laser Doppler data were obtained using Model BPM2 (Vasamedics), which provides seperate outputs for rbc velocity (U), volume (V), and perfusion (Q). This particular instrument uses conversion factors based on correlative data obtained in other tissues such that U, V, and Q are expressed in units of mm/sec, percentage, and ml/min/100 g, respectively. Velocity is expressed in mm/sec using an internal conversion factor of 2.2 kHz/mm sec^{-1}. Volume is expressed as the ratio of moving rbc volume to tissue volume in percentage by multiplying the detected mean number of Doppler events per photon by the factor 7.5. Results of the present study are, as previously reported (Mayrovitz and Regan, 1993), in the units expressed by this instrumentation with the caution that estimates of true flow are ambiguous with laser Doppler methods (Mayrovitz, 1994) and the cited conversion factors have not yet been fully validated. However, use of these machine units is at least as good as using "relative units." All data were acquired directly by computer for postprocessing and simultaneously displayed on a monitor and chart paper (Gould, WindoGraf). For purposes of reference, values obtained with the spacer are referred to as U, V, and Q and corresponding values without the spacer (standard LDF measurement) as UU, VV, and QQ. For each measurement the time average value over the 6-min sampling interval is used. Skin temperature at the measurement site was recorded (Bailey, BAT8, Saddle Brook, NJ) each minute during the LDF data acquisition and the average used to characterize the dorsum skin temperature T_{skin}.

Transcutaneous Oxygen Tension

Transcutaneous oxygen tension ($TcPO_2$) was measured (Novametrix Model 811) at a site near the LDF measurement at a probe temperature setting of 45°. Values obtained at steady state (20 min) were used.

Leg Blood Flow

Leg blood flow was measured using magnetic resonance flowmetry (Metriflow, AFM100) at five below-knee sites corresponding to 10, 25, 50, 75, and 90% of the malleolar-knee length as measured proximal to the medial malleoulus. Principles of operation, validation studies, and applications of this method have been reported (Battocletti, 1986; Kerr *et al.*, 1991). Blood flow data are obtained on a beat-by-beat basis and an ensemble average of an operator selected number of beats is automatically determined. Thirty beats were used in the present study. Flow is determined in absolute units (ml/min) and subsequently calculated as blood perfusion expressed as ml/min/100 cc of distal tissue based on leg circumference and foot size measurements and use of an extensively tested algorithm used by the instrumentation. In addition, an arterial status index (ASI) is derivable

as the ratio of the flow pulse half height to the pulse width at half height. This index has been shown to discriminate between the presence and the absence of LEAD (Rice *et al.*, 1994).

ABI

Brachial and ankle systolic pressures were determined using Doppler ultrasound, and the ankle/brachial index (ABI) was determined.

Subjects and Groupings

The protocol used was approved by the institutional review board and all participating subjects received and signed an informed consent. A total of 44 subjects were evaluated. Subjects with diabetes mellitus (DM, $N = 21$) had been diagnosed at least 5 years prior to evaluation and were all under treatment. Peripheral neuropathy was present in 17 of the DM subjects. Subjects free of diabetes (NO-DM, $N = 23$) either had symptoms of intermittent claudication ($n = 11$) or were free of symptoms ($n = 12$). No subject had rest pain. A total number of 71 limbs were evaluated (ALL), 33 in subjects with DM and 38 in subjects with NO-DM. Limbs were classified as having LEAD based on the limb average ASI value; values <5.0 were so classified ($n = 39$) whereas ASI values ≥ 5.0 were classified as normal (NORM, $n = 32$). These initial classifications were made independently of whether diabetes or symptoms were present. Limbs classified as NORM included 12 from DM subjects and 20 from NO-DM subjects. Limbs classified as LEAD included 21 from DM subjects and 18 from NO-DM subjects.

Statistics

Analyses of differences between paired values obtained with and without the spacer were carried out using the nonparametric Wilcoxon test with a P level <0.05 being considered statistically significant. All other comparisons were based on the nonparametric Mann–Whitney test with a P level <0.05 being considered statistically significant.

RESULTS

Overall Comparison of Spacer and Standard LDF Values

Table 1 summarizes the LDF data as obtained from ALL legs and for the subset groupings described under Methods. Overall, and for each subset, the values obtained with the spacer for almost all of the LDF parameters differed significantly from the corresponding values obtained using the standard method. With the spacer, the velocity component was somewhat greater, whereas the volume component and the net perfusion were much less.

Comparisons Among Subgroups

Table 2 summarizes and compares the supplementary and LDF data for NORM and LEAD limbs within DM and NO-DM patients and between DM and NO-DM patients for corresponding limb classifications.

Supplementary parameters. In patients with or without DM, the limbs classified

TABLE 1
COMPARISON OF FOOT DORSUM LDF VALUES WITH AND WITHOUT SPACER

Grouping	Volume (%)			Velocity (mm/sec)			Perfusion (ml/min/100 g)		
	V	*VV*	*V/VV*	*U*	*UU*	*U/UU*	*Q*	*QQ*	*Q/QQ*
ALL	0.063	0.633[c]	0.12	1.01	0.89[b]	1.19	0.251	1.88[c]	0.16
(n = 71)	(0.043)	(0.339)	(0.10)	(0.31)	(0.27)	(0.37)	(0.190)	(1.21)	(0.11)
NORM	0.081	0.702[c]	0.14	1.05	0.89[b]	1.22	0.323	2.17[c]	0.18
(n = 32)	(0.045)	(0.369)	(0.12)	(0.29)	(0.25)	(0.31)	(0.218)	(1.49)	(0.12)
LEAD	0.049	0.576[c]	0.10	0.98	0.89[a]	1.17	0.193	1.65[c]	0.13
(n = 39)	(0.035)	(0.306)	(0.09)	(0.32)	(0.28)	(0.42)	(0.141)	(0.88)	(0.09)
NO-DM	0.059	0.569[c]	0.12	0.93	0.79[b]	1.23	0.209	1.47[c]	0.16
(n = 38)	(0.045)	(0.262)	(0.11)	(0.19)	(0.20)	(0.35)	(0.155)	(0.82)	(0.15)
DM	0.068	0.716[c]	0.12	1.11	1.00	1.14	0.300	2.36[c]	0.15
(n = 33)	(0.041)	(0.399)	(0.10)	(0.38)	(0.29)	(0.39)	(0.216)	(1.42)	(0.10)

Note. Values are mean and (sd), n = number of legs. [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ vs value with spacer. Single-letter parameters are with spacer.

TABLE 2
PARAMETER COMPARISONS

Parameter	Patients with diabetes		Patients without diabetes	
	NORM	LEAD	NORM	LEAD
n (legs):	12	21	20	18
	Supplementary data			
ABI (mm Hg/mm Hg)	1.15	0.83[b]	1.09	0.77[c]
	(0.14)	(0.29)	(0.16)	(0.16)
TcP02 (mm Hg)	58.0	57.0	67.0	49.1[c]
	(10.8)	(13.1)	(12.3)	(09.0)
Skin temp (°C)	31.4	29.9[a]	30.7	28.5[a]
	(1.60)	(1.20)	(02.0)	(02.3)
Foot perfusion	2.30	1.20[c,*]	1.87	0.87[c]
(ml/min/100 cc)	(0.60)	(0.50)	(0.49)	(0.87)
	LDF data			
Q (ml/min/100 g)	0.418	0.233[b]	0.266	0.146[a]
	(0.245)	(0.170)	0.184)	(0.079)
V (%)	0.091	0.055[b]	0.075	0.041[a]
	(0.036)	(0.039)	(0.050)	(0.030)
U (mm/sec)	1.22*	1.05[a]	0.95	0.90
	(0.33)	(0.40)	(0.21)	(0.16)
QQ (ml/min/100 g)	3.34**	1.80[b]	1.47	1.48
	(1.55)	(1.00)	(0.93)	(0.71)
VV (%)	0.91*	0.60[a]	0.57	0.54
	(0.36)	(0.38)	(0.32)	(0.18)
UU (mm/sec)	1.08**	0.96	0.78	0.81
	(0.27)	0.30)	(0.16)	(0.25)
Q/QQ	0.15	0.15	0.20	0.10[b]
	(0.12)	(0.11)	(0.13)	(0.07)
V/VV	0.13	0.12	0.15	0.07[b]
	(0.11)	(0.11)	(0.13)	(0.05)
U/UU	1.14	1.15	1.26	1.20
	(0.21)	(0.25)	(0.35)	(0.36)

Note. Values are mean and (sd). [a]$P < 0.05$. [b]$P < 0.01$. [c]$P < 0.001$ for LEAD vs NORM within patient groups, *$P < 0.05$, **$P < 0.01$ for DM vs NO-DM. Single-letter LDF parameters are with spacer. NORM = ASI $\geq$ 5.0, LEAD = ASI < 5.0.

as LEAD based on the leg average ASI value had significantly lower ABI, dorsum skin temperature, and leg perfusion than did the NORM limbs. The perfusion value in the table is that measured at the 10% site and approximates the arterial inflow to the foot. Dorsum TcPO_2 differences were dependent on patient status. Thus, in patients with NO-DM, the dorsum TcPO_2 was significantly lower on the LEAD limb but for DM patients TcPO_2 values were nearly identical for LEAD and NORM limbs. Comparison between DM and NO-DM shows that legs with a normal ASI are not significantly different in any supplementary parameter, but the diabetic limbs tended to have a lower TcPO_2 and greater perfusion. Similar tendencies were present for the limbs classified as LEAD, for which the perfusion of the diabetic limb was found to be significantly higher than in LEAD limbs of the NO-DM patients.

LDF parameters. The dorsum perfusion, as measured with the spacer, revealed significantly lower values in the LEAD foot of both DM and NO-DM patients, but only in the DM patients was this difference detected using the standard LDF method. The decreased perfusion in the LEAD limb was attributable to a decrease in the laser Doppler measured volume component in both DM and NO-DM subjects, whereas the velocity component was also reduced in the LEAD limb of the DM subjects. This reduced velocity was not detected without the spacer use. In the NO-DM patients, but not in the DM patients, the ratio of spacer perfusion to standard perfusion was found to be significantly decreased in the LEAD limb.

With spacer use, comparisons of corresponding limb-types between the DM and the NO-DM subjects show that except for a larger velocity component in the NORM limb of DM subjects, no significant differences in the remaining parameters are present. There is, however, a tendency for a larger perfusion in the NORM limb of DM limbs. Without the spacer, all NORM limb LDF parameters of the DM subjects were significantly greater than those measured on the NORM limb of the NO-DM subjects.

DISCUSSION

Appropriate assessment of microvascular function is now recognized as an important adjunct to the diagnostic workup and medical follow-up for a variety of conditions. Although laser Doppler-derived skin blood perfusion has shown itself to be useful in this regard, the fact that the sampled volume includes both nutritional and nonnutritional components may limit its specificity and range of usefulness. The purpose of this study was to compare LDF parameters as determined using a simple modification to those obtained with the standard technique. It was reasoned that if the laser probe was elevated from the skin surface without altering the essential optical transmission features, then the resultant detected signal would be more representative of the nutritional component. Such a spacer was fabricated and used to compare the LDF values obtained with and without its use on the foot dorsum of diabetic and nondiabetic patients with and without lower extremity arterial disease.

All data with spacer use were obtained with a spacer thickness of 0.68 mm. This thickness was chosen somewhat empirically as an initial value in part based on considerations of what is known concerning laser Doppler penetration, but it is not claimed to be optimum. Using a model optically similar to human skin, it was shown that the maximum laser Doppler sensitivity is recorded at a depth of about 0.6 mm, below which the sensitivity falls off in an exponential manner (Nilsson and Tenland, 1980) with sensitivities of about half maximum recorded at depths of about 0.2 and 1.2 mm. In general, however, the depth of maximum sensitivity and its pattern are dependent on the wavelength of laser light used, the diameter of transmitting and receiving fibers, and the separation distance between these. *In vitro* measurements of human skin indicate that red light transmission is exponential, with an effective penetration of about 0.6 mm (Bachem and Reed, 1931; Hardy *et al.,* 1956). The question of the precise penetration depth remains elusive and is an ongoing research issue not yet adequately resolved, although new approaches help to clarify the issue (Jakobsson and Nilsson, 1993).

The present findings provide initial data on the possible utility of the modified

LDF method and also shed some new light on the interpretation of the laser Doppler-derived parameters. One important issue to consider is the relative values of the two components of the laser Doppler signal as obtained with and without the spacer.

Volume Component

An estimate of the "expected" volume component for selective nutritional capillary sampling can be made using human dermal papillary loop length and diameter data (Braverman and Yen, 1977), which yields a loop total volume of 2.3×10^{-8} cm^3; multiplication by capillary hematocrit (0.25) (Fagrell *et al.*, 1980) and density estimates (45–65 loops/mm^2) (Franzec *et al.*, 1984) yields a moving rbc volume/tissue volume ranging from 0.026 to 0.037%. The mean overall value herein measured was 0.06% and was as high as 0.08% in the NORM subset and as low as 0.05% in the LEAD subset (Table 1). This may indicate that even with spacer use, vessels in addition to the capillary loops are being sampled. Use of a slightly thicker spacer may improve this situation if this is the reason. It is also possible that the estimate of capillary hematocrit and/or density was low, causing a low estimate of the expected volume component. In any case, the data show that without the spacer the measured volume component is almost an order of magnitude greater than would be expected based on capillary sampling, whereas with its use the values are at worst close to the expected values.

Other evidence of the utility of the spacer is that only with its use could a lower volume component in the foot of nondiabetic patients with LEAD be detected. This may be explained as a masking effect of the volume deficit when the standard method is used. Based on the previous calculation, the nutritional volume component is small with respect to the remainder of the sampled volume and thus capillary dropout due to decreased perfusion pressure would not be detectable with the standard method.

In diabetic patients, the volume components of LEAD limbs were significantly less than NORM limb values, with and without the spacer. With the spacer, the detected difference probably reflects a true decrease in the LEAD limb; without the spacer the detected difference may be due to the elevated NORM value.

Velocity Component

Overall measured mean velocity was close to 1 mm/sec both with and without the spacer. However, the value obtained with the spacer was statistically greater than without its use (Table 1). This may indicate that the velocity distribution in near-surface vessels is greater than that of those at greater depth. Exclusion of venules with lower rbc velocites from the sampling volume might account for the consistently found higher velocities when the spacer was used, but direct evidence of this is lacking. Interestingly, the velocity component was not useful by itself in detecting differences between LEAD and NORM limbs in either of the patient groupings.

Patient Group Comparisons

In patients without diabetes, limbs classified as having LEAD based on ASI stratification have reduced values of most perfusion indices including ABI, TcPO2, skin temperature, and leg blood perfusion at the 10% site. With spacer use, the

resting dorsum skin LDF perfusion was also significantly reduced but the standard LDF measurement indicated no difference. Use of resting LDF values without additional vascular provocations as a means to detect the presence of LEAD other than in cases of resting ischemia have had mixed success. Contributing to the limited success are probably the intrinsic spatial variability (Tenland *et al.*, 1983; Mayrovitz, 1992) and the likelihood that unless significant disease (resting symptoms) is present, skin perfusion decrements would be below detection by the standard method. The present results suggest that when resting LDF perfusion is restricted to include a significantly lesser contribution of the nonnutritive circulation, these limitations may be overcome.

In patients with diabetes, as with patients without diabetes, most perfusion indices were found to be lower in the LEAD limb. However, no detectable difference in TcPO2 was present although they tended to be less than those measured in normal limbs of NO-DM patients. Spacer use in the DM patients increased the discrimination between NORM and LEAD limbs slightly above that obtained with the standard method in that a decreased velocity was detected only with spacer use.

In summary, use of a modified LDF procedure has shown both utility and promise as a method for evaluation of skin microcirculation and appears to offer a number of potential benefits as compared with the currently used standard method. Previously undocumented differences between LEAD and NORM limbs in DM and NO-DM patients as herein described represent the initial findings using a 0.68-mm spacer. This may not be the optimum configuration for microperfusion discrimination, and further work is needed using different spacer thicknesses.

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