Impaired Peripheral Vasomotion in Diabetes

KEVIN B. STANSBERRY, BS
SHANE A. SHAPIRO, BS
MICHAEL A. HILL, PHD
PATRICIA M. MCNITT, RN
MARTIN D. MEYER, PHD
AARON I. VINIK, MD, PHD

OBJECTIVE — To test the hypothesis that vasomotion, the rhythmic contraction exhibited by small arteries and arterioles, is impaired in diabetic subjects compared with healthy control subjects.

RESEARCH DESIGN AND METHODS — We mathematically modeled the oscillations in laser Doppler microvascular measurements taken from the pulpar surface of the index finger in 20 healthy control subjects and 20 age-matched diabetic subjects (8 with type I and 12 with type II diabetes). The mean duration of diabetes was 17.1 ± 2.3 years, and mean HbA_{1c} was 9.1 ± 0.4%. Blood flow was measured for 5 min as subjects rested quietly in a closed room. Fast Fourier transformation was performed to provide the frequency power spectrum of each recording. Amplitude of vasomotion was correlated with six quantitative measurements of neuropathy.

RESULTS — Diabetic subjects had impaired low-frequency oscillation vasomotion in 75% of age-matched patients (15 of 20 patients), with mean amplitudes of 24.9 ± 6.4 vs. 129.0 ± 33.2 ($P < 0.0039$). Of six somatic and autonomic neuropathy variables, only the warm thermal sensory threshold correlated significantly with the mean amplitude of vasomotion ($r = -0.75$, $P < 0.0009$).

CONCLUSIONS — Patterns of peripheral vasomotion are clearly disordered in diabetes. The loss of low-frequency oscillations observed here suggests a peripheral vascular abnormality that extends past the capillary network to arterial vessels. It is uncertain whether the accompanying small unmyelinated nerve C-fiber dysfunction is a cause or consequence of the impaired microvascular function. Measurement of vasomotion may prove useful as a novel test for peripheral neurovascular function.

Impaired regulation of blood flow in the diabetic periphery has been implicated in the development of diabetic neuropathy (1). Despite this, it is uncertain whether the vascular component represents a primary mechanism, plays a facilitative role, or itself occurs as a result of subclinical neural dysfunction. To date, measurements of skin blood flow in human subjects have produced conflicting results (2,3) and have not demonstrated an obvious relationship with the presence of neuropathy. As such, it may be instructive to consider other components of microvascular perfusion.

Recent attention has been given to one such component of vascular function that may help provide insight to this question: blood vessel vasomotion. Vasomotion is the spontaneous rhythmic oscillation of vessel diameter in arterioles and venules. Microscopic photometry of bat wing vessels provided comprehensive documentation of the existence of vasomotion (4). Fagrell et al. (5) found these same rhythmic variations in flow present in human skin microvasculature when the surface temperature of the tissue was <34°C. While the exact physiological role of vasomotion remains uncertain, it has been hypothesized that the periodic opening and closing of vessels may serve to direct the distribution of blood flow within the capillary network (6). Furthermore, Wilkin (7) uses Poiseuille's law to explain that the resistance of a vessel whose diameter changes sinusoidally is characteristically less than a similar vessel of equal mean diameter but whose diameter does not change. Collectively, the data suggest that vasomotion may be responsible for lowering effective vessel resistance and increasing flow to allow for proper tissue perfusion and oxygen supply.

Vasomotion appears to be a periodic phenomenon, characterized not by a unique frequency, but rather a distribution of frequencies centered around a fundamental frequency with the highest amplitude (6,8). Because of this property, power spectral analysis (usually Fourier transformation) of microvascular blood flow measurements has been used to detect certain frequencies of vasomotion in various models. Two distinct ranges of fundamental frequencies have been found to be the most prominent: a low-frequency band and a high-frequency band. The low-frequency band is composed of oscillations of 1–2 cpm and has been termed "slow-wave" vasomotion, while the high-frequency band contains oscillations of 10–20 cpm, labeled "fast-wave" vasomotion (9).

In various animal models of vasomotion, certain properties have been established under normal conditions. One such model uses the hamster skinfold window and video microscopy for recording images. This enables the quantification of rhythmic diameter changes in arterial microcirculation (6). These findings have revealed that frequency and changes in peak-to-peak amplitude correlate to mean diameter. That is, the fundamental frequency of vasomotion increases as the mean diameter of the vessel decreases, and amplitude of variation increases along with mean diameter.

More recently, laser Doppler fluximetry (LDF) techniques have been used to measure variations in flow motion in vivo in human subjects. The emergence

From The Diabetes Institutes (K.B.S., S.A.S., P.M.M., A.I.V.) and the Departments of Internal Medicine, Physiology (M.A.H.), Anatomy/Neurobiology (A.I.V.), Eastern Virginia Medical School, Norfolk, and the Department of Electrical and Computer Engineering (M.D.M.), Old Dominion University, Norfolk, Virginia.

Address correspondence and reprint requests to Aaron I. Vinik, MD, PhD, The Diabetes Institutes, 855 West Brambleton Ave., Norfolk, VA 23510.

Received for publication 16 August 1995 and accepted in revised form 22 February 1996.

ANOVA, analysis of variance; CV, coefficient of variation; FFT, fast Fourier transformation; LDF, laser Doppler fluximetry.

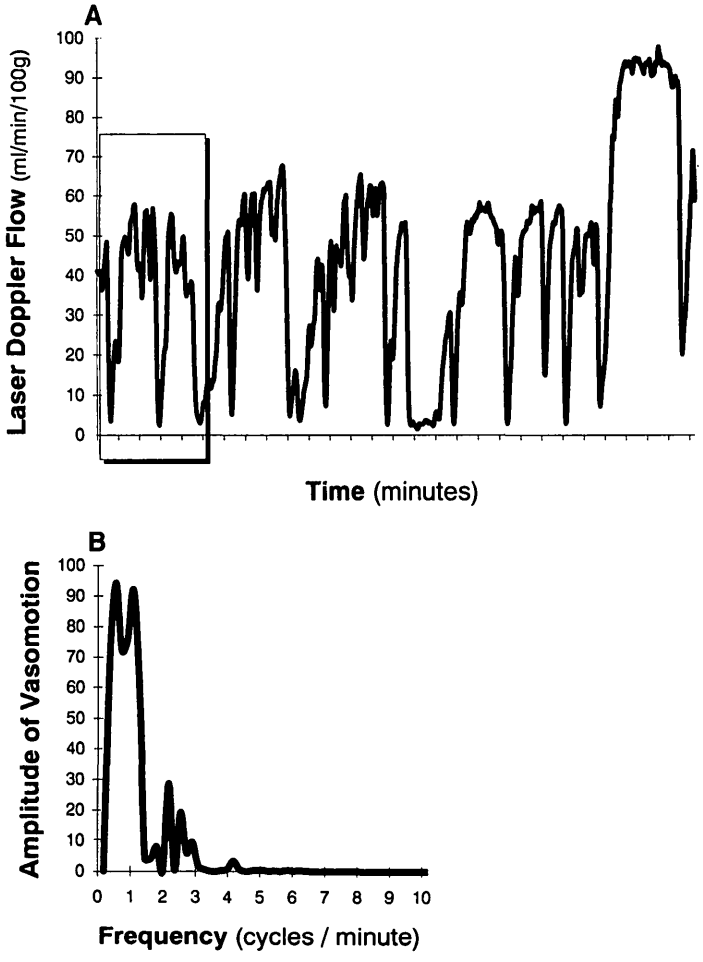

Figure 1—*A: an example of an actual laser Doppler blood flow recording. The blocked portion signifies a 5-min period that was subjected to FFT for vasomotion analysis.* B: *an example of FFT of the 5-min blood flow recording blocked in Fig. 1A.*

of LDF as a technique for vasomotion analysis has led to new information regarding the role of vasomotion in microvascular control as well as the effect of pathological states on cardiovascular hemodynamics (9). For example, using LDF, finger cutaneous blood flow in patients with acute heart failure has been shown to be significantly reduced. With respect to vasomotion, this reduction in flow was accompanied by sudden interruptions of the rhythmic oscillations (10).

Fourier transformation of laser Doppler skin blood flow measurements is capable of identifying certain frequencies of spontaneous vasomotion, as described in other models. Using this approach, we report here that vasomotion is profoundly altered in diabetic subjects and, further, that decreased vasomotor amplitude correlates with the loss of thinly myelinated C-fiber function.

RESEARCH DESIGN AND METHODS

Subjects

Forty subjects for the study included 20 normal healthy control subjects aged 18–72 years and 20 age-matched diabetic subjects. All subjects were volunteers for a larger study investigating cutaneous blood flow reactivity in diabetic neuropathy. Diabetic subjects included in the present study were selected by age matching to within ± 2 years the age of their respective control subjects. Data from all of the control subjects in that larger study were included here, and diabetic subjects were selected solely on the availability of an age-matched control subject, then alphabetically in the case of duplicate matches. Of the diabetic subjects, 8 had IDDM, while the other 12 had NIDDM. Mean duration of disease for the diabetic patients was 17.1 ± 2.3 years, while mean HbA_{1c} was 9.1 ± 0.4%. We excluded subjects with any clinical signs of peripheral vascular disease such as absent or diminished pedal pulses, lower extremity skin changes or hair loss or ulcerations of any kind. Of the 20 diabetic subjects, 8 had retinopathy on funduscopic examination. Of those, four diabetic subjects were considered to have background, one to have preproliferative, and three to have proliferative retinopathy. Three of them (the preproliferative and two of the proliferative patients) had had previous laser treatment.

All diabetic patients had clinically diagnosed neuropathy according to the American Diabetes Association and the American Academy of Neurology criteria as demonstrated by the presence of at least one of the following: clinical signs and/or symptoms, abnormal nerve conduction velocities, abnormal quantitative sensory testing at the dominant great toe, or abnormal quantitative autonomic function tests (11,12). Based upon the neurological symptom score, the neurological disability score, the quantitative sensory tests, and the quantitative autonomic function tests, these patients have been classified as having mild-to-moderate neuropathy (13). None of the subjects had clinical symptoms or signs of ulnar or median entrapment syndromes.

Skin blood flow

Laser Doppler techniques were used to assess cutaneous microvascular flow from the pulpar surface of the left index finger. The Laserflo Blood Perfusion Monitor (BPM 403A, Vasamedics, St. Paul, MN) uses laser Doppler noninvasively to measure erythrocyte volume and velocity in the upper horizontal plexus of the skin. The upper horizontal plexus contains lower-order nonelastic terminal arterioles, capillaries, and postcapillary venules (14). From these measurements of velocity and volume, the monitor calculates an overall measure of flow in units of milliliters per minute per 100 g of tissue.

Subjects were acclimated for at least 10 min in a quiet well-lit room. Five-minute blood flow recordings were taken as each subject rested alone in the closed room. All subjects listened to the same classical music through headphones at a volume that they could control.

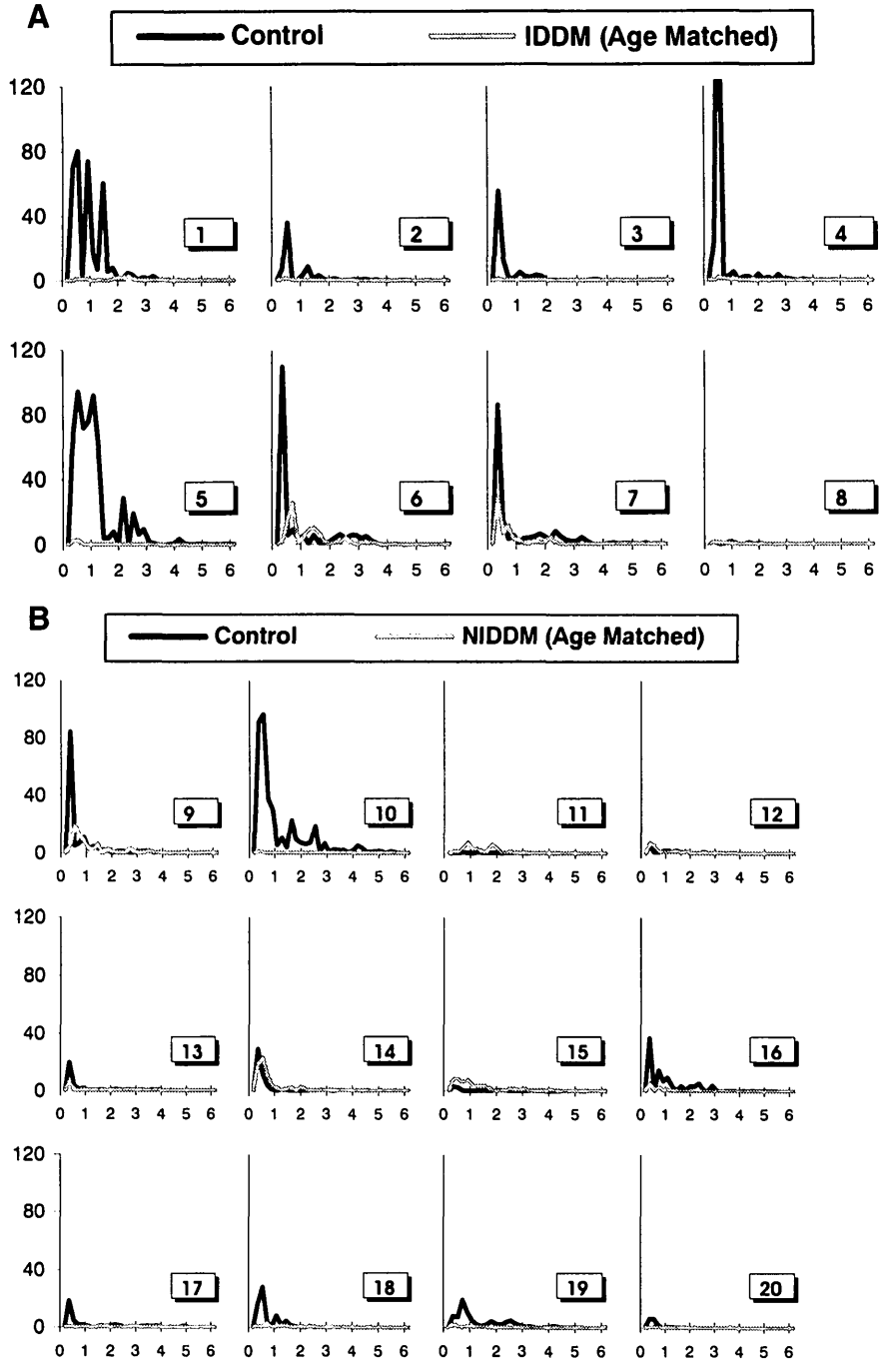

Figure 2—A: *FFT of blood flow from subject pairs 1–8. Diabetic subjects included in these subject pairs are all insulin dependent. Control subjects had greater amplitudes of vasomotion in 7 of 8 subject pairs.* B: *FFT of blood flow from subject pairs 9–20. Diabetic subjects in these subject pairs are non-insulin-dependent. Control subjects had greater amplitude of vasomotion in 8 of 12 subject pairs.*

Central autonomic function

The QMED Monitor One ND_X was used to assess cardiovascular reflex responses via three chest electrodes. The monitor measures the R-R responses to deep breathing (the E:I index), a Valsalva maneuver, and a change in posture. Detailed methods for these tests have been described previously (15).

Sensory function

Vibration and thermal sensory function were measured at the plantar surface of the dominant hallux. A two-alternative forced-choice psychophysical procedure was used that has also been previously described (15). The Physitemp Vibratron II vibrometer (Physitemp, Clifton, NJ) was used to present the vibration stimuli. The stimulus intensity (peak-to-peak amplitude in microns) was subjected to the established algorithm until the threshold was achieved. The Physitemp Thermal Sensitivity Tester NTE-2 (Physitemp) was used to present the thermal stimuli. The null (comparison) stimulus for warm thermal sensation was 35°C, and the positive stimulus was between 35 and 45°C. For cold thermal sensation, the comparison stimulus was 25°C and the positive stimulus was between 0 and 25°C.

Data analysis

Flow values recorded by the blood flow monitor were temporally averaged during data collection over 5 s to act as a high-pass filter. The output voltage data from the blood flow monitor was sampled every 2 s by custom-written data acquisition software that interfaces with an analogue to digital data acquisition board (DAS-8 board, Keithley Metrabyte, Taunton, MA). Voltage data were converted mathematically back to flow values then were subjected to spectral analysis by using fast Fourier transformation (FFT). FFT serves to identify rhythmic components (imbedded sine waves) of changes (oscillations) in the data over time (Fig. 1*A* and *B*). This provides a measurement of the frequency as well as the amplitude of oscillations.

Before statistical analysis, the amplitude at each frequency was divided by each subject's mean blood flow over the 5-min period to adjust for mean flow level during the session. Total vasomotion for the session was then calculated as area under the curve. The data were found to be parametric, and a univariate analysis of variance (ANOVA) was performed to test for a between-subjects effect of diabetes on amplitude of vasomotion. Correlation coefficients were generated comparing vasomotion amplitude with each index of neuropathy, age, duration of diabetes, and diabetes control (HbA_{1c}).

A pilot study to determine repeatability of vasomotion was conducted in three young healthy control subjects. Quadruplicate measurements of vasomotion were conducted on separate days and for longer intervals up to 10 min. Vasomotion was essentially identical when measured for 5 or 10 min in the same session, with a coefficient of variation (CV) of 10.4% (range 0.9–47.4%). When duplicated on the same day, vasomotion showed a CV of 37% (range 3.3–62.3%).

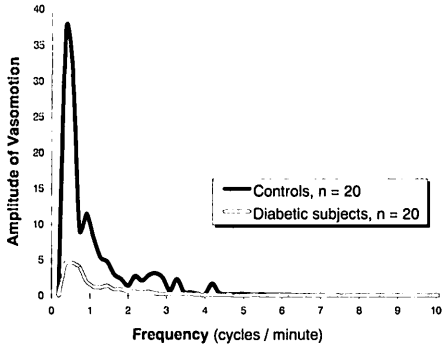

Figure 3—*Mean vasomotion for control and diabetic subjects. The difference between groups was significant (P = 0.0039, ANOVA).*

When repeated on different days, the CV was 45.7% (range 35.9–55.8%).

RESULTS— FFT of blood flow data recorded with laser Doppler techniques was sensitive to low-frequency vasomotion oscillations of <6 cpm. Amplitude of vasomotion for diabetic subjects (mean 24.9 ± 6.4) was significantly less than the group mean for their respective age-matched control subjects (129.0 ± 33.2, $P < 0.0039$). We considered vasomotion to be impaired when a diabetic subject's vasomotion (as area under the curve) was less than one-third that of his or her respective age-matched control subject. Using this criterion, impaired vasomotion was expressed in 75% (15 of 20) of the diabetic subjects (Fig. 2*A* and *B*).

IDDM subjects (25.0 ± 11.9) did not differ significantly from NIDDM subjects (24.8 ± 7.5) in amplitude of vasomotion ($P > 0.9850$). Diabetic subjects with retinopathy were not significantly different from those without retinopathy in amplitude of vasomotion (36.5 ± 9.7 vs. 17.1 ± 7.9, $P > 0.1214$). Complete FFT of the 5-min blood flow recordings for each of the 20 subject pairs can be found in Fig. 2*A* and *B*; the mean amplitudes for control subjects and diabetic patients are depicted in Fig. 3.

Means for all three autonomic function tests were significantly lower in diabetic subjects compared with control subjects ($P < 0.05$). Vibratory, warm thermal, and cold thermal sensation thresholds were all significantly higher in diabetic neuropathic patients as expected ($P < 0.01$). Summary data (means and SE) for the 20 subject pairs are listed in Table 1.

To determine the possible relationships between alterations in vasomotion and the existence of neuropathy, correlation analyses were performed. Of the six neuropathy variables, only warm thermal sensation correlated significantly with subjects' vasomotion amplitude ($r = -0.75$, $P < 0.0009$). Pearson's coefficients are listed for each correlation in Table 2, and the significant ($P < 0.0009$) negative correlation ($r = -0.75$) between warm thermal sensation and vasomotion amplitude is graphically depicted in Fig. 4.

CONCLUSIONS

Laser Doppler flow vasomotion

We have shown that peripheral vasomotion is clearly impaired in the diabetic microvasculature. The loss of vasomotion observed in diabetic subjects examined in this study is manifested particularly in the region of low-frequency oscillations (slow-wave flow motion). It remains questionable whether higher-frequency oscillations can be detected using laser Doppler blood flow measurements. To date, fast-wave vasomotion in the human skin and some animal models has not been found under control conditions using laser Doppler techniques (8,16) but can be induced during reactive hyperemia and occurs in patients with limb ischemia (17). It is possible that in future studies, increasing the sampling rate of the blood flow monitor will allow data collection that is sensitive to higher-frequency oscillations. It is conceivable that fast-wave vasomotion also is impaired in disease states such as diabetes.

The differences in low-frequency oscillations reported here suggest that diabetes not only affects the exchange vessels, but is also the result of dysfunction at the level of the upstream arterioles. This is apparent because the vascular components directly sampled using laser Doppler techniques are predominantly capillaries and postcapillary venules of the upper horizontal plexus that exhibit little variation in vessel diameter and hence do not contribute to rhythmic changes in blood flow. Thus, the vasomotor waveforms observed in the blood flow measurements appear to result from the propagation of contraction/relaxation cycles in arteriolar smooth muscle (6). Studies using experimental animal microcirculatory preparations have shown that vasomotion frequency decreases with increasing arteriolar diameter (6,8,18,19). If these experimental studies are applicable to the present data, it is possible that altered function of arterioles, several branch orders before the capillary bed, contributes to the altered vasomotion pattern seen in the diabetic subjects.

Vasomotion and C-fiber dysfunction

Studies examining the mechanisms underlying the development of diabetic neuropathy have suggested that decreased nutritive capillary blood flow results from shunting of blood directly from the arterial to venous circulations (20). The

Table 1—*Summary data for 40 age-matched subjects*

	Control subjects	Diabetic subjects	*P* value
n	20	20	
Age (years)	45.9 ± 3.2	45.9 ± 3.2	
Duration of disease (years)		17.1 ± 2.3	
HbA_{1c} (%)		9.1 ± 0.4	
Autonomic variables			
E:I ratio	1.26 ± 0.05	1.12 ± 0.03	0.056
Valsalva ratio	1.53 ± 0.07	1.28 ± 0.07	0.0189
Postural ratio	1.44 ± 0.06	1.13 ± 0.05	0.0008
Sensory variables			
Vibratory threshold (log microns)	2.85 ± 0.40	8.58 ± 1.37	0.0003
Warm thermal threshold (°C)	3.32 ± 0.417	7.79 ± 0.85	0.0001
Cold thermal threshold (°C)	0.43 ± 0.05	4.90 ± 1.54	0.0062
Vasomotion			
Amplitude (area under curve)	129.0 ± 33.2	24.9 ± 6.4	0.0039

Data are means ± SE.

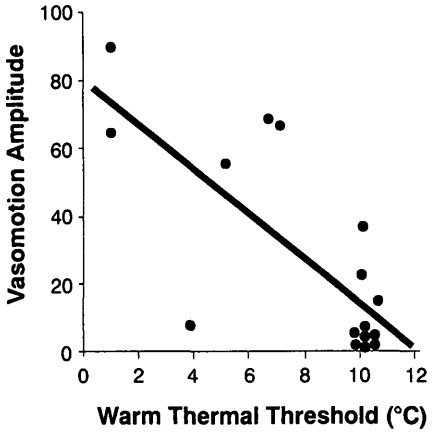

Figure 4—*A scatter plot of warm thermal sensation threshold correlated with amplitude of vasomotion. Pearson's* r = −0.75 (P < 0.0009).

mechanism for this shunting action is presumably via arteriovenous anastomoses (A-V shunts), which run parallel to the nutritive capillary bed in the distal finger. At the site we have sampled (pulpar aspect of left index finger), laser Doppler skin blood flow values of vessels of the upper horizontal plexus are greatly affected by flow through A-V shunts. It is known that these A-V shunts are subject to autonomic regulation because they are highly sympathetically innervated (21). Flow is decreased through A-V shunt closure upon sympathetic activation, while opening occurs when basal sympathetic tone is withdrawn. Thus, total digital cutaneous flow is controlled by sympathetic nerves via mostly α_1- and α_2-adrenergic receptors (21,22). The adrenergic receptors, usually signaled by thermoreceptors in the skin, access both centrally and locally mediated reflex pathways to transmit information (23). We have previously shown α_2-adrenergic receptor downregulation associated with orthostasis in autonomic neuropathic patients (24), and it seems feasible that disordered neural regulation may contribute to the vasomotor disturbance.

Unmyelinated C-fibers, which constitute the central reflex pathway, are assumed to be damaged in diabetic neuropathy, leaving only the local axon reflex to control cutaneous vasculature (25). We have previously shown that abnormalities in C-fiber function correlate with abnormalities in total skin blood flow in diabetic patients with neuropathy (26), and hence, we examined relationships between indicators of neuropathy and vasomotion patterns. That vasomotion amplitude had a negative correlation with warm thermal sensation threshold in diabetic subjects with neuropathy further supports this underlying relationship. The warm thermal threshold is a functional measure of small sensory afferents (C-fibers) in the periphery, and impairment of this function was paralleled by a reduction of vasomotion. That is, the lower the amplitude of vasomotion, the higher the warm thermal sensory threshold. This is to be expected because a high degree of C-fiber dysfunction leaves sympathetic adrenergic receptors constituting the central reflex pathway without thermal input. Thus, only local myogenic regulation of tone remains in the vessels upstream of those being sampled, depriving them of sympathetic discharge and, consequently, vasomotion. Further support for this notion will require testing the effects of a specific blockade of C-fiber function on vasomotion in healthy control subjects.

Predictably the function of larger (myelinated) sensory fibers reflected by vibration and cold sensation was not related to vasomotion. Surprisingly though, we did not find a relationship between vasomotion and three measures of cardiac autonomic function. One plausible explanation for this lack of correlation is that peripheral autonomic function, possibly responsible for control of vasomotion in these peripheral vessels, may be independent of our measures of central (vagal and sympathetic) autonomic function. It will be interesting to correlate these findings with other measures of peripheral sympathetic tone such as quantitative sudorimetry, a measure of local sympathetic regulation of sweat gland regulation (12).

Vasomotion regulatory mechanisms

Much like the debate over dysregulated blood flow in diabetic patients with neuropathy, be it central/neural or local/myogenic, a similar argument exists over the origin and control of vasomotion. Schechner and Braverman (27) found synchrony of vasomotion in two comparable sites on contralateral limbs of human subjects. Synchrony is defined as the superimposability of two separate waveforms with respect to shape, periodicity, and phase. The presence of contralateral synchrony is presumed to be due to regulation by the same autonomic signal, while bursts of asynchrony represent brief interruptions by local influences. In addition, Bernardi (28) has shown the ability to blunt rhythmic vasomotor oscillations via pharmacological sympathectomy, also suggesting a centralized control of vasomotion. However, potent local vasodilators such as acetylcholine and sodium nitroprusside have been shown to reduce the percentage of amplitude vasomotion, and local suppression of endothelium-derived relaxing factors has caused changes in vasomotion without altering mean arterial pressure (19). Furthermore, patterns of vasomotion have been observed in single cannulated arteries isolated from the hamster cheek pouch (29). In light of such findings, it has been suggested that vasomotion originates from local pacemakers within the arteriolar smooth muscle and is modulated by local vasoactive factors and central neural influences. Since studies of both human and experimental diabetes have demonstrated alterations in microvascular reactivity and the production/availability of vasoactive factors (30–33), it remains feasible that abnormalities in vasomotion could result from nerve dysfunction, vascular abnormalities, or both.

Changes in the structure of endoneurial capillaries have been reported in human diabetes (34–36), and diabetes has been shown to lead to reduced endothelial antioxidant capacity (37) and production of nitric oxide (31). Because en-

Table 2—*Neuropathy variables correlated with amplitude of vasomotion*

	Control patients	Diabetic patients
E:I index	0.11	−0.02
Valsalva maneuver	−0.25	−0.21
Postural maneuver	−0.10	−0.05
Vibratory sensation	−0.17	−0.09
Warm thermal sensation	−0.30	−0.75*
Cold thermal sensation	−0.06	−0.09

Correlation coefficients are Pearson's *r*. *Significant correlation, $P < 0.0009$.

dothelial function is important (essential) for the regulation of the delivery of oxygen and nutrients to a peripheral nerve, it is feasible that the abnormalities in vasomotion are related to endothelial dysfunction and thus are a cause, rather than a consequence, of disordered nerve function in diabetes. Somewhat less likely as a cause of impaired vasomotion, but nonetheless feasible, is the possibility that organic structural vascular disease is a consequence of the accumulation of advanced glycosylation end products in the vessel wall or of obstruction of the lumen by microthrombi as a consequence of the high levels of phospholipid antibodies in diabetic patients with neuropathy (38).

Regardless of the origin of vasomotion, it is evident that low-frequency oscillations are nearly nonexistent in the upper limbs of diabetic patients with mild peripheral neuropathy affecting mostly the lower limbs. Because of the perceived role of vasomotion in regulation of microvascular pressure and perfusion state of peripheral tissues, the relationship of this disturbance to local blood pressure control and diabetic microvascular disease (i.e., nephropathy and retinopathy) should be further investigated. Although the present data do not define the mechanism for the alteration in vasomotion, it is of interest to note that we have shown in experimental diabetes that aminoguanidine prevents diabetes-induced alterations in the mechanical properties of arterioles (32). Such studies, however, are yet to be performed in human subjects. Clarification of the extent to which functional and structural microvascular abnormalities, in addition to neural influences, affect vasomotion may provide insight into potential sites for pharmacological intervention.

Acknowledgments— This study was supported by a grant from the Diabetes Institutes Foundation.

References

1. Tesfaye S, Malik R, Ward JD: Vascular factors in diabetic neuropathy. *Diabetologia* 37:847–854, 1994
2. Rendell MS, Bergman T, O'Donnel G, Drobny E, Borgos J, Bonner RF: Microvascular blood flow, volume and velocity measured by laser Doppler techniques in IDDM. *Diabetes* 38:819–824, 1989
3. Belcaro G, Nicolaides AN, Volteas N, Leon M: Skin flow: the venoarteriolar response and capillary filtration in diabetics: a 3 year follow-up. *Angiology* 43:490–495, 1992
4. D'Agrosa LS: Patterns of venous vasomotion in the bat wing. *Am J Physiol* 218: 530–535, 1970
5. Fagrell B, Intaglietta M, Ostergren J: Relative hematocrit in human skin capillaries and its relationship to human blood flow velocity. *Microvasc Res* 20:327–335, 1980
6. Colantuoni A, Bertuglia S, Intaglietta M: Microvascular vasomotion: origin of laser Doppler flux motion. *Int J Microcirc Clin Exp* 14:151–158, 1994
7. Wilkin JK: Poiseuille, periodicity, and perfusion: rhythmic oscillatory vasomotion in the skin. *J Invest Dermatol* 93: 113S–118S, 1989
8. Bertuglia S, Colantuoni A, Coppini G, Intaglietta M: Hypoxia- or hyperoxia-induced changes in arteriolar vasomotion in skeletal muscle microcirculation. *Am J Physiol* 260:H362–H372, 1991
9. Intaglietta M: Arteriolar vasomotion: implications for tissue ischemia. *Blood Vessels* 28:1–7, 1991
10. Seino Y, Tsukamoto H, Ohki K, Nakamura T, Kashiwagi M, Takano T, Hayakawa H: Abnormal cutaneous vasomotion and reduced cutaneous blood mass remain in congestive heart failure even with normalized cardiovascular hemodynamics. *Am Heart J* 126:887–895, 1993
11. American Diabetes Association: Report and recommendation of the San Antonio Conference on Diabetic Neuropathy. *Diabetes Care* 11:592–597, 1988
12. Proceedings of a consensus development conference on standardized measures in diabetic neuropathy. *Muscle Nerve* 15:1143–1170, 1992
13. Dyck PJ: Detection, characterization and staging of polyneuropathy: assessed in diabetes. *Muscle Nerve* 11:21–32, 1988
14. Braverman IM, Keh A, Goldminz D: Correlation of laser Doppler wave patterns with underlying microvascular anatomy. *J Invest Dermatol* 95:283–286, 1990
15. Vinik AI, Suwanwalaikorn S, Stansberry KB, Holland MT, McNitt PM, Colen LE: Quantitative measurement of cutaneous perception in diabetic neuropathy. *Muscle Nerve* 18:574–584, 1995
16. Borgstrom P, Lindbom L, Meyer JU, Sjoquist M, Arfors KE, Intaglietta M: Hemodynamic responses in rabbit tenuissimus muscle arterioles during local reduction in perfusion pressure. *Int J Microcirc Clin Exp* 9:175–186, 1990
17. Seifert H, Jager K, Bollinger A: Analysis of flowmotion by laser Doppler technique in patients with peripheral arterial occlusive disease. *Int J Microcirc Clin Exp* 7:223–236, 1988
18. le Noble JL, Smith TL, Hutchins PM, Struyker Boudier HA: Microvascular alterations in adult conscious spontaneously hypertensive rats (see comments). *Hypertension* 15:415–419, 1990
19. Bertuglia S, Colantuoni A, Intaglietta M: Effects of L-NMMA and indomethacin on arteriolar vasomotion in skeletal muscle microcirculation of conscious and anesthetized hamsters. *Microvasc Res* 48:68–84, 1994
20. Boulton AJM, Scarpello JHB, Ward JD: Venous oxygenation in the diabetic neuropathic foot: evidence of arteriovenous shunting? *Diabetologia* 22:6–8, 1982
21. Coffman JD, Cohen RA: α-Adrenergic and serotonergic mechanisms in the human digit. *J Cardiovasc Pharmacol* 11 (Suppl. 1):S49–S53, 1988
22. Hales JRS, Foldes A, Fawcett AA, King RB: The role of adrenergic mechanisms in thermoregulatory control of blood flow through capillaries and arteriovenous anastomoses in the sheep hind limb. *Pfluegers Arch Eur J Physiol* 395:93–98, 1982
23. Crockford GW, Hellon RF, Parkhouse N: Thermal vasomotor responses in human skin mediated by local mechanism. *J Physiol* 161:10–20, 1962
24. Abrahm DR, Hollingsworth PS, Smith CB, Jim L, Zucker LB, Sobotka PA, Vinik AI: Decreased alpha$_2$ adrenergic receptors on platelet membranes from diabetic patients with autonomic neuropathy and orthostatic hypertension. *J Clin Endocrinol Metab* 63:906–912, 1986
25. Watkins PJ: Clinical observations and experiments in diabetic neuropathy. *Diabetologia* 35:2–11, 1992
26. Stansberry KB, Hill M, McNitt PN, Bhatt BA: Cutaneous blood flow reactivity and neuropathy (Abstract). *Diabetes* 43:A107, 1994
27. Schechner JS, Braverman IM: Synchronous vasomotion in the human cutaneous microvasculature provides evidence for central modulations. *Microvasc Res* 44:27–32, 1992
28. Bernardi L, Rossi M, Fratino P, Finardi G, Mevio E, Orlandi C: Relationship between phasic changes in human skin blood flow and autonomic tone. *Microvasc Res* 37:16–27, 1989
29. Davis MJ, Sikes, PJ: Myogenic responses of isolated arterioles: test for a rate-sensitive mechanism. *Am J Physiol* 259: H1890–H1900, 1990
30. Hill, MA, Larkins RG: Altered microvascular reactivity in streptozotocin-induced diabetes in rats. *Am J Physiol* 257:H1438–H1445, 1989
31. Azadzoi KM, Saenz de Tejada I: Diabetes mellitus impairs neurogenic and endothelium-dependent relaxation of rabbit corpus cavernosum smooth muscle. *J Urol*

148:1587–1591, 1992
32. Hill MA, Ege EA: Active and passive mechanical properties of isolated arterioles from STZ-induced diabetic rats: effect of aminoguanidine treatment. *Diabetes* 43: 1450–1456, 1994
33. Jaap AJ, Hammersley MS, Shore AC, Tooke JE: Reduced microvascular hyperemia in subjects at risk of developing type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 37:214–216, 1994
34. Johnson PC, Beggs JL: Pathology of autonomic nerve innervating the vasa nervorum in diabetic neuropathy. *Diabetic Med* 10 (Suppl. 2):56S–61S, 1993
35. Britland ST, Young RJ, Sharma AK, Blarke BF: Relationship of endoneurial capillary abnormalities to type and severity of diabetic polyneuropathy. *Diabetes* 39:909–913, 1990
36. Malik RA, Tesfaye S, Thompson SD, Veves A, Sharma AK, Boulton AJM, Ward JD: Endoneurial localisation of microvascular damage in human diabetic neuropathy. *Diabetologia* 36:454–459, 1993
37. Taniguchi N, Arai K, Kinoshita N: Glycation of copper/zinc superoxide dismutase and its inactivation: identification of glycated sites. *Methods Enzymol* 179:570–581, 1989
38. Vinik AI, Leichter SB, Pittenger GL, Stansberry KB, Holland MT, Powers AC, Suwanwalaikorn S: Phospholipid and glutamic acid decarboxylase autoantibodies in diabetic neuropathy. *Diabetes Care* 18: 1225–1232, 1995