Noninvasive interrogation of microvasculature for signs of endothelial dysfunction in patients with chronic renal failure

Julian Stewart, Adam Kohen, Daniel Brouder, Fahim Rahim, Stephen Adler, Renee Garrick, and Michael S. Goligorsky. Noninvasive interrogation of microvasculature for signs of endothelial dysfunction in patients with chronic renal failure. *Am J Physiol Heart Circ Physiol* 287: H2687–H2696, 2004. First published August 5, 2004; doi:10.1152/ajpheart.00287.2004.—Endothelial cell dysfunction (ECD) has been demonstrated in patients with end-stage renal disease (ESRD) who have cardiovascular disease (CD) or diabetes mellitus (DM). While techniques to examine conduit arteries have been adapted to these patients, evaluation of microvascular function has lagged behind. Therefore, we used laser Doppler flowmetry (LDF) and scanned laser Doppler imaging (LDI) to quantify parameters of the postocclusion reactive hyperemia and thermal hyperemic responses (local heating to 43°C) in ESRD patients (n = 63) and healthy individuals (n = 33). Patients with ESRD were partitioned among those with either CD or DM or both (designated CDorDM, n = 30), patients with both CD and DM (designated CD+DM, n = 12), statistically similar to CDorDM, and patients with neither CD or DM (designated ¬CDorDM, n = 33). LDF during thermal hyperemia showed a decrease in the thermal peaks and plateau as well as a delay in plateau compared with control, consistent with ECD. LDF during reactive hyperemia showed a decrease in the pay-back area under the curve, also consistent with ECD. ¬CDorDM were heterogeneous: almost 50% contained flow abnormalities similar to CDorDM. There was also a reduction in the number of functional arterioles on LDI images. Fourier analysis of LDF oscillations showed that low-frequency oscillations characterizing endothelial function were impaired in CDorDM and in many ¬CDorDM. The data demonstrate that ESRD patients with expected ECD (CDorDM) are characterized by distinct abnormalities in LDF parameters. However, similar abnormalities are found in approximately one-half of ESRD patients without evidence for CD or DM. Postocclusive and thermal interrogation of the microvasculature with laser Doppler-resolved parameters of the microcirculation, followed by Fourier analysis of the very slow oscillations, may provide a valuable adjunct to early noninvasive diagnosis of ECD in ESRD, especially important in a subpopulation of ESRD patients with no known CD or DM, which could be at increased risk of impending clinical manifestations of vasculopathy.

laser Doppler flowmetry; imaging; microcirculation; coronary artery disease; diabetes mellitus

CARDIOVASCULAR COMPLICATIONS have emerged as the most serious life-threatening accompaniment of end-stage renal disease (ESRD) (23). Widespread macrovascular endothelial cell dysfunction (ECD), diagnosed in large conduit vessels as paradoxical vasoconstriction to acetylcholine occurring before angiographic stigmata of atherosclerosis are detectable, is believed to be ultimately responsible for the development of many cardiovascular complications (24, 33, 43). Patients with ESRD are at additional risk for ECD because the uniform and concomitant presence of hyperhomocystenemia, accumulation of asymmetric dimethylarginine, anemia, oxidative stress, and reduction of bioavailable nitric oxide (NO) facilitate the development of endothelial dysfunction and, ultimately, cardiovascular complications in this cohort (9, 10, 23, 32). This explains why investigators worldwide are intensely searching for noninvasive surrogate markers of endothelial dysfunction.

However, ECD is foremost a microvascular disease, and although macroscopic tests of vascular function such as plethysmographic forearm blood flow measurement have been advocated as tests for detection of endothelial dysfunction in ESRD patients (4, 7), they cannot accurately reflect microvascular function. Similarly, surrogate markers such as plasma levels of plasminogen activator receptor-1, selectins, von Willebrand factor, prostaglandin metabolites, or C-reactive protein are used but remain nonspecific. Although all these tests carry certain informational value, none is capable of reporting directly the status of microcirculation and its regulation. This existing gap in testing microcirculatory profile is being filled by studies using noninvasive laser Doppler flowmetry (LDF), including single point laser Doppler (LDPD) and/or high-resolution laser Doppler perfusion imaging (LDPi) (3, 12–14, 16–20, 22, 25, 27–31, 35).

The surface area of dermal capillaries provides for ~30 m² of endothelial coverage. Blood flow, usually averaging 10–20 ml·min⁻¹·100 g⁻¹, may vary from 1 to 200 ml·min⁻¹·100 g⁻¹ (5), indicating a remarkable plasticity of regulatory control in this vascular bed. The skin, therefore, is highly vascular and very accessible. However, the key question confronting any study of surrogate tissues including laser Doppler studies of cutaneous microvasculature is does information obtained from cutaneous microcirculation correlate with changes in coronary, renal, or muscle vascular beds, which ultimately produce myocardial infarction, insulin resistance, hypertension, and progression of renal failure? There is positive evidence: it has been demonstrated that cutaneous microvascular dysfunction correlates with blood pressure and insulin resistance (11, 36, 37). Patients with cardiac allograft vasculopathy or with coronary three-vessel disease have been found to exhibit abnormalities of the cutaneous microcirculation (15). Diabetic patients show cutaneous microangiopathy which is more pro-

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Microcirculatory Function and Endothelial Dysfunction in ESRD

Microcirculation in other vascular beds including cardiac muscle. Proof of this thesis is missing in patients with ESRD, who are at ~20-fold increased risk of cardiovascular mortality compared with the multiparameter-matched general population (23). We hypothesized that LDF and high-resolution imaging of the cutaneous microvasculature could supply important preclinical information on the state of the microcirculation in ESRD. Therefore, the purpose of this study was to examine the value of laser Doppler methods for functional screening of endothelial dysfunction in patients with ESRD.

MATERIALS AND METHODS

Subjects and Experimental Outline

We studied 63 male and female patients between the ages of 18 and 60 yr with renal failure who were attending our dialysis unit. We recruited 33 healthy male and female subjects between the ages of 18 and 60 yr willing to undergo testing and capable of following the study protocol. Control and ESRD groups had matching age, ethnic, and gender distribution. ESRD patients examined had no surgical exclusion criteria precluding the subject following the protocol. Exclusion criteria were as follows: peripheral vascular disease, porphyria, peripheral neuropathy, liver failure, neoplastic disease, Cushing syndrome, collagen vascular disease, dermatologic problems, chronic angioedema or lymphedema, uncontrolled hyperthyroidism, chronic substance abuse, or any condition precluding the subject following the protocol. Exclusion criteria were as detailed below while LDPM was performed throughout.

Table 1. Anthropometric parameters and laboratory data in ESRD patients

<table>
<thead>
<tr>
<th></th>
<th>ESRD</th>
<th>CD</th>
<th>DM</th>
<th>CD+DM</th>
<th>~CDorDM</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>63</td>
<td>20</td>
<td>22</td>
<td>12</td>
<td>33</td>
</tr>
<tr>
<td>Age, yr</td>
<td>56.62±13.80 (24–85)</td>
<td>64.60±12.33 (42–85)</td>
<td>61.09±11.78 (41–81)</td>
<td>60.00±10.72 (42–78)</td>
<td>50.03±11.54*(24–80)</td>
</tr>
<tr>
<td>BMI</td>
<td>26.16±5.29 (18.5–40.3)</td>
<td>26.66±4.73 (19.2–37.8)</td>
<td>26.57±4.27 (19.2–37.8)</td>
<td>27.33±4.71 (19.2–37.8)</td>
<td>26.00±6.10 (18.5–40.3)</td>
</tr>
<tr>
<td>SBP</td>
<td>145.83±28.81 (86–201)</td>
<td>148.60±28.21 (105–201)</td>
<td>156.09±28.46 (101–201)</td>
<td>159.25±29.22 (115–201)</td>
<td>142.18±29.57 (86–200)</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>148.97±32.22 (78–260)</td>
<td>156.45±30.09 (117–217)</td>
<td>152.77±37.49 (109–260)</td>
<td>153.25±29.08 (117–195)</td>
<td>143.10±27.44 (78–197)</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>71.42±29.92 (25–165)</td>
<td>83.83±30.46 (44–145)</td>
<td>71.33±34.76 (26–165)</td>
<td>75.45±27.99 (44–141)</td>
<td>65.31±22.64*(25–116)</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>145.48±86.13 (43–624)</td>
<td>149.56±60.01 (71–285)</td>
<td>144.82±58.64 (71–285)</td>
<td>154.92±64.41 (71–285)</td>
<td>147.30±107.60 (43–624)</td>
</tr>
<tr>
<td>Hct</td>
<td>36.49±4.11 (27–47)</td>
<td>36.63±4.09 (28–46)</td>
<td>34.70±4.18 (27–47)</td>
<td>34.83±3.31 (28–41)</td>
<td>37.01±3.68*(31–47)</td>
</tr>
</tbody>
</table>

Values are means ± SD, with the range in parentheses; n, n of patients. ESRD, end-stage renal disease; BMI, body mass index; SBP, systolic blood pressure; TG, triglycerides; Hct, hematocrit. Mean age in coronary artery disease (CAD) (P < 0.0001), diabetes mellitus (DM) (P < 0.01), and CD+DM (P < 0.05) groups is significantly different from the group with no CD or DM. (~CDorDM). *In addition, mean LDL in CD (P < 0.05) differs significantly from the group with no CAD or DM, and mean HCT in DM (P < 0.05) differs significantly from the group with no CAD or DM.

Protocol

Test began at 9:30 AM in a temperature-controlled room kept at 24°C with a relative humidity of 40%. All tests were performed while subjects sat in a comfortable armchair. After a 30-min acclimatization period, we measured blood pressure and heart rate, and subjects were acclimated to the laser equipment, monitoring equipment, and environment. The arm selected for investigations was positioned at heart level and gently immobilized using a vacuum pillow containing polyurethane beads, which moulds to the shape of the arm (Germa). Two areas of the volar aspect of the forearm were chosen: one was a 2 × 2-cm² site for laser Doppler perfusion imaging (LDPI; Perimed PeriScan PIM II; Stockholm, Sweden), with each scan taking approximately 10 s; the second site spaced ~6 cm from the scanning site was used to continuously measure skin blood perfusion using a laser Doppler perfusion monitor (LDPM) with single-point LDF probes (Perimed PeriFlux System 5000). Continuous LDPM employed coherent laser light at a wavelength of 780 nm, which was sampled at a rate of 32 samples/s. LDF was measured in arbitrary perfusion units (pfu). Sampled LDF was interfaced to a personal computer through an analog-to-digital converter (DATAQ) using PeriSoft data-acquisition software. LDPM data were electronically readjusted such that baseline flow fell on the y-axis for perfusion units and changes in LDF were measured thereafter. A representative experiment is shown in Fig. 1. As shown in Fig. 1, we measured LDPI at baseline. We applied ischemia, measured LDPM during 10-min postocclusion reactive hyperemia (PORH), and remeasured LDPI immediately after the release of ischemia. After a 10-min postischemic period, thermal hyperemia (TH) was performed as detailed below while LDPM was performed throughout.

Details of the Method

Postocclusive reactive hyperemia. After the acclimatization period, LDPM and LDPI of the forearm were recorded. An occlusion cuff placed around the upper arm was inflated to a pressure 15 mmHg above the systolic pressure and remained inflated for 4 min. Absence of cutaneous flow was documented by LDF. The PORH response (pay-back response) elicited upon release of arterial occlusion was pronounced in those with advanced retinopathy (41). Furthermore, cutaneous microcirculatory responses are blunted in patients with angiographically demonstrated coronary artery disease (38).
measured LDF parameter. or systolic blood pressure (SBP) as well as laboratory data and each unpaired comparisons between control and ESRD were performed using an Tukey test. Differences were considered significant at patients were performed using one-way ANOVA followed by the

Same volar forearm 2 x 2-cm² area was performed. Data analysis was performed using PeriSoft software (Perimed). We measured peak flow, time to peak flow, and area under the curve (AUC) (Fig. 1).

Thermal hyperemia. Local heating of nonglabrous skin such as the forearm evokes vasodilation that is mediated by neurogenic reflexes and locally produced endothelium-dependent vasodilatory substances (12, 16, 25, 18). The LDPM probe was heated to 43°C over a 2-min period. Laser Doppler flow was measured over the next 30 min to record flow patterns characterized by an initial peak, followed by a nadir, and finally rising to a second peak, which continues as a sustained plateau, as depicted in Fig. 1. Kellogg and colleagues (16) first showed and Minson (25) corroborated that the dilator response to local heating is related to NO, which plays a major role in the sustained cutaneous vasodilation (i.e., the secondary plateau) of prolonged heating. The first peak is blunted by NO synthase inhibitors, but the second peak and plateau are more markedly reduced.

Fourier Analysis

LDPM signals recorded at the baseline and throughout experiments were digitized at 32 Hz. Hamming-windowed fast Fourier transform was performed, and digital power spectra were calculated from the squared amplitude at each frequency. The spectral power within a given band was computed by taking the power in the actual frequency band. Spectral power was partitioned into power bands of very low frequency of 0.0095–0.021 Hz, low frequency of 0.021–0.052 Hz, midrange 0.052–0.145 and 0.145–0.6 Hz, and high frequency of 0.6–1.6 Hz (21). The total power was also calculated as the sum over all bands and used as a denominator to obtain normalized power in a given band.

Statistical analyses. Data in tables are presented as means ± SD. Graphical results are presented as frequency distributions for control, CDorDM, and ~CDorDM subjects. CDorDM was used rather than the sicker CD+DM group because these were not significantly different (see below) and to achieve a sufficiently large patient group. Multiple χ²-tests were used to compare these graphical data. Tabular comparisons between control and ESRD and CDorDM were performed using an unpaired t-test; multiple comparisons between subgroups of ESRD patients were performed using one-way ANOVA followed by the Tukey test. Differences were considered significant at P < 0.05. Regression analysis was performed between body mass index (BMI) or systolic blood pressure (SBP) as well as laboratory data and each measured LDF parameter.

RESULTS

The data showed no apparent dependence on the gender and age and therefore were averaged for each group of subjects.

Postocclusive Reactive Hyperemia

Figure 1 demonstrates the nonhomogeneity of perfusion best depicted in the second color inset. This inset shows distinct spots of high perfusion ("hot spots") intermixed with the areas of lower perfusion and stands in contrast to the first inset obtained before ischemia. Hot spots are areas of high Doppler shift (i.e., increased LDF) over sites of entry of cutaneous arterioles. We counted the numbers of hot spots found in a given LDPI scan before and after ischemia and the percent increase in mean perfusion during the scan in the various groups. Mean perfusion within the scanned area was found using the Perimed LDPIwin software (version 2.1). Table 2 shows that there were significantly more hot spots during the postocclusive period. Table 2 also shows that the number of hot spots pre- and postocclusion was uniformly reduced in ESRD patients compared with control subjects regardless of CD or DM status.

Turning to LDPM results, whereas the peak hyperemic flow and the posthyperemic flow AUC was not different between the ESRD and control subjects as a whole, the AUC was significantly reduced (P < .005) for CD+DM while the peak hyperemic flow was also decreased (P < 0.01) in this subgroup compared with control. Further observations emerged when data were graphically analyzed as frequency distributions, as shown in Fig. 2. The distribution of the AUC was significantly different for CDorDM compared with control (P = 0.01 by χ²-analysis). However, ~CDorDM was not different from either control (P = 0.21 by χ²-analysis) or from CDorDM (P = 0.37 by χ²-analysis).

Thermal Hyperemia

There was no significant difference in the first thermal peak (measurement d in Fig. 1) among control and ESRD subgroups. On the other hand, the nadir of thermal hyperemia (measurement e in Fig. 1) was significantly decreased in amplitude for most ESRD subgroups, whereas the second

![Fig. 1. Representative tracing of control postocclusive hyperemia (PORH) and thermal hyperemia (TH). Insets, representative laser Doppler imaging of the microcirculation, showing its nonhomogeneity and the presence of areas of high perfusion. Measurement a, peak hyperemic flow; measurement b, time to peak hyperemic flow; measurement c = area under the curve (AUC); measurement d, first thermal peak; measurement e, thermal nadir; measurement f, second thermal peak; measurement g, time to first thermal peak; measurement h, time to thermal nadir; measurement i, time to second thermal peak; FU, perfusion units. Laser Doppler scanning images obtained at baseline and at the peak of hyperemic response reveal the nonhomogeneity of skin perfusion and the existence of “hot” spots (red color on the pseudocolor images), which represent sites of enhanced perfusion near functioning arterioles.](http://ajpheart.physiology.org/)

Downloaded from http://ajpheart.physiology.org/ by 10.220.33.4 on October 1, 2017
Fig. 2. Frequency distributions for the PORH AUC. This represents the “payback response” to ischemia. Top: distribution for control subjects; middle: distribution for cardiovascular disease (CD) or diabetes mellitus (DM) (CDorDM) patients; bottom: distribution for patients with neither CD or DM (~CDorDM). The distribution of CDorDM is different from control. The distribution of ~CDorDM is neither different from control nor different from CDorDM and therefore contains elements of each. pfu, Arbitrary perfusion units.

Table 2. Post occlusive reactive hyperemia

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 33)</th>
<th>ESRD (n = 63)</th>
<th>CD (n = 20)</th>
<th>DM (n = 22)</th>
<th>CD+DM (n = 12)</th>
<th>~CDorDM (n = 33)</th>
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</thead>
<tbody>
<tr>
<td>LDPI data</td>
<td></td>
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<td></td>
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<tr>
<td>Percent increase</td>
<td>91 ± 35</td>
<td>82 ± 50</td>
<td>68 ± 40</td>
<td>85 ± 46</td>
<td>79 ± 46</td>
<td>92 ± 56</td>
</tr>
<tr>
<td>Hot spots</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preocclusion</td>
<td>7.7 ± 2.5</td>
<td>4.6 ± 2.3b</td>
<td>5.2 ± 2.9d</td>
<td>3.7 ± 1.6c</td>
<td>5.1 ± 2.2e</td>
<td>4.6 ± 2.1f</td>
</tr>
<tr>
<td>Postocclusion</td>
<td>10.1 ± 2.2</td>
<td>8.2 ± 3.3a</td>
<td>7.5 ± 2.9c</td>
<td>7.1 ± 2.9d</td>
<td>6.8 ± 2.3d</td>
<td>9.1 ± 3.5</td>
</tr>
<tr>
<td>LDPM data</td>
<td></td>
<td></td>
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<tr>
<td>Peak hyperemic flow</td>
<td></td>
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<tr>
<td>Arbitrary perfusion units</td>
<td></td>
<td></td>
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<tr>
<td>Time to peak hyperemic flow, s</td>
<td>30 ± 12</td>
<td>24 ± 17</td>
<td>22 ± 15</td>
<td>23 ± 16</td>
<td>18 ± 15e</td>
<td>27 ± 18</td>
</tr>
<tr>
<td>Preocclusion reactive hyperemic flow AUC, arbitrary perfusion units/s</td>
<td>696 ± 287</td>
<td>666 ± 704</td>
<td>473 ± 481a</td>
<td>540 ± 417</td>
<td>323 ± 330a</td>
<td>766 ± 856</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD; n, no. of patients. LDPI, laser Doppler perfusion imaging; LPDM, laser Doppler perfusion monitor; AUC, area under the curve. LDPM peak hyperemic flow is shown in measurement a in Fig. 1, time to peak hyperemic flow is shown in measurement b in Fig. 1, and postocclusion reactive hyperemic flow is shown in measurement c in Fig. 1. Significant difference compared are as follows with compared: *P < 0.01, **P < 0.001 (unpaired t-tests used for comparisons between control and ESRD); †P < 0.05, ‡P < 0.01, and §P < 0.001 (one-way ANOVA used for comparisons between subgroups and control).
Table 3. Thermal hyperemia

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 33)</th>
<th>ESRD (n = 63)</th>
<th>CD (n = 20)</th>
<th>DM (n = 22)</th>
<th>CD+DM (n = 12)</th>
<th>~CDorDM (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First thermal peak</td>
<td>81±27</td>
<td>66±51</td>
<td>61±43</td>
<td>85±80</td>
<td>64±47</td>
<td>67±32</td>
</tr>
<tr>
<td>Nadir</td>
<td>40±17</td>
<td>23±24</td>
<td>15.7±13.8</td>
<td>30±29</td>
<td>17±12</td>
<td>26±21</td>
</tr>
<tr>
<td>Second thermal peak</td>
<td>132±33</td>
<td>106±70</td>
<td>100±50</td>
<td>101±56</td>
<td>102±55</td>
<td>106±50</td>
</tr>
</tbody>
</table>

Thermal response times, s

- Time to first thermal peak: 164±44 s (Control), 210±97 s (ESRD), 202±52 s (CD), 218±59 s (DM), 213±58 s (CD+DM), 233±137 s (~CDorDM)
- Time to nadir: 282±55 s (Control), 356±1230 s (ESRD), 357±114 s (CD), 348±85 s (DM), 316±117 s (CD+DM), 373±146 s (~CDorDM)
- Time to second thermal peak: 1,426±422 s (Control), 1,662±317 s (ESRD), 1,827±171 s (CD), 1,387±445 s (DM), 1,838±101 s (CD+DM), 1,660±275 s (~CDorDM)

Data are presented as means ± SD. The first thermal peak is shown in measurement d in Fig. 1, nadir in measurement e in Fig. 1, and second thermal peak in measurement f in Fig. 1. Significant differences compared with control are as follows: *P < 0.05, †P < 0.01, ‡P < 0.001 (unpaired t-tests used for comparisons between control and ESRD); ³P < 0.05, ⁴P < 0.01, and ⁵P < 0.001 (one-way ANOVA used for comparisons between subgroups and control).

The thermal peak was decreased in ESRD patients with either CD or DM, as shown in Table 3. Again, further clarification emerged when data were graphically analyzed as frequency distributions, as shown in Figs. 3 and 4. The distribution of the amplitude of the first thermal peak shown in Fig. 3 was significantly different for CD or DM compared with control (P = 0.02 by χ²-analysis). However, ~CDorDM was not different from either control (P = 0.17 by χ²-analysis) or from CD or DM (P = 0.32 by χ²-analysis). Similarly, the distribution of the amplitude of the second thermal peak shown in Fig. 4 was significantly different for CD or DM compared with control (P = 0.025 by χ²-analysis). However, ~CDorDM was not different from either control (P = 0.10 by χ²-analysis) or from CD or DM (P = 0.69 by χ²-analysis).

Times to thermal events were generally delayed in ESRD compared with control (Table 3) including the first thermal peak (measurement g in Fig. 1), nadir (measurement h in Fig. 1), and the second thermal peak values for TH (measurement i in Fig. 1). Time to nadir data were graphically analyzed as frequency distributions in Fig. 5. The distribution was significantly different for CD or DM compared with control (P = 0.02 by χ²-analysis). However, ~CDorDM was not different from either control (P = 0.12 by χ²-analysis) or from CD or DM (P = 0.34 by χ²-analysis).

Hence, whereas clear-cut distinctions existed between CD or DM and control group, patients with CD or DM were distributed such that abnormalities in thermal hyperemia appeared in a large fraction of patients leading to frequency distributions sharing features of control and CD or DM groups. Roughly one-half of the otherwise “healthy” ESRD patients displayed the same (endothelial) abnormalities that were detected in the patients with the known CD or DM.

Relation Between Laser Doppler Parameters and Lipid Abnormalities, Blood Pressure, Hematocrit, and Obesity

In an attempt to gain pathophysiological insights into the potential significance of abnormalities in LD parameters, as related to the disturbances in systemic circulation, lipid metabolism, or anemia, we performed regression analyses between each of LDF parameters and results of clinicolaboratory testing at the time of examination. These are shown in Table 4.

Body mass index. BMI directly correlated with the increase in postocclusive perfusion; directly correlated with the amplitude of the nadir in ESRD patients with CAD, and directly correlated to the time to first thermal peak and time to nadir of TH in ESRD patients with diabetes and CD+DM.

Blood pressure. Regression analysis also revealed correlation between SBP and LDF parameters. The increase in postocclusive perfusion was directly related to SBP in ESRD as an
entire group and in ~CDorDM patients. There was an inverse relation between SBP and the number of preocclusive functioning arterioles in the entire ESRD group.

**Hematocrit.** Hematocrit was inversely correlated with the time to the second thermal peak in ESRD patients with CD, but directly correlated with time to the first thermal peak in ~CDorDM patients, and with preocclusion hot spots in CD patients.

**Lipids.** Cholesterol and LDL levels did not correlate with any LDF parameters. However, HDL directly correlated with preocclusion hot spots in CD patients and inversely correlated with time to second thermal peak in the whole ESRD group and with preocclusion hot spots in the ~CDorDM group. Triglycerides directly correlated with time to postocclusive peak in the CD+DM group.

**Fourier Analysis of Frequency Distribution of Laser Doppler Flowmetry**

A more discriminating analysis was accomplished by comparing the frequency distribution of oscillations in baseline nonhyperemic laser Doppler blood flow (LDPM) using Fourier transformation (21, 39). A more complex time-dependent analysis during hyperemic stimulation could be interesting but is beyond the scope of present investigation. Results are presented in Table 5. The values in the database represent the average spectral amplitude taken over the 10-min baseline recording period. Most notable differences occurred in the normalized amplitudes in three frequency domains: very low frequency of 0.0095–0.021 Hz, low frequency of 0.021–0.052 Hz, and high frequency of 0.6–1.6 Hz. In the 0.0095- to 0.021-Hz frequency domain, which is dependent on endothelial function (21), the normalized amplitude of oscillations in control subjects differed significantly from the ESRD group as a whole as well as from the CD, DM, and CD+DM subgroups. Although in this very low frequency domain the ESRD patients with ~CDorDM showed no differences in normalized spectral power compared with the control group, once a frequency distribution is performed as in Figs. 2–5, it is once again clear that approximately one-half of ~CDorDM patients have abnormal endothelial function measured by this index as well. In
the 0.021- to 0.052-Hz domain, the controls differed significantly from the whole ESRD group and the subgroup without \( \sim \text{CDorDM} \). At frequencies of 0.052–0.145 Hz, significant differences from control were apparent in the whole ESRD group as well as the DM group. At frequencies of 0.145–0.6 Hz, the \( \sim \text{CDorDM} \) group differed significantly from both the CD and DM groups. Finally, in the high-frequency oscillations, the 0.6- to 1.6-Hz band, reflective of the vascular wall transmission of the stroke volume (34, 35), controls differed significantly from the total ESRD group and the CD, DM, and \( \sim \text{CDorDM} \) subgroups, whereas \( \sim \text{CDorDM} \) was not different from controls but did differ significantly from the CD and DM groups.

**DISCUSSION**

Cutaneous blood flow and its recovery after ischemia have recently become employed to assess the microcirculation. PORH has been used as a tool for general assessment of vascular and metabolic reactivity. In conduit arteries, its magnitude is partially NO and prostaglandin dependent, and the time to peak value has been suggested to correlate with coronary microcirculatory dynamics (19, 20, 22). However, in the cutaneous microvasculature, NO dependence is controversial (1). Our data suggest that reactive hyperemia does depend on endothelial function but does not posit any specific biochemical mechanism that could, for example, involve prostaglandins and EDHF as well as NO-dependent responses. Thermal hyperemia has been used as a more specific means to assess endothelial function, allowing the assessment of NO-dependent vasorelaxation reported as the amplitude of the second peak (3, 17, 25). Our study uniquely combined both sets of vasomotor challenges. An additional important feature of our study was that information obtained from a spatially limited Doppler probe (LDPM) was supplemented with the data collected using a high-resolution LDPI, which scans a much larger area of the skin and allows data integration.

The data provide the following important insights into the microcirculation of ESRD: 1) testing local postocclusive and thermal microvascular responses with LDF discloses several parameters, which cocluster with ESRD, CD, and DM; 2) LDF measures of endothelial dysfunction are present in a subset of ESRD patients even in the absence of CD and DM; 3) testing the microcirculation with high-resolution LDPI reveals the functional state of cutaneous arterioles, which tend to display

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**Fig. 5.** Frequency distributions for the time to the nadir (measurement h in Fig. 1) during TH. Top: distribution for control subjects; middle: distribution for CDorDM patients; bottom: distribution for \( \sim \text{CDorDM} \) patients. The distribution of CDorDM is different from control. The distribution of \( \sim \text{CDorDM} \) is neither different from control nor different from CDorDM and therefore contains elements of each.
Table 4. Regression analysis summary for LDF subjects

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 33)</th>
<th>ESRD (n = 63)</th>
<th>CD (n = 20)</th>
<th>DM (n = 22)</th>
<th>CD+DM (n = 12)</th>
<th>~CDorDM (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperemic response</td>
<td>* + R² = 0.2225</td>
<td>* + R² = 0.4466</td>
<td>* + R² = 0.2927</td>
<td>* + R² = 0.6921</td>
<td>£ + R² = 0.1319</td>
<td>£ + R² = 0.4010</td>
</tr>
<tr>
<td>Thermal response perfusion</td>
<td>d</td>
<td>e</td>
<td>f</td>
<td>g</td>
<td>h</td>
<td>i</td>
</tr>
<tr>
<td>Thermal response times</td>
<td>* + R² = 0.2602</td>
<td>* + R² = 0.2262</td>
<td>* + R² = 0.2262</td>
<td>* + R² = 0.2625</td>
<td>* + R² = 0.2262</td>
<td>* + R² = 0.2625</td>
</tr>
<tr>
<td>LDPI scan, mean perfusion</td>
<td>* + R² = 0.06438</td>
<td>* + R² = 0.09894</td>
<td>* + R² = 0.09894</td>
<td>* + R² = 0.06438</td>
<td>* + R² = 0.09894</td>
<td>* + R² = 0.06438</td>
</tr>
<tr>
<td>Hot spots or welling points</td>
<td>Preocclusion</td>
<td>Postocclusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| LDF, laser Doppler flowmetry. a, peak hyperemic flow; b, time to peak hyperemic low; c, area under curve (AUC); d, first thermal peak; e, thermal nadir; f, second thermal peak; g, time to first thermal peak; h, time to thermal nadir; i, time to second thermal peak. *Significant correlation with BMI; †significant correlation with HDL; §significant correlation with TG; £significant correlation with Hct.

significantly prolonged periods of cessation of blood flow in ESRD patients, thus masquerading as the decreased density of arterioles; and 4) Fourier analysis of the frequency distribution of oscillations in blood flow revealed substantial differences in endothelium-dependent and -independent harmonics, as detailed below.

Perhaps one of the most revealing finding is point 3, which indicates that a significant proportion of ESRD patients without any clinical manifestations of CD or DM still display abnormalities in LDF parameters consistent with endothelial dysfunction. This may be an indication that 1) the observed findings could reflect the state of global endothelial dysfunction and/or 2) the observed abnormalities could be reflective of the state of insulin resistance, which characterizes majority of ESRD patients. Regardless of the ultimate cause, abnormalities seen in otherwise healthy ESRD patients might be viewed as an early sign for impending clinically important cardiovascular disease or diabetes in this subgroup and prompt the consideration of additional therapeutic modalities such as statins and/or
N-acetylcysteine (40). The proof of this thesis will require longitudinal analysis of morbidity and its modification by therapeutic intervention, which are currently in progress.

Several studies have already characterized macrovascular blood flow responses in ESRD patients (2, 26), but characterization of the microvascular blood flow has been lacking. Shamim-Uzzaman et al. (38) have compared brachial artery flow-mediated dilatation (FMD) with LDPM in patients with CD and demonstrated no differences in FMD between CD and control subjects, whereas there was a significant prolongation of time to peak responses in CD (17 vs. 9 s), significant decrease in PORH, and a trend toward lower AUC at 5 min in patients with CD. Thus the authors concluded that 1) no strong correlation exists between the LD-derived parameters of cutaneous microcirculatory blood flow and the plethysmographic results from conduit vessels and 2) that LD-derived time to peak of more than 10 s appeared to be the most predictive parameter for the presence of CD in the general population. In our ESRD patients, a distinct picture emerges: patients with the existing CD showed profound abnormalities in the TH response but less striking in the parameters of the PORH response. Because thermal responses are mediated by the axon reflex (first peak) and NO-dependent and -independent mechanisms of vasomotor control (second peak), these findings may implicate sympathetic nervous system and endothelium-dependent vasomotion in the observed abnormalities of LDF parameters in ESRD patients. Indeed, autonomic dysfunction in ESRD, both parasympathetic and sympathetic, as well as endothelial dysfunction have been well documented (6, 34). Taking into consideration previous mechanistic studies (17, 25), our data strongly implicate endothelium- and NO-dependent and -independent vascular control in the observed abnormalities in ESRD patients.

In the present study, high-resolution LDPI revealed, for the first time, the functional state of cutaneous arterioles, which tend to display significantly prolonged periods of closed state and cessation of blood flow associated with an impaired postocclusion recruitment in ESRD patients. The apparent "rarefaction" of functional dermal arterioles in patients with ESRD compared with control population may reduce oxygen delivery to the tissues. Interestingly, somewhat similar findings have been obtained using nail fold capillaroscopy (15) in patients with cardiac allograft vasculopathy: investigators have found reduced red blood cell velocity under resting conditions, loss of postischemic reactive hyperemia, and spontaneous temporary cessation of cutaneous blood flow. Similarly, De Backer et al. (8), using orthogonal polarization spectral imaging of the sublingual microcirculation, reported that the density of vessels and the proportion of perfused vessels are reduced in patients with sepsis. Irving et al. (11) have studied capillary density on the dorsum of a finger and demonstrated that insulin resistance and high blood pressure were associated with lower capillary density and impaired increase in the blood flow after heating. Hypertensive subjects with reduced insulin sensitivity displayed deficient capillary recruitment after arterial occlusion as well as defective acetylcholine-mediated and insulin-mediated vasodilation (36).

To determine the origin of the observed decrease in the apparent arteriolar density in patients with ESRD, we considered the possibility that spontaneous pacemaker-like vasoreactivity at the terminal precapillary vascular bed may be impaired. Fourier analysis was employed to resolve the contribution of vascular endothelium to this activity. Oscillations of microvascular blood flow are induced by the heart and respiratory rate, sympathetic tone (higher frequencies), and the intrinsic very-slow-frequency endothelium-dependent pacemaker tone (21, 39). Fourier analysis of the frequency domains in our patient population revealed important hitherto hidden information: endothelium-dependent very-low-frequency oscillations were capable of discriminating between patients without preexisting CD and/or DM from ESRD patients with no clinical manifestations of CD, suggesting that this type of regulation of microvascular perfusion is most profoundly perturbed in the former subgroups of patients.

Another aspect of this study is the search for relation of laser Doppler-determined parameters of blood flow regulation and the levels of lipidemia, anemia, and obesity in ESRD patients. Interestingly, none of these parameters showed correlation with cholesterolemia or levels of LDL, but the level of HDL exhibited a strong correlation with the time of the first thermal peak, the amplitude of the first thermal peak, and the nadir of thermal hyperemia only in patients with diagnosed CD. In patients with CD, correlation existed between the hematocrit and the timing of the second thermal peak. The same group of patients, with or without DM, was characterized by the tight relation between the triglyceridemia and the amplitudes of first and second thermal peaks. BMI correlated with the amplitude of the first thermal peak and with the nadir of thermal hyperemia in patients with CD. These findings further emphasize the importance of controlling anemia, hyperlipidemia, and obesity in vasomotion (1), yet their relation to parameters of LDF seem to be much more complex.

A limitation to our study was the inclusion of cigarette smokers in both ESRD and control groups who were asked to abstain from tobacco use for only 3 h. Cigarette smoking may affect endothelial function. Future studies will employ a longer period of abstention and partitioning of subject groups by tobacco use as a confounding variable.

In conclusion, LDPM and LDPI, followed by Fourier analysis of the very slow oscillations, appear to provide valuable noninvasive tools for diagnosing abnormalities in the regulation of the microcirculation. This technique may be especially valuable for the early detection of ECD and impending CD or DM in ESRD patients with no previously known CD or DM, who are nonetheless at high risk for developing clinical manifestations of vasculopathy and could potentially benefit from a prophylactic therapy with statins, angiotensin-converting enzyme inhibitors, and/or antioxidants (40). The data also demonstrate that the entire cohort of ESRD patients is characterized by distinct abnormalities in LDF parameters. Future studies should also validate LDPM/LDPI as a potentially useful tool for noninvasive assessment of the efficacy of therapeutic interventions designed to reduce microvascular complications in ESRD patients.

GRANTS

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