Altered cutaneous microvascular responses to reactive hyperaemia in coronary artery disease: a comparative study with conduit vessel responses

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ABSTRACT
Cutaneous microvascular responses to physiological stimuli are currently being investigated as indices of vascular function and to monitor responses to therapy. We attempted to systematically assess various microvascular cutaneous flow indices in response to reactive hyperaemia in control subjects and in patients with coronary artery disease (CAD), and to correlate these with brachial artery flow-mediated dilation (FMD). Groups of 24 healthy controls and 24 subjects with CAD underwent sequential brachial FMD determination in the dominant arm, and laser Doppler imaging to assess skin blood flow in the contralateral arm in response to reactive hyperaemia induced by cuff inflation and release. Laser Doppler values were expressed as: (a) AUC5 min (area under the curve over 5 min of release), (b) time to peak response, (c) % reactive hyperaemia, and (d) peak perfusion ratio. As expected, FMD was attenuated in CAD patients compared with controls (1.85±4.29% compared with 4.30±4.00%; P<0.05). Percentage reactive hyperaemia (CAD, 294±290%; controls, 501±344%; P=0.04) and the time to peak response as measured by laser Doppler imaging (CAD, 16.84±9.61 s; controls, 9.13±4.43 s; P=0.001) were significantly different between the CAD and control groups, while AUC5 min and the peak perfusion ratio did not show significant differences. Receiver operator curves for sensitivity/specificity analysis suggested that the time to peak response derived by laser Doppler imaging was superior to FMD for the diagnosis of CAD, with an overall specificity of 91.3% (positive predictive value of 89.4%) and a sensitivity of 73.7% (negative predictive value of 77.6%). In conclusion, laser Doppler-derived indices of microvascular flow do not correlate with conduit vessel responses. However, a time to peak response of >10 s as measured by laser Doppler imaging offers superior specificity for the diagnosis of CAD compared with brachial FMD.

INTRODUCTION
The structure and function of blood vessels and the mediators of flow in the vessels differ according to the size of the vessels, their hierarchy in the circulatory system, the risk factors that the vessels are exposed to and the agonists used to assess their function in experimental protocols. The overall response in any circulatory bed

1These authors contributed equally to this work.
Key words: endothelium, laser Doppler, nitric oxide, reactive hyperaemia.
Abbreviations: AUC5 min, area under the curve over 5 min of release; CAD, coronary artery disease; FMD, flow-mediated dilation; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ROC, receiver-operating characteristic.
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depends on a composite of these factors, and it is therefore not surprising that some studies have found concordance between conduit artery and resistance vascular function, while others have not [1–4]. The relationship between conduit endothelial function in the brachial artery and the resistance circulation of the skin is similarly not clearly understood. A number of studies have utilized laser Doppler perfusion imaging techniques to evaluate cutaneous vessel function [5–9]. The sensitivity, specificity and reproducibility of these techniques for the diagnosis of vascular disease and the relationship with non-invasively determined measures of peripheral conduit (brachial) endothelial function using ultrasound is unknown. Accordingly, we have determined various laser Doppler-derived parameters of skin flow to reactive hyperaemia and compared these with traditional brachial artery ultrasound-derived measures of endothelial function in control subjects and in patients with coronary artery disease (CAD).

METHODS

Study subjects
The study protocol was approved by the Institutional Review Board of the University of Michigan. Subjects were recruited in a prospective fashion through advertisements to undergo these studies at the non-invasive vascular research laboratory based at the Clinical Research Center. Two groups of subjects were studied: a control group and a group with CAD. All subjects gave informed consent.

The control group consisted of 24 healthy individuals (12 adult males and 12 non-pregnant, non-lactating female volunteers), comprising employees belonging to the University of Michigan. Exclusion criteria included: (1) smoking; (2) presence of diabetes (fasting blood glucose > 110 mg/dl); (3) hypertension (systolic blood pressure > 140 mmHg and/or diastolic blood pressure > 90 mmHg) or drug therapy for hypertension; (4) hyperlipidaemia (total cholesterol > 240 mg/dl); (5) history of CAD or peripheral vascular disease; (6) family history of premature CAD.

The CAD group comprised 15 men and nine women who had established CAD. The diagnosis of CAD was defined as the presence of angiographic CAD on a cardiac catheterization and/or the presence of an ischaemic defect on a pharmacological stress modality. Patients in the CAD group were recruited from the cardiology clinics at the University of Michigan, as well as including subjects participating in an ongoing study (CATS – Coronary Artery Disease Alternative Treatment Strategies). As one would expect, all patients in the CAD group received aggressive risk factor modification, including low-density lipoprotein (LDL)-lowering therapy (according to NCEP/ATP III guidelines) and treatment of concomitant risk factors for at least 6 weeks prior to participation in the study. Of the CAD patients, 88% were on acetylsalicylic acid (aspirin) therapy, 83% were on some form of lipid-lowering therapy (statins 67%, niacin 8%, fibric acid derivatives 8%, bile acid sequestrants 6%), 58% were on β-blockers, 67% were on angiotensin-converting enzyme inhibitor therapy, 29% received a diuretic for the treatment of hypertension, and 65% were on nitrates for angina. Four of the nine women (44%) were on hormone replacement therapy. None of the patients had heart failure. In addition, patients in this group also underwent therapeutic lifestyle changes that included dietary counselling, exercise rehabilitation and stress reduction therapies, including meditation. Ongoing smoking was an exclusion criterion.

Brachial artery vasoreactivity studies
All studies were conducted in a darkened, temperature-controlled room, with the temperature set at 20°C (68°F), between 08:00 and 12:00 hours. Most studies were performed after an overnight fast, and in a minority of subjects after a 4-h fast in the morning. All morning medications were withheld prior to the studies.

Flow-mediated dilation (FMD) of the brachial artery was determined from two-dimensional ultrasound images according to established and validated methodology [10,11]. Images were obtained with a Hewlett Packard 10 mHz linear array transducer and an HP Image Point ultrasound system (Hewlett Packard, Andover, MA, U.S.A.). Imaging was performed with the subject resting quietly in a supine position for at least 10 min in a light- and temperature-controlled room. After baseline measurements of brachial artery diameter, a blood pressure cuff was inflated to 50 mmHg above systolic pressure over the proximal portion of the dominant forearm for 4 min. FMD was determined 1 min after release of the cuff. Brachial artery diameter was then allowed to return to baseline over a period of 15 min. Endothelial-independent responsiveness was evaluated with 0.4 mg of nitroglycerin administered sublingually. Brachial artery images were obtained 3 min after nitroglycerin administration.

Two triggered events (occurring after the peak of the R wave on the ECG) for each intervention were recorded and acquired through a frame grabber attached to a computer. Each triggered event consisted of six sequential frames. The media-adventitia interface in a linear portion of the vessel was chosen for analysis. The end point of measurement was the percentage change in diameter in response to reactive hyperaemia (FMD) or to nitroglycerin-mediated dilation. For follow-up studies, the images from the subject’s initial study were recalled on a Matrox secondary monitor (Matrox, Quebec, Canada) and the vessel wall segments were matched.
Laser Doppler studies
Laser Doppler studies were performed using a Lisca laser Doppler imager (PIM II system; Lisca AB, Linköping, Sweden) on the non-dominant hand 10 min after the brachial reactive hyperaemia studies. Briefly, a cuff was applied to the subject’s non-dominant forearm. The hand and middle finger were taped flat in the prone position to an examination table. After positioning the laser beam 15 cm above the centre of the proximal phalanx of the middle digit, baseline measurements were obtained over 2 min (Doppler wavelength 960 nm). Baseline perfusion was expressed in perfusion units as the mean of three to six measurements. Each measurement was performed over 960 ms in an area measuring 16 mm². The cuff was then inflated to a pressure that was 50 mmHg above the subject’s resting systolic blood pressure and held for 4 min, at the end of which the pressure was released. Serial laser Doppler scans were obtained immediately on release and at 5 s intervals for the first 20 s, 20 s intervals for the next 3 min, and 1 min intervals for an additional 2 min (total of 5 min post-release). Reactive hyperaemia measures were expressed as follows. (a) AUC₃₃₅ₖₘₜ, defined as the area under the curve of perfusion measurements (adjusted for baseline) obtained over a 5 min period at pre-defined time points (described above). (b) Time to peak response, i.e. the time to attain maximal cutaneous perfusion. (c) Peak perfusion ratio: the peak perfusion value obtained with reactive hyperaemia was divided by baseline perfusion in each patient to yield the peak perfusion ratio (in arbitrary units). (d) Percentage reactive hyperaemia. This was calculated as: [ (peak perfusion−baseline perfusion)/ baseline perfusion] × 100.

Statistics
All values are expressed as means ± S.D. Group differences in baseline variables, laser Doppler indices and brachial artery studies between controls and CAD patients were compared using two-tailed unpaired t tests. Significance was defined by P < 0.05. Univariate analyses were performed using Pearson correlation coefficients, and multivariate models were constructed using general linear regression analyses. Receiver-operating characteristic (ROC) curves were constructed for sensitivity/ specificity analysis. ROC analysis was used with calculation of AUC [12]. All statistical analyses were performed using Graph Pad Prism statistical software (version 3.02; Graph Pad, San Diego, CA, U.S.A.) and MedCalc software (version 6.01.001).

RESULTS

Baseline clinical characteristics
Table I lists the baseline characteristics of the subjects. As one would expect, age, weight, body mass index, systolic blood pressure and plasma glucose were higher in the CAD group, while high-density lipoprotein (HDL) cholesterol values tended to be lower, compared with the control cohort (P < 0.05 for all variables by unpaired t test). LDL cholesterol values were marginally lower in the CAD group compared with the control group, reflecting aggressive treatment in this cohort, while triacylglycerols tended to be higher in the CAD group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls</th>
<th>CAD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>40.04 ± 15.52</td>
<td>64.67 ± 9.33*</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.71 ± 0.10</td>
<td>1.72 ± 0.10</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.66 ± 11.37</td>
<td>90.91 ± 24.62*</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.85 ± 3.03</td>
<td>30.77 ± 6.96*</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>115.50 ± 11.51</td>
<td>128.60 ± 21.12</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>66.61 ± 7.29</td>
<td>66.13 ± 9.35</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>184.94 ± 39.32</td>
<td>164.00 ± 46.65</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>97.95 ± 70.58</td>
<td>140.40 ± 72.50</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>55.65 ± 15.54</td>
<td>41.08 ± 15.58*</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>112.50 ± 26.94</td>
<td>93.63 ± 37.16</td>
</tr>
<tr>
<td>VLDL cholesterol (mg/dl)</td>
<td>21.87 ± 15.15</td>
<td>31.28 ± 15.25</td>
</tr>
<tr>
<td>Total/HDL cholesterol</td>
<td>3.50 ± 1.04</td>
<td>6.10 ± 9.23</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>88.70 ± 8.08</td>
<td>109.70 ± 29.13*</td>
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Table 2 Brachial artery ultrasound-derived conduit vessel measurements compared with cutaneous responses measured by laser Doppler perfusion imaging in CAD patients and control subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>CAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brachial artery measurements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline diameter (mm)</td>
<td>3.89 ± 0.60</td>
<td>4.38 ± 0.93 *</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>4.30 ± 4.00</td>
<td>1.85 ± 4.29 *</td>
</tr>
<tr>
<td>Nitroglycerin-mediated dilation (%)</td>
<td>18.58 ± 7.12</td>
<td>14.60 ± 8.57</td>
</tr>
<tr>
<td>Laser Doppler microvessel measurements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline flow (perfusion units)</td>
<td>0.30 ± 0.29</td>
<td>0.26 ± 0.19</td>
</tr>
<tr>
<td>AUC5 min (arbitrary units)</td>
<td>595 ± 417</td>
<td>429 ± 199</td>
</tr>
<tr>
<td>Reactive hyperaemia peak perfusion ratio (arbitrary units)</td>
<td>5.11 ± 3.6</td>
<td>3.75 ± 2.95</td>
</tr>
<tr>
<td>Time to peak response (s)</td>
<td>9.13 ± 4.43</td>
<td>16.84 ± 9.61 **</td>
</tr>
<tr>
<td>Reactive hyperaemia (%)</td>
<td>501 ± 344</td>
<td>294 ± 290 *</td>
</tr>
</tbody>
</table>

Conduit vessel responses in treated CAD patients

Despite aggressive LDL reduction according to NCEP guidelines in the CAD group for at least 6 weeks prior to undertaking these studies, brachial FMD was attenuated (CAD patients, 1.85 ± 4.29%; controls, 4.30 ± 4.00%; \( P = 0.05 \); Table 2). In contrast, endothelium-independent dilation to nitroglycerin was not different between CAD patients and controls (14.60 ± 8.57% and 18.58 ± 7.12% respectively; \( P = 0.10 \); Table 2).

Cutaneous microvascular responses in treated CAD patients

Baseline perfusion in the CAD patients as assessed by laser Doppler perfusion was not significantly different from that in the control subjects (0.26 ± 0.19 and 0.30 ± 0.29 perfusion units respectively; \( P = 0.61 \)). The extent of the cutaneous vasodilatory reserve in CAD patients, as measured by AUC5 min, was not significantly altered compared with that of controls (CAD patients, 429 ± 199 units; controls, 595 ± 417 units; \( P = 0.09 \) by \( t \) test). Figure 1 demonstrates the average responses over time in controls and CAD patients. Table 2 summarizes the various microvascular perfusion indices. Time to peak response was roughly doubled in CAD patients (16.84 ± 9.61 s) compared with control subjects (9.13 ± 4.43 s; \( P = 0.001 \)), indicating markedly altered responsiveness in the CAD patients. There was a statistically significant decrease in the percentage reactive hyperaemia response in CAD patients (294 ± 290%) compared with controls (501 ± 344%; \( P = 0.04 \)). Peak perfusion ratios showed no significant difference between the CAD patients and control subjects (3.75 ± 2.95 and 5.11 ± 3.60 arbitrary units respectively; \( P = 0.17 \)).

Sensitivity and specificity analysis for laser Doppler measurements compared with FMD

ROC curves were constructed for sensitivity and specificity analysis of brachial artery ultrasound-derived FMD and laser Doppler measures (time to peak response and AUC5 min) (Figure 2). FMD was associated with an overall sensitivity and specificity of 71.4% and 73.9% respectively for the diagnosis of CAD. The positive and negative predictive values were 73.2% and 72.1% respectively. In contrast, time to peak response was more specific (91.3%) than FMD, with similar sensitivity (73.7%). Positive and negative predictive values were 89.4% and 77.6% respectively (Figure 2, upper panel). The cut-off value associated with the time to peak response was 10 s. AUC5 min as an index of microvascular perfusion was non-specific, but offered comparable sensitivity to FMD and time to peak response (sensitivity...
Cutaneous microvascular function in coronary artery disease

Correlation between conduit and microvascular function

Simple Pearson correlation coefficients were calculated between FMD and two laser Doppler-derived measures (AUC$_{5 \text{min}}$ and time to peak response) as indices of microvascular endothelial function (Figure 3). There was no correlation between FMD and either of the laser Doppler measures.

Determinants of conduit and microvascular function in subjects

On univariate analysis, age was the only significant determinant of time to peak response (Pearson correlation coefficient = 0.30, $P < 0.001$), but was only a weak predictor of baseline FMD (Pearson correlation coefficient = 0.08, $P = 0.05$). Systolic blood pressure and HDL were weak predictors of AUC$_{5 \text{min}}$ responses, but not time to peak response or FMD. On a multivariate model, which included systolic blood pressure, total cholesterol and HDL, systolic blood pressure continued to be correlated significantly with AUC$_{5 \text{min}}$ ($P = 0.04$).

DISCUSSION

The key observations of the present study are the following: (1) laser Doppler-derived measures of cutaneous blood flow in response to reactive hyperaemia do not correlate with conduit vessel responses; (2) the discriminatory ability of laser Doppler-derived measures for CAD is comparable with that of FMD obtained by brachial artery ultrasound; and (3) a laser Doppler-derived time to peak response of $>10$ s appears to be the most predictive among various microvascular measures for the presence of CAD or a higher age profile.

To our knowledge, this is the first descriptive account of the comparative abilities of laser Doppler-derived skin perfusion indices and brachial artery responses to a reactive hyperaemic stimulus of a similar magnitude in patients with CAD and in a pre-specified control population. Dissimilar stimuli used for probing endothelial function in various circulatory beds may account for the lack of correlation between conduit and resistance vessels, at least in some circulatory beds [4].
this variable, we were able to systematically assess the relationship between small and large vessels. There was no relationship between changes in conduit endothelial function and those in the cutaneous microcirculation. This finding is consistent with emerging data indicating that the regulators of microvascular function, in contrast with those in conduit vessels, are not nitric oxide-dependent [13–16]. These differences are likely to have accounted for the lack of correlation. Furthermore, on repeat testing in a cohort of control subjects, this appeared to be a reproducible measure, in keeping with earlier reports [17]. Age was the single consistent predictor of both conduit and cutaneous microvascular responses in univariate analysis, suggesting that it exerts a powerful influence on the responsiveness of the vasculature, independent of size. Indeed, it would be impossible to dissociate the effects of aging from those of CAD in the present study, and this could potentially account for some or all of the differences. Systolic blood pressure in our series did not correlate with brachial FMD, but was the sole correlate of AUC_{\text{min}} that persisted on multivariate analysis. The reasons for this are unclear.

The CAD patient population selected to participate in the present study is representative of patients seen at a tertiary care centre. The majority of subjects were on risk factor reduction therapies, and mean LDL levels and blood pressures were well within JNC VI and NCEP guidelines. As expected, the CAD subjects were older and had additional ongoing risk factors that were less satisfactorily addressed, such as obesity, hyperglycaemia, lower HDL levels and a trend towards higher triacylglycerol levels. These concomitant factors have all been shown to affect conduit vessel endothelial responses, and could have played a role in the differences in conduit endothelial parameters noted between control subjects and those with CAD, despite maximal LDL reduction. As noted by previous groups, brachial artery diameters appeared to be a reproducible measure, in keeping with emerging data indicating that the regulators of microvascular function, in contrast with those in conduit vessels, are not nitric oxide-dependent [13–16]. These differences are likely to have accounted for at least some of the differences noted between the groups [11]. The sensitivity and specificity of FMD measures in our patient population were comparable with those in previously published reports [11,18]. Baseline perfusion as determined by laser Doppler was highly variable and not different between the two groups. Time to peak response was, however, strikingly different, despite comparable AUC_{\text{min}} values. A time to peak response of >10 s was better than FMD in predicting CAD in an older patient population. A simple peak perfusion ratio (peak perfusion/baseline perfusion) was not a useful discriminator; however, once peak perfusion values were corrected for baseline perfusion values, as reflected in the percentage reactive hyperaemic response, differences were again noted between the two cohorts. It is interesting that we continued to see differences between the two groups in the above measures despite aggressive risk factor reduction therapies, and this is likely to reflect the effects of the age difference between the two groups, a factor well known to influence endothelial function [19].

The present study was not designed to control for all the variables that could account for differences across the two groups, but rather to address the utility of two techniques in two discreetly different populations. One could argue that even had we chosen to find control subjects in the same age bracket as that of patients with CAD, without evidence of overt atherosclerosis, these individuals are likely to have harboured evidence of covert atherosclerosis [20].

In conclusion, there is no relationship between changes in conduit function and those in the cutaneous microcirculation either in control subjects or in a treated cohort of CAD patients. Laser Doppler-derived time to peak flux was more specific than brachial FMD in predicting the presence of CAD. These findings have implications for the use of these measures as clinical indices of vascular function.

REFERENCES


