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BRIEF COMMUNICATION

Laser–Doppler Imaging of Forearm Skin: Perfusion Features and Dependence of the Biological Zero on Heat-Induced Hyperemia

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INTRODUCTION

When measuring skin blood perfusion with laser–Doppler methods in a limb, a nonzero perfusion is sometimes registered even when the arterial inflow to, and venous outflow from, the site of measurement is arrested using proximal cuff compression pressures well in excess of systolic blood pressure (Wahlberg *et al.*, 1992; Abbot and Beck, 1993; Zhong *et al.*, 1998; Kernick *et al.*, 1999; Mayrovitz *et al.*, 1999). This residual signal is referred to as the biological zero (BZ) and is present with both laser–Doppler flowmetry (LDF) (Caspary *et al.*, 1988) and laser–Doppler imaging (LDI) (Kernick and Shore, 2000). The BZ thus contributes a confounding background signal that is not directly related to the perfusion within blood vessels. Features of the BZ, based on single-point laser–Doppler studies show that the confounding effects of the BZ are most important when measuring skin areas with relatively low blood perfusion. However, at least two issues remain unresolved. The first concerns the magnitude of the BZ that is associated with the use of laser–Doppler imaging. The perfusion profile obtained with LDI incorporates far more information than the single point method and at present there is sparse data available on the magnitude of the BZ with this method. Second, it is unclear as to the extent to which the magnitude of the BZ depends on the magnitude of the blood perfusion present while the BZ determination is made. Herein, we determined the magnitude of the LDI-BZ and its relationship to various blood perfusion levels associated with local skin heating of volar forearm skin.

METHODS

Subjects

Thirty postmenopausal women who were part of a larger cardiovascular study participated in this separate microvascular study and were evaluated after signing an institutionally approved informed consent. Average age (mean ± SD) was 61.4 ± 7.4 years and the group had been postmenopausal for an average of 14.4 ± 8.6 years. None of this group were taking

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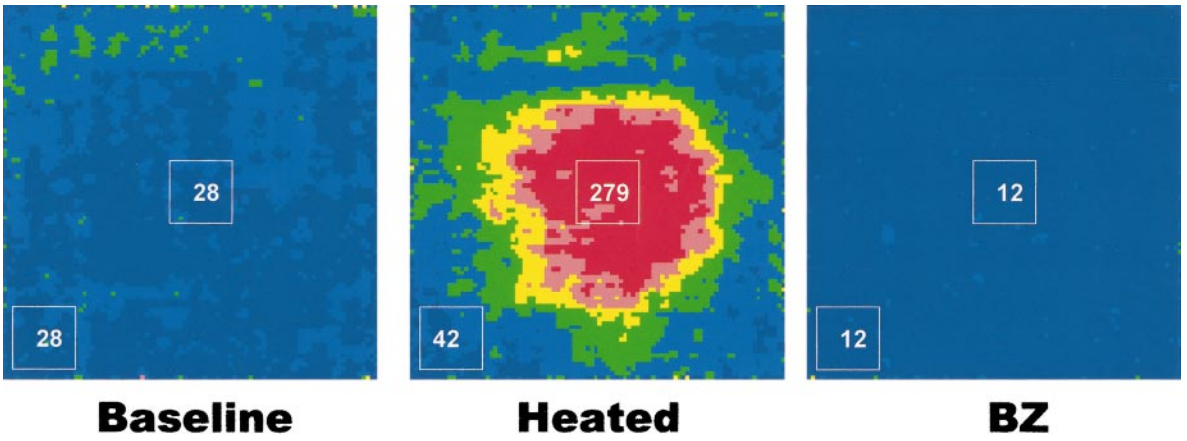

FIG. 1. LDI scan sequence and images. A baseline scan is followed by a heat scan initiated after 2 min of heating at 44°. Blood flow is then arrested with an arm cuff for 4 min. The BZ scan is initiated during the flow arrest starting 1.5 min after cuff inflation. The boxes illustrate two of the areas used for analysis. The central one surrounds the center of the heating site midpoint and the one in the corner of each image is the distant area. The numbers inside of the boxes are the average perfusions in a.u. for this subject. Red is the highest perfusion indicator and dark blue is the lowest. The gradient in perfusion away from the center may be seen in the heat scan image.

hormone replacement therapy. Overall, the group was considered normotensive, with systolic and diastolic blood pressures of 139.2 ± 14.9 and 78.7 ± 7.9 mm Hg, respectively.

Initial Procedures

Subjects were studied while supine, starting after they had been quietly resting for 20 min. The skin target area for LDI was a standardized 7.3 cm^2 rectangular area, with its center point midway between the antecubital space and the wrist on the right volar forearm. Occasionally, the target site was slightly shifted to avoid including large veins. Blood pressure cuffs were placed on right and left arms and blood pressure was measured using the left arm. The right arm cuff was used later to produce a suprasystolic occlusion (systolic + 50 mm Hg) to obtain the BZ. A laser–Doppler imager (Moor Instruments) was positioned so that the center of its scan was at the center of the target area. In all cases the vertical distance from the skin surface to the LDI head was 30 cm and all scans were done at 4 ms pixel and a 0.250- to 15-KHz bandwidth. The scan area encompassed the target area.

Measurements and Sequence

Three LDI scans were done on each subject: a baseline for nonheated skin, a heat scan that was initiated immediately after the central portion of the target area was heated to 44° for 2 min (circular heater with a heating area of 1.1 cm^2), and a third scan, the BZ scan, was initiated at $1\frac{1}{2}$ min into a 4-min suprasystolic arm cuff occlusion, which was started immediately after completion of the heat-scan (Fig. 1).

Analyses

The mean perfusion within the target area was analyzed for nonheated, heated, and occluded (BZ) conditions using progressively increasing concentric areas (0.5, 1, 1.5, 2, 3, 4, 5, and 7 cm^2) that surrounded the midpoint of the heated area. In this way a range of spatially averaged blood perfusion magnitudes were obtained from each heat scan. For each of these areas, mean perfusion was determined and compared between nonheated, heated, and occluded conditions. The BZ was determined in relation to the heated perfusion for each area. In addition, for baseline and BZ scans, a separate 0.5 cm^2 area, that was approximately 2 cm distant from the center of heat area, was used to estimate BZ in relation to nonheated baseline perfu-

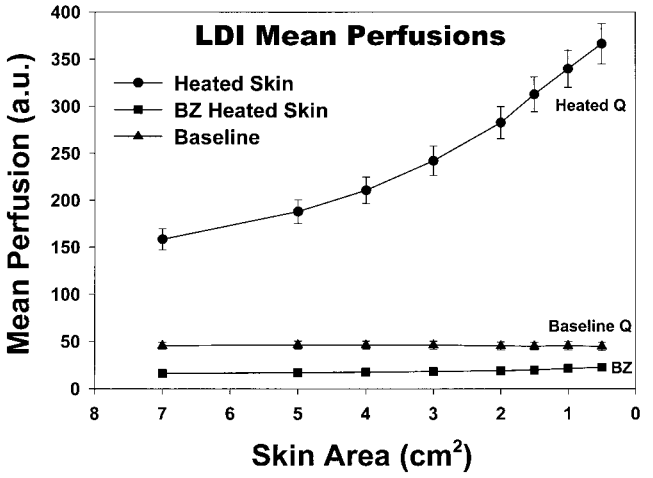

FIG. 2. Mean LDI perfusion. Perfusion ($Q \pm$ SEM) is shown as a function of sampled area for baseline, heated, and BZ scans. Note that heated skin Q was least for the largest sample area and greatest for the smallest area surrounding the heat application midpoint. Baseline Q was independent of sample area. Although not well visualized at this scale, the BZ of heated skin increases, reaching its maximum at the central smallest area.

sion. This area is referred to as the distant area. Values in the text are reported as mean ± SD.

RESULTS

Forearm Perfusion Levels

Comparisons of absolute mean LDI perfusion (Q in a.u.) shows that baseline Q was essentially independent of sample area (Fig. 2), with an average value across all subjects and areas of 45.6 ± 22.4 a.u. Heated skin Q was least (158.3 ± 61.4 a.u.) for the largest sample area and greatest (366.3 ± 117.3 a.u.) for the smallest area surrounding the center of the heat application zone. For the BZ of heated skin, the lowest value (16.3 ± 6.2 a.u.) was achieved for the largest area and the largest value (22.6 ± 17.3 a.u.) for the central area. When perfusion values were normalized to the values obtained using the largest area, a progressive increase in both Q and BZ occurs as smaller concentric sample areas are used (Fig. 3). Overall, the heat-induced perfusion within the central area increased above baseline by a factor of 10.2 ± 5.8 and the distant area Q increased by a factor of 2.2 ± 0.4. The relationship between BZ and Q was also evaluated separately for each subject, and in 24 of the 30 subjects (80%) this relationship was highly significant ($P < 0.001$). In the other 6 subjects BZ did not significantly change.

Relationship of BZ to Heated Perfusion Levels

Although both Q and BZ individually increased with decreasing area, Q increased by a greater relative amount causing the ratio (BZ / Q) to decrease (Fig. 4). The smallest ratio (6.6 ± 4.6%) occurred for the greatest heated perfusion (central area), whereas the largest (11.8 ± 5.8%) occurred for the least heated perfusion (largest area). Overall, the relationship between BZ and Q is described by the linear regression as $BZ = 0.029Q + 11.5$ a.u. ($r^2 = 0.969$, $P < 0.0001$; Fig. 5).

Relationship of BZ to Baseline Perfusion Levels

Within the distant sample areas, BZ was 36.0 ± 13.3% of the baseline perfusion within the same sample area (11.9 ± 3.3 a.u. vs 38.1 ± 19.5 a.u., $P < 0.001$). Baseline perfusion in the distant area (0.5 cm^2) was insignificantly different from the full 7 cm^2 baseline

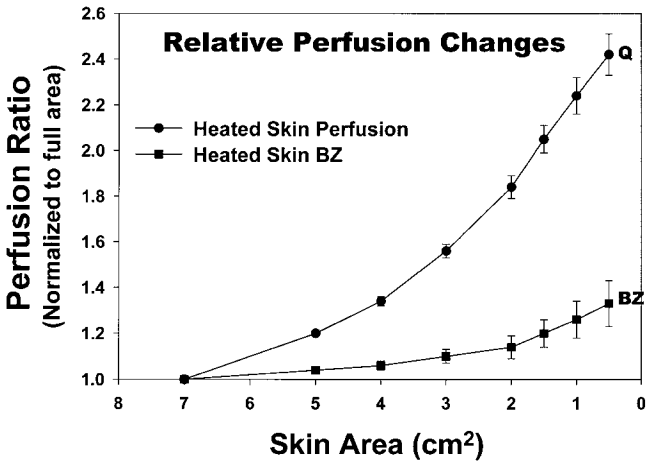

FIG. 3. Relative perfusion and BZ changes. Mean perfusion values are shown normalized to the mean perfusion values within the largest sampled area (7 cm^2). The progressive increase in both Q and BZ, which is here well seen, occurs as sample area decreases.

area (44.8 ± 25.1 a.u.). When the distant area was analyzed for the heat-scan, the BZ fraction in relation to baseline was less (23.7 ± 14.2%) due to a higher perfusion in the heat-scan area compared with corresponding baseline areas (38.1 ± 19.0 a.u. vs 74.5 ± 56.5 a.u., $P < 0.001$). Interestingly, the baseline BZ value (11.9 ± 3.3 a.u.) is strikingly close to that predicted by the regression equation constant (11.5 a.u.) even though this prediction is based only on heated perfusion data.

DISCUSSION

Although the source of BZ is still under investigation, recent work suggests that Brownian motion of macromolecules within the interstitium plays an important role (Kernick *et al.,* 1999). These authors indicate that BZ adds to the perfusion signal but that the relationship between BZ and perfusion levels depends on the extent to which the perfusion levels affect interstitial conditions. Local tissue heating, which effects both perfusion and Brownian motion, might thus differentially affect BZ and its relationship to perfusion. The present results show that local tissue heating causes a progressive and statistically significant increase in BZ that parallels the blood perfusion increase. However, the BZ increase is slightly less than 3% of each unit increase in heat-induced perfusion. Over the full range of heat-induced hyperemia (158.3 ± 61.4 to 366.3 ± 117.3 a.u.), which is a 2.2 ± 0.4 to 10.2 ± 5.8 increase from baseline, the BZ increased from 16.3 ± 6.2 to 22.6 ± 17.3 a.u., respectively. These results are consistent with those obtained using LDF during hyperemic inflammatory reactions (Abbot and Beck, 1993) and suggest that for procedures in which LDI is used to assess acute blood perfusion increases, the confounding effect of BZ over this range results in a relatively small error. Thus assessments of hyperemic perfusion levels, when not expressed relative to baseline, may be suitably adjusted for BZ by subtracting the BZ as determined at any perfusion level.

Contrastingly, BZ was found to be 36.0 ± 13.3% of the average baseline perfusion and, although considerably less than the 65.2% value reported using a different LDI system (Kernick and Shore, 2000), it is still a substantial fraction. This indicates that when possible, adjustments for BZ should be made for all cases in which baseline perfusion is of direct interest and also in cases in which baseline perfusion is used as a normalizing factor for assessing changes.

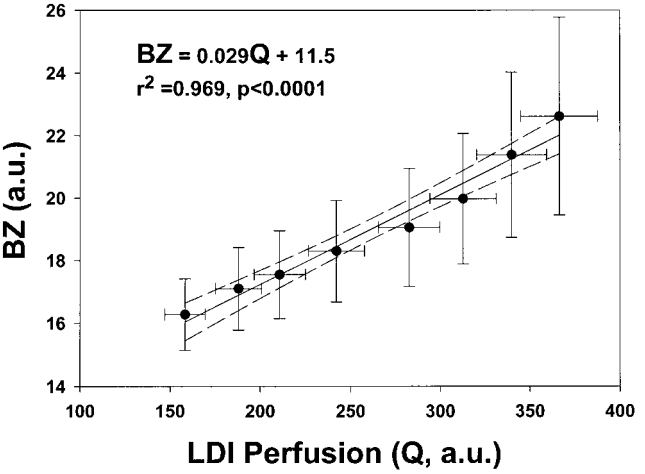

FIG. 5. Relationship between biological zero and perfusion. Points are mean values for $N = 30$ subjects and error bars are ±1 SD. Solid line is regression equation with parameters shown in the figure. Dashed lines are 95% confidence bands.

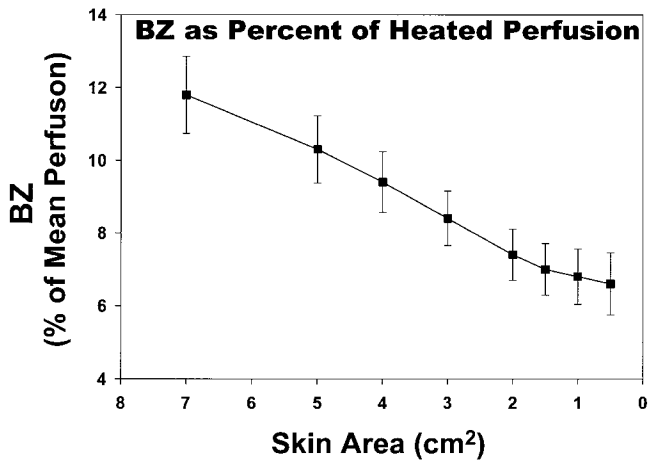

FIG. 4. Biological zero as percentage of heated perfusion. The BZ expressed as a percentage of the mean perfusion within corresponding sampled areas decreases at higher perfusion areas.

CONCLUSIONS

The BZ of LDI increases with heat-induced blood perfusion hyperemia although the amount of the increase is small relative to blood perfusion changes that occur. This fact thereby allows its confounding effect to be handled by simple subtraction of a single BZ value. The BZ value may be obtained during or after the hyperemia with only a small error when quantifying absolute hyperemic perfusion. However, if the hyperemic response is to be characterized in terms relative to resting baseline, then the BZ of the resting state should be used to avoid a possible significant overestimation of the response. A single BZ value so obtained may be applied to the full response. For resting blood perfusion assessments in forearm skin, and likely other regions with relatively low basal perfusion, use of the BZ as an adjustment to raw perfusion data is indicated when comparisons of resting perfusion among groups or treatments are undertaken. In situations in which such BZ determinations cannot be made or, for clinical reasons, should not be made, then detection of differences between groups is limited by the BZ confounding effect.

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