Variability in skin microvascular vasodilatory responses assessed by laser-doppler imaging

THE SYMPOSIUM ON ADVANCED WOUND CARE

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Abstract

Skin blood perfusion (SBP) responses to pressure loading and other traumatic and noxious stimuli are used to help identify patients at–risk of skin breakdown, evaluate preventive strategies and help clarify patho–physiological mechanisms in pre–ulcerative and ulcerative conditions. Often, laser–Doppler methods are used to compare vasodilatory responses at differing skin sites to evaluate skin parameter changes. Significant variations in skin microvasculature are known to be normally present, even in closely separated skin zones. In this study, spatial variability and temporal responses of SBP were evaluated with a widely used topical vasodilator (methylnicotinate, MN). A mask with nine holes (1.25cm² each) was placed on the volar forearm of ten volunteers. SBP was measured with laser–Doppler Imaging (LDI) prior to applying MN (15ul, 50mM) to six zones and 5, 10, 15, 20 and 30 minutes afterwards. Inter–zone mean SBP and inter– and intra–zone coefficients of variation (CV) were determined at each time. Results show that MN responses, when determined as zone LDI means, reached maximum at 15 minutes with no significant differences in relative responses among treated zones. Inter–zone perfusion CV's (range 0.11 – 0.13) were about 50 percent of intra–zone CV's ($p < 0.01$). We conclude that LDI perfusion responses can be obtained at different forearm skin sites with reasonable and acceptable levels of spatial variation if zone mean SBP values are used.

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Introduction

Tests of skin vasodilatory responses are of potential use to help identify patients at risk for skin breakdown, to help clarify patho–physiological mechanisms and to help assess impacts of preventative strategies. Many provocations cause blood flow increases and erythema, but optimal tests should be easily used, minimally injurious and reproducible at varying skin sites.

Topical use of Methylnicotinate (MN), a lipid soluble ester of nicotinic acid, (a relative of Niacin ingested for triglyceride

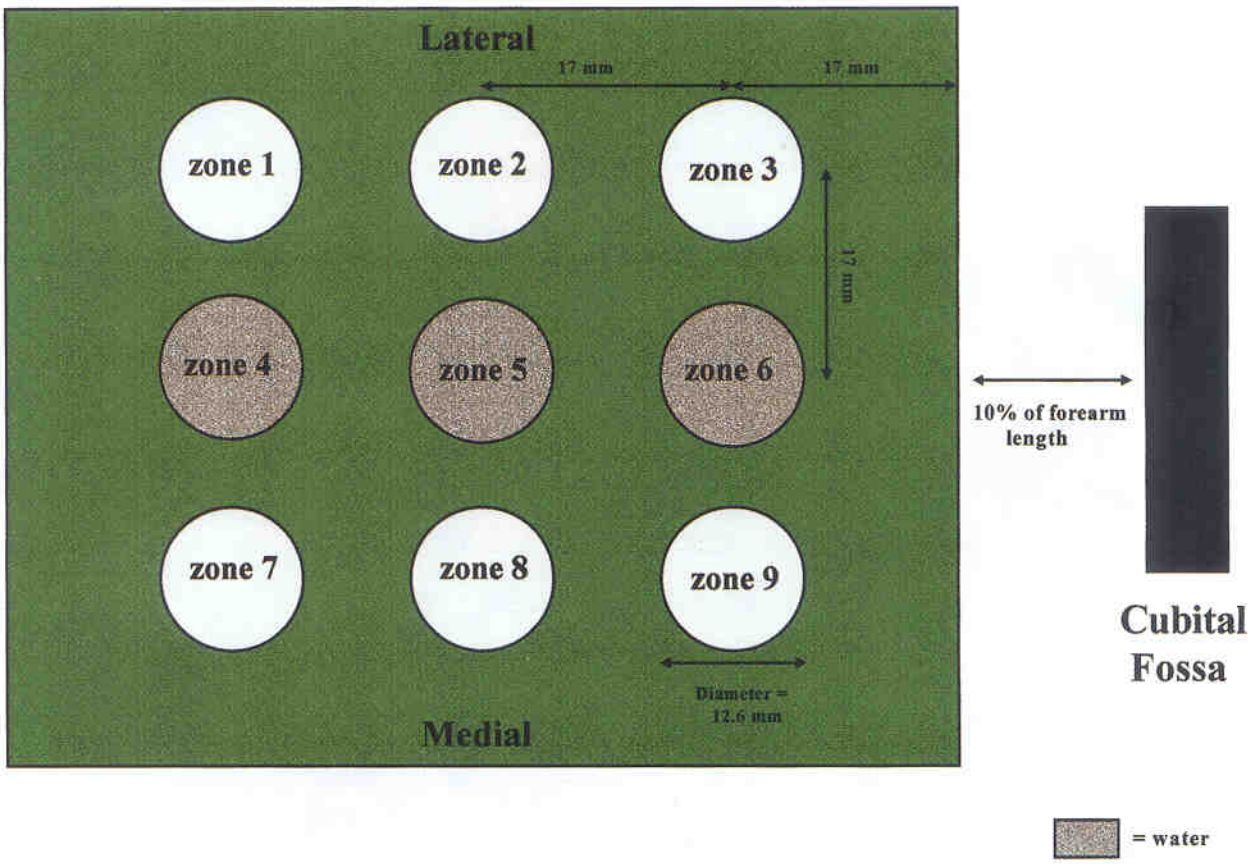

Figure 1. *Experimental design for methylnicotinate application.* This template was affixed to the volar forearm of each subject at a distance of 10 percent L from the cubital fossa. 15 ul of 50 mM methylnicotinate was applied to zones 1, 2, 3, 7, 8, and 9 while 15 ul of distilled water was applied to zones 4, 5, and 6. Printed with permission from H.N. Mayrovitz, PhD.

reduction) effects prostaglandin biosynthesis and release to induce skin microvascular vasodilation and erythema.[1-3] MN has been used to characterize vascular responsiveness in patients with Raynaud's disease[4] and to measure changes in skin vascular reactivity and erythema by laser–Doppler[5] and skin color measurements.[6] Often blood perfusion assessments are made at various sites on the volar forearm most frequently with laser– Doppler fluxmetry. This method requires removal and reattachment of laser–Doppler probes to measure pre– and post–MN application responses, whereas laser–Doppler imaging requires no skin contact and produces perfusion data for a much larger skin area.[7] In view of the well known spacial variability in laser– Doppler perfusion values and heterogeneity of skin microvessel distribution, it is uncertain as to how much laser–Doppler "point" measured responses depend on a specific forearm test zone or a particular site within that zone.

Because the response to MN in some ways appears to mimic some features associated with stage 1 skin breakdown, it was reasoned that it may be possible to use it to simulate certain stage 1 features, thereby permitting the study of certain aspects of this process in a controlled manner using laser–Doppler imaging. However, prior to its direct use for that purpose it was first necessary to characterize the inter–site and intra–site variability in MN responses. This was the primary initial aim of the present study.

Methods

Subjects and preliminary setup

Ten volunteer subjects (age 39 +/– 4, range 24 to 62, 6 female) participated after reading and

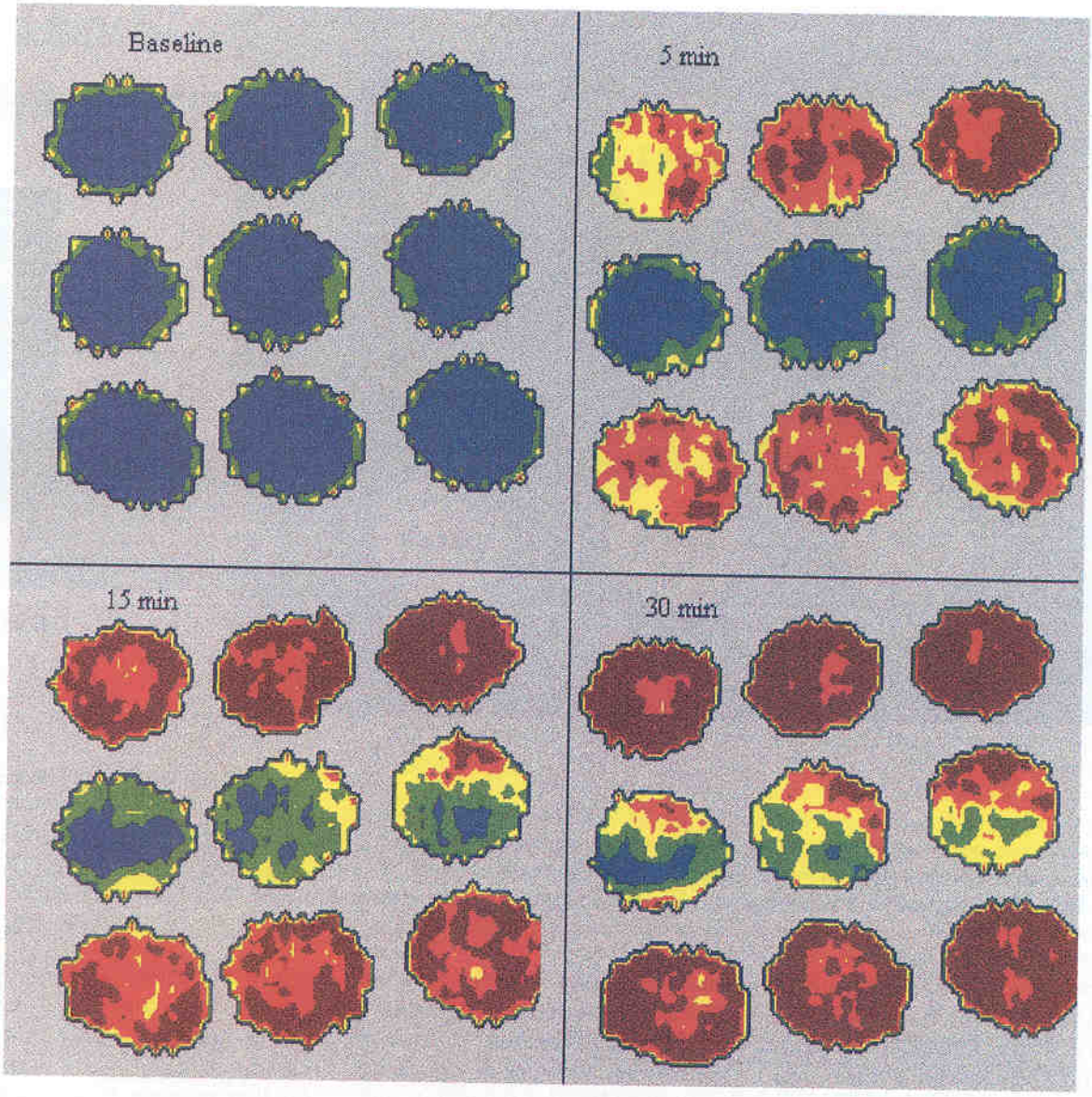

Figure 2. *Example of LDI scans for one subject.* Perfusion responses to a vasodilatory stimulus over time are displayed as color–coded images. In order of increasing perfusion values, colors are dark blue, light blue, green, yellow, and red. By 15 minutes, the response is near maximum in MN treated sites although some run–over to water treated sites is noted. Printed with permission from H.N. Mayrovitz, PhD.

signing an Institutional Review Board approved informed consent. All testing was conducted in a temperature controlled room maintained at 22 +/– 1° C. Each subject assumed a supine position on an exam table with the right arm comfortably extended at approximately 90° with the volar surface facing upward. The arm was supported by a specially designed padded surface that was attached to the exam table. The length (L) of the arm between wrist and elbow was measured using a tape measure, and a mark was made on the arm at a distance of 10 percent of L as measured from the elbow. A rectangular template (Figure 1) consisting of nine uniformly spaced

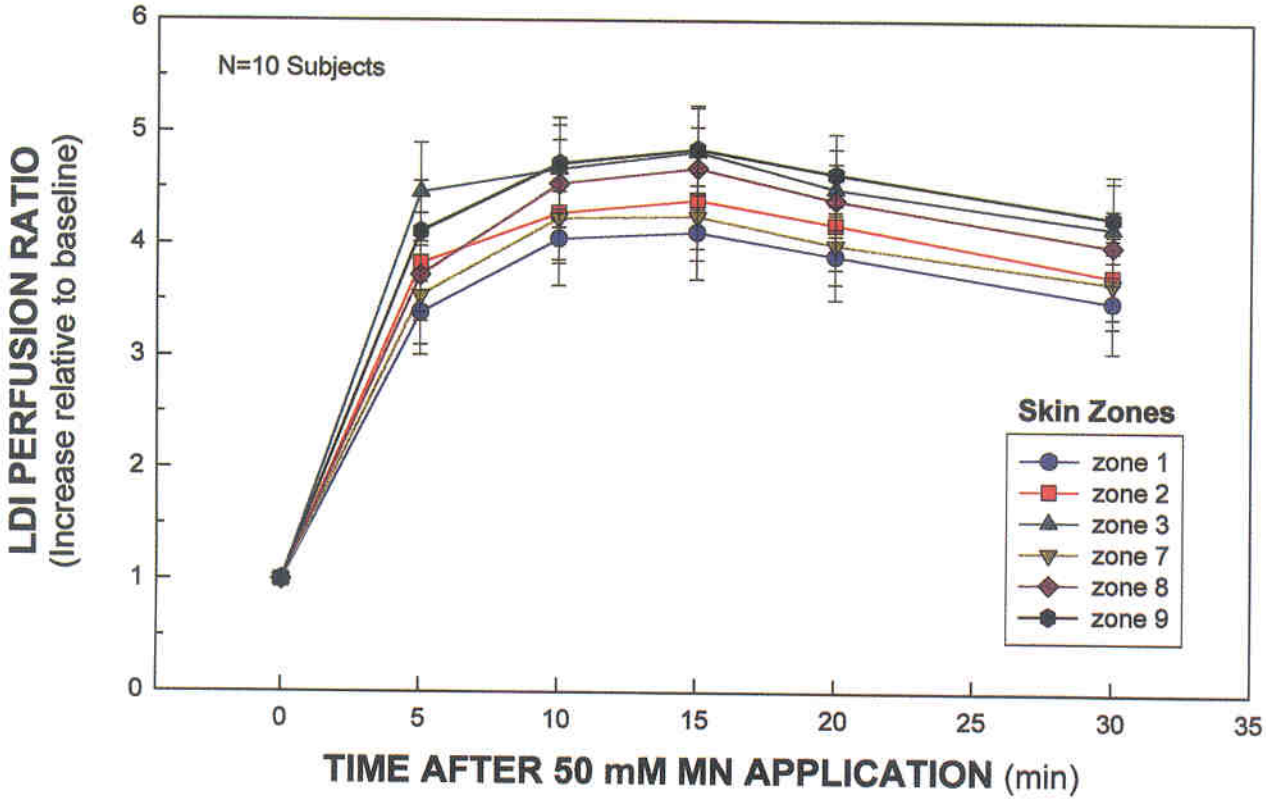

Figure 3. *Temporal response to methylnicotinate.* Perfusion responses are reported relative to their baseline average. All zones shown were treated with 50 mM MN. A rapid response is observed with a gradual decline in perfusion beginning at 30 minutes. Printed with permission from H.N. Mayrovitz, PhD.

holes, each approximately 12.6 mm in diameter previously cut into a dark green felt material, was placed centered on the forearm with the proximal template edge located at the standardized 10 percent L position. A laser–Doppler Imager (LDI) solid state laser head* was positioned 18 cm above the center of the template. The scanning area of the LDI was tested and adjusted so that it just encompassed the template area and the included nine skin areas at a high resolution setting. The orientation of the scanning head produced a lateral–to–medial scanning pattern which progressed up the arm from the distal to proximal template edges. All LDI data was obtained at a gain setting of unity, and values are reported as arbitrary units as is standard for laser–Doppler.

*LISCA, Linnoping Sweden

Protocol

After 15 minutes of supine rest the arm was scanned three times to obtain baseline averages for each of the nine exposed skin zones. The use of the green template allowed all nine sites to be scanned in less than one minute with good contrast of the image to clearly define each zone. Distilled water (15 ul) was applied to zones 4, 5 and 6, (middle row of Figure 1) and aqueous methylnicotinate (15 ul of a 50 mM solution) was applied to the remaining six zones (top and bottom rows of Figure 1) using a micropipete and gently spread over the exposed skin. The order of application for water was 4, 5, 6 and for MN, 1, 7, 2, 8, 3, 9. This particular sequence was adopted since distal zones (1,7) are scanned slightly earlier than the most proximal sites (3, 9). At 5, 10, 15, 20 and 30 minutes after application of MN, the arm was re–scanned.

Analyses

Baseline perfusion (pre–MN application) for each zone was determined by first computing the mean and standard deviation

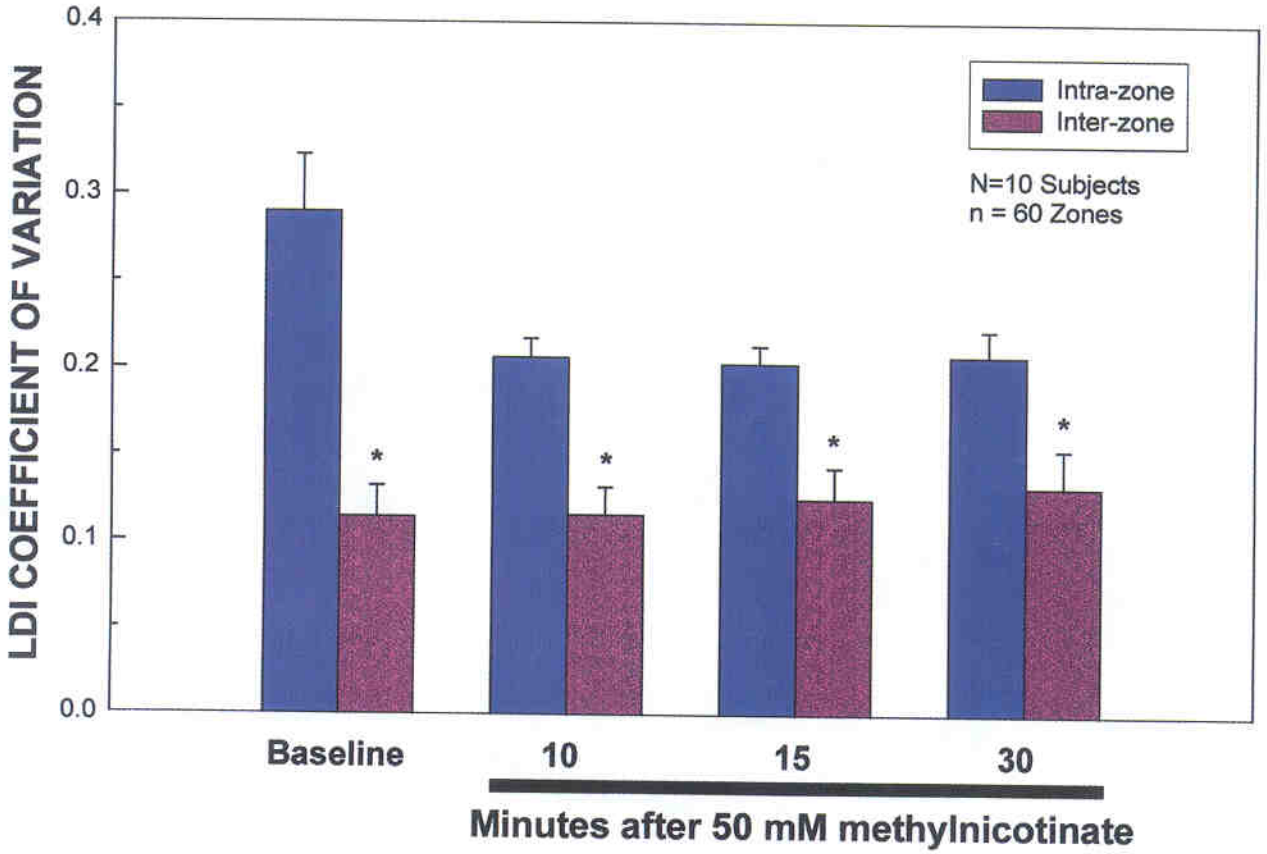

Figure 4. *Variability within and among skin zones.* Inter-zone coefficient of variation remains near 10 percent at all times. Intra-zone (within a zone) perfusion is typically twice a variable. Printed with permission from H.N. Mayrovitz, PhD.

of all intra-zone sites. The data from the three baseline scans were then averaged to yield a single mean baseline value for each zone. Responses to MN were determined similarly but were based on one scan at each of the five post-MN times. Overall zone differences in baseline perfusion were tested using non-parametric Kruskal-Wallis and Median tests of absolute (arbitrary) perfusion values among zones. Overall differences in MN responses among MN treated zones were tested similarly at each time point using absolute perfusion values as well as ratios to each zone's baseline average. Statistical significance was inferred if $p < 0.05$.

Results

Figure 2 illustrates a typical LDI perfusion image before (baseline) and after application of 50 mM MN in six skin regions (top and bottom three holes) and water in the center three holes. In this figure, which provides a rapid visual characterization, skin blood perfusion is represented by different colors with regions of highest relative flow in red and lowest flow in deep blue. Intervening colors in increasing flow order are light blue, green and yellow. From this example it is noted that baseline perfusions are in each site low but that by five minutes, perfusion in the MN treated sites are already increasing. By 15 minutes after application the response is at or near maximum in the MN treated sites. It should also be noted that the perfusion in the water treated sites is also increasing due to the effect of the MN on the vasculature that feeds the middle row of sites. The amount of this effect was variable with the chosen example representing the maximum interaction observed of the ten subjects.

The quantitative perfusion response of all subjects and sites is shown in Figure 3 in which the magnitude of the response is given relative to the pre-treatment baseline. It is to be noted

that the response is rapid, remains relatively uniform between 10 and 20 minutes and begins to decline at about 30 minutes. Quantitative assessments of intra–site and inter–site variabilities for all subjects, based on analyses of the coefficient of variation (CV, standard deviation/mean) are summarized in Figure 4. The important points to note are that when the average perfusion within each site is used as the primary assessment parameter the CV is uniformly about 10 percent at all times and is at least half the variation associated with intra–site perfusion. The intra–site perfusion is that which would most closely resemble perfusion variations as measured by standard laser–Doppler probes.

Summary and conclusions

Topical application of methylnicotinate is associated with dose, time and site dependent increases in forearm skin blood perfusion and erythema. A suitable dose for assessing microvascular responses and skin erythema is 50 mM with response optimally determined 10 to 30 minutes after application although longer times can be used. Inter–site response variations among multiple forearm skin zones are about 10 percent; this is about half that of intra–site (within–zone) variations and is thus adequate for many skin study purposes. Combined use of laser–Doppler imaging together with relatively innocuous vascular probes such as methylnicotinate offer reasonable potential for clarifying many microvascular functional aspects and appear suitable to study issues which impact skin function and skin breakdown.

Acknowledgment

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