Skin Blood Flow Measured by Laser-Doppler Flowmetry and Venous Occlusion Plethysmography: Methodological Considerations

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Cutaneous laser-Doppler flowmetry use includes assessing thermoregulatory effector function and evaluation of clinical and pathologic conditions of the skin circulation. Skin blood flow (SkBF) on the forearm was measured simultaneously by venous occlusion plethysmography (VOP) and laser-Doppler flowmetry (LDF) during leg exercise. Fifteen subjects were studied at T_e = 30°C or 35°C for 30 minutes at 50 to 75% peak Vo_2. LDF was measured using the Med Pacifc LD6000, the TSI Laserflo® BPM403A or Laserflo BPM® with no local heating of the forearm. After the initial vasoconstriction with exercise onset, SkBF increased linearly during the exercise transient. During steady-state exercise, a non-linear relationship between LDF and VOP was observed in all experiments as SkBF measured by VOP continued to increase as SkBF measured by LDF became stable. Second order regression coefficients of LDF and VOP data were r ≥ 0.92 for 13 experiments and r ≥ 0.88 for four experiments. These data suggest that LDF may be limited by the anatomically small area for skin blood flow measurement. Alternately, VOP may detect greater arteriolar and resistance vessel vasoconstriction which occurred later in exercise. These observations are limited to leg exercise under conditions where skin temperature averaged 33-35°C.

Skin blood flow, Exercise, Body temperature regulation, Laser-Doppler flowmetry, Venous occlusion plethysmography

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by

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FOREWORD

This report describes a series of studies done at the U.S. Army Research Institute of Environmental Medicine which characterize skin blood flow and forearm blood flow responses in healthy male and female subjects. The results are applicable to studies using either venous occlusion plethysmography or laser-Doppler flowmetry to estimate skin blood flow in exercising subjects.
ACKNOWLEDGEMENTS

This work would not have been possible without the volunteers who participated in the tests described in this report. We wish to thank them for their time and effort. We especially thank Dr. T. Doherty, G. Sexton and L. Trad for their contributions to these studies.
EXECUTIVE SUMMARY

Cutaneous laser-Doppler flowmetry use includes assessing thermoregulatory effector function and evaluation of clinical and pathologic conditions of the skin circulation. Skin blood flow (SkBF) on the forearm was measured simultaneously by venous occlusion plethysmography (VOP) and laser-Doppler flowmetry (LDF) during leg exercise. Fifteen subjects were studied at $T_a = 30^\circ C$ or $35^\circ C$ for 30 minutes at 50 to 75% peak $\dot{V}O_2$. LDF was measured using the Med Pacific LD6000, the TSI Laserflo® BPM403A or Laserflo BPM²® with no local heating of the forearm. After the initial vasoconstriction with exercise onset, SkBF increased linearly during the exercise transient. During steady-state exercise, a non-linear relationship between LDF and VOP was observed in all experiments as SkBF measured by VOP continued to increase as SkBF measured by LDF became stable. Second order regression coefficients of LDF and VOP data were $r \geq 0.92$ for 13 experiments and $r \geq 0.88$ for four experiments. These data suggest that LDF may be limited by the anatomically small area for skin blood flow measurement. Alternately, VOP may detect greater arteriolar and resistance vessel vasodilation which occurred later in exercise. These observations are limited to leg exercise under conditions where skin temperature averaged 33-35°C.
INTRODUCTION

Historically, the measurement of cutaneous blood flow has been important in assessing thermoregulatory effector function. The volume plethysmograph, combined with venous occlusion, was used to measure rates of limb blood flow (Hewlett et al., 1909). Whitney (1953) introduced an alternative to measuring limb volume directly when he showed a direct relationship between the rate of limb volume increase and the rate of limb girth increase measured by resistance changes in a mercury-in-rubber strain gauge placed around the limb. It was subsequently verified that this strain gauge plethysmograph or venous occlusion plethysmography (VOP) measured similar values for limb blood flow as the volume plethysmograph (Burger et al., 1959; Clarke et al., 1958; Clarke and Hellon, 1957; Whitney, 1953). The measurement of forearm blood flow provided an index of skin blood flow, even as the forearm blood flow measured included flow through the skin, muscle, adipose tissue and bone. However of these components, only skin blood flow increased in an inactive limb (arm) during dynamic leg exercise (Johnson et al., 1974; Rowell, 1977; Rowell, 1983; Rowell, 1986).

Laser-Doppler flowmetry (LDF), as a measure of skin blood flow, has been qualitatively compared to VOP during dynamic exercise and hyperemia. The two indices have been satisfactorily correlated in assessing thermoregulatory effector function with certain limitations. Studies done by Johnson and colleagues (Johnson et al., 1984; Saumet et al., 1988; Taylor et al., 1988; 1989) characterized the responses of skin blood flow measured by VOP and laser-Doppler flowmetry during dynamic exercise or hyperemia (Johnson et al., 1984; Saumet et al., 1988) or changes in skin blood flow during dynamic or isometric exercise (Taylor et al., 1988; 1989). In general, the conclusions from these studies were: 1) laser-Doppler flowmetry was adequate to measure the patterns of increases in skin blood flow, although voltages measured were variable from site to site and subject to subject; 2) laser-Doppler flowmetry measured skin blood flow (1.0±0.5 mm depth) and this technique was sensitive to muscular contraction; 3) for measurement of cutaneous vascular conductance (skin blood flow/mean arterial pressure), LDF and VOP must be linearly related and the intercept should be near the origin.
Specific discussions on the use of the laser-Doppler to measure skin perfusion or skin blood flow have been published elsewhere (see references, Bonner and Nossal, 1981; Borgos, 1990; Johnson et al., 1984; Schabauer et al., 1994). We are presenting aspects of the relationship between laser-Doppler flowmetry and venous occlusion plethysmography during dynamic exercise under conditions where both the local skin temperature and the mean skin temperature averaged 30°C to 35°C. VOP data include blood flowing in arterioles and larger resistance vessels in the skin, and these vessels might exhibit different response characteristics than surface vessels contributing to LDF data.

STATEMENT OF PURPOSE

The purpose of this manuscript is to examine LDF:VOP as the relationship of the two indices of skin blood flow might provide additional information about skin blood flow responses to exercise than either index alone.
METHODS

Fifteen healthy subjects (3 women, 12 men) completed experiments at exercise intensities between 50 to 70% of peak oxygen utilization (\(\dot{V}O_2\)) at either 30°C or 35°C after they were verbally apprised of the nature and risks of the study. Two of the subjects exercised at both 30°C and 35°C on separate test days. Females were studied between days 1-7 of their menstrual cycles.

In all experiments, each subject sat in a chair positioned behind the cycle ergometer, such that during leg exercise, the legs were parallel to the floor. The subject swallowed a catheter containing a thermocouple which was adjusted to approximate heart level based on 25% of each subject’s height. Surface thermocouples were placed at eight skin sites, one site being the forearm adjacent to the strain gauge and Doppler flow probes, to estimate mean skin temperature. A mercury-in-silastic strain gauge was placed on the forearm for the measurement of forearm blood flow (FBF) by venous occlusion plethysmography (Doherty et al., 1993; Hokanson et al., 1975; Whitney, 1953). The strain gauge was placed around a section of forearm distal to the main mass of the muscles to decrease the proportion of muscle in the whole arm cylinder measured. The forearm was suspended by the wrist with a sling apparatus anchored at two points minimizing movement artifact during exercise as the arm and gauge moved in translation with the torso and the strain gauge position on the arm was near the height of the heart.

Perfusion of the skin of the forearm, also used as an index of skin blood flow (SkBF), was estimated by laser-Doppler flowmetry (Med Pacific, LD6000; 2mW HeNe laser at 632.8 nm). In some experiments, SkBF of the forearm was also measured using a Transonic (TSI, Laserflo® BPM 403A; 1.6 mW at 780 nm) laser-Doppler system. In other experiments, SkBF on the forearm was also measured by a Vasamedics Laserflo® BPM² (2.0 mW at 760-800 nm). The Med Pacific system is an FDA Class II laser product which has potential as an optical hazard. The TSI Laserflo® 403A system and the Vasamedics Laserflo® BPM² present no optical hazard. Briefly, the Med Pacific laser presents "flow" in mV proportional to the quantity of moving red blood cells multiplied by the mean velocity of the red blood cells. The TSI Laserflo® and Vasamedics Laserflo® BPM² present "flow" (calculated by a
patented algorithm) which is proportional to the mean frequency of the red blood cells multiplied by the mean number of Doppler shifts per photon.

Skin temperature at the site of skin blood flow measurement was not independently heated or cooled, but was strictly a function of the ambient temperature at rest and changed only slightly during exercise. However, any measured change in the local or mean skin temperature during dynamic exercise was similar for the measurement of skin blood flow by venous occlusion plethysmography and laser-Doppler flowmetry. Laser probe and strain gauge placement was determined by anatomical measurements and was similar for the two experiments for the two subjects who were tested at the two ambient conditions. Heart rate (HR) was measured from the EKG and arterial pressure was measured by automated auscultation (Accutorr).

Temperatures, FBF and SkBF were measured twice each minute. HR and arterial pressure were recorded every 2.5 minutes and metabolic heat production was estimated from oxygen utilization (open circuit spirometry) at rest and during exercise. Dynamic exercise lasted thirty minutes.

Subject Safety

Informed consent was obtained. All of the procedures in this report were within the framework, restrictions and safety limitations of the USARIEM Type Protocol for Human Research Studies in the areas of Thermal, Hypoxic and Operational Stress, Exercise, Nutrition and Military Performance.¹

**STATISTICAL ANALYSES**

Data from VOP and LDF were analyzed by least squares regression coefficients fit to a second order equation.

¹ Approved 14 Dec 1994. The type protocol provides information and explanations about conditions, standards and safeguards, in order to serve as an encompassing framework for specific in-house studies in its general subject area. It is to be used as a reference to facilitate the understanding and review of specific study protocols which conform to its provisions, and thus do not exceed the degree of risk, and safety limits herein stipulated (reference para 18, USAMRDC Reg 70-25).
RESULTS

Figure 1 shows forearm skin blood flow (SkBF) by laser-Doppler flowmetry and forearm blood flow (FBF) by venous occlusion plethysmography at rest and during thirty minutes of dynamic exercise for a representative subject at 30°C and at 35°C. Local skin temperature in all experiments was 33-35°C. Mean skin temperature measured during the experiments conducted at 30°C and 35°C averaged 33°C and at 35°C respectively. As expected, both skin and forearm blood flow were higher at the higher skin temperature.

In Figures 2-6, the data collected from the two techniques are plotted for all experiments. Cutaneous perfusion from laser-Doppler flowmetry is the dependent variable (Y-axis) and forearm blood flow from venous occlusion plethysmography is the independent variable (X-axis). In all experiments SkBF, as measured by laser-Doppler flowmetry, was attenuated as FBF, as measured by VOP, continued to increase during seated cycle exercise. The regression coefficients for the second order equations which describe the individual data are given on each panel of each of the five figures. The regression coefficient between VOP and LDF was r≥0.92 for 13 of the 17 experiments and r=0.88-0.91 for the remaining four experiments.

The data from Figures 2-5 were obtained with the MedPacific LD6000 instrument. The data presented in Figure 2 are the SkBF responses of four men during seated exercise at 60% peak VO₂ at an ambient temperature of 30°C. Figure 3 shows the SkBF responses of six subjects (4 men, 2 women) during seated cycle exercise at 50% peak VO₂ at an ambient temperature of 30°C. Figure 4 shows SkBF responses of two men exercising at 70% peak VO₂ at 35°C. Figure 5 shows the SkBF responses of three women exercising at 50% peak VO₂ at 35°C. The data from Figure 6 represent cutaneous perfusion from the Vasamedics Laserflo® BPM² system. Figure 6 shows the SkBF data from two men exercising at 60% peak VO₂ at 30°C. All experiments at 35°C utilized both the MedPacific LD6000 and the TSI Laserflo® BPM 403A in parallel. Cutaneous perfusion measured by these instruments was highly correlated (r=0.80-0.90), even though the site of measurement was different between instruments.
Figure 7 shows SkBF data collected during exercise at both 30°C and 35°C for the two individuals. The curvilinear relationship showing attenuated cutaneous perfusion as measured by LDF was similar for all four experiments on these subjects. Both methods were sensitive enough to show increased skin blood flow at an elevated skin temperature. As expected with higher skin temperature at the higher ambient temperature, both skin perfusion and forearm blood flow were higher in the experiment with higher local skin temperatures.

The data shown in Figure 8 shows how the relationship between LDF and VOP might be used to detect effects of clinical treatment on skin blood flow responses. The five panels present SkBF and VOP data before, during and after systemic chemotherapy with cyclophosphamide, methotrexate and 5-fluorouracil. The relationship observed in the seventeen untreated "normal" subjects was also seen in the "PRE" chemotherapy experiment. Although the magnitude of the LDF data change after both one and three chemotherapy treatments (approximately ten weeks) the characteristic relationship persisted. The relationship between LDF and VOP was linear after the seventh chemotherapy treatment (approximately 21 weeks). Eight weeks "POST" chemotherapy the pattern of LDF to VOP was similar to that observed before chemotherapy.
Figure 1: Time course of skin blood flow measured by laser-Doppler flowmetry (mV) and venous occlusion plethysmography (ml·100ml⁻¹·min⁻¹) in a representative subject at two ambient temperatures.
Figure 2. Individual subject skin blood flow measured by laser-Doppler flowmetry (mV) as a function of skin blood flow measured by venous occlusion plethysmography (ml\(\cdot\)100ml\(^{-1}\)\(\cdot\)min\(^{-1}\)). These data represent the responses of four men (subjects A, B, J, P) during seated exercise at 60% peak \(\text{VO}_2\) at an ambient temperature of 30°C.
Figure 3. Individual subject skin blood flow measured by laser-Doppler flowmetry (mV) as a function of skin blood flow measured by venous occlusion plethysmography (ml•100ml⁻¹•min⁻¹). These data represent the responses of six subjects (men = subjects J, L, Q, T; women = subjects K, S, T) during seated cycle exercise at 50% peak VO₂ at an ambient temperature of 30°C.
Figure 4. Individual subject skin blood flow measured by laser-Doppler flowmetry (mV) as a function of skin blood flow measured by venous occlusion plethysmography (ml*100ml^2*min^-1). These data represent the responses of two men (subjects B,H) during seated cycle exercise at 70% peak \( \text{\textit{VO}}_2 \) at 35°C.
Figure 5. Individual subject skin blood flow measured by laser-Doppler flowmetry (mV) as a function of skin blood flow measured by venous occlusion plethysmography (ml•100ml⁻¹•min⁻¹). These data represent the responses of three women (subjects K,S₁,T) during seated cycle exercise at 50% peak VO₂ at 35°C.
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DISCUSSION

This study shows that skin blood flow data measured by venous occlusion plethysmography and laser-Doppler flowmetry were not linearly related during thirty minutes of dynamic leg exercise when the local skin temperature was between 33-35°C. Skin blood flow increased linearly for both methods during the initial minutes of exercise before levelling off (see Figure 1). The conclusion that skin blood flow is not linearly related during the entire thirty minutes of dynamic exercise does not affect the analysis of the initial exercise transient, but rather those data that are measured during the later stages of dynamic leg exercise in these experiments.

Skin blood flow increases during heat stress and during exercise (Brengelmann et al., 1977; Brengelmann, 1977; Johnson, 1986; Johnson, 1992; Johnson et al., 1974; Johnson et al., 1984; Roberts and Wenger, 1980; Rowell, 1977; 1983; 1986; Wenger et al., 1975). Elegant studies throughout the 1960's and 1970's characterized the measurement of skin blood flow during exercise and/or heat stress, and these responses have been thoroughly reviewed (Brengelmann, 1977; Johnson, 1992; Rowell, 1977; 1983; 1986). The recent work by Johnson and associates using bretylium tosylate has added to this body of knowledge, greatly through the use of laser-Doppler flowmetry (Johnson, 1992; Kellogg et al., 1993). As presented in the introduction, the use of laser-Doppler flowmetry for the measurement of the pattern of skin blood flow changes during dynamic exercise, heat stress, reactive hyperemia or a combination of these was established (Johnson et al., 1984; Saumet et al., 1988; Taylor et al., 1988; 1989). In those studies in which dynamic exercise was studied, local or whole body skin heating was used such that local temperature at the site of FBF and/or LDF was 38 to 39°C. We chose not to heat the local area of measurement and allowed the skin temperature to be influenced by the ambient conditions of the test chamber because a mean skin temperature equal to 38 - 39°C is unusual. For example, mean skin temperatures during walking or cycling exercise averages 38-39°C at $T_a > 45°C$ or under very hot, very humid conditions. Therefore, the skin temperatures of the forearm in the present study ranged from 33-35°C. Even though the local skin temperature and the mean skin temperature were not "controlled" to a specific level during the present experiments, the strain gauge and laser-
Doppler probe were juxtaposed during all experiments. Therefore, any local skin influences would be similar for both techniques.

We used LDF together with VOP to assess components of cutaneous vasodilation associated with clinical treatment for breast adenocarcinoma during an exercise-heat stress. We had thought that possible changes in the relationship between LDF and VOP during prolonged treatment, might provide insight into changes in vasculature responses resulting from chemotherapy. In this case study, before and during early treatment, the relationship between LDF and VOP during exercise was similar to that observed for healthy subjects although the relationship was not static. After twenty-one weeks or seven cycles of intravenous chemotherapy (cyclophosphamide, methotrexate and 5-fluorouracil), not only was skin blood flow reduced, but the relationship between LDF and VOP had become linear. During this experiment, forearm blood flow was also attenuated during exercise (to a similar core temperature drive) as a result of systemic chemotherapy. Attenuated FBF could result from reduced arteriolar and resistance vessel vasodilation and would thereby affect smaller arteriolar and capillary blood flow as measured by LDF. The progression of reduced skin blood flow as measured by LDF after the third cycle of chemotherapy to attenuated SkBF and FBF after the 7th cycle could be explained by treatment effect on the smaller caliper vessels first, with a later effect on the larger caliper vessels.

The measurement of skin blood flow by venous occlusion plethysmography or laser-Doppler flowmetry requires an understanding of the limitations for each technique. Precision during probe or strain gauge placement is an essential part of the experimental design to evaluate treatment effects on SkBF data. For example, both techniques are extremely sensitive to movement artifact, and venous occlusion plethysmography must be used on an inactive limb or digit to assure that observed changes in limb blood flow are confined to the skin. Since in our experiments, limb blood flow measured by VOP continued to increase after skin blood flow measured by LDF became stable (Figures 2-6), and assuming that only skin blood flow was measured by VOP in the inactive limb (Johnson, 1992; Rowell, 1983; 1986), we can then assume that the measurement of skin blood flow by LDF during dynamic exercise was limited. Inherent in LDF measurements is a small anatomical area of measurement, which has been estimated at ~1mm² (Bonner and Nossal, 1981; Borgos, 1990; Johnson et al., 1984; Schabauer et al., 1994).
Contribution to skin blood flow in this area of skin is limited to those vessels directly in the viewing area. In contrast, the VOP technique measures limb (skin) blood flow around the circumference of the forearm, in this case. There are possible differences in the contribution of resistance vessels and capillaries to measured skin blood flow between the two techniques. Whether the observations from the current experiments are seen at high skin temperature (≥38°C) must yet be determined. However, under the conditions of dynamic leg exercise in a physiologic range of local skin temperature imposed by this study, a non-linear relationship between forearm skin blood flow measured by venous occlusion plethysmography and skin blood flow measured by laser-Doppler flowmetry was consistently observed.

CONCLUSIONS

In summary, the data from these studies suggest that using both venous occlusion plethysmography and laser-Doppler flowmetry in experimental and clinical settings might provide additional insight into the interpretation of skin blood flow responses to an exercise, heat or pharmaceutical challenge. The pattern of the vascular response is similar between these techniques, although the relationship between measurement techniques may be of additional clinical or scientific importance.
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