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REVIEW

Skin blood flow measurements during heat stress: technical and analytical considerations

Georgia K. Chaseling,[1,2] Craig G. Crandall,[3] and Daniel Gagnon[1,2]

[1]*Cardiovascular Prevention and Rehabilitation Centre, Montreal Heart Institute, Montréal, Québec, Canada;* [2]*Département de Pharmacologie et Physiologie, Faculté de Médecine, Université de Montréal, Montréal, Québec, Canada; and* [3]*Institute for Exercise and Environmental Medicine, Texas Health Presbyterian Hospital Dallas, and University of Texas Southwestern Medical Center, Dallas, Texas*

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Chaseling GK, Crandall CG, Gagnon D. Skin blood flow measurements during heat stress: technical and analytical considerations. *Am J Physiol Regul Integr Comp Physiol* 318: R57–R69, 2020. First published October 9, 2019; doi:10.1152/ajpregu.00177.2019.—During heat stress, the skin vasculature can greatly increase conductance secondary to vasodilation. The subsequent increase in skin blood flow allows for convective heat transfer from the core to the skin and between the skin surface and the surrounding environment. Measurement of skin blood flow, therefore, provides valuable information regarding heat exchange between the body and the environment. In addition, assessment of skin blood flow can be used to study vascular control mechanisms. Most often, skin blood flow is measured by venous occlusion plethysmography, Doppler ultrasound, laser-Doppler flowmetry, and, more recently, optical coherence tomography. However, important delimitations to each of these methods, which may be dependent on the research question, must be considered when responses from these approaches are interpreted. In this brief review, we discuss these methods of skin blood flow measurement and highlight potential sources of error and limitations. We also provide recommendations to guide the interpretation of skin blood flow data.

conductance; cutaneous; laser-Doppler; plethysmography; ultrasound

INTRODUCTION

The measurement of skin blood flow provides important information regarding heat exchange between the body and the environment at rest and during thermal challenges (59). During heat stress, in particular, there is a great potential for skin blood flow to increase: maximal increases of up to ~8 L/min have been reported in healthy young adults (59). Such an increase in skin blood flow allows for optimal regulation of core body temperature, but it also poses a challenge to the maintenance of blood pressure. In addition, the skin is an easily accessible vascular bed, such that measurements of skin blood flow can be used to study microvascular control mechanisms (35). Several noninvasive techniques can be used to estimate skin blood flow. These techniques have been employed to investigate/assess basic thermoregulatory mechanisms (36), as well as microvascular control mechanisms (58), to monitor diseases (1), clinical treatments (4), and vascular function (68).

During the 1900s, most studies investigating the control of skin blood flow during heat stress measured limb blood flow by venous occlusion plethysmography (12, 25, 45). Doppler ultrasound is now more commonly used than venous occlusion plethysmography to measure limb, or even organ-specific, blood flow (70, 71). Furthermore, laser-Doppler flowmetry was developed to specifically estimate blood flow from the cutaneous microvasculature (38). Most recently, optical coherence tomography has also been used to evaluate changes in skin microvascular blood flow during heating (16). Notwithstanding the possible divergence of responses between these techniques during heat stress (19, 28), each technique provides fundamentally different estimations of skin blood flow. For this reason, several factors must be considered when skin blood flow measurements via each of these approaches are interpreted. In this brief review, we will *1*) describe the basic principles for the techniques of venous occlusion plethysmography, Doppler ultrasound, laser-Doppler flowmetry, and optical coherence tomography; *2*) provide technical considerations for each method; *3*) compare skin blood flow responses obtained between certain methods that have been directly compared with one another, highlighting various limitations that should be considered; and *4*) provide recommendations for quantifying and interpreting measurements of skin blood flow during heat stress. Pros and cons of each method, as

Address for reprint requests and other correspondence: D. Gagnon, Cardiovascular Prevention and Rehabilitation Centre, Montreal Heart Institute, 5055 rue St-Zotique Est, Montréal, QC, Canada H1T 1N6 (e-mail: daniel.gagnon.3@umontreal.ca).

well as general guidance for the interpretation of skin blood flow measurements, are summarized in Tables 1 and 2, respectively.

FUNDAMENTAL PRINCIPLES FOR MEASUREMENT OF SKIN BLOOD FLOW

To fully appreciate technical and interpretative considerations for each measurement technique, a brief review of the fundamental principles underlying each technique is warranted. The reader is encouraged to consult review articles specific to each technique for more details (18, 31, 62, 69).

Venous Occlusion Plethysmography

Plethysmography is generally defined as the measurement of changes in limb volume due to changes in the flow of blood passing through that limb. The first description of venous occlusion plethysmography dates back to 1905 (10). To determine changes in limb volume, the limb of interest is first "isolated" by placement of a cuff distal to the area of measurement and inflation of the cuff to suprasystolic levels to cause circulatory arrest distal to that cuff. For example, when forearm blood flow is measured by plethysmography, the circulation to the hand is arrested by a cuff placed around the wrist and inflated to ≥200 mmHg. A second cuff, placed proximal to the limb of interest, is inflated to impede venous return without interfering with arterial inflow (Fig. 1*A*). For forearm blood flow measurements, this cuff is placed on the upper arm and typically inflated to 50 mmHg. As blood "enters" and becomes "trapped" within the limb of interest, the volume of the limb will increase. This change in volume is calibrated to provide a measure of volume change. Several approaches have been designed to quantify volume changes by venous occlusion plethysmography. Initial plethysmographs utilized the displacement of water (31). This approach was used in studies

Table 1. *Summary of pros and cons to consider before employing common techniques to measure skin blood flow during heat exposure*

Technique	Technical Considerations	Interpretation	Comparison with Other Methods
VOP			
Pros	*1*) Largely operator-independent	*1*) Provides measures of absolute blood flow *2*) An average measure can be taken from multiple traces	*1*) Information regarding change in blood flow is similar to that provided by Doppler ultrasound *2*) Changes in blood flow during passive heating correlate with changes in red blood cell flux measured by LDF probes
Cons	*1*) Limb must remain still to avoid movement artifacts *2*) Limb must be elevated above heart level to avoid venous congestion	*1*) Does not provide a continuous measure of limb blood flow *2*) Changes in blood flow could be due to changes in vascular beds other than the skin	*1*) Potentially greater variability than Doppler ultrasound *2*) Occlusion of the hand is often employed for VOP but is not employed for Doppler ultrasound *3*) Changes in blood flow between conditions/populations can differ from those observed with LDF probes
Ultrasound			
Pros	*1*) Can be performed at rest or during exercise, provided the limb remains still	*1*) Provides a continuous, beat-by-beat measure of absolute blood flow *2*) Provides a measure of artery diameter and blood velocity	*1*) Information regarding change in blood flow is similar to that provided by VOP and LDF probes
Cons	*1*) Highly operator-dependent *2*) Generally limited to measures of conduit artery blood flow	*1*) Changes in blood flow could be due to changes in vascular beds other than the skin	*1*) Occlusion of the hand is rarely employed for ultrasound but is for VOP *2*) Changes in blood flow between conditions/populations can differ from those observed with LDF probes
LDF			
Pros	*1*) Operator-independent *2*) LDF probes provide a continuous estimate of skin blood flow *3*) LDF probes can be placed on nearly all areas of the body *4*) LDF imaging can be used to sample from a larger surface area	*1*) Provides an estimate of blood flow that is restricted to the skin microcirculation	*1*) Single-fiber probes, multifiber probes, and LDF imaging provide similar information regarding relative changes in red blood cell flux *2*) Changes in red blood cell flux during passive heating correlate with changes in blood flow measured by VOP *3*) Changes in red blood cell flux during passive heating are similar to changes in blood flow measured by ultrasound
Cons	*1*) LDF probes cannot confidently be repositioned at the same site for repeated measurements *2*) Prone to movement artifacts *3*) LDF imaging provides poor temporal resolution	*1*) Does not provide a quantitative measure of skin blood flow *2*) Absolute values depend on underlying vascularization	*1*) Absolute values of red blood cell flux differ between single-fiber probes, multifiber probes, and LDF imaging *2*) Pattern of change during passive heating has not been compared with other methods *3*) Differences in red blood cell flux between conditions/populations can differ from those observed with VOP or ultrasound

LDF, laser-Doppler flowmetry; VOP: venous occlusion plethysmography.

Table 2. *Guidelines for interpretation of skin blood flow data measured during heat exposure*

Technical Considerations	Interpretation	Comparison Between Methods
To ensure that VOP or ultrasound measures of blood flow reflect skin blood flow, measurements must be performed during passive heating from a limb that is not directly exposed to the heating stimulus	When the objective is to investigate heat exchange between the body and the environment, absolute flow/flux values, along with the skin-to-air temperature gradient, should be reported	Caution should be taken when LDF findings are extrapolated from one measurement site to the whole body. Preferably, multiple measurements of red blood cell flux will be recorded across the body
When the objective is to study reflex-mediated changes in skin blood flow, VOP, LDF, or ultrasound measurements should be obtained from a limb that is not exposed to the heating stimulus heating stimulus should be performed	When the objective is to investigate neural control of skin blood flow, cutaneous vascular conductance should be reported to provide an indication of changes in vasomotor tone	Changes in LDF are not always paralleled by similar changes in limb blood flow
When the objective is to evaluate how changes in skin blood flow or vasomotor tone affect systemic hemodynamics, VOP, LDF, or ultrasound measurements should be obtained from an area directly exposed to the heating stimulus should be performed	LDF flux values should be normalized to a maximally induced value to avoid ascribing differences in skin blood flow between groups or in response to an intervention that are due to variations in probe placement	
	Flux/conductance values should be normalized when the objective is to study neural control of the skin circulation	
	Both normalized and unnormalized LDF data should be reported when the objective is to study heat exchange between the body and the environment	

LDF, laser-Doppler flowmetry; VOP, venous occlusion plethysmography.

demonstrating that increases in limb blood flow during passive heating are due to changes in skin blood flow and that skin contains an active vasodilator system (22, 56, 57). However, the most widely employed technique to measure limb volume is currently strain-gauge plethysmography, originally developed by Whitney (76) and later integrated into commercial systems (34). This approach utilizes a strain gauge that is placed around the largest part of the limb of interest. The strain gauge allows for measurements of limb circumference based on the principle that changes in limb circumference during venous occlusion are proportional to changes in resistance to the current within the strain gauge (76). To relate changes in resistance to a change in volume, current commercial systems employ an electrical calibration, whereby a 1% change in resistance is equal to a 1% change in limb volume (34).

Doppler Ultrasound

Doppler ultrasound-based measures of arterial diameter and blood velocity allow for the calculation of blood flow primarily through a conduit vessel (e.g., brachial artery or femoral artery) (Fig. 1*B*). Ultrasound works by emitting longitudinal sound waves that travel through various media, such as tissue, liquids, and gases (66, 72). Sound waves are generated from a transducer containing piezoelectric crystals that convert electrical energy to mechanical vibrations for a given voltage (72). The frequency of the voltage passing through the piezoelectric crystals directly influences the frequency at which a sound wave is emitted through a given medium. When a sound wave comes into contact with a large surface, a portion of that sound wave is reflected. This reflection produces an echo amplitude, which is relayed back to the transducer, causing a vibration and, in turn, generating energy across the piezoelectric crystals. The speed at which an echo travels is directly related to the density of the tissue. When the sender (transducer) and the observer move together at a relative pace, the frequency of the sound wave remains constant. The apparent difference in frequency between the sender and the observer caused by relative motion (i.e., moving red blood cells) is known as the Doppler effect. If the sender remains stationary and the observer is moving forward, the frequency of the returned sound wave will increase. Similarly, when the observer is moving away from the sender, the frequency of the returning sound wave will decrease. The change in frequency between an emitted and a returned sound wave is proportional to the velocity of the moving reflector (blood cells). This change in frequency is called the Doppler shift and is determined using the following equation

$$Df = \frac{2 \cdot f \cdot v \cdot \cos\theta}{c}$$

where Df is Doppler shift frequency, f is transmitted frequency, v is blood velocity, θ is the angle between the sound beam and the direction of moving blood, and c is the speed of sound. To solve for blood velocity, the above equation can be rearranged as follows

$$v^{\circ} = \frac{Df \cdot c}{2 \cdot f \cdot \cos\theta}$$

Based on this equation, it is evident that the ability to detect a Doppler shift depends on the angle of insonation between the transducer axis and the direction of blood flow within a vessel. The insonation angle will depend on vessel orientation. The Doppler shift is most accurately detected when the angle of insonation is 0° (cos = 1), as the Doppler beam is traveling in the same direction as blood flow. However, an insonation angle

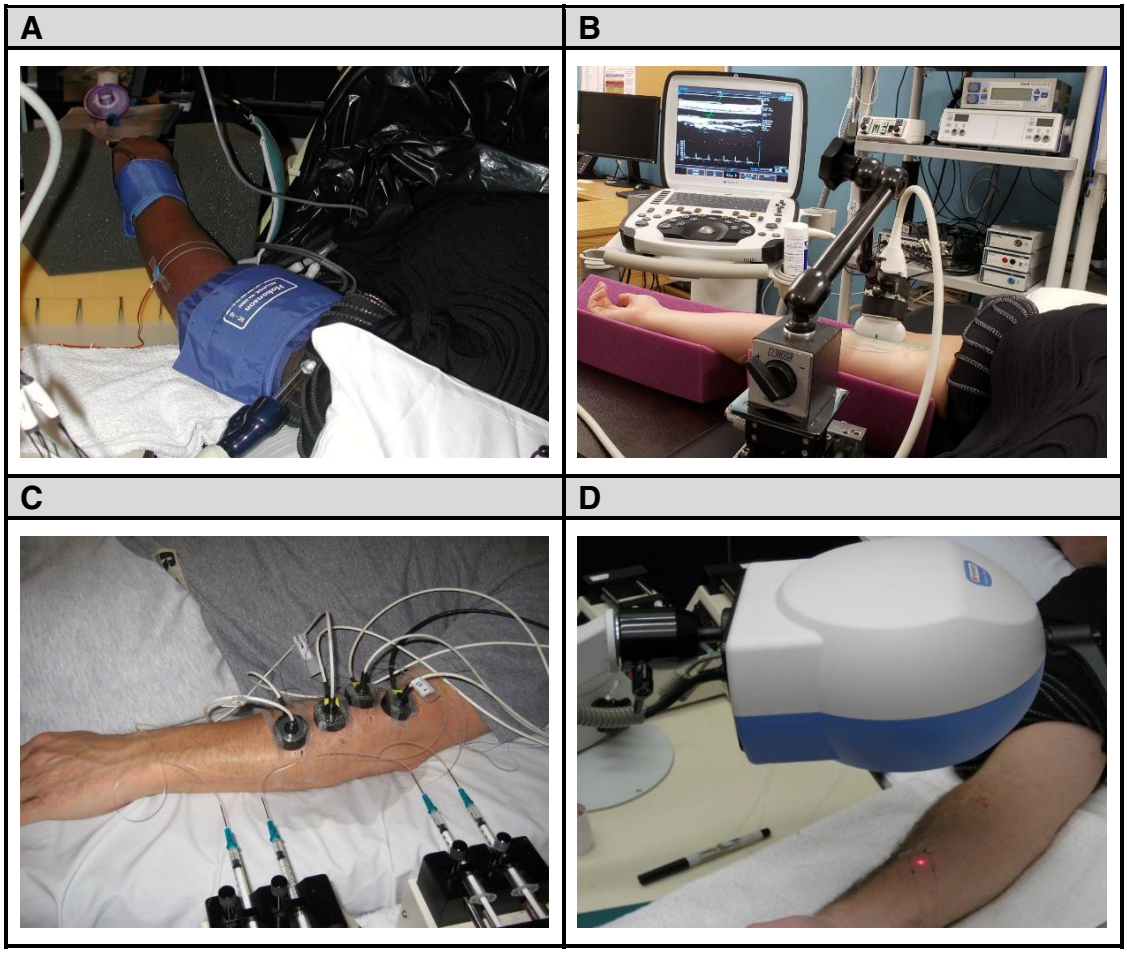

Fig. 1. Typical setup of venous occlusion plethysmography (*A*), Doppler ultrasound (*B*), laser-Doppler flowmetry (*C*), and laser-Doppler imaging (*D*) during passive heat stress protocols.

of 0° is unachievable, as most vessels lay parallel to the skin surface. Conversely, to obtain a clear image of a vessel wall, the Doppler beam would ideally be at a 90° angle to the vessel of interest. However, an insonation angle of 90° (cos = 0) means that the Doppler beam is perpendicular to the observer, resulting in an undetectable Doppler shift. Therefore, a compromise must be made by maintaining an angle of ≤60° to ensure that the Doppler shift is adequately reflected and to minimize error associated with blood velocity calculations (70). Doppler ultrasound measurements require a transducer with two piezoelectric elements, creating a double Doppler effect. A second piezoelectric element allows the transducer to receive echoes from moving blood cells, making it the observer for returning sound. Acoustic impedance describes the resistance encountered by a sound wave when it travels through a tissue. A large change in acoustic impedance for a given medium means that a larger proportion of the ultrasound will be reflected (13). There are large differences in the acoustic impedance between different tissues, and it follows that tissues with a larger density will attenuate the energy of a sound wave to a greater extent, limiting the possibility of obtaining an ultrasound image and Doppler information, such as in bones and calcified arterial walls (72). The frequency at which a sound wave travels will also determine the depth at which a Doppler image is obtained, such that higher frequencies will propagate more shallow sound waves, providing Doppler images for more superficial arteries, in some instances, the microvasculature (17).

Laser-Doppler Flowmetry

Laser-Doppler flowmetry also relies on the Doppler effect, such that the change in frequency of laser light emitted at a known wavelength (rather than sound) is used to estimate skin blood flow. Commercially available systems employ probes containing optical fibers to emit and receive laser light (Fig. 1*C*). Although probes initially contained one emitting and one receiving fiber (single-fiber probes), more recent multifiber probes contain one or more emitting fibers and multiple receiving fibers. Laser-Doppler imaging (Fig. 1*D*) can also be used to estimate skin blood flow. Laser-Doppler probes or imaging devices emit laser light at a known wavelength over a surface area of the skin. As the light penetrates underlying tissue, most of it will be absorbed and/or reflected without undergoing a change in wavelength. The remaining fraction will undergo a change in wavelength as it is scattered from hitting red blood cells circulating within the underlying skin microvasculature. This will cause a change in wavelength of the laser light, which is quantified through receiving fibers placed in close proximity to the emitting fiber. Based on the

principle that the magnitude of change in wavelength is proportional to the concentration of red blood cells and the velocity at which they are moving through the area of measurement, red blood cell flux (in perfusion units) is calculated as

$$\text{flux} = \text{RBC}_{\text{concentration}} \times \text{RBC}_{\text{velocity}}$$

where RBC is red blood cell.

Therefore, it is important to consider that laser-Doppler flowmetry does not provide a quantitative measure of blood flow but, rather, a qualitative estimate based on changes in red blood cell flux. The main advantage of laser-Doppler flowmetry probes is the ability to continuously measure red blood cell flux at a high temporal resolution, specifically within the skin microvasculature. The skin microvasculature is located within the dermal layer and is composed of arterioles and venules, which form an upper and a lower plexus. The upper plexus is located ~1–2 mm below the epidermal layer and contains the nutritive capillaries of the dermal papillae (5). The lower plexus consists of vertical arterioles and venules that connect with the upper plexus, as well as branching vessels that supply the sweat glands (5). The measurement depth and sampling volume of laser-Doppler flowmetry probes depend on the laser light wavelength and the distance separating emitting and receiving fibers (24). At the wavelength employed by currently available commercial systems (780 nm), it has been estimated that laser-Doppler probes placed on forearm skin will sample at a maximum depth of 0.73 mm and volume of 2.7 mm^3 (24). As such, measurements of red blood cell flux primarily reflect flux through vessels of the upper dermal plexus and are unlikely to be influenced by changes in flux through underlying nonskin tissues (>3 mm).

Optical Coherence Tomography

Optical coherence tomography is a noninvasive sampling technique that provides a cross-sectional image of the skin microvasculature by generating images based on backreflected near-infrared light from a sampling area. The main advantage of optical coherence tomography is that it provides information regarding vessel number, depth, and diameter at high spatial and temporal resolutions. A low-coherence interferometer uses near-infrared light waves to provide a structural image of the vessel determined by light backscattered from different tissues. A one-dimensional scan for depth (A mode) is combined with a two-dimensional cross-sectional scan (B mode) to form a three-dimensional image of the vessel. To obtain a cross-sectional image of a vessel, the interferometer measures the difference of the optical path length between the sample and the reference point. This technique has been used for dermatological applications, such as burn assessments (47), cancer (23, 75), and other skin diseases (50). When optical coherence tomography is combined with Doppler velocimetry (i.e., optical Doppler tomography), high-resolution images of blood velocity and a structural image of blood vessels can be obtained from the same area (74, 79). Optical coherence tomography demonstrates good reproducibility for measurements of vessel density (64, 78). However, Smith et al. (64) reported a greater increase in estimated flow during 30 min of local heating measured by laser-Doppler flowmetry (1,360 ± 412%) than by optical coherence tomography (234 ± 118%). Additional studies assessing the reliability and reproducibility of optical coherence tomography are limited and mostly relate to measurements taken from the retina (15, 46). Recently, optical coherence tomography was used to demonstrate that whole body heat stress increases vessel number, depth, and diameter within the skin microvasculature (16). As optical coherence tomography is a relatively new measurement technique for assessing blood flow during heat stress, insufficient literature prevents further discussion of this technique in this review.

TECHNICAL CONSIDERATIONS

Some technical aspects should be considered when deciding on the technique of choice to measure skin blood flow. Here, we explore some of these aspects. The reader is encouraged to consult review articles specific to each method for a more detailed description of these and additional technical considerations (31, 53, 70, 77).

Venous Occlusion Plethysmography

The main advantage of venous occlusion plethysmography is that it provides a measure of absolute limb blood flow. However, a few points must be considered to ensure that valid measurements are obtained. A typical plethysmography trace is characterized by an initial volume change with a slope that is linear as a function of time (31). The slope of the initial, linear increase in volume is taken as arterial inflow. As venous occlusion is prolonged, the change in volume eventually reaches a plateau. For this reason, plethysmography measurements are typically performed in cycles of varying durations that should be determined based on the rates of arterial inflow and venous outflow. Although the duration of these cycles is typically 15 s (8 s of occlusion and 7 s of release), it should be considered that venous outflow can be affected by certain physiological conditions, such as venous congestion (3). Regardless of cycle length, the limb of interest must be elevated relative to heart level to avoid venous congestion and a subsequent attenuation of arterial inflow upon the release of the upper "venous" cuff. Although these cycles do not allow for continuous measurements of limb blood flow, they can be repeated as required to provide changes in limb blood flow at relatively good temporal resolution. It should be noted that an arterial inflow that noticeably decreases on a beat-by-beat basis due to movement artifacts, cuff leakage, or baseline shifts may be less reliable than a steady arterial inflow (31). Care should also be taken to avoid movement artifacts caused by arterial or venous cuff inflation that produce a shift in the displacement signal and an invalid plethysmography measurement. Any movement of the limb of interest while the venous cuff is inflated will also result in signal artifacts that prohibit arterial inflow analyses. A final technical point to consider is the time taken for the cuff to inflate, as it may influence the slope of arterial inflow (40).

Doppler Ultrasound

The main advantage of Doppler ultrasound is that it provides beat-by-beat measures of absolute blood flow. The main considerations related to Doppler ultrasound stem from operator error or differences in operator technique (48). Briefly, the image needs to be optimized to ensure a clear demarcation between the vessel walls and lumen to adequately determine diameter. The sample gate should be

optimized within the vessel lumen and placed at an adequate angle to ensure valid velocity values. For a repeated-measures study design, the reproducibility of the measurement increases with operator experience as well as marking the anatomical position of the Doppler probe (48). The latter can be done by external marking of the skin and photographing the position of the ultrasound probe. Visual landmarks on the imaged vessel (e.g., veins) can also be noted and used to ensure that repeated measurements are taken from the same section of the vessel. To avoid movement artifacts from the operator, a probe-holding device can be considered. For additional information, the reader is encouraged to consult detailed guidelines for Doppler ultrasound measurements (68, 70).

Laser-Doppler Flowmetry

The main advantage of laser-Doppler flowmetry is that it provides an estimate of blood flow that is specific to the skin microvasculature. However, because laser-Doppler flowmetry measurements are prone to movement artifacts, the area of interest should remain as still as possible to ensure stable recordings. When movement cannot be avoided (e.g., dynamic exercise), care should be taken to affix the laser-Doppler probes to areas that will remain relatively still. Practically, the small sampling area of laser-Doppler probes is advantageous for measuring red blood cell flux from any area of the body surface and combining the measurement with intradermal microdialysis to investigate microvascular control mechanisms (35). The small sampling area also represents a limitation to the method, in that local measures of red blood cell flux may not reflect those at the level of the limb and/or of the whole body. Furthermore, the small sampling area makes laser-Doppler measurements highly dependent on the anatomy of the underlying microvasculature (6–8). Finally, because of the small sampling area, it is difficult to confidently assume that repeated measurements of red blood cell flux occur from the same location when laser-Doppler probes are removed between repeated measurements, despite the use of strategies to minimize differences in probe placement (e.g., photographing the probe position and marking the skin surface). Limitations related to sampling area can be overcome with laser-Doppler imaging, since it measures red blood cell flux over a larger surface area; however, this method provides poor temporal resolution. A final consideration is that care should be taken to avoid placement of laser-Doppler probes directly over superficial veins, freckles, hair, or boney areas to ensure that recordings are representative of changes in cutaneous microvascular blood flow.

COMPARISON OF METHODS

Studies investigating skin blood flow responses to various perturbations may employ different measurement techniques. When interpreting such studies, it is important to know if the various measurement techniques provide similar information regarding changes in skin blood flow in response to a given perturbation. In this section, we review the few studies that have directly compared skin blood flow data between certain techniques, with an emphasis on studies that compared these techniques during heat exposure.

Limb Blood Flow: Venous Occlusion Plethysmography Versus Doppler Ultrasound

A direct comparison of limb blood flow between venous occlusion plethysmography and Doppler ultrasound is difficult, given differences in the units each device provides (ml·100 ml tissue^{-1}·min^{-1} for plethysmography vs. ml/min for Doppler ultrasound) and the continuous (Doppler ultrasound) vs. intermittent (plethysmography) nature of each measurement. Nonetheless, a number of studies have demonstrated similar relative changes from baseline in limb blood flow between these two methods during exercise (51, 73), lower body negative pressure (11, 44), and whole body heating and cooling (11). Brothers et al. (11) observed similar relative increases in limb blood flow measured by venous occlusion plethysmography and Doppler ultrasound during skin surface cooling (−21 vs. −25%), whole body heating (~550 vs. ~450%), and baroreceptor unloading under heat stress (~350 vs. ~300%). However, there was greater variability in the change in blood flow during heat stress when measured by venous occlusion plethysmography (550 ± 250%) than when measured by Doppler ultrasound (450 ± 150%) that may have masked statistical differences between methods. Indeed, the reported magnitude of difference for change in blood flow ($d = 0.44$) suggests a moderate difference between the two methods. Tschakovsky et al. (73) also demonstrated a linear relationship ($r = 0.93$) between mean blood velocity measured by Doppler ultrasound and forearm blood flow measured by venous occlusion plethysmography during handgrip exercise. Notably, Tschakovsky et al. (73) commented that it is important to quantify beat-by-beat limb blood flow/velocity to effectively compare Doppler ultrasound with plethysmography and to avoid under- or overestimating forearm blood flow due to cuff inflation. The beat-by-beat comparison presented by Tschakovsky et al. (73) also demonstrated a similar temporal pattern of change in limb blood flow between Doppler ultrasound and plethysmography. Another interesting note is that distal cuff occlusion, typically employed with plethysmography, is less likely to be employed during Doppler-derived measurements of limb blood flow. Thus, despite similarities in the relative changes in blood flow between these two approaches, physiologically relevant differences may exist if the applied perturbation alters hand blood flow.

Laser-Doppler Flowmetry: Single-Fiber Versus. Multifiber Probes Versus Imaging

A contributing factor to differences in red blood cell flux measured using single-fiber or multifiber probes or laser-Doppler imaging systems is the different surface area covered by each measurement technique, as well as the heterogeneity of the underlying microvasculature where sampling occurs (6, 7). Brothers et al. (11) reported differences in absolute red blood cell flux between single-fiber probes, multifiber probes, and laser-Doppler imaging that sampled from the same forearm during passive heating (58 ± 29 vs. 148 ± 28 vs. 206 ± 47 flux units), passive cooling (7 ± 2 vs. 13 ± 5 vs. 28 ± 8 flux units), and lower body negative pressure during passive heating (49 ± 27 vs. 125 ± 43 vs. 177 ± 54 flux units). However, when values were normalized to percentage of maximal red blood cell flux achieved during local skin heating, changes in red blood cell flux were similar during passive heating

(45 ± 25 vs. 55 ± 25 vs. 45 ± 12% maximum), passive cooling (7 ± 4 vs. 6 ± 4 vs. 7 ± 2% maximum), and lower body negative pressure during passive heating (58 ± 40 vs. 65 ± 20 vs. 55 ± 7% maximum). Although these observations suggest that single-fiber probes, multifiber probes, and laser-Doppler imaging provide similar information regarding the relative change in red blood cell flux during thermal perturbations, it is unknown if the pattern of change in skin blood flow was similar between measurement techniques.

Laser-Doppler Flowmetry Versus Limb Blood Flow

Skin blood flow can be estimated from limb blood flow measurements, if the increase in limb blood flow during heat stress is exclusively due to changes in skin blood flow. Notwithstanding limitations to this assumption, as discussed in the next section, limb blood flow measurements can be used to estimate skin blood flow only when the limb is inactive, such as during passive heating, to avoid contraction-induced increases in muscle blood flow. Perhaps the first methodological comparison between laser-Doppler flowmetry and plethysmography was carried out by Johnson et al. (38), who demonstrated a linear relationship between the two methods during a bout of whole body passive heating. However, the slopes depicting these responses were very different between subjects (mean 0.078, range 0.040–0.122), possibly due to interindividual variability in the anatomical placement of the laser-Doppler probe and/or vascularization underneath the probe. Brothers et al. (11) further reported that laser-Doppler flowmetry (single-fiber and multifiber probes) and limb blood flow measurements (plethysmography and Doppler ultrasound) demonstrate comparable changes following 45 min of whole body heating (~600 ± 300% vs. ~550 ± 250%). Although these studies suggest that limb blood flow and local measurements of red blood cell flux provide similar information regarding overall changes in skin blood flow during heat exposure, they did not ascertain if the pattern of change was similar between techniques, nor did they examine if each technique provides a similar interpretation of changes in skin blood flow between interventions or populations.

We observed that changes in laser-Doppler red blood cell flux are not always reflected in measurements of limb blood flow. For example, fan use (27) and 6 wk of folic acid supplementation (28) enhanced red blood cell flux in healthy older adults exposed to a 42°C environment, yet this was not observed with Doppler ultrasound measurements. Similarly, we observed attenuated red blood cell flux, but not forearm blood flow (plethysmography), during heat exposure in combination with dehydration induced via oral administration of the diuretic furosemide (19). Finally, Stanhewicz et al. (65) demonstrated that an oral dose of the nitric oxide synthase essential cofactor sapropterin acutely increased red blood cell flux during passive heating in older adults, yet the magnitude of this increase was larger than that observed with forearm blood flow (plethysmography). Based on these observations, it is possible that interventions or conditions that influence red blood cell flux do not always reflect blood flow at the limb level. Alternatively, changes in red blood cell flux that are not accompanied by similar changes in limb blood flow could be the result of a compensatory response in other vascular beds that cancels out the greater/lower red blood cell flux measured by laser-Doppler flowmetry. Finally, differences between laser-Doppler and whole-limb-derived measurements may be related to a limited depth (i.e., sample volume) during laser-Doppler assessments relative to whole limb measurements. It remains unknown which technique accurately reflects actual differences (or the lack thereof) in skin blood flow responses between populations and/or due to perturbations.

INTERPRETING SKIN BLOOD FLOW MEASUREMENTS

Once skin blood flow data have been collected, several aspects should be considered when interpreting the results. This section aims to highlight some important considerations that can affect the interpretation of skin blood flow measurements, depending on the question of interest.

Flow Versus Conductance

Measurements of limb blood flow or red blood cell flux provide information regarding the "amount" of blood that is flowing through the area of measurement. Alone, these measurements cannot provide information as to the mechanisms resulting in such changes in flow. Flow through any vascular bed is predicated on Poiseuille's law

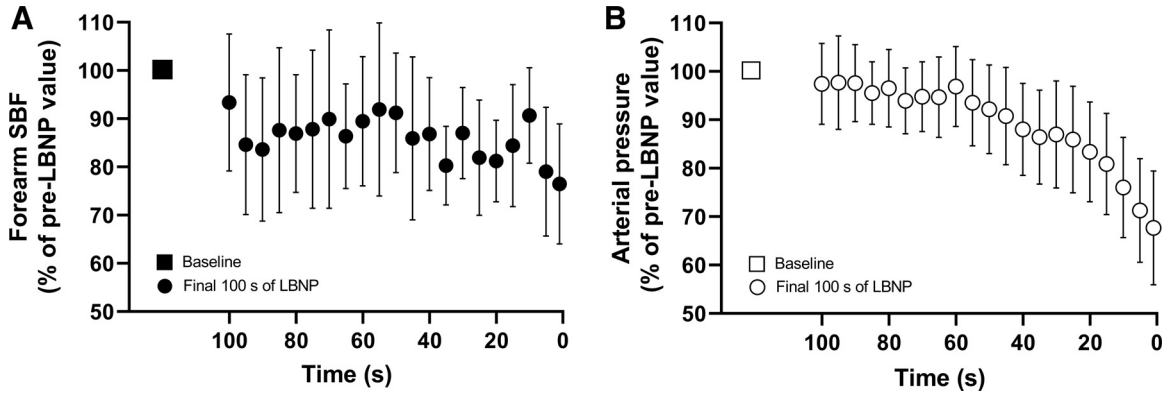

Fig. 2. Relative decreases in forearm skin blood flow (SBF; *A*) and mean arterial pressure (*B*) during the last 100 s of incremental lower body negative pressure (LBNP) performed to presyncope in a normothermic condition. Data are presented as percentage of values measured before lower body negative pressure (Baseline). Values are means ± 95% confidence interval. Note that the decrease in forearm skin blood flow parallels the decrease in mean arterial blood pressure. In this example, 70% of the reduction in skin blood flow could be accounted for by the reduction in arterial blood pressure. [Redrawn from Gagnon et al. (26).]

$$Q = \frac{(P_2 - P_1)}{\mathcal{R}}$$

where Q is the flow rate, $P_2 - P_1$ is the difference in pressure at two points along a vessel, and R is the resistance to laminar flow, which is determined by

$$\mathcal{R} = \frac{8_{\eta l}}{\pi r^4}$$

where 8 is a mathematical constant, r is the radius of the vessel, ŋ is the viscosity of the fluid, and l is the length of the vessel.

Flow is therefore dependent on both the pressure gradient and the vessel radius. Notably, changes in limb blood flow or red blood cell flux do not necessarily reflect changes in vasomotor tone that impact vessel radius. A good example is the reduction in red blood cell flux when individuals undergo lower body negative pressure to presyncope. In Fig. 2*A*, a large reduction in red blood cell flux is observed at the point of presyncope. In this case, laser-Doppler-derived red blood cell flux decreased ~30–40%. Although it might be tempting to attribute this decrease in red blood cell flux to vasoconstriction, the reduction in flux was, rather, primarily due to the decrease in arterial blood pressure caused by lower body negative pressure (Fig. 2*B*). In fact, ≥70% of the reduction in red blood cell flux was accounted for by the decrease in blood pressure, such that a maximum of 15–30% could be attributed to changes in vasomotor tone (20, 26). To determine if changes in flow or flux are due to changes in vasomotor tone, limb blood flow or red blood cell flux must be normalized to arterial blood pressure and expressed as vascular conductance, where

$$\text{conductance} = \frac{\text{flow}}{\Delta\text{pressure}}$$

When limb blood flow, for example, forearm blood flow, is the variable of interest, this measure is termed forearm vascular conductance, whereas the term cutaneous vascular conductance is used when laser-Doppler flowmetry is employed. Reporting data as conductance, rather than resistance, is preferred due to the linear relationship between conductance and blood flow for a given pressure, whereas the relationship between blood flow and resistance is nonlinear (39). The choice to report flow/flux values, rather than vascular conductance, should be driven by the question of interest. When neural control of blood flow is being investigated, vascular conductance provides an index of changes in vasomotor tone independent of potential changes in blood pressure. Alternatively, when the thermoregulatory impact of changes in flow or flux is being investigated, flow or flux values provide an estimate of blood volume directed to the skin circulation. Absolute flow or flux will influence skin temperature and, therefore, the skin-to-air temperature gradient that determines dry heat exchange between the body and the environment. In this case, it can be argued that it does not matter how flow or flux "gets there" but, rather, how much and whether it affects skin temperature.

Limb Blood Flow Measurements

When measurements of limb blood flow during heat stress are interpreted, the potential distribution of flow to various vascular beds must be considered. Changes in blood flow to a limb during heat exposure could reflect changes in skin, muscle, adipose, and/or bone blood flow (32). During passive heat stress, there is conflicting evidence to support or refute the notion that increases in limb blood flow are restricted to the skin. Roddie et al. (57) first evaluated this question by inserting catheters into superficial and deep veins of the antecubital fossa during a bout of whole body passive heating to compare changes in oxygen saturation of superficial vs. deep venous blood. With the assumption that arterial oxygen content remains constant during whole body passive heating, changes in venous oxygen saturation were used to reflect changes in blood flow. Superficial venous oxygen saturation was assumed to reflect changes in skin blood flow, whereas deep venous oxygen saturation was used to reflect changes in muscle blood flow. During heating, deep venous oxygen saturation remained stable at ~40%, yet superficial oxygen saturation increased from ~40% to ~99% (Fig. 3). In parallel, an increase in limb blood flow (plethysmography) was observed on the opposite arm. Based on these observations, it was concluded that increases in forearm blood flow during passive heating reflect changes in skin, but not muscle, blood flow.

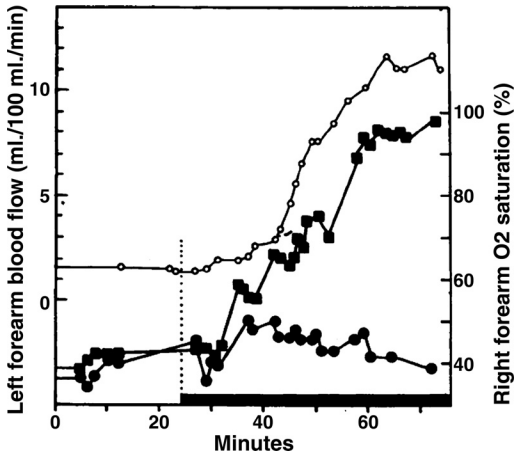

Fig. 3. Effect of body heating (solid bar from *minute 25* to *75*) on oxygen saturation of deep (●) and superficial (■) venous blood and forearm blood flow (○). Traces represent typical data from 1 participant. Note how superficial venous oxygen saturation reaches ~99% at the end of heating, whereas deep venous oxygen saturation remains stable at ~40–50% throughout heating. These findings argue that muscle blood flow, represented by a lack of change in deep venous oxygen saturation, is unchanged during passive heating. [Reproduced with permission from Roddie et al. (57).]

Detry et al. (21) corroborated these findings by measuring forearm muscle blood flow during passive heating and handgrip exercise via the clearance rate of 4-iodoantipyrine-^{125}I ([^{125}I]Ap) injected 1 cm into the brachioradialis muscle. [^{125}I]Ap is a radioactive isotope that, once injected into a tissue, can be detected with a scintillation detector placed above the skin at the injection site. Scintillation detectors contain materials (in this case, a crystal) that absorb the radiation of an isotope (52). As the radiation is proportional to the amount of isotope within the tissue, the clearance rate of an injected isotope provides an index of blood flow through that tissue. Using this technique, Detry et al. (21) observed no change in the clearance rate of [^{125}I]Ap during 45 min of passive heating, suggesting the absence of a change in muscle blood flow in response to that heating perturbation. In contrast,

the clearance rate of [^{125}I]Ap increased 20-fold during handgrip exercise after passive heating. In a separate trial, Detry et al. (21) compared limb blood flow (plethysmography) in a forearm treated with epinephrine iontophoresis with that in the contralateral untreated forearm during passive heating. Since epinephrine iontophoresis caused profound cutaneous vasoconstriction, any changes in limb blood flow in that treated arm during heat stress would be indicative of muscle blood flow. Consistent with the findings of the first trial, heat stress did not change limb blood flow in the epinephrine-treated arm. It was thus concluded that increases in limb blood flow during passive heating solely reflect changes in skin blood flow. Together, the studies by Roddie et al. (57) and Detry et al. (21) led to the long-standing notion that changes in limb blood flow during passive heating are restricted to the skin circulation. However, both studies examined reflex changes in limb blood flow, that is, changes in blood flow within limbs not directly exposed to the heating stimulus. Most recently, Heinonen et al. (32) investigated the influence of local heating on limb blood flow. Calf blood flow was measured by positron-emission tomography during local heating and whole body passive heating. An increase in muscle blood flow was observed when the calf was locally heated, yet no increase in muscle blood flow from the calf not exposed to the heating source was observed during whole body passive heating. A notable difference between conditions was the ~3°C greater calf skin temperature during local heating than during whole body heating, although calf muscle temperature was the same for the two protocols. Therefore, changes in limb blood flow during passive heating cannot be solely attributed to changes in skin blood flow when skin temperatures are high, such as exposure to hot environmental temperatures (9, 32).

Laser-Doppler Measurements

Perhaps the greatest limitation to interpreting laser-Doppler measures of red blood cell flux is the unknown sample volume being measured. This characteristic not only limits laser-Doppler flowmetry to a qualitative estimate of skin blood flow (e.g., in flux units), but it also leads to marked spatial heterogeneity in the data. Braverman and Schechner (7) reported that red blood cell flux varied up to fivefold on forearm skin when measurements were taken contiguously at 1-mm^2 intervals within a total area of 1 cm^2. This variation became even more pronounced (~8-fold) during local heating of the forearm to a skin temperature of 40°C. Such spatial heterogeneity has been attributed to the anatomy of the skin microvasculature within the sampling area of the probe. As mentioned previously, laser-Doppler probes sample primarily from the upper dermal plexus, which is composed of multiple umbrella-shaped networks of arterioles, capillaries, and venules connected to a single ascending arteriole and an accompanying descending venule (8). Importantly, ascending arterioles are separated at random (1.5- to 7-mm) intervals (5). Studies combining laser-Doppler flowmetry with skin biopsies have demonstrated that spatial heterogeneity of red blood cell flux can be correlated to these random separations (6, 8). It should also be considered that red blood cell flux is characterized not only by spatial heterogeneity, but also by temporal heterogeneity, as measurements vary when performed over hours or days at a given site (7). Given the heterogeneity of laser-Doppler flowmetry values, it is often difficult to determine if different flux values represent different flows or different sampling areas. For example, two individuals, *A* and *B*, may display noticeably different flux values when exposed to whole body heat stress: an increase to 210 flux units for *individual A* vs. an increase to 70 flux units for *individual B*. In this case, does *individual A* have a greater capacity than *individual B* to increase skin blood flow during heat exposure? Or is the probe sampling from an area that is relatively more vascularized for *individual A* than *individual B*? Or is it a combination of both possibilities? If one assumes that red blood cell flux before heat exposure was 30 flux units for *individual A* and 10 flux units for *individual B*, then the relative increase in flux during heat exposure would be the same between these individuals (600%). Should it be concluded that both individuals have the same skin blood flow response to heat stress, despite different absolute flux values?

To account for heterogeneity in laser-Doppler flowmetry values, several normalization procedures have been employed. First, perhaps the simplest is to normalize values relative to a baseline measure. However, it should be considered that this approach is highly dependent on baseline values (2, 33). For example, *individuals A* and *B* in the scenario described above have the same relative increase in red blood cell flux, yet absolute values differ by threefold. A second approach is to normalize red blood cell flux to the maximal dilation of the sampling area. This can be achieved by eliciting "maximal" vasodilation of the skin vasculature and subsequently expressing values attained during an experiment as percentage of the maximal value obtained. When limb blood flow is measured, this can be achieved by spraying hot water onto the skin surface or placing the limb beneath a radiant lamp (14, 37, 60). When laser-Doppler flowmetry is used, probes can be housed within a local heater that is placed on the skin surface. Evidence that this protocol elicits maximal vasodilation comes from studies demonstrating that limb blood flow and red blood cell flux cannot be further increased with whole body heating when the skin is locally heated to 42°C (67) and that postocclusive reactive hyperemia is greater when the forearm is heated to 42°C than when it is maintained at 33°C (37). A third approach to obtain maximal skin vasodilation is to locally infuse the nitric oxide donor sodium nitroprusside via intradermal microdialysis, while some studies have combined local skin heating with sodium nitroprusside infusion. Regardless of the method employed, the theory is that maximal flux values should be greater in highly vascularized than less vascular areas. By normalizing values as percentage of maximum, different sites are compared relative to their maximal ability to dilate. Although this is likely the strongest approach to account for intersite or day-to-day variability in absolute flux values, it should be considered that solely presenting data as percentage of maximum may mask some important differences between conditions or groups. For example, consider that *individual A* in the above-mentioned example has a maximal vasodilation of 410 units compared with 140 units for *individual B*. Based on the flux values each individual reached during heat exposure (210 units for *individual A* and 70 units for *individual B*), both individuals would have reached 50% of maximum red blood cell flux. Although both individuals reached a similar level of red blood cell flux when values were normalized relative to the respective maximal values, markedly different absolute values are observed between individuals. If only values expressed as

a percentage of maximum are presented, should it be concluded that *individuals A* and *B* have the same skin blood flow response to heat exposure? Care should also be taken in interpreting skin blood flow responses that are normalized to maximum if an applied perturbation (e.g., chronic drug administration) or physiological condition (e.g., disease state) has the potential to alter that maximal response. There is little interpretive value of reporting skin blood flow responses relative to a site's maximal response when the question of interest pertains to heat dissipation, since absolute skin blood flow will ultimately affect skin temperature and, therefore, the skin-to-air temperature gradient, which dictates dry heat exchange between the body and the environment. Lastly, it should be considered that maximal values are relative to the method employed and may not necessarily reflect "true" maximal vasodilation. For example, we have observed that cutaneous vascular conductance can exceed 100% of maximum, as determined by local skin heating during rapid saline infusion (61).

Covered Versus Uncovered

Studies investigating skin blood flow responses to passive heating often use a tube-lined suit perfused with water or lower limb immersion in a water bath. Under these heating conditions, most studies will measure skin blood flow on a limb that is not directly exposed to the heating stimulus. During passive whole body heat stress, increases in skin blood flow are mediated by both neural and local mechanisms (41, 55). However, the mechanisms that mediate skin vasodilation to local and reflex-induced whole body heating are different. When the skin is directly exposed to a heating stimulus, vasodilation is largely nitric oxide-dependent (41), whereas reflex-induced skin vasodilation is primarily mediated by neurotransmitter release from sympathetic cholinergic nerves, with a smaller contribution from nitric oxide (42, 63). Although measuring skin blood flow from a region not directly exposed to the heating stimulus (i.e., the uncovered forearm during a whole body passive heat stress protocol) is important in studying reflex-mediated control of skin vasodilation, it should be emphasized that this unexposed region represents a very small skin surface area relative to the area covered by the water-perfused suit. Consequently, skin blood flow measurements from the uncovered area may not reflect blood flow over the majority of the skin that is exposed to the heating stimulus. Pearson et al. (54) observed a greater reduction in cutaneous vascular conductance during a simulated hemorrhagic challenge in hyperthermic individuals (passively heated in a water-perfused suit) when red blood cell flux was measured from a forearm site where local temperature was maintained at ~35°C than at an adjacent site that was locally heated to match the temperature of the skin beneath the suit (~38°C) (Fig. 4). Also, differences in limb blood flow will be observed when measurements are obtained from a covered vs. an uncovered limb. During whole body passive heating, Heinonen et al. (32) observed a greater increase when calf blood flow was measured underneath a water-perfused suit than when the calf was not covered by the suit.

Related to the aforementioned discussion, it is important to emphasize that cardiovascular responses to whole body passive heating are primarily driven by changes in cutaneous vascular

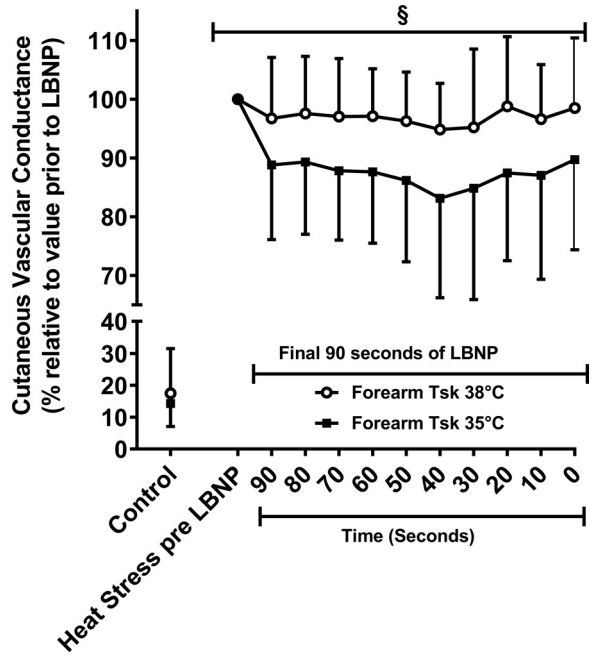

Fig. 4. Relative changes in forearm cutaneous vascular conductance during the final 90 s of lower body negative pressure (LBNP) to presyncope at mild and moderate forearm skin temperature (T_{sk}) sites. Values are means ± SD. §Significant interaction between local skin temperature and time during the final 90 s of lower body negative pressure. Note that cutaneous vascular conductance remained unchanged at the site locally heated to ~38°C (moderate), whereas it declined at the site with a skin temperature of ~35°C (mild). [Reproduced with permission from Pearson et al. (54).]

conductance under the water-perfused suit. For example, older adults display attenuated vasodilation in uncovered skin during passive heat exposure (43). In parallel, older adults also display an attenuated increase in cardiac output during passive heating (29, 30, 49). Based on these observations, Greaney et al. (30) hypothesized that central cardiovascular limitations may contribute to the attenuated skin blood flow response of older adults during passive heating. To address this possibility, they examined if acute augmentation of cutaneous vascular conductance in older adults would result in a greater cardiac output during whole body passive heat stress. Despite greater skin vasodilation measured on uncovered skin following an acute dose of sapropterin, cardiac output was not further increased relative to a control (placebo) condition. Although these observations suggest that central cardiovascular limitations contribute to the attenuated skin blood flow response of older adults, it is important to consider that skin vasodilation was measured from an area that was not exposed to the heating stimulus. It is possible that sapropterin does not result in greater vasodilation of the skin exposed to high local temperatures beneath the suit, which represents ~90% of body surface area. If this is the case, a lack of increase in cardiac output with sapropterin could be explained by a lack of additional vasodilation within the skin under the water-perfused suit.

Perspectives and Recommendations

The choice of technique to investigate skin blood flow and the approach taken for subsequent data interpretation will ultimately be driven by the research question at hand. To provide guidance and to promote standardization of skin blood

flow measurements, we propose the following recommendations.

1) When the objective is to measure skin blood flow during passive heat stress by venous occlusion plethysmography or Doppler ultrasound, available data indicate that an increase in limb blood flow reflects the amount of blood directed toward the skin vasculature. When the limb is directly heated, an increase in muscle blood flow may contribute to the increase in limb blood flow, thereby providing an inaccurate estimation of skin blood flow. Consequently, measurements of skin blood flow recorded by plethysmography or Doppler ultrasound must be obtained from a limb that is not exposed to a direct heating stimulus.

2) When deciding to report flow/flux or vascular conductance, it should be considered that flow/flux values provide an estimate of the amount of blood that is directed to the skin circulation without information regarding how that blood is getting there. In contrast, vascular conductance provides an index of changes in flow/flux that are independent of changes in blood pressure. When the objective is to investigate heat exchange between the body and the environment, absolute flow/flux values, along with the skin-to-air temperature gradient, should be reported. When the objective is to investigate locally derived or neural control of skin blood flow, cutaneous vascular conductance should be reported to provide an indication of changes in vasomotor tone.

3) When deciding to report normalized or unnormalized laser-Doppler values, it should be considered that absolute flux can vary greatly depending on the placement of the laser-Doppler probe due to the spatial heterogeneity of the underlying microvasculature. For this reason, flux values should be normalized to avoid ascribing differences in skin blood flow between groups or in response to an intervention that is due to variations in probe placement. Of the normalizing procedures available, we recommend normalizing flux values to a maximally induced value, as normalization to baseline values does not provide an indication of the extent of underlying vascularization. Furthermore, we recommend that flux/conductance values should be normalized when the objective is to study neural control of the skin circulation. When the objective of the protocol is to study heat exchange between the body and the environment, we recommend that both normalized and unnormalized data be reported to provide information regarding the absolute amount of skin blood flow.

4) When measuring red blood cell flux, caution should be taken when findings are extrapolated from one measurement site to the whole body. Some evidence suggests that differences in red blood cell flux between populations or in response to interventions are not always paralleled by similar changes in limb blood flow. Preferably, multiple measurements of red blood cell flux would be performed across the body to determine if changes in one area (e.g., forearm) are consistent with measurements across other areas (e.g., back, chest, thigh, and forehead). Furthermore, for alterations in red blood cell flux to meaningfully alter dry heat exchange between the body and the environment, they must ultimately affect the skin-to-air temperature gradient. Therefore, when the objective is to determine if differences in red blood cell flux between populations or in response to interventions alter thermoregulatory control, the skin-to-air temperature gradient should also be reported.

5) When considering to measure skin blood flow from an area directly exposed or not exposed to the heating stimulus, measurements from a limb that is not exposed to the heating stimulus should be performed if the objective is to study reflex-mediated changes in skin blood flow (to avoid locally mediated changes in vasomotor tone). However, a limitation that should be recognized with this approach is that skin blood flow from the unexposed limb may not represent skin blood flow across the rest of the body that is directly exposed to the heating stimulus. If the objective is to evaluate how changes in skin blood flow or vasomotor tone affect systemic hemodynamics, measurements should be obtained from a limb directly exposed to the heating stimulus.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.G.C. and D.G. conceived and designed research; G.K.C. prepared figures; G.K.C. and D.G. drafted manuscript; G.K.C., C.G.C., and D.G. edited and revised manuscript; G.K.C., C.G.C., and D.G. approved final version of manuscript.

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