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Noninvasive examination of endothelial, sympathetic, and myogenic contributions to regional differences in the human cutaneous microcirculation

Gary J. Hodges [a,*], Andrew T. Del Pozzi [b,c,d]

[a] Department of Kinesiology, Brock University, St. Catharines, ON L2S 3A1, Canada
[b] Department of Kinesiology, The University of Alabama, Tuscaloosa, AL 35487, USA
[c] Department of Pediatrics, New York Medical College, Hawthorne, NY 10532, USA
[d] Department of Physiology, New York Medical College, Hawthorne, NY 10532, USA

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ABSTRACT

The aim of this study was to examine whether there are regional differences in the cutaneous microvascular responses of the forearm and the leg. Utilizing a non-invasive measure (spectral analysis),we looked at the influence of the endothelial, sympathetic, and myogenic function between regions at thermoneutral conditions (33 °C) and in response to local skin warming (42 °C) using laser-Doppler flowmetry (LDF). We recruited 18 young, healthy participants, who visited the lab on 2 separate occasions. Participants were instrumented with LDF probes and local skin heater probe-holders, placed on the forearm or the leg. Blood pressure was recorded by oscillometry. At both 33 °C and during local skin warming to 42 °C, skin vasomotion for the forearm and leg were evaluated using spectral analysis of the LDF recordings. There were significant differences among all frequencies of interest between the forearm and the leg. At 33 °C the leg presented with higher ($P = 0.003$) activity for endothelial (0.009–0.021 Hz), sympathetic ($P = 0.002$) (0.021–0.052 Hz), and myogenic ($P = 0.004$) (0.052–0.145 Hz) activity when compared to the forearm. In contrast, following 35 min of local skin warming, the forearm had greater endothelial ($P = 0.019$), sympathetic ($P = 0.006$), and myogenic ($P = 0.005$) vasomotion than the leg. These outcomes indicate regional differences in the cutaneous microcirculation. The current results are similar to our previous work using invasive methods and pharmacological agents, indicating that non-invasive analyses may be useful in the diagnoses and understanding of the mechanisms that control the microvascular function of pathological conditions.

Introduction

Control of the cutaneous microcirculation is primarily mediated by the autonomic nervous system (Hodges and Johnson, 2009; Hodges et al., 2009a; Johnson and Kellogg, 2010a, 2010b). The rhythmic variations in cutaneous blood flow are under the influence of autonomic innervations of the skin microvasculature (Bernardi et al., 1997; Rossi et al., 2006, 2008). A non-invasive approach to assess these control systems as well as peripheral sympathetic neuropathy is the analysis of the spectral components embedded in the oscillatory flowmotion of the microvasculature, known as vasomotion (Rossi et al., 2008). Various frequency contents are observed in the blood flow signals spanning from the cardiac rhythms (1 Hz) down to the innate endothelium-related oscillations (0.01 Hz) (Rossi et al., 2006, 2008; Stefanovska et al., 1999). Spectral analysis of laser-Doppler signals is performed using the fast Fourier transform algorithm or wavelet analysis (Rossi et al., 2006). The frequency spectrum can be divided into a predetermined range of interest; enabling the filtering of signals at higher or lower frequencies in order to analyze what is happening at the frequencies of interest. The 0.1 Hz microcirculatory fluctuations appear to be determined mainly by peripheral autonomic control (Bernardi et al., 1997). Wavelet analysis has been suggested to have good resolution in the low frequency domain of non-stationary perfusion signals. According to previous studies using wavelet transformation (Soderstrom et al., 2003; Urbancic-Rovan et al., 2004), the frequency intervals of 0.0095–0.021, 0.021–0.052, and 0.052–0.145 Hz are particularly interesting for the investigation of independent contributions of endothelial, neural, and myogenic activity of the microcirculatory regulation, respectively.

Using laser-Doppler flowmetry and the invasive procedure of intradermal microdialysis infusions of various pharmacological agents, we have previously demonstrated regional differences between the mechanisms of regulation between the skin microcirculation of the forearm

Abbreviations: CVC, cutaneous vascular conductance; eNOS, endothelial nitric oxide synthase; LDF, laser-Doppler flowmetry; Tloc, local skin temperature; MAP, mean arterial blood pressure; PAD, peripheral arterial disease.

* Corresponding author.
E-mail address: ghodges@brocku.ca (G.J. Hodges).

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and leg (Del Pozzi et al., 2013). While these data are valuable for scientists and important in advancing our understanding on the mechanisms of control in the cutaneous microcirculation, these invasive procedures are not practical for the standard clinical diagnostic setting (Cracowski et al., 2006) and are inappropriate for some pathologies (e.g. scleroderma). For example, scleroderma, a disease that is characterized by a hardening of the skin, would make placement of intradermal microdialysis fibers both difficult and perhaps ineffective. Laser-Doppler flowmetry is a non-invasive and simple technique that is not subject to investigator-dependent reliability issues as are other vascular measurement tools (e.g. ultrasound). As a result, laser-Doppler flowmetry is a potentially powerful tool for the examination of microvascular function. A large proportion of the work examining the mechanisms involved under basal conditions and in cutaneous vasodilator responses have been performed in the skin of the forearm — this is typically because the area is easily accessible. However, complications with diseases such as diabetes and peripheral arterial disease (PAD) typically present themselves first in the lower-limbs. Consequently, it is important to establish whether the mechanisms involved in the regulation of skin microvasculature are the same between different regions of the body, if clinical use of laser-Doppler and appropriate examination of the cutaneous vasculature are to be properly analyzed. Furthermore, if laser-Doppler measures of the cutaneous circulation are to be useful in a standard clinical setting, they will need to be performed with as little disruption to the patient as possible. Finding a reliable, non-invasive measure of vascular function, capable of distinguishing mechanisms of action (e.g. endothelial function and sympathetic activity) is of high importance.

The aim of this study was to examine whether or not regional differences in vascular responses while using a non-invasive spectral analysis measures of endothelial, sympathetic, and myogenic function occurs between the skin microcirculation of the forearm and leg. We measured skin blood flow under thermoneutral conditions (33 °C) and in response to local skin warming (42 °C) in skin of the forearm and leg. Previously, we showed that the NO plays a larger role in basal vascular function in the legs than in the arms (Del Pozzi et al., 2013). However, we used the broad isoform inhibitor L-NAME, thus we were unable to determine which isoform plays the largest role. eNOS has been identified as of great importance in the forearms (Kellogg et al., 2008a) while it has been proposed that nNOS is important in the leg (Stewart et al., 2007). Additionally, it has been shown that the sympathetic neurotransmitters do play a role in the vasodilator response (Hodges et al., 2008; Houghton et al., 2006).

Based on the aforementioned previous work (Del Pozzi et al., 2013), we hypothesized that endothelial (eNOS), myogenic (nNOS) and sympathetic function would be higher in the microvascular of the leg than the arm during thermoneutral conditions (33 °C), but lower in the leg than the arm during skin warming (42 °C).

Methods

Ethical approval

The current study was approved for completion by the local Institutional Review Board at The University of Alabama. All participants were fully informed of the experimental methods as well as the associated risks prior to their volunteering to be a participant. Verbal as well as written informed consent was obtained from each participant. All experimental protocols conformed to the guidelines set forth by the Declaration of Helsinki.

Participants

It was determined through an a priori power analysis (nQuery Advisor, v. 3) that 18 participants would be required with an α of 0.05 and 90% power. Standard deviations and expected required mean differences were established through our laboratory's previous work within the field (Carter and Hodges, 2011; Del Pozzi et al., 2013; Hodges and Sparks, 2013a, 2013b; Tew et al., 2011a).

18 healthy, active, but not trained participants (25 ± 3 years, 11 men and 7 women) volunteered for this study. Only participants who were 19 to 35 years of age, and not diagnosed with any metabolic or cardiovascular disease were included. Participants were excluded from participation if they were currently using tobacco or taking any medication other than prescription birth control. As per our previous experiments (Carter and Hodges, 2011; Del Pozzi et al., 2013), female subjects were all using oral contraceptives and currently in the low hormone phase of their routine as verified through self-report (Charkoudian and Johnson, 2000; Stephens et al., 2002). Participants were instructed to suspend the use of caffeine and alcohol for 24 h prior to testing. Additionally, the participants were instructed to not eat for 2 h prior to the testing session, but were instructed to drink water ad libitum.

Instrumentation and experimental procedures

Throughout the study participants laid supine on an adjustable gurney. Two skin sites were prepared for each session, one on the dorsal aspect of the forearm and another on the lateral aspect of the leg (Del Pozzi et al., 2013). The sites for each test session (in counterbalanced order) were prepared for the placement of local skin heaters and laser-Doppler probes. During pilot work and our previous study (Del Pozzi et al., 2013) examining forearm and leg skin blood flow, we found that immobilizing half the body (an arm and a leg) was uncomfortable for the participant. As a result, we performed assessments of arm and leg skin blood flow sequentially, randomizing the order of assessments: arm-then-leg or leg-then-arm. Local skin temperature was monitored and adjusted using an integrated skin heater and temperature monitor (model SH02, Moor Instruments Devon, UK). Red blood cell flux was measured via laser-Doppler flowmetry (MoorVMS-LDF2, Moor Instruments, Devon, UK) and was used to provide an index of skin blood flow in perfusion units (PU; 1 PU = 10 mV). Laser-Doppler flow (LDF) probes (VP12, Moor Instruments, Devon, UK) were placed in a small aperture in the skin heaters to monitor skin blood flow. Local skin heaters were set at 33 °C (thermoneutral) and placed on the participant to control and monitor local skin temperature (Tloc) at the site of measurement. The participant was then allowed to rest quietly for 15-min while the LDF measurement came to a stable reading, after confirming that the recorded measurements were stable, baseline measurements were recorded. After 10 min of baseline data recording, a standard local warming protocol was initiated by increasing Tloc by 0.5 °C · 10 s^{-1} until Tloc had reached 42 °C (Carter and Hodges, 2011; Del Pozzi et al., 2013; Hodges and Sparks, 2013a). Tloc was maintained at 42 °C for 35 min at which time a stable plateau had been reached and verified by a trained researcher, which represented a physiological maximum (Taylor et al., 1984).

Direct (Park and Guntheroth, 1970) and indirect (Sareen et al., 2012) methods of mean arterial pressure measurement have shown that supine mean arterial pressure does not differ between the forearm and the leg. Because of this, blood pressure was measured by oscillometry in the contra-lateral arm every 5-min throughout the duration of the study protocol. Mean arterial pressure (MAP) was calculated as:

$$((2 \cdot \text{diastolic blood pressure} + \text{systolic blood pressure}) \div 3)$$

Data collection and analysis

Data were collected at 50 Hz and stored on a personal computer to be analyzed offline using signal-processing software (Acqknowledge v4.2, Biopac MP150, Camino Goleta, CA). LDF data were converted to cutaneous vascular conductance (CVC) by dividing LDF (PU) by the calculated MAP (mm Hg).