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The effect of heating rate on the cutaneous vasomotion responses of forearm and leg skin in humans

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ABSTRACT

We examined skin blood flow (SkBF) and vasomotion in the forearm and leg using laser-Doppler fluxmetry (LDF) and spectral analysis to investigate endothelial, sympathetic, and myogenic activities in response to slow (0.1 °C·10 s^{-1}) and fast (0.5 °C·10 s^{-1}) local heating. At 33 °C (thermoneutral) endothelial activity was higher in the legs than the forearms ($P \leq 0.02$). Fast-heating increased SkBF more than slow heating ($P = 0.037$ forearm; $P = 0.002$ leg). At onset of 42 °C, endothelial ($P = 0.043$ forearm; $P = 0.48$ leg) activity increased in both regions during the fast-heating protocol. Following prolonged heating (42 °C) endothelial activity was higher in both the forearm ($P = 0.002$) and leg ($P < 0.001$) following fast-heating. These results confirm regional differences in the response to local heating and suggest that the greater increase in SkBF in response to fast local heating is initially due to increased endothelial and sympathetic activity. Furthermore, with sustained local skin heating, greater vasodilatation was observed with fast heating compared to slow heating. These data indicate that this difference is due to greater endothelial activity following fast heating compared to slow heating, suggesting that the rate of skin heating may alter the mechanisms contributing to cutaneous vasodilatation.

Introduction

The skin circulation plays a significant role in thermal homeostasis (Johnson et al., 2014). *In vivo* studies have confirmed that humans are able to regulate body temperature properly under a variety of environmental conditions by regulating the level of perfusion to the skin (Charkoudian, 2010; Cheung and Daanen, 2012; Hodges and Johnson, 2009; Johnson et al., 2014). The regulation of the cutaneous perfusion is achieved through a combination of local and reflex activity, the mechanisms of these systems have been studied at length and have been the subject of numerous recent reviews (Brunt and Minson, 2011; Hodges and Johnson, 2009; Johnson, 2010; Johnson et al., 2014; Simmons et al., 2011; Tew et al., 2012).

It has been shown that following either pre- or post-synaptic adrenergic blockade or sensory nerve blockade, the initial vasodilatation to local skin warming response can be either abolished (Hodges et al., 2009; Houghton et al., 2006) or diminished (Carter and Hodges, 2011; Hodges and Sparks, 2013a; Minson et al., 2002; Tew et al., 2011b). These studies suggest that sensory and sympathetic nerves are required to achieve a complete initial vasodilator response. However, the sustained vasodilatation achieved in response to increased local skin temperature appears to be heavily dependent on endothelial nitric oxide synthase (NOS) (Hodges and Sparks, 2013b, 2014; Kellogg et al., 2008, 2009) and endothelial derived hyperpolarizing factor (EDHF) (Brunt and Minson, 2012).

Previously, we investigated the regional differences in endothelial (Hodges and Del Pozzi, 2014) and NOS activity (Del Pozzi et al., 2013), determining that cutaneous vasodilatation in response to an increased local temperature was greater in the legs than in the forearms (Del Pozzi et al., 2013). We observed that endothelial activity was higher in the legs (Hodges and Del Pozzi, 2014) at 33 °C (thermoneutral) while the contribution of NOS activity was similar between the forearms and legs (Del Pozzi et al., 2013) following prolonged local heating. This trend was also exhibited during baseline where vascular tone (as measured by laser-Doppler) was higher in the forearms than in the legs; appearing to fully explain the observed differences in these circulations (Del Pozzi et al., 2013; Hodges and Del Pozzi, 2014). Thus, the withdrawal of the myogenic and sympathetic influences (a reduction in tone) in response to local heating seemed to explain the observed differences. Using spectral analysis of the vasomotion signals we also found that the endothelial, myogenic, and sympathetic activities were higher in the cutaneous circulation of the legs than that of the forearms during thermoneutral (33 °C) conditions however, after prolonged local skin heating the opposite was found.

The rhythmic contractions of the vascular smooth muscle leading to oscillations in blood flow are termed vasomotion and occur in all

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vascular beds (Funk et al., 1983). These contractions result in variations in the cutaneous blood flow which are under the influence of the aforementioned local factors and autonomic innervations (Bernardi et al., 1997; Hodges and Del Pozzi, 2014; Rossi et al., 2006, 2008). A non-invasive method to assess the control systems of the cutaneous circulation can be achieved *via* the examination of the spectral components entrenched within the oscillatory vasomotion of the vascular beds (Rossi et al., 2008). Numerous frequencies are observed within vasomotion signals. Ranging from 1 Hz, the cardiac rhythm, all the way down to the endothelium-related oscillations at 0.01 Hz (Rossi et al., 2006, 2008; Stefanovska et al., 1999). The frequency spectrum can be divided into predetermined ranges, enabling the filtering of signals higher or lower than that of the frequency range of interest. For this study those would be 0.0095–0.021, 0.021–0.052, and 0.052–0.145 Hz (Soderstrom et al., 2003; Urbancic-Rovan et al., 2004). These ranges are used to determine the independent contributions of endothelial, sympathetic, and myogenic activities, respectively. While the physiological importance of these oscillations remain unclear (Aalkjaer et al., 2011), it seems that vasomotion plays an important role in oxygenation and tissue dialysis (Aalkjaer et al., 2011; Hapuarachchi et al., 2010; Sakurai and Terui, 2006; Thorn et al., 2011; Welsh et al., 2010).

Previously, Hodges et al. (2009) examined the effect of differing rates of heating on the forearm skin blood flow response. They found that a very slow (0.1 °C·min^{-1}) local heating protocol significantly reduced the initial peak and sustained plateau responses when compared to a rapid (2 °C·min^{-1}) heating protocol. Thus, the aim of this study was to examine the impact of heating rate on the cutaneous blood flow and vasomotion in the forearm and leg.

Based on the aforementioned work, we hypothesized that the rate of heating would affect both the forearm and the leg equally, with higher initial and sustained vasodilator responses achieved in response to faster local heating. We hypothesized that the greater initial vasodilator responses to fast heating compared to slow heating would be due to increased endothelial and sympathetic activity, while the greater sustained vasodilation would be due to elevated endothelial activity.

Methods

Participants

All experimental protocols conformed to the guidelines set forth by the Declaration of Helsinki and were approved for completion by the local Institutional Review Board at The University of Alabama. Participants were informed of the experimental procedures and possible risks prior to obtaining verbal and written informed consent.

Power analysis (α of 0.05 and a β of 0.80) determined that a minimum of 12 participants would be necessary to determine differences in the rate of heating, with standard deviations and expected mean differences gathered from our laboratory's previous publications in the field (Del Pozzi and Hodges, 2015a; Hodges and Del Pozzi, 2014; Tew et al., 2011a) (nQuery Advisor, v.3).

Seven male and 7 female, healthy and active, yet not athletically trained participants volunteered for this study (Table 1). Participants were excluded if they had been previously diagnosed with any metabolic, cardiovascular or other systemic disease, were current tobacco users, or taking any medication other than prescription birth control. As in our previous experiments (Del Pozzi et al., 2013; Del Pozzi and Hodges, 2015a; Hodges and Del Pozzi, 2014) all female participants were using oral contraceptives. Data collection occurred during the low hormone phase of their birth control regimen and was verified through self-report (Charkoudian, 2001; Charkoudian and Johnson, 2000; Stephens et al., 2002). Participants abstained from caffeine and alcohol for 24 h and fasted for 2 h prior to testing, but were allowed to drink water *ad libitum*.

Table 1
Subject characteristics.

	Male	Female	*P*	Combined
Stature (cm)	178 ± 4	165 ± 3.0	0.027	171 ± 5
Mass (kg)	82.2 ± 1.5	58.2 ± 3.9	<0.001	68 ± 6
Age (year)	28 ± 1	25 ± 2	0.061	27 ± 2
SBP (mm Hg)	100 ± 3	99 ± 2	0.433	100 ± 3
DBP (mm Hg)	53 ± 2	53 ± 2	0.421	53 ± 3
MAP (mm Hg)	68 ± 2	67 ± 2	0.413	68 ± 3

Instrumentation

Participants reported to laboratory at 07:00 h. All protocols were performed in the same manner in a temperature controlled room ranging from 20 to 22 °C. For the duration of all study procedures the participants laid supine on an adjustable hospital gurney. Skin sites were prepared for each testing session on the dorsal aspect of the forearm and the lateral aspect of the leg (Del Pozzi et al., 2013; Del Pozzi and Hodges, 2015a; Hodges and Del Pozzi, 2014; Hodges et al., 2015a). 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 fluxmetry (MoorVMS-LDF2, Moor Instruments, Devon, UK), this was used to provide an index of skin blood flow in perfusion units (PU; 1 PU = 10 mV). Laser-Doppler flux (LDF) probes (VP12, Moor Instruments, Devon, UK) were placed within a small aperture on the local skin heaters to monitor skin blood flow. The local heaters were set at 33 °C (thermoneutral) and placed on the participant to control and monitor local skin temperature (T_{loc}) at the site of measurement. The participant then rested quietly in a dimly lit room for 15 min while the LDF measurements stabilized. After a trained researcher confirmed that the recorded measurements were stable, baseline measurements were recorded (see Fig. 1 for an outline of the protocol). After 10 min of baseline data recording, T_{loc} was increased to 42 °C using one of two heating protocols: the slow protocol (0.1 °C·10 s^{-1}, 15 min in duration) or the fast protocol (0.5 °C·10 s^{-1}, 3 min in duration). T_{loc} was held at 42 °C for 35 min at which point stable plateaus were confirmed by a trained researcher, representing a physiological maximum (Taylor et al., 1984).

Previous work has shown that, while supine, mean arterial pressure (MAP) does not differ at the level of the arm or the leg (Park and Guntheroth, 1970; Sareen et al., 2012). Therefore, blood pressure was measured in the contra-lateral arm every 5 min throughout the duration of the study. Calculation of MAP used the following equation;

$$((2 \cdot \text{Diastolic blood pressure} + \text{Systolic blood pressure}) \div 3).$$

Data collection and analysis

Data were collected at 50 Hz and stored securely on a personal computer to be analyzed offline using signal-processing software (Acqknowledge v4.2, Biopac MP150, Camino Goleta, CA).

Statistical analysis was completed using SAS v9.13 (SAS institute Inc., Cary, NC, USA). Stable 5 min periods of LDF data were used for both the baseline, the first 5 min of local skin heating, and the final 5 min (plateau phase) (Fig. 1). LDF data were normally distributed as determined by the Kolmogorov–Smirnov goodness of fit test. Text and illustrative results for LDF are reported as mean ± standard deviation, analysis was performed using a paired t-statistic.

Skin vasomotion was assessed by analyzing the spectra of the skin LDF signal. The same 5 min periods of LDF data were used for these analyses. The power spectral density of the LDF signal oscillations was determined using a fast Fourier transformation algorithm. To avoid the well establish leakage phenomenon (frequency components in the