Microvascular Research 112 (2017) 47–52

Contents lists available at ScienceDirect

Microvascular Research

journal homepage: www.elsevier.com/locate/ymvre

Effect of age on cutaneous vasomotor responses during local skin heating

Gary J. Hodges [a,*], Matthew M. Mallette [a], Garry A. Tew [b], John M. Saxton [b], James Moss [c,d], Alan D. Ruddock [e], Markos Klonizakis [e]

[a] Environmental Ergonomics Laboratory, Brock University, St Catharines, Ontario L2S 3A1, Canada
[b] Department of Sport, Exercise and Rehabilitation, Faculty of Health and Life Sciences, Northumbria University, Newcastle Upon Tyne NE1 8ST, UK
[c] Academic Unit of Sleep and Breathing, National Heart and Lung Institute, Imperial College London, SW7 2AZ, UK
[d] NIHR Respiratory Disease Biomedical Research Unit, Royal Brompton and Harefield, SW3 6NP, UK
[e] Sheffield Hallam University, Centre for Sports and Exercise Science, Collegiate Hall, Collegiate Crescent, Sheffield S10 8BP, UK

ARTICLE INFO

Article history:
Received 30 September 2016
Revised 6 March 2017
Accepted 6 March 2017
Available online 09 March 2017

Keywords:
Skin blood flow
Wavelet analysis
Ageing
Endothelial
Spectral analysis

ABSTRACT

This study examined the effect of ageing on the low-frequency oscillations (vasomotion) of skin blood flow in response to local heating (LH). Skin blood flow was assessed by laser-Doppler flowmetry on the forearm at rest (33 °C) and in response to LH of the skin to both 42 °C and 44 °C in 14 young (24 ± 1 years) and 14 older (64 ± 1 years) participants. Vasomotion was analyzed using a wavelet transform to investigate power of the frequency intervals associated with endothelial, neural, myogenic, respiratory, and cardiac activities of the laser-Doppler signal. Laser-Doppler flux increased in both groups with LH (both $d > 1.8$, $p < 0.001$). Endothelial activity increased in both groups following LH to 42 °C (young $d = 1.4$, $p < 0.001$; older $d = 1.2$, $p = 0.005$) and 44 °C (young $d = 1.4$, $p = 0.001$; older $d = 1.5$, $p = 0.005$). Endothelial activity was higher in the young compared to older group during LH to 42 °C ($d = 1.4$, $p = 0.017$) and 44 °C ($d = 1.5$, $p = 0.004$). In response to LH to 42 °C and 44 °C, neural activity in both groups was decreased (both groups and conditions: $d > 1.2$, $p < 0.001$). Myogenic activity increased in the younger group following LH to 44 °C ($d = 1$, $p = 0.042$), while in the older group, myogenic activity increased following LH to 42 °C ($d = 1.2$, $p = 0.041$) and 44 °C ($d = 1.1$, $p = 0.041$). Respiratory and cardiac activities increased in both groups during LH to 42 °C and 44 °C (All: $d > 0.9$, $p < 0.017$). There were no differences in wavelet amplitude between younger and older in the neural ($d = 0.1$, $p > 0.7$), myogenic ($d = 0.3$, $p > 0.7$), respiratory ($d = 0.4$, $p > 0.6$), and cardiac ($d = 0.1$, $p > 0.7$) frequency intervals. These data indicate that LH increases cutaneous endothelial and myogenic activity, while decreasing neural activity. Furthermore, ageing reduces the increase in cutaneous endothelial activity in response to LH.

1. Introduction

Two strengths of laser-Doppler flowmetry are that it is non-invasive and provides a continuous measurement. The majority of studies examining the mechanistic control of the cutaneous vasculature have used needle-based invasive techniques (e.g. intradermal microdialysis) to administer pharmacological agents (Johnson et al., 2014). While artifacts associated with needle trauma can be limited by temporary anaesthesia (Hodges et al., 2009a), it is clear that implantation affects vasodilator function (Hodges et al., 2009a), and there is the chance that the invasive procedures affect vascular function more than realized (Groth, 1998; Groth et al., 1998; Groth and Serup, 1998; Sjogren and Anderson, 2009).

Examination of low-frequency oscillations inherent to the skin blood flow signal enables a non-invasive assessment of regulatory mechanisms of the cutaneous circulation (Bracic and Stefanovska, 1998; Rossi et al., 2006; Rossi et al., 2008; Stefanovska et al., 1999). Strengths of this approach compared to other non-invasive or pharmacological-dependent methods of assessing mechanistic control of the vasculature is that, unlike a procedure such as flow-mediated dilatation, there is no need for a trained sonographer or expensive edge-tracking software. Furthermore, wavelet analysis can be used for microcirculatory function and provide information on not only endothelial activity, but also, neural, myogenic, respiratory and cardiac influences (Bracic and Stefanovska, 1998; Kvandal et al., 2006; Kvandal et al., 2003; Soderstrom et al., 2003). Indeed, characteristic peaks have been identified within the vasomotion signal ranging from the cardiac and respiratory rhythms at approximately 1.6 Hz and 0.6 Hz respectively. Endothelium-related oscillations of approximately 0.01 Hz have been shown via applying both agonists and antagonists (Kvandal et al., 2006; Kvandal et al., 2003; Rossi et al., 2006; Rossi et al., 2008; Stefanovska et al., 1999). Furthermore, the neural influence on skin blood flow has been shown to be associated with a frequency range of 0.02–0.05 Hz (Soderstrom et al., 2003). Thus, wavelet analysis can

* Corresponding author at: Brock University, Faculty of Applied Health Sciences, Niagara Region, 1812 Sir Isaac Brock Way, St. Catharines ON L2S 3A1, Canada.
E-mail address: ghodges@brocku.ca (G.J. Hodges).

http://dx.doi.org/10.1016/j.mvr.2017.03.002
0026-2862/

provide a considerable amount of mechanistic information regarding microvascular function.

Ageing has been reported to decrease both the initial peak (Minson et al., 2002; Tew et al., 2011a; Tew et al., 2011b), and the sustained vasodilatation to prolonged local skin heating (Hodges et al., 2010a; Hodges et al., 2010b; Martin et al., 1995; Minson et al., 2002; Tew et al., 2012a). Evidence indicates that the age-related reduction in the initial peak response is due to impaired neural mechanisms, while the impaired plateau phase is due to reduced endothelial activity (Tew et al., 2012b). Indeed, work in other vascular beds demonstrates reduced endothelial activity with increasing age (Green et al., 2011; Thijssen et al., 2010). To date, no study has examined the effect of ageing on the cutaneous vasomotor response to local skin heating.

Thus, wavelet analysis of the laser-Doppler signal offers the opportunity to develop a relatively short, painless, non-invasive procedure capable of providing mechanistic insight to microcirculatory function. Not only can flux and reactivity responses be measured, but endothelial, neural, myogenic, respiratory, and cardiac mechanisms can also be assessed. If this approach can be shown to be sensitive enough to examine the effects of ageing and disease processes affecting microvascular function (Podtaev et al., 2015), this would be a highly useful clinical tool in diagnosing gross microvascular (dys)function, as well as characterising the endothelial and neural changes that accompany ageing.

Therefore, we sought to determine the effect of ageing on the low-frequency oscillations in cutaneous blood flow at rest and during local skin heating. We hypothesized that local heating would increase endothelial activity in both younger and older individuals, with the increase being greater in the younger compared to the older individuals. We also hypothesized that neural activity during the initial peak phase would be increased in the younger group versus the older group, but that neural activity would be reduced during the local skin heating to 42 °C and 44 °C in both groups.

2. Methods

2.1. Ethical approval

This study was approved by the local research ethics committee at Sheffield Hallam University. All volunteers provided written, informed consent prior to participation. This study was carried out in accordance with the Declaration of Helsinki.

2.2. Participants

Power analysis (α of 0.05 and β of 0.20) determined that 12 participants were needed to determine differences in vasomotion between age groups. Fourteen young and 14 older men without cardiovascular disease, hypertension, diabetes, or cancer, who were not smokers or taking any form of medication were recruited. Each participant visited the laboratory on two occasions. For both sessions, they were asked to refrain from caffeine, alcohol, and exercise for 24 h prior. The physical characteristics of the two groups are presented in Table 1.

Table 1
Participant characteristics. Mean ± standard deviation.

	Young	Older
Age (years)	24 ± 1	64 ± 1*
Body Mass (kg)	76 ± 6	80 ± 6
Stature (cm)	179 ± 2	178 ± 3
Resting heart rate (beats·min^{-1})	56 ± 2	52 ± 4
Resting blood pressure (mm Hg)		
Systolic	118 ± 4	122 ± 4
Diastolic	66 ± 4	76 ± 3*
$\dot{V}O_2$max (ml·kg^{-1}·min^{-1})	50 ± 5	36 ± 5*

* indicates $P < 0.05$ between young and older groups.

2.2.1. Visit 1: cardiopulmonary fitness assessment

All participants performed a continuous, incremental cycling test to volitional exhaustion on an electronically braked cycle ergometer (Excalibur Sport, Lode, The Netherlands). Pedalling frequency was self-selected within 60 to 90 rpm. Following a 2 min warm-up at 0 W, resistance was increased by 20–30 W · min^{-1}, with participants continuing until volitional exhaustion or a plateau in oxygen uptake. Heart rate was collected via ECG. The volume of oxygen consumed during exercise was calculated from minute ventilation, measured using a pneumotachometer, and simultaneous breath-by-breath analysis of expired gas fractions (Ultima CardioO_2; MedGraphics, St. Paul, MN, USA). Gas analysers and flow probes were calibrated before each test. Oxygen uptake was expressed relative to body mass (ml kg^{-1} min^{-1}). Maximal oxygen uptake ($\dot{V}O_2$max) was calculated as the highest 20 s period of gas exchange data in the last minute before the end of the test.

2.2.2. Visit 2: microvascular assessment

2.2.2.1. Instrumentation and experimental procedure. Experiments were performed in a temperature-controlled room (22–24 °C), with participants resting supine and the experimental arm (left) positioned at heart level for the entire protocol. A site on the ventral aspect of the left forearm was chosen, avoiding visible veins, damaged or irritated skin, and hair. Skin blood flow was measured as cutaneous red blood cell flux using a laser-Doppler fluxmeter (LDF; Periflux system 5000, Perimed AB, Järfälla, Sweden) and a 7-point integrating LDF probe (Probe 413, Perimed AB). Local skin heating was performed using a heating disc surrounding the probe (Model 455, Perimed AB), connected to a heating unit (Model 5020, Perimed AB). Recordings of the laser-Doppler signal were made using PeriSoft for Windows 9.0 software (PSW 9.0, Perimed AB). Blood pressure was measured using an automated blood pressure cuff every 2 min (Dinamap Dash 2500; GE Healthcare, Waukesha, WI, USA).

2.2.2.2. Wavelet transform. A Morlet mother wavelet was used to perform wavelet analysis on the LDF signal. Wavelet analysis was chosen as it provides good time and frequency resolution within the uncertainty principle. It uses an adjustable window to provide good frequency resolution for lower and higher frequencies by using a longer and shorter analysis window, respectively. This method characterizes the dynamics of signals overs a wide frequency range, from 0.0095 to 1.6 Hz.

Analysis of time segments of 20 min is needed for good low-frequency resolution and detection of oscillations in the low-frequency range (Stefanovska et al., 1999). We ran the wavelet transform on a minimum of 55 min of data. While we have chosen short time windows for certain phases to extract median amplitudes of the vasodilator responses (initial peak and nadir), the fact that the wavelet was run on the entire data laser-Doppler signal ensured that even these smaller extracted portions had appropriate resolution and power to examine the low-frequency bands.

2.3. Data collection and statistical analysis

LDF data were collected at 32 Hz (PSW 9.0, Perimed AB). The data from the entire protocol ~60 min was exported and run through the wavelet transform in a custom written computer script (Iatsenko et al., 2013; Iatsenko et al., 2015; Iatsenko et al., 2016) (Matlab®, The MathWorks Inc., Natick, MA, USA). Subsequently, median amplitudes were chosen from the appropriate place (Fig. 1) such that data for basal (4 min), initial peak (30 s), nadir (30 s), plateau at 42 °C (4 min), and maximum at 44 °C (4 min) were extracted.

Vasomotion data (AU) were not normally distributed as assessed by the Shipiro-Wilk test of normality. A Friedmann one-way ANOVA was used to examine local heating responses (baseline, initial peak, nadir,