Oscillatory pattern of acral skin blood flow within thermoneutral zone in healthy humans

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Our poster received the award for BEST POSTER ON NEW THEORETICAL OR EXPERIMENTAL METHODS FOR BIOLOGICAL OSCILLATIONS.
Abstract

Objective: The acral skin contains arteriovenous anastomoses, which probably is the main part of skin microcirculation for blood flow adjustments and thermoregulation in the thermoneutral zone. The objective was to investigate if an increase in sympathetic activation during cooling influences the oscillatory pattern of acral skin blood flow.

Approach: We had measurements of bilateral acral skin blood flow (n=12) during lowering of ambient temperature from 32°C to 18°C. We quantified the oscillatory pattern as the time averaged wavelet spectral powers, coherence and phase angles in three frequency intervals (0.01-0.02 Hz, 0.02-0.05 Hz and 0.05-0.08 Hz). The differences were tested by Wilcoxon signed rank sum method between adjacent intervals.

Main results: The absolute fluctuations in laser Doppler flux at 0.01-0.02 Hz, 0.02-0.05 Hz and 0.05-0.08 Hz were similar at 32°C and 25°C, and decreased at 18°C (p<0.001). The relative fluctuations (amplitude of the fluctuations relative to median flux value) in laser Doppler flux at 0.01-0.02 Hz, 0.02-0.05 Hz and 0.05-0.08 Hz were higher at 25°C and 18°C as compared to 32°C (p<0.002). The coherence between the oscillations of signals from right and left finger tips was highest (median coherence>0.89) at 25°C, and lower at 32°C and at 18°C. The median phase angles between the flux signals from right and left finger tips were close to 0 radians.

Significance: We found that the relative fluctuations in acral skin blood flow increased during vasoconstriction due to cooling. Wavelet analysis of acral skin blood flow oscillations could serve as a future clinical tool.
Keywords: acral skin, arteriovenous anastomoses, laser Doppler flux, skin blood flow, thermoregulation, wavelet analysis
Introduction

Regulation of skin blood flow is essential in maintaining core temperature. Acral skin (palms and soles) has a special role in the thermoregulation and has highly variable blood flow. The central nervous system regulates the blood flow fluctuation frequency and amplitude in a very fine tuned feedback system according mainly to the general heat balance of the body. Thermoneutral zone is the ambient temperatures that the lightly dressed subject’s metabolism is unchanged (Kingma et al., 2012). Within the thermoneutral zone, the subject experience neither sweating nor shivering. Core temperature within the thermoneutral zone is probably maintained stable by adjustments of amplitude and oscillations in acral skin blood flow (Romanovsky, 2014). The acral skin contains arteriovenous anastomoses (AVA), which are shunts between arteries and venules. The blood flow through AVAs is adjusted by sympathetic vasoconstrictor nerve impulses. By changing the frequency of this vasomotor activity the AVAs may regulate body temperature within the thermoneutral zone at low energy expenditure.

The AVAs vasoconstrict simultaneously and bilaterally in hands and feet (figure 1b&c). The vasoconstrictions appear at a frequency of 2 to 3 per minute (0.03-0.05 Hz) while the subject is resting comfortable in thermoneutral (Thoresen and Walloe, 1980, Bergersen, 1993). However, the sympathetic vasoconstrictor signal has a great response on skin blood flow around 0.08 Hz (Stauss et al., 1998). The vasoconstrictor signal may also appear at lower frequencies than 0.03 Hz. We wanted to investigate if an increase in sympathetic activation as occur during cooling influences the oscillatory pattern of acral skin blood flow. There are some pathologic conditions that are only manifested in acral skin, with Raynaud’s
phenomenon as the most common. A better understanding of the healthy response to cooling may improve the treatment for acral vascular syndromes.

Most of the previous investigations are from non-acral skin (such as hairy skin on extremities and truncus) and the oscillatory pattern of the non-acral skin blood flow is previously described by Stefanovska et al. (1999). In non-acral skin, blood flow fluctuations around 0.01 Hz are derived from endothelial activity, around 0.04 Hz from neurogenic vasomotor control and around 0.1 Hz from myogenic control (Kvernmo et al., 1998). A similar division of the oscillatory pattern is not clear for acral skin, as much of the oscillations are caused by the thermoregulatory sympathetic vasoconstrictor signal. The skin blood flow oscillations <0.145 Hz are expected to be of local origin (Sheppard et al., 2011).

Local cooling was previously shown to move the myogenic peak frequency from 0.084 Hz to 0.06 Hz in non-acral skin (Sheppard et al., 2011), one reason for us to study the frequency interval 0.05-0.08 Hz.

In the thermoneutral zone the non-acral skin blood flow oscillations are smaller -in terms of amplitude- compared to the blood flow oscillations in acral skin (Elstad et al., 2014) and the coherence between oscillations within non-acral skin is typically low. They do not exhibit the same simultaneous vasoconstriction response, as observed in acral skin, due to the fact that the non-acral sympathetic signals are random and low within the thermoneutral zone (Bini et al., 1980).

Wavelet analysis is the preferred method to analyze infrequent events as the vasoconstrictions of AVA within the thermoneutral zone (Stefanovska et al., 1999). Wavelet analysis has mainly been used while investigating non-acral skin (Hafner et al., 2007, Hafner et al., 2009), with few investigating the acral skin (Dunaev et al., 2014, Jan et al., 2013). To
the best of our knowledge the oscillatory pattern has not been described for acral skin during changing ambient temperatures.

In this study we describe the oscillatory pattern of acral skin blood flow in the upper to lower part of the thermoneutral zone in healthy subjects. We hypothesized that when exposed to a decreasing ambient temperature within the range of [32°C, 18°C], the corresponding oscillatory pattern -as quantified by wavelet spectral analysis- would subsequently shift the oscillations to other frequency intervals as observed in non-acral skin during local cooling (Sheppard et al., 2011). We hypothesized that the relative variability would remain unchanged in these healthy subjects regardless of the ambient temperature. We also expected the coherence between the signals from the finger tips to be reduced during cooling if the control is local at lower frequencies as compared to centrally mediated. We chose to present results from three intervals; i) 0.01-0.02 Hz which may be of metabolic origin, ii) 0.02-0.05 Hz, which is of neurogenic origin, mainly thermoregulatory sympathetic vasoconstrictor control, and iii) 0.05-0.08 Hz which may represent sympathetic or myogenic control. We avoided >0.08 Hz as these frequencies may be of baroreflex origin (Toska and Eriksen, 1994).
2. Methods

Subjects and experimental protocol

In the present study we reanalysed data obtained in the work by Elstad et al (Elstad et al., 2014): Thirteen young, healthy volunteers participated in an experimental protocol with changing ambient temperature (Elstad et al., 2014). In the present study we reanalysed the experimental data using wavelet analysis. Data from one of the thirteen subjects, who completed the original experimental protocol, could for technical reasons not be reanalysed. The twelve subjects were all healthy, between 19 and 23 years median of age, average of 67 kg (range 53-83) and 173 cm (range 162-183), and seven of the 12 were females. None had any self-reported disorders or were under medication.

The subjects were rested supine in a climate chamber. Laser Doppler fluxes (DRT4, Moor instruments, Devon, UK) were obtained from pulps of the right and left third fingers as a measure of acral skin blood flow. The noise-limiting filter was set at 21kHz and the emitted wavelength was 820 nm. The flux output signal was filtered at time constant 0.1 s.

The ambient temperature was lowered from 32°C to 18°C with three plateaus; 32°C, 25°C and 18°C (figure 1a). At each plateau, the temperature was retained constant for 15 min, while each transition phase lasted for approximately 10-15 minutes.

Analysis

The wavelet transform technique, a time-frequency method with logarithmic frequency resolution, was used to analyse the acral skin blood flow oscillations. The time-frequency analysis tools employed in our study are implemented in MATLAB® R2015b (The MathWorks Inc., MA, US) and have been developed by the Department of Physics, Lancaster
University, UK (Iatsenko et al., 2015). The Morlet mother wavelet (Morlet, 1983) was selected for the wavelet transform analysis of the acral skin blood flow at five distinct time intervals - of approx. 800 s each- including three plateaus and their corresponding transition zones (figure 1a).

For each time interval and for both right and left fingertips signals, the time averaged wavelet spectral powers were calculated and divided into three frequency intervals of i) 0.01-0.02 Hz, ii) 0.02-0.05 Hz and iii) 0.05-0.08 Hz respectively. For each of the aforementioned time intervals the integral under the curve of the wavelet spectral power at both frequency intervals was numerically calculated using Simpson’s rule. The spectral powers are reported as the absolute fluctuations. The relative fluctuations are calculated as the square root of the spectral power of the frequency interval divided by the median of the absolute laser Doppler flux in the time interval.

Furthermore, the coherence and the phase angles of the two fingertip signals were also evaluated. The medians of the coherences and phase angles (reported in figure 4) were computed based on their time-averaged values for each one of the investigated frequency intervals.

**Statistics**

Most of the cardiovascular data reported here showed a non-normal distribution, and in addition we have a small sample. We therefore performed non-parametric estimation and statistics on the data set. The data are reported as Hodges-Lehmann estimates of median with 95% confidence interval (Hollander et al., 2013). The significance levels of differences were tested by Wilcoxon signed rank sum method between adjacent intervals. Since the phase angles are on a closed circle, circular statistics were applied when estimating median
direction (Mardia, 1972, Elstad, 2012). The relation between absolute laser Doppler flux from left and right finger tips was estimated by calculation of R and unstandardized B coefficient not including a constant by the linear regression for the full recording period (SPSS 24, IBM Corp.).
3. Results

The laser Doppler flux measurements from the two finger tips exhibit similar behaviour (figure 1b). There are large synchronous oscillations in the blood flow flux to both finger tips in the temperature range 32-25°C (figure 1b). Towards the lower end of the cooling ramp, the AVAs became more vasoconstricted (figure 1b), corresponding to the lower part of the thermoneutral zone. Their oscillations were still in phase at ambient temperatures between 32°C (figure 1c) and 18°C (figure 1d). The appearance of the vasoconstricted episodes changed characteristics and the vasodilatory part of the episode was more visible (figure 1d). The absolute amplitude of the oscillations was significantly decreased at 18°C (figure 1d) as compared to at 32°C (figure 1c).

Both fingertip signals were analysed using the wavelet transformation method and every occurred vasoconstrictor episode was depicted on the time-frequency contour plots (Figure 2). The absolute flux values showed a decrease during cooling of the room temperature (table 1) and this was expected as a sign of vasoconstriction. The absolute flux values were lower for left hand than right hand (table 1), which is commented in study limitations.

**Absolute fluctuations in acral skin blood flow**

The absolute fluctuations in laser Doppler flux showed large individual variability for all three frequency intervals and were of similar amplitude at 32°C and 25°C (figure 3a, c and e). During the transition 25°C-18°C the fluctuations decreased (p=0.005) and then decreased further at 18°C (p=0.0005 as compared to 32°C) (figure 3a, c and e). The fluctuations were
similar between the two finger tips ($R = 0.98$ (95% confidence interval 0.98-0.99)),
unstandardized B coefficient 0.92 (95% confidence interval 0.69-1.07).

*Relative fluctuations in acral skin blood flow*

The relative fluctuations in laser Doppler flux, with which we controlled for the large
individual variability in absolute flux values, showed less individual variability for all three
frequency intervals (figure 3b, d and f). The two finger tips had very similar relative
amplitude. During the transition from 32°C to 25°C, the relative fluctuations in laser Doppler
flux increased for all three intervals (figure 3b, d and f, $p<0.0015$ for all). The relative
fluctuations remained high at 18°C and were unchanged from 25°C ($p>0.45$).

*Coherence and phase angle between the right and left finger tips*

For all three frequency intervals the coherence between the oscillations of the signals from
right and left finger tips were high at 25°C (figure 4a, b, c) and lower at 32°C ($p<0.02$). At
18°C the coherence between the signals from the two finger tips was lower for 0.02-0.05 Hz
and 0.05-0.08 Hz ($p=0.03$, figure 4b and c) but remained unchanged for 0.01-0.02 Hz
($p=0.09$, figure 4a).

The median phase angles between the laser Doppler flux signals from right and left finger
tips were close to 0 radians, which in conjunction with the high coherence, illustrates the
strong central regulation of blood flow through AVAs in acral skin for all three frequency
intervals (figure 4). The phase angles are similar at the ambient temperatures in the range
32-18°C and for all three intervals.
Comparison of relative fluctuations at the three intervals

We did not find a larger increase in relative fluctuations in any one of the three intervals. The relative fluctuations increased for all three intervals when reducing ambient temperature from 32°C to 25°C and 18°C.
4. Discussion

We found that absolute values of fluctuations decrease while relative fluctuations increase in acral skin blood flow during reduction of ambient temperature. We found that the vasoconstriction episodes of AVAs are similar within the three investigated frequency intervals (0.01-0.02 Hz, 0.02-0.05 Hz and 0.05-0.08 Hz) indicating that acral skin is largely under central control in all these frequency intervals. Towards the lower end of the thermoneutral zone, the AVAs became more vasoconstricted but the relative amplitude of the oscillations was increased.

We found that the relative variability of acral skin blood flow increased during cooling, which was a greater response than we anticipated. We still do not have a full explanation for this new finding. The acral skin circulation is mainly under sympathetic control, however the sympathetic tone and its variability may have different origins and different functions. It was recently documented for rats that the brainstem source for the parasympathetic tone, which acts on mean heart rate, differs from the brainstem source for its variability, which decides the amplitude of heart rate variability around respiratory frequency (Farmer et al., 2016).

The contrasting finding that absolute variability in acral skin blood flow was reduced at 25°C and 18° as compared to 32°C while the relative variability was increased is important to document and study further. A reduced absolute skin blood flow suggests vasoconstriction due to the ambient room temperature in a healthy subject. Clinically, low blood flow amplitudes with low variability as seen in the lower part of thermoneutral zone may be misinterpreted as low acral flow with pathologic high sympathetic activity and reduced sympathetic variability. We speculate that the increase in relative variability may indicate a
healthy skin circulation, while pathologic conditions such as secondary Raynaud’s phenomenon may have reduced relative variability.

The characteristics of the vasoconstrictory-vasodilatory episodes were quantified by the wavelet analysis. The wavelet analysis was able to quantify these oscillatory patterns of acral skin blood flow. Wavelet analysis of skin blood flow has already been introduced into clinical observations studies of diseases of metabolic and neurogenic nature. Patients with plantar ulcers, a common complication to diabetes mellitus, have been found to have different response to mechanical pressure than normal skin with wavelet analysis (Jan et al., 2013). Patients with digital ulcers, a complication to systemic sclerosis (Bergersen et al., 2014), may benefit from better tools for examining the dynamics of the acral skin circulation.

We found similar increase in relative fluctuations in all three frequency intervals. This suggests that the effect of general cooling on acral skin blood flow oscillations is apparent in the lower frequencies (0.01-0.08 Hz). We showed again that the acral skin blood flow oscillations depend on the ambient temperature (Bergersen et al., 2016) and the selection of different ambient temperatures may cause conflicting results in the literature.

We did find that the relative fluctuations in acral skin blood flow increased at 25 °C and remained unchanged at 18 °C, despite the general skin vasoconstriction during ambient lowering of temperature. This supports that AVA is an important organ for temperature regulation within the thermoneutral zone (Elstad et al., 2014, Vanggaard et al., 2012).

The coherence between the bilateral acral skin blood flow oscillations was highest at 25°C, and somewhat lower at 32°C and 18°C. The coherence is an indication of the strength of the
central signal through sympathetic nerves to the AVAs which causes simultaneous and synchronous acral skin blood flow oscillations. At higher and lower ambient temperatures the sympathetic signal is either attenuated or counteracted by local processes that override the simultaneous vasoconstriction-vasodilation pattern in AVAs. For the 0.01-0.02 Hz frequency interval the coherence remained high even at 18 °C, which may indicate that sympathetic control is increased at this frequency. For acral skin the frequency interval 0.01-0.08 Hz is thus largely subjected to central control, probably mediated by sympathetic nerves, in contrast to what is argued for the non-acral skin during local cooling (Sheppard et al., 2011). Similar to this latter study we found an increase in the relative fluctuations 0.05-0.08 Hz with cooling, but we interpret this as a sympathetic signal rather than local myogenic component, since the coherence was high.

**Future directions**

A better understanding of normal variability of hand blood flow in different ambient temperatures increases understanding of acral vascular syndromes such as Raynaud’s phenomenon, acrocyanosis and chilblain (Lutolf et al., 1993, Rosato et al., 2009). With wavelet analysis we propose that it is possible to establish a tool for predicting when a patient enters the vasoconstrictor state in the lower part of thermoneutral zone. This phase may be of potential risk for patients with cardiovascular disease since vasoconstrictions increase the arterial blood pressure (Kingma et al., 2011). Even patients with secondary Raynaud’s phenomenon may benefit from such prediction scheme, by preventing cold-induced vasospasms that may lead to digital ulcers (Bergersen et al., 2014).
Study limitations

We did not randomise the order of the ambient temperatures due to technical limitations of the climate chamber. All subjects had the same laser Doppler apparatus attached to the right hand. Any difference in calibration between the two apparatus (even if they were calibrated before each experiment) may show as a difference in the two sides’ absolute flux values. We did not ask for the subject’s handedness, thus in retrospect this may also explain differences in absolute flux values. Our protocol investigated the effect of general skin cooling. The effect of local cooling may involve a more local control of skin circulation which we did not elicit in the current study. Laser Doppler flux measures the variability in skin blood flow reliable; however has not yet the possibility to estimate absolute flow. Thus, we think the relative measure of variability is the preferred method. This need to be confirmed in future studies, both for healthy subjects and for patients with compromised acral skin circulation.

5. Conclusions

The skin blood flow through arteriovenous anastomoses is modified by ambient temperature within the thermoneutral zone. The oscillatory pattern of acral skin blood flow is most prominent at 25°C ambient temperature. With wavelet analysis we described the oscillatory pattern of acral skin blood flow at three frequency intervals (0.01-0.02 Hz, 0.02-0.05 Hz and 0.05-0.08 Hz). We found that acral skin blood flow oscillations in these three intervals show high coherence and zero phase shift in the thermoneutral zone, which indicates that they are centrally controlled. Wavelet analysis of acral skin blood flow may serve as a future non-invasive procedure for patients with compromised skin circulation.
6. Acknowledgement

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References


Table 1 Absolute laser Doppler flux values from the two finger tips at the three temperature plateaus

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Laser Doppler flux from right finger tip (a.u)</th>
<th>Laser Doppler flux from left finger tip (a.u)</th>
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</thead>
<tbody>
<tr>
<td>32°C</td>
<td>494 [387, 518]</td>
<td>448 [322, 499]</td>
</tr>
<tr>
<td>25°C</td>
<td>267 [107, 338]</td>
<td>236 [94, 312]</td>
</tr>
<tr>
<td>18°C</td>
<td>82 [30, 111]</td>
<td>61 [18, 84]</td>
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Values are given as Hodges-Lehmann’s estimate of median and 95% confidence interval
**Figures’ captions**

Figure 1 (a) Measured ambient temperature inside the climate chamber. The 10 min plateau periods at 32°C, 25°C and 18°C correspond to the 1\textsuperscript{st}, 3\textsuperscript{rd} and 5\textsuperscript{th} interval. (b) Simultaneous bilateral recording from the pulp of the third fingers (acral skin) in one subject during the whole body surface cooling in a climate chamber from 32°C to 17°C. The first 200s are part of the stabilisation period and is not used for analysis. The above two signals curves correspond to Laser Doppler flux left fingertip (red) and right fingertip (green). The shaded areas are zoomed in on in figures 1c-d. (c) Zoom in on highlighted region at 32 °C in Fig. 1b to illustrate the simultaneous vasoconstrictory episodes in the bilateral finger tips. (d) Zoom in on highlighted region at 18 °C in Fig. 1b to illustrate the simultaneous flux oscillations in the bilateral finger tips. The dip in the flux shows one vasoconstriction (c) and the increase in signal show the characteristic pattern of AVA events during mild cold-induced vasoconstriction (d). The two signals curves correspond to Laser Doppler flux left fingertip (red) and right fingertip (green).

Figure 2 (a) Time-averaged Wavelet (WT) power and (b) WT contour plots of the right fingertip for the 3\textsuperscript{rd} time interval at 25°C for one subject (same as figure 1). This subject had more vasoconstrictory events (yellow) in [0.02-0.05 Hz] compared to [0.05-0.08] at this temperature. (c) Time-averaged WT power and (d) WT contour plots of the right fingertip for the 1\textsuperscript{st} time interval at 32°C for same subject as in a) and b). This subject had more vasoconstrictory events (yellow) in [0.05-0.08 Hz] compared to [0.02-0.05] at 32°C. The A\textsubscript{1}, A\textsubscript{2} and A\textsubscript{3} denote the areas under the curve that correspond to the 1\textsuperscript{st} frequency interval [0.01-0.02Hz], 2\textsuperscript{nd} frequency interval [0.02– 0.05Hz] and 3\textsuperscript{rd} frequency interval [0.05Hz – 0.08Hz] respectively.
Figure 3 Absolute and relative acral skin blood flow fluctuations

Absolute fluctuations (a, c and e) and relative fluctuations (b, d and f) in the laser Doppler flux from right (R) and left (L) finger tips at 0.01-0.02 Hz (a and b), 0.02-0.05 Hz (c and d) and 0.05-0.08 Hz (e and f) at the three plateau temperatures. Horizontal line indicates median value. There is similar response of the absolute fluctuations in both right and left finger tips, but large inter-individual variability (a, c and e). At all frequencies the absolute amplitude of the frequencies decrease as the subjects move to the colder part of their thermoneutral zone. The relative fluctuations show a different pattern, with increased relative fluctuations at all frequencies at 25°C and 18°C.

Figure 4 Phase angles and coherence of the skin blood flow oscillations from the two finger tips.

Individual phase angles and coherence of the skin blood flow oscillations from the two finger tips at 0.01-0.02 Hz (a), 0.02-0.05 Hz (b) and 0.05-0.08 Hz (c) at three room temperatures. The upper circles show the individual angles between laser Doppler flux of the right and left finger pulp. The angle of each circle’s radius corresponds to the median phase angle. The lower graphs depict the individual coherence between laser Doppler flux of the right and left finger pulp. The horizontal lines indicate the median coherence and 95% confidence interval.
Relative variability at 0.01-0.02 Hz

Relative variability at 0.02-0.05 Hz

Relative variability at 0.05-0.08 Hz