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Using wavelet analysis to characterize the thermoregulatory mechanisms of sacral skin blood flow

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Abstract—Pressure-induced skin blood flow responses measured via laser Doppler flowmetry are commonly reported in the time domain. The usefulness of spectral analysis in examining blood flow control mechanisms has been demonstrated, but traditional Fourier analysis does not provide sufficient resolution to reveal characteristic low frequencies. Time-frequency (wavelet) analysis was performed on 10 subjects' sacral skin blood flow responses to heating (45 degrees C) with improved resolution. Five frequency bands were identified (0.008–0.02 Hz, 0.02–0.05 Hz, 0.05–0.15 Hz, 0.15–0.4 Hz, and 0.4–2.0 Hz) corresponding to metabolic, neurogenic, myogenic, respiratory, or cardiac origins. Significant differences were observed in the mean normalized power of the metabolic ($p < 0.01$) and myogenic frequency bands ($p < 0.01$) between preheating and maximal heating and preheating and postheating periods. Power increased for the metabolic frequency and decreased for the myogenic frequency. Wavelet analysis successfully characterized thermoregulatory control mechanisms by revealing the contributions of the physiological rhythms embedded in the blood flow signal.

Key words: endothelial vasoregulation, heating, laser Doppler flowmetry, skin blood flow, time-frequency analysis, vasomotion, wavelet analysis.

INTRODUCTION

Most researchers agree that prolonged exposure to high-pressure gradients causes tissue necrosis via occlusion of capillary blood flow [1]. Therefore, interventions that have been designed to reduce both the magnitude and duration of pressure are an essential component of pressure ulcer prevention and/or treatment regimens, i.e., support surfaces, repositioning, and turning and weight-shifting protocols. For many years, interface pressure has been used as an indicator of tissue loading tolerance. While interface pressure mapping can identify localized high-pressure areas and evaluate the pressure distribution achieved for a particular individual using a support surface, physiological responses or differences in the mechanical properties of soft tissue either among individuals or at different tissue sites cannot be detected. As these and other systemic factors affect tissue loading tolerance, the range of interface pressures capable of occluding capillary blood flow varies widely [2–3]. Therefore, simultaneous measures of multiple tissue responses have recently been adopted to indicate tissue loading tolerance rather than the solitary use of interface

Abbreviations: ANOVA = analysis of variance, BPM^2 = Blood Perfusion Monitor 2, LDF = laser Doppler flowmetry, SD = standard deviation, TSI = thermal stress index.

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pressure. Future research must seek to quantify and determine the relative significance of the physiological, biochemical, and biomechanical tissue responses to mechanical deformation. The monitoring of skin microcirculatory responses via laser Doppler flowmetry (LDF) has potential for use in this manner.

Thermoregulation and nutrition are the two primary functions of the skin microcirculation. Skin microcirculatory changes are controlled by a combination of complex central and local mechanisms. These mechanisms modulate microcirculatory vascular smooth muscle cell activity, thereby regulating the periodic constriction/dilation patterns (oscillations) known as *vasomotion* [4]. While the physiological mechanisms controlling vasomotion are not well known, vascular smooth muscle activity has been shown to be roughly proportional to the tissue's metabolic demand for oxygen [5]. Vasomotion may also be mediated by metabolic factors such as nitric oxide produced in response to endothelial cell shear stress [6]. Based on Poiseuille's law, increased amplitude in vasomotion acts to decrease flow resistance with resultant enhancement of local perfusion [7]. Changes in vessel transmural pressure resulting from mechanical deformation have also been shown to decrease smooth muscle cell contractility, resulting in vasodilation and enhanced blood flow. This response has been demonstrated in the absence of neural regulation and is mediated by fluctuations in the ion concentrations, mainly Ca++, across the membrane of vascular smooth muscle cells [8–9]. Enhanced perfusion appears to increase tissue viability and tolerance to loading [10–11].

LDF has been used extensively to quantify skin perfusion responses to compressive loading [3,12–14]. LDF is noninvasive, requires no heating of the skin (as transcutaneous oximetry does) and can detect microcirculatory changes near a depth of ~1 mm below the surface of the skin. LDF skin capillary blood flow parameters, flow (mL_{LD}/min/100 g tissue or arbitrary units), velocity (mm/s), and/or volume (% red blood cell volume) have been used to monitor responses to loading regimens. Traditionally, flow parameters have been reported only in the time domain. Because the physiological rhythms associated with blood flow control mechanisms are embedded in the blood flow signal, decomposing the signal via spectral analysis reveals various characteristic frequencies and may contribute to our understanding of these complex mechanisms. Unfortunately, few investigators have used spectral analysis to date and a systematic methodology appears to be lacking [15]. To this end, we have designed a series of experiments using this method to investigate skin microcirculatory control mechanisms in response to various stimuli and loading conditions.

Decomposing the LDF blood flow signal into its characteristic frequencies has previously proven useful in studies of physiological control mechanisms. In an early study, Bernardi et al. used autoregressive analysis of the blood flow signal to demonstrate a number of frequency bands [16]. One low-frequency band (0.017–0.028 Hz) was revealed in 5 of 10 normal subjects. The authors speculated that these fluctuations were due to thermoregulatory influences [16]. In a second study, Bernardi et al. noted that another frequency band with a peak of ~0.1 Hz had decreased amplitude in diabetics compared to nondiabetics. They theorized that this reduction was due to autonomic neuropathy, and thus the frequency band reflected a neurogenic origin [17]. Kastrup et al. also used LDF to study the rhythmical oscillations of blood flow [18]. They classified the observed fluctuations into two categories: (1) α-oscillation (0.0667–0.4333 Hz, median 0.1133 Hz) and (2) β-oscillation (0.0083–0.0467 Hz, median 0.025 Hz). The amplitude of β-oscillation was two to four times greater than that of α-oscillation. Local administration of lidocaine anesthesia extinguished β-oscillation without affecting α-oscillation; thus, Kastrup et al. concluded that β-oscillation was neurogenic in origin while α-oscillation was nonneurogenic [18]. This result contradicted Bernardi's previous work in which oscillations in the 0.1 Hz range were attributed to neurogenic origins.

Traditionally, Fourier transform-based power spectrum analysis has been used to study blood flow that is characterized by two peaks. However, Fourier transform analysis does not provide sufficient time resolution for analysis of nonstationary physiological signals, such as heart rate and myoelectric signals [19–20]. Although windowed Fourier transform method permits time-frequency analysis, obtaining adequate precision in both domains requires selection of a proper window that balances time and frequency resolution. For complex signals with several mixed frequency components (e.g., blood flow), this balance is not possible [21]. To overcome the limitations of the Fourier method, Morlet first conceptualized wavelet analysis in 1983; later Grossman and Morlet laid the mathematical foundation for the wavelet transform permitting multiresolution, time-frequency analysis of the blood flow signal [22].

Stefanovska and Bracic used the wavelet transform to analyze simultaneously measured cardiovascular signals (respiration, electrocardiogram, blood pressure, and LDF), restricting their analysis to the processes associated with one cycle of blood through the system [23]. Stefanovska and Bracic recorded responses at rest and during exercise in subjects with normal, well-conditioned, and pathological cardiovascular systems. According to their eloquently reported results, wavelet analysis of blood flow reveals various peaks in the power spectrum corresponding to specific origins: (1) heart rate (0.4–2.0 Hz), (2) respiratory activity (0.15–0.4 Hz), (3) vascular myogenic responses (0.06–0.15 Hz), (4) neurogenic responses (0.02–0.06 Hz), and (5) metabolic responses (0.0095–0.02 Hz) [15,21,23]. Since wavelet analysis permits examination of the contributions of the myogenic, neurogenic, and metabolic components of vasomotion relative to an exercise stimulus, the authors theorized that this method might also be useful in differentiating the effects of thermal stress versus tissue loading. In addition to being used in the study of exercise effect, wavelet analysis has also been used in the study of (1) the cutaneous microcirculation of free flap tissue [24] and (2) skin blood flow responses to acetylcholine (endothelium-dependent vasodilator) and sodium endothelium (endothelium-independent vasodilator) [25].

This paper describes the first in a series of studies investigating the skin's microcirculatory response to various stimuli and loading conditions as measured by LDF. This study aimed to (1) thermally induce maximal sacral skin blood flow responses, (2) identify the characteristic frequency bands in the blood flow signal using the wavelet transform, and (3) determine the relative power or contribution of the various bands to the total blood flow. In this manner, the response to thermally induced maximal blood flow could be characterized. Thus, in subsequent studies, the control mechanisms associated with the effects of heating could be differentiated from those associated with tissue loading.

METHODS

Ten unimpaired subjects (5 male and 5 female) were recruited into the study. The demographic data (mean ± standard deviation [SD]) were as follows: age 30.0 ± 3.1 years, height 162.9 ± 6.8 cm, and weight 58.3 ± 8.6 kg. The following conditions constituted exclusion criteria: the presence of pressure ulcers on the sacrum, diabetes, vascular disease, hypertension, or use of vasoactive medications. An informed consent approved by the University of Pittsburgh Institutional Review Board was obtained from each subject prior to testing. All tests were performed in the Soft Tissue Mechanics Laboratory, University of Pittsburgh, Pittsburgh, Pennsylvania. Room temperature was maintained at 24 ± 1 °C. For at least 30 minutes prior to testing, all subjects assumed a recumbent, relaxed position in the laboratory to acclimate to the room temperature and achieve a baseline blood flow level.

A Laserflo Blood Perfusion Monitor 2 (Vasamedics, Eden Praire, Minnesota) and Softip pencil probe (P-435, Vasamedics) were used to measure capillary blood perfusion (mL_{LD}/min/100 g tissue). We used a temperature-control module with heater probe (P-422, Vasamedics) to heat the skin to 45 °C to obtain a maximal skin blood flow response. Laser Doppler skin blood flow was sampled at 20 Hz with the use of a 16-bit data acquisition card (PCI-MIO-16XE, National Instruments, Austin, Texas).

With subjects lying prone on a customized, conforming support surface, we recorded blood flow over the sacrum at rest for 10 minutes to establish baseline flow (preheating period). This was followed by 15 minutes of heating, consisting of 3 minutes at 35 °C, 9 minutes of incremental heating (1 °C increase per minute) from 35 °C to 45 °C, and a final period of 3 minutes at 45 °C. Skin blood flow monitoring continued throughout a 10-minute postheating period.

The thermal stress index (TSI) has been used to screen for abnormal microcirculatory functional status in peripheral vascular diseases and diabetes [26]. The procedure consists of measuring the skin blood flow at 35 °C for 2 minutes, followed by heating the skin at 44 °C for 20 minutes, and then recording blood flow for 2 minutes. TSI is defined as blood flow at 44 °C/35 °C and scores ≥ 5 are considered normal. A modified TSI was designated as skin blood flow at 45 °C/35 °C following the heating protocol described previously.

Wavelet analysis provides a multiresolution, time-frequency analysis of sacral skin blood flow. Wavelet transform decomposes a signal over dilated and translated wavelets [27]. Continuous wavelet transform of a signal $f(u)$ was defined as

$$\hat{f}(s, t) = \int_{-\infty}^{\infty} \psi_{s,\, t}(u) f(u)\, du \ , \qquad (1)$$

where $\hat{f}(s, t)$ is a wavelet coefficient and $\psi_{s,t}(u)$ is a wavelet function and was defined as

$$\psi_{s,t}(u) = \frac{1}{\sqrt{s}}\psi\left(\frac{u-t}{s}\right) . \quad (2)$$

A family of time-frequency wavelets is obtained by scaling function ψ by parameter s (scale factor) and translating it by t (time factor). Continuous wavelet transform data are easier to interpret and/or are amenable to pattern recognition because their complete scales tend to reinforce the traits and make all information more visible than data from discrete wavelet transform [28]. Thus continuous wavelet transform was selected to perform time-frequency analysis of skin blood flow in this study.

We used the Morlet wavelet model to perform wavelet transform analysis. Morlet wavelet is a Gaussian function defined as

$$\psi_{s,t}(u) = \frac{1}{\sqrt[4]{\pi}}.\left(e^{-i\omega_0 u} - e^{-\omega_0^2/2}\right). e^{-u^2/2} , \quad (3)$$

where ω_0 was designated as 2_π Therefore, a simple relationship between frequency and scale could be expressed as [15]

$$\text{frequency} = \frac{1}{\text{scale}} .$$

Morlet wavelet, a Gaussian function, allows the best time-frequency localization according to the Heisenberg uncertainty principle [15,29].

To quantify the amplitude of power within the characteristic frequency bands, we calculated the average amplitude of each frequency band using

$$A_i(f_{i1}, f_{i2}) = \frac{1}{t}\int_0^t \frac{1}{f_{i2}-f_{i1}}\int_{1/2\pi f_{i2}}^{1/2\pi f_{i1}} \frac{1}{S^2}\hat{f}(s,t)\,dsdt , \quad (4)$$

where f_{i1} and f_{i2} are the limits of a given frequency band, e.g., myogenic characteristic frequency band.

To permit comparisons of the subjects' power distributions, we used the relative contribution of each frequency band (myogenic, neurogenic, etc.) in this study and defined it as ratio of the average amplitude of total frequency range (0.008–2.0 Hz) for each condition, i.e., preheating, heating, and postheating:

$$a_i(f_{i1}, f_{i2}) = \frac{A_i(f_{i1}, f_{i2})}{A_{\text{total}}} . \quad (5)$$

Because physiological signals in the skin blood flow rarely have frequencies higher than 2 Hz [23], the specified range of frequencies for the wavelet analysis was established at 0.007 Hz to 2.5 Hz. The limits of each frequency band were chosen based on the ranges previously reported by Stefanovska et al [23] and our research data. Selection was also contingent upon the peak frequency for each heating condition and each subject falling within each band.

All mathematical functions were developed with MATLAB 5.2 and Wavelet Toolbox (MathWorks, Natick, Massachusetts). We used a skewness test to assess the normality of the data. We used a Mauchly's test of sphericity to test the equal variances of the repeated measures data. A one-way analysis of variance (ANOVA) with repeated measures compared differences among preheating, heating, and postheating periods. Where skewed distributions existed, we used a Kruskal-Wallis one-way ANOVA to compare differences among frequency bands during preheating, heating, and postheating periods. The level of significance for post hoc multiple comparisons of repeated measures was adjusted to 0.017 based on the Bonferroni correction [30].

RESULTS

The mean skin blood flow for each temperature was plotted (**Figure 1**). Skin blood flow shows steep increases for skin temperature exceeding 42 °C. The mean blood flow during the postheating period was higher than that at 45 °C (maximum thermal stress). The increase in skin blood flow during postheating compared to maximal heating was observed in seven subjects.

A modified TSI was calculated for each subject. Based on our definition, all subjects demonstrated normal microcirculatory function with modified TSI scores ≥5 and a group mean ± SD = 8.44 ± 2.43.

The wavelet transforms of blood flow signal are expressed in the time-frequency domain (Scalogram, the squared magnitude of the wavelet transform) (**Figure 2**).

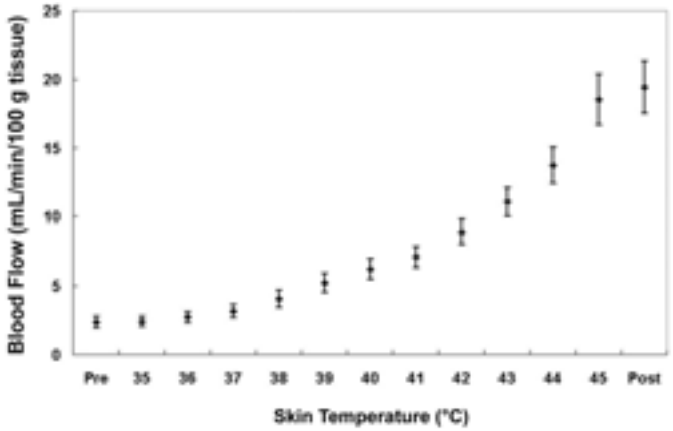

Figure 1.
Mean skin blood flow at different skin temperatures (values are mean ± standard deviation [SD]). (Time period is 10 minutes for preheating and postheating, 3 minutes for 35 °C and 45 °C, and 1 minute for other temperatures.)

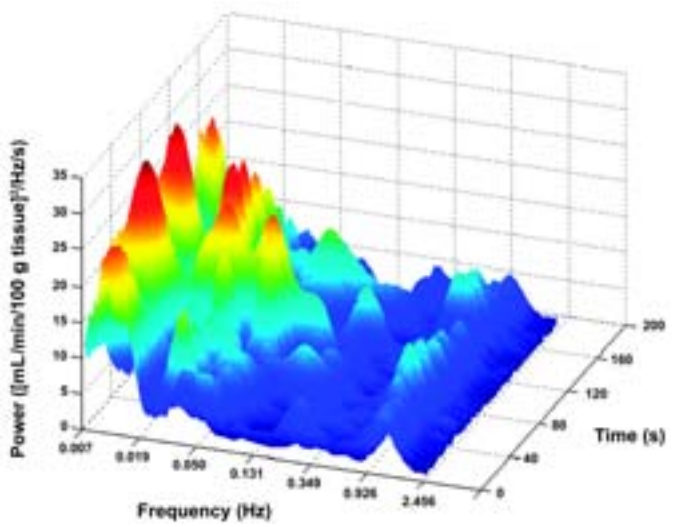

Figure 2.
Typical example of Scalogram of skin blood flow at 45 °C.

Five characteristic frequency bands were observed in the time-frequency domain. We are particularly interested in describing changes of power within each characteristic frequency band in response to thermal stress. In this form, the data cannot be easily compared or quantified. However, by averaging the data points in the time domain during a specific time event, one may reduce two-dimensional time and frequency data to one-dimensional frequency domain data. All participants' time-averaged wavelet transforms of skin blood flow at 45 °C are shown in **Figure 3**.

Based on the distribution of power, five frequency modes were identified (**Figure 3**). (Since intersubject peak frequency location varied slightly for each degree of thermal stress, we determined that a frequency band that captured the peaks for all the subjects would more accurately characterize the blood flow frequency elements. All subjects' power spectra during maximal heating were plotted for comparisons.) Five frequency bands were chosen to represent the frequency elements for all subjects: (1) metabolic (0.008–0.02 Hz), (2) neurogenic (0.02–0.05 Hz), (3) myogenic (0.05–0.15 Hz), (4) respiratory (0.15–0.4 Hz), and (5) cardiac (0.4–2.0 Hz) (**Figure 3**).

To compensate for variability in total flow, we normalized the power for each frequency band to the total power for each condition (**Figure 4**) (the data set in **Figure 4** was calculated from the same subject as in **Figure 2**). A skewness test for each frequency band showed significant differences in the respiratory frequency band during

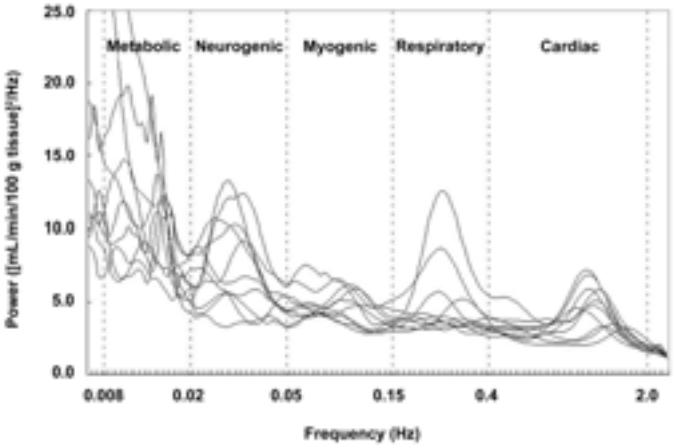

Figure 3.
Time-period-averaged wavelet transforms of skin blood flow at 45 °C for all participants' spectra.

preheating and heating periods ($p < 0.05$). A Mauchly's test of sphericity for each frequency band demonstrated no statistically significantly differences ($p > 0.05$). A one-way ANOVA with repeated measures demonstrated a significant difference between the mean metabolic and myogenic frequency bands for all conditions ($p < 0.001$). A Kruskal-Wallis one-way ANOVA tested the respiratory frequency band and showed no significant differences among preheating, heating, and postheating periods. Following post hoc analysis, however, statistically significant differences

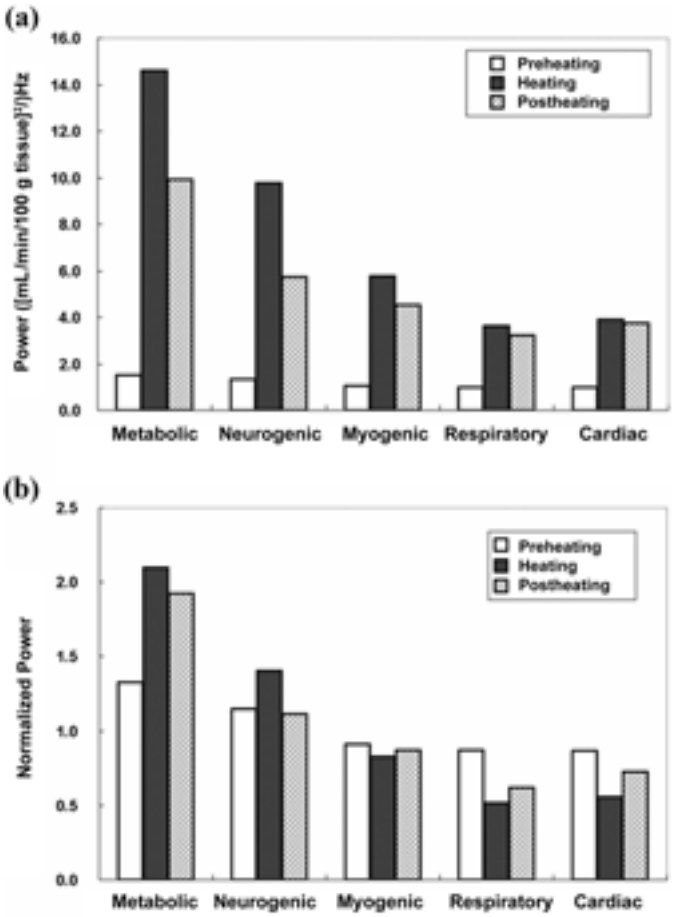

Figure 4.
Typical example of **(a)** nonnormalized and **(b)** normalized power of each characteristic frequency band during different periods.

were demonstrated only between (1) the mean preheating and 45 °C heating period and (2) the preheating and postheating period for the metabolic and myogenic frequency bands. The relative contribution in the metabolic frequency band was increased ($p < 0.01$), while the myogenic frequency band decreased ($p < 0.01$) (**Figure 5**).

DISCUSSION

The vasodilation induced by local heating is primarily mediated by neurogenic reflexes and locally released substances. However, the interactions between these mechanisms are complex and poorly understood [31–33]. To further clarify and objectively characterize these mechanisms, we used wavelet analysis to reveal five different frequency bands associated with the various control mechanisms of thermally induced vasomotion embedded in the maximal blood flow signal.

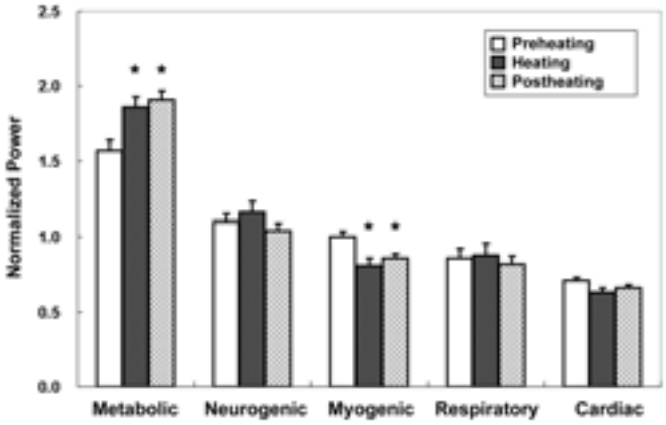

Figure 5.
Comparisons of each characteristic frequency band during different periods for all participants (values are mean ± standard deviation [SD]); $^{*}p < 0.01$.

Skin blood flow and control mechanisms of vasomotion in response to local heating depend on the temperature, the duration of exposure, and the rate of heat application. Varying these factors stimulates different control mechanisms resulting in different perfusion responses [34]. For example, increasing the temperature quickly or beyond 45 °C could stimulate nociceptive receptors and increase the neurogenic component of the signal. In other studies of thermally induced maximal blood flow (including TSI), the skin is heated rapidly (over 2–3 minutes) to 42 °C and the temperature is held constant for 20 to 40 minutes [35–36]. This rapid heating of the skin produces two signal peaks: the first due to local sensory nerve activity (axon reflex) and the second due to release of endothelial nitric oxide [33–34]. In this study, we applied a stepwise heating from 35 °C to 45 °C to the skin over the sacrum to avoid nociceptive stimulation and to permit analysis of the blood flow response at each temperature.

Because our rate of heating was much slower than the rapid increase in heat conventionally used to achieve maximal vasodilation, we did not observe the typical biphasic blood flow response to heating. In our study, only the second peak was observed during maximal heating. This peak extended into the postheating period. The nitric oxide phenomenon may account for the increased flow observed during the postheating recovery period.

We believe that the slower, incremental heating rate in this study may account for the absence of the first peak (local sensory nerve activity).

Direct and indirect evidence supports the designation of the frequency bands' origins. While direct evidence supports the designation of the higher frequencies to cardiac and respiratory origins [21,37], the evidence supporting assignment of the lower frequencies to metabolic, neurogenic, or myogenic origins is primarily indirect [18,21,24–25].

Several reports provide indirect evidence to support the designation of the ~0.1 Hz peak frequency to local control mechanisms [18]. The smooth muscle cells respond continually to changes in transmural pressure [38–39]. This ion-mediated response has been demonstrated in isolated animal vessels either by dynamically measuring the change in vessel diameter [40] or by ion concentration [41]. This myogenic theory appears to be the most accepted explanation for local control of vasomotion [42]; therefore, the 0.05 Hz to 0.15 Hz frequency band was attributed to myogenic control mechanisms.

Stefanovska et al. and Kvernmo et al. demonstrated an isolated peak at ~0.01 Hz in all cardiovascular signals with a variance in its peak location characteristic of local phenomena. They cited indirect evidence for attributing this frequency to endothelial cell metabolic processes [23,43]. These processes may be mediated by oxygen demand or nitric oxide release. Kvernmo et al. demonstrated that the 0.008 Hz to 0.02 Hz frequency band is an endothelial-dependent response by comparing the skin blood flow responses of acetylcholine and sodium nitroprusside [25].We also hypothesize that the 0.008 Hz to 0.02 Hz frequency band is metabolic in origin, i.e., mediated by any locally released vasodilator.

Kastrup's blood flow recordings under both anesthesia and denervation clearly identified the neurogenic origin of the 0.0083 Hz to 0.0467 Hz (β-oscillation) frequency range [18]. However, because of the superior resolution provided by wavelet transform at low frequencies, our data revealed two peaks within this range. We chose to associate the higher frequency band, 0.02 Hz to 0.05Hz with neurogenic origins and, as previously stated, the lower frequency band, 0.008 Hz to 0.02 Hz, with metabolic effects [5]. Although designation of the frequency bands in this study is based solely on the data obtained from 10 subjects, we believe this method will prove useful in determining the relative significance of the various control mechanisms of skin blood flow in response to loading or other stimuli.

The increased metabolic influence was anticipated and may be explained by the increase in metabolic activity associated with tissue heating. Higher ambient temperatures have been shown to cause an increase in tissue metabolism and oxygen consumption on the order of 10 percent for every 1 °C [44].

The decreased myogenic activity was also anticipated because heat produces a mild inflammatory reaction in which local chemical mediators act to decrease smooth muscle tone and increase capillary and postcapillary venule permeability, resulting in vasodilation of resistance vessels [45]. Local myogenic control is considered independent of any neural or humoral influences and has been associated with the application of mechanical force to the vascular smooth muscle cell [38–39]. In this study, thermal stress was applied with minimal mechanical force and deformation to the soft tissue. Therefore, compared to preheating blood flow, heat stress accounts for the observed decreased power in the myogenic frequency band shown in the heating and postheating periods.

Because of the increase in total blood flow resulting in increased total power for the maximal heating period, significant differences between the preheating and maximal heating period were demonstrated for each frequency band. To permit comparisons of the energy contributions from each frequency band for all subjects during all periods, we normalized the power of each frequency band (myogenic, neurogenic, etc.) to the total power for each period, i.e., preheating, heating, and postheating. Subsequently, statistically significant differences between the preheating and heating periods and the preheating and postheating periods were demonstrated for only the frequency bands at 0.008 Hz to 0.02 Hz (metabolic) and 0.05 Hz to 0.15 Hz (myogenic). Wavelet transforms must be normalized to the total power for a specific period to reveal the relative contribution of the physiological rhythms embedded in the blood flow.

Skin blood flow response to local heat appears to increase the amplitude of vasomotion without affecting the characteristic frequencies. This finding contradicts the traditional view that the frequency of the flow signal shifts in response to stimulation [46–47]. Our analysis supports the evidence reported by Stefanovska and Bracic demonstrating that the peaks of the average wavelet transforms from all cardiovascular signals (electrocardiogram, blood pressure, and LDF) appear at similar or, in some

instances, exactly the same frequencies [21]. Thus, thermal stress enhances and maintains vasomotion in the microcirculatory flow. The increased amplitude of vasomotion results in decreased effective vascular resistance resulting in increased blood flow based on Poiseuille's law [7].

CONCLUSIONS

Our long-term goal is to improve our understanding of the control mechanisms of blood flow associated with tissue loading to optimize critical parameters that enhance tissue viability. Through wavelet analysis of the LDF skin blood flow signal, our study identified five characteristic frequency bands. Our findings indicate that (1) local heating increases skin blood flow at the sacrum and (2) the relative contribution in the metabolic frequency band increased while the myogenic frequency band decreased. Thus, our study has established a means of evaluating the relative influence of specific control mechanisms in response to local heating. With this method, differentiating heating effects from loading effects will be possible in future studies. Our results encourage the use of this method for differentiating the control mechanisms of blood flow in response to a variety of stimuli. For example, since the degree of vasodilation induced by local heating has been used as a clinical diagnostic tool for evaluation of sympathetic function [48,49], this method could further aid in the diagnosis of specific pathologies affecting the vasodilatory response.

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