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Wavelet Analysis of the Effects of Static Magnetic Field on Skin Blood Flowmotion: Investigation Using an *In Vivo* Rat Model

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Abstract. *Background: In the literature, various in vivo studies on animals have demonstrated that a static magnetic field (SMF) might maintain microvascular tone in the cutaneous microcirculatory system by its biphasic effects on vasomotion. Here, the effects of locally applied SMF on skin blood flowmotion within the stressed or unstressed skin in the trochanter area were evaluated using wavelet analysis of skin blood perfusion as measured by laser Doppler flowmetry (LDF) in anesthetized rats. Materials and Methods: Forty-eight experimental trials were carried out on twelve Sprague-Dawley rats. Four experimental groups were formed at random: i) Group CNL (no loading or SMF exposure; n=12 trials); ii) Group SMF (SMF exposure only; n=12 trials); iii) Group L (stressed skin without SMF exposure; n=12 trials); iv) Group L+SMF (stressed skin with SMF exposure; n=12 trials). Results: SMF significantly enhanced endothelial related metabolic activity (0.01-0.05 Hz) in the stressed skin (p=0.03). However, SMF did not induce significant change in the flowmotion amplitude in the unstressed skin (p=0.22). Conclusion: The modulating effect of SMF on skin blood flowmotion might be related to the vascular tone modified by prolonged loading.*

Vasomotion is the rhythmic contraction and dilatation of arterioles (1, 2). Rhythmic variations of the blood flow in the vascular network are referred to as flowmotion, which can be detected non-invasively using laser Doppler flowmetry (LDF) (3). By evaluation of the skin flowmotion in areas susceptible to pressure ulcers, disturbed vascular tone has been found in persons with spinal cord injury (SCI) (4, 5). A disturbed vascular tone has been considered to have pronounced influence on capillary perfusion (5).

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In the literature, various *in vivo* studies on animals (6-9) have demonstrated that a static magnetic field (SMF) might maintain microvascular tone in the cutaneous microcirculatory system by its biphasic effects on vasomotion, *i.e.* it could influence the homeostasis of vasomotion in the cutaneous microcirculatory system. For example, locally applied SMF of 1 mT for 10 minutes on the cutaneous microcirculation in a rabbit ear chamber under noradrenaline-induced high vascular tone, could significantly enhance vasodilation with increased vasomotion. When low vascular tone was induced by acetylcholine, SMF could cause increased vasoconstriction with reduced vasomotion (9). This work suggests that when the vascular tone was low, SMF caused vasoconstriction and, in contrast, when the vascular tone was high, SMF induced vasodilatation. Ohkubo and Xu (6) have found that SMF locally applied to the cutaneous tissue in a rabbit ear chamber for 10 minutes at 1, 5 and 10 mT induced a biphasic variation of vasomotion in a non-dose dependent manner. However, there is little information available concerning the effect of SMF on flowmotion in tissues that have been subjected to prolonged compressive loading. The understanding of this physiological response is of particular interest because of its potential contribution to the prevention of pressure ulcers.

Spectral analysis of the skin flowmotion signal deals with changes in the dynamics of blood flow and has been introduced as an approach for the evaluation of microvascular control mechanism (3, 10, 11). The flowmotion signals consist of notably different features in both time and frequency and, typically, the high-frequency components have a shorter time span than the low-frequency components. To measure these features actually, time-frequency domain method with adjustable time and frequency resolution is required.

The method of wavelet analysis has been applied to the study of cardiovascular signals (12). Five characteristic frequencies of blood perfusion signals have been identified in the human or rat cutaneous circulation using LDF (13-16). These oscillations manifested the influence of heart beat, respiration, intrinsic myogenic activity of the vascular

smooth muscle, neurogenic activity on the vessel wall and endothelial related metabolic activity (10, 12, 17-20).

Prolonged loading of vascularized soft tissues is well accepted to be the most important mechanical cause for the onset of tissue breakdown in the skin, subcutaneous tissues and underlying striated muscles (21). Prolonged surface loading has been shown to induce disturbed skin flowmotion in the stressed skin in anesthetized rats (22, 23). Since prolonged loading leads to a disturbance in microvascular tone, the biphasic effects of SMF on vasomotion might be used to counteract such stresses. In the current study, it has been hypothesized that SMF would modify vascular tone in the stressed skin and thus improve tissue perfusion.

Materials and Methods

Animals. Sprague-Dawley rats weighing 400-500 g were used for all experiments. These rats were fed with a standard nutrient diet, with no extra vitamins supplement. Each rat was anesthetized by intraperitoneal delivery of a combination of xylazine (0.4 ml, 20 mg/ml) and ketamine (0.2 ml, 100 mg/ml). Additional small doses (0.2 ml) were administered at appropriate intervals during the study to maintain a proper level of anesthesia and an insensate state of the animal during the experiment.

Static magnetic field (SMF). A SMF was generated by neodium-iron-boron alloy (Nd_2-Fe_{14}-B) magnets (Ø10x5 mm, Aim Industrial Co., HK) incorporated in the indentor (Figure 1). The flux density of the SMF was measured with a Gauss/Tesla meter (Model 5070, F.W. Bell, Orlando, USA). The accuracy of the gaussmeter probe was 0.01 mT. The actual dosage delivered to the trochanter skin was 30 mT. The background intensity of the geomagnetic field was 0.05 mT. The relative orientations of the SMF and the geomagnetic field were roughly perpendicular to each other. No ferromagnetic components were located in the field area of the magnet.

Experimental set-up and procedure. In this study, an experimental rat model established by the research team (24) was adopted. Two specifically designed pneumatic indentors of 1.5 cm^2 area with incorporated probe and magnet were used in this study (Figure 1). Forty-eight experimental trials were carried out on 12 Sprague-Dawley rats. The hair at the loading site was carefully shaved and care was taken not to damage the skin during shaving. All animals were under anesthesia throughout the loading or measurement period. Four experimental groups were formed at random: i) Group CNL (no loading or SMF exposure) (n=12 trials); ii) Group SMF (SMF exposure only) (n=12 trials); iii) Group L (stressed skin without SMF exposure) (n=12 trials) and iv) Group L+SMF (stressed skin with SMF exposure) (n=12 trials).

The experiment period lasted for six days on each rat. On day 1, one side of the trochanter area served as Group SMF and the contralateral side as Group CNL. From day 2 to day 5, a precalibrated average pressure of 13.3 kPa (100 mmHg) was applied to the trochanter areas of both sides via two specifically designed pneumatic indentors (Figure 1a). The loading duration was six hours/day for four consecutive days. On day 6, no pressure was applied to the trochanter areas. One side of the trochanter area in the stressed skin served as Group L and the contralateral side as Group L+SMF.

Laser Doppler perfusion measurements. Blood perfusion measurements were performed using a laser Doppler flow monitor (Moor Instruments DTR4, software version 4.1, Axminster, UK) with a contact probe (DP1T/7-V2) with a power of 1.0 mW at a wavelength of 780 nm. This probe was incorporated in the indentor for real time monitoring of local tissue blood perfusion in the trochanter area (Figure 1). The LDF signal was sampled at 40 Hz and the measurements were expressed in arbitrary units in the tissue sample volume (flux) for comparative study. A time constant of 0.1 sec was selected. The calibration of the laser Doppler flowmeter was performed regularly using a standard reference (Flux Standard) provided by Moor Instruments. The standard uses the Brownian motion of polystyrene microspheres in water to produce the reference signals.

To examine the effect of SMF on unstressed tissue, SMF was applied to the tissues overlying the trochanter area for 40 minutes (25) and the contralateral side served as its own control on day 1. This measurement interval was equally divided into two 20 minutes exposures (EXP1 and EXP2 for analysis). Peripheral circulations were measured and recorded for 120 minutes that included an initial 20 minutes (PRE) interval before SMF application and 60 minutes (divided into three 20 minutes intervals, namely POST1, POST2 and POST3) after cessation of the SMF on day 1 of the experiment (Figure 2). The same SMF exposure protocol as on day 1 was repeated on day 6 to examine the SMF effect on stressed tissues. During the experiments, peripheral circulation at the tissues overlying both trochanters was measured. The trochanter areas were marked before the experiments and to make sure the LDF probe was in the same site on the four consecutive days. The experimental protocol was reviewed and approved by the Department of Health and the Hong Kong Polytechnic University Ethical Committee for the use of animals in research.

Spectral analysis. Spectral analyses have commonly been performed using Fourier or short-time Fourier methods. However in the blood perfusion signal both low and high frequency components are present and a Fourier transformation fails either in following the time evolution of the high frequency events or the estimation of the frequency content of the low frequency band (19). The method of wavelet analysis offers a solution to this problem.

The idea of the continuous wavelet transform is to project a signal on a family of zero-mean functions, the wavelets, deduced from an elementary function, called the mother wavelet $\Psi(u)$, by translations and dilations. Continuous wavelet transform of a signal s is defined as

$$\tilde{g}(s,t) = \int_{-\infty}^{+\infty} \Psi_{s,t}(u) g(u) du \quad (1)$$

where $\tilde{g}(s,t)$ is a wavelet coefficient and $\Psi_{s,t}(u)$ is a wavelet function and was defined as

$$\Psi_{s,t} = \frac{1}{\sqrt{s}} \Psi\left(\frac{u-t}{s}\right) \quad (2)$$

where t is time, s is scale related to the frequency f as $f=f_0/s$, and f_0 determines the current frequency resolution. By choosing $f_0=1$, we obtain the simple relation $f=1/s$.

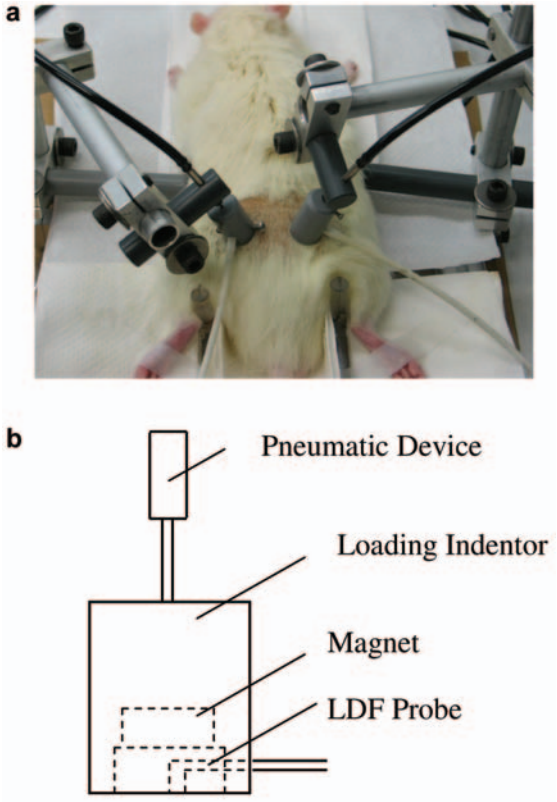

Figure 1. *Experimental setup (a) for pressure loading over the trochanter area and (b) schematics of indentor with incorporated probe (Ø14x12.5 mm) and magnet (Ø10x5 mm). LDF: laser Doppler flowmeter.*

The continuous wavelet transform is a mapping of the function onto the time-frequency plane. By adjusting the window used in wavelet transform, slower and faster events can be categorized accordingly (26). In this study, Morlet wavelet was chosen for the wavelet transform analysis. Morlet wavelet (27) is a Gaussian function that allows the best time-frequency localization within the limits given by the uncertainty principle (12, 19, 26).

The average values of all LDF signals were removed before further analysis. The wavelet transform was calculated in the frequency interval from 0.01 to 5 Hz. The upper limit was set to include the heart rate frequency, while the lower limit was chosen to include all three regulatory mechanisms of the blood flow, the myogenic, neurogenic and metabolic.

Typical wavelet transforms of LDF signal in the time-frequency plane (a) and averaged over time (b) are presented in Figure 3. The wavelet transform is calculated with a logarithmic resolution, and the frequency axes are therefore presented logarithmically. This is particularly appropriate for the estimation of the low frequency component. In this interval, periodic oscillations with five different characteristic frequencies (around 2.8, 0.96, 0.23, 0.1, and 0.028 Hz, respectively) were observed (Figure 3). All mathematical algorithms were developed using Matlab (version 6.5).

In rats, the dynamics of the cardiovascular-respiratory system possess features similar to those observed in humans (13, 28). However, cardiac and respiratory rhythms are approximately 2.5 times faster than in humans as shown in this study. This factor

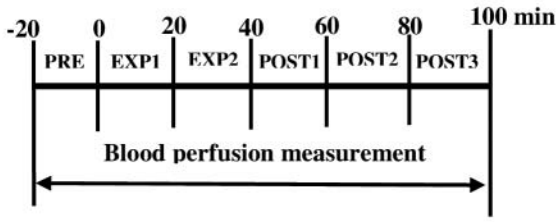

Figure 2. *Experimental procedures for Group SMF (SMF exposure) and Group L+SMF (stressed skin with SMF exposure) and measurement of the skin blood perfusion. A period of 20 min just before exposure (PRE) was compared with those of two 20 minute consecutive intervals during exposure (EXP1 and EXP2) and thereafter with three periods (POST 1-3), 20 min each, starting at the cessation of SMF exposure.*

corresponds to the ratio of total blood volume to the cardiac output (13, 16). The position of each peak differs among rats and changes with time in a given rat, but they are found to be within the following frequency intervals: 0.01-0.05; 0.05-0.15; 0.15-0.4; 0.4-2.0···2-5 Hz, which correspond to endothelial related metabolic activity, neurogenic activity, myogenic activity respiratory and cardiac activities respectively (12-14, 16, 19, 20).

Quantitative measurements. An oscillatory component in a signal can be characterized by its instantaneous frequency and corresponding amplitude. Quantitative measures were introduced to make comparisons between sets of signals (12). The average amplitude within each interval was used to characterize the spectral components. The average amplitude of a spectral component in a given frequency interval can be determined as

$$A_i(f_{i1}, f_{i2}) = \frac{1}{t_w} \int_{1/f_{i1}}^{1/f_{i2}} \frac{1}{s^2} \tilde{g}(s,t) ds dt \qquad (3)$$

The relative amplitude, or normalized amplitude, is then

$$a_i(f_{i1}, f_{i2}) = \frac{A_i(f_{i1}, f_{i2})}{A_{total}} \qquad (4)$$

where a_i is the normalized average amplitude within the i th frequency interval and A_{total} is the average amplitude obtained over the entire frequency range under observation.

Statistical analysis. All values were expressed as means with standard deviation. One-way ANOVA analysis was used to study the differences in the blood oscillations within the groups. Two-way repeated measure ANOVA analysis was used for comparison among groups (SPSS version 11.5.1). A difference of $p<0.05$ was considered statistically significant. *Post-hoc* analyses within the groups were done with LSD's multiple comparison tests.

Results

Average value of the blood perfusion. The values of the blood perfusion averaged over the total time of observation before SMF exposure (PRE), during SMF exposure (EXP1 and

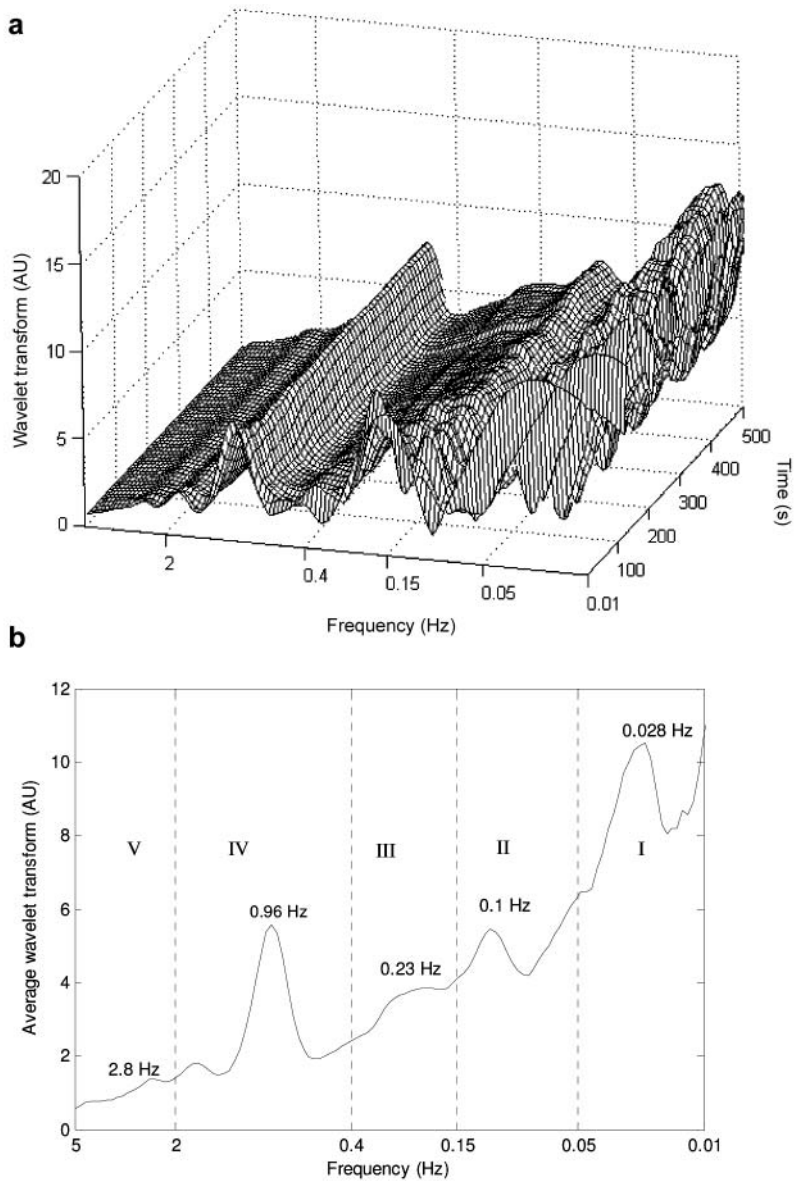

Figure 3. *The wavelet transform (a) and average spectrum (b) of the laser Doppler perfusion signal in the resting skin at the trochanter area, which are shown on a log scale. The vertical lines indicate the outer limits of each frequency interval from I to V, which correspond to endothelial related metabolic, neurogenic, myogenic, respiratory and cardiac activities respectively.*

EXP2) and post SMF exposure (POST1-3) are presented in Figure 4. The results showed that SMF significantly increased the blood perfusion of stressed skin by 19% during the exposure period compared to PRE values ($p<0.05$) in the stressed skin in the Group L+SMF. The normalized perfusion exhibited a significant difference between the Group L and Group L+SMF during the exposure periods ($p<0.05$) (Figure 4a). However, SMF did not induce a significant change in the blood perfusion in the unstressed skin ($p=0.57$) (Figure 4b).

Spectral amplitudes within each frequency interval. The results showed that prolonged loading induced a reduction of 28% in the spectral amplitude in the frequency interval I at the trochanter area in the stressed skin during the PRE period on day 6 ($p=0.01$). Analysis of variance revealed a significant interaction between surface loading and SMF ($F=4.60$, $p=0.03$) in the skin over the trochanter area in the frequency interval of 0.01-0.05 Hz. The normalized amplitude exhibited a significant difference between Group L and Group L+SMF during the SMF exposure period

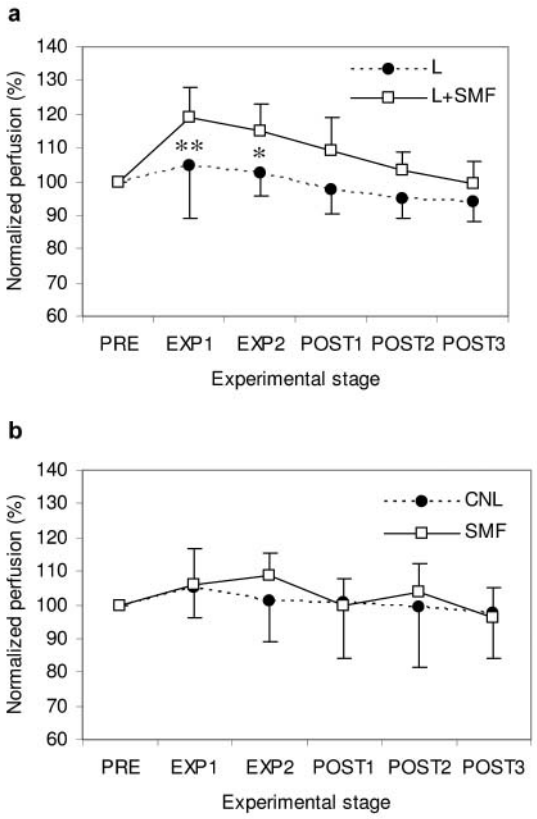

Figure 4. *Comparison of the average values of skin blood perfusion for the resting skin at the trochanter area. Significant differences are marked with *p<0.05, **p<0.01 between Group L and Group L+SMF (a), and Group SMF and Group CNL (b). Each value was calculated as a percentage of each PRE value in the same group.*

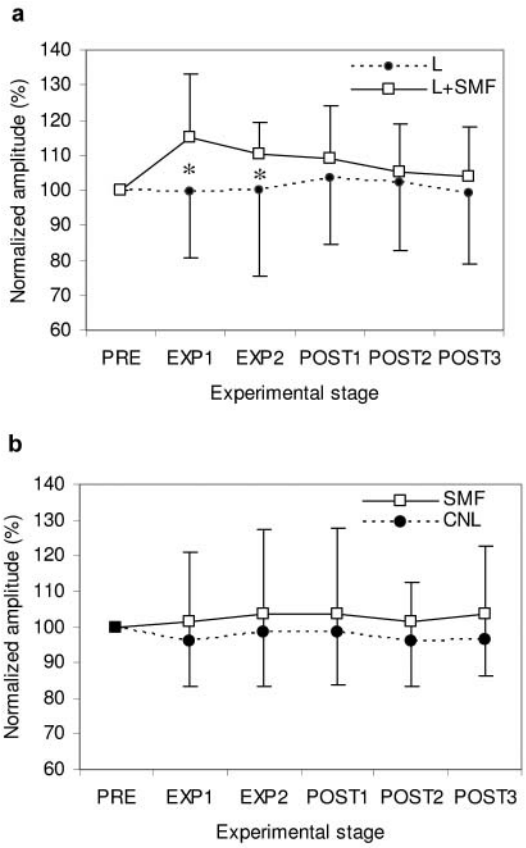

Figure 5. *Changes of skin blood flowmotion of the normalized amplitude in the frequency interval of 0.01-0.05 Hz for the resting skin at the trochanter area. Significant differences are marked with *p<0.05 between Group L and Group L+SMF (a) and Group SMF and Group CNL (b). Each value was calculated as a percentage of each PRE value in the same group.*

($p<0.05$) (Figure 5a).The results suggested that SMF significantly enhanced endothelial related metabolic activity in the stressed skin ($p=0.03$). However, SMF did not induce significant changes in the flowmotion amplitude in the unstressed skin ($p=0.22$) (Figure 5b).

Figure 6 shows typical examples of time-averaged wavelet transform calculated from signals measured in the unstressed skin, stressed skin and stressed skin with SMF exposure in the trochanter area.

Discussion

In this study, wavelet transform analysis of skin blood perfusion was employed to demonstrate the effects of SMF on skin flowmotion. A locally applied SMF significantly induced an increase of endothelial related metabolic activity (0.01-0.05 Hz) in the stressed skin. However, SMF did not induce significant changes in this activity in the unstressed skin.

In the present study, the applied surface magnet flux density was 30 mT. The locally applied SMF intensity of 1mT has been considered as a threshold level for enhancing muscle microcirculation (6, 29). For a biological tissue sample (magnetic permeability $\mu_r \approx 1$) (30), the magnetic field penetrates all tissues in exactly the same manner as in air dosimetry. In the present study, the average depth of trochanter skin was 1.5 ± 0.2 mm measured using a Sonosite ultrasound system (Ultronics Enterprise Limited Co., HK). The actual dosage of magnetic energy delivered to the tissue *i.e.* skin, subcutis and skeletal muscle, was in the range of 15-30mT as measured by the Gauss/Tesla meter.

With spectral analysis of the periodic oscillations in the cutaneous circulation based on wavelet transformation, endothelial function may be evaluated non-invasively (12-14, 19, 20). The endothelial related metabolic activity has been found to be within the frequency interval of 0.01-0.02 Hz for human and 0.01-0.06 Hz for rat. An enhanced resting

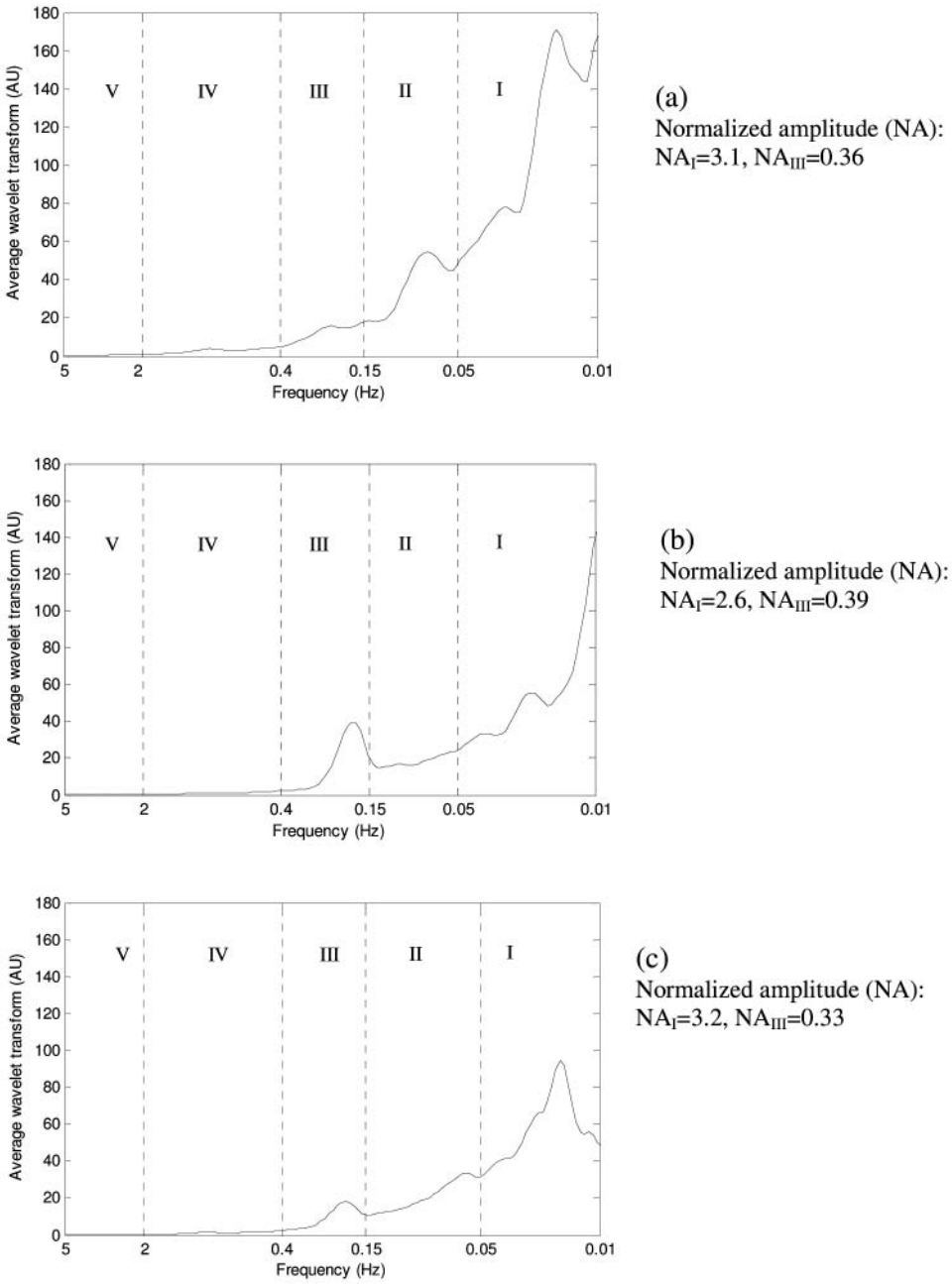

Figure 6. *Typical examples of time-averaged wavelet transform calculated from signals measured in the unstressed skin (a), stressed skin (b) and stressed skin with SMF exposure (c) in the trochanter area. The vertical lines indicate the outer limits of each frequency interval from I to V, which coincided with the frequency range of endothelial related metabolic, neurogenic, myogenic, respiratory and cardiac activities respectively.*

endothelial activity in cutaneous blood flow oscillations of athletes has been found by spectral analysis (15). Humeau *et al.* (16) have found that the relative contribution of the endothelial related metabolic activity significantly increased in the regulation of the blood flow after application of pressure in anesthetized rats. In the present study, prolonged surface loading caused significant reduction of the endothelial related metabolic activity and increased the myogenic activity, namely, induced a higher vascular tone in tissues that had been stressed as compared to the unstressed ones. In contrast, SMF significantly increased the endothelial dependent vasodilation and subsequently increased blood flow in the stressed skin. The current study has further confirmed the biphasic effect of SMF on vasomotion in stressed skin.

An increased workload of the vascular smooth muscle would increase the oxygen consumption rates of arteriolar walls (31). Significantly increased endothelial dependent vasodilation after locally applied SMF indicated reduced vascular tone and workload of the vascular smooth muscle. The reduction of vascular tone of the arteriolar walls might facilitate an efficient supply of oxygen to the surrounding tissue (31).

The result of this study demonstrated that SMF significantly enhanced endothelial related metabolic activity in the stressed skin but not in the unstressed skin. Locally applied SMF could modulate vascular tone, primarily due to the modification of hemodynamics biphasically in the cutaneous tissue (6, 9, 32). However, under normal vascular tone conditions, locally applied SMF may not induce significant changes of the vasomotion in the normal cutaneous tissue (29). Our findings suggest, therefore, the modulating effect of SMF on skin blood flowmotion might be related to the modified vascular tone. This may in part explain why no significant effect of SMF on resting blood flow has been found in some studies (25, 33, 34).

The regulation mechanism of flowmotion/vasomotion during SMF exposure has not been completely elucidated. Endothelial cells are the source of several vasoactive substances which control the contraction and relaxation of smooth-muscle by releasing vasodilators, such as nitric oxide (NO), as well as vasoconstrictors including endothelin (ET) and platelet-activating factor (PAF) (35-37). The modulating effect of SMF on vascular tone might be related to the regulation of vasomotion by endothelium-derived NO during SMF exposure. When the vascular tone was lower compared to the normal conditions, SMF might have promote the release of vasoconstrictors, as well as inhibited the production of NO, and thus induced vasoconstriction, and, in contrast when the vascular tone was higher such as modified vascular tone by prolonged loading in this study, SMF might have promoted the production of NO and facilitated vasodilation. Further studies are necessary to clarify the role of NO in flowmotion/vasomotion regulation under SMF intervention.

In conclusion, SMF significantly enhanced endothelial related metabolic activity only in the stressed skin. This suggests, therefore, that the modulating effect of SMF on skin blood flowmotion might be related to the vascular tone modified by prolonged loading. The results suggest that SMF might have potential clinical significance for pressure ulcer prevention and therapy. In this study systemic effects might not be evident since the effect of SMF on skin flowmotion was determined in relation to the pre- and post-loading periods in the rat.

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