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Local cooling reduces skin ischemia under surface pressure in rats: an assessment by wavelet analysis of laser Doppler blood flow oscillations

Yih-Kuen Jan, PT, PhD^{1,2}, Bernard Lee, BS², Fuyuan Liao, PhD¹, and Robert D. Foreman, PhD²

¹Rehabilitation Research Laboratory, Department of Kinesiology and Community Health, University of Illinois at Urbana-Champaign, Champaign, IL, USA

²Department of Physiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Abstract

The objectives of this study were to investigate the effects of local cooling on skin blood flow response to prolonged surface pressure and to identify associated physiological controls mediating these responses using wavelet analysis of blood flow oscillations in rats. Twelve Sprague Dawley rats were randomly assigned into three protocols, including pressure with local cooling ($\Delta t = -10^{\circ}\text{C}$), pressure with local heating ($\Delta t = 10^{\circ}\text{C}$), and pressure without temperature changes. Pressure of 700 mmHg was applied to the right trochanter area of rats for 3 hours. Skin blood flow was measured using laser Doppler flowmetry. The 3-hour loading period was divided into non-overlapping 30 min epochs for analysis of the changes of skin blood flow oscillations using wavelet spectral analysis. The wavelet amplitudes and powers of three frequencies (metabolic, neurogenic and myogenic) of skin blood flow oscillations were calculated. The results showed that after an initial loading period of 30 min, skin blood flow continually decreased in the conditions of pressure with heating and of pressure without temperature changes, but maintained stable in the condition of pressure with cooling. Wavelet analysis revealed that stable skin blood flow under pressure with cooling was attributed to changes in the metabolic and myogenic frequencies. This study demonstrates that local cooling may be useful for reducing ischemia of weight-bearing soft tissues that prevents pressure ulcers.

Keywords

cooling; heating; laser Doppler; pressure ulcers; wavelet

Introduction

Pressure ulcers are a common and serious problem for people with limited mobility (Byrne and Salzberg 1996, Chen et al. 2005). A pressure ulcer is described as a localized injury to the skin and/or underlying tissues that usually occurs over a bony prominence. Prolonged unrelieved pressure is widely accepted as the primary causative factor of pressure ulcers (Yarkony 1994, Allman 1997). Other factors such as temperature, moisture and shear also contribute to tissue ischemia and subsequent pressure ulcers (Dinsdale 1973, Nixon et al. 2005). The Agency for Healthcare Research and Quality lists pressure ulcers as one of the

seven most important health issues in the United States (Agency for Health Care Policy and Research 1994).

Recent studies conducted by Iaizzo, Kokate and colleagues have provided confirmative evidence on the use of local cooling to reduce tissue damage in the weight-bearing soft tissues (Iaizzo et al. 1995, Kokate et al. 1995, Iaizzo 2004). Their results showed that severity of tissue damage, under the same loading conditions at various skin temperatures, was highly correlated with the skin temperature. When the skin was cooled to 25°C, no tissue damage was observed after 5-hour loading; however, full-thickness tissue damage occurred when the skin was heated to 45°C. Their studies suggest that lowering skin temperature may be beneficial to preserve tissue viability in both the skin and muscles. However, the use of histological analysis in these animal studies limits the method for use in human beings. Based on the available research findings, Lachenbruch performed a theoretical discussion about the influences of skin temperature on pressure ulcer development and concluded that lowering skin temperature from 36°C to 28°C is equivalent to reducing the interface pressure from 56 mmHg to 40 mmHg (Lachenbruch 2005). Tzen et al. observed a reduced reactive hyperemic response to an ischemic stimulus (60 mmHg for 30 min) when local cooling (25°C) was simultaneously applied with pressure in healthy young subjects (Tzen et al. 2010). However, whether the differences of skin temperatures during the reactive hyperemic response affecting the skin blood flow response requires further investigation. Nevertheless, this finding implies a protective effect of skin cooling on the weight-bearing tissues.

Presently, sensitive methods are lacking to monitor the status of the skin and/or muscles under pressure and the progression of pressure ulcer development (Jan and Brienza 2006). Kokate et al. have suggested that analyses of skin blood flow are more effective than local skin temperature measurements or visual observations of wounds (Kokate et al. 1995). Because skin blood flow can be monitored noninvasively by using laser Doppler flowmetry (LDF), its responses to surface pressure and/or thermal stresses (cooling and heating) have a potential to assess the efficacy of cooling on reducing the susceptibility to pressure ulcers (Meijer et al. 1994, Kwan et al. 2007). Meijer et al. found that susceptible patients have substantially prolonged recovery times of blood flow after pressure relief (Meijer et al. 1994). Kwan et al. investigated the time effect of pressure on tissue viability using a rat model and suggested that postocclusive reactive hyperemia and distress of tissues under loading are related (Kwan et al. 2007).

Spectral analysis has recently been introduced to investigate the regulatory mechanisms of skin blood flow in response to pressure or thermal stress (Li et al. 2006, Jan et al. 2008). bBlood flow oscillations measured with spectral analysis in human beings has revealed five characteristic frequencies (Stefanovska et al. 1999). The oscillations around 1.0 Hz and 0.3 Hz reflect the influence of heart beats and respirations, respectively and around 0.1 Hz, 0.04 Hz, and 0.01 Hz are associated with myogenic activity of vascular smooth muscles, neurogenic activity of the vessel wall, and vascular endothelium related metabolic activity, respectively. In anaesthetized rats, five characteristic frequencies have also been identified but in different frequency ranges between 0.01 Hz and 5 Hz (Bajrovic et al. 2000, Humeau et al. 2004). Based on an analogy to human physiology, the frequency ranges are divided into five intervals, including 0.01–0.076 Hz for metabolic control, 0.076–0.2 Hz for neurogenic control, 0.2–0.74 Hz for myogenic control, 0.74–2 Hz for respiratory activities and 2–5 Hz for cardiac activities. The response of these characteristic frequencies embedded in skin blood flow oscillations can be used to study the physiological regulation to ischemic stresses under local cooling.

The objectives of the present study were to investigate the effects of local cooling on skin blood flow response to prolonged pressure and to identify associated physiological controls mediating these responses using wavelet analysis of blood flow oscillations. This requires an animal model because prolonged pressure may cause soft tissue damage to human subjects. We studied skin blood flow oscillations in rats in three experimental conditions: pressure with cooling, pressure with heating, and pressure without temperature changes. We hypothesized that in the condition of pressure with cooling, the skin would exhibit higher blood flow during prolonged pressure as compared to the conditions of pressure with heating and of pressure without temperature changes.

Materials and Methods

Animals

Twelve 8–12 week old male Sprague Dawley rats weighing between 300 g and 400 g were used. The rats were randomly assigned into three protocols, including pressure with cooling ($\Delta t = -10^{\circ}\text{C}$, skin temperature around 27°C), pressure with heating ($\Delta t = +10^{\circ}\text{C}$, skin temperature around 47°C), and pressure without temperature changes (skin temperature around 37°C). Infection of the rats was tested by measuring body temperature with a rectal thermometer, and all rats had temperature at $37.5\text{--}37.8^{\circ}\text{C}$. The animal protocol for this study was approved by the Institutional Animal Care and Use Committee.

Data collection

Each rat was anesthetized with xylazine (33 mg/ml) and ketamine (67 mg/ml) injected intraperitoneally. The degree of anesthesia was determined by a moustache dithering test. Once the rat was unconscious, the hairs on the right trochanter area were carefully shaved without damage to the skin (Salcido et al. 1995). Then the rat was positioned prone with its hind legs splayed out and taped (Figure 1). A temperature control probe (Probe 415–242, Perimed AB, Sweden) was placed over the shaved skin of the right trochanter area. Skin blood flow and temperature were recorded at a sampling rate of 32 Hz using laser Doppler flowmetry with temperature control capacity (PF 5001, Perimed AB, Sweden). A custom designed indenter was used to apply a pressure of 700 mmHg to the skin surface on the right trochanter area. The protocol included a 20 min baseline, a 180 min pressure loading period, and a 20 min recovery period. During the loading period, the temperature was set to either reduce 10°C ($\Delta t = -10^{\circ}\text{C}$), increase 10°C ($\Delta t = +10^{\circ}\text{C}$), or without changes. Room temperature was maintained at $24 \pm 2^{\circ}\text{C}$. Figure 2 shows examples of skin blood flow in response to pressure without temperature changes, pressure with heating, and pressure with cooling.

The rationales for determining the parameters of the protocols are based on the previous studies (Iaizzo et al. 1995, Kokate et al. 1995, Linder-Ganz and Gefen 2004, Kwan et al. 2007). Kwan et al. applied pressures of 100 mmHg to the trochanter and tibialis areas of rats for 6 hours each day for 4 consecutive days (Kwan et al. 2007). Baseline blood flow and reactive hyperemia were recorded for 10 min and 20 min, respectively. After 2 days of loading, cutaneous tissue damage was observed at the trochanter area but not at the tibialis area. Linder-Ganz and Gefen applied pressure of 86 mmHg, 262 mmHg, and 525 mmHg to gracilis muscles of rats for 2 hours, 4 hours, and 6 hours for a total of 9 conditions (Linder-Ganz and Gefen 2004). They found that muscles exposed to 262 mmHg and 525 mmHg became stiffer and the stiffening was accompanied by extensive necrotic damage. In order to induce skin damage, we selected pressure of 700 mmHg for 3 hours that would induce gradual tissue damage based on the larger pressure-duration value of the reported studies ($700\text{ mmHg} \times 3\text{ hours} = 2,100\text{ mmHg-hour}$). As for the skin temperature, in the studies by Kokate et al. (Kokate et al. 1995) and Iaizzo et al. (Iaizzo et al. 1995), a series of skin

temperatures of 25°C, 35°C, 40°C, and 45°C were used, which had distinguishable effects on the severity of tissue damage. Since the body temperature of rats is around 37°C, we selected three conditions, including $\Delta t = -10^\circ\text{C}$ (cooling), $\Delta t = 0$ (no temperature change), and $\Delta t = +10^\circ\text{C}$ (heating). These temperatures (27°C, 37°C, and 47°C) allowed us to study the influences of temperature changes on skin blood flow response to prolonged surface pressure in rats.

Normalized skin perfusion

To study the evolutionary changes in skin blood flow, the 3-hour loading period was divided into non-overlapping 30 min epochs (each epoch had the same length of the baseline). For each epoch, skin perfusion was normalized by the mean value of skin blood flow during the first 30 min epoch of the loading period. The reason for the choice of 30 min epochs is as follows. Herrman et al. suggested that the record length of skin blood flow data should include at least two or more cycles of the lowest frequency for analysis (Herrman et al. 1999). In their study, spectral analysis was initially performed on 30 min data records. Then the record length was divided into shorter time periods, e.g., 20 min, 15 min, 10 min, 6 min, 3 min, and 2 min, to determine the frequency content of each time period. They found that 10 min was the shortest record length that provided values consistent with those obtained from the total 30 min period. In this study, our selections met the recommendation.

Wavelet analysis and quantification methods

Continuous wavelet transform of a signal $x(u)$ is defined as

$$w(s, t) = \int_{-\infty}^{\infty} \psi_{s,t}(u) x(u) du, \quad (1)$$

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

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

where t is time, s is the scale related to the frequency. In this study, the Morlet wavelet was used, defined as

$$\psi_0(u) = \pi^{-1/4} e^{j\omega_0 u} e^{-u^2/2}, \quad (3)$$

where ω_0 is the nondimensional frequency. By choosing $\omega_0 = 2\pi$, the relation between the scale and the central frequency is $f = 1/s$ (Bracic and Stefanovska 1998). The Morlet wavelet allows the best time-frequency localization according to the Heisenberg uncertainty principle (Bracic and Stefanovska 1998).

To study the physiological mechanisms of blood flow oscillations during the loading period, we performed continuous wavelet transforms on the blood flow signal during baseline and each epoch of the loading period. The frequency regions between 0.01 Hz and 5 Hz were analyzed, and the limits of each frequency band were chosen based on the ranges reported by previous studies (Bajrovic et al. 2000, Humeau et al. 2004). Briefly, the local mechanisms of skin blood flow oscillations were considered: frequency interval 0.01–0.076 Hz for the endothelial related metabolic activity, 0.076–0.2 Hz for the neurogenic activity, and 0.2–0.74 Hz for the myogenic activity (Humeau et al. 2004). Figure 3 shows the wavelet transform of the blood flow signal shown in Figure 2a (baseline). To quantify the contribution of the mechanisms to skin blood flow, we calculated two measures of the corresponded characteristic frequencies: relative amplitude and relative power (Bracic and

Stefanovska 1998, Bajrovic et al. 2000, Humeau et al. 2004). The relative amplitude in the frequency interval $[f_1, f_2]$ is defined as

$$A_{relative} = \frac{A_{f_1, f_2}}{A_{0.01, 5}}, \quad (4)$$

where A_{f_1, f_2} and $A_{0.01, 5}$ are the mean amplitudes of the wavelet transform in the frequency intervals $[f_1, f_2]$ and $[0.01, 5]$, respectively. Analogously, the relative power is defined as

$$P_{relative} = \frac{P_{f_1, f_2}}{P_{0.01, 5}}, \quad (5)$$

where P_{f_1, f_2} and $P_{0.01, 5}$ are the power of the wavelet transform in the frequency intervals $[f_1, f_2]$ and $[0.01, 5]$, respectively.

All values were expressed as means with standard errors. One way analysis of variance (ANOVA) was used to examine the difference in skin blood flow among three experimental conditions. Descriptive statistics were used to describe the evolutionary changes of amplitudes and powers in three frequencies (ie. metabolic, neurogenic, and myogenic). All statistical tests were analyzed using SPSS 16 (SPSS, Chicago, IL), and was performed at an alpha level of 0.05.

Results

Normalized skin perfusion

Figure 4 shows normalized skin blood flow in three experimental conditions during the loading period. For all the conditions, blood flow in the second 30 min epoch was much lower than that in the first 30 min epoch, especially using pressure with heating. During the loading period, blood flow continually decreased under both pressure with heating and pressure without temperature changes, whereas blood flow was rather stable under pressure with cooling. ANOVA indicated a statistically significance in three conditions, and the post hoc analysis (multiple paired t tests with Bonferroni correction) showed skin blood flow using pressure with cooling was higher than that of the condition of pressure with heating after 30 min loading pressure ($p < 0.05$).

Relative amplitude and relative power of wavelet transform

Figure 5 shows the relative amplitudes of the metabolic, neurogenic, and myogenic components during the baseline and loading periods. In the condition of pressure without temperature changes, metabolic amplitudes decreased sharply in the first 30 min loading epoch, then increased and reached a higher level as compared to the baseline. On the contrary, myogenic amplitudes initially increased and then decreased. In the condition of pressure with heating, changes in metabolic amplitudes were similar to that of pressure without temperature changes. However, myogenic amplitudes rapidly decreased during the initial 30 min epochs and then maintained at a lower level as compared to that in the other two conditions. In the condition of pressure with cooling, both the metabolic and myogenic amplitudes underwent only small changes throughout the whole loading period.

Figure 6 shows the relative powers of the metabolic, neurogenic, and myogenic components of three conditions during the baseline and loading period. The trends of the changes of relative powers of the three characteristic components were similar to that of the relative amplitudes shown in Figure 5.

Figure 7 shows the normalized amplitudes of three conditions during the loading period. Neurogenic components of both the conditions of pressure with heating and of pressure with cooling show a similar contribution to skin blood flow during the loading period. Myogenic components using pressure with cooling show a higher value as compared to those of both conditions of pressure with heating and of pressure without temperature changes. Metabolic components of the condition of pressure with heating show a higher value as compared to those of both conditions of pressure without temperature changes and of pressure with cooling.

Discussion

We have demonstrated that local cooling reduces ischemia and preserves metabolic and myogenic activities of the skin during the loading period. To the best of our knowledge, this is the first study to investigate time-evolutionary changes in skin blood flow oscillations during prolonged pressure with cooling and heating stresses in rats. Our results suggested that local heating aggravates ischemia of weight-bearing soft tissues, whereas local cooling provides a protective effect on reducing ischemia. Our results illustrated that evolutionary change in skin blood flow oscillations may be a promising indicator for assessing risk for pressure ulcers. The detailed findings are summarized here. (1) In the three experimental conditions, i.e., pressure with cooling, pressure with heating, and pressure without temperature changes, skin blood flow rapidly decreased during the first 30 min episode. Then blood flow continually decreased in both the conditions of pressure with heating and of pressure without temperature changes, whereas it remained stable in the condition of pressure with cooling. (2) In the conditions of pressure with heating and of pressure without temperature changes, the contribution of the endothelial related metabolic activity to blood flow oscillations initially decreased during the first 30 min period, then increased and maintained at a higher level as compared with the baseline; on the contrary, the contribution of the myogenic activity to blood flow oscillations initially increased and then decreased. (3) In the condition of pressure with cooling, the contribution of both metabolic and myogenic activities to blood flow oscillations only underwent small changes.

A common feature of skin blood flow in the three experimental conditions was that blood flow during the second 30 min epoch of the loading period was much lower than in the first 30 min epoch (Figure 4), indicating that local skin blood flow was not completely occluded. This was somewhat different from the reports by Herrman et al. (Herrman et al. 1999) and Patel et al. (Patel et al. 1999). Herrman et al. reported that skin blood flow in rats could reach the minimum at a surface pressure of about 58 mmHg (Herrman et al. 1999). Patel et al. reported a surface pressure over 55 mmHg that totally occluded skin blood flow in rats (Patel et al. 1999). However, Kwan et al. reported that a pressure of 100 mmHg did not cause a complete occlusion of skin blood flow (Kwan et al. 2007). The differences among the reported surface pressures that caused skin blood flow occlusion were likely due to the differences of the internal mechanical stress surrounding blood vessels. The issue was discussed by Bader and Oomens (Le et al. 1984, Bader and Oomens 2006).

A significant phenomenon was that skin blood flow remained stable after the first 30 min epoch for the condition of pressure with cooling but not for the conditions of pressure with heating and of pressure without temperature changes (Figure 4). This means after the first 30 min period pressure slightly decreased blood flow due to skin cooling. Because tissue ischemia is not likely to occur during the first 30 min period, skin cooling could preserve tissue viability and reduce ischemia of weight-bearing tissues. In the condition of pressure with heating, skin blood flow showed the largest decrease during the second 30 min epoch. One possible explanation was that surface pressure resulted in progressively increased tissue deformation due to its viscoelastic characteristics (Kwan et al. 2007). During the first 30 min

episode, tissue deformation might not be enough to completely suppress heating-induced vasodilation. Thus, local heating might lead to an increase in skin blood flow. During the second 30 min epoch, a larger deformation of the tissue might completely suppress heating-induced vasodilation. Hence, skin blood flow showed the greatest decrease as compared to that in the condition of pressure or pressure with cooling. Another explanation for this observation may be that local heating caused the tissue to be stiffer (Patel et al. 1999) and thus compromised its ability to dissipate the stress resulting from the surface pressure. Therefore, local heating could lead to larger internal stresses, which resulted in a larger decrease in skin blood flow.

The results of wavelet analyses showed that relative amplitudes of the metabolic component slightly increased with time during the pressure with the cooling condition and were similar to that of the baseline (Figure 5). If we consider the relative amplitude of metabolic component as a representation of metabolic demands of local cells, our results implied that during pressure with cooling the metabolic demands of local cells and blood supply were almost matched for a rather long period. Our results supported the theory that cooling of weight-bearing soft tissues decreases the metabolic rate of the tissues and relatively increases tissue viability over time. Li et al. reported that prolonged compression leads to a decrease in the power of skin blood flow oscillations in the frequency interval 0.01–0.04 Hz, which corresponds to the endothelial related metabolic activities (Li et al. 2006). In their study, the term ‘power’ actually referred to the ratio of wavelet amplitude of metabolic component to the mean amplitude in the frequency interval 0.01–5 Hz (Li et al. 2006). Indeed, as shown in Figure 7, our data showed that in the conditions of pressure with heating and of pressure without temperature changes, wavelet amplitudes of the metabolic frequency showed a decreasing trend over time, but the relative amplitudes of metabolic frequency that initially decreased and then increased. One possible reason for this contradiction between the two studies may be due to the fact that in the study by Li et al., wavelet amplitudes were computed from 6 hour data series and comparisons were performed between the successive days, whereas in the present study wavelet amplitudes were calculated over 30 min periods.

The relative wavelet amplitudes of the myogenic component can be considered as a measure of the contribution of myogenic activity to skin blood flow. The myogenic activities are the rhythmic constriction and dilation of the smooth muscles of the vessel wall and are mechanical stress dependent (Popel and Johnson 2005). Although blood flow regulation is expected to be correlated to the metabolic needs of the local tissues, the myogenic mechanism is a key determinant of blood flow regulation in many organs (Popel and Johnson 2005). It was reported that power of skin blood flow oscillations in the myogenic frequency band increased for incrementally increased pressure within the range of 25 mmHg, but decreased for incrementally increased pressure above the threshold of 25 mmHg (Brienza et al. 2005). Our results confirmed that prolonged compression would lead to a decrease in the power of the myogenic component (Figure 7c). Moreover, the relative amplitude of myogenic component was lower in the condition of pressure with heating as compared to the condition of pressure alone. This is probably due to a inflammatory reaction induced by prolonged heating and pressure in which local chemical mediators act to decrease smooth muscle tone (Geyer et al. 2004). In the condition of pressure with cooling, either the absolute amplitude or relative amplitude of the myogenic component maintained stable, suggesting local cooling has a protective effect on myogenic oscillations. Sheppard et al. indicated that myogenic oscillations may be the key role on the regulation of skin blood flow in response to local cooling and heating (Sheppard et al. 2011). They demonstrated that under vasoconstriction induced by local cooling, myogenic oscillations became more synchronized; while under vasodilation induced by local heating, myogenic oscillations became small for relaxing vascular smooth muscles and increasing average vessel radius. Based on these evidences, myogenic oscillations, a local blood flow regulatory mechanism,

may be a promising control for determining the efficacy of local cooling regimens on enhancing viability of weight-bearing tissues.

Because prolonged pressure may cause irreversible ischemic injury, an animal model is necessary for the study of pressure ulcer development. We chose a rat model because it has been shown that the microcirculation and effects of thermal stress on blood perfusion in rats and human beings have key similarities (Rendell et al. 1998). For example, the hairless plantar paw surface of rats shows higher blood flow and substantial response to thermal stimulation, whereas hair-covered areas show much lower blood flow and thermal response. These properties are similar to the differences in human beings between skin sites with a high density of arterioles and venules and sites with predominantly nutritive capillary perfusion. Although pig skin is more physiologically similar to the human skin (Hollander et al. 2003), the high cost and our inexperience with porcine husbandry make it prohibitive to use a porcine model. Previous studies in rat models produced valuable results (Herrman et al. 1999, Li et al. 2006, Kwan et al. 2007). Herrman et al. applied a pressure of 92 mmHg to the greater trochanters of rats for 5 hours to study pressure ulcers (Herrman et al. 1999). Kwan et al. applied a pressure of 100 mmHg to the greater trochanter area of rats for 6 hours each day for 1 to 4 days (Kwan et al. 2007). This model was also adopted by Li et al. to study the changes of blood flow oscillations after surface pressure (Li et al. 2006). In the present study, we applied a pressure of 700 mmHg to the trochanter area of rats for 3 hours, which was much higher than in the previous studies. We assumed that higher pressure with thermal stress would result in more obvious changes in skin blood flow oscillations in a shorter period of time. This would provide a more significant difference in skin blood flow among three protocols.

Limitations

This study had several limitations. First, we only performed the tests on healthy rats. This rat model did not mimic pathological conditions such as spinal cord injury, in which skin blood flow shows an attenuated response to loading pressure (Li et al. 2009) or thermal stress (Nicotra et al. 2004). Therefore, our findings cannot be naturally extended to the condition of spinal cord injury. However, the purpose of this preliminary study was to examine the protective effect of local cooling on tissue damage due to prolonged pressure. We showed that local cooling has an effect of preserving skin blood flow and metabolic and myogenic oscillations in healthy rats. Further studies using a rat model of spinal cord injury may be needed to verify our results. The other limitation is a small sample size. The purpose of this study was to explore whether local cooling can reduce tissue ischemia during the loading period. Our results supported this hypothesis and suggest that future studies should be conducted to investigate the physiological mechanisms associated with local cooling on the weight-bearing tissues. In this study, we demonstrated that under such a loading pressure skin blood flow oscillations showed evolutionary changes with time that feasibly reflect the dynamics of skin blood flow. Moreover, changes in blood flow oscillations exhibited consistent trends within each groups, i.e., pressure with cooling, pressure with heating, and pressure without temperature changes. Nevertheless, more studies are needed to confirm our findings.

Conclusions

This study supports the concept of local skin cooling to enhance skin blood flow of the weight-bearing tissues. Wavelet analysis of skin blood flow oscillations indicated that metabolic and myogenic controls may attribute to this protective mechanism.

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Figure 1.
The experimental setting for applying pressure with temperature control to the skin over the trochanter area of the rat.

Figure 2.

Examples of skin blood flow in response to (a) loading pressure, (b) loading pressure with local heating, and (c) loading pressure with local cooling. Data from figures (a), (b), and (c) were obtained from 3 different rats.

Figure 3.
An example of (a) wavelet transforms of blood flow oscillations and (b) time-averaged wavelet amplitudes of blood flow oscillations.

Figure 4.

Normalized skin blood flow during the loading period (normalized by the mean blood flow of the first 30 min epoch of the loading period). Values are represented as means $\pm$ standard errors. From the left to the right, each value corresponds to the mean value of a 30 min epoch.

Figure 5. Relative wavelet amplitudes of (a) metabolic, (b) neurogenic and (c) myogenic components during the baseline and loading periods. Values are represented as means $\pm$ standard errors. From the left to the right, each value corresponds to the mean of a 30 min epoch.

Figure 6. Relative wavelet power of (a) metabolic, (b) neurogenic and (c) myogenic components during the baseline and loading periods. Values are represented as means $\pm$ standard errors. From the left to the right, each value corresponds to the mean value of a 30 min epoch.

Figure 7. Normalized wavelet amplitude during the loading period (normalized by the mean value of wavelet amplitude of the first 30 min epoch of the loading period). Values are represented as means $\pm$ standard errors. From the left to the right, each value corresponds to the mean value of a 30 min epoch.