Skin microvascular vasomotion is synchronous at acupoints of beagle dogs

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Research

Keywords: vasomotion, synchronous, acupoints, meridians, low resistance, frequency, dog

Posted Date: April 21st, 2020

DOI: https://doi.org/10.21203/rs.3.rs-22969/v1

Abstract

Background/purpose: Skin microvessels at acupoints have been documented to be more abundant and well-organized, and the synchronous microvascular vasomotion was detected at acupoints in our previous human study. This present study aimed to characterize the skin microvascular vasomotion at acupoints on the twelve meridians of beagle dogs.

Materials and Methods: Two acupoints were selected on each meridian, and exactly located at the rosy red spots by an electrochemical color-appearing method, where the electrical resistance was measured. The skin blood flow at acupoints was recorded by laser Doppler flowmetry (LDF), and microvascular vasomotion was analyzed according to LDF waveforms.

Results: The skin electrical resistance at acupoints was significantly lower than that at control non-acupoints. The LDF waveforms at acupoints was sinusoidal, which showed the synchronization of the microvascular vasomotion. The spectral analysis revealed that the vasomotion frequencies at acupoints on the same meridian were identical but not among different meridians, and the frequencies on the twelve main meridians displayed a constant order.

Conclusion: The skin microvascular vasomotion is synchronous at acupoints of beagle dogs and has a specific frequency along the meridian, and the electrochemical color-appearing method is a feasible strategy for the precise and visual location of acupoints. The study provides evidence for the universality of synchronous vasomotion of skin microvessels at acupoints and contributes to clarifying the essence of acupoints and their effect mechanism.

Background

Skin microvessels have been documented to be more abundant and more well-organized at acupoints and along meridians [1–3]. The unusual morphology suggests their close relation with acupoints and meridians and that they may have some particular functional characteristics. Higher microcirculation blood perfusion has been well characterized in some studies [4–6]. Vasomotion is the spontaneous rhythmic oscillation in microvascular diameter, independent of heartbeat, respiration or nerve input [7], which at least regulates local blood flow and material exchanges between the vascular system and surrounding tissues [9, 10]. However, as a functional status sign of microcirculation, microvascular vasomotion received little attention.

Microvascular vasomotion is commonly measured using laser Doppler flowmetry (LDF) [11, 12]. Generally, its LDF waveforms are seemingly irregular. Interestingly, however, the sinusoidal waveforms of skin microvascular vasomotion were recorded at human acupoints in our previous studies [13]. We speculated that the special vasomotion may be a physiological property of skin microvessels at acupoints and it should be common in acupoints of various animals.

To verify our speculation, beagle dogs were used in this study to measure the microvascular vasomotion at acupoints, which were precisely located by a color-appearing method based on lower electrical resistance in skin at acupoints. We looked forward to providing evidence for the physiological property and the specificity of skin microcirculatory system at acupoints on different meridians.

Materials And Methods

Animals

Five clinically healthy beagle dogs (2 males and 3 females) aged 6–12 months were used in the experiment and were singly housed in stainless steel cages in an air-conditioned room. They were allowed a standard pellet diet twice daily and had ad libitum water. All experiments were in accordance with the guideline of animal ethics and were approved by the animal ethics and welfare committee of Beijing University of Agriculture.

Acupoint Selection And Location

Considering their common application in clinical practice and the convenient fixation of laser Doppler probes, two acupoints were selected from each one of the twelve main meridians (Table 1). They were firstly located according to traditional criteria [14], and then were precisely determined by an electrochemical color-appearing method as the following. The nearby control points were chosen about 1 cm away from acupoints and approximately 45 degrees off their meridian. The blood flow of skin microvessels at acupoints and control points were measured using LDF, and microvascular vasomotion was analyzed on the basis of LDF waveforms.

Table 1
Selected acupoints on twelve main meridians of beagle dogs.

Meridians	Acupoints
Heart Meridian	Shaohai (HT-3), Tongli (HT-5)
Lung Meridian	Kongzui (LU-6), Chize (LU-5)
Pericardium Meridian	Neiguan (PC-6), Quze (PC-3)
Triple Energizer Meridian	Waiguan (TE-5), Naohui (TE-13)
Small Intestine Meridian	Bingfeng (SI-12), Zhizheng (SI-7)
Large Intestine Meridian	Qiansanli (LI-10), Quchi (LI-11)
Kidney Meridian	Yingu (KID-10), Zhongzhu (KID-15)
Spleen Meridian	Xuehai (SP-10), Yinlingquan (SP-9)
Liver Meridian	Ququan (LIV-8), Ligou (LIV-5)
Gallbladder Meridian	Yanglingquan (GB-34), Qiuxu (GB-40)
Bladder Meridian	Shenshu (BL-23), Pangguangshu (BL-28)
Stomach Meridian	Tianshu (ST-25), Housanli (ST-36)

Electrochemical Color-appearing

According to a previously described method [15], the color-appearing reagent was prepared from 9 gram of methylcellulose (M0294, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), 0.8-gram phenolphthalein (77-09-8, Gracia Chemical Technology Co., Ltd., Tokyo, Japan), 1 ml of ethanol solution with 15% Methyl 4-hydroxybenzoate (M2206, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), 1 ml of ethanol solution with 2.5% ethyl 4-hydroxybenzoate (E0884, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) and 2 ml of 10% benzalkonium bromide (B5776, Sigma, USA) in 100 ml boiled distilled water.

Beagle dogs were shaved around acupoints, and the color-appearing process was conducted as follows. The negative copper sheet (about 20 mm diameter and 0.5 mm thickness) of the power supply (BY3005S, Beijing Boyu Xunming Technology Co., Ltd., Beijing, China) was placed at the acupoint area, and its positive stainless-steel clip (about 40 × 20 × 0.2 mm in dimensions) cushioned with saline-wet gauze was clamped to the dog ear, which was connected in series with a multimeter (UT39, Shanghai Youlide Electronics Co., Ltd., Shanghai, China) set to the 2-mA range. The power supply was turned on, and the current was adjusted to 0.3 mA. The power was cut off 1 minute later, and the negative copper sheet and positive clip were removed off. The color-appearing reagent was applied to the copper sheet-treated area, and one or more rosy-red spots about 1–2 mm in diameter appeared soon (Fig. 1), which were marked with a marker pen as the precise acupoints. To exclude the possible influence of electrochemical

treatment, control points were also handled with the above steps, and the LDF measurement was carried out at least 48 h later.

Electrical Resistance Measurement

To confirm the lower electrical resistance at color-appearing points, the resistance values of them and nearby control points were measured using a multimeter. The reference electrode cushioned with saline-wet gauze was clamped to the dog ear base, which was ipsilateral to the measured points. The multimeter was set to the 200-MΩ range, and the working electrode was placed at acupoints and control points, respectively. Each point was measured five times, and its electrical resistance value was presented as mean plus or minus the standard deviation. Analysis of variance was used to compare the difference of resistance values between color-appearing points and control points, and significant differences were identified by $P < 0.05$.

LDF Measurement

Skin microvascular blood flow at acupoints of beagle dogs was detected using LDF (PeriFlux 5000, Perimed AB, Stockholm, Sweden) with type 407 probes (Perimed AB, Stockholm, Sweden). Dogs were accustomed for about 10 minutes in the testing room with stable temperature and then restrained on a net shelf. One probe was fastened to the acupoint, and the other one was fastened to the control point or another acupoint. The LDF time constant τ was set as 0.03 s, and the LDF data were recorded for at least five minutes when dogs were quiet.

The LDF spectral analysis was performed by PeriSoft for Windows 2.50 (Perimed AB, Sweden). Frequencies of LDF waveforms at different acupoints were compared with each other. Data were presented as mean plus or minus the standard deviation and analyzed using analysis of variance. Significant differences were identified by $P < 0.05$.

Results

Lower electrical resistance at acupoints

The lower skin electrical resistance at acupoints was determined by multimeter measurement. As shown in Table 2, their electrical resistance values were generally higher at least 3 MΩ than those at control points, and analysis of variance showed that there was a significant difference between them (all $P < 0.05$ or $P < 0.01$).

Table 2
Electrical resistance values of color points and control points in beagle dogs (all $P < 0.05$ or $P < 0.01$)

Acupoints	Electrical Resistance Value (MΩ)		Acupoints	Electrical Resistance Value (MΩ)	
	Color-appearing points	Control points		Color-appearing points	Control points
Shaohai	13.1 ± 0.6	16.5 ± 0.7	Yingu	15.4 ± 0.7	17.2 ± 1.6
Tongli	11.7 ± 0.3	17.2 ± 1.1	Zhongzhu	13.8 ± 0.4	17.7 ± 1.1
Kongzui	16.4 ± 0.5	21.5 ± 1.6	Xuehai	16.2 ± 0.5	17.8 ± 0.5
Chize	13.3 ± 0.7	16.9 ± 1.3	Yinlingquan	17.7 ± 0.4	26.5 ± 0.8
Neiguan	15.6 ± 0.5	17.4 ± 1.1	Ququan	15.6 ± 0.4	21.0 ± 1.9
Quze	10.3 ± 0.4	16.2 ± 0.9	Ligou	16.7 ± 0.6	18.6 ± 0.8
Waiguan	15.3 ± 0.5	20.8 ± 1.5	Yanglingquan	16.7 ± 0.5	18.9 ± 1.2
Naohui	10.3 ± 0.6	15.1 ± 1.4	Qiuxu	17.6 ± 0.6	19.1 ± 0.5
Bingfeng	11.4 ± 0.5	15.1 ± 1.2	Shenshu	16.9 ± 0.2	18.9 ± 1.0
Zhizheng	12.6 ± 0.7	15.8 ± 1.2	Pangguangshu	17.9 ± 0.4	19.5 ± 1.1
Qiansanli	16.7 ± 0.4	19.0 ± 1.1	Tianshu	14.2 ± 0.5	18.2 ± 1.4
Quchi	11.9 ± 0.6	16.6 ± 1.2	Housanli	17.6 ± 0.4	19.2 ± 0.9

Characteristic Of Skin Microvascular Vasomotion At Acupoints

To investigate the physiological characteristic of skin microvascular vasomotion at acupoints, their blood flows were measured using LDF. As shown in Fig. 2, the LDF waveforms at acupoints were generally in sinusoidal shape, while the LDF waveform pattern at control points was seemingly random, and their baselines did not display regular fluctuation. The sinusoidal LDF waveforms show that all or the majority of skin microvessels at the acupoint synchronously dilate and constrict.

Specificity of skin microvascular vasomotion at acupoints along the meridian

To further explore the characteristics of skin microvascular vasomotion at acupoints on different meridians, their LDF signals were simultaneously recorded. For both acupoints on the same meridian, their LDF signals synchronously reached peaks and troughs, and their waveforms could overlap with

each other in general (Fig. 3). The spectral analysis showed that there were no significant differences in their frequencies (all $P > 0.05$).

However, the microvascular vasomotion frequencies at acupoints on different meridians were found to be various, and their statistical differences may be significant or not (Fig. 4). And even if their frequencies were statistically identical, their LDF waveforms displayed an obvious phase shift and could not overlap in peaks and troughs. Furthermore, every dog had different vasomotion frequencies at the same acupoint, and their vasomotion frequencies at acupoints on the twelve main meridians were in much the same order (Fig. 5).

Discussion

In the study, the skin microvascular vasomotion was found to be synchronous at acupoints of beagle dogs, which was also synchronized along the meridians and whose frequencies on different meridians were in good order with the minimum frequency on the bladder meridian and the maximum one on the liver meridian. The results are in line with previous findings based on the human study. Moreover, an electrochemical color-appearing method was applied to precisely locate acupoints, which avoided subjectivity in placing the LDF probes.

Microvascular vasomotion is generally thought to be spontaneous and be asynchronous with the adjacent one [16]. Synchronous vasomotion of skin microvessels was only recorded during some special treatments, such as nonpulsatile perfusion, or at sites comprising 17–26 μm ascending arterioles [17–19]. Microvascular vasomotion at acupoints has been reported to be the dominant myogenic oscillation and have the larger amplitude [20–22], while their conclusions came from wavelet or fast Fourier transform analysis based on research in a few acupoints. Sinusoidal LDF waveforms showing synchronous microvascular vasomotion at acupoints on the twelve main meridians of dogs were firstly recorded in this study.

As we know, the basic role of acupoints is Qi and blood infusion. The blood perfusion has been demonstrated to be higher at acupoints and on certain parts of meridians [5, 23]. Although the physiological role of microvascular vasomotion remains unclear, it has been shown to enhance blood flow and nutrient delivery [9, 24]. The synchronous vasomotion suggested that the majority of skin microvessels at acupoints worked as a whole, which would release more energy than an irregular one. More energy and higher blood perfusion should be able to meet the needs of Qi and blood infusion. Thus, the more abundant and well-organized microvessels with synchronous vasomotion maybe are the morphological basis of acupoints.

Vasomotion is the low-frequency oscillation of microvascular walls, which is principally myogenic and independent of heartbeat, respiration, and innervation. The synchronization of skin microvascular smooth muscle cells at the acupoint is believed to be related to rhythmic oscillation of calcium ions and intercellular gap junction [25, 26]. The skin microvessels at different acupoints are not adjacent, whose synchronous vasomotion should have a different mechanism, such as under central control [16]. We

think that more research is needed to investigate the microvascular properties, local microenvironment, and nerve supply. Anyhow, the synchronous vasomotion of microvascular populations at different acupoints of the same meridian suggests that there are better communication and closer cooperation among them. On the contrary, the synchronous vasomotion of skin microvessels at acupoints of different meridians possessed different frequencies, which displayed no interference with each other. Thus, we hypothesize that the frequency specificity along the meridian would bring forth their separate workings and responses to the stimulation, which may underlie the propagation of acupoint effects.

The order of microvascular vasomotion frequencies at acupoints on the twelve main meridians was constant, which was another important finding in this study, although its physiological significance in the meridian theory is yet unclear. The frequencies on the lung meridian of Dog 1 and on the liver meridian of Dog 4 were the exceptions to their order, whether it suggested the abnormal physiological status of the meridians is still open. Overall, frequencies on meridians affiliated with Zang-organs (liver, heart, spleen, lung, kidney, and pericardium) were higher than their corresponding Fu-organs (gallbladder, small intestine, stomach, large intestine, bladder, and triple energizer). However, the lowest and highest frequencies were on the bladder meridian and the liver meridian, respectively, which have mutual connections of meridians by an exterior-interior relationship. Whether the microvascular vasomotion frequencies on the twelve main meridians of the human being and other animals have the same order still needs further studies.

Although the synchronous microvascular vasomotion was detected in the previous human study, the position of acupoint located by traditional methods was not exactly suitable for the tiny LDF probe, which is so sensitive that a micro displacement would result in a significant change of LDF waveforms. The objective and precise location of acupoints is necessary to ensure good repeatability in measuring the synchronous microvascular vasomotion. Considering the wide acceptance of lower electrical resistance at acupoints [27, 28], an electrochemical color-appearing method was used as described previously with some modification in this study [15]. The results indicated rosy red spots displayed at all acupoints of beagle dogs, and there was one spot at most acupoints and two or more spots at a few acupoints. The rosy red spots lasted about dozens of minutes, where the electrical resistance was proved to be lower than the adjacent control points. The electrochemical color-appearing method was confirmed to be a feasible strategy for detecting synchronous microvascular vasomotion at acupoints, and its underlying mechanism remains to be investigated.

Conclusions

In conclusion, the synchronous microvascular vasomotion at acupoints and its specificity along meridians were confirmed in beagle dogs, and the electrochemical color-appearing method is feasible for the precise and visual location of acupoints. This present study firstly presents the synchronous characteristic of microvascular vasomotion at acupoints of dogs and shows the universality of the synchronous vasomotion. The results strengthen the correlation between acupoints and microvessels and contribute to clarifying the essence of acupoints and their effect mechanism.

Abbreviations

LDF
laser Doppler flowmetry.

Declarations

Acknowledgements

We gratefully acknowledge all graduate students who participated in feeding and management for the beagle dogs.

Author Contributions

TZ and XM conceived and designed the research framework; TZ, CL and GH performed the experiments; TZ and CL wrote the paper and analyzed the data; All authors read and approved the final manuscript.

Funding

The work was supported by the National Key Basic Research Program of China (No. 2012CB518502), National Natural Science Foundation of China (No. 31672597) and Beijing Natural Science Foundation (No. 6202003).

Availability of data and materials

All the data used to support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

The experimental protocol was in accordance with the guideline of animal ethics and was approved by the Animal Ethics and Welfare Committee of Beijing University of Agriculture.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Figures

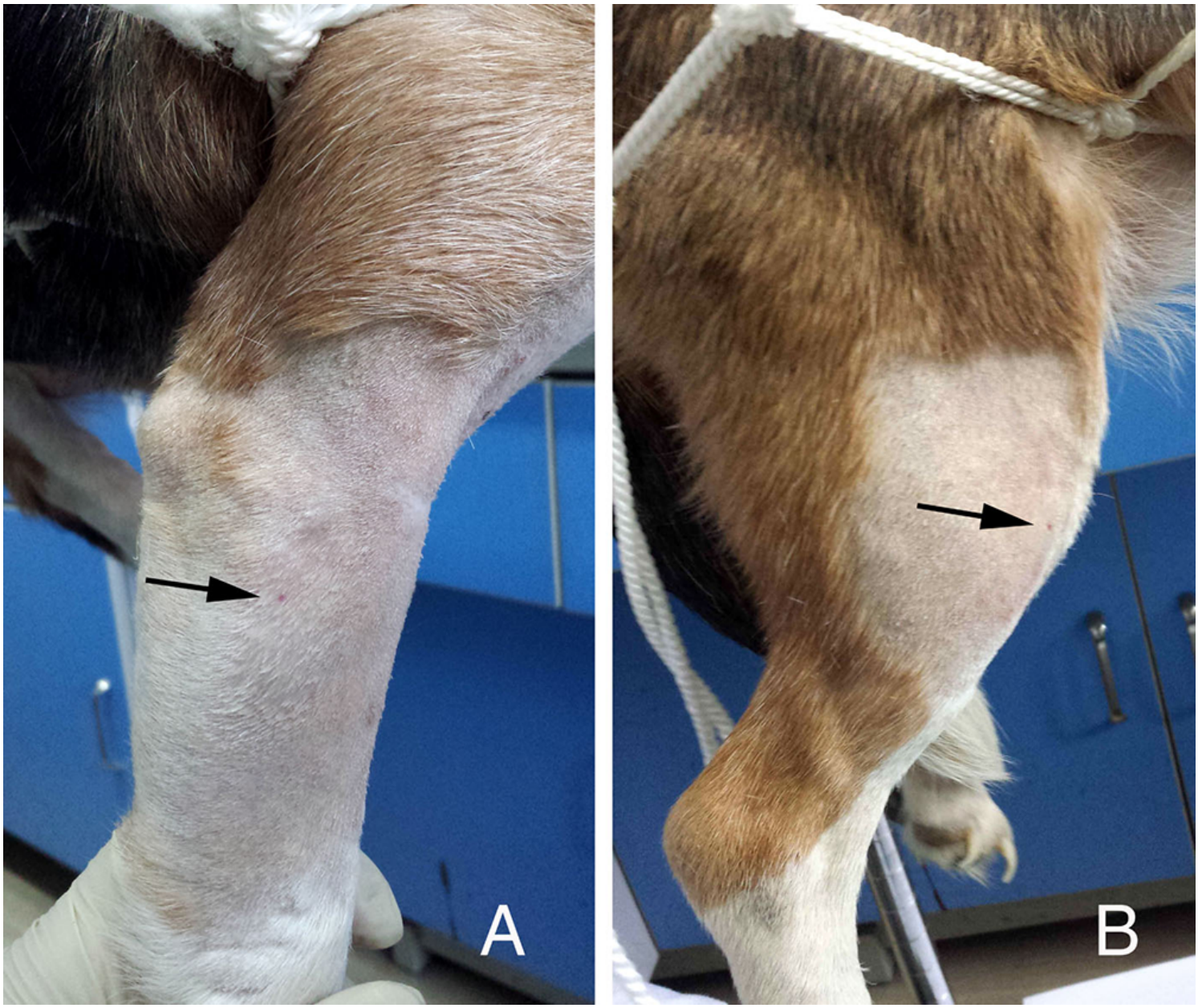

Figure 1

Electrochemical color-appearing spots at acupoints of beagle dogs. Acupoints firstly were located according to a traditional method, where the hairs were shaved. The color-appearing process was conducted as described previously with some modification (Zhang et al., 1991). The rosy red spots (arrows) about 1-2 mm in diameter appeared soon after the color-appearing reagent was applied. A: Qiansanli (LI10), B: Housanli (ST36).

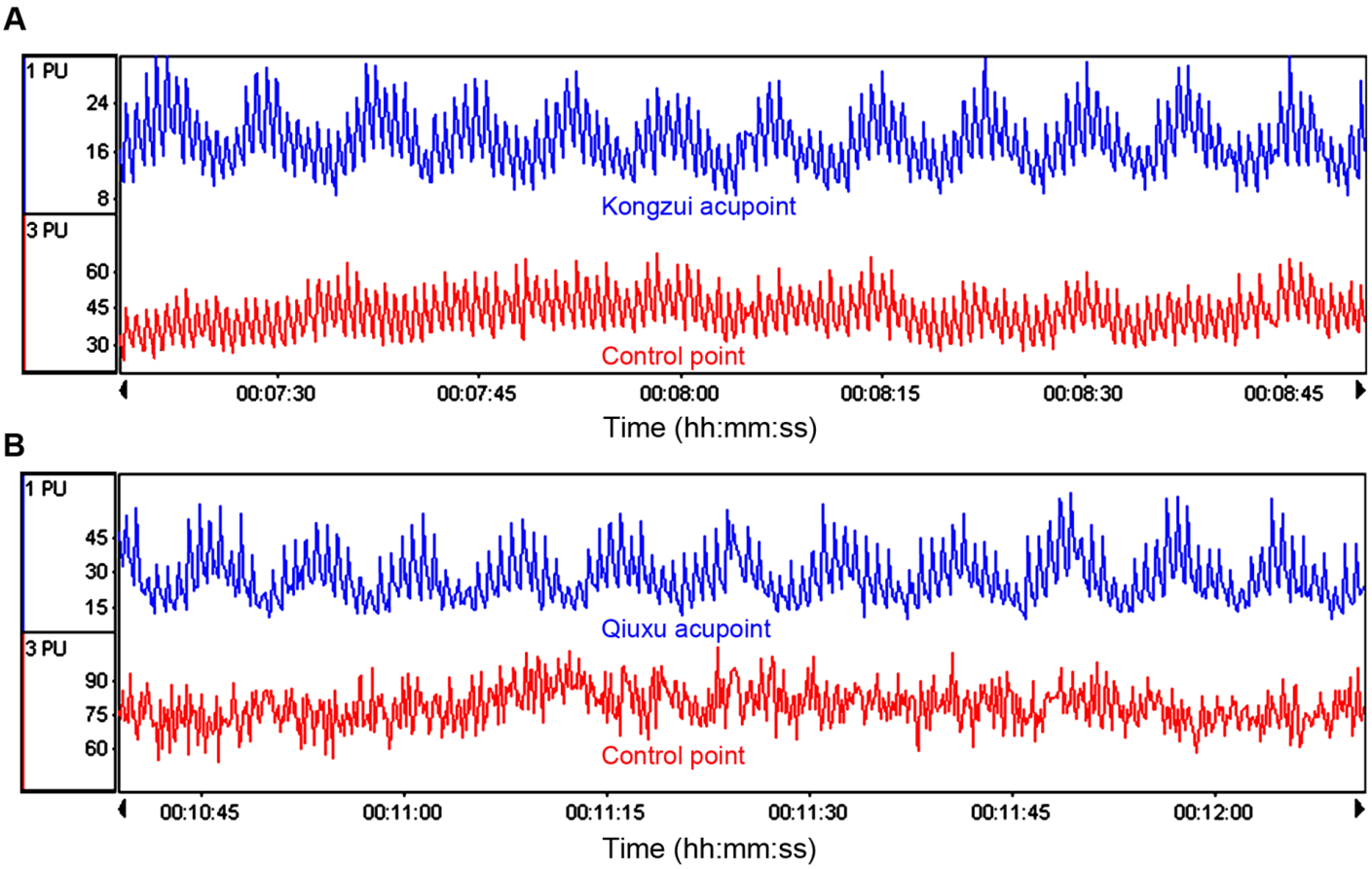

Figure 2

Typical LDF waveforms at acupoints and control points of beagle dogs. The perfusion at acupoints and control points was recorded simultaneouslly using PeriFlux 5000 LDF with type 407 probes. Its time constant τ was set as 0.03 s, and the recording was at least five minutes when dogs were quiet. A: the Kongzui acupoint (above blue) and the control point (below red); B: the Qiuxu acupoint (above blue) and the control point (below red).

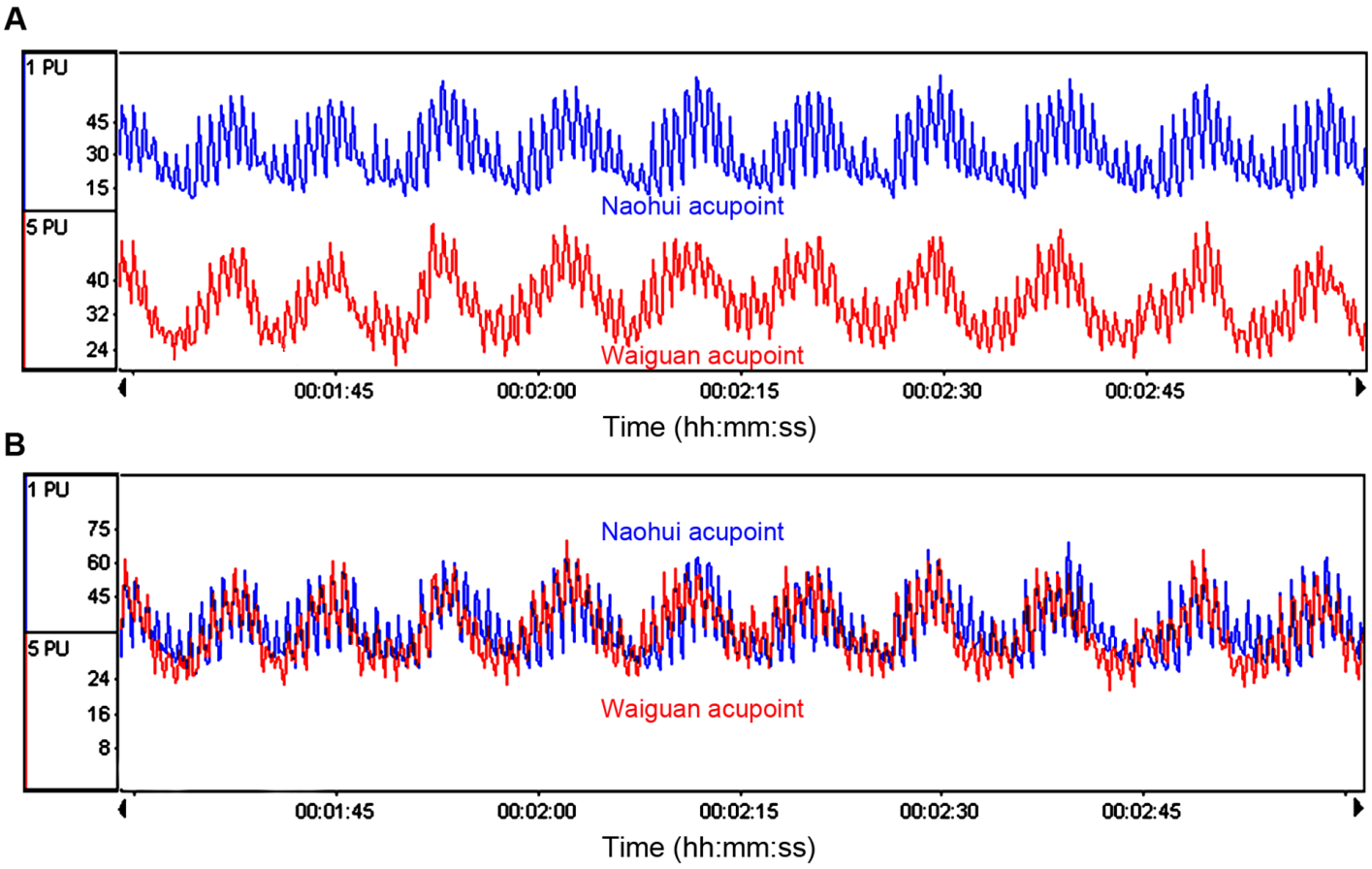

Figure 3

Synchronous vasomotion LDF waveforms at acupoints on the same meridian of beagle dogs. The perfusion at both acupoints of one meridian was recorded simultaneouslly using PeriFlux 5000 LDF with type 407 probes. Its time constant τ was set as 0.03 s, and the recording was at least five minutes when dogs were quiet. A and B were the same recording, and the Naohui acupoint and Waiguan acupoint in the triple energizer meridian were shown blue line drawing and red line drawing, respectively.

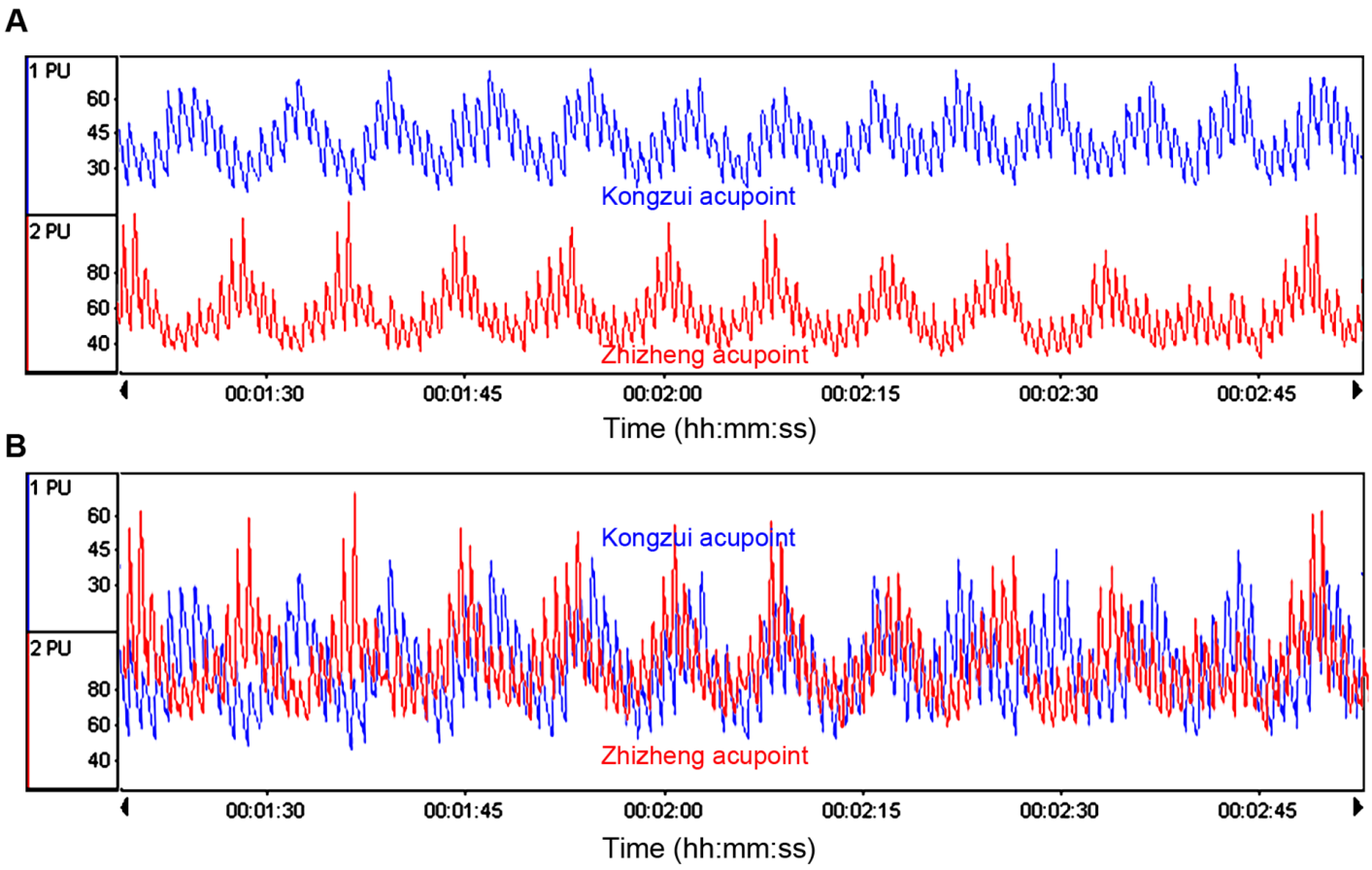

Figure 4

Synchronous vasomotion LDF waveforms at acupoints on different meridians of beagle dogs. The perfusion at both acupoints of different meridians was recorded simultaneouslly using PeriFlux 5000 LDF with type 407 probes. Its time constant τ was set as 0.03 s, and the recording was at least five minutes when dogs were quiet. A and B were the same recording, and the Kongzui acupoint in the lung meridian and the Zhizheng acupoint in the small intestine meridian were shown blue line drawing and red line drawing, respectively.

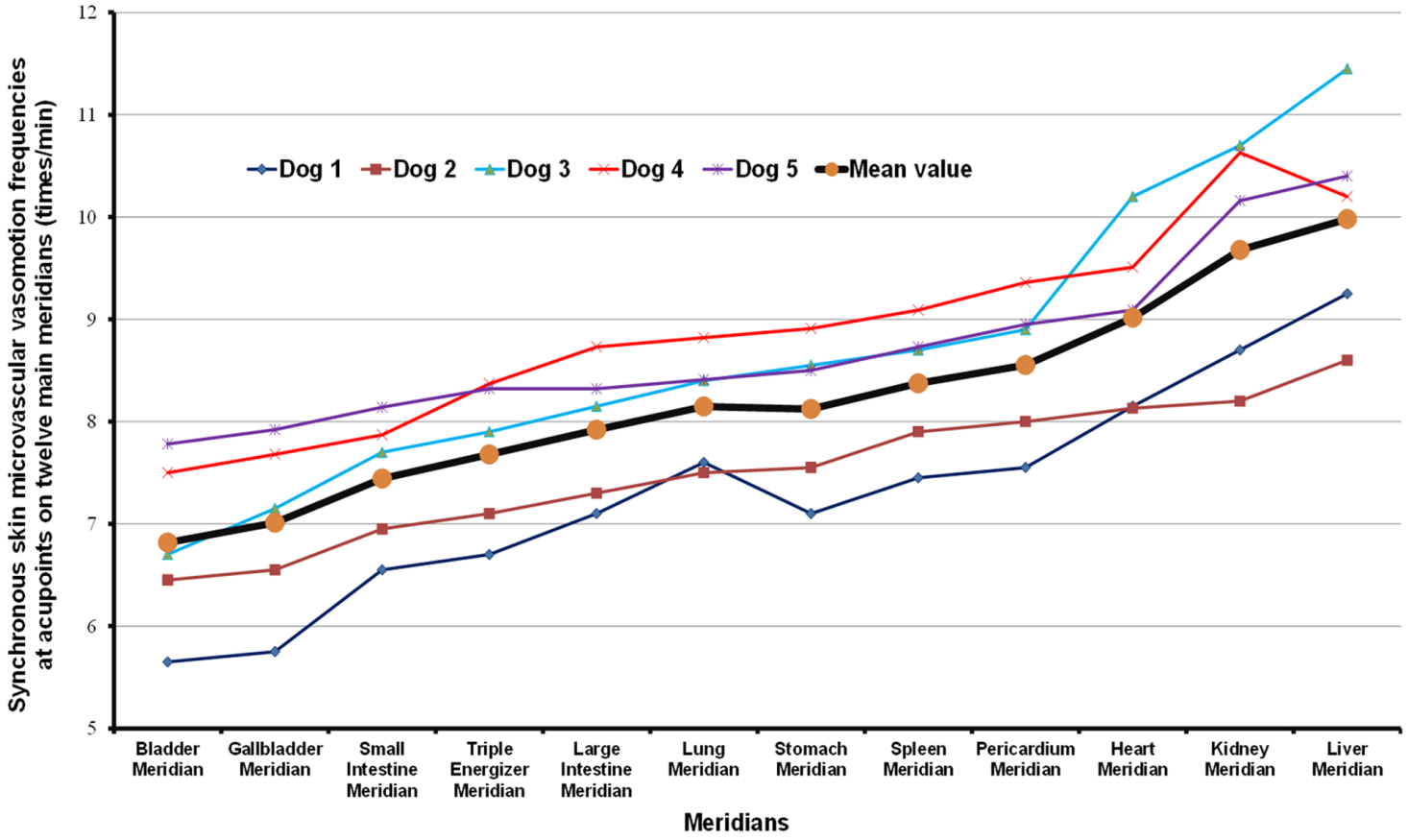

Figure 5

Frequencies of synchronous microvascular vasomotion at acupoints on the twelve main meridians of five beagle dogs. The LDF waveforms at acupoints on the twelve main meridians were recorded using PeriFlux 5000 LDF with type 407 probes, and their spectral analysis was performed by PeriSoft for Windows 2.50. The frequency of any meridian of each dog were calculated from the waveforms of both acupoints on it, and data of the mean value line were the average frequencies of 5 dogs.