

Dear Dr. Cooper,

Thank you for taking our paper into your consideration. We deeply appreciate the comments from you and all the reviewers for they are so valuable to our current and further studies.

Following your indications, we took out all the phrases 'responses of' from the subheadings under the section of Results, so that the subheadings are much concise.

Secondly, referring to the papers by Vojdani and by Salvioli, we added the explanations to the diagrams.

Compared with the original version, the revised version we submitted in February has been rewritten a lot in the method paragraph (on page 6-7), and the description has been made much clearer.

On the other hand, reviewer 4 suggested us to submit this article to physiology-related journal. In our opinion, alternative medical science belongs to physiology-related research. We would like to emphasize the evidence-based approach of this study. This is a practical experiment about the effect of heat and cold stress to radial pulse and heart rate variability.

We anticipate the response and indications from you.

Yours truly,

Fun-jou Chen

Radial Pressure Pulse and Heart Rate Variability in Heat and Cold-stressed Humans

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Running Head: Heat and cold stress on radial pressure pulse

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Abstract

This study aims to explore the effects of heat and cold stress on the radial pressure pulse (RPP) and heart rate variability (HRV). The subjects immersed their left hand into 45°C and 7°C water for 2 minutes respectively. Sixty healthy subjects (age 25 ± 4 yr; 29 men and 31 women) were enrolled in this study. All subjects underwent the supine temperature measurements of the bilateral forearms, brachial arterial blood pressure, HRV and RPP with a pulse analyzer in normothermic conditions and thermal stresses. The power spectral low-frequency (LF) and high-frequency (HF) components of HRV decreased in the heat test and increased in the cold test. The heat stress significantly reduced radial augmentation index (AIr) ($p < 0.05$), but the cold stress significantly increased AIr ($p < 0.001$). The spectral energy of RPP did not show any statistical difference in 0~10Hz region under both conditions, but in the region of 10~50Hz, there was a significant increase ($p < 0.001$) in the heat test and significantly reversed in the cold test ($p < 0.01$). The changes in AIr induced by heat and cold stress were significantly negatively correlated with the spectral energy in the region of 10~50Hz ($SE_{10-50\text{Hz}}$), but not in the region of 0~10Hz ($SE_{0-10\text{Hz}}$). The results demonstrated that the $SE_{10-50\text{Hz}}$ which only possessed a small percentage in total pulse energy presented more physiologic characteristics than the $SE_{0-10\text{Hz}}$ under the thermal stresses.

Keywords: spectral energy – 10~50Hz – radial augmentation index – thermal stress.

Introduction

The radial pressure pulse (RPP), which is derived from the heart's pumping action and transmitted from the heart to the end of the radial artery, is regulated by sympathetic nerve activity (SNA). The RPP conveys much information about the physiology of the cardiovascular system. For example, it carries the effects of meditation on the cardiovascular system (1). Dating back two thousand years ago, the Chinese doctors placed their fingertips on patients' wrists to palpate the pulse property for diagnosis. Nowadays, a study has confirmed the quantitative characteristics of the pulse signals from RPP (2). By RPP, we can evaluate the augmentation index, elasticity of the artery, and pulse wave velocity, etc. Studies have indicated that the radial augmentation index (AIr) and radial diastolic augmentation (DAI) can reflect the left ventricular load (3, 4). Peripheral blood pressure pulse transmits along the arterial tree and is reflected to become a retrograde wave when the pulse encounters the resistance of the arterial wall. As a result, increasing the peripheral resistance may induce the higher degree of reflected waves which then results in higher amplitude of AIr (5). The peripheral vascular vasomotion is generated and modulated by the SNA (6, 7). Studies have demonstrated that lowering

the temperature induces peripheral vasoconstriction and decreases vasomotion; these responses are reversed if the temperature is raised (8).

The first procedure of the cold pressor test was to immerse the subjects' arms into 1~7 °C water for 1~6 minutes, which resulted in high-stressed responses including a rise in blood pressure and heart rate (9, 10). The spectral analysis of HRV is a quantitative tool for evaluating the autonomic nervous system of heart (11, 12). The high-frequency component (HF, 0.15~0.4 Hz) of HRV reflects cardiac vagal nervous activity, and the low-frequency component (LF, 0.04~0.15 Hz) is mediated by both cardiac vagal and SNA; therefore, the ratio of low to high frequency component (LF/HF) is an index of cardiac sympatho-vagal balance. The HF power (HF %), normalized to the total spectral power, is regarded as an indicator of parasympathetic activity (13). The spectral analysis of HRV is often used to assess the effects of heat and cold tests (14). Besides the evaluating A_{Ir} in the time domain, the spectra transformed into frequency domain are mostly used for the periodic waves to represent the dynamics carried in the waves by obtaining the sums of the spectral energy (SE). In normal individuals, the spectral energy within 0~10 Hz (SE_{0-10Hz}) took more than 99.1% of the total energy of the radial pressure waveforms, with less than 0.9% of energy within 10~50Hz (SE_{10-50Hz}), and it had large variations above 10Hz for patients who had acute illness or under metabolic stress (15, 16). Spectral

harmonic peaks have been studied to observe how the heart distributes blood into the arterial system under normal and abnormal situations (17). Therefore, RPP always exhibits some specific characteristics in the spectral domain. However, the spectral characteristics of RPP for humans under heat and cold-stress have not been well studied. This study aims to explore the spectral and time-domain effects on the RPP and HRV in heat and cold stress.

Methods

Subjects

Sixty healthy subjects (29 men and 31 women) participated in the study. The mean age in men was 25.1 ± 4.0 yr, and 24.7 ± 4.3 yr in women. The protocol and informed consent were approved by IRB in China Medical University Hospital (DMR97-IRB-191). In order to exclude participants with acute illness, all subjects underwent a detailed medical examination including a medical history survey and a physical examination. A written informed consent was obtained from each participant before the experiment. Consumption of caffeinated and alcoholic beverages as well as smoking were forbidden for a period of 24 hours prior to the test.

Testing protocol

All subjects participated in two tests at normothermic conditions by immersing their left hands into 45°C water and 7°C water, respectively. The experimental

procedures are shown in Fig. 1. During the experiment, the room temperature was maintained at about 26~27°C. The subjects were instructed to lie down in the supine position, and relax for 20 minutes. During the last 2 minutes, the subjects sat up and subsequently lay down again to take the measurements including the temperatures on their both forearms, the blood pressure (BP), heart rate (HR), 5-minute ECG as well as the radial pulse at the right wrist. These data were used as the variables for control-1. Afterwards, the subjects immersed their left hands into 45°C water for a period of 2 minutes. After removing hands out from the water the subjects were gone through the same measurement procedures. When the heat test was finished, the subjects rested for 10 minutes and then repeated the prior procedures with the water temperature being changed from 45°C to 7°C. The measurements before the 7°C water immersing were recorded as control-2 variables.

Measurements

Skin temperature

An infrared thermometer (Omni-Nure TE-HBI, Constant healthcare technology Co., Ltd., Taiwan) was used to record the skin temperature on the inner sides of both forearms 3 cm below the elbow joint by slightly touching the skin.

Hemodynamics

BP (systolic, diastolic blood pressure) and heart rate were measured by the

Panasonic Diagnostec Upper Arm Blood Pressure Monitor (Matsushita electric Works, Ltd. Osaka Japan) through a cuff wrapped around the left upper arm.

Pulse and analysis

The right RPP was recorded by the Pulse analyzer (designed by China Medical University, Taiwan) consisting of a high-fidelity pressure sensor and a stable X-Y-Z axial moveable framework. The examinations were carried out with each subject lying in the supine position, shown in Fig. 2A. When the sphygmogram showed the greatest amplitude, as in Fig. 2B, the RPP was considered suitable to be recorded. The electrical pulse signal from the sensor was digitized and fed into a computer for processing through fast Fourier transformation in order to obtain the sums of the $SE_{0-10\text{Hz}}$ and $SE_{10-50\text{Hz}}$. The corresponding spectrogram of 0~10 Hz and 10~50 Hz bands are shown in Fig. 2C and 2D. In Fig. 3, AIr was calculated as (late systolic pressure – diastolic pressure) / (systolic pressure – diastolic pressure) × 100%, and DAI was calculated as (early diastolic pressure – diastolic pressure) / (systolic pressure – diastolic pressure) × 100% (3).

HRV and analysis

The HRV analyzer (designed by China Medical University, Taiwan) with one-channel electrocardiograph (Lead II) was used to record the surface ECG from the subjects in the lying position for five minutes. The analogue ECG signals were

immediately converted into digital signals. R–R intervals were measured after the R waves were detected. The ECG signals contained abnormal complexes or artifacts were discarded. Parameters of the HRV spectral analysis were computed from the sequence of normal R–R intervals by means of the fast Fourier transformation. LF (0.04~0.15 Hz) and HF (0.15~0.5 Hz) were determined by integrating the power spectrum density in the respective frequency range. Then, the normalized unit, $HF\% = 100 \times HF / (LF+HF)$, and LF/HF were calculated.

Statistical methods

In addition to the calculation of mean \pm SD, the paired t-test was used to compare the temperature difference between two forearms in the baseline condition, and the variance changed from normothermic condition to the thermal stress condition.

Pearson's correlation analysis was used to study the variables between the spectral energy of RPP and AIr induced by heat and cold. All tests were two-sided; $P < 0.05$ was taken as significant. Statistical analysis was performed by the Statistical software SPSS 15.1 for Windows (SPSS Inc.).

Results

All subjects completed the experimental trials and measurements. The responses of physiological variables, HRV and RPP are presented in Table 1. There was significant

difference in the baseline temperatures of the left and right forearms ($p= 0.000043$).

Physiological variables

Changes in physiological variables are shown in Fig. 4. In the heat test, the mean heart beat slowed down, and the systolic and diastolic pressure decreased, but these responses were not significantly different with that in control-1. The temperature in the left hand significantly increased than that in normothermic control-1 ($p<0.01$) and it significantly decreased in the right hand ($p<0.01$). In the cold test, systolic and diastolic pressure significantly increased ($p<0.01$), and temperatures in both hands significantly decreased ($p<0.01$).

Heart rate variability (HRV)

Changes in the spectral components of HRV are shown in Fig. 5. The power spectrum of HRV revealed a decreased power for the LF and HF components after heat stress and an increased power after cold stress. These changes were not significantly different in this experiment. There was no significant difference in LF/HF either, but the HF% significantly increased by comparing the 7°C water immersion with normothermic control-2 ($p<0.05$).

Radial pressure pulse

Changes in the parameters of RPP are shown in Fig. 6. There were no statistical differences in the amplitudes of p1, p2 or p3 in the heat test nor the p2, or p3 in the

cold test, but there was a statistical decrease in the p1 in the cold test ($p < 0.05$). The heat test significantly reduced the AIr and DAI ($p < 0.05$), but the cold test significantly increased the AIr ($p < 0.001$) and DAI ($p < 0.05$). In SE_{0-10Hz} , there was no statistical difference in the heat or cold test. However, $SE_{10-50Hz}$ significantly increased ($p < 0.01$) in the heat test and significantly reversed in the cold test ($p < 0.01$). Pearson's correlation analysis demonstrated that the changes in AIr induced by the heat and cold stress were significantly negatively correlated with the induced changes in $SE_{10-50Hz}$ (Pearson's correlation coefficients: $r = -0.289$, $p = 0.02519$; $r = -0.315$, $p = 0.01424$, respectively), but the changes in AIr were not significantly correlated with that in SE_{0-10Hz} (Pearson's correlation coefficients: $r = -0.112$, $p = 0.39482$; $r = -0.139$, $p = 0.2884$, respectively).

Discussion

The significant findings of this study are that the spectral energy of radial artery can be altered by heat and cold stress from the skin surface, and these changes did not converge in lower frequency bands, 0~10Hz, but in higher frequency bands, 10~50Hz, and the variables induced by heat and cold in AIr and $SE_{10-50Hz}$ were significantly negative correlation. The results demonstrate that the $SE_{10-50Hz}$ which only possesses a small percentage in total pulse energy presents more physiologic characteristics than

the SE_{0-10Hz} in the thermal stresses.

In baseline condition, the mean skin temperature (T_{sk}) of the right hand was significantly higher than that of left hand. The result is consistent with the study by Luis Rodrigues et al. (1998), which revealed that the right forearm was more motoric than the left one (18). It increased during the heat stress while decreased under cold stress (Fig. 4). Although the heat and cold stimulation were carried out only on the left hand, the T_{sk} changes of both hands showed significant difference. The heat stimulation in the left hand induced the T_{sk} to be increased significantly in the hand itself, while the T_{sk} in the right hand significantly decreased. The physiological study has showed that the microcirculation is a functional system by the reflex of the automatic feedback system. For example, the skin blood flow is reflectively modulated by the vasodilator and vasoconstrictor in response to the change in skin temperature, and the subcutaneous vasomotor activity is synchronous on bilateral limbs (19, 20). Therefore, the heat stress induced the vasodilatation of the left hand by directly contacting with hot water, and at the same time it resulted in the loss of dermal heat in the right hand synchronously. The result reveals that the subcutaneous thermoregulation is systemic after undergoing heat and cold stress. This result is similar to the study by Bozdemir H. et al. (1998), that cold stress causes systemic vasomotor changes (21). Figure 5 presents that HF% is a significant increase after $7^{\circ}C$

immersion and both LF and HF increase in the cold test while decreasing in the heat test, which reveal that skin surface cooling can augment the cardiac sympathetic and vagal nervous activity, but the skin surface heating is reversed. The responses in this experiment are consistent with prior study by Hirayanagi et al. (1999). (22)

Skin surface cooling induces peripheral vasoconstriction and decreases vasomotion. According to the Poiseuille's law, the small decrease in the lumen of small arteries and arterioles may significantly increase blood pressure and peripheral vascular resistance which results in an increase of reflection wave. In contrast, heat induces peripheral vasodilatation and increases vasomotion to decrease blood pressure and reflection wave (8, 23, 24). In Fig. 4 and 6, the heat stress decreases systolic and diastolic pressure, and significantly reduces the AIr and the DAI, which are associated with peripheral vasodilatation (3). The cold stress significantly increases the systolic and the diastolic pressure and significantly increases the AIr and the DAI, which are associated with peripheral vasoconstriction (25). Through these results, it is comprehended that the heat decreases the left ventricular load while the cold increases the left ventricular load (3). Figure 6 presents that the $SE_{0-10\text{Hz}}$ showed no statistical change in both experiments. In the heat test, the $SE_{10-50\text{Hz}}$ significantly increased, but the response was significantly reversed in the cold test. In cardiovascular physiology, the vascular smooth muscles are innervated by nerve fibers and exposed to recurrent

oscillated stimuli; thus, the motion of vessels is influenced by nerve activity. Both heat and cold stresses can significantly increase peripheral SNA (9, 26). Most of the sympathetic nerve discharge is between 2 and 6 Hz, and it would be increased to 50 Hz during acute stress, which maximizes the arterial and arteriolar tension (27). The increased impedance of vessels is proportional to the frequency. Such a variation in the tension of vascular wall will lead to the fluctuation of blood pressure pulse with the higher natural frequency response (28). Hence, it is inferred that the tension fluctuation of the time-domain pressure pulse may correspond to the fluctuation of a relatively higher frequency band (10~50 Hz). The result that $SE_{10-50Hz}$ is negatively correlated to A_{Ir} is consistent with the theory.

The RPP measured at the end of the radial artery which is adjacent to the small arteries and arterioles. Hence, the RPP has its own blood pressure, and receives the higher fluctuation frequency of blood flow pressure from the retrograde waves of the small arteries and arterioles. Energy dissipation of blood flow is necessary to overcome peripheral vascular resistance (29). The reductions of peripheral vascular lumen and vasomotion under cold stress induce energy dissipation and increase the amplitude of reflection waves; subsequently higher frequency energy, $SE_{10-50Hz}$, is decreased. In contrast, the increases of peripheral vascular dilatation and vasomotion in heat stress decrease energy dissipation and reduce the amplitude of reflection

waves; higher frequency energy, $SE_{10-50\text{Hz}}$, is increased subsequently.

In conclusion, our study demonstrates the following results: 1) The skin temperature of the right hand is significantly higher than that of the left hand. The change of T_{sk} induced by local skin heat and cold on the left hand is systemic. 2) The heat reduces A_{Ir} and DAI , which indicates a decrease of left ventricular load; the cold increases A_{Ir} and DAI , which indicates an increase of left ventricular load. 3) The $SE_{10-50\text{Hz}}$ which only possesses a small percentage in total pulse energy presents more physiologic characteristics than the $SE_{0-10\text{Hz}}$ under the thermal stresses.

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