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RESEARCH ARTICLE

The Effect of Short-term Bed-rest on Radial Pulse in Healthy Subjects



Yun-Kyoung Yim^{1,*}, Kwang Suk Park²

¹ Department of Meridian and Acupoint, College of Korean Medicine, Daejeon University, Daejeon, South Korea ² Department of Biomedical Engineering, College of Medicine, Seoul National University, Seoul, South Korea

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Abstract

The objective of this study is to investigate the effects of short-term bed rest on the radial pulse in healthy subjects. Twenty-one healthy volunteers participated in this study. Their radial pulse was measured at different measuring positions using a multi-step tonometry system. The participants took 30 minutes of bed rest and their radial pulse was measured before and after the bed rest. The effects of bed rest on the radial pulse were analyzed. The pulse area, the amplitudes of h4 and h5, the pulse period, and the diastolic pulse proportion increased with short-term bed rest, whereas the proportions of systolic and hightension pulse and the fundamental frequency of the pulse wave decreased with short-term bed rest. All the changes were in the same direction in both male and female participants at all measuring positions; however, some parameters changed more in women than in men, and some changed more at the distal position than at the proximal position. In shortly, Short-term bed rest induces significant changes in the radial pulse of healthy subjects. The results of this study could be used as a control reference for clinical acupuncture studies with participants lying on a bed for acupuncture treatment.

* Corresponding author. Department of Meridian and Acupoint, College of Korean Medicine, Daejeon University, 96-3, Yongun-dong, Dong-gu, Daejeon 300-716, South Korea.

E-mail: docwindy@dju.kr (Y.-K. Yim).

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1. Introduction

According to traditional Eastern medical theories, the pulse provides complete information, not only about the cardiovascular system, but also with regard to the entire body, revealing the physiological and pathological disorders of the individual [1]. In this view, the intrinsic and extrinsic factors that affect the body also affect the pulse. For example, on the basis of Eastern medical pulse diagnosis, pregnancy induces specific physiological changes in the radial pulse. Liao et al reported the differences in the radial pulse spectrum between healthy pregnant women and women who were not pregnant, indicating that pregnancy affects the radial pulse and also validating the traditional pulse diagnosis [2]. Other studies have reported the effects of food intake on the pulse [3,4] and a growing number of investigations have described the effects of acupuncture or herbal treatments on the pulse [5-10].

We believe that pulse analysis could be a possible quantitative and objective methodology for use in evaluating the effects of traditional medical treatments as well as in the diagnosis of diseases. We believe that pulse analysis can be used to interpret the disease condition of a patient before treatment and also to evaluate the efficacy of a treatment after it has finished.

Pulse diagnosis has the advantages that it is noninvasive and easy to access, offering real-time results. We therefore suggest that it could be used to monitor the pathophysiological changes that occur in the human body before and after acupuncture treatment.

Although acupuncture is performed in various postures, the supine posture is most often used in acupuncture clinics [11]. The duration of acupuncture treatment also varies, but 20 minutes of needle retention is most often suggested [12]. Considering the extra time required for needle insertion, withdrawal, and manipulation, we have assumed that 30 minutes of lying on a bed is the most common format in acupuncture clinics. We therefore investigated the effects of 30 minutes of bed rest on the radial pulse as a control reference for clinical acupuncture studies using pulse analysis with participants receiving acupuncture treatment while lying on a bed.

2. Methods

2.1. Subjects

Twenty-one healthy volunteers (11 men, mean \pm standard deviation (SD) age 22.00 \pm 2.39 years, height 174.64 \pm 2.48 cm, weight 71.64 \pm 7.86 kg; 10 women, age 21.90 \pm 1.52 years, height 168.60 \pm 7.13 cm, weight 65.60 \pm 8.08 kg) in the age range 20–29 years with no underlying disease and not taking any prescribed medicines were included in this study.

The criteria for exclusion were as follows: arrhythmia, systolic blood pressure >150 mmHg, diastolic blood pressure <60 mmHg, wounds or scars in the region of pulse measurement, body mass index <18 kg/m² or >32 kg/m², pregnancy, or menstruation.

All subjects provided written informed consent. This study was approved by the institutional review board of Daejeon University Hospital (Approval No. P2011-09-03).

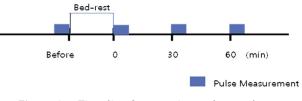


Figure 1 Time-line for experimental procedure.

2.2. Experimental procedure

Fig. 1 shows the time-line of the experimental procedure. The pulse measurement procedure was conducted in a quiet room. All participants took 30 minutes of rest sitting on a comfortable chair before the pulse measurement. The radial pulse was measured in a sitting position. After the first measurement of the radial pulse, the participants lay on a bed next to the pulse-measuring system. After 30 minutes of bed rest the radial pulse was measured again in the same manner as the first measurement, taking three measurements at 30-minute intervals. The participants were asked to remain calm throughout the experiment. Participants were allowed water, but were restricted from consuming food or other drinks. The experimental room was kept at a stable temperature (24–26 °C) and humidity (40–60%).

2.3. Pulse measurement

The radial pulse was measured at three different positions (bilateral *Chon*, *Gwan*, and *Cheock*) on the wrist (Fig. 2). The region opposite the styloid process of the radius is *Gwan*, distal to *Gwan* is *Chon*, and proximal to *Gwan* is *Cheock* [1].

For reliable data acquisition and experimental reproducibility, the position of *Gwan* was marked on the skin in the first measurement. The next measurements were conducted based on this mark, thus guaranteeing that the pulse was measured at the same position in every measurement [13,14].

The pulse was measured using the DMP-3000 system (Daeyomedi Co. Ltd, Ansan, Korea). This device has an arterial tonometry sensor with an array of five piezoresistive semiconductor transducers. After the sensor is placed on the pulse-measuring position, it scans the artery automatically and applies multiple levels of pressure to obtain stable multi-step pulse waveforms [15] (Fig. 3).

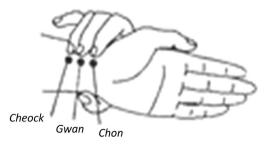


Figure 2 Pulse measuring positions.



Figure 3 Experimental set-up for pulse measurement.

In this study the pressure was applied at five different levels (50 g, 90 g, 140 g, 190 g, and 240 g), and the pulse waves were recorded for 5 seconds at each level. The data at each level included five pulse waves from five piezor-esistive semiconductor transducers. Therefore 25 pulse

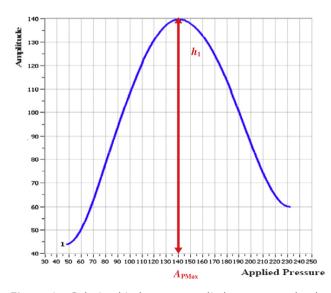


Figure 4 Relationship between applied pressure and pulse amplitude. A_{PMax} is the applied pressure level where the amplitude of h_1 is at a maximum. The pulse wave obtained at pressure A_{PMax} is suitable to observe the pulse characteristics.

waves were obtained from one measuring position [13,14] (Figs. 4 and 5).

2.4. Data analysis

2.4.1. Pulse wave selection

Once the tonometry sensor begins pressing on the skin at the pulse measuring position, the applied pressure gradually increases and the pulse height also increases to some extent, before later decreasing [15] (Fig. 4). In this study, we applied pressure at five different levels. The pulse waveform with the highest h_1 was the most distinct and suitable to observe the pulse characteristics. It was therefore selected as the 'representative pulse wave' among the five pulse waves measured at five different levels [14] (Figs. 5 and 6).

The tonometry system automatically scans the artery and places the sensor above the arterial flow. The sensor has five transducers (medial, lateral, distal, proximal, and central) and the central transducer is placed above the centre of arterial flow. In this study, the representative pulse wave from the central transducer was selected to analyze the pulse parameters [14].

2.4.2. Time domain analysis

The following pulse parameters were obtained from the time domain analysis (Fig. 6): (1) pulse height parameters [division (div) = digital value for pressure], amplitudes of h_1 , h_2 , h_3 , h_4 , and h_5 ; (2) periodic parameters (in seconds), with periods of *T*, t_1 , t_2 , t_4 , and t_5 ; (3) *W* (in seconds), high-tensioned pulse period, i.e. width of main peak where the pulse height is two-thirds of h_1 ; (4) A_p (div²), pulse area estimated with pulse wave; (5) A_s/A_p , percentage of systolic area to total pulse area; (6) A_d/A_p , percentage of diastolic area to total pulse area; (7) *W* area (div²), high-tensioned pulse area, i.e. area of upper one-third of main peak, formed with pulse wave and *W*; and (8) A_w/A_p , percentage of *W* area to total pulse area.

2.4.3. Frequency domain analysis

Time domain pulse data were transformed using Fourier transformation and the fundamental frequency (F_1) was analyzed.

2.4.4. Statistical analysis

The statistical analysis was performed using PASW Statistics 18.0 (IBM, Armonk, NY). The general characteristics of the participants (age, height, and weight) are presented as mean \pm SD. Pulse data are presented as mean \pm standard error (SE). The changes in the pulse parameters with bed rest were analyzed using repeated measures of analysis of variance (ANOVA). All reported *p* values are two-sided and p < 0.05 was considered statistically significant.

3. Results

3.1. Effects of short-term bed rest on radial pulse (independent of gender and measuring position)

Some parameters changed significantly with short-term bed rest, independent of gender and measuring position. The

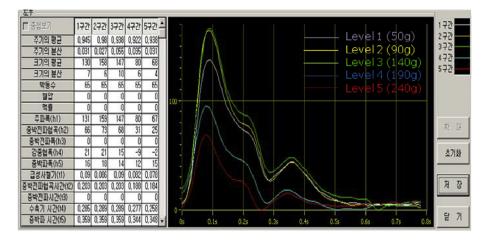


Figure 5 Pulse waveforms measured at five different pressure levels. The pressure was applied at five different levels and the pulse wave with highest h_1 value was selected as the representative pulse wave.

data obtained for both genders in all positions (n = 126) were analyzed together for these parameters (Table 1).

The amplitude of h_4 and the pulse area decreased immediately after short-term bed rest and then increased to a level higher than the baseline after 30 minutes of bed rest, before returning towards the baseline 60 minutes after bed rest. The amplitude of h_5 was increased and the pulse period (T) was lengthened, whereas t_1/T , t_2/T and t_5/T were shortened by short-term bed rest. The fundamental frequency (F_1) decreased with short-term bed rest (Table 1).

3.2. Gender differences in the effects of short-term bed rest on radial pulse

To investigate the gender difference in the change in pulse with short-term bed rest, the data obtained from men (n = 66) and women (n = 60) were analyzed separately (Table 2).

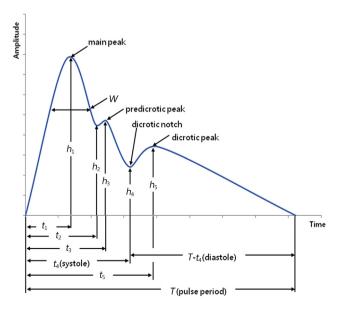


Figure 6 Radial pulse waveform.

In both men and women, t_4/T and $t_4/(T - t_4)$ decreased, and $(T - t_4)/T$ increased; however, the changes were significantly larger in women. A_d/A_p significantly increased and A_s/A_p and A_w/A_p significantly decreased with shortterm bed rest in women, whereas no significant change in these parameters was observed in men. All genderdependent changes with short-term bed rest resulted in reducing the gender differences of the baseline (Table 2).

3.3. Positional differences in the effects of shortterm bed rest on radial pulse

To investigate the effect of different positions on the change in pulse with short-term bed rest, the data from different measuring positions (n = 42) were analyzed separately (Table 3).

The changes in W/T and A_w/A_p were significantly different in different measuring positions. These parameters were significantly reduced with short-term bed rest at the *Chon* and *Gwan* positions, although not at the *Cheock* position (Table 3).

4. Discussion

All the factors that affect the body also affect the pulse [1]. In this study, the radial pulse was measured using an arterial tonometry system and the pulse wave was analyzed to investigate the pulse parameters that changed with short-term bed rest. Some parameters changed regardless of gender and measuring position, whereas others changed differently with these two variables.

It is known that the first peak (main peak) in systole of the pulse is related to the cardiac output and the dicrotic wave in diastole is related to the closure of the aortic valve [16]. In this study, the pulse heights of the main peak (h_1) , dicrotic notch (h_4) , and dicrotic peak (h_5) were analyzed. The pulse area was estimated using the pulse wave, and the fundamental frequency (F_1) of the radial pulse was analyzed using Fourier transformation.

The pulse area and the amplitude of h_4 reduced immediately after bed rest and increased to a level higher than

Table 1	Effects of short-term be	ed rest on pul	se parameters in	dependent of	gender and	l measuring position.

	Measured value at each time point (change from baseline) ($n = 126$)				
	Baseline	0 min	30 min	60 min	
$\overline{h_4}$	47.011 ± 2.198	43.330 ± 2.398	52.192 ± 2.507	50.313 ± 2.238	0.000
		$(\downarrow -3.562 \pm 1.514)$	(†4.873 ± 1.804)	(†3.322 ± 1.739)	
h_5	66.461 ± 2.168	67.195 ± 2.162	$\textbf{73.993} \pm \textbf{2.322}$	$\textbf{72.579} \pm \textbf{2.099}$	0.001
		↑0.733 ± 1.726	\uparrow 6.869 \pm 2.039	↑6.117 ± 2.152	
Т	$\textbf{0.787} \pm \textbf{0.012}$	$\textbf{0.802} \pm \textbf{0.009}$	$\textbf{0.839} \pm \textbf{0.010}$	$\textbf{0.849} \pm \textbf{0.010}$	0.000
		\uparrow 0.015 \pm 0.007	$\uparrow 0.053 \pm 0.008$	$\uparrow 0.062 \pm 0.008$	
t ₁ /T	0.160 ± 0.0025	0.157 ± 0.0021	0.149 ± 0.0017	0.146 ± 0.0017	0.000
		$(\downarrow -0.003 \pm 0.002)$	$(\downarrow -0.010 \pm 0.002)$	$(\downarrow -0.014 \pm 0.002)$	
t_2/T	$\textbf{0.269} \pm \textbf{0.005}$	$\textbf{0.264} \pm \textbf{0.004}$	$\textbf{0.256} \pm \textbf{0.004}$	$\textbf{0.247} \pm \textbf{0.004}$	0.000
		$\downarrow -0.005 \pm 0.004$	$\downarrow -0.013\pm0.005$	$\downarrow-0.022\pm0.004$	
t ₅ /T	$\textbf{0.517} \pm \textbf{0.007}$	$\textbf{0.510} \pm \textbf{0.006}$	$\textbf{0.490} \pm \textbf{0.005}$	$\textbf{0.480} \pm \textbf{0.006}$	0.000
		$\downarrow -0.007 \pm 0.005$	$\downarrow -0.031\pm0.005$	$\downarrow -0.039 \pm 0.005$	
Pulse area	10,876.10 \pm 339.58	10,835.14 \pm 339.45	11,855.11 \pm 395.90	11,370.25 \pm 341.42	0.018
		$(\downarrow -40.96 \pm 281.32)$	(†831.70 ± 323.49)	(†494.14 ± 305.14)	
<i>F</i> ₁	$\textbf{1.259} \pm \textbf{0.017}$	$\textbf{1.168} \pm \textbf{0.017}$	$\textbf{1.142} \pm \textbf{0.014}$	$\textbf{1.118} \pm \textbf{0.015}$	0.000
		$\downarrow -0.091 \pm 0.013$	$\downarrow -0.117 \pm 0.014$	$\downarrow -0.141\pm0.015$	

Values are mean \pm standard error. Parameters analyzed by repeated measures ANOVA. The *p* value is not presented when it is not significant (p > 0.05).

 \downarrow = decrease; \uparrow = increase.

Table 2	Effects of short-term bed rest on pulse parameters dependent on gender

		Measured value at each time point (change from baseline)				p (bed rest)	p (gender)
		Baseline	0 min	30 min	60 min		
t ₄ /T	Μ	$\textbf{0.378} \pm \textbf{0.006}$	0.376 ± 0.005	0.362 ± 0.005	0.353 ± 0.005	0.000	0.038
			$(\downarrow -0.002 \pm 0.005)$	$(\downarrow -0.017 \pm 0.005)$	$(\downarrow -0.025 \pm 0.005)$		
	F	$\textbf{0.435} \pm \textbf{0.006}$	$\textbf{0.418} \pm \textbf{0.006}$	$\textbf{0.402} \pm \textbf{0.004}$	0.401 ± 0.004	0.000	
			$(\downarrow -0.017 \pm 0.005)$	$(\downarrow -0.033 \pm 0.004)$	(↓-0.035 ± 0.005)		
$(T - t_4)/T$	Μ	$\textbf{0.622} \pm \textbf{0.006}$	$\textbf{0.624} \pm \textbf{0.005}$	0.638 ± 0.005	$\textbf{0.647} \pm \textbf{0.005}$	0.000	0.037
			(↑0.002 ± 0.005)	(↑0.017 ± 0.005)	(↑0.025 ± 0.005)		
	F	$\textbf{0.565} \pm \textbf{0.006}$	$\textbf{0.582} \pm \textbf{0.006}$	$\textbf{0.598} \pm \textbf{0.004}$	$\textbf{0.599} \pm \textbf{0.004}$	0.000	
			$(\uparrow 0.017 \pm 0.005)$	$(\uparrow 0.033 \pm 0.004)$	(\uparrow 0.035 \pm 0.005)		
$t_4/(T - t_4)$	Μ	$\textbf{0.619} \pm \textbf{0.017}$	$\textbf{0.611} \pm \textbf{0.013}$	$\textbf{0.572} \pm \textbf{0.012}$	$\textbf{0.552} \pm \textbf{0.013}$	0.000	0.013
			$(\downarrow -0.009 \pm 0.014)$	$(\downarrow -0.049 \pm 0.013)$	$(\downarrow -0.067 \pm 0.014)$		
	F	$\textbf{0.783} \pm \textbf{0.020}$	$\textbf{0.729} \pm \textbf{0.018}$	$\textbf{0.677} \pm \textbf{0.012}$	$\textbf{0.673} \pm \textbf{0.011}$	0.000	
			$(\downarrow -0.054 \pm 0.015)$	($\downarrow -0.107 \pm 0.015$)	$(\downarrow -0.111 \pm 0.017)$		
$A_{\rm s}/A_{\rm p}$	Μ	$\textbf{63.11} \pm \textbf{0.862}$	$\textbf{63.77} \pm \textbf{0.744}$	$\textbf{62.95} \pm \textbf{0.809}$	$\textbf{62.62} \pm \textbf{0.779}$	—	0.000
			$(\uparrow 0.667 \pm 0.846)$	$(\downarrow -0.397 \pm 0.736)$	$(\downarrow -0.485 \pm 0.859)$		
	F	$\textbf{70.92} \pm \textbf{0.737}$	$\textbf{68.83} \pm \textbf{0.741}$	$\textbf{65.52} \pm \textbf{0.658}$	$\textbf{66.02} \pm \textbf{0.554}$	0.000	
			$(\downarrow -2.083 \pm 0.732)$	($\downarrow -5.400 \pm 0.855$)	$(\downarrow -4.900 \pm 0.789)$		
$A_{\rm d}/A_{\rm p}$	Μ	$\textbf{36.89} \pm \textbf{0.862}$	$\textbf{36.23} \pm \textbf{0.744}$	$\textbf{37.05} \pm \textbf{0.809}$	$\textbf{37.38} \pm \textbf{0.779}$	—	0.000
			$(\downarrow -0.667 \pm 0.846)$	(↑0.397 ± 0.736)	(↑0.485 ± 0.859)		
	F	$\textbf{29.08} \pm \textbf{0.737}$	$\textbf{31.17} \pm \textbf{0.741}$	$\textbf{34.48} \pm \textbf{0.658}$	$\textbf{33.98} \pm \textbf{0.554}$	0.000	
			(\uparrow 2.083 \pm 0.732)	(\uparrow 5.400 \pm 0.855)	(\uparrow 4.900 \pm 0.789)		
$A_{\rm w}/A_{\rm p}$	Μ	$\textbf{40.429} \pm \textbf{0.752}$	40.241 ± 0.753	$\textbf{39.179} \pm \textbf{0.782}$	$\textbf{39.051} \pm \textbf{0.793}$	—	0.005
			($\downarrow -0.188 \pm 0.836$)	$(\downarrow -0.763 \pm 0.658)$	$(\downarrow -1.378 \pm 0.729)$		
	F	$\textbf{43.615} \pm \textbf{0.844}$	$\textbf{42.655} \pm \textbf{0.794}$	$\textbf{40.171} \pm \textbf{0.631}$	$\textbf{39.845} \pm \textbf{0.655}$	0.000	
			$(\downarrow -0.960 \pm 0.636)$	(\downarrow -3.444 \pm 0.824)	$(\downarrow -3.770 \pm 0.750)$		

Values are mean \pm standard error. Parameters analyzed by repeated measures ANOVA. The *p* value is not presented when it is not significant (*p* > 0.05).

M = male (n = 66); F = female (n = 60). = decrease; \uparrow = increase \downarrow .

 Table 3
 Effects of short-term bed rest on radial pulse parameters according to measuring position

		Measured value at each time point ($n = 42$) (change from baseline)				p (bed rest)	p (position)
		Baseline	0 min	30 min	60 min		
W/T	Chon	0.161 ± 0.005	$0.154 \pm 0.004 \\ (\downarrow -0.006 \pm 0.003)$	0.154 ± 0.006 (↓-0.007 ± 0.004)	0.147 ± 0.005 (↓-0.014 ± 0.003)	0.001	0.004
	Gwan	$\textbf{0.174} \pm \textbf{0.006}$	$(\downarrow -0.003 \pm 0.005)$ $(\downarrow -0.018 \pm 0.005)$	0.153 ± 0.005	$(\downarrow -0.014 \pm 0.005)$ $(\downarrow -0.026 \pm 0.005)$	0.000	
	Cheock	$\textbf{0.156} \pm \textbf{0.007}$	(1000000000000000000000000000000000000	$(\downarrow -0.022 \pm 0.003)$ 0.151 ± 0.006 $(\downarrow -0.0022 \pm 0.008)$	$(\downarrow -0.025 \pm 0.005)$ 0.150 ± 0.006 $(\downarrow -0.0059 \pm 0.007)$	—	
$A_{\rm w}/A_{\rm p}$	Chon	$\textbf{42.144} \pm \textbf{0.809}$	(1.584 ± 0.780) $(1-0.560 \pm 0.563)$	40.393 ± 0.775	$(1 - 2.653 \pm 0.760)$ (1 - 2.653 ± 0.661)	0.000	0.045
	Gwan	$\textbf{43.268} \pm \textbf{1.024}$		$(1-3.021 \pm 0.994)$	$(1-3.822 \pm 0.928)$	0.005	
	Cheock	$\textbf{40.425} \pm \textbf{1.119}$	(↑ 1.385 ± 1.115 (↑0.959 ± 1.075)	$\begin{array}{c} (1.021 \pm 0.071) \\ 38.567 \pm 0.870 \\ (1.1432 \pm 1.116) \end{array}$	$(1 - 1.076 \pm 1.093)$	_	

Values are mean \pm standard error. Parameters analyzed by repeated measures ANOVA. The *p* value is not presented when it is not significant (*p* > 0.05).

 \downarrow = decrease; \uparrow = increase.

the baseline at 30 minutes, returning towards the baseline at 60 minutes. The amplitude of h_5 and the pulse period increased, whereas the fundamental frequency (F_1) of the pulse decreased with short-term bed rest. No significant difference was observed in the changes of these parameters between men and women, nor between different measuring positions. The correlation analysis showed that the pulse area was positively correlated with the amplitude of h_4 (r = 0.836, p = 0.000) and h_5 (r = 0.917, p = 0.000). Therefore we presume that the changes in the amplitudes of h_4 and h_5 made some contribution to the change in pulse area with short-term bed rest.

In addition, the diastolic pulse proportion increased, while the systolic and high-tension pulse proportions decreased with short-term bed rest. It is noteworthy that the changes in these parameters were significantly larger in women than in men, and these changes resulted in reducing the gender differences of the baseline.

It is widely known that the cardiovascular and autonomic nervous systems are affected by body posture and physical activity and that the mechanisms may be complex, related to reflexes and adjustments [17–21]. Resting in the supine position is believed to increase parasympathetic activity and to consequently lower the heart rate, blood pressure, and LF/HF ratio [21–24]. We presume that the changes observed in the pulse period, periodic pulse proportions, and fundamental frequency of the pulse with short-term bed rest in this study may be related to increased parasympathetic activity and that women are more sensitive to these effects.

In addition to gender differences, some parameters changed according to the measuring position. The proportions of the high-tension pulse period and high-tension pulse area significantly decreased with short-term bed rest at the *Chon* and *Gwan* positions, although not at the *Cheock* position.

Based on Eastern medical theories, each pulsemeasuring position corresponds to different internal organs or body parts. The distal position of the radial pulse belongs to Yang and refers to the condition of Yang, whereas the proximal position of the radial pulse belongs to *Yin* and refers to the condition of *Yin* [1]. The decrease in the proportion of the high-tension pulse at the *Chon* and *Gwan* positions in this study may be interpreted as the effects of short-term bed rest on the internal organs or body parts corresponding to these positions, and it appears that the effects are stronger on *Yang* than *Yin*.

On this basis, we conclude that short-term bed rest induces significant changes in the radial pulse. All the changes were in the same direction in both men and women at all measuring positions. However, some parameters changed more in women than in men, and some changed more at the distal position than at the proximal position.

The mechanisms of pulse changes with short-term bed rest are not completely understood at present. However, we believe that the results of our study may lead to an improved understanding of traditional pulse diagnosis and may be used as a possible control reference for clinical acupuncture studies using pulse analysis with participants lying on a bed for acupuncture treatment.

There are limitations to this study. Firstly, all the subjects in this study were in their 20s. Studies in other age groups will also be needed. Secondly, the sample size was relatively small. A further study with a larger sample size may consolidate the conclusion. Thirdly, we analyzed only the representative pulse from the central transducer of the sensor array. We determined that the representative pulse from the central transducer is an appropriate choice to observe the pulse characteristics. However, for more detailed information and more precise understanding of the pulse, not only the representative pulse from the central transducer, but also the pulse waves at all pressure levels from all transducers of the sensor may be worthy of analysis.

5. Conclusions

Short-term bed rest induces significant changes in the radial pulse. The results of this study may be used as a possible

control reference for clinical acupuncture studies with participants lying on a bed for acupuncture treatment.

Disclosure statement

The author affirms there are no conflicts of interest and the author has no financial interest related to the material of this manuscript.

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