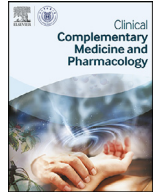




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Full Length Article

The Influence of Age on Blood Flow and Temperature of Acupuncture Points: A Study based on Laser Doppler Flowmetry and Infrared Thermography

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ABSTRACT

Background: To date, there has been a great lack of investigation on the influence of age on blood flow and temperature of acupoints in specific regions.

Objective: This study aimed to determine the association between different age categories and acupoint blood flow/temperature on the forearm.

Methods: Acupoint blood flow and temperature were measured in healthy adults of different age categories using laser doppler flowmetry (LDF) and infrared thermography (IRT), respectively. A total of 60 eligible healthy volunteers were divided into the young group, mid age group and old age group. All groups received LDF and IRT examination. Shenmen (HT7), Shaohai (HT3), Taiyuan (LU9) and Chize (LU5) of the Heart and Lung meridians on the forearm were selected as 4 test acupoints.

Results: Regarding blood flow of the 4 test acupoints, the PU of Taiyuan (LU9) in the old age group was significantly different compared with that of the young age group ($P < 0.05$) and the mid age group ($P < 0.05$), while there was no significant difference in PU of the other acupoints between 3 groups (all $P > 0.05$). Similarly, regarding acupoint temperature of the 4 test sites, the temperature of Shaohai (HT3) in the old age group was significantly different compared with that of the mid age group ($P < 0.05$), while there was no significant difference in the temperature of the other 3 acupoints between 3 age groups (all $P > 0.05$).

Conclusion: Age category tends to have notable influence on the blood flow and temperature in specific acupoints in the forearm. Therefore, particular concerns should be taken into consideration regarding the effect of age differences for future studies in this field. Nevertheless, further studies with a large sample size and more test acupoints are needed to further verify current findings.

1. Introduction

Acupuncture points (acupoints) are major parts of the meridian system that constitutes the most fundamental concept in traditional Chinese medicine (TCM), though the underlying anatomy and physiology of this system remain unclear. In the modern researches of acupuncture, the investigation into the specified characteristics of acupoints has always been a hotspot. With the aid of modern measurement techniques, previous studies in past decades have indicated that acupoints tend to have distinct characteristics when compared with non-acupoints,

such as significantly different microcirculatory, thermal, chemical, and electrical properties (Hsiu et al., 2007a, 2007b; Ding and Jiang, 2018; Zhou et al., 2012; Zheng et al., 2014).

Among them, the microcirculatory characteristics of acupoints can be assessed by laser doppler flowmetry (LDF) (Hsiu et al., 2007a, 2007b). LDF can monitor microcirculatory blood flow by exploiting Doppler shifts in laser light produced by moving red blood cells (Kouadio et al., 2018). Due to its notable clinical advantages, such as safety, noninvasiveness, and rapid responses (Roeykens et al., 2016), it is widely adopted in acupuncture trials and previous studies indi-

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Table 1
The general information of 60 subjects in 3 age groups.

	Gender (n)		Age (mean \pm SD)	Height (mean \pm SD)	Weight (mean \pm SD)	BMI (mean \pm SD)
	Male	Female				
Young age group	10	10	26.65 \pm 5.13	167.55 \pm 8.86	61.85 \pm 13.99	21.78 \pm 2.80
Mid age group	10	10	49.90 \pm 2.36	168.25 \pm 9.77	63.39 \pm 18.04	22.09 \pm 4.26
Old age group	10	10	65.35 \pm 3.95	166.80 \pm 8.41	61.95 \pm 13.16	21.97 \pm 3.28

cated acupoints have more affluent blood flow when compared with non-acupoints (Hsiu et al., 2007a, 2007b).

On the other hand, the thermal property of acupoints is always measured based on infrared thermography (IRT) (Zhou et al., 2012). IRT can visualize heat radiating from the body (Marins et al., 2014), thereby dynamically detecting the temperature of the body surface with the advantages of high sensitivity, good visualization, low cost and non-invasiveness (Ring and Ammer, 2012). Given that skin temperature can reflect the condition in underlying tissues during pathological states (Marins et al., 2014), IRT has been widely used to aid diagnosis of various diseases, including vascular deficiencies (Huang et al., 2011), neurological problems (Zaproudina et al., 2006), and cancer (Rassiwala et al., 2014), or as a tool for evaluation of outcomes in clinical trials (Ring and Ammer, 2012). Meanwhile, it is also often used to investigate the temperature of acupoints and meridians in acupuncture-related researches (Cai et al., 2019).

Although these two modern techniques are widely used in acupuncture trials (Lou et al., 2020; Agarwal-Kozlowski et al., 2009; Hsiu et al., 2011), investigation on the influence of different age categories on acupoint blood flow and temperature has been scarce to date. Since age heterogeneity is common in relevant studies, which might reduce the robustness of corresponding results, it is of great significance to investigate the influence of age on acupoint blood flow and temperature, the results of which would determine whether moderate age differences between different investigation groups can be tolerated, thereby enhancing the credibility of results involving subjects of significantly different age categories in such studies.

Thus, in this study, microcirculatory blood flow and temperature in four acupoints of interest on the forearm were measured and compared between 3 different age groups based on LDF and IRT. The results could provide references for the clinical application of such tests in acupoint studies.

2. Materials and Methods

2.1. Study subjects and grouping

A total of 60 healthy volunteers were recruited to receive examinations of LDF and IRT. All healthy participants were recruited from Zhejiang Chinese Medical University. All participants confirmed that they had no major systemic diseases such as cardiovascular, hematological, endocrine, and neurological diseases based on a recent medical examination report. In addition, they did not take any medication in the past 3 months, nor did they have skin injury or scars in the test sites of the forearm. Participants with alcohol dependence or drug abuse, and lactating or pregnant subjects were excluded. All subjects were fully informed of the study protocol and informed consent forms were signed.

In this study, we defined those aged 20–40, 41–60, and 61–80 years old as the young age group, mid age group, and old age group, respectively. Based on the defined age categories, 60 eligible healthy subjects were divided into 3 different age groups. 20 of them were between 20 and 40 years (26.65 \pm 5.13 years, male/female = 1:1), 20 of them were between 41 and 60 years (49.90 \pm 2.36 years, male/female = 1:1), and the remaining 20 were between 61 and 80 years (65.35 \pm 3.95 years, male/female = 1:1). The general information of the included subjects was displayed in Table 1.

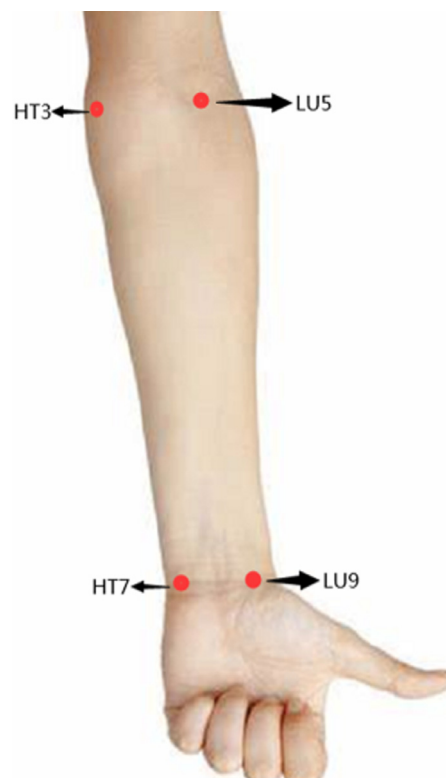


Fig. 1. The four acupoints of interest for LDF and IRT assessment. LDF = laser doppler flowmetry, IRT = infrared thermography.

2.2. Procedures for IRT and LDF examination

All included subjects received a session of LDF and IRT assessment, respectively. To minimize the interference effect induced by confounding factors, all examinations were carried out in a quiet experimental room at about the same time (i.e. 8:30–10:30 am) in July and August, with temperature controlled at 25 \pm 1°C and humidity controlled at 30–40%. For menstruant female subjects, we managed to avoid the test date of LDF and IRT overlapping with the menstrual period. Prior to formal examinations, all subjects were requested to drink no alcoholic, caffeine-containing drinks and avoid exercise during the 24 h before the experiment. The volunteers were asked to rest in the room in a supine position for 30 min with their forearms exposed. Throughout the whole assessment period of formal examination, they were informed to remain silent, keep normal breath and avoid forearm movement.

Four acupoints of interest, including Shenmen (HT7), Shaohai (HT3), Taiyuan (LU9), and Chize (LU5) on the forearm, were selected as test points. The four measurement sites are shown in Fig. 1 and their anatomical locations are displayed in Table 2.

In the first place, the healthy subject received an IRT assessment. A schematic diagram of the IRT assessment was exhibited in Fig. 2. The procedure was as follows. The temperature of the 4 acupoints was measured by a thermography camera (NEC InfRec R450, Avio Infrared Technologies Co., Ltd., Tokyo, Japan) for 10 min, with one infrared thermal

Table 2

Anatomical location of 4 acupoints selected as measurement sites.

Acupoints	Anatomical location
Shenmen (HT7)	On the anteromedial aspect of the wrist, radial to the flexor carpi ulnaris tendon, on the palmar wrist crease.
Taiyuan (LU9)	On the anterolateral aspect of the wrist, between the radial styloid process and the scaphoid bone, in the depression ulnar to the abductor pollicis longus tendon.
Chize (LU5)	On the anterior aspect of the elbow, at the cubital crease, in the depression lateral to the biceps brachii tendon.
Shaohai (HT3)	On the anteromedial aspect of the elbow, just anterior to the medial epicondyle of the humerus, at the same level as the cubital crease.

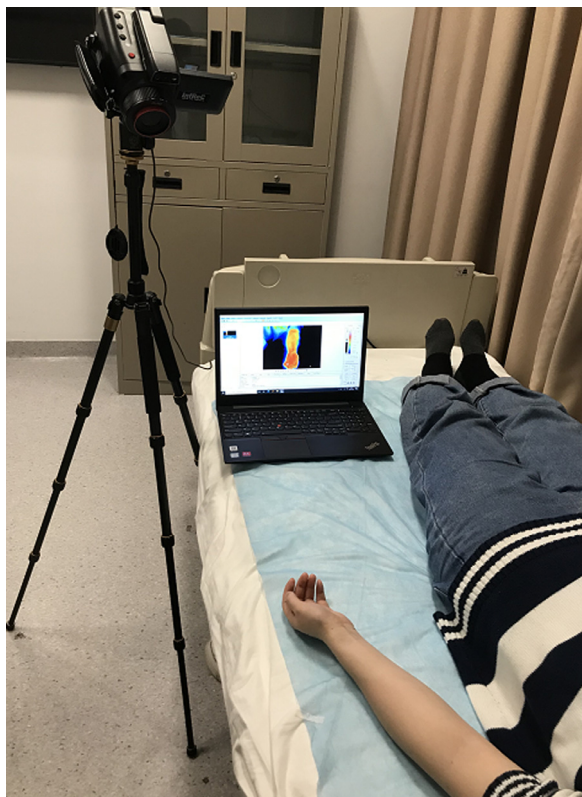


Fig. 2. A schematic diagram of the IRT assessment.

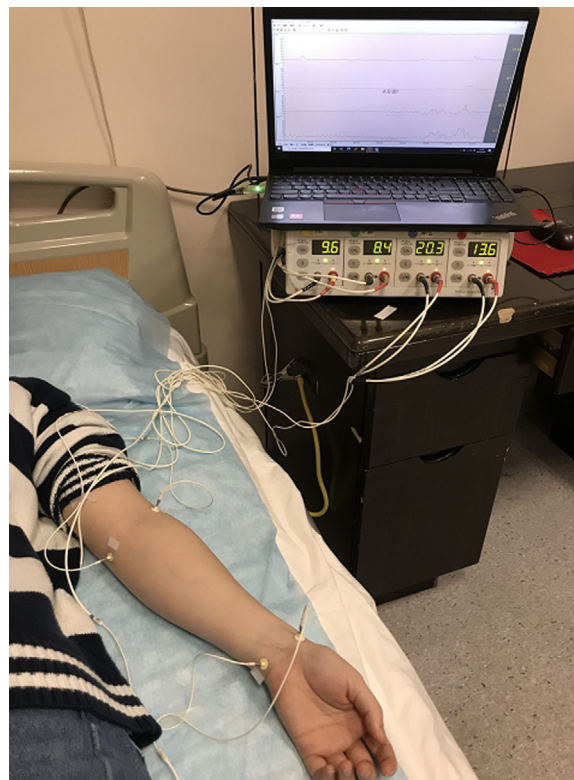


Fig. 3. A schematic diagram of the LDF assessment.

image taken each minute. A researcher placed the blunt head of a cotton swab over the 4 test acupoints in the forearm to help the other researcher locate these sites in the screen of the thermography camera. All acquired infrared thermal images were imported into the analysis software NS9500Std (Avio Infrared Technologies Co., Ltd., Tokyo, Japan) to extract the temperature data.

Subsequently, the healthy subject received a LDF assessment. A schematic diagram of the LDF assessment was exhibited in Fig. 3. In details, a four-channel LDF with a wavelength of 780 nm and time constant 0.2 (PeriFlux System 5000, Sweden) was adopted to measure the microcirculatory blood flow (calculate automatically as perfusion unit (PU) by PeriFlux System 5000) of the 4 test acupoints. The fiber probe model attaching to measurement sites was PeriFlux probe 407, which could detect the surface blood flow. Microcirculatory blood flow at the 4 test acupoints was monitored constantly for 10 min.

2.3. Statistical analysis

Statistical analysis was performed using the statistical software package SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA). Blood flow data and temperature data were displayed in mean \pm standard deviation (SD) if they were in a normal distribution.

Differences regarding PU and temperature of the four acupoints in 3 age groups were evaluated by one-way analysis of variance (ANOVA), and if an overall statistically significant ($P < 0.05$) difference was de-

tected, multiple comparisons between groups were performed using the Bonferroni's post-hoc test.

3. Results

As shown in Table 3 and Fig. 4, regarding microcirculatory blood flow in the four acupoints of interest on the forearm, the PU of Taiyuan (LU9) in the old age group was significantly different compared with that of the young age group ($P < 0.05$) and the mid age group ($P < 0.05$), while there was no significant difference in PU of the other acupoints between 3 different age groups (all $P > 0.05$).

On the other hand, regarding temperature in the four acupoints of interest on the forearm (as shown in Table 4 and Fig. 5), the temperature of Shaohai (HT3) in the old age group was significantly different compared with that of the mid age group ($P < 0.05$), while there was no significant difference in the temperature of the other 3 acupoints between 3 different age groups (all $P > 0.05$).

In addition, with the aim to explore the potential interference of the gender factor on the aforementioned test results, we analyzed them by dividing the included 60 subjects into the male group and the female group, with 30 cases in each group. As shown in supplementary Table 1, there was no statistical difference between the two groups in PU of 4 acupoints of heart and lung meridians (all $P > 0.05$). As shown in supplementary Table 2, there was also no statistical difference between the two groups in the temperature of 4 acupoints of the heart and lung

Table 3
Blood flow of 4 four test acupoints in 3 age groups (mean ± SD, PU).

Group	Taiyuan (LU9)	Chize (LU5)	Shenmen (HT7)	Shaohai (HT3)
Young age group (n = 20)	12.44 ± 2.62	12.47 ± 2.44	18.23 ± 4.70	8.03 ± 1.65
Mid age group (n = 20)	12.21 ± 3.70	11.58 ± 3.03	16.47 ± 7.05	7.56 ± 2.38
Old age group (n = 20)	15.14 ± 4.69*#	12.90 ± 2.88	18.96 ± 6.26	8.30 ± 1.42

Note: Compared with the young age group, there was a statistical difference (**P* < 0.05) in PU of LU9; Compared with the mid age group, a statistical difference (**P* < 0.05) in PU of LU9.

Table 4
Temperature of 4 four test acupoints in 3 age groups (mean ± SD, °C).

Group	Taiyuan (LU9)	Chize (LU5)	Shenmen (HT7)	Shaohai (HT3)
Young age group (n = 20)	31.97 ± 0.70	31.93 ± 0.60	32.59 ± 0.65	31.16 ± 0.71
Mid age group (n = 20)	31.97 ± 0.93	31.88 ± 0.56	32.46 ± 1.07	30.99 ± 0.76
Old age group (n = 20)	32.15 ± 0.98	32.03 ± 0.72	32.57 ± 0.94	31.61 ± 0.73*

Note: Compared with the mid age group, there was a statistical difference (**P* < 0.05) in the temperature of HT3.

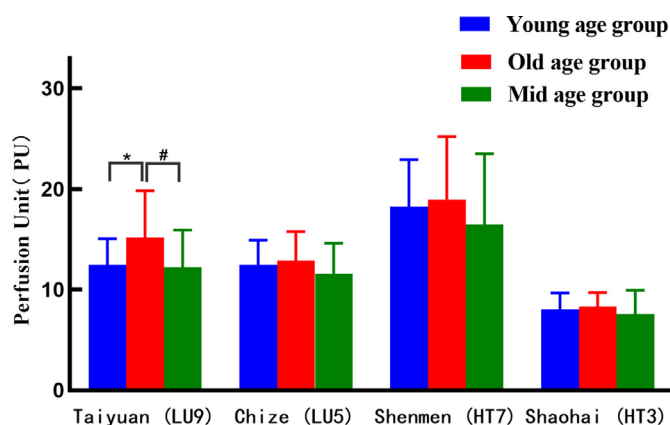


Fig. 4. Comparison of blood flow in 4 acupoints of interest between 3 age groups. **P* < 0.05, compared with the young age group; #*P* < 0.05, compared with the mid age group.

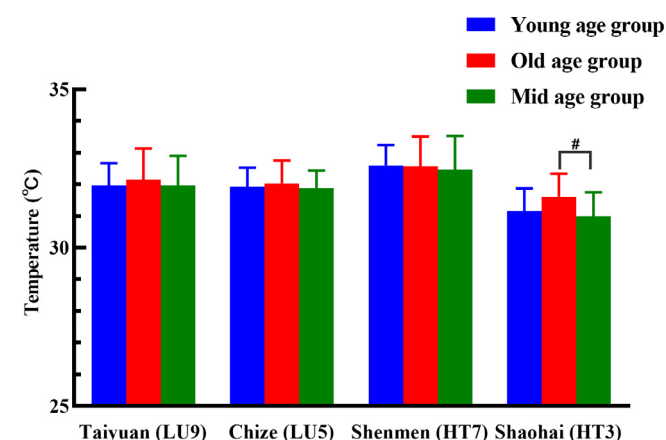


Fig. 5. Comparison of temperature in 4 acupoints of interest between 3 age groups. #*P* < 0.05, compared with mid age group.

second meridians (all *P* > 0.05). Thus, the potential interference caused by gender on the major study results and findings were eliminated to a certain degree.

4. Discussion

Although LDF and IRT have been widely used in acupoint studies (Cai et al., 2019; Lou et al., 2020; Agarwal-Kozlowski et al., 2009; Hsiu et al., 2011; Litscher, 2005), as far as we are concerned, there has been a great lack of investigations on the influence of different age categories on acupoint blood flow and temperature, which might reduce the credibility of study results involving subjects of different age stratifications in such trials. In such a scenario, this present study was conducted.

In this study, it is noteworthy that 4 specified acupoints in the forearm were selected as measurement sites because these 4 acupoints are the primary acupoints for comparisons in our subsequent larger-scale trials, which aims to investigate the specificity for "site-to-site" association on the body surface in the physiological state and the specificity for "meridian-viscera" association in the pathological state by selecting the heart and lung meridians for comparison. The protocols of these larger-scale trials have been previously published (Hu et al., 2020; Li et al., 2021). Thus, prior to our future studies that might inevitably include healthy subjects of significantly different age categories, we conducted this preliminary trial to determine the influence of different age categories on acupoint blood flow and temperature in advance, the results of which would determine whether moderate age differences between different investigation groups can be tolerated in the future study. The reasons for selecting these 4 acupoints in the present study and subsequent trials are expounded as follows. Among them, Shenmen (HT7) and Taiyuan (LU9) are the yuan (source) acupoint of the heart and lung meridians, respectively. Accordingly to theories of Traditional Chinese medicine (TCM), when a specific internal organ is in the pathological state, the yuan (source) acupoint of the corresponding meridian appears to be more sensitive sites to manifest abnormal changes (eg. abnormal microcirculatory blood flow and temperature) on the body surface. Beside, Shaohai (HT3) and Chize (LU5) are located on the same cross-section of the heart and lung meridians, thereby making them more suitable for comparison between these two meridians and their corresponding internal organs.

Based on findings of the present study, regarding the influence of age difference in blood flow of these 4 acupoints of interest, our study showed that the PU of Taiyuan (LU9) in the old age group was significantly different compared with that of the young age group (*P* < 0.05) and the mid age group (*P* < 0.05), while there was no significant difference in PU of the other acupoints among three age groups. Our results indicate that different age categories tend to have a notable influence on the blood flow and temperature in specific acupoints or regions on the forearm. Our results are partly consistent with a previous study (Stücker et al., 2001). In the study of Stücker et al., they mea-

sured the blood flow in more skin regions compared with our study, which included 20 different skin regions almost covering all the surface of the human body. Their study (Stücker et al., 2001) revealed that age difference had a significant effect on the blood flow of various skin regions including the dorsal aspect of the fingers, palms, back of the hands, bottom, thighs, and the lower legs, but no age-related differences were detected in other skin regions (i.e. forehead, cheek, lip, nose, chin, breast, abdomen, upper arm, forearm, neck, back, back of the fingers, palmar aspect of the fingers, soles, back of the foot). In addition, another study (Van den Brande et al., 1997) revealed that younger subjects ($n = 27$, aged 25–29 years) have more affluent blood flow than older subjects ($n = 22$, aged 60–92 years) at the dorsal foot skin.

Similarly, regarding the influence of age difference in temperature of the 4 acupoints of interest, the temperature of Shaohai (HT3) in the old age group was significantly different compared with that of the mid age group ($P < 0.05$), while there was no significant difference in the temperature of the other 3 acupoints between 3 different age groups (all $P > 0.05$). It appears that the temperature of HT3 was significantly higher in the old age group than the mid age group. The reasons might be partly explained as follows. First, the subcutaneous fat in the location of Shaohai (HT3) tends to be thinner in the old age group (Jiang et al., 2008), so it is easier to reveal the vessels (e.g. the basilic veins, ulnar superior and inferior accessory arteries) that underlying the anatomical structure Shaohai (HT3), thereby leading to a higher temperature of Shaohai (HT3) in the old age than the mid age group. This is consistent with previous findings that as the epidermis of the elderly becomes thinner and the light transmittance of the skin increases, the subpapillary vascular plexus is easier to be observed. In addition, due to the dilation and thickening of the blood vessels (Kelly et al., 1995), some small vessels or deeper vessels that are not easily observed in the skin of young people can also be detected in old people. Second, the difference between these two age groups may also be induced by measurement deviation because Shaohai (HT3) is close to the ulnar edge of the elbow joint so that it can be easily affected by ambient temperature during point selection analysis in IRT.

Moreover, it should be noted that multiple measures were adopted to minimize the confounding factors that might affect the results of acupoint blood flow and temperature in our study. First, healthy volunteers were chosen as study subjects because we aim to eliminate changes in blood flow and body temperature caused by disease as much as possible. Previous studies indicated that in pathological states, acupoints tend to represent specified changes in the microcirculatory blood flow (Peng et al., 2020; Ding et al., 2019). Similarly, the temperature of acupoints in specified meridians can increase or decrease if the corresponding viscera (Zang and Fu in TCM concepts) is in disease conditions (Xu et al., 2019; Du et al., 2018; Li et al., 2018). Second, an experimental room with controlled temperature and humidity was set up. Third, all healthy subjects were requested to rest for a long time to adapt to the environment. All LDF and IRT examinations were conducted at about the same period in the morning (Yang et al., 2007). Meanwhile, stimulating food or drink, and exercise were forbidden strictly before examinations. Lastly, the ratio of participant gender in each age category is controlled as 1:1 to avoid the influence of gender difference on results. All these measures could verify the robustness of the current findings of this study.

Nevertheless, several limitations of the present study should be emphasized. First, due to the exploratory nature of a pilot trial, this preliminary study involves a small sample size, which will reduce the robustness of current results. Second, although diverse kinds of measures had been adopted to minimize external and internal factors that affect acupoint blood flow and temperature, given that individual differences might be inevitable in some subjects, it may affect the reliability of results to a certain extent. For example, subjects' constitutions might have a potential influence on test results. Nonetheless, due to the nature of a preliminary trial, this study did not have sufficient research conditions to include a large number of subjects to investigate the interference

caused by different TCM constitution types on test results. Additionally, TCM constitution has numerous interaction effects with other possible confounding factors. Previous studies show that a specific TCM constitution type is unevenly distributed among subjects of different genders and age groups (Di et al., 2014). Therefore, it is difficult to limit all subjects in each age group to one specific TCM constitution type.

Last but not least, because these 4 test acupoints are the primary acupoints for comparisons in our subsequent larger-scale trials and it is due to technical restrictions (i.e. the maximum number of LDF probes is 4), only 4 acupoints in the heart and lung meridians were selected as measurement sites. In the future, more meridians (e.g. the pericardium meridian) and more acupoints will be measured to verify or revise current findings.

5. Conclusion

In conclusion, our study indicates that age category tends to have a notable influence on the blood flow and temperature in specific acupoints in the forearm. Therefore, particular concerns should be taken into consideration regarding the effect of age differences for future studies in this field. Nevertheless, further studies with a large sample size and more test acupoints in diverse regions are needed to further verify current findings.

Ethical Approval

Not applicable.

Data Availability

Nil.

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Declaration of Competing Interest

The authors declare that they have no competing interests.

CRediT authorship contribution statement

Yajun Zhang: Writing – original draft. **Xiaoyu Li:** Investigation, Data curation. **Xiaofen He:** Investigation. **Yongliang Jiang:** Writing – review & editing. **Hantong Hu:** Writing – review & editing. **Jiali Lou:** Writing – review & editing. **Ruijie Ma:** Supervision. **Yi Liang:** Formal analysis. **Xiaomei Shao:** Methodology. **Jianqiao Fang:** Methodology, Supervision.

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Supplementary Materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ccmp.2021.100018.

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