

Effect of Microbubble Bathing of Lower Extremities on Peripheral Circulation

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(Received October 27, 2010, accepted January 3, 2011)

Abstract Purpose: The purpose of the present study was to investigate the effect of microbubble bathing of lower extremities on peripheral circulation. **Methods:** Data were obtained in six healthy subjects under three exposure conditions of footbathing (for a duration of 10 min at a water temperature of 33 ± 1 °C) with microbubbles, macrobubbles, and control or no bubbles. Skin blood flow (measured from right foot) and skin temperature (measured at both hands and left foot) were recorded continuously for a period of 5 min before immersion, during 10-min immersion of legs with feet up to a level of mid-calf into water, and for a further 10 min after-immersion period (when the legs were removed and kept in the baseline posture). **Results:** The values of skin temperature recorded from the right hand during the third measurement period were significantly different ($P < 0.05$) among the three exposure conditions; the value of skin temperature was larger under the microbubble exposure condition compared with the other two exposure conditions. During the immersion period (second and third measurement periods), skin blood flow values differed significantly ($P < 0.01$) while compared among the different exposure conditions. However, compared with the control condition, the values were significantly higher only for exposure with microbubbles ($P < 0.05$). **Conclusion:** Microbubble bathing of lower extremities has a potential role in increasing peripheral circulation as a simple, safe and noninvasive method for this purpose.

Key words: microbubble, footbathing, skin temperature, skin blood flow

Introduction

Microbubbles are defined as the air bubbles which have a diameter of 10-40 micrometers at the time of generation.¹⁾ The generation of microbubbles in a two-step fluid mechanic process is called a high-speed turn method, and this method is characterized by high-speed rotations of gas or liquid.²⁾ The rotation speed is 400-600 rotations per second as observed from the pictures taken with a high-speed camera.³⁾

Microbubbles have a capability to shrink or decrease in size after generation.³⁻⁵⁾ When a microbubble shrinks and becomes smaller than 50 micrometers, its negative charge increases rapidly.³⁾ In contrast, ordinary macrobubbles rapidly float up and burst at the surface. The

special characteristics of the microbubbles indicate a great potential for their use in a variety of practical applications.⁴⁾ Recently, there is a growing concern regarding the development of technology for the generation of microbubbles.

Microbubbles were originally developed as an ultrasound contrast agent for diagnosis purpose, and in recent years, the microbubble technology has been applied to an environmental or industrial field. Application of microbubbles was found to promote the growth of oysters; the weight of the young oysters grew twice as compared with the usual oysters not treated with microbubbles.⁶⁾ A similar effect was found in case of scallop cultivation and pearl farming in Japan.⁶⁾ Park and Kurata in a recent study demonstrated that

microbubbles generated in a hydroponics solution significantly promoted plant growth. The authors speculated that the larger specific surface area of the microbubbles and negative electronic charges of the microbubbles surfaces may promote growth.⁷⁾

As revealed in the previous research works, application of microbubbles in water may have beneficial health effects.³⁻⁷⁾ Warm bathing could be incorporated with the use of microbubbles for a better effect on health. Any noninvasive method of increasing peripheral circulation would be beneficial clinically to many populations, especially with peripheral ulcers and for whom aerobic exercise is contraindicated or is not feasible.

There is no studies examining the effect of microbubble bathing on human peripheral circulation. Hence, the purpose of the present study was to investigate the effect of microbubble bathing of lower extremities on skin blood flow (SBF) and skin temperature (ST) of extremities. We hypothesized that microbubble bathing of lower extremities would enhance peripheral circulation.

Subjects and method

Six healthy nonsmoking male medical students aged 24 to 26 years (mean age \pm SD, 25 ± 0.6 yr; mean BMI \pm SD, 20.7 ± 1.3 kg/m²) were examined. Test room temperature was kept at 25 ± 1 °C. The subjects wore light clothing and rested in the seating position for 30 minutes before the start of the experiment; sensors were attached during this time. At the end of the rest period, each subject was asked to immerse both legs and feet up to a level of mid-calf area in a tank filled with tap water at 33 ± 1 °C, for a duration of 10 minutes. Figure 1 depicts the posture of the subject during exposure. The subjects maintained the posture with their both palms up on their thighs. After the exposure period, both legs were taken out of the tank and wiped lightly with a towel. The subject maintained the previous rest position and measurements were continued for further 10 minutes. The bubbles and the water current were generated two minutes prior to the immersion of the legs.

Each subject had three experiments with footbath under three different exposure con-

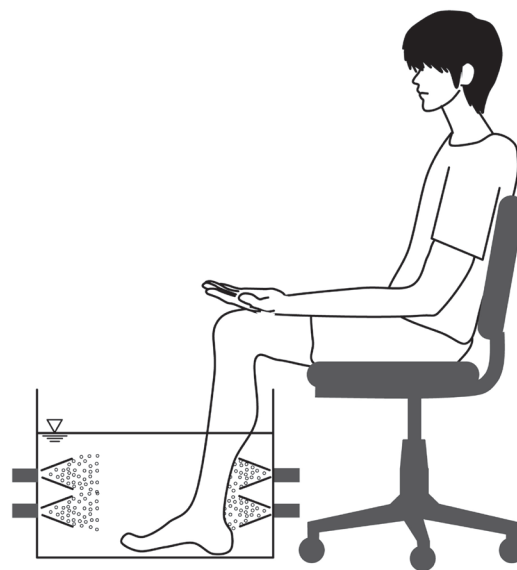


Fig. 1 Posture of the subject during exposure.

ditions: using tank water with microbubbles (experiment 1), macrobubbles (experiment 2) and control or no bubbles (experiment 3). Ten sets of microbubble generators (M2-MS, Nanoplanet Research Institute, Japan) were used. The microbubble generators were attached to the surface of the tank wall. A microbubble generator can generate microbubbles when the water flow to it is maintained at around 7 l/min and the air volume, at 1 l/min. Therefore, for the ten sets of generators in experiments 1 and 2, the water flow and air volume were set at 70 l/min and 10 l/min, respectively. Experiment 3 was without bubbles but with a water flow at 70 l/min. The macrobubbles were generated using the Air Compressor (HP60, Techono Takatsuki, Japan), and a ceramic stone, the small pores of which were able to produce uniform macrobubbles. The size of the macrobubbles generated by the equipments used in this study had a diameter more than 1 millimeter.

The flow velocity (directions X, Y and Z) at the measurement point of blood flow was measured using a three-dimensional electromagnetic current meter (VP3000, KENEK Corporation, Japan). The average velocities of water for the 3 directions (X, Y and Z axes) were 0.04, 0.22 and 0.04 m/sec, 0.38, 0.20 and 0.06 m/sec, 0.01, 0.11 and 0.11 m/sec for microbubble, macrobubble, and control conditions, respectively.

One experiment was conducted on a single

day for each subject; the order of different experimental conditions was controlled to eliminate the order effect. The subjects were not informed of the order. Each subject used ear muffs during all experimental sessions in order to reduce the sound while generating microbubbles and macrobubbles.

To measure SBF, laser tissue blood flowmeter (FLO-C1, Omegawave, Japan) was used. This blood flowmeter can measure the Doppler shift of reflected laser light, and thereby provide a measurement of blood flow at a depth of around 1 mm. Several earlier studies demonstrated the usefulness of such type of blood flowmeter in measuring blood flow.⁸⁾⁹⁾ In this study, the disc-shaped sensors of the blood flowmeter were fixed without exerting any pressure over the skin area of about 1.0 cm in diameter. To measure ST, a digital thermistor (K730, Technolseven, Japan) was used. The measurements were recorded continuously for a period of 5 min before immersion, during 10-min of immersion and for a further 10 min after-immersion period.

The sensor of the blood-flow meter was fixed to the dorsal side of the middle phalanx of middle toe in the right foot. Sensors of the thermistor were attached on the dorsal side at the middle phalanges of right and left hands (middle fingers) and at the middle phalanx of the second toe of right foot. Blood pressure and heart rate were also measured at the right upper arm before and after immersion using a fully automated blood pressure monitor (Dinamap 8100, Critikon, USA).

Statistical analysis

Values of blood pressure and heart rate before and after immersion were compared. For comparison of the SBF and ST values ob-

tained under different exposure conditions, average values of each were calculated for an equal 5 min period before, during and after immersion. Statistical analysis was done with Friedman's test and Wilcoxon signed-ranks test, with Bonferroni correction for multiple comparisons where appropriate, by using the SPSS statistical software version 16.0. Statistical significance was considered at $P < 0.05$.

Ethical consideration

The research outline and methods were explained to the subjects, and their written informed consent was taken. The subjects had the right to interrupt the experiment even if it was in the middle of an experimental session.

Results

The average values of systolic and diastolic blood pressures, and heart rate under the three exposure conditions are shown in Table 1. The values before and after exposure did not differ significantly in any of the exposure condition except for systolic blood pressure under the control condition ($P = 0.040$).

Figures 2 and 3 show the average values of ST at each minute for six subjects under different exposure conditions, at the dorsal side of the middle fingers of right and left hands, respectively. Table 2 shows the average values of ST at different measurement periods under different exposure conditions from the dorsal side of the middle fingers of right and left hands. No statistically significant differences between the three exposure conditions were revealed at any measurement period in any of the measurement locations, except that the values of ST recorded from the right hand during the third measurement period were signifi-

Table 1 Blood pressure and heart rate under three exposure conditions

| | Microbubble | | Macrobubble | | Control | |
|------------------|-----------------|----------------|-----------------|----------------|-----------------|----------------|
| | Before exposure | After exposure | Before exposure | After exposure | Before exposure | After exposure |
| SBP (mmHg) | 115.5±3.8 | 119.2±4.5 | 117.3±2.9 | 114.7±2.1 | 118.0±6.2 | 112.8±3.5* |
| DBP (mmHg) | 67.3±5.5 | 69.5±3.7 | 64.8±5.3 | 66.8±4.4 | 65.0±9.0 | 66.2±5.4 |
| Heart rate (bpm) | 68.0±10.2 | 71.7±8.5 | 64.8±6.6 | 66.3±5.4 | 71.8±4.8 | 71.0±5.06 |

SBP: systolic blood pressure

DBP: diastolic blood pressure

Significantly different from the before exposure value: * $P < 0.05$

Values are shown as mean ± SD for six subjects.

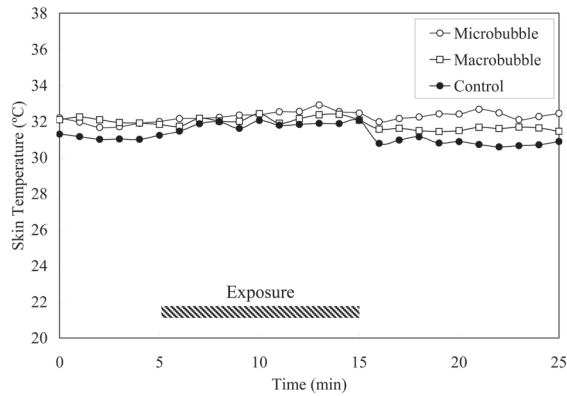


Fig. 2 Changes in average skin temperature (°C) at the middle finger of right hand for six subjects under different exposure conditions.

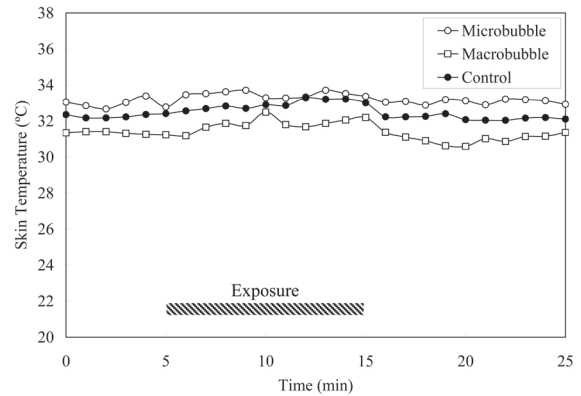


Fig. 3 Changes in average skin temperature (°C) at the middle finger of left hand for six subjects under different exposure conditions.

Table 2 Skin temperature (°C) from the middle finger of right and left hands at different time intervals under three exposure conditions

| Time (min) | Right middle finger in dorsal side (°C) | | | | Left middle finger in dorsal side (°C) | | | | |
|------------|---|-------------|-------------|----------|--|-------------|-------------|----------|---------|
| | | Microbubble | Macrobubble | Control | P value | Microbubble | Macrobubble | Control | P value |
| 1~5 | Before | 32.9±1.2 | 31.3±1.8 | 32.3±1.3 | 0.607 | 31.9±1.7 | 32.0±2.6 | 31.1±1.8 | 0.115 |
| 6~10 | Exposure | 33.5±1.1 | 31.8±1.6 | 32.7±1.3 | 0.846 | 32.3±1.4 | 32.1±2.4 | 31.8±1.4 | 0.069 |
| 11~15 | Exposure | 33.4±1.0 | 31.9±1.6 | 33.1±1.4 | 0.607 | 32.6±1.4 | 32.2±2.4 | 31.9±1.3 | 0.042 |
| 16~20 | After | 33.1±0.9 | 30.9±2.3 | 32.2±1.2 | 0.223 | 32.2±1.6 | 31.5±3.0 | 30.9±1.6 | 0.135 |
| 21~25 | After | 33.1±1.0 | 31.1±2.1 | 32.1±1.3 | 0.223 | 32.4±1.4 | 31.6±3.1 | 30.7±1.7 | 0.135 |

Values are shown as mean ± SD for six subjects.

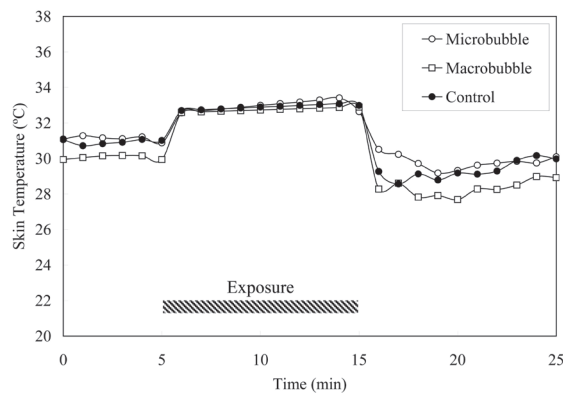


Fig. 4 Changes in average skin temperature (°C) at the second toe of right foot for six subjects under different exposure conditions.

cantly different ($P = 0.042$) among these three conditions; the value of ST was larger under the microbubble exposure condition compared with the other two exposure conditions.

Figure 4 shows the average values of ST at each minute for the dorsal side of the second toe of right foot for six subjects, under different exposure conditions. Table 3 shows the average values of ST at different measurement periods under different exposure conditions from the dorsal side of the second toe of right foot. No statistically significant differences among the three exposure conditions were revealed at any measurement period in any

Table 3 Skin temperature (°C) from the second toe of right foot at different time intervals under three exposure conditions

| Time (min) | | Microbubble | Macrobubble | Control | P value |
|------------|----------|-------------|-------------|----------|---------|
| 1~5 | Before | 31.1±2.4 | 30.1±3.1 | 30.9±3.1 | 0.513 |
| 6~10 | Exposure | 32.8±0.3 | 32.7±0.2 | 32.8±0.1 | 0.607 |
| 11~15 | Exposure | 33.1±0.4 | 32.8±0.2 | 33.0±0.1 | 0.115 |
| 16~20 | After | 29.8±1.1 | 28.1±1.7 | 29.0±2.6 | 0.115 |
| 21~25 | After | 29.8±1.6 | 28.6±2.4 | 29.7±3.0 | 0.846 |

Value are shown as mean±SD for six subjects.

of the measurement location. The ST after immersion decreased under all exposure conditions. Although the condition of microbubble had the tendency for a higher ST compared with other conditions, the difference was not significant.

The average change in SBF from the middle toe of right legs is depicted in Table 4 and Figure 5. During the immersion period (second and third measurement periods), SBF values differed significantly ($P = 0.006$ and $P = 0.009$, respectively) while compared among the different exposure conditions. However, compared with the control condition, the values were significantly higher ($P = 0.040$, $P = 0.014$, respectively) only for exposure with microbubbles. In general there was a trend for an increase in SBF during exposure followed by a decreasing trend after exposure.

Discussion

A number of research works on hot bath has been conducted during the past few decades,

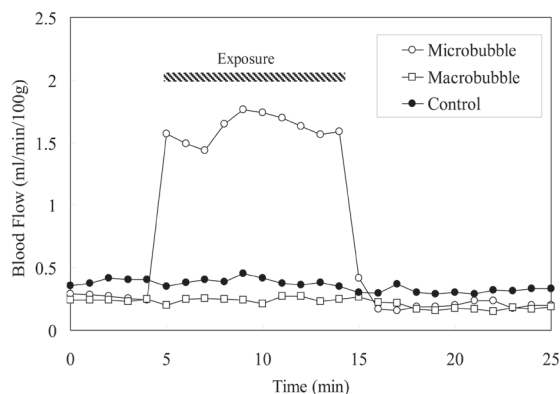


Fig. 5 Changes in average blood flow (ml/min/100g) at the middle toe of right foot for six subjects under different exposure conditions.

Table 4 Blood flow (ml/min/100g) from the middle toe of right foot at different time intervals under three exposure conditions

| Time (min) | | Microbubble | Macrobubble | Control | P value |
|------------|----------|-------------|-------------|-----------|---------|
| 1~5 | Before | 0.52±0.29 | 0.23±0.18 | 0.43±0.23 | 0.115 |
| 6~10 | Exposure | 1.62±0.71* | 0.24±0.12 | 0.45±0.21 | 0.006 |
| 11~15 | Exposure | 1.38±0.44* | 0.26±0.12 | 0.39±0.18 | 0.009 |
| 16~20 | After | 0.18±0.03 | 0.18±0.15 | 0.35±0.20 | 0.513 |
| 21~25 | After | 0.21±0.10 | 0.15±0.14 | 0.35±0.21 | 0.311 |

Significantly different from the corresponding control value: * $P < 0.05$
Value are shown as mean±SD for six subjects.

and in recent years, the health effects of footbathing are being reported. Those studies demonstrated various effects; psychological and immunological effects, effect on sleep, influence on autonomic nerve system, relaxation effect etc.¹⁰⁻¹³ As demonstrated in previous studies, footbath with warm water of certain temperatures caused an increase peripheral blood flow without affecting core body temperature.¹⁴ Warm footbath is also an effective method of relaxation.¹⁵ Moreover, heart rate during footbathing remains within normal range, and hence, footbathing is also a safe intervention for the elderly.¹⁴

Various methods of footbathing have been reported in the literature: footbath simply with warm water; footbath with essential oils and bath salts, with washing and massage, or with exposure to carbon dioxide.¹⁶⁻¹⁸ The water temperature and immersion time vary among studies.¹⁰⁾¹²⁾¹⁴⁾¹⁹⁾²⁰⁾ In this experiment, we used a water temperature which is near to our body skin surface temperature, in order to avoid any confounding influence of heat on peripheral circulation.

As observed in the present study, systolic blood pressure under the control condition differed significantly after exposure compared to the before exposure value. In contrast, there was no remarkable change in subject's blood pressure or heart rate after micro- or macrobubble bathing of lower extremities, which indicates that such bathing did not exert any stress to the cardiopulmonary function of the study population.

During exposure, under all conditions, there was an increasing trend for hand skin temperature, which showed a significant difference during the second half of immersion period; skin temperature was higher under the condi-

tion of bathing with microbubbles. Furthermore, as revealed in this study, a significant difference among the three exposure conditions was observed in blood flow measured with the laser tissue blood flowmeter; compared with the control condition, the difference (increase) was significant only under the condition of bathing with microbubbles. All these findings indicate the capability of microbubble bathing to positively influence the peripheral circulation.

In this experiment, the response patterns in ST and SBF were not similar. Microcirculation in human hand skin is accomplished through the superficial capillary network and the deeper subpapillary network.²¹⁾²²⁾ The blood flowmeter used in this study could record blood flow in the superficial vessels of skin.⁹⁾ In contrast, peripheral skin temperature reflects mainly the volume of blood flow in deeply located vessels responsible for thermoregulation.²³⁾ Therefore, the responses in peripheral ST and SBF may differ from same type of exposures or provocations. Such different patterns of responses in ST and SBF have been observed in previous studies also.²³⁾²⁴⁾

The underlying mechanisms for the above-mentioned responses in SBF and ST are difficult to explain due to limited literatures on the topic. Also, from our study design, it is not possible to explain the mechanisms lying behind the observed effects with the application of microbubbles. As it has been demonstrated earlier, warm footbath increases parasympathetic activity and decreases sympathetic activity.¹⁵⁾ In a study by Shimizu et al., the authors concluded that microbubble bathing provides more comfort and relaxation than the other bathing conditions without microbubbles.²⁵⁾ In another study, microbubble bathing containing oxygen was more effective for refreshing and relaxing for a smooth transition to sleep as well as for soothing dry skin.²⁶⁾ It may be possible that the activity of parasympathetic nervous system is augmented and that of the sympathetic nervous system is diminished further by the footbath with microbubbles. As mentioned by Harada et al., sensory neuron stimulation in the skin from micro-bubble bathing can enhance the release of calcitonin gene-related peptide (CGRP) from the nerve endings.²⁷⁾ There is a possibility that the in-

creased peripheral circulation observed in our study is mediated by the vasodilation induced by the activity of CGRP.²⁸⁾ In future, the local interaction of microbubbles with the skin (e.g. role of microbubbles on skin pores and permeability) needs to be investigated.

During the post-immersion period in the foot, the skin temperature and blood flow decreased after an initial rise during the immersion. Since the room temperature was set at 25 °C, skin temperature and blood flow of the foot probably decreased due to evaporation of heat from the lower extremity. However, it might be interesting to observe the changes in peripheral circulation with microbubble bathing at a higher room temperature or water temperature.

The findings of the current study indicate that warm footbath with application of microbubbles was significantly more effective in improving peripheral circulation compared to only warm footbath or footbath combined with application of macrobubble. However, the optimum water temperature and duration of microbubble bathing of extremities need to be confirmed in future studies.

Limitations to this study

Firstly, a small number of study subjects participated in this study. Secondly, this study was conducted with young healthy subjects and the generalizability of the current findings to other groups is uncertain. Thirdly, microbubble bathing was done with a single water temperature of 33 °C for a fixed duration of exposure (10 min). Future studies should focus on the effects of microbubble bathing on peripheral circulation by changing the density of microbubbles, with a different water temperature and exposure time.

Conclusions

From the observed results it can be concluded that as a simple, safe and noninvasive method, microbubble bathing of lower extremities has a potential role in increasing peripheral circulation. Further research works with a larger sample size are necessary to confirm the findings of this study.

Acknowledgements

The author is grateful to Prof. Noriaki Harada (Department of Hygiene, Yamaguchi University Graduate School of Medicine, Japan) for his academic advice and final revision of this manuscript, and Dr. Hossain Md. Mahbub (Department of Hygiene, Yamaguchi University Graduate School of Medicine, Japan) and Prof. Hirofumi Ohnari (Tokuyama College of Technology, Yamaguchi, Japan) for their valuable advice on statistical analysis and in the preparation of the manuscript.

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