

Effects of Short-Term Forest Bathing on Human Health in a Broad-Leaved Evergreen Forest in Zhejiang Province, China*

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Abstract

Objective To investigate the effects of short-term forest bathing on human health.

Methods Twenty healthy male university students participated as subjects and were randomly divided into two groups of 10. One group was sent on a two-night trip to a broad-leaved evergreen forest, and the other was sent to a city area. Serum cytokine levels reflecting inflammatory and stress response, indicators reflecting oxidative stress, the distribution of leukocyte subsets, and plasma endothelin-1 (ET-1) concentrations were measured before and after the experiment to evaluate the positive health effects of forest environments. A profile of mood states (POMS) evaluation was used to assess changes in mood states.

Results No significant differences in the baseline values of the indicators were observed between the two groups before the experiment. Subjects exposed to the forest environment showed reduced oxidative stress and pro-inflammatory level, as evidenced by decreased malondialdehyde, interleukin-6, and tumor necrosis factor α levels compared with the urban group. Serum cortisol levels were also lower than in the urban group. Notably, the concentration of plasma ET-1 was much lower in subjects exposed to the forest environment. The POMS evaluation showed that after exposure to the forest environment, subjects had lower scores in the negative subscales, and the score for vigor was increased.

Conclusion Forest bathing is beneficial to human health, perhaps through preventive effects related to several pathological factors.

Key words: Forest bathing; Oxidative stress; Inflammation; Endothelin-1; Cortisol level

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INTRODUCTION

During the first 5 million years of their history, humans lived in forest environments, and it was only several thousands years ago that

we started living in urban environments. It is widely understood that forest environments have favorable effects on human physiological functions, and sanatoria are often built in forest environments on mountains^[1]. A number of reports have indicated

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that forest environments exert beneficial physiological effects on patients with allergies or respiratory diseases^[2]. Factors in the forest environment that may provide beneficial physiological effects include the aroma of plants as well as such various factors as temperature, humidity, light intensity, wind, and oxygen concentrations; thus, exercise performed in such environments would appear to offer significant benefits^[3].

In contrast, people living in an urban environment are prone to developing such diseases as chronic fatigue syndrome. Otherwise normal healthy subjects may develop chronic fatigue syndrome as a result of increased fatigue and stress if they are not appropriately treated^[4]. In addition, urban air pollution is a serious environmental problem, especially in many developing countries. Epidemiological and mechanistic animal studies from across the world have shown that both acute and chronic exposure to air pollution is associated with chronic diseases and mortality^[5-7], thus impacting public health. To improve the quality of life of those who live in cities, it has been suggested that society develop better methods to facilitate and promote healthy activities that can be performed in a very short period of time, are inexpensive, and are also enjoyable.

Forest bathing, known as *shinrin-yoku* in Japan, has received increasing attention in recent years for its positive effects on human health. Previous studies on this topic have mostly focused on the capacity of forest bathing to provide relaxation and reduce stress for people living in urban areas, mainly in terms of its physiological effects. However, there have as yet been no direct demonstrations as to whether forest bathing exerts any other effects on human health. In the present study, we aimed to investigate the positive effects of forest bathing on human health in terms of the pathological factors involved in oxidative stress, inflammation, immunity, and cardiovascular diseases in healthy young subjects. We chose a forest site in Hangzhou, Zhejiang Province, China, since forests occupy 60.58% of the land area of this province and there is ready access to forests.

MATERIALS AND METHODS

Experimental Sites

The experiments were conducted in a broad-leaved evergreen forest named Wuchao

Mountain Forest in Hangzhou (Zhejiang, China) from 26 to 28 September 2010. The forest covers an area of about 330 000 m², and the predominant species are *Ormosia hosiei*, *Diospyros glaucifolia*, *Chamaecyparis pisifera*, *Zelkova schneideriana*, *Machilus pauhoi*, *Cladrastis platycarpa*, *Manglietia yuyuanensis*, *Cinnamomum camphora*, *Ormosia hosiei*, *Magnolia officinalis* subsp. *biloba*, and *Nyssa sinensis*. An urban area was used for comparison. The two experimental sites are shown on the map in Figure 1. Hereinafter, the two sites are referred to as the forest area and the city area.



Figure 1. Location of the experimental sites. Wuchao Mountain Forest is located in the suburban district of Hangzhou and is about 15 km from the urban experimental site, which is located in the downtown area.

Subjects

This study enrolled 20 normal male university students (age 20.79±0.54 years). None of the subjects reported any physiological or psychiatric disorders in their personal histories. The study was approved by our hospital's ethics committee, and the procedures were in accordance with the Helsinki Declaration of 1975 as revised in 1983. The study was fully explained to all the subjects in both spoken and written form, specifically focusing on its purpose, the precise procedures that would be used, and any possible adverse events. Signed informed consent was obtained from every subject.

Procedure

The experimental schedule for this study is shown in Figure 2. On the day before the experiments, all the subjects were fully informed about the experimental procedure; blood from each subject was sampled in the morning before breakfast in our hospital, and the samples were tested in the clinical laboratory by technicians. Two hotels offering similar conditions near each experimental site were chosen as the places of accommodation.

To control for environmental conditions, the same single rooms were prepared as lodgings for each subject, and the same meals were offered during the experiments. The subjects were randomly divided into two groups consisting of 10 people each. One group was sent to the forest site, the other to the city site. On the morning of 26 September, each group was taken to its experimental site. The subjects then walked along a predetermined course in each area at an unhurried pace for about 1.5 h, with a 10-minute rest during the walk. In the afternoon, after taking lunch in the resting room, the subjects walked another predetermined course in each area at an unhurried pace for about 1.5 h, with a 10-minute rest during the walk. The subjects were allowed to do as they wished in the hotel, though avoiding strenuous exercise and any stimulating activities in their hours of relaxation before sleeping. The subjects were kept at each experimental site for two days. In the morning before breakfast on 28 September, both two groups were taken to our hospital (it took 30-40 min by car for each group to return from the experimental sites), and blood was sampled for tests in the clinical laboratory by technicians.



Figure 2. The experimental protocol for subjects exposed to the forest or urban environment.

SOD Activity Detection

The activity for serum total SOD (T-SOD) was examined according to the xanthine oxidase method using a standard assay kit (Nanjing Jiancheng Bioengineering Institute, China). The assay employs the xanthine-xanthine oxidase system to produce

superoxide ions, which react with 2-(4-iodophenyl)-3-(4-nitrophenyl-5-phenyltetrazolium chloride) to form a red formazan dye, and the absorbance at 550 nm was determined. One unit of SOD was defined as the amount of SOD inhibiting the rate of reaction by 50%.

Malondialdehyde Level Measurement

Lipid peroxidation was evaluated by measuring malondialdehyde (MDA) concentrations according to the thiobarbituric acid (TBA) method, as commercially recommended (Nanjing Jiancheng Bioengineering Institute, China). The method was based on the spectrophotometric measurement of the color produced during the reaction to TBA with MDA. MDA concentrations were calculated by the absorbance of TBA reactive substances (TBARS) at 532 nm.

Cytokine Production

Serum and plasma samples were analyzed using commercially available radioimmunoassay kits (Poole Albert Biotechnology Co., Ltd., Beijing, P.R. China) for interleukin-6 (IL-6), tumor necrosis factor α (TNF- α), and endothelin-1 (ET-1), according to the manufacturer's protocol.

Serum Cortisol and Testosterone Level

Quantitative determination kits with chemiluminescent immunoassay (Beckman Coulter, Inc.USA) were used to examine the changes in serum cortisol and testosterone levels after forest bathing, according to the manufacturer's protocol.

Lymphocyte Assay

Lymphocyte assay was performed as described previously^[8]. In brief, peripheral blood lymphocyte subsets were assessed using fluorochrome-conjugated monoclonal antibodies specific for lymphocytes from the Simultest IMK-Lymphocyte kit (BD Biosciences, San Jose, CA, USA). A fluorescence-activated cell-sorting (FACS Calibur) flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) was used to determine lymphocyte subsets, CD₅⁺/CD₁₉⁺ (B cells), CD₃⁺ (T cells), CD₃⁺/CD₄⁺ (T-helper cells), CD₃⁺/CD₈⁺ (T suppressor cells), and CD₃⁻/CD₁₆⁺/CD₅₆⁺ (natural killer cells).

POMS Evaluation

The profile of mood states (POMS) assessment provides a rapid, efficient method of assessing

transient, fluctuating active mood states, and it is widely used in mood and mental health assessment. In this study, we used the standard version of POMS^[9], a 65-item self-administered rating scale that measures six dimensions of mood (tension-anxiety, depression-dejection, fatigue-inertia, confusion-bewilderment, vigor-activity, and anger-hostility) to assess the subjects' mood changes on the evening of 27 September; the POMS assessment provides detailed scores for all the scales.

Data Analysis

The results were expressed as ($\bar{x} \pm s$). Final data analysis was performed using SPSS version 17.0 (obtained from SPSS China, Shanghai). Samples were initially analyzed using the Kolmogorov-Smirnov test and Levene's test for, respectively, normality, and homogeneity of variances. If the samples were closed to normal distribution and had homogeneous variance, the *t* test was used for data comparison between the two groups. Otherwise, a non-parametric test (Mann-Whitney test) was used. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Baseline Characteristics of Participants

Twenty normal male university students (age 20.79±0.54 years) participated as study subjects. They were randomly divided into two groups before the experiments. Basic indicators, such as body mass index (BMI), blood pressure, and heart rate (HR), were not significantly different between the two groups (Table 1). In addition, no significant differences in baseline values of biological indicators—including TNF- α , IL-6, T-SOD, MDA, ET-1, cortisol, testosterone, and the distribution of leukocyte subsets—were observed between the two groups (see Table 1).

Effect of Forest Bathing on Serum Pro-inflammatory Cytokine Levels

As shown in Table 2, the serum TNF α and IL-6 levels were significantly reduced in the forest-bathing group compared with the urban group. Reduced levels of pro-inflammatory cytokines indicate that spending time in a forest environment may exert a lower inflammatory response, which may improve pathological conditions. We also monitored the levels of C-reactive protein (CRP) and hypersensitivity C-reactive protein (H-CRP)—two

Table 1. Baseline Levels of Indicators of the Subjects before the Study ($\bar{x} \pm s$)

	City Group (n=10)	Forest Group (n=10)	P Value
High (m)	1.73±0.05	1.73±0.07	0.928
Weight (kg)	63.45±7.03	62.40±7.29	0.747
BMI (kg/m ²)	21.12±1.77	20.82±1.74	0.718
SBP (mmHg)	117.80±8.18	119.60±8.10	0.591
DBP (mmHg)	71.40±9.63	76.80±5.85	0.147
HR (bpm)	67.90±9.39	73.00±11.84	0.300
IL-6 (pg/mL)	67.05±15.14	88.45±59.05	0.481*
TNF- α (ng/mL)	0.65±0.67	0.70±0.64	0.853*
T-SOD (U/mL)	76.48±7.15	75.06±8.87	0.697
MDA (nmol/L)	7.79±3.21	8.39±5.63	0.771*
ET-1 (pg/mL)	63.19±24.00	60.12±16.38	0.742
Cortisol (nmol/L)	577.14±147.85	620.95±204.73	0.590
Testosterone (ng/dL)	541.82±85.61	517.99±132.52	0.639
Total T cell (%)	70.20±5.43	71.60±5.78	0.584
Total B cell (%)	10.90±3.18	11.70±3.65	0.608
Thlymphocyte(%)	35.70±6.22	38.00±6.29	0.422
Tslymphocyte %)	30.80±3.71	29.70±6.88	0.622
NK cell (%)	18.30±7.94	16.00±5.66	0.307
CD ₄ /CD ₈	1.19±0.30	1.35±0.40	0.465
Platelet activation (CD _{42a} ⁺ /CD ₁₄)	10.05±2.81	8.78±1.28	0.212

Note. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate. *Mann-Whitney test was used; others were analyzed using the independent-samples *t* test.

Table 2. Effect of Forest Bathing on Serum Pro-inflammatory Cytokine Levels in Experimental Subjects ($\bar{x} \pm s$)

	City Group (n=10)	Forest Group (n=10)	Z Value	P Value
IL-6 (pg/mL)	138.59±90.42	85.07±56.83	-2.419	0.016
TNF- α (ng/mL)	1.24±0.67	0.33±0.02	-2.457	0.014

Note. The Mann-Whitney test was used for data analysis.

well-established markers of inflammatory status—to check the effect of forest bathing on the inflammatory response. As expected, a mild reduction in the two parameters was also observed in forest-bathing groups, though it was not statistically significant (data not shown).

Effect of Forest Bathing on Oxidative Stress Status

Subjects staying forest area showed a decreased MDA level—an indicator of lipid peroxidation in the serum—compared with urban groups (Table 3). However, no obvious change in the activity of T-SOD was observed.

Table 3. Effect of Forest Bathing on T-SOD Activity and MDA Level in the Experimental Subjects ($\bar{x} \pm s$)

	City Group (n=10)	Forest Group (n=10)	T Value	P Value
T-SOD (pg/mL)	91.99±10.01	72.89±18.78	-1.353	0.193
MDA (ng/mL)	8.65±1.70	5.44±1.34	-4.683	0.001

Note. Independent-samples *t* test was used for data analysis.

Effect of Forest Bathing on Factors Associated with Cardiovascular Disease

ET-1 is by far the most powerful vasoconstrictor known, and it is always involved in the progression of cardiovascular diseases^[10]. The results showed that the ET-1 level of subjects that had experienced forest bathing was significantly lower than that of subjects exposed to the urban environment (Table 4). Platelet activation also plays an important role in the occurrence and development of cardiovascular diseases^[11]. However, no significant changes in this indicator were observed between the two groups.

Table 4. Effect of Forest Bathing on Plasma ET-1 Level and Platelet Activation ($\bar{x} \pm s$)

	City Group (n=10)	Forest Group (n=10)	T Value	P Value
ET-1 (pg/mL)	86.11±12.92	54.05±23.77	-3.747	0.002
Platelet activation (CD _{42a} ⁺ /CD ₁₄)	9.50±2.53	9.42±2.20	-0.663	0.516

Note. Independent-Samples *t* test was used for data analysis.

Effect of Forest Bathing on Distribution of Leukocyte Subsets

The total number of circulating leukocytes in the subjects is shown in Table 5. An obviously higher level of B (CD₃⁻/CD₁₉⁺) lymphocytes was observed in the forest group than in the urban group (*P*<0.05). Additionally, elevated percentages of T (CD₃⁺), T-helper (CD₃⁺/CD₄⁺), and NK (CD₃⁻/CD₅₆⁺/CD₁₆⁺) lymphocytes and a reduced level of T suppressor cells (CD₃⁺/CD₈⁺) were also found in the forest group, though the difference between the two groups was not statistically significant.

Effect of Forest Bathing on Stress Response

Serum cortisol level has been used as a stress marker in various scientific fields. In our field studies, we confirmed that the serum cortisol concentration was significantly lower in subjects exposed to a forest environment than in those exposed to an urban environment (Figure 3). However, the change in another stress-associated indicator, testosterone, was not statistically significant. These findings indicate that subjects staying in a forest environment showed a reduced stress response.

Table 5. Distribution of Total Leukocyte Percentage in the Peripheral Blood of Subjects after Exposure to Forest and Urban Environments ($\bar{x} \pm s$)

	City Group (n=10)	Forest Group (n=10)	T Value	P Value
Total T cell (%)	65.30±5.27	66.30±4.57	0.646	0.527
Total B cell (%)	9.60±2.63	11.90±2.23	2.106	0.049
Th lymphocyte (%)	30.80±6.91	32.70±6.41	1.234	0.233
Ts lymphocyte (%)	29.50±2.84	29.30±3.62	-0.620	0.543
CD ₄ /CD ₈	1.07±0.31	1.14±0.30	1.532	0.143
NK cell (%)	22.50±6.77	24.50±8.34	1.399	0.179

Note. Independent-samples *t* test was used for data analysis.

Mood State Evaluation

In this experiment, we used the POMS standard version to assess the subjects' mood changes. The scores in the negative subscales, such as tension, depression, anger, fatigue, and confusion, were lowered; however, the positive score for vigor was increased (Figure 4).

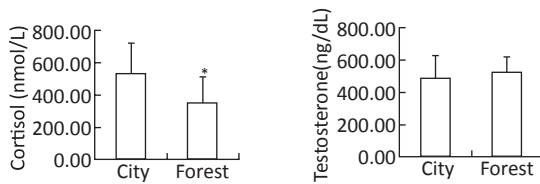


Figure 3. Effect of forest bathing on serum cortisol and testosterone levels ($n=10$). Two quantitative determination kits using chemiluminescent immunoassay were employed to detect the serum cortisol and testosterone levels in the forest-bathing and urban control groups. The Mann-Whitney test was used for data analysis. * $P<0.05$ vs. the city group.

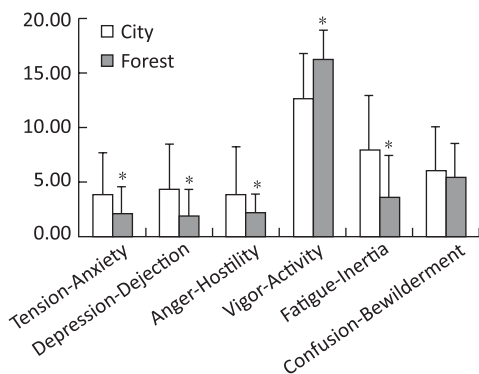


Figure 4. POMS evaluation of subjects exposed to forest and urban environments ($n=10$). The standard version of POMS, including negative subscales (tension-anxiety, depression-dejection, anger-hostility, fatigue-inertia, and confusion-bewilderment) and a positive subscale (vigor-activity) was used to assess the subjects' mood changes at the end of the experiment. The Mann-Whitney Test was used for data analysis. * $P<0.05$ vs. the city group.

DISCUSSION

In modern society, air pollution is an unavoidable problem, especially in many developing countries. It is a complex mixture of particulate matter, gases, and organic compounds especially present in urban areas^[6]. It has been reported that chronic exposure to urban air pollution produces inflammation in rodents. Elevated levels of inflammatory mediator gene expression, including interleukin-1 β (IL-1 β), IL-6, and TNF- α , were observed in mice exposed to polluted areas

compared with those kept in clean air^[6]. As is well known, the air in a forest environment is much cleaner than in an urban area. Thus, our finding of a reduced inflammatory response in subjects exposed to a forest environment compared with those in an urban area is consistent with the results of that previous study^[6]. Air pollution is thought to exert negative health effects through oxidative stress, which causes damage to DNA and lipids. Exposure to inhaled emissions can induce oxidized LDL and vascular reactive oxygen species in both humans and rodents^[12]. Indeed, the present study showed an elevated serum MDA level, which is widely used as an indicator of lipid peroxidation, in the urban group; however, subjects who experienced forest bathing showed a significantly reduced elevation (see Table 3). In this study, we have provided direct evidence that forest bathing is beneficial for reducing oxidative stress in humans. Based on our findings, exposure to forest environments for people living in urban areas is a way to reduce inflammatory reactions and oxidative stress and thus may help to promote health.

Epidemiological studies have shown that traffic-related pollution is a strong predictor for morbidity and mortality related to cardiovascular disease in Asian developing countries^[13]. ET-1 is by far the most powerful vasoconstrictor known and is always involved in the progression of cardiovascular diseases. It has been reported that acute exposure to vehicular air pollution results in elevated plasma ET-1 levels in both humans and rodents^[14-15], and it also up-regulates circulating and vascular factors associated with the progression of atherosclerosis, mediated in part through activation of ET-1 receptor pathways^[15]. In the present study, a significant decrease in the plasma ET-1 level was observed in subjects exposed to the forest environment compared with the urban group, which implies that forest bathing may attenuate the risk of developing cardiovascular diseases.

It is believed that higher levels of negative oxygen ions are beneficial for human health. Investigations into physiological and psychological conditions showed that performance efficiency and mental state were improved by exposure to negative ions in the environment^[16]. In the present study, the concentration of negative air ions was also determined, and a significantly higher daytime level of negative air ions was recorded in the forest environment ($1509.1 \pm 357.0 \text{ cm}^{-3}$) than in the urban area ($263.3 \pm 99.3 \text{ cm}^{-3}$). Thus, our finding about the

beneficial effect of forests for humans may be at least partly due to the abundance of negative air ions.

Several previous studies have also demonstrated that forest bathing enhances immune functions. It has been reported that subjects who took a three-day trip to a forest area showed enhanced immune functions, as evidenced by an increase in natural killer (NK) cell activity and the levels of intracellular granulysin, perforin, and granzymes A and B^[17-18]. Data from an in vitro study showed that the elevated NK cell activity could be induced by the essential oil(s) or odorous components of wood, such as cypress (*hinoki*) stem oil, α -pinene, and 1,8-cineole^[19]. In the present study, increased numbers of NK cells in peripheral blood lymphocytes were also observed in subjects who experienced forest bathing compared with the urban group, though the change was not statistically significant. In addition, the percentage of B lymphocytes in the forest-bathing group was mildly increased compared with the urban group, which may indicate elevated humoral immunity. One explanation for these findings could be that the amount of volatile components of the trees in our experimental forest site may have been lower than in the previous study or the forest in our investigation may have lacked the essential oil(s) or trees supplying the odorous components.

Modern societies are subject to high stress owing to the fast pace of life. In addition, people living in urban areas are prone to irritability and tiredness through being in a crowded, unpleasant environment that is characterized by noisy traffic and the unpleasant smells of automobile exhausts. As anticipated in the present study, a higher level of stress—as evidenced by an elevated serum cortisol level—was observed in subjects exposed to the urban environment than in the forest group. This finding is consistent with that of a previous study, which used the salivary cortisol level as a stress marker^[20]. In addition, subjects staying in the forest environment felt more comfortable: the scores in the negative subscales of the POMS test, such as tension, depression, anger, fatigue, and confusion, were lower than those of the urban group, and the score for vigor was elevated. The POMS test is a well-accepted quantitative means of evaluating mood, widely used in psychological investigations, and our findings are consistent with those of studies conducted in Japan^[21].

However, the present study was limited in

several areas. First, the findings may need to be repeated in a larger experiment because of the limited sample size in this study. Second, it is not known whether our findings in young healthy participants would be reflected in old or infirm individuals. Third, whether the positive effects of forest bathing on human health resulted from better air quality needs to be verified by more quantitative data related to air pollution at each experimental site. Further, the factor of climate also warrants investigation. We chose September in this study because it is a suitable season for outdoor travel in our country. It is not clear whether similar changes would be found in other seasons or other countries.

This study has provided some evidence that living in a forest environment, even for a short time, exerts benefits on human health. In addition, forest bathing may represent a potential medical intervention in several pathologies, including inflammation and cardiovascular and nervous conditions. We intend to conduct a larger study with subjects of different ages and examine the detailed molecular mechanisms regarding the relationship between forest environments and the positive effects on human health.

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