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Randomized controlled trial on the efficacy of forest walking compared to urban walking in enhancing mucosal immunity

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Scientific research on forest therapy's preventive medical and mental health effects has advanced, but the need for clear evidence for practical applications remains. We conducted an unblinded randomized controlled trial involving healthy men aged 40–70 to compare the physiological and psychological effects of forest and urban walking. Eighty-four participants were randomly assigned to either the forest or urban group, with 78 completing 90-min walks and analysis. The primary outcome measured was the change in saliva-secreted immunoglobulin A (sIgA) levels. Evaluating researchers were blinded to the groups, but participants and on-site staff were not. Here, we demonstrate a significant increase in saliva-secreted immunoglobulin A (sIgA) levels in the forest group. Furthermore, forest walking resulted in improved mood, including a reduction in stress hormone levels. In conclusion, mindful forest walking has the potential to enhance immunity and promote health.

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Keywords Forest walking, Forest therapy, Mental health, Mucosal immunity, Randomized controlled trial, Saliva-secreted immunoglobulin A

Human happiness cannot be separated from the natural environment, and in contemporary society, the decreasing daily contact with nature is a matter of concern. The Greek physician Hippocrates stated, "Medicine should be practiced as a scientific discipline based on the natural sciences,"[1] emphasizing the importance of harmonizing with nature. In recent years, as the health benefits of spending time in nature have become a social expectation[2], "forest therapy" has become the most frequently used keyword in nature therapy-related articles[3]. Experiments have been conducted to scientifically verify the health benefits of spending time in the forest, and these experiments have gained attention[4].

Multiple meta-analyses have suggested significant improvements in subjective symptoms, such as anxiety, depression, and negative emotions, by spending time in a forest environment[5–7]. However, there is inconsistency in the results among papers owing to factors, such as moderate to low quality or presence of bias risks[8]. For example, a review suggested that forest environments decrease blood pressure significantly[9], whereas another paper stated that the decrease in blood pressure was not statistically significant[8]. Antonelli et al.[10] reviewed the potential for a significant short-term decrease in cortisol levels, which is a stress biomarker, during forest therapy. However, many studies are noted to have limitations, such as a lack of randomization, lack of controls, or a very limited number of participants. When looking at the immunological effects, many studies had small sample sizes and highly heterogeneous participants with regard to age and sex, making them unsuitable for a meta-analysis[11]. Therefore, high-quality research on forest therapy is warranted.

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Currently, multiple infectious diseases are emerging worldwide, leading to an increased focus on infection prevention. Moreover, the demand for individual-level defenses to boost immune function and protect the body is increasing. Chronic stress caused by a prolonged infection is also a social issue. If forest environment not only reduces stress but also, proves, effective in combating infectious diseases, its significance would be substantial.

Therefore, we conducted a randomized controlled trial to determine whether the forest's environment enhances mucosal immunity, using changes in salivary sIgA levels as the primary outcome measure. To compare the differences between forest and urban environments, we chose walking as the most common behavioral component for this experiment.

Methods

Eligibility and study design

The study was conducted following the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the National Hospital Organization Tokyo Medical Center (Issue no. R21-046, approval date: 19/07/2021). The clinical trial registration was at the University Hospital Medical Information Network (UMIN000045851, 25/10/2021). All participants provided written informed consent including approval of photo publication, and this study followed the Consolidated Standards of Reporting Trials 2010 guidelines[12].

This trial was conducted as an open-label, randomized, parallel-group controlled trial in October 2022. The inclusion criteria were as follows: (1) 40–70-year-old men, (2) no difficulty in walking for approximately 90 min, and (3) living in an urban area in the Kanto region and being able to travel to place where the experiments are conducted on their own without difficulty. The exclusion criteria were as follows: (1) those who were taking medications that may interfere with the study, such as antibiotics, anti-inflammatory, anti-allergic, steroids, and immunosuppressive medications; (2) those who showed related diseases (collagen disease, common cold, and inflammatory diseases); and (3) those who had a smoking habit. To determine eligibility, information, such as age, sex, place of residence, medical history, medication use, and smoking habits, was collected at the time of application. Participants also reported their height and body weight. Body mass index was calculated based on this information.

Randomization

Participants were recruited through the forest-related website "Watashi no Mori" (https://watashinomori.jp/) for 1 month starting on 01/09/2021, and the eligibility criteria for the participants were described. Participants applied online after checking the website and ensuring that they met the eligibility criteria. A physician (H.O.) contacted individuals by phone from the Tokyo Medical Center and determined their eligibility for the study. Subsequently, ID numbers were assigned to each participant. A researcher (G.M.) who only had access to ID numbers and did not have a corresponding table allocated participants to groups randomly using a high-quality pseudorandom number generator, particularly the Mersenne Twister algorithm. Participants were stratified on the basis of age (40–49, 50–59, and 60–69 years), and randomly assigned to two groups within each age stratum.

Sample size

We set the target number of participants for this study at 80. We determined the sample size using an effect size (d) of 0.8, which is commonly referred to as a strong effect size, because no suitable prior studies were available. With a power of 0.8 and a significance level of 0.05, a sample size of 52 (26 + 26) was determined. In addition, the total sample size was 80 by assuming a 30% attrition during follow-up. We used G*Power analysis program version 3.1.9.7 from Heinrich-Heine University in Düsseldorf, Germany.

Intervention methods

The randomly assigned participants were further divided into two subgroups within each intervention and control group to ensure a suitable number for walking sessions. Although the experiment was completed in 1 day, it was conducted over 2 days for each group, with the intervention group on two consecutive days and the control group on another two consecutive days. The experiment was conducted in October, chosen for its relatively stable weather conditions. The "forest walking" and "urban walking" groups walked on a relatively flat course of the same distance, and completed the task within 90 min. The forest where the intervention group walked was a vast mountainous forest park located in Tokyo, with walking trails developed within a forest. These trails have been designated "forest therapy roads" by the nonprofit organization Forest Therapy Society (https://www.fo-society.jp/en/index.html), indicating their suitability for forest therapy. The control group walked in Ginza, a city area in Tokyo known for its luxury boutiques and restaurants. The experiment was conducted in an area that becomes a "pedestrian paradise" on weekends: a car-free zone where people can freely walk and shop. The walking distance for both the forest and urban environments was set at 1.1 km in morning and 1.1 km in afternoon, with a course designed for participants to walk leisurely for approximately 45 min, enabling time to observe the surroundings and enjoy the experience. During the walking sessions, qualified guides with over 10 years of experience accompanied both the intervention (forest) and control (urban) groups. These guides were trained in methods developed by the Japanese nonprofit organization "Forest Therapy Society," but adapted their approach to suit both environments. They adjusted walking speed, distance, and resting times to ensure similar levels of physical activity among all participants. In both groups, participants were encouraged to engage their five senses during the walk, with instructions tailored to each environment. For the forest group: participants were guided to focus on observing the natural scenery, exploring forest elements, and noticing surrounding sounds and smells of nature. For the urban group: participants were instructed to pay attention to the urban landscape, observe architectural features, and notice the sounds and smells characteristic of the city environment. This approach ensured that both groups had a similar sensory engagement experience,

appropriate to their respective environments, while maintaining consistency in the overall structure of the walk. Participants were also encouraged to minimize conversation with each other and the guides while walking. Occasionally, participants paused to concentrate on their five senses without sitting down. The guides led the way, explaining to the participants the importance of maintaining the pace and not falling behind the group. During lunch breaks, participants could sit, eat, and talk, but were asked not to sleep. The specific schedule for the walks is provided in Table 1.

To avoid any effect on the experimental results, participants were instructed to refrain from intense exercise and heavy alcohol consumption the day before the intervention. During the experiment, participants were only allowed to consume predetermined items (water and boxed lunch) and were instructed not to consume anything else.

Meteorological data were obtained from the Japan Meteorological Agency, which provides comprehensive and accurate weather observations across Japan. These data included weather conditions and variables such as temperature and wind speed. Air pollution measurement data were sourced from the Tokyo Metropolitan Government Bureau of Environment. This dataset included key air quality indicators such as nitrogen dioxide (NO_2) and particulate matter (PM2.5 and PM10) concentrations.

Outcomes

Primary outcome

The primary endpoint was salivary sIgA (secretory Immunoglobulin A). Saliva testing was conducted for approximately 30 min before and immediately after the walks. Participants were instructed not to eat any food for 1 h before the test and not to drink any fluids 30 min before the test. Salivette™ (Sarsted, Aktiengesellschaft & Co., Nümbrecht, Germany) was used to collect saliva samples. Participants placed the swab under their tongue, leaving it there for 3 min without chewing. The swab was frozen immediately at − 80 °C and transported to the Hayakawa Institute of Preventive Hygiene Co., Ltd. (Tokyo, Japan). The sIgA concentration in the sample was measured using an enzyme immunoassay (EIA): Salivary secretory IgA indirect enzyme immunoassay kit (Salimetrics, LLC., Carlsbad, CA, USA) according to the manufacturer's instructions.

Secondary outcomes

The secondary endpoints were stress hormone levels and the Profile of Mood Status questionnaire, second version (POMS-2).

Blood testing: blood samples were collected approximately 30 min before and immediately after the walks. Each sample was placed in a tube and centrifuged on-site to analyze CD4, CD8, peripheral blood count, interferon (IFN)-γ, interleukin (IL)-6, cortisol, catecholamine fractions, and lactate. Samples were then stored refrigerated or frozen, according to the testing manual, before being submitted to the testing company. Cortisol (ECLusys cortisol assayII, Roche Diagnostics Co., Mannheim, Germany) and catecholamine (CA Test "TOSOH" reaction solution, D, E, Tosoh Co., Tokyo, Japan) levels of the blood samples were measured using the indicated measurement equipment. IFN-γ (IFN-γ ELISA, Life Technologies Co., Tokyo, Japan), IL-6 (IL-6 LPG Immunoreaction Cartridges, Fujirebio, Tokyo, Japan), and IgG (N-assay TIS IgG-SH, Nittobo Medical, Tokyo, Japan) were assayed by performing ELISA according to manufacturer's manual. Flow cytometry was conducted to count the number of CD4 (Immunofluorescence staining with anti-mouse IgG1, Becton Dickinson Company, CA, USA) and CD8 (Immunofluorescence staining with anti-mouse IgG1, Beckman Coulter, Inc., CA, USA).

Modified version of POMS2 (Profile of Mood States, Second Version)[13]: The POMS2 questionnaire, which included 35 questions, was administered approximately 15 min before and after the walks. The POMS scores were determined for the following seven subscales: Anger-Hostility (AH), Confusion-Bewilderment (CB), Depression-Dejection (DD), Fatigue-Inertia (FI), Tension-Anxiety (TA), Vigor-Activity (VA), and Friendliness (F). The Total mood disturbance (TMD) score was calculated by combining AH + CB + DD + FI + TA-VA. A higher TMD score indicates an unfavorable psychological state.

Other items

Physical activity levels during the forest and urban walks were evaluated using validated, research-based accelerometers (HJA-750 C Active Style Pro, OMRON Healthcare Co., Ltd., Kyoto, Japan)[14,15]. The accelerometer

	Intervention group ($n = 37$)	Control group ($n = 41$)
10:30	Meeting	
10:30~	Questionnaire survey, blood sampling, saliva testing	
11:30–12:15	Forest walking	Urban walking
12:15–13:15	Lunch, break (all participants consume the same boxed lunch)	
13:15–14:00	Forest walking	Urban walking
14:00–15:00	Questionnaire survey, blood sampling, saliva testing	
15:00	End	

Table 1. Intervention schedule.

was attached at a designated position on a waist belt, and the activity levels, [metabolic equivalents (METs)·in hours] during the walks were calculated.

Statistical analysis

First, the characteristics of the intervention and control groups were described. Changes in test values before and after the walks were visualized using histograms to examine the distribution. At that time, we excluded cases that were determined to have abnormal values according to the laboratory manual. The between-group comparison of the change in values was performed using an unpaired t-test, two-sided. Analyses of covariance (ANCOVA) were also performed, adjusting for baseline values, and total physical activity during the walks. A P value of 0.05 was considered statistically significant when performing the statistical analysis using R version 3.6.3 (The R Foundation for Statistical Computing, Vienna, Austria) ("Supplement Information").

Results

Participants (description of flowchart)

A total of 84 eligible individuals participated in the study. Randomization was used to assign these 84 participants to either the intervention group or the control group (42 participants in each group). In the intervention group, five participants withdrew from the study owing to a scheduling conflicts ($n=3$), knee pain ($n=1$), and concerns about contracting COVID-19 ($n=1$). The remaining 37 participants in the intervention group completed the scheduled interventions as planned, and no participants were lost to follow-up. The data of 37 participants were analyzed. In the control group, one participant withdrew from the study owing to scheduling conflict; however, the remaining participants completed the urban walk as scheduled. No participants were lost to follow-up in the control group, and data from 41 participants were analyzed (Fig. 1). Their basic characteristics were shown in Table 2.

Implementation of interventions

Table 3 presents the conditions of the walks, weather conditions, and details of the interventions for each day. The weather conditions were consistent across sites, with sunny weather during the first experimental day and light rain during the second experimental day at both locations. The average temperature difference between the forest and urban sites was 0.7 °C. Air quality indicators at both locations remained within normal ranges throughout the study period. The appearance of the forest and urban walk courses is shown in Fig. 2.

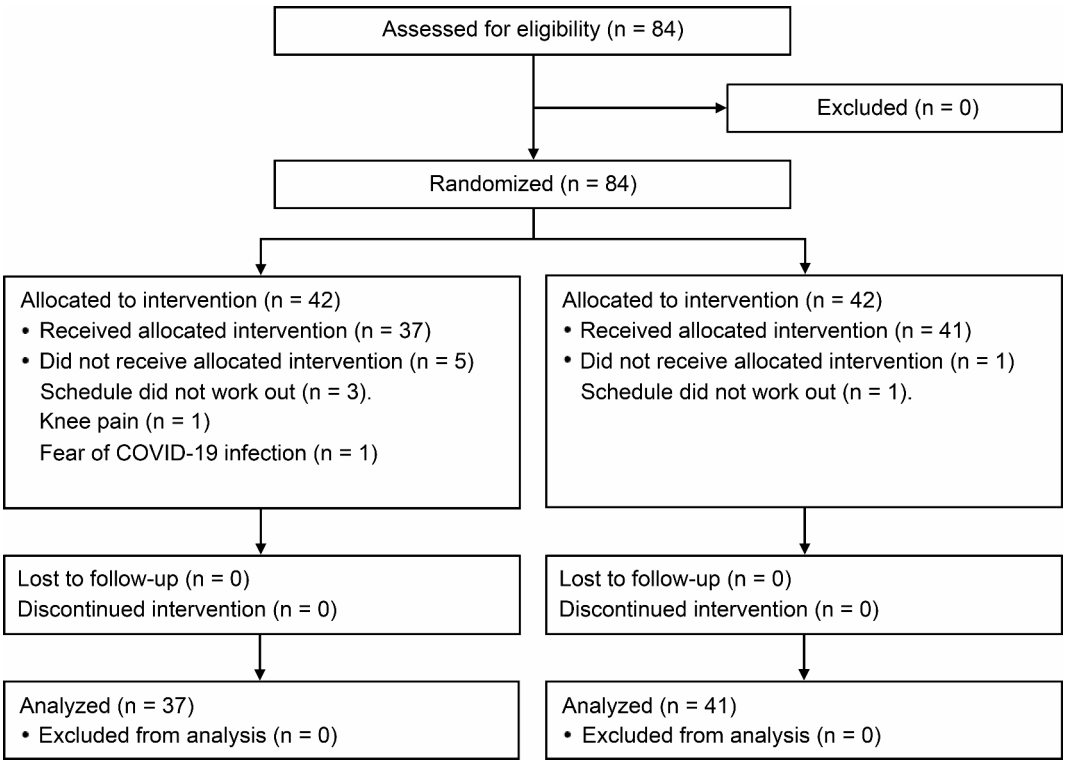

Fig. 1. Participant flow in a study of forest vs. urban walk randomized controlled trial. CONSORT Flow Diagram. 84 participants were randomized into two group. Before the forest walk, five participants dropped out and before the urban walk, one participant dropped out. Remaining 78 paticipants completed the trial and were included in the present analysis.

Characteristics	Intervention (*n* = 37)	Control (*n* = 41)	*P* value
Age, years	52.5 ± 8.7 (40–68)	53.6 ± 8.2 (40–68)	0.584
Sex (men), %	100	100	
Height, cm	171.5 ± 4.8 (162–182)	171.5 ± 8.4 (163–184)	0.996
Body weight, kg	66.7 ± 9.4 (52–88)	70.5 ± 11.7 (53–103)	0.123
Body mass index, kg/m^2	22.7 ± 2.9 (16.5–29.1)	23.9 ± 3.2 (18.8–31.1)	0. 086

Table 2. Basic characteristics of participants. Data were expressed as mean ± standard deviation (range).

		Intervention group (*n* = 37)		Control group (*n* = 41)	
Program		Forest walk		Urban walk	
Date of Implementation		October 9, 2021 (*n* = 20)	October 10, 2021 (*n* = 17)	October 30, 2021 (*n* = 18)	October 31, 2021 (*n* = 23)
Walk duration (min)	AM	48	45	49	49
	PM	47.5	52	49	45
Lunch break duration (min)		63	63	63	64
Examination time		Before walking, 10:30; after walking, 14:00		Before walking, 10:30; after walking, 14:00	
Location		Tokyo, Hinohara Village, Otaki-no-Michi Course		Tokyo, Chuo Ward, Ginza Street	
Weather condition[a]		Sunny	Light rain (1.0 mm/10:00–14:00)[a]	Sunny	Light rain (1.0 mm/10:00–14:00)[a]
Actual average temperature (°C)		19.6	18.2	20.3	17.9
Wind speed (m/s)		1.1	0.9	2	1.6
Nitrogen dioxide (ppb)[b]		4	1	23	15
Particulate matter (μg/m^3)[b]		16	0	15	20
Road condition		Wood chip trail prepared for the forest walk		Paved road	
Total length of the course (km)		1.74		1.7	
Elevation differences in the course (m)		48		1	
Total physical activity level (METs·h)[c]		2.2	2.2	2.3	2.3
Walking style		Participants walked slowly while experiencing the forest using all five senses with the supervision of a guide. They stopped along the way to listen to sounds and smell the scents.		Participants walked slowly while observing buildings with the supervision of a guide. They stopped along the way to look at displays and listen to sounds.	

Table 3. Description of intervention. [a]Weather data obtained from the Japan Meteorological Agency. [b]Air pollution measurement data obtained from the Tokyo Metropolitan Government Bureau of Environment. [c]METs: metabolic equivalents.

Main outcome: salivary sIgA

Table 4 presents the changes in sIgA concentration owing to walking. A change was observed after walking in the forest (+ 14.4 ± 96.6 μg/mL) and (− 28.9 ± 90.1 μg/mL) in the urban area. When adjusting for baseline levels before the walks and total physical activity (METs·hours), the adjusted mean difference was 32.1 μg/mL (95%CI 2.3 to 62.0, *P* = .035), indicating an increase in sIgA concentration after the forest walk (Table 3).

Other results

Other measured indicators are presented in Table 4. Changes in dopamine (plasma) levels were significantly different between the groups, with forest group showing a considerable increase (*P* = .048). The change in plasma cortisol levels of the forest group decreased significantly (*P* = .007). In addition, significant intergroup differences were observed in the number of white blood cells (*P* = .001) before and after the walk, with significant decreases in blood CD4$^+$ T cells (*P* = .022) and blood CD8$^+$ T cells (*P* = .003) in the forest group. Regarding the change of POMS2, the forest group's VA increased dramatically (*P* = .001), whereas the TMD score decreased significantly compared with that of the urban group (*P* = .007).

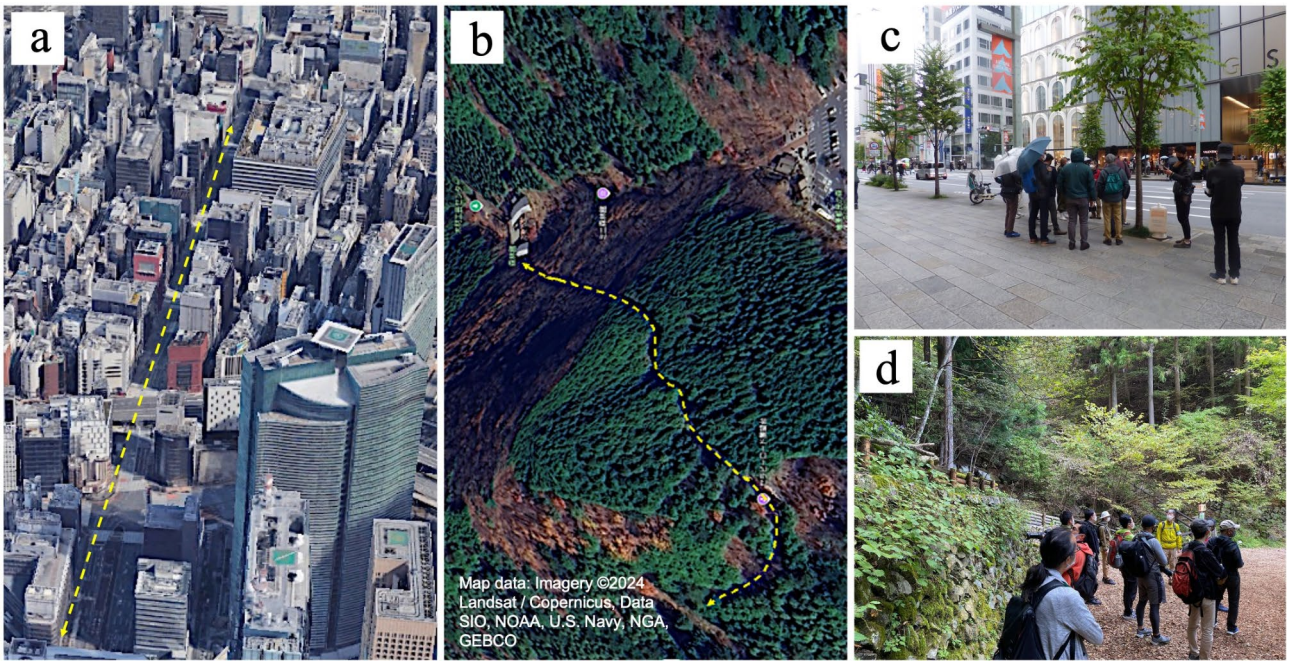

Fig. 2. Walking course of urban (a, c) and forest (b, d). (a) Walking course of Tokyo, Chuo Ward, Ginza Street (yellow dashed line). **(b)** Walking course of Tokyo, Hinohara Village, Otaki-no-Michi Course (yellow dashed line). (**c**) Vegetation in urban: broadleaf trees are planted at 6-m intervals.(**d**) Vegetation in forest: mixed forest with diversity of tree species. (Base maps of (a) and (b) were exported by Google Maps, further edited by Hiroko Ochiai. Map data: Imagery ©2024 Landsat / Copernicus, Data SIO, NOAA, U.S. Navy, NGA, GEBCO)

Discussion

The findings show that, compared with urban walking, forest walking considerably raises sIgA levels and enhances mucosal immune function. Furthermore, forest walking decreased the levels of stress hormone cortisol significantly and positively affect mood profiles. These effects were observed even after adjusting for baseline values and total physical activity during forest and urban walking.

In this study, salivary sIgA was evaluated as a primary outcome measure. IgA exhibits activity against certain pathogens[16–18], including viruses, and sIgA released in secretions[19], such as saliva, indicates an improvement in overall mucosal immune function[20]. Recently, studies have reported that sIgA contributes significantly to the neutralization of SARS-CoV-2[21,22]. Referring to the association with stress, sIgA levels have been shown to decrease in the presence of stress[23,24], and at the same time stress is speculated to affect the hypothalamic–pituitary–adrenal axis and the sympathetic nervous system[25]. Therefore, it is inferred that there is a relationship between stress hormones and sIgA. In this study, cortisol, an indicator of stress, decreased significantly with forest walking. In addition, levels of dopamine, a neurotransmitter associated with pleasure and happiness, increased significantly, and a positive effect on mood status was observed following forest walking. Changes in sIgA, stress-related hormones, and mood status were consistent, suggesting that reducing stress is considered an important factor in enhancing mucosal immunity. The observed changes were within the normal range, indicating that forest walking has beneficial effects on the health promotion of healthy individuals.

Previous studies have also reported relaxing effects, stress-reduction effects, and improvements in depressive symptoms of forest therapy[5–7], as well as decreased stress hormone levels[26–28] and activation of parasympathetic nervous system as indicated by heart rate variability[29,30]. However, conducting experiments in natural environments poses a challenge for establishing rigorous experimental conditions. This study was a well-designed randomized controlled trial that addressed these issues and provided more reliable findings. Based on previous studies reporting a positive correlation between psychological well-being and mucosal immunity[21], it can be argued that the consistency of the results in this study is supported.

Conversely, compared to forest walking, urban walking resulted in a significant increase in white blood cell (WBC) count ($P = .001$). Steenhof et al.[31] reported that air pollution induces acute inflammatory responses that increase the WBC count. However, because other subtypes were not examined, the exact cause is unclear. In addition, a significant rise in $CD4^+$ T cells and $CD8^+$ T cells was observed in the urban group. As trees and forests remove air pollutants[32], exposures to urban air may trigger hypersensitive immune reactions[33].

Forest environments decrease negative mood and increase positive mood, as measured by POMS[8,34]. In this study using a robust research design, we demonstrated that forest walking has a more positive effect on mood than urban walking. Furthermore, the results of subjective symptoms were consistent with the changes in hormone and sIgA levels.

In this study, no participants dropped out, and no negative outcomes were observed in the intervention group; all participants completed the program successfully and according to schedule. Based on the accelerometer data,

	Intervention ($n=37$)				Control ($n=41$)				Adjusted mean difference [a]	*P* value
	n	Mean ± SD			*n*	Mean ± SD				
		Baseline	After walk	Change		Baseline	After walk	Change		
sIgA (saliva, μg/ml)	37	187.1 ± 127.4	201.5 ± 95.9	14.4 ± 96.6	40	197.1 ± 115.6	168.2 ± 75.9	−28.9 ± 90.1	32.1 (2.3, 62.0)	0.035
Adrenaline (plasma, pg/ml)	36	40.1 ± 18.5	29.8 ± 15.6	−10.3 ± 11.0	41	52.0 ± 25.8	40.9 ± 21.7	−11.1 ± 14.2	−1.4 (−6.5, 3.6)	0.568
Noradrenaline (plasma, pg/ml)	37	594.4 ± 205.3	606.5 ± 213.7	12.1 ± 190.3	41	646.4 ± 199.5	582.1 ± 196.2	−64.3 ± 150.5	65.8 (−6.7, 138.2)	0.075
Dopamine (plasma, pg/ml)	37	20.9 ± 13.8	21.6 ± 8.7	0.7 ± 11.6	40	20.8 ± 8.7	19.4 ± 6.2	−1.4 ± 6.5	2.8 (0.0, 5.6)	0.048
Cortisol (plasma, μg/dl)	37	7.2 ± 3.3	5.3 ± 1.9	−1.9 ± 3.2	41	7.6 ± 2.6	6.6 ± 2.5	−1.0 ± 3.3	−1.4 (−2.4, −0.4)	0.007
IgG (serum, mg/dl)	37	1,179.3 ± 158.8	1,145.5 ± 154.1	−33.8 ± 36.0	41	1,164.8 ± 226.9	1,135.7 ± 216.4	−29.1 ± 29.7	−3.9 (−18.4, 10.6)	0.596
White blood cell count (blood, /μl)	37	5,394.6 ± 1,538.4	5,500.0 ± 1,552.4	105.4 ± 579.2	41	4,978.1 ± 1,037.4	5,658.5 ± 1,139.1	680.5 ± 745.7	−532.5 (−844.1, −220.8)	0.001
Percentage of lymphocytes (blood, %)	37	34.7 ± 8.8	33.2 ± 7.5	−1.5 ± 4.9	41	32.1 ± 6.6	31.6 ± 7.1	−0.5 ± 4.9	−0.3 (−2.4, 1.8)	0.803
$CD4^+$ T cell count (blood, /μl)	37	779.2 ± 241.2	833.9 ± 257.7	54.7 ± 99.2	41	704.4 ± 252.2	821.1 ± 296.2	116.6 ± 126.2	−62.9 (−116.4, −9.3)	0.022
$CD8^+$ T cell count (blood, /μl)	37	499.1 ± 207.7	443.1 ± 149.9	−56.0 ± 119.3	41	440.9 ± 183.3	467.3 ± 190.3	26.4 ± 89.2	−66.9 (−110.1, −23.6)	0.003
Interleukin-6 (serum, pg/ml)	37	1.6 ± 3.9	1.7 ± 3.7	0.1 ± 1.2	40	1.2 ± 1.7	1.3 ± 2.6	0.1 ± 1.0	0.0 (−0.5, 0.5)	0.929
POMS2										
Anger-Hostility (AH, score)	37	41.2 ± 4.1	39.3 ± 2.8	−1.9 ± 2.8	41	43.3 ± 6.3	40.8 ± 5.2	−2.5 ± 5.7	−0.3 (−2.0, 1.3)	0.68
Confusion-Bewilderment (CB, score)	37	45.1 ± 6.8	41.5 ± 4.2	−3.5 ± 4.6	41	45.6 ± 6.7	43.0 ± 5.9	−2.6 ± 4.0	−1.1 (−2.7, 0.4)	0.144
Depression-Dejection (DD, score)	37	44.5 ± 65.2	43.0 ± 3.9	−1.4 ± 3.1	41	45.7 ± 6.4	44.1 ± 5.3	−1.6 ± 3.7	−0.1 (−1.4, 1.2)	0.834
Fatigue-Inertia (FI, score)	37	41.6 ± 6.4	39.3 ± 4.4	−2.4 ± 4.4	41	41.1 ± 6.5	39.9 ± 6.2	−1.2 ± 4.1	−0.8 (−2.4, 0.8)	0.332
Tension-Anxiety (TA, score)	37	43.2 ± 8.2	36.9 ± 5.3	−6.3 ± 5.9	41	44.4 ± 8.4	38.8 ± 6.6	−5.6 ± 5.6	−1.5 (−3.4, 0.5)	0.142
Vigor-Activity (VA, score)	37	58.2 ± 10.8	63.3 ± 9.5	5.1 ± 9.0	41	60.4 ± 9.4	59.2 ± 10.3	−1.2 ± 6.4	5.6 (2.3, 8.9)	0.001
Friendliness (F, score)	37	57.7 ± 11.0	60.1 ± 11.3	2.4 ± 6.7	41	59.7 ± 10.3	59.5 ± 12.2	−0.2 ± 7.2	2.8 (−0.3, 6.0)	0.078
Total mood disturbance (TMD, score)	37	40.8 ± 7.0	36.6 ± 4.6	−4.2 ± 4.9	41	41.2 ± 6.6	39.3 ± 6.0	−1.9 ± 5.3	−2.6 (−4.4, −0.7)	0.007

Table 4. Change in forest and urban walk test results. [a]The model was adjusted for baseline levels before the walks and total physical activity (METs·hours).

the average activity intensity was 2.2 METs, which falls under the category of light intensity (< 3 METs) according to the WHO classification of physical activity[35]. This activity intensity is weaker than that of normal walking and is the intensity of activity performed in daily life. Forest and urban walking conducted in this experiment were planned as part of the forest therapy program; as a low-intensity exercise, it was considered a safe health practice. Furthermore, despite minimal differences in weather and standardized walking activities (physical activity levels) between the experimental groups, differences in outcome measures were observed, indicating the effectiveness of the forest's environmental conditions. Various factors such as air composition[33], sensory experiences[36], and presence of phytoncydes[37] and microorganisms[38], can be considered; however, identifying which specific factors have influenced the outcomes is difficult. Incorporating forest-derived stimuli, such as visual and auditory elements, into indoor environments in daily life is expected to help mitigate mental and physical health deterioration[39]. This study supports these results, but more robust research designs have shown clear and definite beneficial effects of experiencing actual forest environments. Moreover, the findings indicate that improving residential environments and urban planning to enable forest therapy-like experiences in daily life could benefit people's health. As part of urban greening initiatives that mimic forest environments, incorporating pathways surrounded by diverse plants, soil, and water features is particularly promising. Specifically, well-maintained areas with good visibility[40] and designs that stimulate the five senses are considered effective[5,36]. This study demonstrated that such efforts could reduce stress and enhance mucosal immunity, thereby supporting healthier lifestyles in urban settings. Furthermore, the findings suggest that introducing forest therapy can contribute to disease prevention, potentially improving public health and healthcare economics. This underscores the need for further quantitative public health research to validate these effects.

This study has several limitations. First, since experiments in the intervention and control groups were conducted on different dates and at different locations, it was impossible to fully match the weather conditions. However, by conducting the experiments over a span of days during stable weather, the differences between the groups were small, and the experiments were conducted under conditions during which the weather conditions were almost matched. Temperature, rainfall, and other conditions were approximately comparable, and we believe that these factors had minimal effects. Second, the external validity of the study was an issue. This study focused on healthy middle-aged and older men. Although it was conducted using courses recognized as suitable for forest therapy and followed a typical implementation method, it remains specific to certain locations and specific programs. Further research under different conditions is necessary. Third, this study examined the

acute effects of forest walking. To better understand the effects of habitual practice, more studies are warranted. Fourth, the control group also engaged in urban walking, and the effect of physical activity, which is inherently present in forest walking, was examined as a covariate. Therefore, the effects demonstrated exclude the potential health benefits associated with the physical activity aspect of forest therapy. If physical activity also has health benefits, the results of this study might underestimate the effects of forest therapy.

Despite the aforementioned limitations, this is a well-designed randomized controlled trial that considerably contributes to the discussion of biases, which has been highlighted in other studies on forest therapy.

Conclusion

This study is a randomized trial comparing forest walking and urban walking among healthy men in their middle to late adulthood. Results of the study showed that forest walking significantly increased salivary sIgA levels, suggesting an improvement in mucosal immune function. It also reduced the levels of stress hormone cortisol significantly and increased levels of dopamine, which contribute to a sense of well-being. Furthermore, a significant increase was noted in the mood dimension of "vigor" and a decrease in the dimension of "general negative mood state" because of forest walking. The increase in salivary sIgA is assumed to be influenced by the decrease in stress hormones and changes in mood. Forest therapy, being a low-intensity physical activity, can thus be recommended as a safe health practice that anyone can engage in.

Data availability

The data that support the findings of this study are available from the corresponding author, H.O., upon reasonable request.

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Author contributions
YN, SI, TS, GM, NY and MI contributed in concept creation and design in this study. HO, SI and TO conducted all experiments. GM and SA performed the data analysis. All the authors contributed to the composition and revision of the manuscript and gave final approval to its content.

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Declarations

Competing interests
The authors declare no competing interests.

Ethical approval
The National Hospital Organization Tokyo Medical Center's Ethics Committee approved this study (Issue no. R21-046, approval date: July 19, 2021).

Informed consent
All participants provided written informed consent for this study following the Consolidated Standards of Reporting Trials 2010 guidelines.

Consent to publish
All participants provided written informed consent for the photo publication.

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