# VISITING A FOREST, BUT NOT A CITY, INCREASES HUMAN NATURAL KILLER ACTIVITY AND EXPRESSION OF ANTI-CANCER PROTEINS

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**We previously reported that a forest bathing trip enhanced human NK activity, number of NK cells, and intracellular anti-cancer proteins in lymphocytes. In the present study, we investigated how long the increased NK activity lasts and compared the effect of a forest bathing trip on NK activity with a trip to places in a city without forests. Twelve healthy male subjects, age 35-56 years, were selected with informed consent. The subjects experienced a three-day/two-night trip to forest fields and to a city, in which activity levels during both trips were matched. On day 1, subjects walked for two hours in the afternoon in a forest field; and on day 2, they walked for two hours in the morning and afternoon, respectively, in two different forest fields; and on day 3, the subjects finished the trip and returned to Tokyo after drawing blood samples and completing the questionnaire. Blood and urine were sampled on the second and third days during the trips, and on days 7 and 30 after the trip, and NK activity, numbers of NK and T cells, and granulysin, perforin, and granzymes A/B-expressing lymphocytes in the blood samples, and the concentration of adrenaline in urine were measured. Similar measurements were made before the trips on a normal working day as the control. Phytoncide concentrations in forest and city air were measured. The forest bathing trip significantly increased NK activity and the numbers of NK, perforin, granulysin, and granzyme A/B-expressing cells and significantly decreased the concentration of adrenaline in urine. The increased NK activity lasted for more than 7 days after the trip. In contrast, a city tourist visit did not increase NK activity, numbers of NK cells, nor the expression of selected intracellular anti-cancer proteins, and did not decrease the concentration of adrenaline in urine. Phytoncides, such as alpha-pinene and beta-pinene were detected in forest air, but almost not in city air. These findings indicate that a forest bathing trip increased NK activity, number of NK cells, and levels of intracellular anti-cancer proteins, and that this effect lasted at least 7 days after the trip. Phytoncides released from trees and decreased stress hormone may partially contribute to the increased NK activity.**

*Key words: anti-cancer proteins, forest bathing, NK activity, granulysin, granzyme, perforin*

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A forest bathing trip, which is similar to natural aromatherapy, involves a visit to a forest field for the purpose of relaxation and recreation by breathing in volatile substances, called phytoncides (wood essential oils), which are anti-microbial volatile organic compounds derived from plants ( trees), such as alpha-pinene and limonene (1-2). Forest bathing trips were first proposed in the 1980s and have become a recognized relaxation and/or stress management activity in Japan (2-5). The forest bathing trip significantly increased the score for vigor and decreased the scores for anxiety, depression, and anger as investigated by the Profile of Mood States (POMS) test (2). Customary forest bathing may help to decrease the risk of psychosocial stress-related diseases (5). Since forests occupy 67% of the land in Japan (6), forest bathing is easily accessible. According to a public opinion poll conducted in Japan in 2003, 25.6% of respondents had participated in a forest bathing trip, indicating its popularity in Japan (5). Moreover, forest bathing is possible in similar environments throughout the world. We previously reported that phytoncides enhance human natural killer (NK) activity and intracellular levels of perforin, granulysin, and granzyme A in NK cells in vitro (1). Komori et al. (7) also reported that citrus fragrance found in forest affects the human endocrine and immune systems as analyzed by the measurement of urinary cortisol and dopamine levels, NK activity, and CD4/8 ratios. These findings strongly suggest that forest bathing may have beneficial effects on human immune function. Thus, we previously investigated the effect of a forest bathing trip on human NK activity, and found increased human NK activity, NK cell numbers, and intracellular levels of perforin, granulysin (GRN), and granzymes A/B (GrA/B) in peripheral blood lymphocytes (2). Nevertheless, two questions remained to be resolved: (i) Will a trip to places without a forest (a city tourist visit) also increase NK activity? (ii) How long does the increased NK activity last after a forest bathing trip or a city tourist visit? In the present study, we addressed these two questions.

## MATERIALS AND METHODS

*Subjects*

Twelve healthy male subjects, aged 35-56 years (mean 45.1±6.7), were selected from four large companies in Tokyo, Japan for the present study. Information gathered from a self-administered questionnaire, including age and lifestyle habits asking about cigarette smoking, alcohol consumption, eating breakfast, sleeping hours, working hours, physical exercise, nutritional balance, and mental stress, have been reported previously (2, 8). Written informed consent was obtained from all subjects after a full explanation of the study procedures. None of the subjects had any signs or symptoms of infectious disease, used drugs that might affect immunological analysis, or were taking any medication at the time of the study. The Ethics Committee of the Nippon Medical School approved this study (approval No. 16-1).

*Forest bathing trip and a trip to places without forest ("city tourist visit")*

In the forest bathing trip, the subjects experienced a three-day/two-night trip to three different forest fields at Agematsu town in Nagano prefecture located in northwest Japan in early September, 2006. On the first day, the subjects walked for two hours in the afternoon in a forest field, and then stayed at a nearby hotel within the forest. On the second day, the subjects walked for 2 hours in the morning and afternoon, respectively, in two different forest fields. On day 3, the subjects finished the trip and returned to Tokyo after drawing blood and completing the questionnaire. In contrast, on the city tourist visit, eleven subjects experienced a three-day/two-night trip to Nagoya city in mid-May, 2006. Nagoya is located in Aichi prefecture roughly in the center of Japan with a population of 2,219,515, and is an important crossroads for transportation in Japan. On the first day, the subjects walked for two hours in the afternoon along a tourist route, which is through an old style district in Nagoya, and then stayed at a hotel in Nagoya. On the second day, the subjects walked for 2 hours around Nagoya baseball Dome in the morning and 2 hours around/in Nagoya airport nearby Nagoya city in the afternoon. On day 3, the subjects finished the trip and returned to Tokyo after drawing blood and completing the questionnaire. There are some areas of trees in Nagoya city, but there are almost no trees in the areas visited. The class of hotels was the same and the hotel experience was the same for the city and the forest trips. The distance between Tokyo and the forest fields at Agematsu town in Nagano prefecture is almost the same as the distance between Tokyo and Nagoya. Each course in both trips was 2.5 km, closely resembling normal physical activity for the subjects on an average of normal working days. Daily physical activity of the subjects was monitored with a pedometer, and the level of background walking steps of the subjects on normal working days were monitored for a week, and the average walking distance of all the subjects was about 5.0 km/day. Then, we set the walking distances on both trips based on the results. The duration of sleep was measured with

a piezo-electric accelerometer, Actiwatch(R) (Mini Mitter Co. Inc., Sunriver), worn on the wrist of the non-dominant arm. A validation study was reported previously (9). Blood was sampled on the second and third days during the trips, and on days 7 and 30 after the forest bathing trip, and three days prior to the trips as a control. Since it has been reported that human NK cell activity shows circadian rhythms (10-11), all samples were obtained at 8:00 am. All blood samples were placed in an ice/water box at 4°C and assays performed within four hours from the blood being drawn. White blood cell (WBC) counts, NK activity, proportions of NK and T cells, and granulysin, perforin, and granzymes A/B-expressing cells in peripheral blood lymphocytes were measured. Adrenaline concentration in urine was also determined.

*NK activity*

Human peripheral blood lymphocytes (PBL) were separated from peripheral blood with a BD Vacutainer CPT (Becton Dickinson, Franklin Lakes, NJ), and then adjusted to $4x10^6$ cells/ml for the assay of NK activity. The viability of the cells, as determined by trypan blue dye exclusion, was more than 95%. NK activity was assayed according to the traditional method (2, 12). Briefly, K-562 target cells were labeled with a sodium $^{51}Cr$-chromate solution (Perkin Elmer, Boston, MA) for 60 min at 37°C in 5% $CO_2$ and washed 4 times in RPMI 1640 containing 10% fetal bovine serum (FBS) (JRH Biosciences, Lenexa, KS). The target cells were plated into round-bottomed 96-well microplates, then the effector cells (PBL) at $4x10^6$, $2x10^6$, and $1x10^6$ cells/ml in 100 µl were added to the wells in triplicate at E:T ratios of 40:1, 20:1, and 10:1. Following a 4-h incubation at 37°C in 5% $CO_2$, the microplates were centrifuged and 100 µl of supernatant from each well was collected and measured in a gamma counter. The NK activity was then calculated as described previously (2, 12). For laboratory controls of the NK activity assay, we used the same K-562 cells as the target in both experiments and always kept the K-562 cells in the same conditions before the experiments, e.g. we always used the K-562 cells 96-hr after thawing out the cells.

*Cell staining and flow cytometric analysis*

The surface markers of PBL were stained with fluorescein isothiocynate (FITC)/ phycoerythrin (PE) -CD16 and PerCP-Cy5.5-CD3 (BD PharMingen, San Diego, CA) for NK and T cells, and FITC/PE/PerCP-Cy5.5-mouse IgG1 as negative controls, for 30 min in the dark, after which the cells were fixed/permeablized with Cytofix/cytoperm solution (BD PharMingen, San Diego, CA) for 20 min at 4°C, and then intracellular perforin and GrA/B were stained with FITC- anti-human perforin, GrA/B, and FITC-IgG2b for perforin and FITC-IgG1 for GrA/B as negative controls (BD PharMingen, San Diego, CA), respectively, for 30 min at 4°C according to the manufacturer's instructions. Intracellular GRN was stained with rabbit anti-human GRN polyclonal Ab and rabbit serum as negative control (1-2, 8) after fixation/ permeablization with Cytofix/cytoperm solution, and then stained with PE-goat anti-rabbit IgG (Vector Laboratories Inc., Burlingame, CA) for 30 minutes at 4°C in the dark. After staining, the cells were washed twice with the fixative solution and once with PBS containing 1% FBS. Flow cytometric analysis was performed with a FACScan flow cytometer as described previously (2, 8, 13). Lymphocytes were identified by their characteristic appearance on a dot plot of FSC versus SSC and electronically gated to exclude dead cells and granulocytes. The fluorescence gates were set using negative controls. For laboratory controls in the cell staining and flow cytometric analysis, we used antibodies from the same lot and we always added the same volume of antibodies to the cells with the same volume of buffer in both experiments, e.g. we added 10 µl FITC-anti-granzyme A into the cell suspension in 30 µl PBS in all experiments.

*Urinary adrenaline measurement*

The levels of adrenaline in urine were measured by an HPLC method using an HLC-725CAII analyzer. The instrument features a column-switching system composed of two pretreatment columns, one separation column, and a high-sensitivity detection unit based on a post-column reaction using a fluorogenic reagent, 1,2-diphenylethyleneamine. The detection limit of adrenaline in urine was 8 fmol/ml (14).

*WBC count*

WBC, RBC, and platelet counts, the percentage of granulocytes, lymphocytes, and macrophages in peripheral blood, and the concentration of Hb, Hct, MCV, MCH, and MHCH were determined by an automatic cell counter (LC-550, Horiba Co., LTD. Kyoto, Japan) as described previously (2).

*Measurements of phytoncides and environmental temperature/ humidity in the forest fields and in the city during the investigation*

The volatile organic compounds (phytoncides) in forest and city air were measured as reported previously (2). The temperature and humidity in the forest fields were measured as reported previously (2).

*Statistical analysis*

It is generally accepted that there is wide variation in the number and activity of NK cells between individuals (15-16). We also found a high variability of the number

**Table I.** *Sleeping hours of the subjects before and during the forest bathing trip and city tourist visit (Mean±SD)*

| | Control day (Before trip) | Day 1 (During trip) | Day 2 (During trip) | 1 Week (After trip) | 1 Month (After trip) |
|---|---|---|---|---|---|
| Forest bathing trip | 6.18±1.63 | 7.29±1.69 | 7.03±1.44 | 5.38±0.88 | 5.63±0.96 |
| City tourist visit | 5.98±1.79 | 7.22±1.52 | 6.59±1.41 | | |

**Table II.** *Concentration of volatile substances (phytoncide) in the air of forest fields and in the air of city calculated as alpha-pinene (ng/m³).*

| | Forest bathing trip | | City tourist visit | |
|---|---|---|---|---|
| Measuring points | Field 1 Day 1 pm | Field 2 Day 2 | Old style district in Nagoya Day 1 pm | Nagoya baseball Dome Day 2 |
| Kind of Trees | *Chamaecyparis obtusa* | *Chamaecyparis obtuse, Thuja occidentalis* | | |
| Tricyclene | 299.7 | 805.5 | n.d | n.d |
| α-Pinene | 2,886.7 | 1,281.7 | 6 | n.d |
| Camphene | 375.6 | 486.8 | n.d | n.d |
| β-Pinene | 137.5 | 66.9 | n.d | n.d |
| Myrcene | 109.4 | 71.8 | n.d | n.d |
| δ-3-Carene | 66.6 | 25.3 | n.d | n.d |
| α-Terpinene | 43.8 | 26.9 | n.d | n.d |
| *p*-Cymene | 109.4 | 67.3 | n.d | n.d |
| Limonene | 111.1 | 48.4 | 17 | n.d |
| γ-Terpinene | n.d | 33.2 | n.d | n.d |
| Terpinolene | 87.5 | 13.4 | n.d | n.d |
| Camphor | 32.8 | 14.8 | n.d | n.d |
| Bornyl acetate | 54.7 | 43.4 | n.d | n.d |

*n.d: not detected*

and activity of NK cells in different individuals in the present study and in our previous study (2, 8, 12). Because the variability between individuals in the number and activity of NK cells was large, we did not compare the difference in NK activity between the forest bathing trip and city tourist visit. Rather, differences in NK activity before and after each type of trip were compared in the present study. Instead, the comparisons between the two trips were conducted using the rates of increased NK activity after the trip. We analyzed the data with two-way ANOVA with no-repeated measures (one-way ANOVA with repeated measures), with the variability among individuals and the different days as two factors. Comparisons between different days were made with the paired t-test if the analysis of variance was significant. Comparisons between the two trips were made with the unpaired t-test. The analyses were performed with the Microsoft Excel software package for Windows. The significance level for p values was set at $< 0.05$.

## RESULTS

### Effect of a forest bathing trip versus a city tourist visit on NK activity

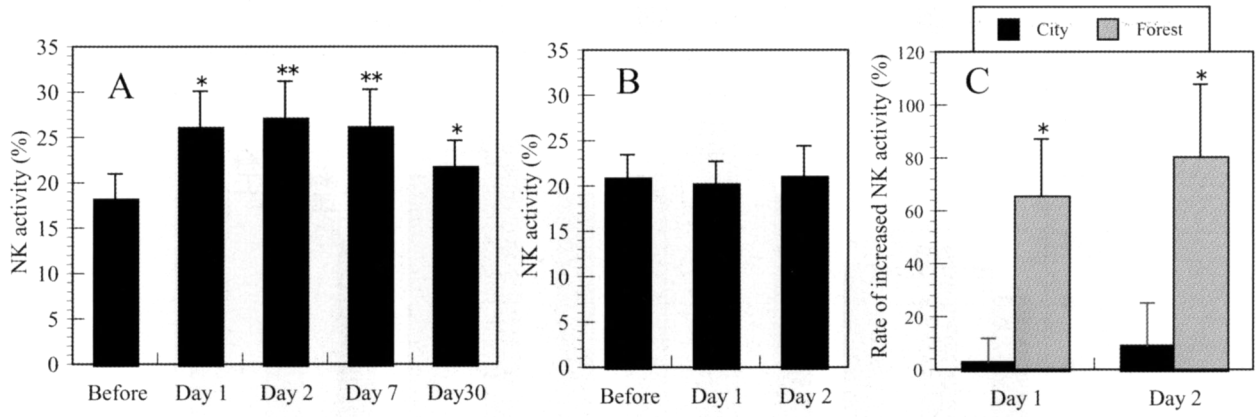


**Fig. 1**. *Effect of the forest bathing trip (A) and the city tourist visit (B) on NK activity. The columns labelled "Before" indicate the NK activity determined before the trips, the columns labelled "Day 1" indicate the NK activity determined after the first day of each trip, the columns labelled "Day 2" indicate the NK activity determined after the second day of each trip, the column labelled "Day 7" indicates the NK activity determined 7 days after the trip, the column labelled "Day 30" indicates the NK activity determined 30 days after the trip. Data are presented as the mean+SE (n=12 in A and n=11 in B). Two-way ANOVA with no-repeated measures indicated that the forest bathing trip and the variability between individuals significantly affected NK activity (all p<0.01). *: p<0.05, **: p<0.01, significantly different from before the trip by paired t-test. C: Comparison of the rate of increase in NK activity between the forest bathing trip and the city tourist visit. Data are presented as the mean+SE (n=12 in forest and n=11 in city). *: p<0.05, significantly different from the city tourist visit by unpaired t-test. The activity values for an E/T ratio of 20/1 are shown, and similar results were also obtained with E/T ratios of 40/1 and 10/1.*

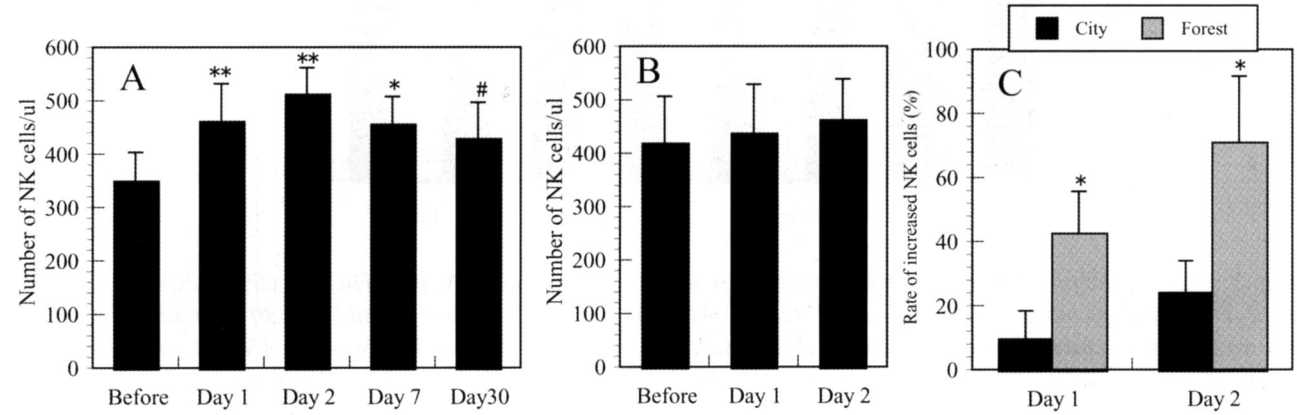


**Fig. 2.** *Effect of the forest bathing trip (A) and the city tourist visit (B) on the number of NK cells. The meanings of the columns labelled "Before", "Day 1", "Day 2", "Day 7", and "Day 30" are the same as those shown in Fig. 1. Data are presented as the mean+SE (n=12 in A and n=11 in B). Two-way ANOVA with no-repeated measures indicated that the forest bathing trip and the variability between individuals significantly affected the percentage and number of NK cells (all p<0.01). *: p<0.05, **: p<0.01, #: p=0.054 significantly different from before the trip by paired t-test. C: Comparison of the rate of increase in NK number between the forest bathing trip and the city tourist visit. Data are presented as the mean+SE (n=12 in forest and n=11 in city). *: p<0.05, significantly different from the city tourist visit by unpaired t-test.*

The forest bathing trip significantly increased human NK activity, and this increase lasted more than 7 days (Fig. 1A). In contrast, the city tourist visit did not increase human NK activity (Fig. 1B). Moreover, the rates of increase in NK activity during the forest bathing trip were significantly greater than those during the city tourist visit (Fig. 1C).

Effect of a forest bathing trip versus a city tourist visit on $CD16^+$ NK cells

The forest bathing trip significantly increased the

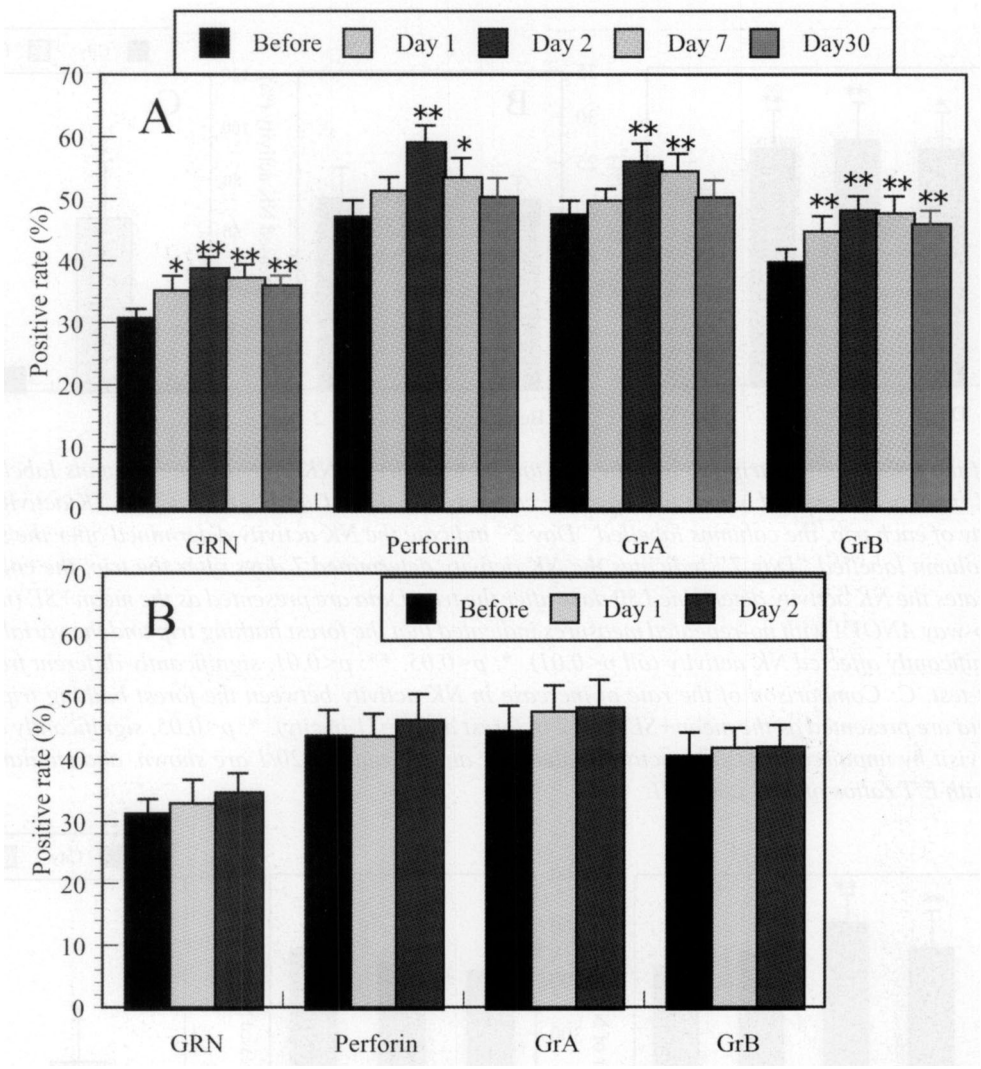


**Fig. 3**. *Effect of the forest bathing trip (A) and the city tourist visit (B) on GRN, perforin, and GrA/B-expressing cells in PBL. The meanings of the columns labelled "Before", "Day 1", "Day 2", "Day 7", and "Day 30" are the same as those shown in Fig. 1. Data are presented as the mean+SE (n=12 in A and n=11 in B). Two-way ANOVA with no-repeated measures indicated that the forest bathing trip and the variability between individuals significantly affected the GRN, perforin, GrA/B-expressing cells in PBL (all p<0.01). *: p<0.05, **: p<0.01, significantly different from before the trip by paired t-test.*

number of $CD16^+$ NK cells, and this increase lasted more than 7 days after the trip (Fig. 2A). Although the city tourist visit also slightly increased $CD16^+$ NK cells, this change was not significant (Fig. 2B). Moreover, the rates of increase in the number of NK cells during the forest bathing trip were significantly greater than those during the city tourist visit (Fig. 2C). The forest bathing trip did not affect lymphocyte or WBC counts.

Effect of a forest bathing trip versus a city tourist visit on the percentage of cells expressing cytolytic molecules

The forest bathing trip significantly increased the percentages of GRN, perforin, and GrA/B-expressing cells in PBL, and this increase lasted more than 7 days after the trip (Fig 3A). Although the city tourist visit also slightly increased the percentages of GRN, perforin, and GrA/B-expressing cells in PBL, these changes were not significant (Fig. 3B).

Effect of a forest bathing trip versus a city tourist

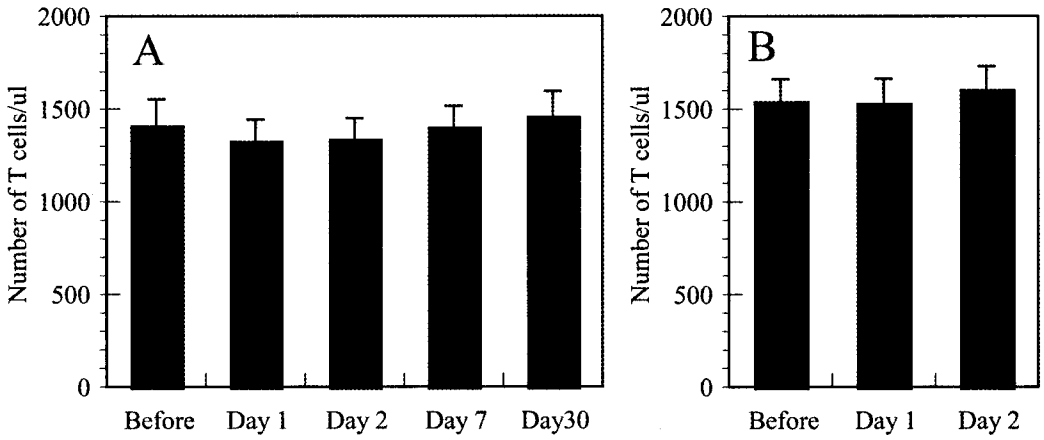


**Fig. 4.** *Effect of the forest bathing trip (A) and the city tourist visit (B) on the number of T cells. The meanings of the columns labelled "Before", "Day 1", "Day 2", "Day 7", and "Day 30" are the same as those shown in Fig. 1. Data are presented as the mean+SE (n=12 in A and n=11 in B). Two-way ANOVA with no-repeated measures indicated that neither the forest bathing trip nor the city tourist visit affected the number of T cells.*

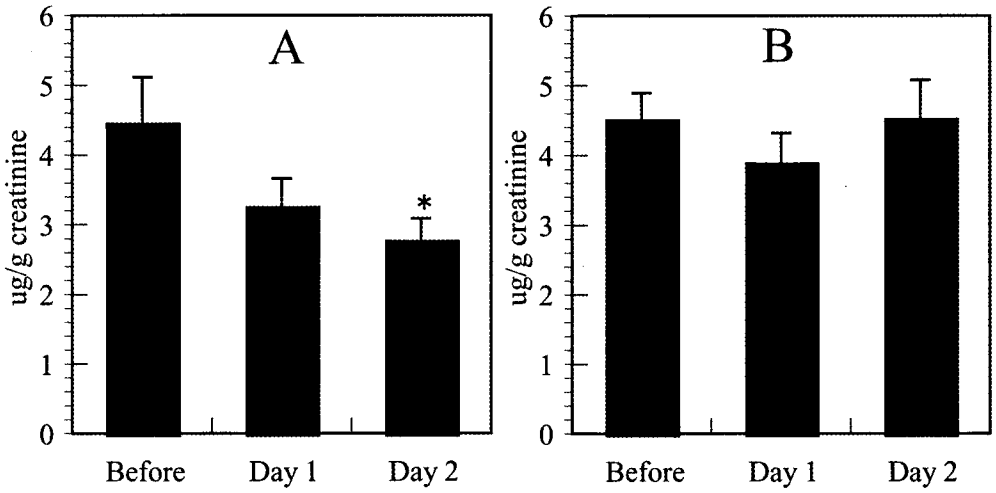


**Fig. 5.** *Effect of the forest bathing trip (A) and city tourist visit (B) on adrenaline concentration in urine. The meanings of the columns labelled "Before", "Day 1" and "Day 2" are the same as those shown in Fig. 1. Data are presented as the mean+SE (n=12 in A and n=11 in B). Two-way ANOVA with no-repeated measures indicate that the forest bathing trip significantly affected the adrenaline concentration in urine ($p<0.05$). *: $p<0.05$, significantly different from before the trip by paired t-test.*

visit on T ($CD3^+$) cells

Neither the forest bathing trip (Fig. 4A) nor the city tourist visit (Fig. 4B) affected T cell number.

*Effect of a forest bathing trip* versus a city tourist visit *on adrenaline concentration in urine*

The forest bathing trip significantly decreased the concentration of adrenaline in urine while the city tourist visit had no effect (Fig. 5A, 5B).

There were no significant differences in daily physical activity before or during the trips, nor significant differences in daily physical activity between the two trips (data not shown). Although the hours of sleep increased during both types of trips compared with control days (Table I), this difference was not significant.

Lastly, phytoncides, such as alpha-pinene, beta-pinene, tricyclene, camphene, and limonene, were

detected in higher concentrations in the forest fields during the investigation, but were not detected in the urban area of Tokyo, or in the city tourist visit on day 2 although only very low concentrations of alpha-pinene and limonene were detected on day 1 (Table II). Weather during the forest bathing trip was excellent with average temperatures and humidity in the forest fields during the walks of 16.31 ± 0.23°C, 99.77 ± 0.44% on day 1 in the afternoon, 19.55 ± 0.61°C, 78.62 ± 3.24% on day 2 in the morning, and 20.78 ± 0.42°C, 74.16 ± 1.94% on day 2 in the afternoon, respectively. The average temperature and humidity in the urban area of Tokyo on the control day was 25.7°C and 62%, respectively. While weather during the city visit tourist in Nagoya also was excellent with average temperatures of 23°C and humidity of 60% on day 1, and 22°C and 37% on day 2. The average temperature and humidity in the urban area of Tokyo on the control day were 19.1°C and 42%, respectively.

## DISCUSSION

The present study confirmed that a forest bathing trip enhances the immune response as measured by human NK activity and the numbers of NK cells, as reported previously (2). In contrast, a trip to places without forests (city tourist visit) had no effect on NK activity or the numbers of NK cells, indicating that forest bathing does indeed enhance human NK activity. Moreover, we also found that the increased NK activity and numbers of NK cells induced by a forest bathing trip lasted more than 7 days, even 30 days, after the trip. This suggests that if people visit a forest once a month, they may be able to maintain increased NK activity. This may be important in health promotion and preventive medicine.

NK cells kill tumor or virus-infected cells by the release of perforin, granzymes (13, 17-20), and GRN (21-22) via the granule exocytosis pathway.

In order to explore the mechanism of enhancement of NK activity by forest bathing, we investigated the effect of forest bathing on the intracellular levels of perforin, GRN, and GrA/B in PBL. We found that the forest bathing trip significantly increased the proportion of PBL expressing these effector molecules, confirming our previous report (2). In contrast, a city tourist visit had no effect on the expression of these proteins. Moreover, we found that increased perforin, GRN, and GrA/B-expressing cells induced by a forest bathing trip lasted more than 7 days, even 30 days, after the trip. These cytolytic molecules contribute to NK and anti-tumor activity (22-23).

Adrenaline is released from the adrenal medulla, and the adrenaline level increases under circumstances of novelty, anticipation, unpredictability, and general emotional arousal, whereas the level of noradrenaline increases during increased physical activity (24). Measurement of free adrenaline in urine provides a reliable measure of the circulating concentration of adrenaline in the bloodstream and thus a measure of adrenal medulla activity (25). The concentration of adrenaline in urine has been used to evaluate work related stress in lorry drivers (26), long distance coach drivers (27), and psychosocial stress (28). We found that a forest bathing trip significantly decreased the adrenaline concentration in urine, while a city tourist visit had no effect, suggesting that the subjects were under conditions of lower stress during the forest bathing trip. It has been reported that adrenaline inhibits human NK activity (29). We found previously that physical and/or psychological stress decreased NK activity, NK receptors, and mRNA transcription of granzymes and perforin in mice (30). The increased NK activity in a forest bathing trip may be related to an attenuated stress hormone response (adrenaline) associated with the forest bathing trip. Previous studies have reported that forest bathing reduces the concentration of cortisol in saliva, reduces prefrontal cerebral activity, reduces blood pressure, and stabilizes autonomic nervous activity in humans (4, 31).

Many factors, including circadian variation (10-11), physical exercise (8, 32), and alcohol consumption (8, 33) can affect human NK activity. In order to control the effect of circadian rhythm on NK activity, we sampled blood at 8 am on all days. To control for the effect of physical exercise on NK activity, we limited the walking steps during the trips to the averaged normal workday distances as monitored by a pedometer. The levels of physical activity between the two trips were also matched. To control the effect of alcohol on NK activity, the subjects did not consume alcohol during the study

period for either trip. The sleeping hours during the trips were a little longer than on average working days (Table I); however, the difference was not significant in either type of trip. Kusaka et al (34) reported that sleeping hours did not affect NK activity, or NK cell numbers under physiological conditions. In fact, we also found that there was no difference in the numbers of NK cells, nor the levels of perforin, GRN, or GrA/B-expressing cells in PBL among the subjects who slept 5, 6, or 7 hours, respectively (8). In addition, although the sleeping hours during the city tourist visit were a little longer than on average working days (Table I), the NK activities during the trip were almost the same as for working days, indicating that the longer sleeping hours did not contribute to NK activity on the city tourist visit. Taken together, although the sleeping hours during the trips were a little longer than those on average working days, this difference did not affect either NK activity or cell numbers in the present study.

As detailed in Table II, we detected several phytoncides, such as alpha-pinene, beta-pinene, tricyclene, camphene, and limonene in higher concentrations in the forest fields during the trip; however, only very low concentrations of alpha-pinene and limonene were detected only in a city area for the city tourist visit. We previously found that such phytoncides significantly enhanced human NK activity and increased the expression of intracellular cytolytic molecules, perforin, GrA, and GRN *in vitro* (1). Komori et al (7) also reported that citrus fragrance found in forests affects the human endocrine and immune systems as analyzed by the measurement of urinary cortisol and dopamine levels, NK activity, and CD4/8 ratios. These findings suggest that phytoncides may partially contribute to the enhanced NK activity during the forest bathing trip.

In summary, a forest bathing trip can increase human NK activity, the number of NK cells, and the expression of intracellular perforin, GrA/B, and GRN. Forest bathing may contribute to decreased stress and improved immunity, and phytoncides from trees may partially contribute to this effect.

## ACKNOWLEDGEMENTS

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