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*Original Research*

# Influence of Aerobic Exercise Combined with Forest Bathing on Immunocytes, Stress Hormones, $VO_2$peak, and Body Composition in Elderly Men: A Randomized Controlled Trial

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**Abstract**

**Background**: Forest bathing and aerobic exercise are known to be factors that increase natural killer (NK) cell, but it is considered to provide a greater effect when the two factors are combined. To date, it has not been studied whether aerobic exercise combined with forest bathing can further increase innate immunocytes, including NK cell. Therefore, this study investigated the effect of aerobic exercise + forest bathing on NK cell and whether stress hormones (epinephrine and cortisol) are involved in this physiological process. In addition, this study tried to confirm whether the mixed effect of the two had a more positive effect on cardiorespiratory endurance as well as body composition in the elderly men. **Methods**: Thirty-two participants were randomly assigned to one of four groups: control group (CON, n = 8) which was not provided with any intervention, exercise group (EXE, n = 8) which performed treadmill exercises without phytoncide, phytoncide group (PHYT, n = 8) which was provided with phytoncide intervention, and exercise + phytoncide group (EXE + PHYT, n = 8) which performed treadmill exercises and was provided with phytoncide. Treadmill exercises and phytoncide exposures were performed for 45~60 min a day, 3 days a week for 12 weeks. **Results**: Compared with CON, (1) the leucocytes and lympocytes of EXE, PHYT, and EXE + PHYT showed positive changes. Specifically, total NK cells, NKT cells, and NKG2D + NK of the EXE + PHYT increased after 12 weeks of intervention. (2) The cortisol concentrations of EXE, PHYT, and EXE + PHYT showed decreasing changes, whereas the epinephrine concentration were significantly increased. (3) Although there were no changes in the muscle mass of EXE, PHYT, and EXE + PHYT, cardiorespiratory endurance was significantly increased in those groups. In addition, the body weight, fat mass, and fat percentage significantly decreased only in the EXE + PHYT. **Conclusions**: This study confirmed that forest bathing and aerobic exercise positively affected immunocyte function in elderly men. It also found that the improved results from forest bathing + treadmill walking were caused by an increase in cardiorespiratory endurance by increased epinephrine concentrations. In addition, this increased cardiorespiratory endurance can be interpreted as significantly reducing the body weight and fat in the group that participated in the exercise combined with phytoncide exposure.

**Keywords:** forest bathing; aerobic exercise; immunocyte; physical fitness; NK cell; elderly

## 1. Introduction

As age increases, the function of immunocytes decreases, leading to cancer, disease, or death [1]. Although the immunosenescence that occurs in the body may be a natural phenomenon, there are many ways to delay or inhibit this process. The human immune system has innate immunity and acquired immunity. However, innate immunity can protect the human body more effectively against cancer that can come from aging or substances that invade from the outside [2]. Upon identification of danger or a foreign invader, innate immunocytes, such as natural killer (NK) cells, respond by destroying infected cells and releasing cytokines and chemokines to recruit additional cells to fight the infection and alter host tissues, a process commonly referred to as inflammation [3]. NK cells are a type of leucocyte in the blood and are innate immunocytes that mainly produce and mature in the bone marrow to directly destroy cancer cells. There are a total of about 100 million NK cells in the body. The anticancer mechanism of NK cells is well known. That is, NK cells recognize abnormal cells, make holes in the cell membrane with perforin, and induce apoptosis by introducing granzyme B into the cytoplasm, or destabilize the osmotic pressure of the cytoplasm and excessive inflow of water and salt to cause cell necrosis [4].

In order to protect the human body from immunosenescence, the amount or sensitivity of innate immunocytes, including NK cells, must be improved. Efforts to increase the number and quantity of innate immune cells from the past to the present have been continued through scientists in many fields such as Immunology, Medicine, Pharmacy, Biology and Exercise Physiology. In particular, phytoncide emitted from trees located in the mountains has attracted much attention in the academic world as it is known to effectively destroy infected cells or tumor cells by increasing NK cells in the human body [5]. Since several

studies have shown that phytoncide increases the activity of NK cells [6–9], phytoncide is expected to have anticancer and antiaging effects by increasing NK cells activity. Phytoncides are volatile substances that are emitted or secreted by plants to resist pathogens. It consists of phenolic compounds, alkaloids, and glycosides, including terpenes, which are the main components. Among the terpenes, monoterpenes have adrenal cortex stimulation, antiseptic, sterilization, and antiviral effects. Tannin, the second most abundant component in phytoncide, has detoxification, sterilization, hemostasis, and anti-inflammatory effects [10]. When phytoncide is inhaled as a gas, it relieves stress, strengthens intestinal and cardiopulmonary functions, and has a sterilization effect. In addition, it is reported that phytoncide has an antibacterial effect, which can increase immunity.

It is also reported that physical exercise slows or prevents the aging process by enhancing cardiorespiratory endurance and basal metabolic rate, as well as improving immunocyte function [11,12]. In particular, moderate aerobic exercise is considered to have anticancer effects because it increases NK cells [13]. Crist *et al*. [14] reported that NK cell tumor cytotoxicity was significantly improved after treadmill exercise was performed by elderly women. Recently, Lee and Jee [15] reported that increasing muscular strength and endurance through a resistance exercise for 12 weeks in cancer survivors could improve adaptive immunocytes. In fact, the increase in NK cell activity due to exercise is explored by regulating the autonomic nervous system and the action of hormones caused by the body's response to exercise. According to Tønnesen *et al*. [16], there was no change in NK cell activity after cortisol alone, but epinephrine alone or cortisol + epinephrine administration increased NK cell activity. It can be considered that the epinephrine regulated by the $\beta$-adrenergic receptor increased the activity of NK cells. It has been explained that NK cells have adrenergic receptors on their cell membranes and thus respond sensitively to changes of cortisol or epinephrine in the blood [16]. However, it is difficult to draw conclusions because the responses and adaptations of hormones due to exercise vary according to various experimental environments, as well as the exercise intensity, time, and period.

Here, we can speculate that exercising in the mountains with a high level of phytoncide can prevent the decline of immune function due to aging and activate the function of innate immune cells that destroy cancer cells. However, it was not easy to establish a scientific basis for such a theory due to the lack of an experimental environment or research manpower. Moreover, it is not known whether exercise while forest bathing is consistent with the exercise mechanism described in previous studies [17,18]. Therefore, this study investigated the effects of aerobic exercise combined with forest bathing on the innate immuocytes including NK cells and receptors in elderly men. In addition, this study investigated how cortisol and epinephrine, which affect innate immunocytes, change as a result of exercise with abundant phytoncides, and whether aerobic exercise combined with forest bathing can improve their effects.

## 2. Materials and Methods

### *2.1 Participants*

The participants were aged between 61 and 79 years from Seoul Seniors Tower in Seoul and Inje, Korea. All participants did not exercise regularly for over six months, but expressed willingness to improve their immunosenecense. The inclusion criteria included (1) no or minor early knee orsteoarthritis that is confirmed by the anteroposterior, weight-bearing, and short knee X-ray; (2) over 60 years of age; and (3) male. Exclusion criteria included: having a deformity of the knee, hip, or back; central or peripheral nervous system involvement; having a history of taking any medication including steroids or intra-articular injection within the previous 6 months; having undergone surgery within the previous 6 months; using a pacemaker; having internal metallic materials; having a history of impairment of a major organ system or a psychological disorder [19].

Specifically, all participants were assigned using random number tables and identification numbers upon recruitment. Initially, forty two elderly men enrolled in this study. Two participants were excluded from the process of grouping, and forty participants were randomized. In the experimental phase, two participants in the control group (CON), two participants in the exercise group (EXE), one participant in the phytoncide group (PHYT), and three participants in the exercise + phytoncide group (EXE + PHYT) were lost. Finally, a total of 32 participants were included in the study as shown in Fig. 1.

Thirty-two participants who met the inclusion criteria were randomly assigned to one of four groups: a CON (n = 8) that was not provided with any intervention, an EXE (n = 8) that performed a treadmill exercise without phytoncide; a PHYT (n = 8) that was provided with phytoncide intervention; and an EXE + PHYT (n= 8) that performed a treadmill exercise with phytoncide provided. Participant characteristics are presented in Table 1.

### *2.2 Experimental Design*

This prospective, cross-sectional randomized controlled trial was conducted in accordance with the Declaration of Helsinki (2013 version) and was approved by the ethics committee (September 1, 2018 to August 31, 2019; 2-1040781-A-N-012021024HR and Seoul Songdo Hospital; 2021-008) This study was registered in the Korean Clinical Research Information Service. Prior to the study, participants received detailed explanations regarding the study procedures and written informed consent was obtained from all participants. The participants were assessed at two different time points and two different places, including Seoul

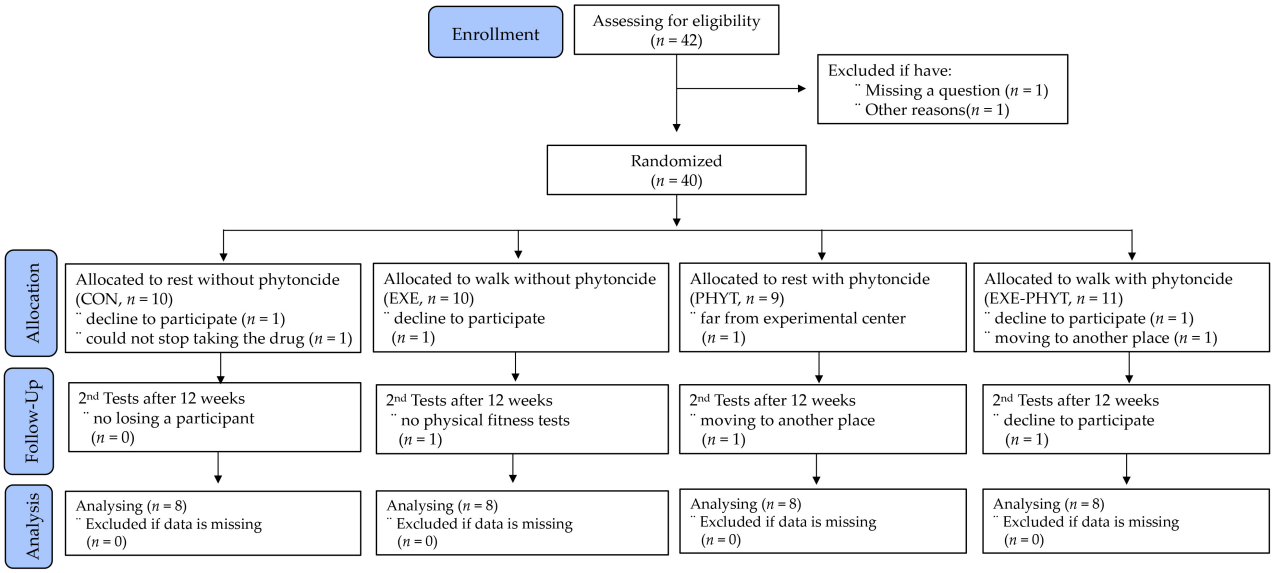


**Fig. 1. Participants' allocation (consolidated standards for reporting of trials flow diagram).**

**Table 1. Physical characteristics of the participants.**

| | Groups without phytoncide | | Groups with phytoncide | | $X^2$ | $p$ |
|---|---|---|---|---|---|---|
| | CON | EXE | PHYT | EXE + PHYT | | |
| | (n = 8) | (n = 8) | (n = 8) | (n = 8) | | |
| Age (y) | 64.25 ± 2.05 | 65.63 ± 2.00 | 64.50 ± 2.14 | 64.63 ± 2.88 | 1.697 | 0.638 |
| Height (m) | 1.71 ± 0.03 | 1.71 ± 0.04 | 1.72 ± 0.02 | 1.71 ± 0.03 | 0.406 | 0.939 |
| Body weight (kg) | 72.98 ± 6.48 | 75.10 ± 3.77 | 73.61 ± 8.81 | 72.86 ± 6.70 | 0.827 | 0.843 |
| Muscle mass (kg) | 34.09 ± 5.50 | 33.75 ± 5.03 | 32.43 ± 4.84 | 33.00 ± 7.51 | 0.816 | 0.846 |
| Fat mass (kg) | 20.18 ± 2.84 | 20.14 ± 2.53 | 19.98 ± 2.55 | 20.66 ± 1.45 | 0.099 | 0.992 |
| Percent fat mass (%) | 28.01 ± 5.50 | 26.90 ± 3.86 | 27.58 ± 5.38 | 28.59 ± 3.45 | 0.738 | 0.864 |
| Percent muscle mass (%) | 47.03 ± 8.84 | 45.03 ± 6.93 | 44.19 ± 5.12 | 45.05 ± 8.11 | 0.574 | 0.902 |
| Body mass index (kg/m$^2$) | 25.00 ± 2.05 | 25.71 ± 2.22 | 24.98 ± 3.34 | 24.98 ± 1.93 | 0.361 | 0.948 |

All data represents mean ± standard deviation. CON, control group; EXE, exercise group; PHYT, phytoncide group; EXE + PHYT, exercise + phytoncide group.

and Inje of Gangwondo, South Korea over 12 weeks. The purpose of this study was to investigate the effects of treadmill walking on cortisol, epinephrine, and NK cells and receptors in two areas with low and high levels of phytoncide, targeting elderly males of similar age groups. The assessment was performed before and after the intervention period. The participants involved in the PHYT and EXE + PHYT left in Inje, Gangwondo the day before the experiment, received intervention for 3 days and were allowed to return to their residence in the afternoon at the end of the week. To prevent communication among the groups at each location, the participants were classified according to their community areas. Moreover, the interventions and measurements were also scheduled at different times to prevent communication among the CON, EXE, PHYT and EXE + PHYT. The CON was scheduled at 10:00, the EXE at 12:00, the PHYT at 10:00, and the EXE + PHYT at 12:00. For the CON and PHYT, they gathered at the immune center at the scheduled time and performed only meditation while lying on a mattress for 60 min at each place. The assessments were performed at Week 0 and at Week 12. The intervention program consisted of treadmill walking, which was conducted 60 min a day, 3 consecutive days a week for 12 weeks. The independent variables in this study included forest bathing and treadmill walking, whereas the dependent variables consisted of leucocytes, lymphocytes, NK cells/receptors, epinephrine, cortisol, body composition, and cardiorespiratory fitness.

### *2.3 Characteristics of Experimental Locations*

The weather and climate conditions were obtained through the Korea Meteorological Administration (https://www.weather.go.kr/w/index.do) [20] and information regarding phytoncide concentrations was obtained through the Korea National Institute of Forest Science (https://www.forest.go.kr/kfsweb/kfs/idx/Index.do) [21].

**Table 2. The environmental characteristics and differences between Seoul and Inje.**

| | Seoul | Injae | Z | *p* |
|---|---|---|---|---|
| Average temperature (°C) | 25.60 ± 2.66 | 23.23 ± 2.50 | –1.091 | 0.400 |
| Average maximum temperature (°C) | 29.83 ± 2.30 | 29.07 ± 2.78 | –0.443 | 0.700 |
| Maximum temperature (°C) | 33.93 ± 2.46 | 34.50 ± 2.04 | –0.655 | 0.700 |
| Average minimum temperature (°C) | 22.10 ± 2.91 | 19.03 ± 2.71 | –1.091 | 0.400 |
| Minimum temperature (°C) | 17.00 ± 3.29 | 14.57 ± 2.35 | –1.091 | 0.400 |
| Average humidity (%) | 72.67 ± 1.53 | 77.67 ± 4.04 | –1.771 | 0.100 |
| Minimum humidity (%) | 37.33 ± 1.53 | 33.33 ± 10.69 | –0.655 | 0.700 |
| Average wind speed (m/s) | 2.10 ± 0.10 | 1.33 ± 0.15 | –1.964 | 0.100 |
| Maximum wind speed (m/s) | 7.83 ± 0.45 | 5.30 ± 0.82 | –1.964 | 0.100 |
| Maximum wind speed/direction (º) | 190.00 ± 135.28 | 73.33 ± 92.38 | –1.550 | 0.200 |
| Maximum instantaneous wind speed (m/s) | 15.97 ± 0.84 | 11.97 ± 2.60 | –1.964 | 0.100 |
| Maximum instantaneous wind speed/direction (º) | 276.67 ± 90.74 | 200.00 ± 0.00 | –0.696 | 0.700 |
| Phytoncide concentration (pptv) | 575.30 ± 361.02 | 828.94 ± 527.54 | 8.842 | 0.031 |

All data represents mean ± standard deviation. CON, control group; EXE, exercise group; PHYT, phytoncide group; EXE + PHYT, exercise + phytoncide group.

The experiments in this study were conducted between June and August 2021. The environmental characteristics related to phytoncide concentration, temperature, humidity, and wind speed in the two locations are shown in Table 2.

### *2.4 Treadmill Exercise Program*

The EXE and EXE + PHYT participated in a treadmill exercise in a supervised exercise program 3 days a week for 12 weeks. The treadmill exercise for EXE was conducted at the Exercise Immunity Center in Seoul where phytoncide was low, while the treadmill exercise for EXE + PHYT was conducted at the Exercise Immunity Center in Gangwon-do where phytoncide was high. During the treadmill exercise for EXE + PHYT, all doors and windows were opened so that fresh air with high phytoncide concentration could be inhaled. The elderly who participated in this study observed whether treadmill exercise was possible in advance, and participants in EXE and EXE + PHYT, who had to do this exercise, were allowed to exercise for a set period on the treadmill assigned to them. During exercise, four exercise prescribers observed the elderly at a close distance, and one observed the data transmitted from the heart rate clock through a laptop placed on the table. The dosage of the exercise regimen was determined through the FITT principle, wherein the frequency, intensity, time (duration), and type of exercise together quantify the total dose [22]. In particular, when designing an exercise program, this study tried to prescribe 150 min of moderate-intensity, or 75 min of vigorous-intensity, exercise per week as suggested by Physical Activity Guidelines Advisory Committee [23], and is shown in Table 3.

As shown in Table 3, EXE and EXE + PHYT began with warm-up conditioning for 10 min as instructed by the researcher. Next, all participants performed the workout. Aerobic exercise training was performed 3 days a week, every other day (Monday, Wednesday, and Friday), and the intensity of exercise included walking or running at 60–85% of $VO_2$peak. For this, a heart monitor was used to maintain a heart rate according to the exercise intensity. Phase I started with 60 min of walking and progressed to 45 min of brisk walking and light jogging in Phase IV, where the program ended. The pulse rate was measured during the treadmill exercise using the Xcoach system (Spornix, Seoul, Korea). The Xcoach system is a device that can analyze the exercise intensity and fatigue recovery ability through real-time heart rate/exercise intensity wireless monitoring of the subject. As shown in Fig. 2, it consists of a heart rate measuring chest belt, data transmitter/receiver, and an exercise intensity display wristwatch, and it is composed of a laptop (program installation) that receives the data transmitted from the transceiver. Before the exercise, all subjects were instructed to place a sensor (heart rate checking) in the center of the chest and fix the torso with a band. And the heart rate transmitted from the sensor on the wristwatch was derived from the exercise intensity, and it was received and quantified by a laptop computer. Lying stretches were performed for 15 min after exercise.

### *2.5 Measurement Methods*

#### 2.5.1 Stress Hormones and Immunocytes Measures

All subjects were prohibited from exercising and taking drugs for more than 12 h before blood collection, and after fasting, about 10 mL of blood was collected from the antecubital vein, centrifuged at 3000 rpm for 15 min, and stored in a –70 °C freezer until analysis. This study tried to analyze cortisol and epinephrine as stress hormones and to analyze leucocyte, lymphocyte, and granulocyte subsets as immune cells. Cortisol concentration was measured using an enzyme-linked immunosorbent assay (ELISA) kit (DRG Instruments GmbH, Hamburg, Germany), and epinephrine was analyzed by HPLC (Hercules, California, USA) using a plasma catecholamine ELISA kit

**Table 3. Treadmill exercise training program.**

| Items | Exercise types | | Exercise training phases | | | |
|---|---|---|---|---|---|---|
| | | | I | II | III | IV |
| | | | 0–3 week | 4–6 week | 7–9 week | 10–12 week |
| Warm-up | Standing stretching | | 10 min | 10 min | 10 min | 10 min |
| Work-out | Aerobic training | F | 3 days | 3 days | 3 days | 3 days |
| | | I | 60% $VO_2$peak | 70% $VO_2$peak | 80% $VO_2$peak | 85% $VO_2$peak |
| | | T | 60 min | 55 min | 50 min | 45 min |
| Cool-down | Sitting/Lying stretching | | 15 min | 15 min | 15 min | 15 min |

F, frequency; I, intensity; T, time; $VO_2$peak, peak oxygen consumption.

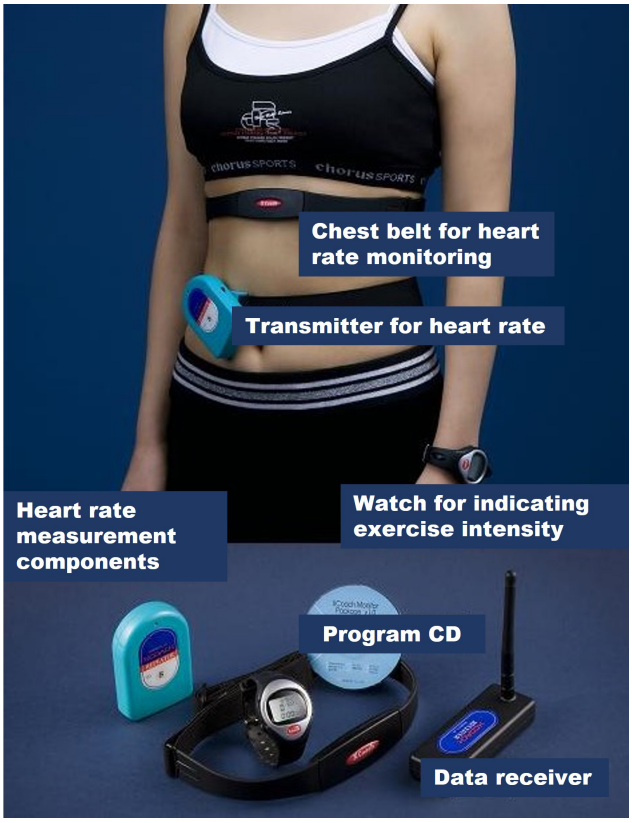


**Fig. 2. Device for checking a heart rate.**

(Labor Diagnostika Nord, Nordhorn, Germany). In the case of immunocytes, peripheral blood samples were obtained in heparinized collection tubes and EDTA tubes. Whole blood samples were used for the automated differential blood cell counts and fluorescence-activated cell sorting (FACS) analysis. EDTA-anticoagulant blood samples for automated leucocyte differential tests were submitted to our hematology laboratory and tested on the Sysmex XN-550 (Sysmex Corporation, Kobe, Japan) as the primary routine method. The samples were stored at room temperature for no longer than 4 hours prior to testing. A lysed whole blood technique with maximum 8-color staining of blood was used on cells for flow cytometry and antibody staining, as shown in Fig. 3.

Subpopulations of human peripheral blood cells were analyzed as follows: 50 $\mu$L of blood were stained with anti-human antibodies against anti-CD56 (NK), anti-CD3, anti-CD314 (NKG2D), and anti-CD158b (KIR2DL3) (BioLegend, San Diego, California, USA), together with appropriate isotype controls. All antibodies were obtained from BD Biosciences (Franklin Lakes, NJ, USA). After incubation for 30 min at room temperature in the dark, the red blood cells were lysed by adding 500 $\mu$L of FACS lysing solution (BD Biosciences) to each test tube for 15 min in the same conditions. After the lysis of red blood cells, the remaining cells were washed in 2 mL of permeabilization buffer (DPBS 1X, Welgene, Gyeongsan, Korea). After staining was completed, cells were analyzed using FACS Canto II (BD Bioscience) and Flowjo software (Treestar, Ashland, OR, USA) and are presented as percentages.

### 2.5.2 Body Composition and Cardiorespiratory Endurance Measures

For the accuracy of the test, participants were asked to limit food, alcohol and caffeine intake prior to the test. They were also asked not to exercise on the day before the test and to urinate just before the test. The body composition including height was measured by Inbody 770 (Biospace Co., Ltd., Seoul, Korea). All participants took off all metal objects attached to their bodies. Participants were asked to stand on the device and hold the handles for 2~3 min. Only body weight, muscle mass, fat mass, percent fat, and body mass index were selected from among various data to be derived from the body composition test and used for analysis. Cardiorespiratory endurance of participants was estimated by obtaining maximum oxygen uptake ($VO_2$peak) through a graded exercise test (GXT) device, which equipped with treadmill (Q65-90, Quinton, USA), electrocardiogram (Q-4500, SunTech Medical, Inc., USA), automatic sphygmomanometer (M-412), and gas tester (QMC4200). Participants placed 10 electrodes on their chest and an automatic sphygmomanometer was worn on their left arm. Prior to this exercise stress test, an examiner placed the participant on a chair on a treadmill and put a mask on the participant to prevent air from leaking from the nose and mouth. The participant was given several explanations to be given dur-

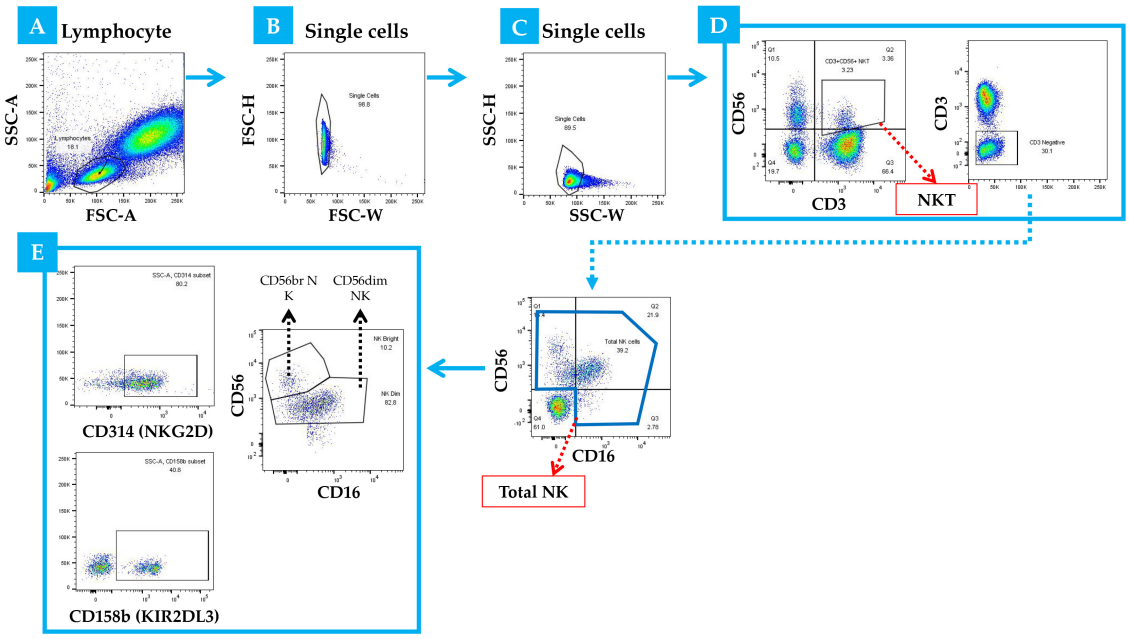


**Fig. 3. Flow cytometry analysis of NK cells and receptors in the elderly.** A front scattered light (FSC)-A and side scattered light (SSC)-A plot was used to identify nucleated cells. (A) A lymphocyte gate was set based on the size and granularity of the cells. (B) Single cells were gated, and doublets were excluded. (C) CD14+ macrophages were excluded. (D) NK cells were defined as CD3-CD56+. (E) Two NK cell subsets were identified: CD16+ and CD16- NK cells. NKG2D and KIR2DL3 were defined as CD314 and CD158b, respectively.

ing the examination. In particular, all participants were instructed to verbally express the degree of fatigue they feel when walking or running while looking at the rating of perceived exertion chart attached to the wall in front of the treadmill during the test. Considering that the elderly were the subjects, the modified Bruce protocol was used as a protocol for exercise stress testing. The modified Bruce protocol has 2 warmup stages, each lasting 3 min. The first is at 1.7 mph and a 0% grade, and the second is at 1.7 mph and a 5% grade [24]. This protocol is recommended for the elderly whose exercise capacity is limited by chronic diseases or who are physically weak. The elderly were advised to proceed with the test until they were able to walk or run to the maximum, and the examiner frequently checked the rating of perceived exertion. This study was conducted after identifying all the $VO_2$peak levels of the elderly in advance and confirming the possibility through preliminary experiments. The test was stopped if the subject complained of leg pain, shortness of breath, dizziness, and chest pain during the test, or if the electrocardiogram showed the following symptoms or signs: a decreasing systolic blood pressure ($\geq$10 mmHg) despite an increase in workload, a sustaining ventricular tachycardia, an elevating ST segment ($>$1 mm), a decreasing ST segment ($<-$3 mm), and technical difficulties in screening electrocardiographic tracings [25].

### *2.6 Measurements of Calorie Intake and Output*

During the entire study period, all subjects consented not to change their daily diet and activities. In order to investigat this, the researchers conducted weekly evaluations using the following two methods and informed them. First, a diet diary was given to them for recording what they had in the morning, afternoon, and evening. A researcher input the type and amount of diet into CAN-Pro 5.0 (http://canpro5.kns.or.kr; Korean Nutrition Society, Seoul) every day, calculated the calorie intake, and evaluated it every 3 weeks. Second, the activity status in daily life was checked with the short-form type of International Physical Activity Questionnaire [26]. Participants' physical activity levels were recorded over the past last weeks. Metabolic equivalents (MET)/min (kcal/kg/min) for daily calorie output was calculated every 3 weeks. The total scores were calculated by adding the time and frequency of walking, moderate-intensity activity, and vigorous activity. The recorded calorie intake and output data were averaged at Week 3, 6, 9, and 12 and analyzed at Week 0 and Week 12.

### *2.7 Data Analyses*

Sample size was determined using G*Power program [27], which calculated with a priori effect size (0.4) of $f^2$ ($V$), $\alpha$ error probability (0.05), 1-$\beta$ error probability (0.8), groups' number (4), and numerator difference (2), respectively. In this calculation, the total number of people was

**Table 4. Changes and differences in mean daily calorie intake and output.**

| | Groups | | | | $X^2$ | $p$ |
|---|---|---|---|---|---|---|
| | CON | EXE | PHYT | EXE-PHYT | | |
| Daily physical activity (kcal) | | | | | | |
| Week 0 | 275.75 ± 10.81 | 258.25 ± 26.03 | 268.50 ± 24.74 | 264.88 ± 26.23 | 1.883 | 0.597 |
| Week 12 | 295.75 ± 23.46 | 278.13 ± 36.57 | 277.63 ± 20.16 | 290.50 ± 37.36 | 1.430 | 0.698 |
| Daily dietary amount (kcal) | | | | | | |
| Week 0 | 1578.88 ± 157.99 | 1590.25 ± 157.88 | 1627.50 ± 191.88 | 1593.13 ± 177.58 | 0.122 | 0.989 |
| Week 12 | 1754.63 ± 144.76 | 1730.50 ± 227.18 | 1630.50 ± 261.74 | 1840.88 ± 135.68 | 3.369 | 0.338 |

All data represents mean ± standard deviation. The data was analyzed by Kruskal-Wallis test among four groups. CON, control group; EXE, exercise group without phytoncide; PHYT, phytoncide group; EXE + PHYT, exercise + phytoncide group with phytoncide.

32, and this study divided them equally into four groups. All data were statistically analyzed using SPSS (ver. 25; IBM Corp., Armonk, NY, USA). The data are presented as mean ± standard deviation and normal distribution was performed using the Shapiro-Wilk test. Prior to the full-scale analysis, this study confirmed the correlation between major variables through Spearman's correlation. This study analyzed the pre-scores of variables among the groups using the Kruskal–Wallis test to ascertain the differences at baseline. The environmental characteristics were analyzed using Mann-Whitney-U rank test. Then, most contribution variables were compared using a repeated-measures (4 × 2) ANOVA with Duncan post-hoc testing. At last, delta (Δ) % was calculated for each period for detailed data analysis.The effect size $(\eta^2)$ was calculated according to Cohen's d, which is equal to the mean difference of the groups divided by the pooled SD [28]. The effect sizes (Cohen's $d$ and $Z/\sqrt{N}$) were calculated and thresholds for small, moderate, and large effects were 0.2, 0.5, and 0.8 (parametric), and 0.1, 0.3, and 0.5 (non-parametric), respectively [29]. Statistical difference was considered significant at $p < 0.05$.

## 3. Results

### *3.1 Analysis of Demographic Data and Controlled Variables*

As shown in Table 1, age, height, and body composition among the four groups were not significantly different prior to the experiment. There were also no significant differences in temperature, humidity, and airflow between the two experimental places, but a significant difference in phytoncide concentration existed as shown in Table 2. That is, the demographic and environmental variables of this study indicated homogeneity of this study. Moreover, there were no significant differences in the controlled variables, such as the daily amount of diet and physical activity, as shown in Table 4.

### *3.2 Relationship between the Variables*

As shown in Table 5, $VO_2$peak before the start of the study showed a significant positive correlation with total NK cells (rho = 0.477) and NK T cells (rho = 0.374). After the study completion, $VO_2$peak showed a significant positive correlation with total NK cells (rho = 0.403), NK T cells (rho = 0.396) and epinephrine (rho = 0.396). Before the start of the study, total NK cells showed no significant correlation with NK T cells, cortisol and epinephrine. After the study completion, total NK cells showed significantly positive correlation with NK T cells (rho = 0.521) and epinephrine (rho = 0.454), whereas a negative correlation with cortisol (rho = –0.500). Moreover, NK T cells showed a positive correlation with epinephrine (rho = 0.603), and cortisol showed a negative correlation with epinephrine (rho = –0.382).

### *3.3 Effect of Aerobic Exercise + Forest Bathing on Immunocytes*

As shown in Table 6, although neutrophils and KIR2DL3 + NK did not show significant interactions between group and time, there were significant changes in leucocytes, lymphocytes, total NK cells, NKT cells, and NKG2D + NK after 12 weeks. Among those that showed an interaction effect, leucocytes showed a similar tendency to increase in EXE-PHYT, EXE, and PHYT, but decreased only in CON in post-hoc test. Lymphocytes increased significantly in EXE-PHYT and PHYT, increased slightly in EXE, and decreased in CON. Total NK cells, NKT cells, and NKG2D + NK in the post-hoc test showed the highest changes in EXE-PHYT, followed by EXE, PHYT, and CON sequentially.

### *3.4 Effects of Aerobic Exercise + Forest Bathing on NK Cells-related Hormones*

After the experiment, cortisol and epinephrine concentrations showed significant changes in the four groups. As shown in Fig. 4 (left), the cortisol concentration of CON at baseline was 16.43 ± 1.72 ug/dL, whereas it increased to 18.81 ± 2.48 ug/dL after 12 weeks (Δ% = 16.59 ± 25.21). The cortisol concentration of EXE at baseline was 16.18 ± 3.64 ug/dL, whereas it decreased to 14.45 ± 2.74 ug/dL after 12 weeks (Δ% = –9.69 ± 9.25). The cortisol concentrations of PHYT and EXE-PHYT measured at baseline were 16.44 ± 2.27 ug/dL and 16.11 ± 2.11 ug/dL, respectively,

**Table 5. Spearman's correlation coefficients for the main variables before and after the experiment.**

| | | $VO_2$peak | | Total NK | | NK T | | Cortisol | | Epinephrine | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | pre | post | pre | post | pre | post | pre | post | pre | post |
| $VO_2$peak | rho | 1.00 | 1.00 | 0.477 | 0.403 | –0.374 | 0.396 | 0.164 | 0.022 | –0.105 | 0.432 |
| | *p* | | | 0.006 | 0.022 | 0.035 | 0.025 | 0.371 | 0.903 | 0.568 | 0.014 |
| Total NK | rho | | | 1.00 | 1.00 | –0.227 | 0.521 | 0.119 | –0.500 | –0.052 | 0.454 |
| | *p* | | | | | 0.212 | 0.002 | 0.517 | 0.004 | 0.778 | 0.009 |
| NK T | rho | | | | | 1.00 | 1.00 | –0.201 | –0.196 | 0.153 | 0.603 |
| | *p* | | | | | | | 0.271 | 0.282 | 0.403 | 0.000 |
| Cortisol | rho | | | | | | | 1.00 | 1.00 | 0.042 | –0.382 |
| | *p* | | | | | | | | | 0.819 | 0.031 |
| Epinephrine | rho | | | | | | | | | 1.00 | 1.00 |
| | *p* | | | | | | | | | | |

$VO_2$peak, maximal oxygen consumption; NK, natural killer cell.

whereas those decreased to 14.41 ± 5.13 ug/dL and 12.76 ± 2.43 ug/dL after 12 weeks (PHYT Δ% = –12.54 ± 25.73; EXE-PHYT Δ% = –20.28 ± 14.65), respectively.

As shown in Fig. 4 (right), epinephrine concentration of CON at baseline was 38.81 ± 8.57 pg/mL, whereas it decreased to 36.51 ± 6.79 pg/mL after 12 weeks (Δ% = –1.80 ± 31.91). Epinephrine concentration of EXE measured at baseline was 38.43 ± 11.51 pg/mL, whereas it increased to 51.78 ± 12.25 pg/mL after 12 weeks (Δ% = 41.74 ± 43.46). The epinephrine concentrations of PHYT and EXE-PHYT measured at baseline were 37.45 ± 9.34 pg/mL and 37.23 ± 4.04 pg/mL, whereas those increased to 52.61 ± 14.32 pg/mL and 64.60 ± 9.41 pg/mL after 12 weeks (PHYT Δ% = 47.24 ± 52.26; EXE-PHYT Δ% = 76.01 ± 37.40), respectively. Cortisol, which can affect innate immune cells in the human body, showed a statistically significant interaction effect ($F = 4.350$; $p < 0.05$) after the end of the experiment, and epinephrine also showed a significant interaction effect ($F = 7.348$; $p < 0.001$) after the end of the experiment similarly to cortisol. In post-hoc test, the cortisol concentration in CON showed the highest value, followed by EXE, PHYT, and EXE-PHYT sequentially. On the other hand, the epinephrine concentration of EXE-PHYT showed the highest value, followed by PHYT, EXE, and CON sequentially.

### *3.5 Effects of Aerobic Exercise + Forest Bathing on Body Composition and Cardiorespiratory Fitness*

As shown in Table 7, although muscle mass did not show the significant interaction between group and time, there were significant changes in body weight, fat mass, percent fat, and body mass index (BMI) after 12 weeks. Although the body weight including fat mass, percent fat, and BMI showed an interaction effect, only fat mass and percent fat showed significant differences between groups in post-hoc test. That is, the fat mass of CON and PHYT was similarly high, while that of EXE and EXE-PHYT was similarly low. Specifically, in the case of fat mass, EXE-PHYT and EXE showed the smallest decrease, while CON and PHYT showed an increase. The percent fat showed similar results to fat mass.

As shown in Fig. 5, $VO_2$peak showed significant changes in the four groups after the experiment. The $VO_2$peak in CON at baseline was 34.58 ± 5.26 mL/kg/min, whereas it decreased to 30.49 ± 5.27 mL/kg/min after 12 weeks (Δ% = –11.19 ± 12.45). The $VO_2$peak of PHYT at baseline was 35.09 ± 4.38 mL/kg/min, whereas it increased to 32.56 ± 5.63 mL/kg/min after 12 weeks (Δ% = –6.94 ± 12.77). The $VO_2$peak of EXE and EXE-PHYT at baseline were 35.48 ± 2.31 mL/kg/min and 35.23 ± 3.76 mL/kg/min, respectively, whereas those increased to 36.44 ± 3.43 mL/kg/min and 38.35 ± 3.83 mL/kg/min after 12 weeks (EXE Δ% = 2.61 ± 4.75; EXE-PHYT Δ% = 9.14 ± 6.23), respectively. Cardiorespiratory endurance also showed a significant interaction ($F = 6.206$; $p < 0.01$) after the end of the experiment. Moreover, it showed a difference between groups in post-hoc test: EXE-PHYT showed the highest value, followed by EXE, PHYT, and CON sequentially.

## 4. Discussion

This study was conducted while controlling for daily activities and diet in elderly men for 12 weeks. During the experiment, aerobic exercise was performed for 45~60 min according to the principle of gradual overload 3 days a week. Characteristically, the following results in this study were obtained as a result of executing the program at the same time in both locations, one with high phytoncide and one low phytoncide concentration. Prior to the full-scale analysis, this study analyzed the correlation between the variables. As a result, $VO_2$peak showed a significant positive correlation with total NK cells and NK T cells before and after the experiment. At the end of the study, total NK cells showed a significantly positive correlation with NK T cells and epinephrine, whereas a negative correlation with cortisol. Moreover, NK T cells showed a positive correlation with epinephrine, and cortisol showed a negative correlation with epinephrine. This study also found that

**Table 6. Differences and changes in immunocytes.**

| | Time | Groups | | | | Repeated ANOVA (F) | | | $\eta^2$ |
|---|---|---|---|---|---|---|---|---|---|
| | | CON | EXE | PHYT | EXE-PHYT | G | T | G*T | |
| Leucocyte | Pre | 5.20 ± 0.57 | 5.58 ± 0.76 | 5.38 ± 0.86 | 5.25 ± 0.59 | 1.458 | 8.659** | 5.675** | 0.046 |
| (×$10^3$ cells/$\mu$L) | Post | 4.96 ± 0.65 $^b$ | 5.65 ± 0.71 $^a$ | 5.91 ± 0.47 $^a$ | 5.88 ± 0.63 $^a$ | | | | 0.302 |
| Δ% | | –4.51 ± 8.08 | 1.70 ± 8.61 | 11.66 ± 13.17 | 12.21 ± 8.33 | | | | |
| Neutrophill | Pre | 45.76 ± 8.27 | 44.20 ± 8.26 | 45.35 ± 7.11 | 45.50 ± 6.58 | 1.286 | 0.417 | 1.409 | 0.007 |
| (%) | Post | 41.36 ± 5.94 | 45.24 ± 5.21 | 47.91 ± 7.65 | 50.71 ± 6.10 | | | | 0.256 |
| Δ% | | –6.10 ± 24.02 | 5.43 ± 23.22 | 7.21 ± 19.66 | 13.01 ± 17.64 | | | | |
| Lymphocyte | Pre | 42.43 ± 6.31 | 41.03 ± 6.58 | 41.68 ± 5.98 | 42.13 ± 8.72 | 1.090 | 5.490* | 3.504* | 0.006 |
| (%) | Post | 38.31 ± 8.25 $^b$ | 45.03 ± 6.08 $^{ab}$ | 47.19 ± 8.11 $^a$ | 49.19 ± 7.11 $^a$ | | | | 0.257 |
| Δ% | | –6.70 ± 29.79 | 10.61 ± 11.73 | 13.46 ± 14.07 | 18.44 ± 13.32 | | | | |
| Total NK cell | Pre | 8.93 ± 2.17 | 8.65 ± 2.08 | 8.78 ± 2.37 | 8.60 ± 2.67 | 2.789 | 1.880 | 6.067** | 0.003 |
| (%) | Post | 6.48 ± 0.66 $^d$ | 10.44 ± 2.07 $^{ab}$ | 9.26 ± 2.40 $^c$ | 11.26 ± 1.65 $^a$ | | | | 0.533 |
| Δ% | | –24.73 ± 14.01 | 27.66 ± 42.70 | 9.34 ± 27.57 | 39.08 ± 36.55 | | | | |
| NKT cell | Pre | 4.31 ± 1.40 | 4.19 ± 2.52 | 4.19 ± 1.63 | 4.25 ± 1.56 | 2.531 | 3.280 | 4.735** | 0.001 |
| (%) | Post | 3.04 ± 0.98 $^d$ | 5.06 ± 1.77 $^{ab}$ | 4.69 ± 1.80 $^c$ | 6.69 ± 0.91 $^a$ | | | | 0.486 |
| Δ% | | –23.49 ± 31.13 | 62.83 ± 102.35 | 19.10 ± 32.11 | 82.81 ± 83.54 | | | | |
| NKG2D + NK | Pre | 53.50 ± 14.22 | 52.69 ± 9.87 | 54.34 ± 11.23 | 52.26 ± 10.66 | 2.117 | 8.373** | 3.647* | 0.005 |
| (%) | Post | 48.85 ± 7.37 $^c$ | 59.48 ± 10.85 $^{bc}$ | 62.13 ± 7.43 $^{ab}$ | 69.51 ± 9.86 $^a$ | | | | 0.436 |
| Δ% | | –4.19 ± 22.21 | 16.24 ± 31.63 | 21.89 ± 43.83 | 36.79 ± 26.95 | | | | |
| KIR2DL3 + NK | Pre | 34.83 ± 8.79 | 33.26 ± 10.82 | 35.84 ± 9.58 | 34.34 ± 5.16 | 0.917 | 3.873 | 2.392 | 0.012 |
| (%) | Post | 38.71 ± 6.04 | 29.51 ± 10.57 | 30.50 ± 8.83 | 27.19 ± 11.25 | | | | 0.197 |
| Δ% | | 16.98 ± 35.80 | –5.83 ± 38.74 | –12.65 ± 19.65 | –23.21 ± 22.36 | | | | |

All data represents mean ± standard deviation. CON, control group; EXE, exercise group without phytoncide; PHYT, phytoncide group; EXE + PHYT, exercise + phytoncide group with phytoncide; G, Group; T, Time. *, $p < 0.05$; **, $p < 0.01$. Symbols $^{a,b,c}$ and $^d$ represent post-hoc test results. The absence of symbols indicates that there is no significant difference between groups. For reference, in the post-hoc test, the largest value is denoted by symbol $^a$, and the smallest value is denoted by $^b$, $^c$ or $^d$. When symbols are combined, for example, the symbol $^{ab}$ means that the value of the group denoted by the symbol $^a$ and the value of the group denoted by the symbol $^b$ are almost the same.

forest bathing and aerobic exercise positively affected immunocyte function and that the improved results from forest bathing + aerobic exercise were caused by an increase in cardiorespiratory endurance and epinephrine. In addition, this increase in cardiorespiratory endurance can be interpreted to have significantly reduced the body weight, fat mass, fat percentage, and BMI of the two groups that participated in the treadmill exercise.

Short-term or long-term exercise and training affect the rise and fall of hormone levels. In particular, these two hormones analyzed in this study are major components of the physiological stress response [30] and are usually excreted in response to exercise. Basically, cortisol is a corticosteroid hormone that promotes gluconeogenesis and acts on fat and water metabolism. It is a stress hormone that promotes muscle contraction due to excitement in nervous tissue. On the other hand, epinephrine is a type of catecholamine and is one of a group of similar compounds that exhibit sympathetic excitatory action. The blood level of these hormones can be interpreted as a change in the secretion rate of the hormone secreted by the endocrine gland. The increase in circulating plasma hormones during exercise depends on an increase in secretion rate, a decrease in circulating or clearance of the hormone, a decrease in plasma volume due to sweating, or one or more of a combination of factors. Among the combined effects, the phytoncide used as an intervention in this study caused significant changes in stress hormones and NK cells, even without exercise. In particular, the leucocyte and lymphocyte concentrations of the PHYT that received only phytoncide increased to a similar degree after 12 weeks as that of the EXE-PHYT that received both phytoncide and aerobic exercise. In other words, although phytoncide can affect leucocytes and lymphocytes to some extent, when combined with aerobic exercise, the degree of exposure to phytoncide increases, suggesting that more beneficial effects can be provided. As shown in Table 5, the leucocytes concentration in CON was 4.96 ± 0.65 × $10^3$ cells/$\mu$L, whereas the leucocytes concentration in EXE was 5.65 ± 0.71 × $10^3$ cells/$\mu$L, the PHYT was 5.91 ± 0.47 × $10^3$ cells/$\mu$L, and EXE-PHYT was 5.88 ± 0.63 × $10^3$ cells/$\mu$L. Lymphocytes, similar to leucocytes, were 38.31 ± 8.25% in CON, 45.03 ± 6.08% in EXE, 47.19 ± 8.11 in PHYT, and 49.19 ± 7.11% in EXE + PHYT.

During exercise, cortisol rises after strenuous exercise. The heart and blood vessels play an important role

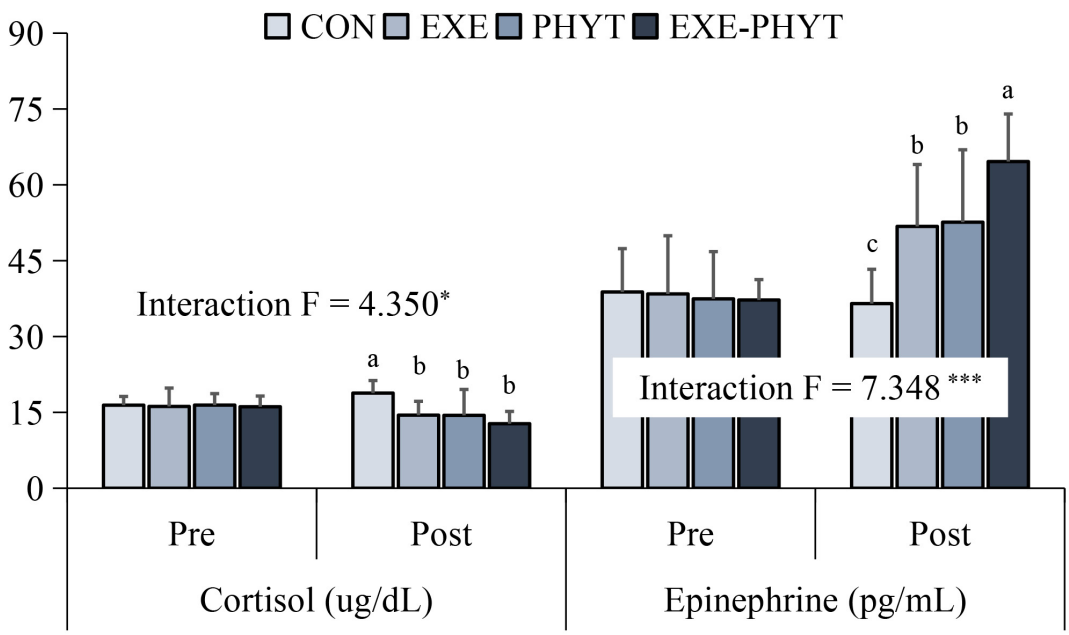


**Fig. 4. Differences of cortisol and epinephrine concentrations between times and groups.** In the figure, CON, control group; EXE, exercise group without phytoncide; PHYT, phytoncide group; EXE + PHYT, exercise + phytoncide group with phytoncide; $^{*}$, $p < 0.05$; $^{***}$, $p < 0.001$. Symbols $^{a,b}$ and $^{c}$ represent post-hoc test results. The absence of symbols indicates that there is no significant difference between groups. For reference, in the post-hoc test, the largest value is denoted by symbol $^{a}$, and the smallest value is denoted by $^{b}$, $^{c}$ or $^{d}$. When symbols are combined, for example, the symbol $^{ab}$ means that the value of the group denoted by the symbol $^{a}$ and the value of the group denoted by the symbol $^{b}$ are almost the same.

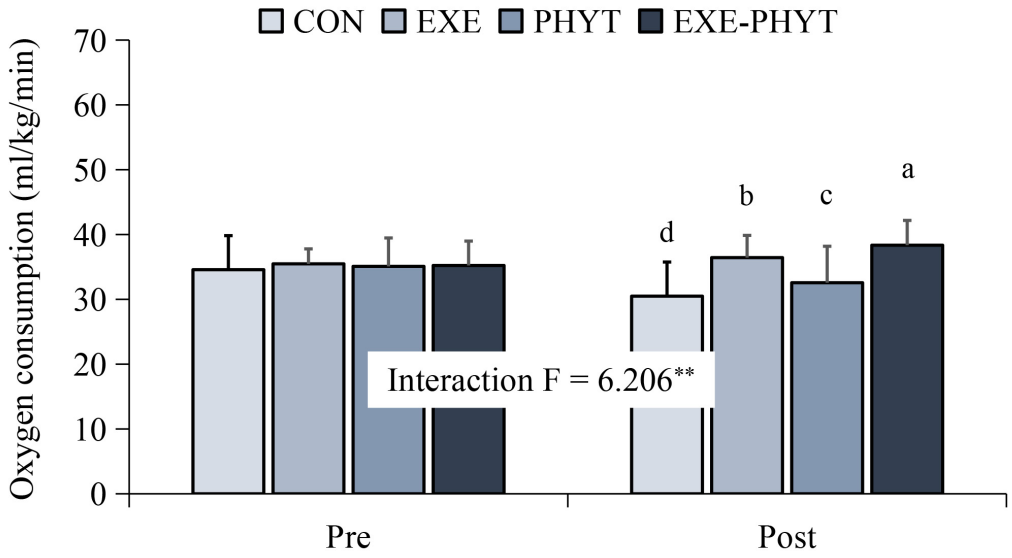


**Fig. 5. Differences of physical fitness between times and groups.** In the figure, CON, control group; EXE, exercise group without phytoncide; PHYT, phytoncide group; EXE + PHYT, exercise + phytoncide group with phytoncide; $^{**}$, $p < 0.01$; $^{***}$, $p < 0.001$. Symbols $^{a,b,c}$ and $^{d}$ represent post-hoc test results. The absence of symbols indicates that there is no significant difference between groups. For reference, in the post-hoc test, the largest value is denoted by symbol $^{a}$, and the smallest value is denoted by $^{b}$, $^{c}$ or $^{d}$. When symbols are combined, for example, the symbol $^{ab}$ means that the value of the group denoted by the symbol $^{a}$ and the value of the group denoted by the symbol $^{b}$ are almost the same.

**Table 7. Differences and changes of body composition.**

| | Time | Groups | | | | Repeated ANOVA (F) | | | $\eta^2$ |
|---|---|---|---|---|---|---|---|---|---|
| | | CON | EXE | PHYT | EXE-PHYT | G | T | G*T | |
| Body weight | Pre | 72.98 ± 6.48 | 75.10 ± 3.77 | 73.61 ± 8.81 | 72.86 ± 6.70 | 0.414 | 2.481 | 7.819*** | 0.020 |
| (kg) | Post | 75.65 ± 5.42 | 72.96 ± 1.85 | 73.35 ± 8.15 | 69.70 ± 5.26 | | | | 0.140 |
| Δ% | | 3.91 ± 5.28 | –2.71 ± 3.68 | –0.24 ± 1.38 | –4.16 ± 2.89 | | | | |
| Muscle mass | Pre | 34.09 ± 5.50 | 33.75 ± 5.03 | 32.43 ± 4.84 | 33.00 ± 7.51 | 0.340 | 0.635 | 2.598 | 0.014 |
| (kg) | Post | 32.49 ± 5.19 | 35.28 ± 6.31 | 31.84 ± 3.86 | 35.54 ± 5.13 | | | | 0.102 |
| Δ% | | –4.43 ± 6.46 | 4.53 ± 10.09 | –1.39 ± 5.49 | 10.45 ± 19.59 | | | | |
| Fat mass | Pre | 20.18 ± 2.84 | 20.14 ± 2.53 | 19.98 ± 2.55 | 20.66 ± 1.45 | 2.186 | 0.480 | 11.541*** | 0.013 |
| (kg) | Post | 24.84 ± 4.27 $^a$ | 18.15 ± 3.89 $^b$ | 21.58 ± 5.22 $^a$ | 17.80 ± 2.50 $^b$ | | | | 0.359 |
| Δ% | | 23.61 ± 19.31 | –10.19 ± 13.77 | 7.16 ± 16.55 | –14.09 ± 8.96 | | | | |
| Percent Fat | Pre | 28.01 ± 5.50 | 26.90 ± 3.86 | 27.58 ± 5.38 | 28.59 ± 3.45 | 1.241 | 0.700 | 6.607** | 0.020 |
| (%) | Post | 33.25 ± 7.41 $^a$ | 24.88 ± 5.29 $^b$ | 29.71 ± 7.84 $^a$ | 25.70 ± 4.40 $^b$ | | | | 0.239 |
| Δ% | | 19.19 ± 19.42 | –7.56 ± 15.29 | 7.50 ± 16.92 | –10.28 ± 9.80 | | | | |
| Body mass index | Pre | 25.00 ± 2.05 | 25.71 ± 2.22 | 24.98 ± 3.34 | 24.98 ± 1.93 | 0.327 | 2.537 | 7.552*** | 0.019 |
| (kg/m$^2$) | Post | 25.90 ± 1.74 | 24.94 ± 1.40 | 24.90 ± 3.14 | 23.91 ± 1.69 | | | | 0.113 |
| Δ% | | 3.91 ± 5.28 | –2.71 ± 3.68 | –0.24 ± 1.38 | –4.16 ± 2.89 | | | | |

All data represents mean ± standard deviation. CON, control group; EXE, exercise group without phytoncide; PHYT, phytoncide group; EXE + PHYT, exercise + phytoncide group with phytoncide; G, Group; T, Time. **, $p < 0.01$; ***, $p < 0.001$. Symbols $^a$and$^b$ represent post-hoc test results. The absence of symbols indicates that there is no significant difference between groups. For reference, in the post-hoc test, the largest value is denoted by symbol $^a$, and the smallest value is denoted by $^b$, $^c$ or $^d$. When symbols are combined, for example, the symbol $^{ab}$ means that the value of the group denoted by the symbol $^a$ and the value of the group denoted by the symbol $^b$ are almost the same.

in stability and reflex control during exercise to increase cardiac output, heart rate, and blood pressure. Generally, cortisol concentration returned to pre-exercise levels within 2~3 hours after a single bout of exercise. Cortisol is a corticosteroid hormone that passively inhibits the secretion of adrenocorticotropic hormones from the hypothalamic-pituitary-adrenal axis [31]. In addition, it is involved in the metabolism of energy substrates, promotion of the synthesis and secretion of other hormones, regulation of reproductive functions, nervous system, immune function, and adaptation to stress such as exercise, while also playing an important role in synthesizing catecholamines at the sympathetic nerve endings [32]. It is also known that an abnormally high concentration of cortisol suppresses fever and causes loss of immunity. High cortisol levels ultimately cause tissue destruction and negative nitrogen balance in the body. Although the effects of exercise on cortisol levels show somewhat contradictory results, it is generally accepted that exercise increases them. On the other hand, around 70~80% of the epinephrine secreted from the adrenal medulla plays a role in the cardiovascular system, expanding cardiac output, myocardial contractility, heart rate, muscles and cardiovascular blood vessels [33]. Epinephrine increases the blood glucose concentration by decomposing the glycogen stored in the liver and muscle while freeing fatty acids to increase the blood fatty acid concentration, thereby promoting the metabolic rate [34]. In this study, while combining forest bathing with aerobic exercise decreased cortisol levels, epinephrine increased. Contrary to the results of previous studies in which epinephrine concentration decreased during long-term training, this study showed increased epinephrine concentration, which is thought to be due to the effect of phytoncide exposure through forest bathing to some extent. In other words, it is thought that long-term forest bathing combined with aerobic exercise affected the metabolic effect of increasing epinephrine and inducing sympathetic nervous system activity, thereby increasing the mobilization of energy substrates and the rate of energy consumption. These results can also be seen in the body weight, fat mass, percent fat, and BMI which were significantly changed after 12 weeks. As shown in Table 7, after the end of the experiment, the body weight of CON increased ~3.91%, while the body weight of EXE, PHTY and EXE + PHTY decreased ~2.71%, ~0.24% and ~4.16%, respectively. In particular, the muscle mass of EXE and EXE + PHTY, which performed treadmill exercise, increased 4.53 ± 10.09% and 10.45 ± 19.59%, while the body fat mass of them decreased –10.19 ± 13.77% and –14.09 ± 8.96%, respectively. In other words, the group exposed to phytoncide showed more positive results. Similar changes were also observed in percentage fat and body mass index.

Previous studies reported that forest bathing enhanced NK cell activity, the number of NK and NKT cells, and intracellular anti-cancer proteins in lymphocytes [35–37]. Li *et al.* [35] reported that the increased NK activity was the leading cause of aromatic volatile substances derived from trees, called phytoncides, such as $\alpha$-pinene and limonene. If phytoncide is inhaled while bathing in the forest, it is

well known that it reduces cortisol, a stress hormone. It has also been known through several scholars that cortisol reduces NK cells. However, the activity of NK cells varies depending on the level of cortisol concentration. When the level of cortisol is high, the NK cell activity decreases, and when the level of cortisol is low, the NK cell activity increases [38,39]. As a result of extracting NK cells from forest bathing humans, NK cell activity increased, and the number of NK cells in the body also increased. Despite forest bathing for 2 days, the effect of increasing NK cell activity lasted for about 30 days [17]. Meanwhile, this study found that the total NK cells, NKT cells, and NKG2D + NK of the PHYT group increased, while KIR2DL3 + NK decreased, similar to the results of the EXE and the EXE-PHYT, compared with those of CON. These results suggest that phytoncide affects immune cells, and when it is combined with exercise, the blood flow of the circulatory system or chemical function in tissues is improved, which may be beneficial to immunocytes function. Similar to this regards, Jia *et al*. [30] investigated the effects of forest bathing on the health of the elderly with chronic obstructive pulmonary disease. The elderly participants for their study design were randomly divided into two groups, which one group was sent to forest and the other was sent to an urban area. They found that the perforin and granzyme B significantly decreased with accompaning by reduced proinflammatory cytokines and stress hormones in the forest. In fact, NK, NK T cell and cytotoxic T-cell are the three main classes of human killer cells, which can cause targeted cell death through perforin/granzyme granule exocytosis pathway [40]. Numerous studies have reported that forest therapy can have positive effects on physical and psychological health [18,41,42]. Forest bathing has also reported to reduce psychological depression by activating the parasympathetic nervous system while inhibiting the sympathetic nervous system due to various chemicals emitted from the forest [17,43]. As such, exposure to phytoncide through forest bathing is thought to provide the effect of stabilizing the body's immunity as well as mentally through various biochemical substances. These results appear to be similar to those of the data that improved the function of innate immune cells presented in this study, but more specific research is needed for the psychological part.

This study also found that the NKG2D + NK concentrations of the EXE, PHYT, and EXE-PHYT increased, while KIR2DL3 + NK receptors were not changed compared with those of CON. NK cell activation receptors recognize ligands expressed when target cells are in an abnormal state. Typically, the NKG2D + NK receptor detects UL16-binding proteins (ULBPs) and major histocompatibility complex (MHC) class-I-chain-related protein (MIC), which are intracellular molecules whose expression is increased during DNA damage, cancer, and infection in the human body [6,42]. On the other hand, inhibitory receptors of NK cells measure the presence or absence of intracellular molecules that are constitutively expressed on the surface of target cells [7]. Typically, NK cells express inhibitory receptors specific to MHC class I. If the target cell is deficient in MHC class I, a signal that can inhibit NK cell activity is not transmitted. An MHC class I-specific inhibitory receptor expressed in NK cells includes human KIR (killer cell immunoglobulin-like receptor). In addition, the existence and role of many other activation/inhibitory receptors that can regulate the activity of NK cells have been reported. Therefore, to understand and effectively regulate the activity of NK cells [44,45], it is essential to elucidate how the activity of NK cells is regulated by the combination of these various receptors [8,9]. In particular, the specific effects that phytoncide or exercise provides in relation to inhibitory receptors remains to be found.

Ultimately, this study detected that aerobic exercise combined with forest bathing positively affected NK cell and NK cell related to receptors through activated epinephrine and inhibited cortisol. Moreover, this change in the immunoendocrine system in the body can be promoted a little more through exercise, and when phytoncide is incorporated, it has been confirmed that desirable changes in body composition can come through the development of cardiorespiratory endurance. However, this study has some limitations. First, the sample size consisted of only elderly men. Second, although hundreds of types of immunocytes exist, this study only observed a select portion of NK cells and related receptors. Considering these limitations, further studies investigating the effectiveness of aerobic exercise + forest bathing are encouraged to include a greater number of participants with diverse demographic backgrounds and to use multiple immunocyte tests.

## 5. Conclusions

This study found that aerobic exercise combined with forest bathing positively affected immunocyte function via controlled hormones in elderly men. It also found that the improved results from forest bathing + treadmill walking were caused by an increase in cardiorespiratory endurance by higher epinephrine concentrations. In addition, this increased cardiorespiratory endurance can be seen to significantly reduce body weight, fat mass, fat percentage, and BMI of the group that participated in exercise combined with phytoncide exposure.

## Abbreviations

NK, natural killer cell; NK T, natural killer T cell; NKG2D, natural-killer group 2, member D; KIR, killer cell immunoglobulin-like receptor; MHC, major histocompatibility complex; RPE, rating of perceived exertion; CON, control group; EXE, exercise group without phytoncide; PHYT, phytoncide group; EXE + PHYT, exercise + phytoncide group with phytoncide.

## Author Contributions

SP and Y-SJ conceived the idea. Y-SJ developed the background and performed the calibration of different devices used in the tests. J-KL and Y-SJ verified the methods section. All authors discussed the results and contributed to the final manuscript. SP and Y-SJ performed the tests. Y-SJ wrote the manuscript with support from SP and J-KL. All authors contributed to the final version of the manuscript. SP and Y-SJ contributed to the interpretation of the results and data analysis, and they drafted the manuscript and designed the figures and tables. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

## Ethics Approval and Consent to Participate

The study was conducted according to the guidelines of the Declaration of Helsinki (2013 version) and approved by the Institutional Review Board of Sahmyook University (2-1040781-AB-N-01-2017083HR) and Seoul Songdo Hospital (2021-008).


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## Conflict of Interest

The authors declare no conflict of interest. Y-SJ is serving as one of the Guest editors of this journal. We declare that Y-SJ had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to DM.

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