Mitigation of heat strain by wearing a long-sleeve fan-attached jacket in a hot or humid environment

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Abstract
Objectives: This study examined whether a fan-attached jacket (FAJ) may mitigate the heat strain in hot or humid environment.

Methods: Nine healthy men engaged in 60-min sessions on a bicycle ergometer (4 metabolic equivalents [METs] workload) in hot-dry (40°C and 30% relative humidity) and warm-humid (30°C and 85% relative humidity) environments. Both are equivalent to an approximately 29°C wet-bulb globe temperature. The experiment was repeated—once wearing an ordinal jacket (control condition) and once wearing a long-sleeve FAJ that transfers ambient air at a flow rate of 12 L/s (FAJ condition)—in both environments.

Results: Increases in core temperatures in hot-dry environment were not statistically different between control and FAJ; however, that in the warm-humid environment were significantly different between control and FAJ (0.96 ± 0.10°C and 0.71 ± 0.11°C in rectal temperature, P < .0001; and 0.94 ± 0.09°C and 0.61 ± 0.09°C in esophageal temperature, P < .0001). Changes in heart rate were different between control and FAJ in both environments (62 ± 3 bpm and 47 ± 7 bpm, P < .0001 in hot-dry environment; and 61 ± 3 bpm and 46 ± 5 bpm, P < .0001 in the warm-humid environment) and decrease of %weight change was different in hot-dry environment (1.59 ± 0.12% and 1.25 ± 0.05%, P = .0039), but not in the warm-humid environment.

Conclusions: Wearing a FAJ may mitigate heat strain both in hot or humid environments.

KEYWORDS
cooling garment, core temperature, fan-attached jacket, heat-related illness, humidity, occupational health service
1 | INTRODUCTION

World Meteorological Organization reported the global mean temperature for 2020 was 1.2 ± 0.1°C above the baseline approximated pre-industrial levels. International Labour Organization (ILO) reported that an increase in heat stress resulting from global warming is projected to lead to global productivity losses equivalent to 80 million full-time jobs in the year 2030. Heat-related illnesses experienced by workers who are exposed to extreme heat have become a major concern in occupational health. In the United States, environmental heat caused 37 work-related deaths and 2830 lost-workday occupational illnesses in 2015. Thirty-three of fatal cases occurred in June through September. In Japan, the average number of heat-related occupational illnesses from 2011 to 2020 came up to 625 including 21 fatalities; approximately 40% occurred in the construction industry or in the manufacturing industry, and 84% occurred in July or August. Performing physically demanding work in extreme heat is often inevitable in work settings; therefore, cooling garments such as those continuously cooled by circulating water connected to a chiller, ice vests, and phase-change materials have been developed and tested. In Japan, fan-attached jackets (FAJs), a thin long-sleeve jacket without cooling-specific materials but with two small fans attached at the waist on the back, have been popular among workers since 2000. This type of jacket is lightweight, does not restrict movement, and was designed to cool the trunk and arms by transferring air from under the clothes to the surroundings, which promotes the evaporation of sweat.

Cooling methods by ventilating air inside clothing have been investigated in military science, in sports science, and in occupational health. Christie et al. reported the improved physiological strain by increased air volume in the environment of 21–43°C ambient temperature and 40%–80% relative humidity. Shapiro also reported the similar effect in 35°C and 75% or in 49°C and 20% environment. Previous studies have examined the efficacy of vest-type fan-attached cooling garments specifically designed for use in the military: Hadid et al. observed improved physiological strain, with lower rectal and skin temperatures in two environments (40°C ambient temperature and 40% relative humidity; 35°C ambient temperature and 60% relative humidity); Chinevere et al. observed improved physiological strain in 40°C ambient temperature and 20% relative humidity, in 35°C ambient temperature and 75% relative humidity, and in 30°C ambient temperature and 50% relative humidity environmental conditions. However, ordinary workers cannot use these military garments. Nor can they use garments without long sleeves for safety reasons. Otani et al. reported a significant decrease in infrared tympanic temperature and skin temperature by wearing a long-sleeve FAJ during the inter-exercise and post-exercise recovery, however, workers may not be able to take breaks every 30 min and rarely wear FAJs only during breaks. One study reported that the air-ventilating clothing is only recommended for indoor and normal office work environments with air temperatures up to 32.0°C. Therefore, it was unknown whether this FAJ could feasibly be used to effectively suppress increases in body temperature in ordinary physical work settings above 32.0°C. Studies using thermal manikin reported that the area-weighted average torso heat flux increased as the fan air-flow rate increased and clothing size had almost no impact on predicted thermophysiological responses in 20L/s ventilation. High-humidity climate conditions start at the beginning of summer and last approximately 1 month; a temperature exceeding 40°C is not rare in the middle of the summer in Japan. When planning measures for preventing heat-related illnesses at workplaces, both extremely humid and extremely hot environments must be considered. Xu et al. used a thermal manikin to evaluate the cooling effect of a FAJ in environments set at 25, 30, 35, or 40°C ambient temperature and 25%, 50%, or 75% relative humidity; Xu et al. reported that the jacket’s cooling capacity was lowest in the hot and humid environment. However, to date and to the best of our knowledge, no study has examined whether a long-sleeve FAJ can prevent increased human core body temperature in a hot or humid climate during 60 min of continuous work. We hypothesized that the sufficient cooling effect of wearing a long-sleeve FAJ cannot be achieved either in the environment hotter than the skin temperature or in the environment too humid to let sweat to evaporate. Heat dissipation may not be sufficient in high-humidity environments, and heat convection from the surroundings to the skin surface may occur in high-temperature environments. Therefore, we performed this study to determine the efficacy of wearing a FAJ in reducing heat-related physiological strain while performing exercise in extremely humid or hot environments.

2 | METHODS

2.1 | Participants

Nine Japanese men who did not have any current (at the time of the study) or previously treated medical conditions, were not currently taking medications, and had no smoking history (age: 24.1 ± 4.3 years; height: 170.1 ± 6.6 cm; body weight: 62.2 ± 6.5 kg) participated in this study.


2.2 Clothing and cooling system

For all experiments, each participant wore a short-sleeve T-shirt (100% cotton) and lightweight work trousers (90% polyester and 10% cotton). During the control conditions (CON), each participant wore a long-sleeve work shirt (70% polyester and 30% cotton) over the T-shirt, and during the FAJ conditions, they wore a jacket (KU90720, 100% polyester; Kuchofuku), with two 10-cm-diameter fans (FAN2100, weight 204 g; Kuchofuku) powered by four Li-ion AA batteries (LI-Pro I, weight 469 g; Kuchofuku) attached on the rear left and right sides of the waist to transfer air from underneath the jacket to the surroundings via the cuffs or collar. The jacket is equipped with four-levels air volume switch at 12, 22, 25, and 30 L/s, and the lowest airflow rate of 12 L/s was chosen in this study because a battery life of 24 h is expected at this airflow rate. The FAJ is made of cloth materials with poor air permeability to circulate the air on the upper body surface, so that, we used an ordinal long-sleeve work shirt for the control condition instead of simply switching off the fan of the tested jacket.

2.3 Experimental procedures

All experiments were conducted between September 2019 and March 2020 in the artificial climate chamber of the Shared-Use Research Center, University of Occupational and Environmental Health, Japan (TBR-8E20W0P2T; Espec).

On the day before the experiment, each participant underwent a gradually increased workload test on an ergometer (T.K.K. 3070; Takei Scientific Instruments) to estimate maximum oxygen uptake; the mean breath-by-breath oxygen uptake was analyzed every 10 s by using an exhaled gas analyzer (Arco-2000; Arco System, Japan). They were asked to sit still for 5 min in the experiment room controlled at 25°C and 50% relative humidity. After 2 min of rest on the ergometer, they started to exercise at 20 W, then increasing by 10 W every minute until the heart rate reached 80% Heart Rate Reserve (80% HRR) or the rate-perceived exertion (RPE) reached to 20. The 80% HRR was calculated by following equation: 24,25:

\[
\text{Maximum heart rate} = 208 - \text{Age} \times 0.7
\]

\[80\% \ HRR = (\text{maximum heart rate} - \text{resting heart rate}) \times 0.8 + \text{resting heart rate}.
\]

Based on the estimated maximum oxygen uptake, workload of 4 metabolic equivalents (METs), when oxygen consumption was 14 ml/kg/min (1 MET = 3.5 ml/kg/min), was set.

Participants were instructed to sleep well and abstain from alcohol on the day before and to refrain from consuming caffeine-rich products and performing exercise on the day of the experiment. The participants were provided with and asked to consume a 400-kcal meal (carbohydrates 42.7 g, protein 8.4 g, fat 22.2 g, salt 0.94 g; Calorie Mate Block, Otsuka Pharmaceutical) and 500 ml of water (Crystal Geyser Water Company) at 8:00 a.m. each day on which they performed an experiment. They were also provided with and asked to consume an 838-kcal meal (carbohydrates 107.3 g, protein 41.5 g, fat 28.1 g, salt 4.2 g) and 500 ml of water at approximately 11:30 a.m. (more than 2 h before the start of the experiment).

Each participant’s body weight and height were measured without clothing. The participants were then asked to put on a T-shirt and work trousers and enter the anterior chamber.

After all sensors were attached and normal real-time monitoring had been confirmed, participants were asked to put on either a long-sleeve work shirt (CON) or a FAJ. They were asked to sit quietly on a chair for 5 min in the anterior chamber before they entered the experimental chamber. The experimental chamber was maintained at 40°C ambient temperature and 30% relative humidity for the hot-dry environment or at 30°C ambient temperature and 85% relative humidity for the warm-humid environment. Both environments are equivalent to an approximately 29°C wet-bulb globe temperature measured by WBGT measuring device (HI-2000SD; CUSTOM) placed at least 2 m apart from the subject. The wind velocity inside the artificial climate room measured by anemometer (AM-4224SD; MOTHERTOOL) was mostly <0.2 m/s throughout the experiment. After resting on a chair for 15 min, they were asked to move to the bicycle ergometer and exercise at a workload of 4 METs for 60 min. When the exercise was finished, they moved back to the anterior chamber and rested on a chair for 15 min. After confirming rectal temperature, $T_{re}$, had peaked, sensors were removed, and body weight was measured without clothing again. In the FAJ conditions, the fan was turned on when the participant entered the experimental chamber and remained on until monitoring was over. During the experiment, we allowed the participants to wipe exposed skin with a towel. We set safety criteria for halting the experiment ($T_{re} > 38.5°C$ or if the participant exhibited headache, dizziness, nausea, or other symptoms that could indicate heat-related illness), and physicians were always present during the experiments to monitor participants and stop the experiment, if necessary, prior to the onset of any heat-related illness. Each participant performed each of the 4 experiments (2 conditions for each of two environments) in a random order and with 72-h intervals between experiments.
2.4 Measurements

Rectal temperature, esophageal temperature, ear temperature, and skin temperatures of the forehead, chest, and back were continuously monitored in real time every 10 s using Class 1, IEC 60584-1, copper-constantan thermocouples (JIS C 1602, type T). A rectal temperature probe (diameter 0.32 mm), covered by a rubber sheath (P249A; Nikkiso-Therm), was inserted approximately 15 cm into the anus, until the monitored temperature stabilized. The esophageal temperature probe (diameter 0.1 mm), covered by a polyethylene tube, was swallowed by mouth; it was located at a depth of 45 cm and incrementally raised by 2 cm to find the correct placement to obtain the maximum temperature. An ear temperature probe (Midori Electronic) was inserted into the right external ear canal. We measured the ear temperature as a surrogate index of esophageal temperature for future investigation in the real workplace because it is almost impossible to measure the core temperature at work. Skin temperature probes (diameter 0.2 mm) were affixed by surgical tape (MESHPORE).

Heart rate was measured using electrocardiography (BSM-2401; Nihon Kohden), and the mean of every eight beats was recorded every 10 s. Sweat volume was estimated by change in nude body weight, which was measured with a scale (IS-150IGG-H; Sartorius Japan K.K.). The subjects were asked to take off all the clothing including underwear after wiping off the sweat and put on a dry body towel to measure their weight. We recorded their nude body weight by deducting the weight of the towel and calculated % weight change.

Participants were asked to RPE using a Borg scale with 15 stages ranging from 6 to 20 (where 7 = very light, 9 = very light, 11 = fairly light, 13 = somewhat hard, 15 = hard, 17 = very hard, 19 = very hard). Participants were asked to rate their perceived thermal comfort using the ISO10551 scale, with 5 stages ranging from 0 to 4 (4 = extremely uncomfortable, 3 = very uncomfortable, 2 = uncomfortable, 1 = slightly uncomfortable, 0 = comfortable). These subjective evaluations were asked 8 times: before the exercise (t1); 10 min (t2), 20 min (t3), 30 min (t4), 40 min (t5), 50 min (t6), and 60 min (t7) after the start of the exercise; and at the end of the experiment (t8).

2.5 Statistical analysis

Statistical analysis was performed using JMP Pro 16 (SAS Institute Japan Ltd.). Mean values and standard error for rectal temperature, esophageal temperature, ear temperature, skin temperature, and heart rate 1 min before and after every 5 min were calculated for all participants. Missing data were supplemented by calculating the mean value of nearby data. Rectal temperature, esophageal temperature, ear temperature, skin temperature and heart rate, subjective symptoms, and changes of values from −20 min in temperatures and heart rate were compared by two-way repeated-measures analysis of variance (ANOVA) in a model including time term and condition term as the main effect along with their intersection term. When the interaction effect turned out to be significant, Tukey-Kramer test was performed. Estimated amount of sweating and % weight change were compared between conditions using the Wilcoxon signed-rank test (significance was set as P < .05). Spearman’s rank correlation coefficient Rho was calculated with the increase in rectal temperature during 65 min of experiment and the % weight change.

3 RESULTS

3.1 Experimental data

All participants completed all four conditions. Five sets of data were excluded from the statistical analysis because of missing data at 75 min: 1 in the FAJ condition for both warm-humid and hot-dry environments and 3 in the CON condition for the warm-humid environment.

3.2 Estimated maximum oxygen uptake

The median of estimated maximum oxygen uptake was 40 ml/kg/min (range: 36–60 ml/kg/min) and the median of physical workload at 4 METs was calculated as 51 W (range: 43–63 W).

3.3 Core temperatures

Mean rectal temperatures, esophageal temperatures, and ear temperatures during the period 20 min before the participants started exercising until 15 min after the end of exercise are shown in Figures 1 and 2. Statistically significant differences between CON and FAJ conditions were found in the absolute values of rectal temperatures in hot-dry environment and in the changes of rectal temperatures in the warm-humid environment. Statistically significant differences were also found in the absolute values and in the changes of esophageal temperatures in the warm-humid environment. In ear temperatures, the differences of the absolute values and of the changes were statistically significant in both environments.
Mean values of skin temperature are shown in Figures 3 and 4. The skin temperatures reflected the ambient temperature, increasing when the participants entered the room for exercise. While the values of skin temperature were elevated during exercise in the CON condition, they increased by a lower amount in the FAJ condition. Statistically significant differences were found for skin temperature of all skin temperatures and both environments. The interaction effects were significant for chest skin temperature in both environments, especially at the late period of exercise.
3.5 Heart rate

Trends for mean heart rate are shown in Figure 5. While heart rate increased at the beginning of the exercise in both environmental conditions, increases were lower in the FAJ condition than in the CON condition, and the difference was statistically significant for both environment (hot-dry $P < .0001$, warm-humid $P < .0001$). The interaction effect was statistically significant for the warm-humid environment; however, post hoc tests did not indicate significant differences between FAN condition and CON condition at any time during the experiment.

**FIGURE 2** Mean core temperature and changes of values from −20 min in CON and FAJ conditions for warm-humid (30°C ambient temperature and 85% relative humidity) environments (in the experimental chamber). The anterior chamber (AC) was 28°C ambient temperature and 50% relative humidity. Values are mean ± SEM ($n = 9$). CON, control condition; FAJ, fan-attached jacket condition; SEM, standard error of the mean. *Different ($P < .05$) in the two-way repeated measures analysis of variance.
Estimated amount of sweating and % weight change

The estimated amount of sweating, 986 ± 64 g and 781 ± 28 g in CON and FAJ conditions, respectively, in the hot-dry environment and 805 ± 76 g and 664 ± 67 g in CON and FAJ conditions, respectively, in the warm-humid environment. % Weight change in the hot-dry environment was significantly lower in FAJ than in CON; 1.59 ± 0.12% and 1.25 ± 0.05% (P = .0039) in hot-dry environment, and
1.29 ± 0.15% and 1.07 ± 0.11% ($P = .1289$) in the warm-humid environment. The Spearman’s rank correlation coefficient Rho between the increase in rectal temperature and the % weight change demonstrated significant inverse correlation in all conditions; −0.6 ($P = .0876$) in CON, −0.85 ($P = .0037$) in FAJ, in hot-dry environment, −0.7667 ($P = .00159$) in CON, and −0.5333 ($P = .1392$) in FAJ, in the warm-humid environment. Rho between % weight change in CON and increase in rectal temperature in FAJ also demonstrated significant inverse correlation; −0.8667 ($P = .0025$) in hot-dry environment and −0.6833 ($P = .0424$) in the warm-humid environment.
Subjective evaluations

The RPE and thermal comfort values are shown in Figure 6. The RPE was higher in CON in hot-dry conditions (hot-dry $P = .0005$, warm-humid $P = .1341$) and the thermal comfort was also higher in CON in both environmental conditions (hot-dry $P < .0001$, warm-humid $P < .0001$).

4 | DISCUSSION

This study confirmed that the use of a long-sleeve FAJ can effectively suppress the increases in core temperature, skin temperature, heart rate, and estimated sweat volume, which could contribute to mitigate heat strain even when the temperature in the surrounding environment is hotter than skin temperature or in humid environments. This is the first study to evaluate the use of the FAJ at the condition applicable to the real work setting—60 min of continuous exercise and an air-flow setting that allows the battery to last for 8 h.

4.1 | Core temperatures

We anticipated that the cooling effect of wearing a long-sleeve FAJ may not be sufficient in the environment hotter than the skin temperature or in the environment too humid to let sweat evaporate. However, by wearing the FAJ, the elevations of ear temperature were reduced.
in the hot-dry environment, wherein the evaporation of sweat must be promoted to allow the amount of heat dissipation by evaporation to exceed the amount of heat convection from ambient air to the body. Increases in rectal temperature, esophageal temperature, and ear temperature were reduced in the warm-humid environment. We expected that the evaporation of sweat would be reduced in the humid environment; however, the moist skin surface might be favorable for heat dissipation from evaporation because of greater heat dissipation by convection (through wind with a lower air temperature).

Previous studies that examined the cooling effect of vest-type FAJs using a thermal manikin determined cooling efficacy with skin temperature, ambient temperature, and relative humidity; however, they indicated that the wet skin surface of a human is different from a manikin’s skin surface and that true effectiveness should be evaluated in experiments with humans. We confirmed that heat dissipation might be weak during early stages of exercise in hot-dry environments because the wearer will begin to sweat.

The temporal trends of rectal temperature differed from those of esophageal temperature because rectal temperature changes slowly, reflecting the temperature of visceral organs, and esophageal temperature changes rapidly, reflecting the temperature of circulating blood. As previous studies performed in similar hot-dry environment have demonstrated statistically significant differences after 60 min from the start of exercise, our study might have been curtailed before the significant difference could be observed. The fact that, in our study, only the upper body was cooled and the exercise involved the limbs of the lower body, is similar to observations stated in the reports from previous studies.
Core temperatures after exercise may not have been lowered to initial values during the resting period spent in anterior chamber because of the constriction of arteriovenous anastomoses in the subcutaneous region, which might inhibit heat dissipation from the skin by heat conduction and convection.

### 4.2 Skin temperatures

We anticipated the heat convection from the surroundings to the skin surface may occur in high-temperature environments; however, all the skin temperatures were reduced in hot-dry environment. Slower skin temperature increases were most evident for skin temperature of the chest, likely because perspiration generally starts from the chest region and the cooling effect of the wind is noticeable. Although the condition factor was significant also in back skin temperatures, the difference in the temperatures between CON and FAJ conditions was smaller than other skin temperatures we measured. A preceding study reported the cooling effect should be most eminent at the back where the air-flow directly blows in; however, in this study, we estimate the air flow might circulate into front abdominal area because the participants bent forward while cycling. Thus, only the chest skin temperature demonstrated the significant interaction effect of time and condition. In some participants, increases in skin temperature on the forehead were reduced, likely because the air was blown out of the collar. The cooling effect became evident in the latter stage of exercise because of the wet skin surface.

### 4.3 Heart rate

We estimated the difference of heart rate increase could not be observed; however, the increase in heart rate was suppressed by wearing a FAJ in both environments. Heart rate increases were significantly reduced in both environments, likely because decreased stroke volume was mitigated by systemic arterial constriction, which facilitates heat dissipation from surface blood circulation by surface cooling from perspiration.

### 4.4 Estimated amount of sweating and %weight change

We hypothesized the sweating might be ineffective in the warm-humid environment compared to the observation in hot-dry environment. We believe that estimated sweat volume was reduced in the FAJ condition because the proportion of evaporated sweat was greater and effectively enhanced heat dissipated by the air flow from the fan. Reduced increases of core temperatures might have also contributed to the reduction. The reduction was significant only in the hot-dry environment because evaporation occurred more easily than in the warm-humid environment. In humid environments, work clothes might stick to the skin surface and the sweat could be wicked away to the clothing so that this enhanced sweating due to enlarged sweating area. In addition, the increase in rectal temperature and the % weight change demonstrated inverse correlation in all conditions. This can be explained by the fact that the sweat effectively reduced the elevation of body temperature. Furthermore, %weight change in CON inversely correlated with the increase in rectal temperature in FAJ in both environments. Participants who already had an ability to sweat abundantly in CON might be more easily benefited by the body-cooling effect from wind in FAJ.

### 4.5 Interviewed subjective evaluations

We predicted the subjective evaluations might not differ by wearing a FAJ in hot-dry or in the warm-humid environment; however, we observed significant differences except for RPE in the warm-humid environment. We presume that the airflow of the jacket in almost windless environment might enhance the thermal comfort of subjects. The Borg scale used in RPE is generally said to reflect one’s heart rate, which was not the case in this study. These subjective evaluations cannot be free from the Hawthorne effect in non-blinded study.

We confirmed that the long-sleeve type FAJ has body-cooling effects, these findings were in agreement with those of previous studies; however, in severe thermal environments or for heavier physical workloads, stronger wind flow, or additional cooling measures such as the use of phase change materials or pre-cooling methods must be considered.

### 4.6 Limitations

Our study had four limitations. First, the participants were all Japanese male adults in their 20s and 30s. This was to ensure uniformity of the physiological responses and because the differences between individuals are generally thought to be less apparent among young people, but the results have limitations in terms of their generalizability to female, younger, or older individuals and to people of different ethnicities. Second, the physical workload primarily targeted the lower extremities, and the artificial environment was almost windless to allow for the standardization...
of the workload with various sensors; therefore, the conditions did not reflect an actual work situation. Third, the study was performed in only two different artificial environments. Fourth, in this study, we conducted a two-way repeated ANOVA in a model including time term and condition term as the main effect along with their intersection term; however, the statistical power might not be enough to examine the combined effect of the time term and the condition term because of the limited number of subjects.

5 | CONCLUSIONS

We confirmed that increases in core body temperature during 60-min continuous exercise at 4 METs were reduced by wearing a long-sleeve FAJ at 40°C ambient temperature and 30% relative humidity or at 30°C ambient temperature and 85% relative humidity environment. We also confirmed that increases in sweat volume are reduced by wearing a long-sleeve FAJ at 40°C ambient temperature and 30% relative humidity. We conclude that wearing a long-sleeve FAJ may help mitigate heat strain of people engaged in physical tasks in hot or humid environments.

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DISCLOSURE

Approval of the research protocol: The study design and procedures were approved by the Ethics Committee of Medical Research of UOEH, Japan (approval number R1-031). All procedures were in accordance with the ethical standards of the Declaration of Helsinki and its amendments. Informed consent: All participants were asked to carefully read a document explaining the purposes and procedures of the experiment and all gave their written informed consent prior to participation. Registry and the registration no. of the study/trial: N/A. Animal studies: N/A. Conflict of Interest: S.H. was supported by donations from Kamakura Seisakusho Co., Ltd., Shigematsu Works Co., Ltd., Kyoto Electronics Manufacturing Co., Ltd., and Midori Electronics Manufacturing Co., Ltd. However, these funding sources had no role in the study design, practice, or analysis. The authors declare that they have no competing interests and have received no financial support from manufacturers of the fan-attached jacket.

AUTHOR CONTRIBUTIONS

Seichi Horie, KImiyo Mori, Kimie Fukuzawa, and Chikage Nagano conceived and designed the research. Kimiyo Mori, Kimie Fukuzawa, Chikage Nagano, and Seichi Horie jointly conducted all experiments. Kimiyo Mori, Kimie Fukuzawa, Seichi Horie, and Chikage Nagano performed statistical analyses. Kimiyo Mori created the tables and the figures. All authors were involved in interpreting the data. Seichi Horie, Kimiyo Mori, Kimie Fukuzawa, and Chikage Nagano drafted the manuscript. All authors contributed to revising the manuscript and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

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

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