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ORIGINAL ARTICLE

# Comprehensive assessment of the impact of horticultural activities on salivary stress biomarkers, psychological status, and the autonomic nervous system response visualized using a wearable biosensor

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**Abstract**

**OBJECTIVES:** This study aims to comprehensively investigate the changes of salivary stress biomarkers, psychological status, and autonomic nervous system (ANS) response due to horticultural activities (HAs).
**DESIGN AND METHODS:** A prospective observational study was conducted in twenty Japanese healthy adults (mean age, 58.4 years). Flower appreciation, flower arrangement, and farm work experience were performed as three HAs with different working concepts. Five salivary stress biomarkers (cortisol, α-amylase, S-IgA, chromogranin A, and oxytocin) were measured to quantify the stress levels before and after each HA. The Profile of Mood Status 2nd edition (POMS2) was used as a subjective psychological evaluation. Wearable biosensors were used to visualize the continuous ANS response throughout the process.
**RESULTS:** In the POMS2 investigation, the negative factors, which included Anger-Hostility, Confusion-Bewilderment, Depression-Dejection, Tension-Anxiety, and Total Mood Disturbance, were significantly decreased ($p$=0.0135, $p$=0.0004, $p$=0.0024, $p$=0.0015, $p$=0.0063, respectively). In the measurement of salivary stress biomarkers, flower appreciation decreased cortisol ($p$=0.0134), and farm work experience not only decreased cortisol but also increased oxytocin ($p$=0.0041, $p$=0.0128 respectively). In the visualization results of the ANS response, a graph demonstrated that the difference in activity between the sympathetic nerve and the parasympathetic nerve was narrowed by a series of HAs.
**CONCLUSIONS:** In healthy adults, HAs had a stress-reducing effect, which was evidenced by neuroendocrinological and psychological evaluations, a study of POMS2, salivary stress biomarkers, and visualization of the ANS response.

## INTRODUCTION

It has been empirically known that horticultural activity (HA), wherein an individual interacts with plants such as flowers and trees, alleviates psychological stress. In recent years, HA has been attracting attention as a complementary and alternative therapy for patients with specific psychiatric disorders, such as dementia and depression, and the effects of these activities have been investigated by measuring immune biomarkers and endocrine biomarkers, as well as by various psychological tests. Previous research has shown that HA enhances euphoria and social connections in patients with brain injury (Soderback *et al.* 2004). A review of reports on people living with dementia revealed that HA improved self-esteem and social interaction, eliminated negative emotions, enhanced positive emotions, and increased engagement in HA (Blake & Mitchell, 2016). A recently reported randomized controlled trial revealed that HA improved the inflammation caused by a reduction in plasma IL-6 levels in healthy Asian elderly people without mental illness and that it also had a protective effect on the nervous function via the maintenance of plasma CXCL5 and CXCL12 (SDF-1α) and Brain-derived neurotrophic factor (Ng *et al.* 2018). HA is expected to reduce psychological stress regardless of the presence or absence of mental illness.

Psychological stress, which is one of the factors that causes the onset of mental illness, occurs in modern times irrespective of the environment (e.g., the workplace or home), and people tend to accumulate stress unconsciously (Esch & Stefano, 2010). In such a stress-filled society, the importance of establishing a voluntary method of stress control and creating an environment that leads to stress reduction is becoming more important (Esch & Stefano, 2010). In this background, HA may be useful as a primary preventive measure for psychological stress reduction, however, the accumulation of quantified information regarding its effects is scarce. Thus, to comprehensively investigate the changes in stress caused by HA in healthy adults, we conducted a conventional psychological test, measured the levels of five salivary stress biomarkers that have been reported to be related to stress, and visualized the autonomic nervous system (ANS) response in real-time using wearable biosensors.

## MATERIAL AND METHODS

*Study Design and Participants*

Twenty healthy Japanese adults (male, n=3; female, n=17; mean age, 58.4 ± 23.8 years) who provided their informed consent were enrolled in this study. A prospective observational study was conducted on a farm (Floral Production Corporation, SCRE LCC/Ltd.) in Okayama City, Okayama Prefecture, Japan in November 2017. A flow chart of the study is shown in Figure 1. HA consisted of the following three parts: 1) Part A: flower appreciation (orchid flower appreciation), 2) Part B: flower arrangement (*Cymbidium* and genus gypsophilia), and 3) Part C: farm work experience (loading rice into a threshing machine). These three horticultural activities were considered to not require a high degree of physical stress and they tend to be less demanding on an individual's sympathetic tone. All protocols of this study were approved by the review board and ethics committee of Juntendo University (No. B-15-016), and conformed to the tenets of the Declaration of Helsinki. Informed consent for the measurements was obtained from each of the participants.

*Measurement of the psychological status and salivary stress biomarkers*

The saliva component was measured before and after each part. As for the measurement items, five salivary biomarkers, cortisol, chromogranin A, α-amylase, S-IgA, and oxytocin, were measured as stress indexes.

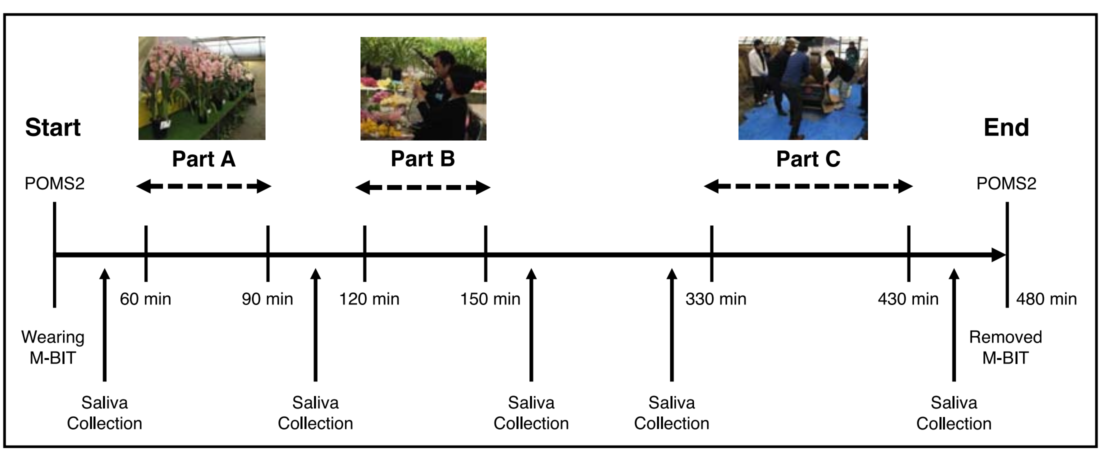


**Fig. 1.** The study procedure. Participants wore an M-BIT biometric sensor and their electrocardiogram was continuously recorded from the start to the end of the study. Salivary samples were collected at five time points: before and immediately after each part. The Profile of Mood State 2nd edition (POMS2) was administered before and after the series of all parts.

**Tab. 1.** Changes in POMS2 parameters with HA

| POMS 2 | Baseline | after HA | *p* value |
|---|---|---|---|
| Anger-Hostility | 46.7 ± 9.73 | 42.2 ± 7.65 | 0.0135* |
| Confusion-Bewilderment | 53.3 ± 7.03 | 47.8 ± 7.75 | 0.0004*** |
| Depression-Dejection | 50.4 ± 7.56 | 45.8 ± 6.50 | 0.0024** |
| Fatigue-Inertia | 46.0 ± 8.23 | 44.4 ± 7.22 | 0.4941 |
| Tension-Anxiety | 52.3 ± 7.51 | 45.4 ± 8.15 | 0.0015** |
| Vigor-Activity | 54.9 ± 6.35 | 53.9 ± 7.48 | 0.5333 |
| Friendliness | 57.0 ± 10.2 | 54.8 ± 9.60 | 0.0668 |
| Total Mood Disturbance | 48.7 ± 7.51 | 44.1 ± 6.63 | 0.0063** |

Data are means ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with baseline by paired t-test. HA, horticultural activity; POMS2, Profile of Mood Status 2$^{nd}$ edition; SD, standard deviation.

About 1 ml of saliva was collected using Saliva Collection Aid (SCA: Salimetrics, USA). Subjects put a straw, which was inserted in a vial (preservation tube), into their mouth, and the saliva was taken into the vial as it drooped. The obtained saliva was frozen at -20 °C until the analysis. As for the salivary components, α-amylase was measured using a saliva amylase monitor (Nipro Corporation), and cortisol, S-IgA, chromogranin A and oxytocin were measured using a Salivary Cortisol Enzyme Immunoassay Kit (Salimetrics, USA), a Salivary Secretory IgA Indirect Enzyme Immunoassay Kit (Salimetrics, USA), Human Chromogranin A ELISA Kit (Yanaihara Institute Inc., Japan) and Oxytocin ELISA kit (Enzo, USA), respectively. As an evaluation of the psychological state, the Profile of Mood Status 2nd edition (POMS2) (Heuchert & McNair, 2012; Yokoyama & Watanabe, 2015) was conducted at the beginning and after HA. POMS2 is one of the questionnaire forms developed for the purpose of subjectively evaluating human emotions, such as mood, feeling and emotion. It can measure seven mood scales: "Anger-Hostility", "Confusion-Bewilderment", "Depression-Dejection", "Fatigue-Inertia", "Tension-Anxiety", "Vigor-Activity", and "Friendliness". In addition, "Total Mood Disturbance", which is a score that comprehensively represents a negative mood state, can be measured at the same time.

*Evaluation of the ANS response*

Wearable biometric sensors M-BIT (Institute of Man and Science, Inc.) were used for real-time recording of the ANS response. The M-BIT is a system that can be attached to the left chest and simultaneously monitor biological information, such as an electrocardiogram (ECG), thermometer (infrared temperature sensor), and 3-axis accelerometer. To classify and quantify autonomic activity, a heart rate variability analysis of ECG RR interval was performed. First, an accurate RR interval is calculated from the ECG waveform. Next, the Smoothed Pseudo Wingner-Ville distribution (Pereira de Souza Neto E *et al.* 2001), a time-frequency analysis, is used to calculate the RR Interval Variation (RRIV). In the RRIV frequency band, the low frequency region (Low Frequency: LF) is defined as from 0.04 to 0.15 mm and while the high frequency region (High Frequency: HF) is defined as from 0.15 to 0.40, respectively, and the BITAS software program (Institute of Man and Science, Inc.) was used to evaluate HF as an index of the parasympathetic nerve activity and LF/HF as an index of the sympathetic nerve activity. Since this device performs a frequency analysis once every minute, it is thus

**Tab. 2.** Changes in salivary stress biomarkers with each part of HA

| Salivary stress biomarker | Part A | | Part B | | Part C | |
|---|---|---|---|---|---|---|
| | pre | post | pre | post | pre | post |
| Cortisol (μg/dL) | 0.19 ± 0.35 | 0.14 ± 0.28* | 0.14 ± 0.28 | 0.14 ± 0.21 | 0.12 ± 0.14 | 0.09 ± 0.12** |
| Chromogranin A (pmol/mL) | 6.06 ± 3.44 | 7.30 ± 5.50 | 7.30 ± 5.50 | 6.41 ± 3.99 | 5.76 ± 5.61 | 4.67 ± 4.62 |
| α-Amylase (KIU/L) | 93.9 ± 79.6 | 56.2 ± 55.4 | 56.2 ± 55.4 | 53.2 ± 51.7 | 73.7 ± 78.5 | 70.6 ± 95.8 |
| S-IgA (μg/mL) | 36.3 ± 62.0 | 22.7 ± 41.6 | 22.7 ± 41.6 | 19.4 ± 24.8 | 21.9 ± 34.0 | 24.2 ± 31.4 |
| Oxytocin (pg/mL) | 33.9 ± 29.7 | 33.9 ± 26.3 | 33.9 ± 26.3 | 27.1 ± 16.9 | 27.0 ± 21.9 | 38.1 ± 28.9* |

Data are means ± SD. * $p < 0.05$, ** $p < 0.01$ compared with baseline by paired t-test. HA, horticultural activity; SD, standard deviation. Part A: flower appreciation, Part B: flower arrangement, Part C: farm work experience.

possible to measure the ratio of HF and LF components in detail within that minute.

*Statistical analyses*

Continuous variables were compared between two corresponding groups by a paired t-test. *P* values of <0.05 were considered to indicate statistical significance. All statistical analyses were performed using the JMP® 14 software program (SAS Institute Inc., Cary, NC, USA).

## RESULTS

*Changes in psychological status using POMS2*

Table 1 shows the changes in the psychological state due to HA. After HA, negative psychological factors, which are Anger-Hostility, Confusion-Bewilderment, Depression-Dejection, and Tension-Anxiety, were significantly decreased ($p$=0.0135, $p$=0.0004, $p$=0.0024, $p$=0.0015, respectively). Total Mood Disturbance, which is a score that comprehensively represents a negative mood state, also was decreased significantly ($p$=0.0063).

*Changes in salivary stress biomarkers*

Table 2 shows the changes in salivary stress biomarkers before and after the three HA parts. Cortisol was significantly decreased by flower appreciation and the farm work experience ($p$=0.0134, $p$=0.0041, respectively). In addition, the farm work experience significantly increased oxytocin ($p$=0.0128). There were no significant changes by HA on chromogranin A, α-amylase, and S-IgA.

*Changes in ANS response*

Figure 2 shows the chronological changes in the ANS response due to HA. Before and after the flower appreciation and the flower arrangement activities and during these experiences, there were no significant changes in sympathetic or parasympathetic nerve activities, which were observed as a parallel graph. In the farm work experience, a temporary decrease in sympathetic nerve activity and an increase in parasympathetic nerve activity were observed at the end and after the end of the activity. Furthermore, throughout the series of experiences of all parts, a graph demonstrated that the difference between sympathetic nerve and parasympathetic nerve activities narrowed.

## DISCUSSION

In this study, we evaluated the effect of HA on psychological status using POMS2 and five salivary stress biomarkers, and at the same time, visualized an ANS response using a wearable biosensor. Negative factors in POMS2 were significantly decreased by a series of HA, cortisol was significantly decreased by the flower appreciation and the farm work experience, and oxytocin was significantly increased by the farm work experience. Regarding the ANS response, although there was no change in the predominance of the sympathetic and parasympathetic in the flower appreciation and the flower arrangement activities, a temporary decrease in sympathetic nerve activity and an increase in parasympathetic nerve activity were observed at the end of and after the end of the farm work experience activity. A graph demonstrated that through the series of all parts of the HAs, the difference in activity between the sympathetic nerve and the parasympathetic nerve narrowed. In terms of the neuroendocrinological and psychological aspects, these results indicated that HA may help alleviate stress in healthy adults.

Cortisol is a steroid hormone released from the adrenal cortex. It is called a stress hormone because it is released via the HPA system by various stress stimuli. It

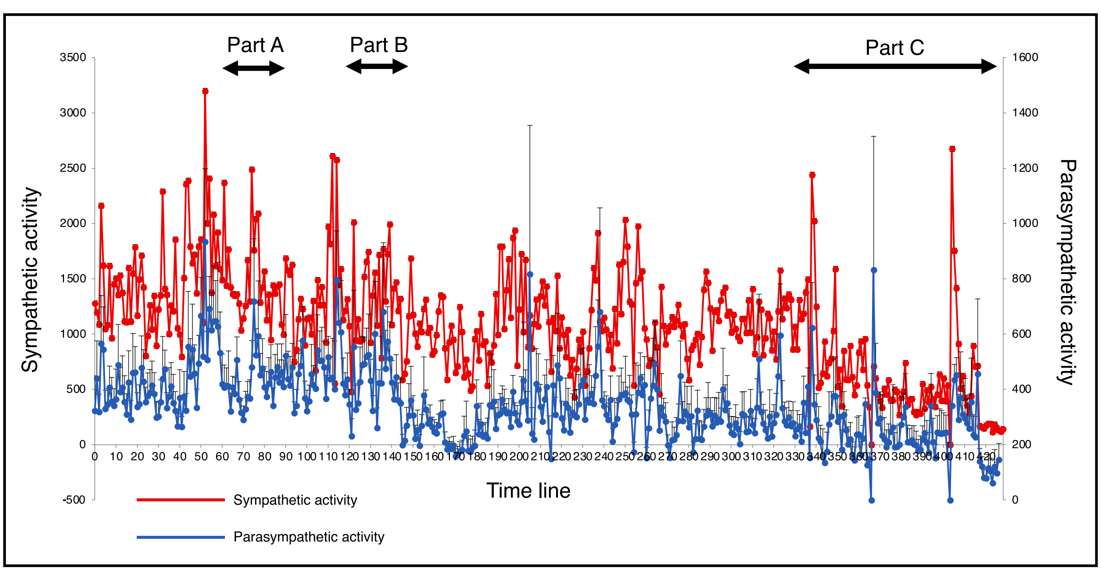


**Fig. 2.** The effects of horticultural activities on sympathetic and parasympathetic activities visualized by a wearable biometric sensor (M-BIT).

is known that stress increases the cortisol concentration in blood or saliva, and—in particular—a saliva sample, which is a simple measurement that is very highly correlated with the cortisol concentration in blood, is often used (Izawa *et al.* 2008). In our current study, a significant difference was observed in the flower appreciation and farm work experience, suggesting that stress was relieved. On the other hand, no significant difference was observed in a flower arrangement. Unlike the other tasks, flower arrangement requires dexterity, thought, and concentration on the hands. The possibility is considered that for some people the stimuli caused by the color, shape, size, and scent of the plants in front of the eyes brought stress rather than pleasant feelings but stress. During the task of freely arranging the flowers, it was considered necessary to select flower materials and to work in consideration of individual tastes and aptitudes, including types, colors, and aromas of flowers.

Oxytocin, which is a peptide consisting of nine amino acids, has been identified as a peptide with a similar amino acid sequence not only in vertebrates (including humans) but also in invertebrates, and is considered to be among the most classical hormones. Oxytocin is produced in the paraventricular nucleus and suprachiasmatic nucleus of the hypothalamus, and is either secreted from the posterior lobe of the pituitary gland via axons, or it acts on the target cells from the hypothalamus through axons without passing through the pituitary gland (Ludwig & Pittman, 2003). Oxytocin has been reported to have various effects and has many functions, including facilitating labor, maternal behavior, forming trust (Rilling & Young, 2014), improving communication disorders (Meyer-Lindenberg *et al.* 2011), and regulating appetite and energy (Maejima *et al.* 2009). It has been reported as well that the secretion of oxytocin reduces the secretion of ACTH, which is a stress-related hormone (Uvnas-Moberg, 1998). In this study, a significant increase in oxytocin was observed in the farm work experience part, but no significant difference was observed in the flower appreciation or flower arrangement parts. It is possible that the activation of serotonin nerves due to exercise associated with the farm work experience and sunshine exposure, and that stress alleviation due to aroma components generated from plants and soil had an effect on mental stability and increased the secretion of oxytocin.

Although chromogranin A is one of the soluble proteins secreted together with catecholamines from adrenal medulla chromaffin cells and sympathetic neurons, it is also contained in saliva and is an index substance that reflects sympathetic nerve activity. It does not respond to physical stress due to exercise load, but specifically responds to mental stress (Nakane *et al.* 2002). It is reported that the diurnal fluctuation of chromogranin A is highest at the time of waking, decreases rapidly 1 hour after waking, and subsequently stabilizes (Den *et al.* 2007). Since the secretion of α-amylase is promoted by the action of the sympathetic nerve, it responds well to stress loads, and the time lag is approximately one minute. It is known as an index for evaluating the neural activity of the sympathoadrenal medullary system, and the α-amylase activity in saliva increases due to mental stress. It is reported that the diurnal fluctuation reaches its lowest value immediately after waking up and reaches its highest value at 8:30 p.m. (Yamaguchi *et al.* 2006). S-IgA is a type of antibody that has the function of preventing the growth of pathogens in the mucous membranes of the oral cavity, respiratory tract, intestinal tract, and other locations. Acute stress increases s-IgA and chronic stress decreases s-IgA (Bosch *et al.* 2002), and the salivary s-IgA subclass was negatively associated with psychological stress (Engeland *et al.* 2016). One of the advantages of using saliva is the ability to simultaneously measure various stress-related substances. Acute and short-term stress is reflected in chromogranin A and α-amylase, and cortisol and s-IgA are considered as candidate indexes to measure long-term stress coping. Cortisol has a slow response peak, and cortisol and s-IgA have been indicated to be associated—to some extent—with long-term stress. In this study, although the chromogranin A, α-amylase, and s-IgA did not show any significant changes, evaluations by these indicators may be important depending on the start time and duration of the experiment.

The present study was associated with several limitations. First, this was a single arm study with a relatively small number of Japanese participants. Further validation of the effectiveness of the findings requires RCTs, including studies with large sample sizes and control settings. Secondly, we did not investigate the quantification or subject recognition of how much the scent was affected. The entire greenhouse on the farm, which was the experimental site, was filled with the scent of flowers, and it is probable that some people felt pleasant in the presence of the scent and that some did not. In the future, it will be necessary to quantify the experimental site and the scent of flowers during the experiment and to include questions including about unpleasant feelings. Thirdly, the interpretation of the significant changes in salivary oxytocin levels in this study is controversial. There is a report that saliva and plasma oxytocin levels are not correlated in healthy adult men (Javor *et al.* 2014). However, there are reports that plasma and salivary oxytocin are correlated in healthy mothers at 1–3 months after childbirth (Grewen *et al.* 2010), and that the salivary level of oxytocin better reflects the cerebrospinal fluid concentration in comparison to the plasma levels (Martin *et al.* 2018). The evaluation of the stress response by salivary oxytocin levels may need to be validated in a study with a large cohort in the future to determine its validity.

In conclusion, a study on POMS2, salivary stress biomarkers, and the visualization of the autonomic nervous function indicated the effect of HA on stress reduction in healthy adults. Hence, it might be desirable to accumulate objective data using physiological indicators in combination with subjective evaluations in a larger study population to accelerate the systematization of the theory of stress reduction by HA. In the future, HA may play a role in the stress management of modern people living in a stress-filled society through collaboration with the medicine, welfare, and agriculture sectors.

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