

42.7 years old, SD: 8.8). The mean CAPS score of the loss group was 32.8 ± 6.1 points. The number of subjects in the non-loss group was 32 (mean age: 43.8 years, SD: 8.9). The mean CAPS score of the non-loss group was 30.41 ± 6.6 points. Other information for each group is shown in Table 1. Written informed consent was obtained from each subject in accordance with the Declaration of Helsinki (1991). This study was approved by the Ethics Committee of Tohoku University School of Medicine.

B. Experimental procedure

The scores on the psychological measures were compared between the two groups. Subjects received instructions from the experimenter about the experiment and completed psychological measures and saliva sampling in the time allotted. The subjects were given 120 minutes to complete the psychological measures. Then, the subjects underwent saliva sampling at 4:00 p.m. for five minutes. Therefore, the experimental time was 125 minutes. The subjects also completed certain psychological tests and an MRI scan that were not used in this study while they waited for the next procedure.

C. Psychological measures

To assess any effects in terms of the quality of life, happiness and mental health of the subjects, we administered the following psychological measures: (a) a face sheet consisting of facts such as age, home damage and a lifestyle evaluation after the earthquake (responses rated from 0 [worst] to 8 [good]); (b) the World Health Organization Quality of Life 26 (WHO-QOL26) questionnaire, which measures a survivor's quality of life [47]; (c) the General Health Questionnaire 30 (GHQ30), which measures psychological distress and physical symptoms [48, 49]; and (d) the Center for Epidemiologic Studies Depression Scale (CES-D), which measures the level of depressive symptoms occurring within the past week [50, 51].

D. Saliva sampling

We collected saliva samples from subjects to measure their levels of salivary cortisol. Distressing psychological stimuli are associated with increased cortisol levels [52]. Taking into consideration the subjects' circadian cortisol rhythms, we collected all saliva samples at 4:00 p.m. on weekdays, as people are less affected by circadian cortisol rhythms at this time of day [53]. The collection time for the saliva samples was determined in reference to a previous study showing that it is preferable to collect saliva samples during a timeslot with little circadian variation (for example, 3:00 p.m. or 4:00 p.m.) when performing saliva sample collection two or more times [54]. The subjects also refrained from drinking, eating [55] and exercise [56] for two hours before the saliva sampling.

E. Sampling strategy

(1) Measurement of salivary cortisol

To assess physiological stress, we used the same technique to measure salivary cortisol as that used in a previous study [57]. The saliva samples were collected

using a salivette apparatus (Sarstedt, Nümbrecht, Germany) and centrifuged at 3,000 rpm for five minutes. We stored the supernatant solutions in airtight containers at -80°C and measured the salivary cortisol levels in these solutions. We measured salivary cortisol using a semi-microcolumn high-performance liquid chromatography (HPLC) system (Shiseido, Tokyo). The following conditions were used for the HPLC analysis: the mobile phase for preprocessing used a 5-mmol/L phosphoric acid buffer solution (pH = 6.9) and acetonitrile at a ratio of 98:2, with the solution flowing through the columns at a rate of 1 mL/min. The mobile phase for measurement used a 10-mmol/L phosphoric acid buffer solution (pH = 6.9) and acetonitrile at a ratio of 78:22, with the solution flowing through the columns at a rate of 0.1 mL/min. The column temperature was maintained at 35°C , and the detection wavelength was 242 nm. Increased cortisol levels are associated with distressing psychological stimuli [52].

(2) Statistical analyses

The psychological and salivary data were analyzed using the PASW statistical software package (ver. 18 for Windows; SPSS, Inc., Chicago, IL, USA). Comparisons of demographic and clinical data, psychological measures and the salivary cortisol levels between the loss group and the non-loss group performed with a two-sample t-test (marital status, family composition after the earthquake, and damage to housing performed with Pearson's chi-square test). To evaluate the correlations between each psychological variable and the salivary cortisol levels, Pearson's correlation coefficients were determined. All *p* values were two-tailed, and values of ≤ 0.05 were considered to be significant.

III. RESULTS

A. Psychological measures

The demographic and clinical data of the subjects are shown in Table 1. Age, the CAPS scores, marital status, and of the percentage of semi-destroyed house, which is part of damage to housing did not differ significantly between the loss and non-loss groups. The loss group exhibited significantly lower lifestyle evaluations after the earthquake compared to the non-loss group [$t = 4.83$, degrees of freedom [df] = 52, $p < 0.001$]. Additionally, the loss group exhibited significantly higher the percentage of living alone and completely-destroyed house which is part of damage to housing compared to the non-loss group [the percentage of living alone: $\chi^2=8.505$, $df=1$, $p<.01$; the percentage of completely-destroyed house which is part of damage to housing: $\chi^2=13.12$, $df=1$, $p<.001$]. The loss group also exhibited significantly lower the percentage of living together with a spouse or a relative and no damage which is part of damage to housing compared to non-loss group [the percentage of living together with a spouse or a relative: $\chi^2=8.505$, $df=1$, $p<.01$; the percentage of no damage which is part of damage to housing: $\chi^2=9.82$, $df=1$, $p<.01$]. The comparison results for the data obtained from these tests are shown in Table 2. The loss group demon-

TABLE 1. BASELINE DEMOGRAPHIC AND CLINICAL DATA OF THE STUDY SUBJECTS

Factor	loss group (N = 22)		non-loss group (N = 32)		<i>p</i> ^a
	Mean	SD	Mean	SD	
Age (years)	42.7	8.8	43.8	8.9	0.646 ^a
CAPS score	32.8	6.1	30.4	6.6	0.178 ^a
Marital status (% married)	94.5		96.9		0.786 ^b
Family composition after the earthquake (%)					
Alone	31.8		3.1		0.004 ^b
With spouse or relatives	68.2		96.9		0.004 ^b
Damage to housing (%)					
Completely destroyed house	59.1		12.5		0.001 ^b
Semi-destroyed house	31.8		37.5		0.667 ^b
No damage	9.1		50.0		0.002 ^b
Lifestyle evaluation after the earthquake	1.4	1.3	3.5	1.7	0.001 ^a

^a Two sample t-test (two-tailed), ^b Pearson's chi-square test (two-tailed)
SD, standard deviation; CAPS, Clinician-Administered Post-Traumatic Stress Disorder Scale

TABLE 2. PSYCHOLOGICAL MEASURES FOR EACH GROUP

Factor	loss group		non-loss group		<i>p</i> ^a
	Mean	SD	Mean	SD	
Physical health subscale (WHO-QOL26)	3.29	0.42	3.33	0.51	0.761
Psychological health subscale (WHO-QOL26)	2.95	0.45	3.26	0.41	0.012
Social relationship subscale (WHO-QOL26)	3.21	0.48	3.56	0.42	0.006
Environment subscale (WHO-QOL26)	2.71	0.49	3.12	0.50	0.004
GHQ score	8.59	6.67	5.78	3.97	0.058
CES-D score	15.95	7.49	12.66	6.33	0.087
Salivary cortisol level	9.80	8.23	6.64	5.97	0.107

^a Two sample t-test (two-tailed)

SD, standard deviation; WHO-QOL26, World Health Organization Quality of Life 26; GHQ, General Health Questionnaire; CES-D, Center for Epidemiologic Studies Depression Scale

strated significantly lower scores on the psychological health, social relationship and environment subscales of the WHO-QOL26 than the non-loss group [psychological health score: $t = 2.593$, $df = 52$, $p < 0.05$; social relationship score: $t = 2.854$, $df = 52$, $p < 0.01$; environment score: $t = 3.008$, $df = 52$, $p < 0.01$]. The loss group had higher GHQ and CES-D scores than the non-loss group; however, these results were only marginally significant [GHQ score: $t = -1.939$, $df = 52$, $p = 0.058$; CES-D score: $t = -1.746$, $df = 52$, $p = 0.087$].

B. Salivary stress markers

The comparison results of the salivary cortisol levels are shown in Table 2 and Figure 1. The loss group had higher salivary cortisol levels than the non-loss group. However, this result was not significant [$t = -1.638$, $df = 52$, n.s.].

C. Correlation analyses

A Pearson correlation analysis for each group is shown in Tables 3 and 4. In the loss group, the salivary cortisol levels were negatively correlated with the physical and psychological health scores of the WHO-QOL26. Furthermore, the physical health scores of the WHO-QOL26 were positively correlated with the psychological health, social relationship and environment WHO-QOL26 scores and negatively correlated with the GHQ scores. The psychological health scores of the WHO-QOL26 were positively correlated with the social relationship and environment WHO-QOL26 subscales and negatively correlated with the GHQ and CES-D scores. The environment scores of the WHO-QOL26 were negatively

correlated with the GHQ and CES-D scores. Finally, the GHQ scores were positively correlated with the CES-D scores. On the other hand, in the non-loss group, the salivary cortisol levels were not significantly correlated with any psychological measures. The physical health subscale of the WHO-QOL26 was positively correlated with the WHO-QOL26 psychological health and environment scores and negatively correlated with the CES-D scores. The psychological health subscale of the WHO-QOL26 was negatively correlated with the CES-D scores and positively correlated with the social relationship subscale of the WHO-QOL26. The CES-D scores were positively correlated with the GHQ scores.

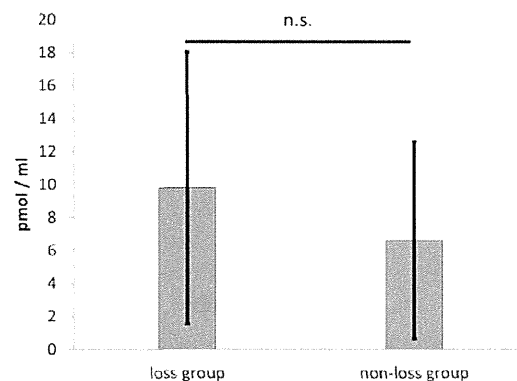


Fig. 1. Comparisons of the salivary cortisol levels between the loss group and the non-loss group

The salivary cortisol levels of the loss group were high compared with those of the non-loss group. However, there were no statistically significant differences.

TABLE 3. PEARSON CORRELATIONS AMONG STUDY VARIABLES—LOSS GROUP (N = 22)

	CAPS score	Physical health score (WHO-QOL26)	Psychological health score (WHO-QOL26)	Social relationship score (WHO-QOL26)	Environment score (WHO-QOL26)	GHQ score	CES-D score	Salivary cortisol level
1. CAPS score	1.00							
2. Physical health score (WHO-QOL26)	.15	1.00						
3. Psychological health score (WHO-QOL26)	-.03	.82***	1.00					
4. Social relationship score (WHO-QOL26)	-.06	.56**	.62**	1.00				
5. Environment score (WHO-QOL26)	-.03	.50*	.49*	.13	1.00			
6. GHQ score	.45	-.45*	-.43*	-.36	-.45*	1.00		
7. CES-D score	.12	-.38	-.52*	-.23	-.44*	.56**	1.00	
8. Salivary cortisol level	.04	-.54**	-.53*	-.38	.04	.04	-.02	1.00

* $p < .05$, ** $p < .01$, *** $p < .001$ (two-tailed)
 CAPS, Clinician-Administered Post-Traumatic Stress Disorder Scale; WHO-QOL26, World Health Organization Quality of Life 26; GHQ, General Health Questionnaire; CES-D, Center for Epidemiologic Studies Depression Scale

TABLE 4. PEARSON CORRELATIONS AMONG STUDY VARIABLES—NON-LOSS GROUP (N = 32)

	CAPS score	Physical health score (WHO-QOL26)	Psychological health score (WHO-QOL26)	Social relationship score (WHO-QOL26)	Environment score (WHO-QOL26)	GHQ score	CES-D score	Salivary cortisol level
1. CAPS score	1.00							
2. Physical health score (WHO-QOL26)	.17	1.00						
3. Psychological health score (WHO-QOL26)	-.23	.59***	1.00					
4. Social relationship score (WHO-QOL26)	-.02	.29	.41*	1.00				
5. Environment score (WHO-QOL26)	-.19	.54***	.16	.06	1.00			
6. GHQ score	.06	-.38*	-.28	-.12	.05	1.00		
7. CES-D score	.21	-.61***	-.54***	-.24	.25	.58***	1.00	
8. Salivary cortisol level	.27	-.05	-.05	-.17	-.24	.03	-.03	1.00

* $p < .05$, ** $p < .01$, *** $p < .001$ (two-tailed)
 CAPS, Clinician-Administered Post-Traumatic Stress Disorder Scale; WHO-QOL26, World Health Organization Quality of Life 26; GHQ, General Health Questionnaire; CES-D, Center for Epidemiologic Studies Depression Scale

IV. DISCUSSION

The novelty of this study is that there are identified the psychological effects on women with mild PTSD derived from natural disasters such as earthquakes. The objective of the present study was to investigate the psychological effects on women who live in the disaster area of the Great East Japan Earthquake using psychological data collected six months after the earthquake, particularly comparing women who lost a member of their household and/or a

relative and women who did not. The present study revealed heavy psychological effects in women who live in the disaster area affected by the earthquake, especially those who lost a member of their household and/or a relative. These results are consistent with our hypothesis that women who lose a member of their household and/or a relative suffer worse psychological effects than women who do not. In addition, based on these results, the relationship between higher cortisol levels and psychological stress suggests that high-stress conditions

induce alterations in the HPA axis that stimulate the release of cortisol [61–63].

In terms of the results of the psychological measures, the loss group had significantly lower WHO-QOL26 subscale scores (psychological, social relationship, environment) compared to the corresponding scores of the non-loss group. It was estimated that the women in the loss group were strongly influenced with respect to their quality of life, health and psychology by negative experiences of the loss of a relative in the disaster. Previous studies have reported that women' mental health and quality of life were worse after the earthquake [61–63]. These studies suggest that a poor QOL is a consequence of the earthquake. Although previous studies have not reported much in detail regarding the background of women' mental health, the results of our study clarified the psychological effects in women under more concrete situations in which they lost a relative in the disaster. In this disaster, many areas along the Sanriku shoreline sustained damage from the tsunami rather than the earthquake. Many people who live in the disaster area had negative experiences such as being in buildings broken by tremendous shaking. Moreover, many people who live in the coastal area of the disaster experienced the tsunami; their family members and others were carried away by the tsunami in front of their eyes and they witnessed people dying in front of them after the tsunami. This experience led to a sense of loss of the family and significant others that were part of the mental bases the subjects had thus far built. These are called object losses [64] and losses [65]. This loss experience includes negative feelings such as anger, pain, a sense of powerlessness, despair and sadness. A previous study suggested that a series of psychological reactions triggered by loss leads to mourning [64]. Previous studies also suggest that such persons undergo several stages of mourning [66–68]. First, the loss and mourning results in shock about the experience and feelings of sadness and pain. Then, a condition of spiritless persists. After a while, people gradually respond to the fact of the loss and begin to develop positive feelings. A previous study suggested that psychological recovery can be delayed, so that the attachment to the loss remains strong [69]. Additionally, a previous study suggested that women tend to be in a depressive condition for a longer time after separation than men [70]. We considered that the women in this study responded to the fact that their loss caused by the disaster had occurred in the stage of six months after the disaster; however, the subjects had not yet relinquished their sense of loss.

Additionally, psychological responses in times of disaster can be categorized into three steps: 1) reactions, 2) factors, and 3) psychodynamics [71, 72]. The period of this study was six months after the earthquake. We believe that the residents of the disaster areas of six months after the disaster were in transition from factors to psychodynamics. Although there were cases of spontaneous psychological recovery, many survivors still had strong negative feelings such as depression, anger and discontentment regarding the earthquake during this period. Living conditions changed dramatically soon after the earthquake due to the need to live in temporary evacuation centers, the inconvenience of

obtaining things, the lack of essential utilities and so on. Moreover, as the affected people were forced to live in complex and unfamiliar environments, it is believed that they felt more anxiety and fatigue than usual in their daily living and human relationships [73]. Although this study was conducted six months after the earthquake disaster, the subjects were still living with anxiety due to continuing aftershocks and unstable life security.

Social support is also disrupted after an earthquake disaster. Many of the survivors of this period were forced to move to a permanent or temporary house from a shelter. They broke off personal relationships they formed in the shelter and had to remake personal relationships in a new environment. When living in new environment such as a temporary residence, people often avoid telling others about having lost a family member because they think that there are many people who went through terrible experiences. Therefore, they cannot create relationships easily and make only necessary and minimum contact with others. Therefore, people are still isolated from people. A previous study suggested that the existence of social support has a positive effect on psychological recovery from loss [74]. However, it is assumed that social support in the aftermath of disaster may not be adequate. With these points in mind, we believe that the altered daily life environment and psychological stress caused by the disaster resulted in increases in the salivary cortisol levels in our study.

The salivary cortisol levels of both groups were higher than the standard values for women (3.46 ± 3.20 pmol/ml, 4:00 p.m.) reported in a previous study [75], and the average salivary cortisol level of the loss group was higher than that of the non-loss group. This finding is consistent with the results of previous studies, such as those of victims of the Hanshin-Awaji Earthquake and the Wenchuan Earthquake, which also reported higher cortisol levels [2, 76]. Cortisol is an indicator of psychological and physiological stress that can be used to examine PTSD pathophysiology [77]. Anxiety may have exacerbated the salivary cortisol levels due to disruption of access to daily information, damage to homes or businesses and safety-related concerns after the earthquake. Many people affected by the disaster still feel stress, fear, fatigue, helplessness and disappointment [78] due to the earthquake, and it appears that these factors continue to influence psychological responses six months after the disaster.

To summarize the above, although half a year has passed since the earthquake, psychological damage remains strong among women who live in the hardest-hit coastal areas, especially women who lost a relative in the disaster. We believe that enriching mental health, livelihood and social support for women in the disaster area such as preventing social isolation and hardship as quickly as possible is necessary. These kinds of activities will aid the early detection of women's PTSD symptoms and provide psychological care and livelihood support for women who have not recovered from negative experiences.

Finally, this study is associated with some limitations: 1) the sample size of this study was small because the number of subjects in the overarching interventional study was 54;

2) There was a clear difference between the loss group and the non-loss group. It was loss experience of family members by the Great East Japan Earthquake. The loss group consisted of women who lost a member of their household and/or a relative by the earthquake. On the other hands, the non-loss group consisted of women whose a member of their household and/or a relative was safe in the earthquake; and 3) we were unable to conduct follow-up with these women because they participated in a scheduled interventional study after this examination and were affected by that intervention. Therefore, in the future, we will review the number of respondents to surveys and will investigate the trends of the recovery process of psychological and physical health by studying women survivors' mental health continuously in the disaster area.

V. CONCLUSION

The purpose of this study was to investigate psychological effects in women who live in the disaster area using psychological data collected six months after the Great East Japan Earthquake. Our research demonstrated that women who live in the disaster area and lost a member of their household and/or a relative were strongly affected by the earthquake (i.e., they have a lower QOL and poorer mental health than those who did not suffer such a loss). In the future, we would like to examine further time-dependent changes in people living in the affected areas while simultaneously supporting the survivors.

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Research Article

The Psychological Effect of Horticultural Therapy Intervention on Earthquake-Related Stress in Women of Earthquake-Related Areas

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Abstract

In this study, we provided horticultural therapy as an intervention to 54 adult women with earthquake-related stress from the disastrous Great East Japan Earthquake along the coastline of Miyagi Prefecture. After two months of horticultural intervention, scores on the Clinician-Administered PTSD Scale (CAPS), Positive and Negative Affect Schedule (PANAS), Positive Affect (PA), and the Japanese version of the Posttraumatic Growth Inventory (PTGI-J) improved significantly in the intervention group, and salivary cortisol concentration decreased significantly. Although subsequent retesting after a two-month follow-up period revealed no major changes in CAPS or PANAS-PA scores or salivary cortisol concentration, there was an increase in PTGI-J score. These findings suggest that horticultural therapy has an effect on the symptoms of earthquake-related stress in women, and that this effect may sustain.

INTRODUCTION

The Great East Japan Earthquake on March 11, 2011 was the largest earthquake and tsunami to have occurred in the postwar period. It devastated large swathes of eastern Japan, with particularly severe damage in the prefectures of Iwate, Miyagi, and Fukushima on the Pacific coast. Despite the passage of time, some people are still living with the emotional trauma and stress experienced, when lifelines were disrupted immediately after the earthquake, and the stress caused by directly experiencing or witnessing the devastating structural, human, and other damage that occurred in coastal areas. Previous studies reported about mental health problems of survivors after a disaster such as a temporary increase in cortisol level [1-3]. Horticultural therapy (HT) is an intervention method for post-traumatic stress disorder (PTSD) that was developed in the United States for the psychological care and social rehabilitation of disabled soldiers and war veterans with PTSD symptoms after World War II [4]. In Europe, HT was developed as a program for elderly people. HT interventions are led by professionals trained to incorporate the use of plants and horticultural education into rehabilitation therapies. HT typically involves seeding and growing vegetables and flowers, actions that have been observed to improve the participant's mood and attentiveness. In addition, therapy in a

group setting improves the participant's communication skills through collaborative horticultural activities. It has been reported that participants begin to identify with plant growth, regain health and motivation, and have a chance to be happy again. Through such experiences and their association with nature, participants are thought to experience improvement [5]. HT in Japan became the focus of attention, when it was implemented as assistance for rehabilitation after the Great Hanshin-Awaji Earthquake; it has mainly been developed for elderly adults and people with disabilities [6].

Previous studies of patients, veterans, and older persons have suggested that HT and exposure to nature can have cognitive [7,8], psychological [9-12], social [13,14], and physical [10,12] benefits. Studies of allotment gardeners have also suggested that HT has a positive effect on physiological factors, such as heart rate and salivary cortisol levels [15]. In this way, most research on the effect of HT has utilized psychological measures and observational data, and the effect of HT on earthquake-related stress and the maintenance of this effect have not been fully investigated. The purpose of this study was to verify the reduction in the symptoms of earthquake-related stress in women who live in disaster areas through HT intervention using psychological measures and salivary cortisol level. Additionally,

we investigated the effect of HT on the symptoms of earthquake-related stress and the maintenance of its effect after a two-month follow-up (FU) period using psychological measures and salivary cortisol level.

METHODS

Participants

The participants were women aged 23–55 years who were residents of the coastal areas of Miyagi Prefecture and had experienced the Great East Japan Earthquake of March 11, 2011. We targeted the adult woman from 20 years old to 60 years old in this study. Review of the epidemiology of PTSD after Disasters reported that women are more likely than men to have PTSD after natural disasters, and low social support is associated with a higher likelihood of PTSD [16]. We focused this point. As the first step of horticultural therapy study, we performed an experimental study aimed at adult woman from 20 to 60 years old because there was no study of effect verification of horticultural therapy for a woman who lives in disaster area until now.

They were recruited through newspaper advertisements distributed in the earthquake-affected areas, to which 106 residents of the coastal areas (from Kesennuma City to Iwanuma City) responded.

These 106 applicants were screened for PTSD using a combination of the Mini-International Neuropsychiatric Interview (M.I.N.I.) [17,18] and the Clinician-Administered PTSD Scale (CAPS) [19-21]. In the CAPS, the F1/2 method was used for evaluation, with applicants regarded as symptomatic if they scored ≥ 1 on frequency and ≥ 2 on intensity. After the exclusion of 52 applicants who had no PTSD symptoms and a CAPS score of 40, 54 healthy, right-handed women participated in this study as part of our ongoing project to investigate the associations between brain structure and mental health. All participants who took part in this study also participated in our interventional studies and underwent psychological tests and MRI scans that are not described in this study but were performed together with those described in this study. All participants were diagnosed with a symptom of PTSD on the M.I.N.I., and they had one to two symptoms of all three PTSD symptom clusters, including re-experiencing the event, avoidance, and hyperarousal. The CAPS and M.I.N.I. were administered before and after the intervention. This study was approved by the Research Ethics Committee of Tohoku University Graduate School of Medicine after an ethical screening. Informed consent in writing was obtained from the experimental participants before the start of the experiment. The intervention period was from September 2011 to March 2012.

The study was a randomized, open-label assessor-blinded, crossover trial (RCT), and it was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000006170). Testers are blind to the study's hypothesis and the group membership of participants. The participants were divided into two groups, an intervention group ($n = 27$) and a control group ($n = 27$), by the permuted block method, and the intervention group underwent eight weeks of horticultural intervention followed by an eight-week FU period. The control group underwent eight weeks of stress control education, followed by eight weeks of horticultural intervention.

Although the intervention group had a follow-up period after the intervention period, participants were allowed to keep growing plants during the follow-up period because it could have been stressful or created negative emotions, if we required participants to stop their horticultural activities.

Description of interventions

Horticultural intervention (Intervention group): The HT intervention was designed in collaboration with a horticultural therapist and clinical psychologists. The intervention comprised a total of eight weekly sessions (60 min each) at a university lab and 15 minutes per day at participants' homes. The lab sessions were comprised of interactive lectures and practical training. The participants then attended six horticultural lessons, including topics such as designing a garden planter, seeding, watering, weeding, and picking flowers. Participants filled out an HT intervention session checklist after each session as a self-assessment. Participants took care of plants for 15 min per day at their convenience with horticulture kits provided by the experimenters, and recorded the completion of this task daily on forms provided by the experimenters at the intervention sessions. The participants submitted these forms to the experimenters at the HT intervention session each week.

Stress control education intervention (Control group): The SE intervention session was a 60-minute session consisting of a lecture regarding stress education, and it was managed by teaching assistants who served as psychological testers. The participants in the control group attended the SE intervention sessions once each week (a total of eight lessons). The video series used in the SE intervention sessions taught participants about the human body, such as stress mechanisms, psychology, and stress management. Participants filled out an SE intervention session checklist after each session. The 2nd session and the 6th session of the HT intervention session and the SE intervention session used the same teaching aid.

Follow-up (Intervention group): At the end of the two-month horticultural intervention, the experimental participants allocated to the intervention group entered a two-month FU period (Figure 1). During this period, they did not receive any specific instructions from the investigators and were asked to lead their normal lives.

PTSD screening: M.I.N.I. and CAPS

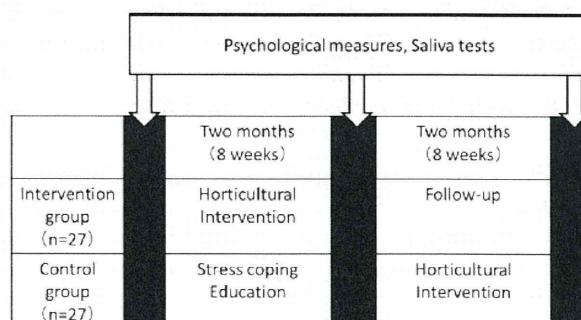


Figure 1 Intervention design. ** $p < .01$, * $p < .05$.

In this study, structured interviews were performed by six clinical psychologists and psychologists with experience in assisting earthquake victims, who underwent training before carrying out the actual interviews.

Psychological measures

The following questionnaires were administered three times (pre-intervention, post-intervention, and FU): (a) the World Health Organization Quality of Life 26 instrument questionnaire (WHO-QOL26) [22], (b) the Center for Epidemiologic Studies Depression Scale (CESD) [23,24], (c) the General Health Questionnaire 30 (GHQ30) [25,26], (d) the Positive and Negative Affect Schedule (PANAS) [27,28], and (e) the Posttraumatic Growth Inventory (PTGI) [29,30]. We used the Japanese version of these psychological measures.

Saliva sampling

We collected saliva samples from participants to measure the salivary cortisol levels. Distressing psychological stimuli are associated with an increased cortisol level [1]. In consideration of the participants' circadian cortisol rhythms, we collected all saliva samples at 4:00 p.m. on weekdays both before and after the intervention. We selected 4:00 p.m. because humans are less affected by circadian cortisol rhythms at this time of day [31]. Participants were asked to refrain from drinking, eating [32], and exercising [33] for two hours before saliva sampling.

Measurement of salivary cortisol

To assess physiological stress, we used the same technique to measure salivary cortisol as described in a previous study [2]. Saliva samples were collected using the salivette apparatus (Sarstedt, Nümbrecht, Germany). We stored the supernatant solutions in airtight containers at -80°C and measured salivary cortisol using the solutions. We measured salivary cortisol with a semi-microcolumn high-performance liquid chromatography (HPLC) system (Shiseido, Tokyo).

ANALYTICAL METHODS

The psychological and salivary data were analyzed using the PASW statistical software package (ver. 18 for Windows; SPSS Inc., Chicago, IL, USA). Demographic and clinical data were subjected to the one-way analyses of variance. The one-way analyses of covariance were conducted with the differences between the pre- and post-intervention scores included as dependent variables and pretest scores as covariates of each psychological measurement. Because our primary endpoint of interest was the beneficial effect of intervention training, test-retest changes were compared between the intervention and control groups using one-tailed tests ($p < 0.05$), in the same manner as in previous studies [34].

Moreover, changes in the intervention group were confirmed by the analysis of variance of psychological measure scores and salivary cortisol level at three points (pre-intervention, post-intervention, and FU) using a mixed model. Changes in each measure over time in the intervention and control groups were then compared by means of analysis of variance using repeated measures.

RESULTS

A. Comparison of an intervention group with a control group (Pre vs. Post)

Psychological measures: The demographic and clinical data for the study participants are given in (Table 1). The participants age and Clinician-Administered PTSD Scale (CAPS) scores did not differ significantly between the intervention group and control group. Comparisons of the psychological changes before and after the intervention between the two groups are shown in (Table 2). The intervention group had a significant decrease in the post-intervention CAPS score ($F[1,51] = 13.526, p < 0.001$). The PTSD symptoms reduced more in the intervention group than in the control group. The intervention group also showed a significantly larger increase in the post-intervention Posttraumatic Growth Inventory (PTGI)-J total scores [$F(1,51) = 4.315, p < 0.05$] (Figure 2). The PTGI-J total score was significantly higher in the intervention group than in the control group, and the PTGI-J score improved more in the intervention group compared with the control group. Moreover, the intervention group showed a significantly larger pre- to post-intervention increase in the Positive and Negative Affect Schedule-Positive Affects (PANAS-PA) scores ($F[1,51] = 5.66, p < 0.05$). The PANAS-PA score was significantly higher in the intervention group compared to the control group, and the PA increased in the intervention group compared with the control group.

Salivary cortisol level: Comparisons of salivary cortisol level pre- and post-intervention are summarized in (Table 2). The intervention group had a significantly larger pre- to post-intervention decrease in salivary cortisol ($F[2, 52] = 14.077, p = 0.001$), indicating that stress was more effectively reduced in the intervention group compared with the control group.

B. Annual changes of an intervention group (The effectiveness of HT intervention)

Psychological measures: Changes in the various psychological measures of the intervention group are shown in (Table 3). Analysis of CAPS scores showed that the main effect of time (pre-intervention, post-intervention, and FU) was significant [$F(2,52) = 459.12, p < 0.001$]. Bonferroni's multiple comparison showed a significant decrease in the post-intervention score compared with the pre-intervention score ($p < 0.001$), with the FU score also significantly lower than the pre-intervention score ($p < 0.001$) and post-intervention score ($p < 0.05$), confirming that this effect was maintained (Figure 3).

The main effect of time was significant for PANAS-PA score ($F[2,52] = 8.40, p < 0.001$). Multiple comparisons showed that the post-intervention score was significantly lower than the pre-intervention score ($p < 0.05$), and that the FU score was also

Table 1: Baseline demographic and clinical data of the participants.

Factor	Intervention group		Control group		p^a
	Mean	SD	Mean	SD	
Age (years)	42.48	9.72	44.22	7.78	0.884
CAPS score	31.52	6.5	31.25	6.47	0.471

^aOne-way analysis of variance.

HT, horticultural therapy; SE, stress education; SD, standard deviation; CAPS, Clinician-Administered Post-Traumatic Stress Disorder Scale.

Table 2: Psychological measures pre- and post-intervention.

Measurements	Intervention group				Control group				p ^a
	Pre		Post		Pre		Post		
CAPS score	31.5	6.5	10.0	7.1	31.3	6.5	16.1	9.3	<0.001
WHO-QOL26 total score	52.5	5.8	53.9	6.5	52.3	6.3	51.0	5.9	0.297
CES-D score	13.4	7.1	11.8	7.4	14.6	6.9	12.5	5.2	0.934
GHQ score	7.1	5.1	4.4	4.2	6.8	5.7	5.0	4.7	0.248
PANAS positive affect	20.5	6.4	23.3	7.4	23.6	7.8	21.0	7.2	0.011
PANAS negative affect	18.9	7.7	15.1	6.0	21.8	7.1	18.5	6.4	0.071
PTGI total score	66.6	18.1	72.3	15.7	68.4	18.3	66.5	17.9	0.022
Salivary cortisol level	8.3	6.4	3.0	4.9	7.5	7.8	11.9	11.6	0.001

^aOne-way analyses of covariance with pre-post differences in psychological measures as dependent variables and pre-intervention scores as covariates (one-tailed). HT, horticultural therapy; SE, stress education; SD, standard deviation; CAPS, Clinician-Administered Post-Traumatic Stress Disorder Scale; WHO-QOL26, World Health Organization Quality of Life 26; CES-D, Center for Epidemiologic Studies Depression Scale; GHQ, General Health Questionnaire; PANAS, Positive and Negative Affect Schedule; PTGI, Posttraumatic Growth Inventory.

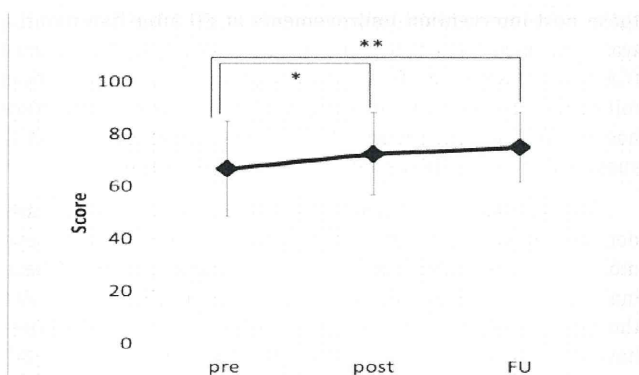


Figure 2 The change in PTGI-J score of the intervention group. *** p < .001, * p < .05.

Table 3: Psychological changes of Intervention group (Pre, Post, and FU).

Measurements	Pre		Post		FU	
	Mean	SD	Mean	SD	Mean	SD
CAPS score	31.5	6.5	10.0	7.1	8.7	6.2
WHO-QOL26 total score	52.5	5.8	53.9	6.5	54.2	6.3
CES-D score	13.4	7.1	11.8	7.4	12.0	6.8
GHQ score	7.1	5.1	4.4	4.2	4.3	4.3
PANAS positive affect	20.5	6.4	23.3	7.4	24.2	6.4
PANAS negative affect	18.9	7.7	15.1	6.0	15.2	5.9
PTGI total score	66.6	18.1	72.3	15.7	74.9	13.4
Salivary cortisol level	8.3	6.4	3.0	4.9	2.0	3.2

HT, horticultural therapy; SD, standard deviation; CAPS, Clinician-Administered Post-Traumatic Stress Disorder Scale; WHO-QOL26, World Health Organization Quality of Life 26; CES-D, Center for Epidemiologic Studies Depression Scale; GHQ, General Health Questionnaire; PANAS, Positive and Negative Affect Schedule; PTGI, Posttraumatic Growth Inventory.

significantly lower than the pre-intervention score ($p < 0.01$) and post-intervention score ($p < 0.01$), confirming that this effect was maintained (Figure 4). The main effect of time was significant for total PTGI score ($F[2,52] = 7.54, p < 0.001$). Bonferroni's

multiple comparison showed that the post-intervention score was significantly higher than the pre-intervention score ($p < 0.05$). The FU score was also significantly higher than the pre-intervention score ($p < 0.01$), confirming that this effect was maintained.

Salivary cortisol level: Changes in salivary cortisol level

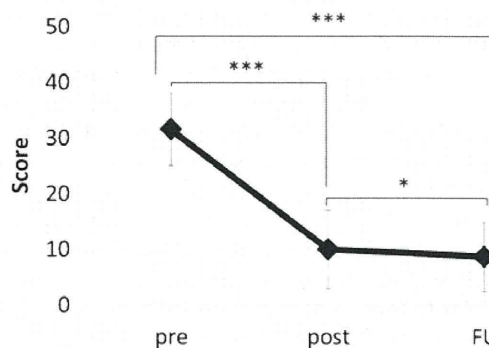


Figure 3 The change in CAPS score of the intervention group. ** p < .01, * p < .05.

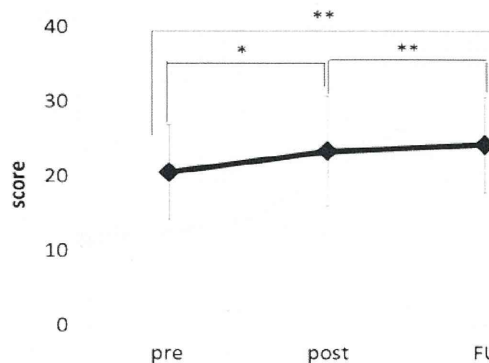


Figure 4 The change in PANAS-PA score of the intervention group. *** p < .001, * p < .05.

in the intervention group are shown in (Table 3) and (Figure 5). The main effect of time was significant for salivary cortisol ($F[2,52] = 25.88, p < 0.001$). Multiple comparisons showed that the post-intervention score was significantly lower than the pre-intervention score ($p < 0.001$), and the FU score was significantly lower than the pre-intervention score ($p < 0.001$).

DISCUSSION

The purpose of this study was to verify the reduction in the symptoms of earthquake-related stress in women who live in disaster areas using HT intervention, and to investigate the effect of HT on the symptoms of earthquake-related stress and the maintenance of its effect after a two-month follow-up period. The present study revealed that HT affected the psychological changes and salivary cortisol level in women with earthquake-related stress. These results are consistent with our hypothesis that HT may help women with earthquake-related stress improve their mental and physical functioning affected due to the traumatic experience.

Compared to the intervention group and control group, the intervention group showed improved CAPS scores, indicating that HT reduced PTSD symptoms. This finding extends the previous findings of the effect of HT on severe PTSD in males, by showing its efficacy with women with earthquake-related stress. In addition, the intervention group showed improved PTGI-J total scores and PANAS-PA scores, and reduced salivary cortisol levels after intervention compared with the control group, indicating that HT reduced stress levels. Findings related to salivary stress marker are consistent with previous research [15,35]. Previous studies reported that HT intervention for patients with depression improves mental health indices including the PANAS scores [36]. We believe that the CAPS and PANAS-PA scores of intervention group improved through stress reduction and elevated mood brought about by the HT [10-12]. About the result of PTGI, a study of PTG process suggests that people suffer emotional pain due to disruptions of their personal growth resulting from traumatic experiences [37]. However, people use PTG to cope in diverse ways, such as remembering their status before the event, referring to their own personality characteristics, relying on the support of others, and self-

disclosing their own experiences with the negative event [37,38]. Horticulture activity involves instinctive and creative action and leads to improvement of humanity [39]. PTG and horticulture share a key feature. We suggest that people feel their own growth overlap with the growth process of the plant while cultivating the plant. This could be considered a psychological effect because horticulture work offers fulfillment, pleasure, challenges, and a sense of accomplishment.

The salivary cortisol levels decreased after HT in this study. Cortisol is considered an indicator of psychological and physiological stress and can be used to examine the pathophysiology of PTSD [40]. Previous studies reported that people with severe PTSD due to the Hanshin-Awaji earthquake had significantly higher cortisol levels than unaffected individuals [3]; salivary cortisol levels were significantly decreased and the PANAS-PA scores were fully restored after horticultural activity [15]. We think that the improvement of the earthquake-related stress by HT was reflected in the endocrine system, specifically in salivary cortisol. In addition, the maintenance of these post-intervention improvements at FU after two months was confirmed from the results of the psychological measures (CAPS, PANAS-PA, and PTGI-J) and salivary cortisol levels after follow-up. These results indicate that horticultural intervention has an effect on earthquake-related stress, and that this effect is sustained for a certain period, i.e., two to four months.

The generally recommended treatments for PTSD that have demonstrated validity are cognitive behavioral therapy or eye movement desensitization and reprocessing (EMDR). These may also be accompanied by drug therapy in some cases. Art therapy, music therapy, and other complementary therapies have also been reported as effective. However, there is still little scientific evidence of the effectiveness of these methods for earthquake-related stress. The results of our present study suggest the possibility of HT as an effective intervention against the earthquake-related stress.

There are some limitations. The major limitation of this study was the small sample size. In the results of analysis of variance (ANOVA) for sensitivity, the effect size of this study was 0.39 and power was 0.8, and α err prob was 0.05. Therefore, a possible future direction would be to replicate and extend the results of current study with larger sample and a lighter (more casually controlled) trial design. Second limitation is the study participants were all women participants. Therefore, it is difficult to say whether a similar effect would be observed in male participants, if horticultural interventions were provided for men. We believe that further investigation is required on this issue. Third, although our study showed that coming into contact with plants through horticultural activities had a beneficial effect on earthquake-related stress, we are not able to consider which horticultural activities (e.g., touching the ground, planting a seed and a seedling) produce the strongest benefit. We intend to carry out further studies to explore this limitation immediately. The final limitation is the verification of the difference in the effect of the horticultural therapy by interpersonal relations (communication), such as group intervention and individual intervention. We are experimenting about this problem now. In the near future, we will report the results of this research.

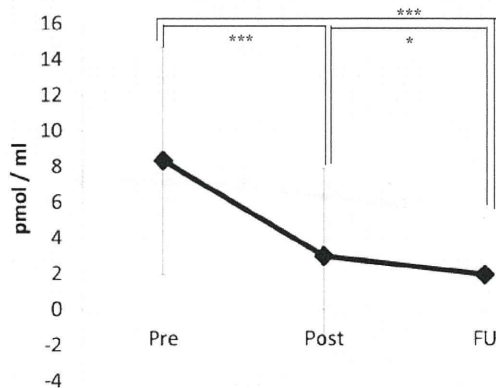


Figure 5 The change in salivary cortisol level of the intervention group. *** $p < 0.001$, * $p < .05$.

In conclusion, the present study suggested that, the present study suggested that it has been improved earthquake-related stress of women who live in disaster area by our HT intervention for two months from the result of a CAPS score, PANAS PA score, PTGI-J score, and a salivary cortisol level. Additionally, it suggested that these effects may sustain after intervention from the result of a psychological measures and a salivary cortisol level. We believe horticultural therapy may be able to suggest the possibility is one of the effective interventions for earthquake-related stress.

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Effects of the Higashi-Nihon Earthquake: Posttraumatic Stress, Psychological Changes, and Cortisol Levels of Survivors

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Abstract

On March 11, 2011, the Pacific side of Japan's northeast was devastated by an earthquake and tsunami. For years, many researchers have been working on ways of examining the psychological effects of earthquakes on survivors in disaster areas who have experienced aftershocks, catastrophic fires, and other damage caused by the earthquake. The goal of this study is to examine scores on psychological measures and salivary cortisol level in these individuals both before and three months after the earthquake. The participants had been measured for these variables before the earthquake. After the earthquake, we carried out PTSD screening using CAPS for participants for another experiment, and then again conducted the aforementioned tests. We collected saliva samples from all survivors. Our results show that social relationship scores on the WHO-QOL26, negative mood scores of the WHO-SUBI, total GHQ score, POMS confusion scores, and CMI emotional status score after the earthquake showed scores indicating significantly decreased compared to before the earthquake. On the other hand, salivary cortisol levels after the earthquake was significantly increased compared to before the earthquake. Moreover, the result of a multiple regression analysis found that negative mood score on the WHO-SUBI and social relationship score on the WHO-QOL26 were significantly related to salivary cortisol levels. Our results thus demonstrate that several psychological stress induced by the earthquake was associated with an increase in salivary cortisol levels. These results show similar findings to previous study. We anticipate that this study will provide a better understanding of posttraumatic responses in the early stages of adaptation to the trauma and expand effective prevention strategies and countermeasures for PTSD.

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Introduction

On March 11, 2011, at 2:46 pm, the Higashi-Nihon Earthquake (the Great East Japan Earthquake), the largest disaster to occur in Japan since World War II, hit three prefectures (Miyagi, Iwate, and Fukushima) on the Pacific side of northeastern Japan, with lesser damage in several other prefectures. These regions were severely damaged by the magnitude 9 earthquake and ensuing tsunami. The coastal communities of Kesennuma, Ishinomaki, and Minamisanriku were particularly devastated by the earthquake. More than 20,000 people were killed or went missing as a result of the tsunami and fires after the earthquake, and over 240,000 homes were damaged or destroyed. Immediately after an earthquake, people affected are damaged both physically and emotionally. Even now, the region is fighting intermittent aftershocks.

Over the past dozen years or so there have been several serious disaster events in Japan, including the Hokkaido Nansei-oki Earthquake of 1993, the Great Hanshin-Awaji earthquake of 1995, shocking cases such as the sarin gas attack on the Tokyo subway system in 1995, and so on. Many researchers have studied survivors' mental health and physical changes after such events

[1–6]. According to many previous studies of survivors of disaster areas, prevalence of posttraumatic stress disorder (PTSD) ranging from approximately 5 percent to 60 percent is seen in the first 1–2 years after a disaster [7,8]. Further, more than 60 percent of survivors of disasters are at high risk of PTSD [9]. Fujimori and Fujimori (1996) have researched the mental health of survivors of the Hokkaido Nansei-oki Earthquake and pointed out that survivors can be in critical psychological condition six months after such a disaster [6]. Thus, it has been pointed out that mental health problems of survivors are most evident a certain amount of time after a disaster [10].

Previous studies have reported that based on individual difference and the type of disaster, the rate of psychiatric disorders among survivors either decreased after the second year or was prolonged and became chronic [5,6,11–13]. There are growing concerns about the development of PTSD in people from the disaster-affected areas. In the Great Hanshin-Awaji Earthquake in 1995, many survivors were injured in mind and body and had mental health problems [9]. Even now, some survivors of this earthquake suffer some sort of trauma.

With regard to the relationship between stress and the body, Cannon (1929) and Selye (1956) provide the foundation for the

current interest in this physiological phenomenon [14–15]. In addition, McEwen and Stellar suggests that chronic stress responses involve actual physiological changes to body systems and organs, and considerable attention has been paid to acute physiological stress responses and how they might possibly lead to subsequent chronic stress responses [16]. Other previous studies suggest that PTSD is associated with behavioral and physiological pathology, which includes disruption of the hypothalamic–pituitary–adrenal (HPA) axis [15,17]. The HPA axis is involved in mediating physiological responses to stress and the secretion of the stress hormone cortisol [18]. Cortisol is considered an indicator of psychological and physiological stress and can be used in examining the pathophysiology of PTSD [19]. Some previous studies on cortisol data in PTSD report that cortisol level are high when people feel heavily stressed, a symptom of PTSD that can result from events including but not limited to earthquakes, war, accidents, abuse, or radioactive damage [20–22]. During the Hanshin-Awaji earthquake, people who had severe PTSD were found to have significantly higher cortisol levels [2]. Cortisol can be extracted from blood, urine, and saliva. Sampling by saliva collection has attracted attention for being a less stressful and invasive method for estimating cortisol levels than other methods of extraction [23–26]. Salivary cortisol level have been reported to reflect unbound forms of blood cortisol in particular, and a very high correlation has been reported between plasma and salivary cortisol levels [25–27]. To our knowledge, no study has yet examined psychological and physiological changes in survivors before and after the Great East Japan Earthquake.

In this study, we examined that these changes by collecting samples before the earthquake and three months after the earthquake. We hypothesized that psychological measures regarding the mental health of survivors, such as depression, anxiety would worsen and the saliva cortisol level of survivors would increase, three months after the earthquake, compared to before the earthquake. Our hypothesis was based on the previous studies mentioned above.

Methods

Ethics Statement

Written informed consent was obtained from each subject, in accordance with the Declaration of Helsinki (1991). This study was approved by the Ethics Committee of Tohoku University.

Participants

The study was performed three months after the Great East Japan Earthquake occurred. A total of 14 participants (men: 7; women: 7; age range: 19–26) were recruited from among undergraduate and postgraduate students at Tohoku University. All participants experienced the Great East Japan Earthquake. They live in Sendai and the surrounding areas, including the areas closest to the epicenter of the earthquake. All participants had participated in past psychological experiments and saliva cortisol experiments conducted in our laboratory, and had undergone psychological tests and saliva cortisol tests within the past six months and before the earthquake (experimental period: February 8 to March 7, 2011). All participants were screened for absence of neuropsychiatric disorders using the Mini-International Neuropsychiatric Interview (MINI) [28,29] and all participants were also interviewed by trained psychologists (initials: AO, NA, NS, and YW) using the Clinician-Administered PTSD Scale (CAPS) [30,31], a structured interview for screening for posttraumatic stress symptoms. CAPS generally requires that the interviewer undergo a psychiatric diagnostic interview. In accordance with the

MINI, no participant was diagnosed as having PTSD. Of the 14 participants, each filled more than one but not all the criteria of three clusters of PTSD symptoms, including re-experiencing of the event, avoidance, and hyperarousal.

Psychological Measures

To assess any change in quality of life, happiness, and mental health of participants from before to after the earthquake, we administered the following questionnaires to participants.

World Health Organization Quality of Life 26 (WHO-QOL26). The World Health Organization Quality of Life 26 (WHO-QOL26) is a 26-item self-report measure designed to assess quality of life (QOL). Twenty-four items measure the four domains of QOL—physical, psychological, social, and environmental—and the other two items measure overall QOL and general health. The score for each question ranges from 1 to 5; higher scores reflect higher QOL. This study used the Japanese version of the WHO-QOL26, which was created by Tasaki and Nakane (1997) [32].

World Health Organization Subjective Well-being Inventory (WHO-SUBI). The World Health Organization Subjective Well-being Inventory (WHO-SUBI) is a 41-item self-report measure designed to assess subjective well-being [33,34]. The score for each question ranges from 1 to 3. The WHO-SUBI measures two types of subjective well-being. One is “positive affect,” which is measured by the indices of good psychological health (19 items), while the other is “negative affect,” which is measured by the indices of poor psychological health (21 items). Thus, the test evaluates the positive and negative aspects of 11 factors: sense of satisfaction, sense of achievement, self-confidence, sense of happiness, support of close relatives, social support, family relationships, sense of spiritual control, sense of physical ill health, and dissatisfaction with social ties. The reliability and validity of the Japanese version have been demonstrated by Ono et al. [35,36].

General Health Questionnaire (GHQ30). The General Health Questionnaire (GHQ30) is self-report measure designed to assess underlying psychological distress. This questionnaire comprises 30 questions covering a range of neurotic symptoms, with an emphasis on those typical of anxiety and depression and with a deliberate avoidance of those that might also reflect physical illness [37]. The responses are made on a four-point ordinal scale. The response for each item is scored from 0 to 3 and then summed over the 30 items (range 0–90). This study used the Japanese version of the GHQ30, which was created by Nakagawa and Daibo (1996) [38].

Profile of Mood States (POMS). The Profile of Mood States (POMS) is a 65-item self-report measure designed to assess seven aspects of mood (anxiety/tension, depression/dejection, anger/hostility, confusion/bewilderment, vigor/activity, fatigue/inertia, and friendship). Factor analyses by the developers failed to confirm the friendship domain, and although the guidelines for administration that were followed in this trial continue to include the seven friendship items, they are no longer reported as a POMS subscale or included in the TMD score [39]. Responses to each item range from 0 to 4, with higher scores indicating a more negative mood. This study used the Japanese version of the POMS, which was created by Yokoyama and Araki (1994) [40].

Cornell Medical Index (CMI). The Cornell Medical Index (CMI) consists of 18 sections and 195 items. The A–L sections (144 items) represent physical state and the M–R sections (51 items) represent mental state. Participants answered “yes” or “no” to indicate the presence or absence of a symptom or disorder. If the answer was “yes,” it indicated that the patient had symptoms and

received a score of 2. On the other hand, a “no” answer indicated that the patient had no symptoms and was scored at one point [41]. This study used the Japanese version of the CMI, which was created by Kanehisa and Fukamachi (1972) [42].

Saliva sampling. We collected saliva samples from participants to measure salivary cortisol level. In consideration of the circadian cortisol rhythms of the participants, we collected saliva samples across the board at 5 pm on weekdays, both pre-examination (before the earthquake) and post-examinations (after the earthquake). The reason we selected 5 pm is because people at this time of day are less affected by circadian cortisol rhythms [43] and we wished to consider participants' experimental spread-over. We also ordered participants to refrain from drinking, eating [44] and exercise [45] for two hours before saliva sampling.

Sampling Strategy

Saliva samples were collected using the salivette (Sarstedt, Nümbrecht, Germany) and centrifuged at 3,000 rpm for 5 minutes. We stored the supernatant solution in an airtight container at minus 80 degrees and measured cortisol using the solution. We measured the cortisol with a semi-microcolumn High Performance Liquid Chromatography (HPLC) system (Shiseido, Tokyo). For reagents, we used cortisol and cortisone (Nacalai Tesque, Inc., Kyoto).

The acetonitrile and methanol used were those available on the market. As for the standard solution of CS and CZ, the CS and CZ were dissolved in methanol so that 0.1 mg/ml each (CS: 275.9 nmol/ml, CZ: 277.5 nmol/ml) was used as the original liquid; this was then diluted with 100% methanol or 10% methanol (for CS and CZ, respectively) to be the standard solution used. As for the semi-microcolumns, we used the Capcell Pak MF Ph-1 (4.6 micrometer i.d. times 50 mm) from Shiseido Co., Ltd. as the semi-microcolumn for the preprocessing column, Capcell Pak C18 UG120 (1.5 micrometer i.d. times 250 mm) of Shiseido Co., Ltd. as the semi-microcolumn for the analytical column, and Capcell Pak C18 UG120 (2.0 micrometer i.d. times 35 mm) of Shiseido Co., Ltd. as the semi-microcolumn for the concentrating column.

For HPLC analysis, we set the following conditions: the mobile phase for preprocessing used 5 millimoles per liter phosphoric acid buffer solution (pH = 6.9) and acetonitrile at a ratio of 98/2, and the solution was sent at the flow rate of 1 ml/min. The mobile phase for measurements used 10 millimoles per liter phosphoric acid buffer solution (pH = 6.9) and acetonitrile at a ratio of 78/22, and the solution was sent at the flow rate of 0.1 ml/min. The column temperature was kept constant at 35 degrees Celsius with the detection wavelength set at 242 nm. The duration of salivary cortisol levels was 50 min per sample.

Experimental Procedure

In this study, participants were measured within subject, pretest (before the earthquake) and posttest (after the earthquake). Both of these experiments followed the same procedure. Participants were tested in the lab of university at weekday afternoon. Participants received experimental suggestions from experimenter and were answered all psychological measures prepared by experimenters in the time given. Participants were allowed 60 min as response time to psychological measures. Then, participants took a saliva sampling at 5 pm. Saliva sampling time was for about five minute. Total experimental time was 65 minutes.

Statistical Analyses

The statistical analyses of psychological and salivary cortisol data between participants the high PTSD score group and the low PTSD score group were analyzed using the statistical software PASW Statistics 18 for Windows (SPSS Inc, Chicago). Pre- and post-levels of measured variables were analyzed by a paired t-test, and exacerbating factors in salivary cortisol were analyzed by multiple regression analysis. We set the significance level at $p < 0.05$.

Results

First, we examined sex differences in salivary cortisol level, because several previous studies have indicated that it shows a sex difference in stress response [46–48]. The result showed no significant differences between males and females (Table 1). We thought that males and females might not differ in the face of the statistics, so we put all of the data together and analyzed it.

Next, the comparison results for the data obtained from these tests are shown in Table 2. First, we examined changes in the scores of the psychological measurements before and after the earthquake. According to the results of the paired t-test, positive scores on the WHO-SUBI, negative scores on the WHO-SUBI, and the social relationship score of the WHO-QOL26 significantly increased (positive score of the WHO-SUBI: $t = 2.166$, degree of freedom [df] = 13, $p = 0.05$; negative score of the WHO-SUBI: $t = 3.183$, $df = 13$, $p = 0.01$; social relationship score of the WHO-QOL26: $t = 2.222$, $df = 13$, $p = 0.05$). Emotional status per the CMI and confusion score per the POMS also significantly increased (emotional status: $t = -2.471$, $df = 13$, $p < 0.05$; confusion score: $t = -3.633$, $df = 13$, $p < 0.01$).

Next, we measured cortisol levels from after the earthquake. The results of the comparison of salivary cortisol level before and after the earthquake are given in Table 2; they show that salivary cortisol level had increased by three months after the earthquake, a difference with statistical significance ($t = -2.745$, $df = 13$, $p < 0.05$).

To pinpoint the exacerbating factors in salivary cortisol level, a multiple regression analysis was conducted using the difference between salivary cortisol level before and after the earthquake as the objective variable. The CAPS score, positive score on the WHO-SUBI, negative score on the WHO-SUBI, and the social relationship score of the WHO-QOL26 were used as explanatory variables. The results of the multiple regression analysis are shown in Table 3. It was found that negative scores on the WHO-SUBI and the social relationship score of the WHO-QOL26 were factors significantly related to salivary cortisol level.

Discussion

To investigate psychological changes among the survivors in the disaster area before and three months after the earthquake, we

Table 1. Sex differences of the participants.

	Men (N=7)	Women (N=7)	P value
Before the earthquake (mean±SD)	4.3529±2.8122	4.153±2.8992	0.898 ^a
After the earthquake (mean±SD)	14.3546±12.6064	9.2219±5.5099	0.343 ^a

^a: an independent-samples t-test.

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Table 2. Characteristics of the participants.

Age [years], (mean±SD)	20.64±2.53		
CAPS Total score, (mean±SD)	17.07±9.38		
	Before the earthquake	After the earthquake	P value
The WHO-QOL26 Physical functioning, (mean±SD)	3.07±0.63	2.81±0.38	0.09 ^b
The WHO-QOL26 Psychological functioning, (mean±SD)	3.26±0.6	3.27±0.59	0.865 ^b
The WHO-QOL26 Social relationship, (mean±SD)	3.67±0.87	3.26±0.83	0.045^b
The WHO-QOL26 Environmental functioning, (mean±SD)	3.63±0.64	3.46±0.5	0.202 ^b
The WHO-QOL26 Global functioning, (mean±SD)	3.54±0.5	3.61±0.86	0.789 ^b
The WHO-SUBI positive score, (mean±SD)	41.43±7.71	37.5±5.68	0.05^b
The WHO-SUBI negative score, (mean±SD)	52.36±6.54	47.14±5.63	0.007^b
GHQ score, (mean±SD)	5.64±5.88	7.79±6.59	0.375 ^b
POMS Tension-Anxiety score, (mean±SD)	53.36±10.4	54.36±12.29	0.77 ^b
POMS Depression-Dejection score, (mean±SD)	51.71±10.58	46.0±17.47	0.381 ^b
POMS Anger-Hostility score, (mean±SD)	44.57±7.14	47.21±17.26	0.615 ^b
POMS Vigour-Activity score, (mean±SD)	50.86±7.42	47.86±15.33	0.577 ^b
POMS Fatigue-Inertia score, (mean±SD)	52.64±10.12	53.07±15.83	0.915 ^b
POMS Confusion score, (mean±SD)	51.0±9.54	65.14±13.92	0.003^b
POMS Total Mood Disturbance score, (mean±SD)	202.43±38.84	217.93±29.96	0.3 ^b
CMI somatic status score, (mean±SD)	14.57±8.67	18.21±15.74	0.278 ^b
CMI emotion status score, (mean±SD)	7.64±6.33	19.14±21.07	0.028^b
Salivary cortisol level, (mean±SD)	4.25±2.45	11.79±9.72	0.17 ^b

^b: Paired t-test.

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used psychological measures and salivary cortisol level. The results show that salivary cortisol levels increased after the earthquake. The exacerbating factors in this increase were negative scores on the WHO-SUBI and (higher) social relationship score of the WHO-QOL26. Our findings were consistent with the results of previous studies, such as those on the Hanshin-Awaji earthquake of 1995 and the Wenchuan earthquake of 2008 which were associated with higher cortisol level [2,4]. The relationship between higher cortisol levels and psychological stress suggests that high stress conditions induce an alteration in the HPA axis and stimulate the release of cortisol [49–51].

Some exacerbating factors in salivary cortisol level may have included anxiety as a result of the disruption of access to daily information, damage to homes or businesses, and lifeline damage such as interruption of water supply, gas supply, or electrical power supply. Another possible factor was logistical disruption and resultant shortages of food, drinking water, and gasoline. Many people who were in the disaster still spend their days feeling stress, fear, fatigue, helplessness, disappointment [11], and so on. It would appear that these factors still influence psychological responses to the disaster three months after the earthquake. Previous studies have suggested that psychological responses in times of disaster can be categorized into three steps: “Reactions,”

Table 3. Results of a multiple regression analysis of change in salivary cortisol levels before and after the earthquake.

Explanatory variable	Objective variable	
	Change in salivary cortisol levels before and after the earthquake	
	β	P
CAPS score	1.02	0.004
Positive score of the WHO-SUBI	−0.022	0.923
Negative score of the WHO-SUBI	0.717	0.021
Social relationship score of the WHO-QOL26	0.653	0.029
R ²	0.696	

*β: Standardized Coefficients.

R²: R Squar.

P: Significance probability.

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“Factors,” and “Psychodynamics” [52,53]. “Reactions” are the psychological states associated with the stress situation, “Factors” are the various kinds of circumstance said to contribute to the reactions observed, and “Psychodynamics” are the character and mode of operation of the alleged relationships between the normal or abnormal reactions observed and the factors contributing to them [52]. The current situation in the disaster area is in transition from Factors to Psychodynamics. There are cases of spontaneous psychological recovery; however, many survivors still have strong negative feelings such as depression, anger, and disconcertment around the earthquake. It would appear that living conditions changed dramatically soon after the earthquake disaster because of temporary living in evacuation centers, the inconvenience of getting things, lack of essential utilities, etc. Moreover, as people had to live in an environment that was complex and unfamiliar, it is considered that they felt more anxiety and fatigue than usual in their daily living and human relationship [54]. With these points in mind, we consider that the altered daily life environment and psychological stress caused by the disaster influenced an increase in salivary cortisol level. In summary, our study demonstrated that the severe psychological stress induced by the Great East Japan Earthquake was associated with salivary cortisol level and that psychological changes resulted from this earthquake, although the difference was not significant.

In the way of limitations of this study, four points should be noted. 1) The sample size of this study was very small. This is because the psychological and saliva cortisol data from before the earthquake were conducted for the purpose of collecting baseline data for other preliminary experiments, and the number of participants in the preliminary experiment was 14. This study compared the data of these 14 participants before the earthquake and three months after the earthquake. 2) We could not consider gender-differentiated salivary cortisol levels or psychological measures, because the numbers of men and women in participants was very small and because the salivary cortisol levels of participants showed no significant difference by gender. 3) Because this study compared data before the earthquake three months after the earthquake, we did not use new depression scales such as the Center for Epidemiologic Studies Depression scale (CES-D) or the Self-rating Depression Scale (SDS). 4) We did not check or consider coping behavior or resilience of survivors, for the same reason as in point 3.

In the future, we plan to examine the following three points: 1) First, we will examine change in activity of natural killer cells before and after the earthquake, because these cells are associated with cortisol levels and immune activity in stress conditions [55]. We already have blood samples from participants, obtained before

and three months after the earthquake. Therefore, we will immediately analyze the change in activity of natural killer cells in the blood. 2) Second, we will examine the same variables in the same participants six months, twelve months, or three years after the earthquake, as a follow-up study. Some previous studies about earthquakes have done follow-ups a few years later [56–59]. We consider it very important for the mental health of survivors to track psychological changes and salivary cortisol levels over time. 3) As an additional experiment, we are considering studying survivors living in the coastal area devastated by the earthquake. This study was conducted with survivors living in Sendai and the surrounding, areas which were less affected by the earthquake. The reason we were unable to study coastal survivors was that we did not have psychological and saliva data from them before the earthquake. However, time has passed since the Great East Japan Earthquake, and we think that it is important to conduct a longitudinal study of the mental health of survivors living in the area most severely affected by the earthquake. And not only that, we need to consider the importance of examining the new lifestyles and psychological support for survivors based on the outcomes of studies. To gather further data, we feel that a cohort study of these individuals is important; although we will not have data from before the earthquake to fall back on, we will be able to assess changes over time using the longitudinal cohort model. Therefore, we want to examine these issues after improvements of the methodology used in this study.

In conclusion, more than half a year after the earthquake, rebuilding efforts in the disaster area are very slow. Many survivors still have to confront painful situations such as life stress after the earthquake, disparity between survivors in the disaster area, employment problems, and the effect of radiation. Especially in the worst-affected coastal area, survivors have become extremely unstable. Our overarching mission is to assess about change over time in physical and mental health of many people damaged by earthquake using scientific methods.

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Author Contributions

Conceived and designed the experiments: YK RK. Performed the experiments: YK. Analyzed the data: YK. Contributed reagents/materials/analysis tools: YK. Wrote the paper: YK.

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