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# Heat-inactivated *Bacillus subtilis* subsp. *natto* strain QOL (QOL bacillus natto) improves negative mood states: A randomized, double-blind, and placebo-controlled parallel study

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**ABSTRACT**

**Background/Objectives:** Heat-inactivated *Bacillus subtilis* subsp. *natto* strain QOL (QOL bacillus natto) is a bacterial strain isolated from the fermented food natto and is produced through heat sterilization following pure culture. QOL bacillus natto has been reported to improve sleep quality in healthy adults, and to improve depression-like behavior caused by social defeat stress in animal models. However, there is a lack of research investigating the effects of QOL bacillus natto on psychological stress related to desk work. The purpose of this study was to evaluate the effects of QOL bacillus natto intake on psychological stress in healthy adults prone to fatigue and stress caused by daily desk work.

**Methods:** A randomized, double-blind, placebo-controlled, parallel-group study was conducted on 112 healthy adult males and females aged 24 to 89 years. Participants were randomly assigned to receive either a test food containing QOL bacillus natto (100 mg) or a placebo daily for 8 weeks. Participants were assessed at weeks 0 and 8 using the Profile of Mood States 2nd Edition (POMS2), the Ogu-ri-Shirakawa-Azumi Sleep Inventory MA version (OSA-MA), and stress intensity using visual analog scale (VAS).

**Results:** After 8 weeks of intervention, the Total Mood Disturbance (TMD) score in POMS2 for the QOL bacillus natto group was below the mean value, and significantly lower than that of the placebo group. Additionally, the Vigor-Activity

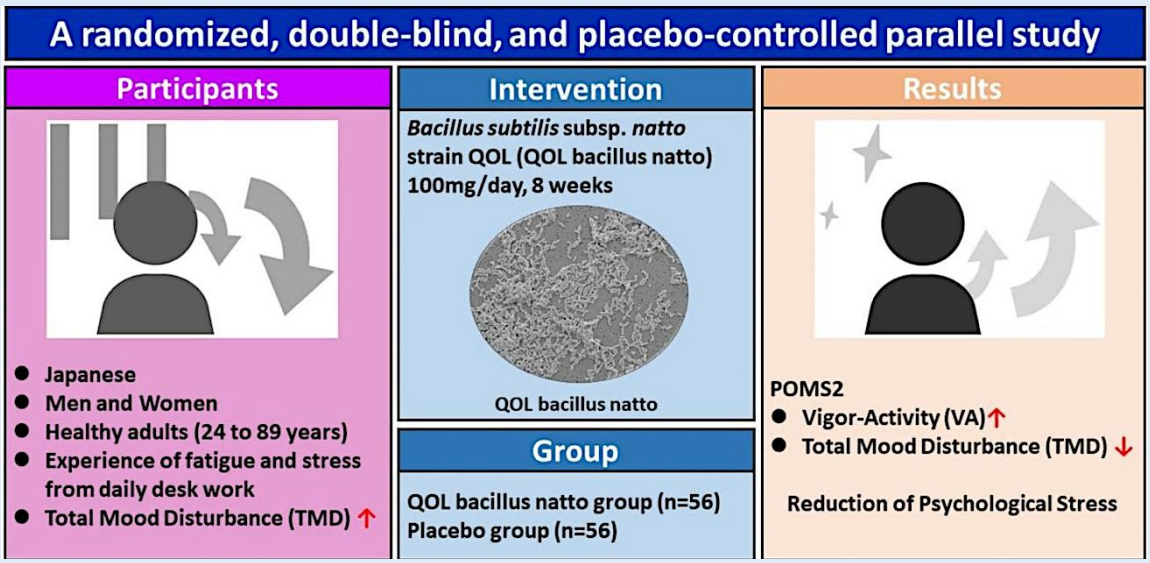
score in POMS2 for the QOL bacillus natto group was above the mean value, and significantly higher than that of the placebo group.

**Conclusions:** The results suggested that QOL bacillus natto intake enhances vigor and activity, improves negative mood states, and is effective in reducing psychological stress.

**Trial registration:** jRCT1030240202

**Keywords:** Bacillus subtilis; QOL bacillus natto; Postbiotics; Stress; Negative mood states; POMS

Graphical Abstract: QOL bacillus natto improves negative mood states






## INTRODUCTION

The gut-brain axis is a bidirectional pathway connecting the gut and brain via the vagus nerve, and has been shown to play a crucial role in maintaining gut homeostasis and brain function [1-3]. In recent years, postbiotics containing inactivated microbial cells have been reported to influence psychological stress responses via the gut-brain axis [4-6]. Postbiotics are defined as a "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host." Compared to probiotics, they have a lower risk of contamination and inactivation, making them easier to incorporated into a variety of food and drink products. Consequently, research in this field has been actively progressing [4, 7-8].

Heat-inactivated *Bacillus subtilis* subsp. *natto* strain QOL (QOL bacillus natto), produced through pure culture, heat sterilization, and powderization of a bacterial strain isolated from natto, a traditional Japanese fermented food, functions as postbiotics. QOL bacillus natto intake has been reported to enhance wake-up satisfaction and improve sleep quality in healthy adults [9]. It was also reported that QOL bacillus natto improved depression-like behavior in mice subjected to 10 days of social defeat stress [10]. These results suggest that QOL bacillus natto intake may help alleviate psychological stress via the gut-brain axis.

In recent years, work-related stress has increased exponentially worldwide, with many workers experiencing high levels of daily stress [11-12]. Work-related stress not only impairs everyday workplace performance but also worker health, potentially leading to mental disorders such as depression, anxiety, cognitive impairment, and obesity, all of which highlight a significant social issue [13-14]. Moreover, chronic psychological stress in today's high-stress society can lead to depression, profoundly diminishing an individual's quality of life [15]. Depression is a mental disorder characterized by decreased mood, loss of interest or pleasure, and increased fatigue. According to the World Health Organization (WHO), depression currently affects 350 million people worldwide [16] with a particularly significant impact on women, underlining a major social issue [17].

This study aimed to investigate the effects of continuous intake of QOL Bacillus natto for 8 weeks on reducing psychological stress. The participants were healthy adults prone to fatigue and stress caused by daily desk work and were confirmed not to have depression based on the results of the Beck Depression Inventory 2nd Edition (BDI-II). In this study, the primary outcome was the Total Mood Disturbance (TMD) score from the Profile of Mood States 2nd Edition (POMS2), measured at 8 weeks of intervention. Additionally, mood states were evaluated using POMS2, sleep quality was evaluated using the Ogu-ri-Shirakawa-Azumi Sleep Inventory MA version (OSA-MA), and stress intensity was measured using a visual analog scale (VAS).

## METHODS

**Study design:** This study used a randomized, double-blinded, placebo-controlled, parallel-group design with an intervention period of 8 weeks. Participants were randomly assigned to either the test food or placebo group in a 1:1 allocation ratio using a computer. Throughout the study, the principal investigator, participants, medical staff, and all other personnel involved in the study were blind to the treatment provided. The primary outcome was the TMD score, measured at 8 weeks. The secondary outcomes included the POMS2 scores (excluding TMD) at 8 weeks and their changes, the OSA-MA scores at 8 weeks and their changes, and the VAS scores for stress intensity at 8 weeks and their changes. This study was conducted at Medical Corporation Seishinkai, Takara Clinic (Tokyo, Japan) from July 2024 to February 2025 by a contract research organization ORTHOMEDICO Inc. (Tokyo, Japan).

**Ethics Statement:** The study complied with the Declaration of Helsinki (2013) and the Ethical Guidelines for Medical Research Involving Human Subjects. The study was approved by the Ethics Committee of Medical Corporation Seishinkai, Takara Clinic (Tokyo, Japan) under approval number 2406-04426-0052-27-TC and registered with the Japan Registry of Clinical Trials (jRCT1030240202).

**Participants:** The participants were healthy Japanese adult men and women who had received a prior explanation of the study, demonstrated comprehension of its contents, and provided written consent to participate. In addition, the participants experienced fatigue and stress from their daily desk work, and their TMD scores before the intervention were relatively high. Participant eligibility for the study was determined by the principal investigator based on their Beck Depression Inventory-II (BDI-II) results. The exclusion criteria were recorded in the Japan Registry of Clinical Trials (jRCT1030240202).

**Intervention:** The intervention was two foods: a test food and a control food (placebo), and participants took one capsule daily after breakfast. The test food contained 100 mg of QOL bacillus natto (inactivated bacteria: 10 billion cells; Ikeda Tohka Industries Co., Ltd., Hiroshima, Japan) per capsule, while the placebo contained 100 mg of

dextrin per capsule. The intervention was encapsulated in brown No. 2 capsules, rendering them indistinguishable in appearance, shape, color, odor, and taste. The capsules were provided by Ikeda Food Research Co., Ltd. (Hiroshima, Japan).

**POMS2:** The POMS2 was used to assess psychological state at pre-intervention (baseline) and at 8 weeks of intervention. The POMS2 consisted of 65 questions, requiring participants to assess their current mood on a 5-point scale. Scores were standardized with a mean of 50 and a standard deviation of 10, and categorized into seven factors: Anger-Hostility (AH), Confusion-Bewilderment (CB), Depression-Dejection (DD), Fatigue-Inertia (FI), Tension-Anxiety (TA), Vigor-Activity (VA), and Friendliness (F). In addition, the TMD score (TMD = AH + CB + DD + FI + TA - VA) was calculated based on the results of the six factors. High scores on AH, CB, DD, FI, TA, and TMD reflect negative mood states, whereas high scores on VA and F reflect positive mood states.

**OSA-MA:** The OSA-MA was used to assess sleep quality at baseline and at 8 weeks of intervention. The OSA-MA consisted of 16 questions, requiring participants to assess their sleep state on a 4-point scale immediately upon awakening. The average values over three days (the day of the test, the day before, and two days prior) were categorized into five factors: (1) sleepiness upon waking, (2) initiation and maintenance of sleep, (3) frequent dreaming, (4) refreshing on rising, and (5) sleep length, to evaluate sleep quality.

**VAS:** The VAS was used to assess subjective stress intensity at baseline and at 8 weeks of intervention. Participants were asked to mark their current level of stress on a 100 mm line, where 0 (far left) represented no stress and 100 (far right) represented maximum stress. The participant's stress intensity can be evaluated based on the position of the mark on the scale.

**Safety Evaluation:** At both baseline and 8 weeks, physical measurements, physical examinations, urine parameters, and blood parameters were assessed. Physical measurements included height, weight, and body mass index (BMI), while physical examinations included systolic and diastolic blood pressure. Urine parameters included protein, glucose, occult blood, and pH levels. Blood parameters included white blood cell count, red blood cell count, hemoglobin, hematocrit, platelet count, aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, total bilirubin, total protein, urea nitrogen, creatinine, uric acid, sodium, potassium, chlorine, serum amylase, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, glucose, and hemoglobin A1c. Urine parameters and blood parameters were measured by the LSI Medience Corporation (Tokyo, Japan).

**Statistical Analysis:** All statistical analyses were performed using two-sided tests, and the significance level was set at 5%, conducted with IBM SPSS statistics software (version 23 and higher; IBM Japan, Ltd., Tokyo, Japan). Outcomes were presented as mean and standard deviation (SD) for baseline data, and as estimated marginal means (EMM) and standard error (SE) for data at 8 weeks. Statistical analyses of outcomes were performed using Welch's t-test for comparisons between groups at baseline, and analysis of covariance (ANCOVA) with baseline as a covariate for comparisons between groups at 8 weeks. The incidence rates of adverse effects and adverse events were aggregated by group, and 95% confidence intervals were calculated for incidence rates within each group and for differences in incidence rates between groups. The proportion of cases in which urinary and blood parameters deviated from reference values at 8 weeks was also calculated. Statistical analyses were conducted using Fisher's exact test, the chi-square test, and other methods.

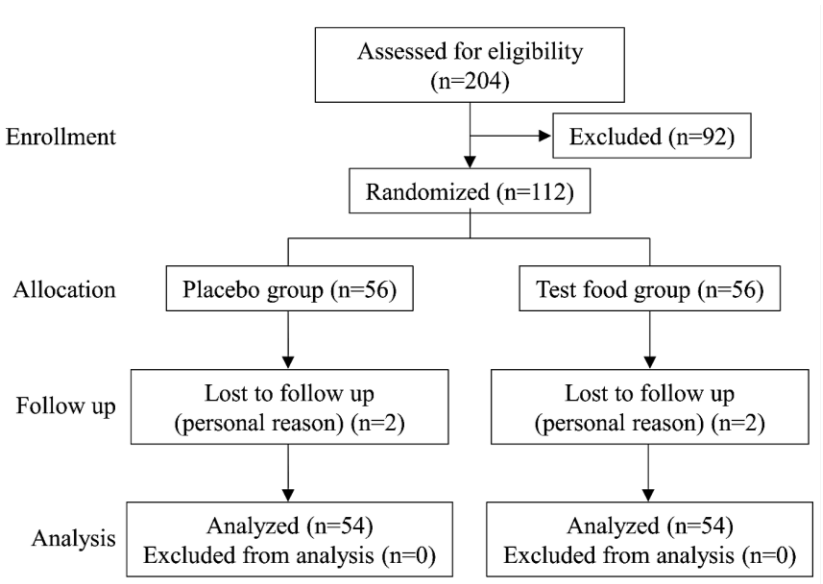



**Figure 1**. Flow of the participants.

## RESULTS

**Participants:** The flow of participants is shown in Figure 1. Among the 204 individuals who consented to participate in the study, 112 participants meeting the inclusion criteria and without any exclusion criteria were enrolled. Participants were randomly assigned to either the test food group or the placebo group, with 56 participants in each group. 4 participants who did not receive the intervention were excluded from the analysis. The efficacy assessment analysis dataset was defined as the Full Analysis Set (FAS), consisting of 108 participants (54 in the test food group and 54 in the placebo group). The safety assessment analysis dataset was defined as the Safety Analysis Population (SAF), and the participants were the same as those in the FAS. The background characteristics of the participants are shown in Table 1. No significant differences were observed in the participant background characteristics.

**Table 1.** Background characteristics of the participants. BMI: Body Mass Index, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, BDI- II: Beck Depression Inventory-Second Edition, POMS2: Profile of Mood States 2nd Edition, TMD: Total Mood Disturbance.

| | Placebo Group (n=54) | | Test food Group (n=54) | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| Sex (n, Male/Female) | 17/37 | | 15/39 | |
| Age (years) | 50.8 | 12.7 | 52.2 | 6.5 |
| Height (cm) | 160.4 | 7.8 | 162.4 | 6.2 |
| Weight (cm) | 56.0 | 12.7 | 59.3 | 9.8 |
| BMI (kg/m2) | 21.6 | 3.5 | 22.4 | 3.1 |
| SBP (mmHg) | 116.9 | 16.4 | 114.5 | 13.7 |
| DBP (mmHg) | 76.7 | 12.9 | 75.7 | 10.3 |
| BDI-II score | 14.1 | 5.4 | 13.6 | 5.2 |
| POMS2 TMD | 53.4 | 6.2 | 53.1 | 6.4 |

**POMS2:** The results of POMS 2 are shown in Table 2. The primary outcome, the TMD score at 8 weeks of intervention, was significantly lower in the test food group compared to the placebo group ($p = 0.046$). The change in TMD score from baseline to 8 weeks was also significantly lower in the test food group compared to the

placebo group (p = 0.046). Additionally, the VA score at 8 weeks of intervention and the change from baseline to 8 weeks were significantly higher in the test food group compared to the placebo group (p = 0.031 for both).

**OSA-MA:** The results of OSA-MA are shown in Table 3. There were no significant differences observed between groups across the five factors. However, the frequent dreaming score at 8 weeks of intervention showed a tendency to be higher in the test food group compared to the placebo group (p = 0.072).

**VAS:** There were no significant differences observed between groups in the VAS assessment of subjective stress intensity.

**Safety:** Under the conditions of this study, no adverse events were observed during the study period. At 8 weeks of intervention, some urinary and blood parameters deviated from the reference range; however, no significant differences were observed between the groups (Table 4). The principal investigator reviewed the deviations and confirmed that the intervention did not result in any medically concerning variations.

**Table 2.** Results of the POMS 2.

| | | Placebo group (n=54) | | | | Test food group (n=54) | | | | *p* value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | EMM | SE | Mean | SD | EMM | SE | |
| **TMD** | Pre | 53.4 | 6.2 | - | - | 53.1 | 6.4 | - | - | 0.819 |
| | Post | 51.0 | 8.4 | 50.9 | 0.8 | 48.5 | 7.3 | 48.6 | 0.8 | 0.046 |
| | Change | −2.4 | 5.2 | −2.4 | 0.8 | −4.6 | 6.2 | −4.6 | 0.8 | 0.046 |
| **AH** | Pre | 50.2 | 8.4 | - | - | 51.2 | 9.0 | - | - | 0.539 |
| | Post | 49.1 | 9.6 | 49.5 | 0.9 | 48.9 | 8.3 | 48.6 | 0.9 | 0.452 |
| | Change | −1.0 | 6.9 | −1.2 | 0.9 | −2.3 | 7.1 | −2.1 | 0.9 | 0.452 |
| **CB** | Pre | 53.7 | 8.0 | - | - | 53.7 | 7.7 | - | - | 0.980 |
| | Post | 51.4 | 8.4 | 51.4 | 0.8 | 49.4 | 8.9 | 49.4 | 0.8 | 0.097 |
| | Change | −2.3 | 5.8 | −2.3 | 0.8 | −4.3 | 6.5 | −4.3 | 0.8 | 0.097 |
| **DD** | Pre | 52.0 | 7.4 | - | - | 50.4 | 6.6 | - | - | 0.225 |
| | Post | 51.1 | 9.0 | 50.5 | 0.8 | 48.6 | 6.7 | 49.2 | 0.8 | 0.256 |
| | Change | −0.9 | 6.2 | −0.7 | 0.8 | −1.8 | 5.4 | −2.0 | 0.8 | 0.256 |
| **FI** | Pre | 54.4 | 8.8 | - | - | 53.7 | 7.8 | - | - | 0.662 |
| | Post | 50.6 | 8.9 | 50.3 | 0.8 | 50.0 | 8.5 | 50.2 | 0.8 | 0.962 |
| | Change | −3.8 | 6.0 | −3.7 | 0.8 | −3.7 | 7.1 | −3.8 | 0.8 | 0.962 |
| **TA** | Pre | 53.6 | 7.4 | - | - | 55.3 | 8.4 | - | - | 0.263 |
| | Post | 51.9 | 8.6 | 52.3 | 1.0 | 50.2 | 8.3 | 49.7 | 1.0 | 0.062 |
| | Change | −1.7 | 7.2 | −2.1 | 1.0 | −5.1 | 8.8 | −4.8 | 1.0 | 0.062 |
| **VA** | Pre | 46.4 | 8.6 | - | - | 48.2 | 8.4 | - | - | 0.285 |
| | Post | 48.9 | 9.0 | 49.6 | 0.9 | 53.1 | 9.3 | 52.5 | 0.9 | 0.031 |
| | Change | 2.5 | 7.1 | 2.3 | 0.9 | 4.9 | 7.4 | 5.2 | 0.9 | 0.031 |
| **F** | Pre | 50.1 | 8.2 | - | - | 52.4 | 8.4 | - | - | 0.153 |
| | Post | 51.3 | 10.0 | 52.2 | 1.0 | 55.5 | 9.3 | 54.6 | 1.0 | 0.092 |
| | Change | 1.2 | 7.7 | 1.0 | 1.0 | 3.1 | 7.2 | 3.4 | 1.0 | 0.092 |

Pre: pre-intervention (baseline), Post: 8 weeks of intervention, Change: change score from baseline to 8 weeks, EMM: Estimated Marginal Means, POMS2: Profile of Mood States 2nd Edition, TMD: Total Mood Disturbance, AH: Anger–Hostility, CB: Confusion Bewilderment, DD: Depression–Dejection, FI: Fatigue–Inertia, TA: Tension–Anxiety, VA: Vigor–Activity, F: Friendliness, p value: intergroup comparison with Welch's t-test of baseline data (Pre), and analysis of covariance (ANCOVA) with baseline as a covariate and for comparisons between groups at 8 weeks data (Post, Change).

**Table 3.** Results of the OSA-MA.

| | | Placebo group (n=54) | | | | Test food group (n=54) | | | | *p* value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | EMM | SE | Mean | SD | EMM | SE | |
| **Sleepiness on rising** | Pre | 13.7 | 4.5 | - | - | 15.1 | 4.3 | - | - | 0.097 |
| | Post | 17.8 | 4.8 | 18.1 | 0.6 | 18.3 | 5.0 | 18.0 | 0.6 | 0.952 |
| **Initiation and maintenance of sleep** | Pre | 13.7 | 4.4 | - | - | 15.2 | 4.9 | - | - | 0.101 |
| | Post | 17.4 | 5.1 | 17.6 | 0.7 | 17.6 | 5.0 | 17.4 | 0.7 | 0.819 |
| **Frequent dreaming** | Pre | 21.4 | 7.1 | - | - | 21.1 | 7.3 | - | - | 0.832 |
| | Post | 21.3 | 7.0 | 21.3 | 0.8 | 23.2 | 6.6 | 23.3 | | 0.072 |
| **Refreshing on rising** | Pre | 13.0 | 4.4 | - | - | 14.1 | 4.1 | - | - | 0.164 |
| | Post | 17.6 | 5.3 | 17.7 | 0.7 | 17.2 | 4.4 | 17.1 | | 0.557 |
| **Sleep length** | Pre | 15.7 | 5.3 | - | - | 16.1 | 4.4 | - | - | 0.717 |
| | Post | 19.8 | 4.9 | 19.8 | 0.6 | 19.3 | 5.3 | 19.3 | | 0.522 |

Pre: pre-intervention (baseline), Post: 8 weeks of intervention, Change: change score from baseline to 8 weeks, EMM: Estimated Marginal Means, p value: Inter-group comparison with Welch's t-test of baseline data (Pre), and analysis of covariance (ANCOVA) with baseline as a covariate and for comparisons between groups at 8 weeks data (Post, Change).

**Table 4.** Urine parameters and blood parameters after the intervention.

| | Placebo Group (n=54) | | Test food Group (n=54) | | *p* value |
|---|---|---|---|---|---|
| | Number | Rate (%) | Number | Rate (%) | |
| **Quantitative urinary protein** | 2 | 3.7 | 3 | 5.6 | 0.647 |
| **Quantitative urinary sugar** | 0 | 0.0 | 0 | 0.0 | NA |
| **Urinary pH** | 1 | 1.9 | 0 | 0.0 | 0.315 |
| **Urinary occult blood** | 3 | 5.6 | 1 | 1.9 | 0.308 |
| **White blood cell count (WBC)** | 4 | 7.4 | 1 | 1.9 | 0.169 |
| **Red blood cell count (RBC)** | 0 | 0.0 | 0 | 0.0 | NA |
| **Hemoglobin (Hb)** | 1 | 1.9 | 2 | 3.7 | 0.558 |
| **Hematocrit value (Ht)** | 2 | 3.7 | 5 | 9.3 | 0.241 |
| **Platelet count (PLT)** | 3 | 5.6 | 3 | 5.6 | 1.000 |
| **Aspartate aminotransferase (AST)** | 1 | 1.9 | 0 | 0.0 | 0.315 |
| **Alanine aminotransferase (ALT)** | 1 | 1.9 | 1 | 1.9 | 1.000 |
| **γ-Glutamyltransferase (γ-GT)** | 4 | 7.4 | 2 | 3.7 | 0.401 |
| **Total bilirubin (T-BIL)** | 0 | 0.0 | 0 | 0.0 | NA |
| **Total protein (TP)** | 4 | 7.4 | 2 | 3.7 | 0.401 |
| **Urea nitrogen (UN)** | 3 | 5.6 | 4 | 7.4 | 0.696 |
| **Creatinine (CRE)** | 3 | 5.6 | 3 | 5.6 | 1.000 |
| **Uric acid (UA)** | 1 | 1.9 | 0 | 0.0 | 0.315 |
| **Sodium (Na)** | 2 | 3.7 | 0 | 0.0 | 0.153 |
| **Potassium (K)** | 1 | 1.9 | 5 | 9.3 | 0.093 |
| **Chlorine (Cl)** | 0 | 0.0 | 1 | 1.9 | 0.315 |
| **Serum amylase (AMY/S)** | 2 | 3.7 | 3 | 5.6 | 0.647 |
| **Total cholesterol (T-Cho)** | 4 | 7.4 | 11 | 20.4 | 0.051 |
| **HDL- cholesterol (HDL-Cho)** | 1 | 1.9 | 0 | 0.0 | 0.315 |
| **LDL-cholesterol (LDL-Cho)** | 4 | 7.4 | 4 | 7.4 | 1.000 |
| **Triglycerides (TG)** | 5 | 9.3 | 3 | 5.6 | 0.462 |

| | Placebo Group (n=54) | | Test food Group (n=54) | | *p* value |
|---|---|---|---|---|---|
| | Number | Rate (%) | Number | Rate (%) | |
| **Glucose (GLU)** | 1 | 1.9 | 3 | 5.6 | 0.308 |
| **Hemoglobin A1c (HbA1c: NGSP)** | 0 | 0.0 | 0 | 0.0 | NA |

Number: The number of adverse events, Rate: The rate of adverse events, NA: Not Available, p value: Asymptotic significance obtained using the chi-square test.

## DISCUSSION

In this study, we evaluated the effects of QOL bacillus natto on psychological stress in healthy adults who experienced fatigue and stress from daily desk work, using the TMD score of POMS2 as the primary outcome measure at 8 weeks of intervention. The POMS2, used to assess psychological stress, is a test that can swiftly assess not only relatively long-lasting emotional states but also fluctuating transient emotions. It comprises seven factors (AH, CB, DD, FI, TA, VA, F) and a comprehensive factor known as TMD. The scores for TMD, AH, CB, DD, FI, and TA, which represent negative mood states, indicate a more favorable condition when lower, whereas the scores for VA and F, which represent positive mood states, indicate a more favorable condition when higher [18, 19]. The participants in this study consisted of 112 individuals whose baseline scores for TMD, AH, CB, DD, FI, and TA were above the mean value, while their VA score was below the mean value. This group was considered to exhibit relatively strong negative moods and weaker positive moods. At 8 weeks, the test food group showed significantly lower TMD scores than the placebo group, both for absolute scores and change in scores from baseline ($p = 0.046$; Table 2). In addition, although there was no statistically significant difference, the AH, CB, and DD scores of the test food group at 8 weeks were found to fall below the mean value (Table 2). The test food group at 8 weeks also displayed significantly higher VA scores than the placebo group, having both higher absolute scores and changes in scores from baseline ($p = 0.031$; Table 2). Collectively, these results suggest that continuous intake of QOL bacillus natto may help alleviate psychological stress by reducing negative moods such as anxiety, depression and anger, while also enhancing vitality and promoting activity.

In recent years, the intake of probiotics has been reported to influence stress responses via the gut-brain axis by alleviating psychological stress [20-24]. This mechanism is thought to involve the secretion of serotonin by enterochromaffin (EC) cells, a type of endocrine cell. Serotonin secreted by EC cells activates the afferent fibers of the vagus nerve, transmitting signals to the nucleus of the solitary tract in the brainstem [25]. Subsequently, the nucleus of the solitary tract is thought to integrate serotonergic signals and project to higher brain regions involved in autonomic regulation and mood [25]. Therefore, the intake of components that promote serotonin secretion by EC cells is expected to alleviate psychological stress via vagus nerve activation. In addition to probiotics, postbiotics are also considered to promote serotonin secretion by EC cells [4]. Potential mechanisms for the mediation of health effects by postbiotics, which contain inactivated microbial cells, are thought to be similar to those of probiotics [4, 26]. For example, postbiotics containing heat-inactivated *Lactobacillus helveticus* CP790 are suggested to improve overall mood, expressed as total mood disturbance, and reduce depression–dejection scores [6]. Similarly, the continuous intake of QOL Bacillus natto, a postbiotic, has been reported to improve depression-like behavior observed in socially defeated model mice [10]. These findings suggest that postbiotics, like probiotics, may influence stress responses via the gut-brain axis. Therefore, QOL Bacillus natto may have alleviated psychological stress through this mechanism.

If the intake of QOL bacillus natto alleviates psychological stress via the gut-brain axis, it is inferred

that it may influence sleep by modulating the autonomic nervous system through the same pathway. A previous study conducted on healthy individuals experiencing sleep issues reported that continuous intake of QOL bacillus natto at a higher dosage (200 mg/day) than in this study improved sleep quality, specifically sleepiness upon waking, as assessed by the OSA-MA [9]. Similar results were expected in this study; however, no significant difference in sleepiness upon waking was observed. However, the frequent dreaming score of the test food group at 8 weeks exhibited a tendency toward higher values compared to the placebo group ($p = 0.072$; Table 3). The frequent dreaming score was assessed based on two questions: 'Frequent nightmares vs. no nightmares' and 'frequent dreaming vs. no dreaming.' Previous reports indicate that individuals experiencing frequent nightmares tend to have poor sleep quality due to excessive stress, and that frequent dreaming, as a form of sleep introspection, is commonly associated with shallow sleep and insufficient rest [27]. Sleep is influenced not only by psychological stress but also by physical stressors such as light and noise [28, 29]. One of the responses to these stressors is REM sleep rebound, a temporary increase in REM sleep that is thought to induce dream recall, including nightmares [30, 31]. Therefore, reducing stress is thought to decrease dream recall. Indeed, a study investigating dream recall among healthy adults upon waking in sleep-deprived and recovery sleep conditions reported that dream recall decreased during recovery sleep, associated with an increase in deep sleep and a reduction in REM sleep duration [32]. Based on these reports, the reduction of stress and depression is suggested to not only decrease the severity of nightmares but also reduce dream recall, potentially contributing to the normalization of sleep patterns and enhancing the overall sense of sleep satisfaction. Therefore, the tendency for improvement in frequent dreaming observed in this study may be attributed to the stress-reducing effects of QOL bacillus natto intake.

A limitation of this study is that it did not consider factors such as the menstrual cycle and premenstrual syndrome (PMS), which are thought to influence psychological stress in women. It has been reported that there are no significant differences in POMS2 scores between women with and without PMS during the follicular phase; however, during the luteal phase, women with PMS exhibit significantly higher TMD and DD scores [33]. Women with PMS tend to experience intensified depressive symptoms during the luteal phase, which are believed to be reflected in an overall negative mood state. In this study, 70% of the participants were women, and no PMS-specific interviews were conducted, making it possible that women with PMS were included. This study confirmed that continuous intake of QOL bacillus natto reduces psychological stress, which may have a positive impact on sleep quality. However, to strengthen the reliability of this evidence, further studies that consider the menstrual cycle are necessary.

## CONCLUSION

In conclusion, the intake of QOL bacillus natto improved negative mood states in healthy adults prone to fatigue and stress caused by daily desk work. The results of this study suggest that QOL bacillus natto enhances vigor and activity, improves negative mood states, and effectively reduces or alleviates psychological stress. No adverse events were observed under the conditions of this study, and there were no safety issues.

**List of Abbreviations:** QOL bacillus natto, Bacillus subtilis subsp. natto strain QOL; WHO, World Health Organization; BDI-II , Beck Depression Inventory 2nd Edition; TMD, Total Mood Disturbance; POMS2, Profile of Mood States 2nd Edition; OSA-MA, Ogu-ri-Shirakawa-Azumi Sleep Inventory MA version; VAS, visual analog scale; AH, Anger-Hostility; CB, Confusion-Bewilderment; DD, Depression-Dejection; FI, Fatigue-Inertia; TA, Tension-Anxiety; VA, Vigor-Activity; F, Friendliness; BMI, body mass index; SD, standard deviation; EMM, estimated marginal means; SE, standard error; FAS, Full Analysis Set; SAF, Safety Analysis Population; EC, enterochromaffin; PMS, premenstrual syndrome.

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