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ORIGINAL CONTRIBUTION

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Pre-germinated brown rice could enhance maternal mental health and immunity during lactation

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Abstract *Background* Rice is a dietary staple worldwide, especially pre-germinated brown rice has recently been widely served in Japan because of its abundant nutrition. Relationship between lactation and pre-germinated brown rice has attracted interest in terms of mental health and immunity. *Aim of the study* To demonstrate that Japanese foods are beneficial for psychosomatic health, the effects of pre-germinated brown rice on the mental status and immunological features during lactation were investigated. *Methods* Forty-one breast-feeding mothers were recruited, and randomly divided into two groups. One group took pre-germinated brown rice and the other white rice (control) as their staple diet for 2 weeks. The Profile of Mood States (POMS) and salivary amylase activity as psychological indices and secretory IgA (s-IgA) and lactoferrin (LTF) in breast milk as immunological indices were determined before and after dietary intervention, and changes were investigated. *Results* In the psychological assessment, the scores of depression, anger-hostility, and fatigue were decreased on POMS analysis in the pre-germinated brown rice diet group, resulting in a significant decrease in total mood disturbance (TMD). The salivary amylase activity measurement suggested that resistance to stress was increased in the pre-germinated brown rice diet group. On the immunological assessment, the s-IgA level was significantly increased in the pre-germinated brown rice diet group. *Conclusion* We have shown that pre-germinated brown rice may have beneficial effects on psychosomatic health.

Key words pre-germinated brown rice – mental health – POMS – salivary amylase – immunity – secretory immunoglobulin A – Lactoferrin

Introduction

Dietary components influence the physiological and psychosomatic state by means of modifying the various regulatory molecules in the neuro-endocrine-immune system [15]. Recent studies have reported that mental health is associated with dietary habits [18], and maternal diets during pregnancy and lactation are associated with allergic manifestations in infants [3, 4, 8, 22]. One of the studies reported that the beneficial effects of maternal antigen avoidance were mainly observed in breast-fed infants [2]. Several molecules play immunological roles in milk. Secretory immunoglobulin A (s-IgA) and lactoferrin

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Table 1 General nutrients of pre-germinated brown rice and white rice

Nutrient	Pre-germinated brown rice	White rice
Energy (kcal/100g)	350	356
Protein (g/100g)	6.8	6.1
Fat (g/100g)	2.9	0.9
Carbohydrate (g/100g)	72.5	77.1
Fiber (g/100g)	3.0	0.5

Composition data of pre-germinated brown rice and white rice are quoted from the technical data of FANCL Corp. and the standard table of food composition in Japan (fifth revised and enlarged edition) issued by MEXT, respectively

(LTF) exert protective effects against microbial and viral infection [14, 23]. Especially, an alternative effect of the former molecule is related to the exclusion of foreign food antigens at the intestinal surface of breast-fed infants [13].

Pre-germinated brown rice has been developed to enhance the nutritional value of brown rice, manufactured by soaking brown rice in water to induce slight germination according to a patented method (JP3738025), and become popular because of its nutritional functions and taste. The general nutritional contents of pre-germinated brown rice and white rice are shown in Table 1. Many physiological effects of pre-germinated brown rice have been reported. Pre-germinated brown rice includes substantially higher content of dietary fiber and exhibits the suppression of an increase in postprandial blood glucose [12, 19] and plasminogen activator inhibitor [7] in diabetes, the inhibition of cancer cell proliferation [17], and the prevention of Alzheimer's disease [16].

In this study, we examined the effects of pre-germinated brown rice on maternal mental health status and immunological features during lactation.

Methods

Subjects

For this study, we recruited 41 healthy mothers whose lactation stages ranged from 2 to 11 months (a mean ± SD: 6.1 ± 2.4) after delivery, with a mean (±SD) age of 32.2 ± 3.9 years and a body mass index of 20.4 ± 2.6 kg/m^2. No subjects were receiving any medication known to interfere with lactation, and did not take supplements or vitamins 1 week before the study until the end of sampling. They have no dietary habits leading to them avoiding or favoring selected food items before the study.

Experimental design

The study was performed with a randomized, controlled, comparison design. Subjects were divided into two groups by the envelope method. One group (n = 21) took pre-germinated brown rice (FANCL Corp., Japan) and the other (n = 20) white rice (control) as their every staple diet for 2 weeks. Specific information regarding pre-germinated brown rice was not provided to the participants. Before and after dietary intervention, the Profile of Mood States (POMS) test and measurement of salivary amylase activity were performed for mental health assessment, and s-IgA and LTF in milk were measured for immunological assessment.

The experimental design was registered with UMIN-CTR (Trial number: UMIN463) (University hospital medical information network-control trials registry) and the protocol was approved by the medical ethics committee of the University of Tsukuba (Ibaraki, Japan). Prior to the study, the purpose and possible risks of the experiment were carefully explained to all subjects, and written consent was obtained from them.

Sample collection

Each mother breast-fed her infant and any remaining milk was completely emptied 1 h before sampling. After the breast was wiped with wet cotton, a 25 mL milk sample was collected from the same breast of each mother by a manual breast pump into clean plastic containers, followed by dividing it into aliquots. All aliquots were frozen immediately and stored at −80°C until assaying.

Profile of Mood States test

The Profile of Mood States (POMS)-Brief Form Japanese Version was used to assess the mental health status of subjects. The brief form consist of 30 questions, and subjects were instructed to self-assess their current mood state by a five-point scale ranging from "not at all" to "quite frequently", assigned from 0 to 4 respectively. The mood states of subjects were classified into six states: tension–anxiety (T–A), depression (D), anger–hostility (A–H), vigor (V), fatigue (F), and confusion (C). The scores of each category were summed up to obtain the total mood disturbance (TMD). The TMD was calculated by subtracting V score from the total of T–A, D, A–H, F and C scores.

Amylase activity measurement

Salivary amylase activity was measured using the Dry Chemistry System (Nipro Corp., Japan) according to the manufacturer's protocol. Saliva was sampled by directly immersing a saliva-sampling strip in saliva under the tongue for 30 s. The strip was immediately placed in an automatic saliva transfer system, and saliva was transferred by compression to an amylase test paper on the reverse side of the strip sleeve. The amylase test paper contained the substrate (2-chloro-4-nitrophenyl-4-O-β-D-galactopyranosylmaltoside, Gal-G2-CNP). The enzyme reaction started upon the transfer by compression, and the free CNP level was optically measured after 20 s. The amylase activity that produces reduced sugars equivalent to 1 μmol/min of maltose was defined as 1 unit.

s-IgA measurement

Secretory immunoglobulin A (s-IgA) in milk was measured by the EIA s-IgA test (Medical & Biological Laboratories Co., Ltd., Japan) according to the manufacturer's protocol. Each milk sample was diluted 510-fold with reaction buffer. About 10 μL of s-IgA standard or diluted milk sample was pipetted into a test tube, together with 400 μL of reaction buffer. Subsequently, one bead coated with anti-secretory component antibody was added to each tube and incubated for 1 h at 37°C. After the beads were washed with PBS, they were added to 300 μL of anti-human IgA antibody coupled with horseradish peroxidase (HRP) solution for 1 h at room temperature. The beads were then washed again with PBS, and transferred to another test tube containing 500 μL of substrate solution (*o*-phenylenediamine and hydrogen peroxide). After standing for 30 min at room temperature, the reaction was stopped by 2 mL of 2 mol/L sulfuric acid, followed by reading the absorbance at 490 nm. The standard curve was obtained by plotting the absorbance as a function of the logarithm of s-IgA standard concentrations in mg/L. The sample values were then obtained by interpolation.

Lactoferrin measurement

Lactoferrin (LTF) in each milk sample was measured using a Lactoferrin ELISA Kit (EMD Biosciences, Inc., Germany) according to the manufacturer's protocol. The milk sample was diluted 125,000-fold with sample diluting buffer. About 100 μL of LTF standard or diluted milk sample was applied to a microplate coated with a primary monoclonal antibody to LTF, and incubated for 1 h at 37°C. After unbound components were removed with a washing buffer, each well was incubated with 100 μL of biotinylated monoclonal antibody solution to LTF for another 1 h at 37°C. Each well was then washed again with the washing buffer, followed by incubation with 100 μL of avidin-coupled HRP solution for 15 min at 37°C. After washing each well, the bound HRP activity was measured by a colorimetric assay. The colorimetric assay was started by adding 100 μL of substrate solution (*o*-phenylenediamine and hydrogen peroxide). After standing for 15 min at room temperature, the reaction was stopped by 50 μL of 1 mol/L sulfuric acid, followed by reading the absorbance at 490 nm within 30 min after termination of the reaction. The standard curve was obtained by plotting the absorbance as a function of the logarithm of LTF standard concentrations in μg/L. The sample values were then obtained by interpolation.

Statistical analysis

Values of results are expressed as the mean ± SD. Significant differences of values between before and after intervention were analyzed by the Wilcoxon signed-rank test.

Results

The participants in the pre-germinated brown rice diet group and white rice diet group took 238.0 ± 35.3 g and 270.6 ± 57.6 g of each rice (dry weight) in a day, respectively, as their staple diet for 2 weeks.

TMD was calculated from the scores of the six mood parameters of POMS. Dietary intervention significantly decreased the TMD value from 11.5 ± 10.0 to 5.6 ± 8.9 in the pre-germinated brown rice diet group ($n = 21$, $P = 0.01$) (Fig. 1A). The value

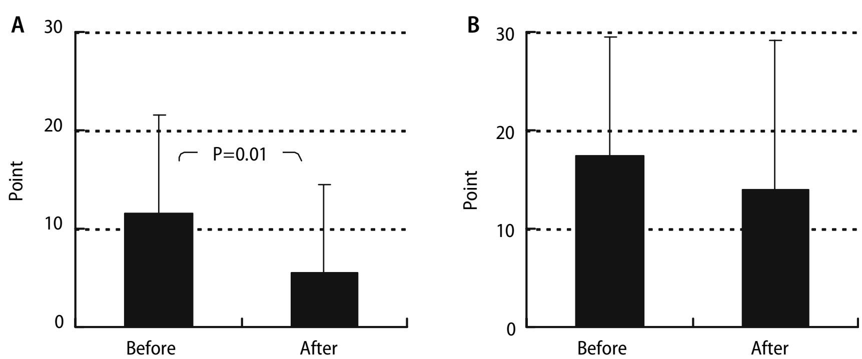

Fig. 1 Total mood disturbance (TMD) scores in the pre-germinated brown rice diet group (**A**, $n = 21$) and white rice diet group (**B**, $n = 20$) before and after intervention. The longitudinal axis shows the TMD score calculated from six mood states, and the horizontal axis shows the time points of assessment. Data for each group, expressed as the mean ± SD, are shown by black box columns. In the pre-germinated brown rice diet group (**A**), the TMD score after intervention was significantly lower than that before it [$P = 0.01$, Wilcoxon signed-rank test]

Table 2 Changes in POMS scores before and after dietary intervention

Parameter		Pre-germinated brown rice diet group (n = 21)	White rice diet group (n = 20)
Tension–anxiety	Before	3.9 ± 2.9	4.5 ± 2.7
	After	3.3 ± 2.7	4.0 ± 3.3
Depression	Before	1.8 ± 1.6	2.6 ± 2.1
	After	1.0 ± 1.8*	2.4 ± 3.0
Anger–hostility	Before	3.9 ± 2.1	4.8 ± 3.8
	After	2.7 ± 1.8**	3.6 ± 2.6
Vigor	Before	9.3 ± 3.6	7.7 ± 3.4
	After	9.8 ± 3.3	8.2 ± 3.3
Fatigue	Before	5.8 ± 3.6	7.3 ± 3.6
	After	3.6 ± 2.5**	6.9 ± 3.9
Confusion	Before	5.5 ± 2.9	6.1 ± 2.4
	After	5.1 ± 2.7	5.3 ± 3.2

Data are expressed as the mean ± SD. The values between before and after dietary intervention were subjected to the Wilcoxson signed-rank test. * $P < 0.05$, ** $P < 0.01$, significantly different from before intervention

was also slightly decreased (from 17.5 ± 12.1 to 13.9 ± 15.2) in the white rice diet group (n = 20) (Fig. 1B). As for the individual POMS parameters, dietary intervention significantly decreased the scores of depression, anger-hostility, and fatigue in the pre-germinated brown rice diet group (Table 2), but none of the parameters were significantly changed in the white rice diet group.

In the analysis of a stress marker, salivary amylase activity, one subject in the pre-germinated brown rice diet group was excluded from Smirnov's rejection test as an outlier. The salivary amylase activities before intervention were 24.9 ± 10.4 and 28.2 ± 22.9 KU/L in the pre-germinated brown (n = 20) and white rice diet groups (n = 20), respectively, showing a stress-free condition (30 KU/L or lower). The enzyme activity tended to decrease (17.2 ± 11.3 KU/L) in the pre-germinated brown rice diet group after 2-week dietary intervention (Fig. 2A), whereas that was significantly increased (47.5 ± 44.2 KU/L, P = 0.02) in the white rice diet group (Fig. 2B), showing a stressful condition (46–60 KU/L). Regarding changes in salivary amylase activity in individual subjects, the stress level was increased in 6 subjects in the pre-germinated brown rice diet group, and 13 subjects in the white rice diet group.

As immunological components in breast milk, the s-IgA and LTF levels were measured. One subject in the pre-germinated brown rice diet group was excluded from the analysis of dietary intervention-induced changes in these component levels because she was under medication for viral infection at the time of sample collection. The level of breast milk s-IgA, which protects infants from bacterial and viral infections, was significantly increased by dietary intervention from 788.0 ± 498.7 to 893.2 ± 579.1 mg/L (P = 0.04) in the pre-germinated brown rice diet group (n = 20) (Fig. 3A), whereas no change was recognized in the white rice diet group (n = 20) [Fig. 3B]. LTF in breast milk is involved in intestinal immunity in infants. The LTF level measured by EIA was slightly higher than that measured by the conventional method [10]. The level tended to be increased by dietary intervention in both groups: from 7.74 ± 4.72 to 9.07 ± 5.06 g/L in the pre-germinated brown rice diet group (n = 20) (Fig. 4A) and from 9.31 ± 4.79 to 10.35 ± 4.89 g/L in the white rice diet group (n = 20) (Fig. 4B).

Discussion

The significant decreases in the depression, anger-hostility, and fatigue scores in the pre-germinated brown rice diet group on POMS analysis may have been due to components rich in pre-germinated brown rice, compared to white rice. γ–Amino butyric acid (GABA) [150 mg/kg: pre-germinated brown rice (Technical data from FANCL Corp.), 20 mg/kg: white rice (Technical data from National Agriculture and Food Research Organization)], abundant in pre-germinated brown rice, is known to exhibit antidepressant and mood-stabilizing effects [20], and vitamin B1

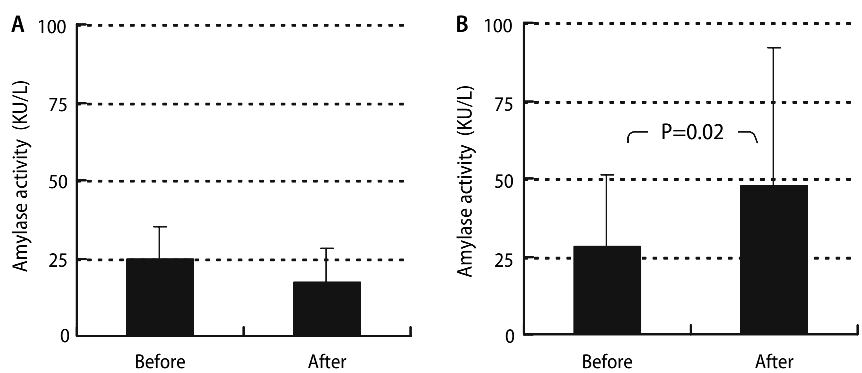

Fig. 2 Salivary amylase activities in the pre-germinated brown rice diet group (**A**, n = 20) and white rice diet group (**B**, n = 20) before and after intervention. The longitudinal axis shows amylase activity determined using the Dry Chemistry System, and the horizontal axis shows the time points of sample collection. Data for each group, expressed as the mean ± SD, are shown by black box columns. Salivary amylase activity tended to decrease after intervention in the pre-germinated brown rice diet group (**A**), but was significantly higher than that before it in the white rice diet group (**B**) [P = 0.02, Wilcoxon signed-rank test]

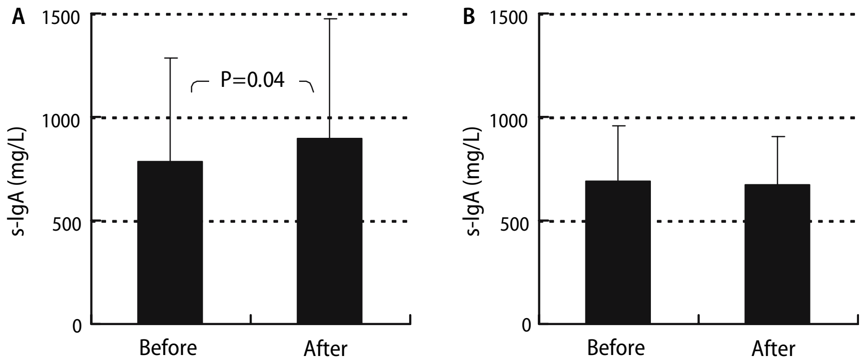

Fig. 3 Secretory IgA (s-IgA) levels in milk in the pre-germinated brown rice diet group (**A**, $n = 20$) and white rice diet group (**B**, $n = 20$) before and after intervention. The longitudinal axis shows the s-IgA concentration determined by enzyme immunoassay, and the horizontal axis shows time points of sample collection. Data for each group, expressed as the mean ± SD, are shown by black box columns. In the pre-germinated brown rice diet group (**A**), the s-IgA level after intervention was significantly higher than that before it [$P = 0.04$, Wilcoxon signed-rank test]

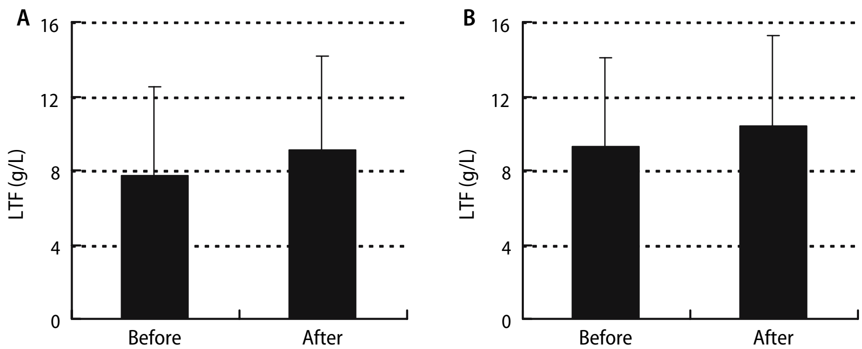

Fig. 4 Lactoferrin (LTF) levels in milk in the pre-germinated brown rice diet group (**A**, $n = 20$) and white rice diet group (**B**, $n = 20$) before and after intervention. The longitudinal axis shows the LTF concentration determined by enzyme immunoassay, and the horizontal axis shows the time points of sample collection. Data for each group, expressed as the mean ± SD, are shown by black box columns. In both diet groups, the LTF level tended to increase after intervention

[4.2 mg/kg: pre-germinated brown rice (Technical data from FANCL Corp.), 0.8 mg/kg: white rice ("Standard table of food composition in Japan (fifth revised and enlarged edition)" issued by MEXT)] promotes to recover mental and physical fatigue [1, 21].

Salivary amylase activity rapidly and sensitively reflects the stress condition [24], compared to cortisol, which has been used as a salivary stress maker. Since breast-feeding inhibits the hypothalamic–pituitary–adrenal (HPA) axis, which responds to psychosocial stress [9], breast-feeding mothers have less stress than mothers using formula milk [5]. All subjects in this study were breast-feeding mothers, and the stress-free condition with regard to salivary amylase activity was confirmed before dietary intervention. The stress index tended to decrease after intervention in the pre-germinated brown rice diet group, but the condition became stressful in the white rice diet group, significantly. The subjects are considered to have been subjected to stress caused by restrictions regarding selection of the staple type due to their participation in this study, but the anti-stress potentiality of pre-germinated brown rice may have made the subjects stress-resistant.

The central nervous system and the immune systems are closely related. The s-IgA in breast milk is decreased under perceived stress, and increased by positive life events [6]. The significant increase in the breast milk s-IgA level in the pre-germinated brown rice diet group may have been due to the anti-stress effect of pre-germinated brown rice. It is known that the breast milk LTF level is high in mothers in good nutritional status [11]. The increases in the breast milk LTF level in both groups may have been attributed to not only the effect of the staple diet on the mental status through the neuroendocrine immune system but also its nutritional ingredients.

It is possible that the psychosomatic effects of pre-germinated brown rice on lactating mothers was due to the changes in dishes accompanied with the staple food, in addition to the direct effect. However, no distinguishable alteration was observed in non-staple foods after intervention, but another effect of the increased grain energy ratio to the total energy intake was supposed to be emerged. In addition to the direct effect, the synergy effects via psychological process of pre-germinated brown rice on the breast milk ingredient could also be considered. In conclusion, pre-germinated brown rice may have beneficial effects on psychosomatic health.

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