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Correlation Between Lactic Acid Bacteria Beverage Intake and Stress Resilience

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We investigated the effect of lactic acid bacteria-containing beverage intake on the level of resilience against stress in male university students. Forty male university students were recruited into the study and randomly assigned into two groups. They were instructed to consume lactic acid bacteria-containing beverage or water twice a day for 28 days. The level of stress resilience, stress reaction, and anxiety were evaluated by a series of questionnaires conducted at three time points (T1: day 0, T2: day 14, and T3: day 28). The stress response was also assessed by measuring their salivary amylase levels. The variance analysis of each group showed a significant increase in stress resilience at T3 compared with T1 in the group of participants who consumed the lactic acid bacteria-containing beverage. Our results suggest that lactic acid bacteria-containing beverage intake could affect resilience against stress positively.

INTRODUCTION

In amidst today's stressful environment, the identification of the factors which enhance the ability to cope stress can contribute to maintaining and promoting mental health. It can improve the individual's quality of life and also increases health expectancy, leading to reduced social welfare burden, which is a significant problem faced by public health financial institutions. Therefore, from the standpoint of salutogenesis (1), which is the leading framework used in contemporary health promotion theories, we focused on lactic acid bacteria-containing beverage in this study as part of a diet influencing resilience against stress. We thus conducted an interventional study to investigate the effect of lactic acid bacteria-containing beverages on resilience against stress.

The core theory of salutogenesis is the sense of coherence (SOC), which is a concept of resilience against stress that is composed of the elements of comprehensibility, manageability, and meaningfulness to maintain health in stressful circumstances. The theory of salutogenesis demonstrates that proper coping against stressful stimuli can aid in maintaining and promoting mental health (2). Several studies have reported that SOC correlates with mental health and health behaviors (3, 4) and related physical health (5). It has been also reported a correlation between food intake and the SOC, such as those by Wainwright et al. (6) and Lindmark et al. (7), which indicated that there is a correlation between vegetable, fruits, and dietary fiber intake with SOC. These studies discussed that a stronger SOC contributed to healthy diet management.

In contrast, specific food intake correlates with mental health, and diet management is gaining attention as a means for restoring mental health. In an epidemiological study on the correlation between dairy product intake and the SOC, middle-aged and older participants were asked a series of questions, including the following: "Do you keep it in mind to take a sufficient amount of dairy products or calcium?" The results showed that the participants who answered positively to this question had a stronger SOC compared with those who answered with "I try, but not enough," or "I really do not care much about it," suggesting that there is a correlation between the frequency of dairy product intake and the SOC (8). The effect of dairy product intake on SOC has been examined among middle school students by longitudinal questionnaire survey (9).

In particular among dairy products, the effects of probiotic microorganisms considered to promote mental health, such as lactic acid bacteria or bifidobacteria, have been widely investigated. Although there were slight differences in results, the consumptions of probiotic microorganisms have positive effects on human mental health (10–12). These studies were performed in healthy participants and appear to have been motivated from the

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standpoint of salutogenesis to promote good health, rather than from that of pathogenesis, which is a framework that seeks to restore good health by controlling disease mechanisms. Even so, these studies did not investigate the effect of the core idea of salutogenesis SOC. Therefore, we aimed to investigate the effect of lactic acid bacteria beverage intake on stress resilience, SOC, in this interventional study.

MATERIALS AND METHODS

Participants

We explained the purpose and mechanisms of the investigation to student members of two sports clubs at a university in the Kansai region and we recruited 40 male students who agreed to participate in the study. They were divided into two groups of 20 students each: those who consumed lactic acid bacteria beverage (LAB group) and those who consumed water as the control (water group). The sample size was determined according to previous studies with similar experimental designs (9). The expected outcomes of the hypothesis were not known to the participants.

All experimental protocols were approved by the Human Ethics Committee of Graduate School of Human Development and Environment Kobe University and all methods were carried out in accordance with relevant guidelines and regulations.

Protocol

This interventional study was conducted at the university in the Kansai region from November to December in 2018. A blinded investigator randomly allocated the participants to one of the two groups by blocking on the affiliation club. After obtaining informed consent from all 40 participants, they were asked to consume lactic acid bacteria beverage or water regularly over 28 days, according to their group assignment, in order to compare the fluctuation patterns in resilience against stress and the relevant indexes.

We delivered lactic acid bacteria beverage (Yakult 400LT and Milmil S, Yakult, Tokyo) or water to the participants. One bottle of Yakult 400 LT (80 ml) contains Lactobacillus casei strain Shirota (4 × 1010 bacteria). One bottle of Milmil S (100 ml) contains Bifidobacterium breve strain Yakult (1.2 × 1010 bacteria), Lactococcus lactis (1 × 1010 bacteria), and Streptococcus thermophilus (1 × 1010 bacteria). The participants were asked to consume two bottles of Yakult 400LT after waking up in the morning and one bottle of Milmil S before bedtime. The study was randomized but unblinded for participants. Those who allocated the group randomly and assessed the outcomes were blinded; however, those who distributed the materials were unblinded.

Prior to the intervention, a dietician examined the students' meals and confirmed that none of them consumed Yakult 400LT and Milmil S. Throughout the study, participants did their normal diet or exercise.

Figure 1 showed the study schedule. The intervention period lasted four weeks, during which we confirmed the intake daily practice using social networking services. We conducted questionnaires on stress and their salivary amylase measurement three times: before the intervention (T1; day 0), two weeks after the intervention start (T2; day 14), and four weeks after the intervention start (T3; day 28). We conducted the questionnaires from seven to nine in the evening after their club activity.

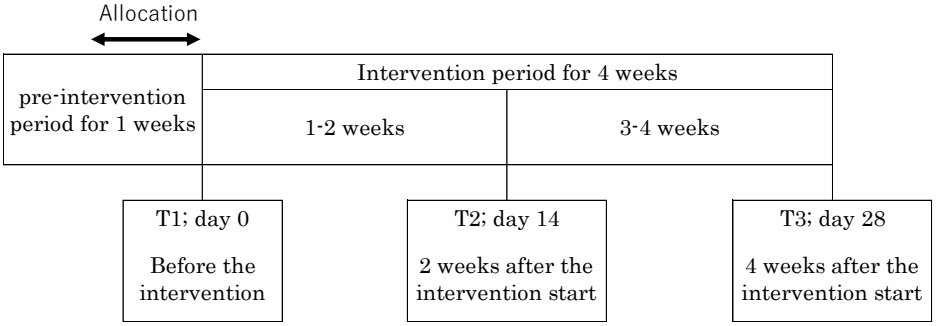

Figure 1. Study schedule

Statistics

Throughout the study, five participants (two water group members, three LAB group members) were not compliant with the intervention. These data were excluded from the analysis. All measurements were performed at T1, T2, and T3 and we calculated the mean value and standard deviation for each item. Next, we conducted a one-way repeated measures analysis of variance (one-way ANOVA), followed by Dunnett's test, in each group

to investigate the measurement fluctuation patterns during the intervention period. p-values < 0.05 were considered statistically significant. SPSS ver. 21 was used to analyze the data.

Measurement

Stress resilience: We measured the level of SOC as the stress resilience factor to maintain health under stressful situations using the Japanese version of SOC-13 (13), which consists of 13 items. The SOC-13 (2) is a shorter version of the SOC-29 and consists of three subscales; namely, comprehensibility, manageability, and meaningfulness.

Stress reaction: We measured the level of stress reaction using the Japanese version of the Stress Arousal Check List (SACL) (14), composed as J-SCAL (15). The scale consists of two subcategories; namely, stress-inducing oppression (18 subscales) and feelings of liveliness evaluating the favorable state of consciousness (12 subscales).

Anxiety: We measured the levels of state and trait anxieties (20 items each) using the new version of the State-Trait Anxiety Inventory, STAI (16), which was developed from the STAI-Y (17) with the addition of emotional characteristics typical for the Japanese population.

Salivary amylase: We measured salivary amylase levels using the Salivary Amylase Monitor (NIPRO CORPORATION, OSAKA) (18). Amylase contained in the saliva hydrolyzes the α-2-chloro-4-4-galactopyranosyl maltoside (Gal-G2-CNP) contained in the tip of the test paper; the amylase level in the saliva is measured by converting the fluctuation of reflected light intensity induced by the α-2-chloro-4-nitrophenol (CNP) produced in the test paper into amylase activity values.

RESULTS

After the 28-day investigation period, there were a total of 35 subjects without insufficient data in the survey sheets; 17 in the LAB group (mean age, 20.2 ± 1.0 years; mean body mass index, 25.6 ± 4.2) and 18 in the water group (mean age, 20.0 ± 1.0 years; mean body mass index, 25.8 ± 3.9).

Table 1 shows study results. We found significant differences in the mean values of the SOC total and comprehensibility subscale scores between T1 and T3 in the LAB group, showing score improvement in both items after four weeks of regular intake of lactic acid bacteria-containing beverage. Regarding the level of trait anxiety, the results at T3 showed an inclination towards a decrease in the mean value. The results at T2 showed a significant decrease in the mean value in terms of the salivary amylase level. In the water group, there was an inclination towards increase in the favorable state of awareness at T3.

We did not confirm any significant fluctuation in the scores of meaningfulness, manageability, state anxiety, and stress-inducing oppression.

DISCUSSION

Several studies have investigated the effects of lactic acid bacteria on stress levels or emotional states and how the probiotic microorganisms affect human health favorably. However, these studies did not investigate the effect of lactic acid bacteria on the stress resilience index SOC (10–12). The results of our study showed that regular intake of lactic acid bacteria-containing beverage for four weeks increased the SOC level, suggesting the possibility of enhanced stress resilience induced by the regular intake of lactic acid bacteria-containing beverage. In contrast to this, we were unable to identify any evident effects on state anxiety and subjectively assessed stress reactions, if a decreasing trend was observed. These results suggest that lactic acid bacteria-containing beverage promotes better stress resilience but does not directly affect induced anxiety or stress reaction. Previous studies on the effect of probiotic microorganism intake on depression/anxiety have shown a considerable range of diversity among the research projects or participants groups included in the study (19). For example, a study on the effect of Lactobacillus casei strain Shirota (LcS) showed that improvement of depression was found only in the group with a high level of depression prior to the intervention (10). The result is in agreement with that of our study using LcS regarding the possibility that probiotic microorganisms, including lactic acid bacteria, would have a positive effect only on the group exposed to a sustained level of stress because it can improve depression/anxiety through stress resilience enhancement.

The effects of probiotics on salivary stress markers have been reported (20, 21). In this study, we measured the salivary amylase level as an objective stress indicator, as sympathetic activation increases salivary amylase in a manner correlated to plasma noradrenalin concentration in humans (22). Notably, lactic acid bacteria-containing beverage decreased the salivary amylase earlier than subjective indicators including SOC. This finding suggests that salivary stress markers can be used as a predictive marker for SOC alterations.

However, the mechanism by which lactic acid bacteria-containing beverage promotes resilience against stress remains unknown, and warrants further investigations using animal models or human brain functional imaging. A recent study using an animal model has indicated that the neuroinflammation might be a critical

factor for depression/anxiety promotion by chronic stress (23). In addition, a substance in whey, the byproduct of fermented dairy products, suppresses neuroinflammation and the depression/anxiety promoting effect of chronic stress (24). Although there are only limited studies on the correlation of the stress/depression/anxiety level of healthy participants with neuroinflammation, increased inflammatory reaction in the brain or blood has been repeatedly reported in cases of mental diseases closely related to stress, such as depression (25). The intestinal environment including microorganism metabolites, such as those of lactic acid bacteria, could affect brain function through hormones or the vagal nerves. Indeed, it was reported that the oral administration with LcS stimulates the activity of vagal nerves and reduces stress-induced activation of the hypothalamic-pituitary-adrenal axis for glucocorticoid release in rats (26). It was reported that the consumption of LcS and Bifidobacterium breve strain (BbS) in combination for four weeks improved gastrointestinal functions (27). We used this probiotics regimen to test the effect of probiotics on SOC. Further investigation is needed on the fluctuation of the inflammatory reaction or brain functions in response to the stress resilience enhancement induced by lactic acid bacteria beverage intake.

Table I. Results of ANOVA and multiple comparison test

		Water Group（n = 18）					LAB Group (n = 17)				
	Time	M	SD	F	p	Dunnett's multiple comparison	M	SD	F	p	Dunnett's multiple comparison
SOC	T1	53.5	10.2	0.42	0.66	ns	51.8	8.6	5.89	0.01	T1 < T3**
	T2	55.6	11.8				52.4	9.2			
	T3	54.2	7.4				55.6	9.5			
Meaningfulness	T1	16.3	1.2	1.96	0.16	ns	16.6	2.7	0.57	0.57	ns
	T2	18.4	1.0				17.2	3.4			
	T3	17.3	0.7				17.2	3.6			
Comprehensibility	T1	20.4	4.4	0.25	0.78	ns	19.5	5.6	5.96	0.01	T1 < T3*
	T2	20.1	5.2				19.1	3.9			
	T3	19.8	3.6				21.4	4.9			
Manageability	T1	16.8	3.9	0.05	0.95	ns	15.8	3.2	2.26	0.12	ns
	T2	17.1	4.3				16.1	3.3			
	T3	17.1	3.0				17.0	2.8			
State Anxiety	T1	37.8	8.5	0.06	0.94	ns	39.2	8.9	0.91	0.42	ns
	T2	37.8	12.6				42.0	11.2			
	T3	38.7	11.7				39.7	10.4			
Trait Anxiety	T1	46.2	10.3	0.67	0.52	ns	48.0	8.4	2.72	0.08†	T1 > T3†
	T2	43.6	12.3				44.9	9.1			
	T3	43.8	6.4				44.6	9.7			
Stress-inducing Oppression	T1	−7.8	10.6	0.89	0.42	ns	−8.4	10.2	0.45	0.64	ns
	T2	−9.4	10.6				−7.8	11.1			
	T3	−10.7	8.1				−6.8	11.1			
Favorable State of Consciousness	T1	3.7	7.4	2.85	0.07	T1 < T3†	3.9	7.4	0.31	0.73	ns
	T2	6.1	7.5				5.0	6.6			
	T3	6.7	6.8				5.2	7.5			
Salivary Amylase	T1	17.7	17.2	0.44	0.65	ns	22.1	17.3	3.64	0.04	T1 > T2†
	T2	14.6	10.5				14.7	13.1			
	T3	17.8	12.2				15.9	9.7			

†: $p < 0.1$, *: $p < 0.05$, **: $p < 0.01$,
M, mean value; SD, standard deviation; F, F value; ns, not significant.

The strength of the present study is that we conducted the investigation after the sports club activity of the students, which enabled us to regulate the activity levels of our participants. However, sports activities have been reported to have stress-reducing effects (28). Thus, it is possible that the activities reduced the stress level of our

participants. Future studies on this matter should be performed in participants who do not exercise on a daily basis.

Furthermore, there have been only few studies on the correlation between the SOC and food intake; thus, investigating the relation between the SOC and dairy product intake have been reported only in Japan (8, 9). Hence, the effect of food culture should also be investigated.

This study also had limitations. First, the study was an open-label interventional study; hence, we could not eliminate the possibility of placebo effect. Second, the number of participants was relatively small, which limited the generalizability of the findings. In this study, we analyzed male participants, as the gender effect on SOC has not been determined. Many studies did not find apparent differences in SOC between men and females (29–31). Some studies reported its gender difference, but the results are inconsistent across studies. For example, Horiguch et al. (32) reported higher SOC in females than in males, whereas other studies reported males showed higher SOC than females (33, 34). It was reported that stress response varies according to menstrual cycle (35), which may also contribute to SOC variability. Future multicenter double-blind studies with a larger sample size including females are necessary to confirm the results of the present study and analyze the gender difference.

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