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Effects of lactic acid bacteria-containing foods on the quality of sleep: a placebo-controlled, double-blinded, randomized crossover study

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ABSTRACT

Background: There are various types of sleep disorders, such as insomnia, hypersomnia, and rhythm disorder, which are attributed to diverse and complex background factors. Recently, many studies have reported that lactic acid bacteria, bifidobacteria, and lactoferrin, are related to fatigue and sleep.

Objective: To prepare test food samples containing lactic acid bacteria ingredients (fermentation products, living bacteria, and ground bacteria) derived from the lactic acid bacterium strain *Lactobacillus helveticus* MIKI-020 (LBH MIKI-020) and theanine, which is known to have a relaxing effect. Then testing and observing the effects of lactic acid bacteria on the quality of sleep.

Methods: In this placebo-controlled, double-blinded, randomized crossover study, we randomly selected 40 male and female subjects (aged 20-64 years) to consume four-weeks test food (lactic acid bacteria ingredients –containing) tablets and placebo control food. The physical examination and laboratory test, sleep electroencephalography, Oguri-Shirakawa-Azumi sleep questionnaire

(OSA sleep questionnaire), Pittsburgh Sleep Quality Index (PSQI), and Visual Analogue Scale (VAS) questionnaire were measured.

Results: Sleep electroencephalography: the intergroup comparison in changes of the sleep efficiency (SE) until the 4th week showed a significant increase (improvements) compared with the placebo control food group. Furthermore, SE was compared within the group. In the test food group, the SE increased (improved) significantly in 4 weeks. OSA sleep questionnaire: in intragroup analyses between 0 and 4 weeks, significant increases (improvements) were found in the test food group. PSQI: in intragroup comparisons between week 0 and week 4, significant decreases (improvements) were found in the test food group. VAS questionnaire: the intergroup comparison in changes of Feeling of physical fatigue, Motivation (liveliness), and Calmness until the 4th week showed significant decreases (improvements) compared with placebo control food group.

Conclusion: The sleep efficiency (SE) and Feeling of physical fatigue, Motivation (liveliness), and Calmness were improved by continuous consumption of test food (lactic acid bacteria-containing food). Among various sleep disorders, a large population in Japan has trouble with sleeping quality. Accordingly, consumption of lactic acid bacteria-containing foods can be a safe and effective method to improve sleep quality.

Keywords: *Lactobacillus helveticus*, lactic acid bacteria, clinical trial, quality of sleep, sleep EEG, OSA sleep questionnaire, PSQI

INTRODUCTION

There are various types of sleep disorders, such as insomnia, hypersomnia, and rhythm disorder, which are attributed to diverse and complex background factors. Insomnia is the most common type of sleep disorder. In Japan, 1 in 5 individuals are estimated to suffer from insomnia. 1 in 20 individuals use sleeping pills. These figures reportedly increase with increasing age. Previously reported causes of insomnia include aging, sleep apnea syndrome, atopic dermatitis, depression/anxiety disorder, inappropriate use of caffeine/alcohol, inappropriate use of electronic devices, climacteric disorder, increased urinary frequency, night shift, etc. [1].

While complex exogenous and endogenous factors are involved in sleep disorder, there are many research reports on the circadian sleep rhythm. For example, blood levels of endocrine substances, such as melatonin and cortisol, undergo diurnal changes as they are deeply involved in arousal from sleep [2, 3].

Recently, many studies have reported that lactic acid bacteria, bifidobacteria, and lactoferrin are related to fatigue and sleep. In a human clinical study, Ooki et al. reported that 8-week consumption of a bifidobacteria-containing food improved QOL, quality of sleep, and mood state [4]. These effects are attributable to the secretion of serotonin by *Bifidobacterium*, which acts as a neurotransmitter. In a human clinical trial, Uesaki et al reported that a lactoferrin-containing health food reduced stress, improved drowsiness on awakening, and had a positive effect on recovery from fatigue [5]. The improvement of intestinal flora by lactoferrin may be responsible

for these effects, suggesting that the intestinal environment and brain function affect each other (gut–brain interaction).

Considering these factors, we prepared test food samples containing lactic acid bacteria ingredients (fermentation products, living bacteria, and ground bacteria) derived from the lactic acid bacterium strain *Lactobacillus helveticus* MIKI-020 (LBH MIKI-020) and theanine, which is known to have a relaxing effect [6]. We then tested its effects on the quality of sleep using subjective and objective measures.

STUDY DESIGN

Eligibility criteria

Individuals who satisfied the inclusion criteria and did not meet the exclusion criteria were eligible to participate in this study. Inclusion criteria were men and women aged 20–64 years who have a problem with everyday sleep. Exclusion criteria were the following: (1) history of diabetes, hepatic disease, renal disease, gastrointestinal disease, peripheral vascular disease, or other serious diseases; (2) impaired cardiopulmonary function; (3) abnormal test results for liver function and kidney function; (4) had undergone surgery of the gastrointestinal tract; (5) had a disease under treatment during the study period; (6) allergic to food or drugs; (7) engagement in intense sports or diet; (8) consumption of a health food, quasi-drug, or drug (including OTC and prescription drugs) expected to improve bowel movement or insomnia; (9) consumption of excessive alcohol or unable to abstain from the consumption alcohol during a measurement period of sleep encephalograms or daytime activity meter (from the day before examination to the day of the examination); (10) pregnancy, expected pregnancy, or breast-feeding during the study period; (11) participation or planning to participate in any other clinical trials at the commencement of this study; (12) individuals whom investigators consider inappropriate for participation.

Targeted sample size

78 participants were recruited and underwent a preliminary survey. The study included 40 subjects (20 subjects in the test food group, and 20 subjects in the placebo control food group) who met all the selection criteria and none of the exclusion criteria.

Study outline

This study was conducted in compliance with the spirit of Declaration of Helsinki (adopted in 1964, Fortaleza amendment in 2013) and ethical guidelines on medical research on humans (2014 Ministry of Education, Culture, Sports, Science and Technology/Ministry of Health, Labor and Welfare Notification No. 3). This study was approved by the Ethical Review Board of Miura Clinic (approval number: R1604). Prior to participation, subjects received adequate oral and written explanations about this study, including its purpose and procedures, and provided written consent to participation.

This was a placebo-controlled, double-blinded, randomized crossover study. After obtaining written consent, the assigner randomly allocated 20 subjects each to the test food group and the placebo control group using random numbers. The subjects underwent medical interviews and

laboratory tests on VISIT 1 and VISIT 2 (which was 4 weeks after VISIT 1). After a 6-week washout period, subjects underwent similar tests on VISIT 3 and VISIT 4 (which was 4 weeks after VISIT 3) (Fig1) (Table 1). Additionally, each subject was provided with a sleep electroencephalograph to measure sleep electroencephalogram (EEG) at home. The subjects were instructed to comply with restrictions (as described below) and prohibitions during the study period.

Restrictions

Investigators instructed the subjects to comply with the following restrictions during the study period:

1. Lifestyle such as diet, drinking, exercise, sleeping, and smoking should not be remarkably changed during the study period from those before study participation.
2. Avoid excessive exercise and abstemious diet or overeating that deviates from the range of daily life.
3. Consumption of pharmaceutical products (including those for external use), newly designated quasi-drugs, traditional Chinese medicines, health foods, and supplements are prohibited. If the use of any of these products is necessary for unavoidable reasons (such as poor physical condition) subjects should consult the contract research organization in advance.
4. The amount or frequency of consumption of foods/beverages expected to improve insomnia or regulate the function of intestine (e.g., yoghurt, lactic acid bacteria, *Bifidobacterium*, kimchi, natto, oligosaccharides, and indigestible dextrin) should not be changed during the study period from what it was before study participation.
5. Alcohol consumption and excessive exercise are prohibited from the day before the examination until the examination is completed.
6. No eating or drinking (except for water and lukewarm water) from after 10 PM of the day before the examination until the examination is completed.
7. Visit the hospital without eating the test food on the day of examination.

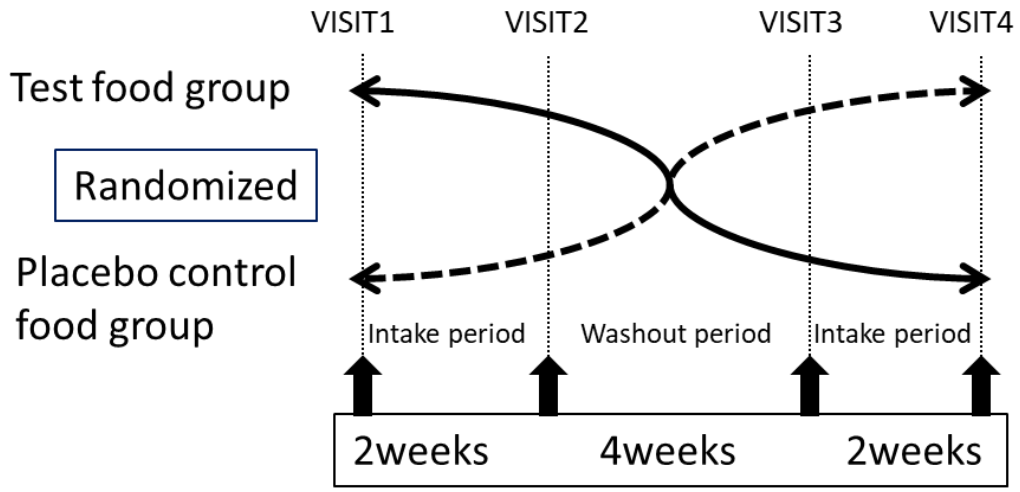

Figure 1. Timeline of this placebo-controlled, double-blinded, randomized crossover study.

Table 1. Schedule of interventions and assessments.

Study phase	**Treatment 1**		**Wash out**	**Treatment 2**	
	Visit 1	**Visit 2**		**Visit 3**	**Visit 4**
Sleep EEG measurement	●●●*1	●●●*2		●●●*1	●●●*2
OSA sleep questionnaire	●	●		●	●
PSQI sleep questionnaire	●	●		●	●
VAS questionnaire	●	●		●	●
Blood and urine sampling	●	●		●	●
Physical examination	●	●		●	●

*1: Sleep EEG measurements were performed for the first 3 days after visit 1 and visit 3.
*2: Sleep EEG measurements were performed for the last 3days before visit 2 and visit 4.

Intervention with the test food or placebo control food

Subjects in the test food group ingested 8 tablets (total 4.8 g) of the lactic acid bacteria-containing food (MIKI Corporation, Osaka) daily. The test food contained 900 mg of fermented products of LBH_MIKI-020, 200 mg of live lactic acid bacteria powder, 200 mg of ground lactic acid bacteria powder, and 200 mg of theanine in a daily amount in addition to maltitol, lactose, calcium stearate, lactic acid, flavor, and yeast extract (0.3 g of proteins, 0.2 g of lipids, 4.2 g of carbohydrates, 0.4mg of sodium, and calorific value of 14 kcal). Subjects in the control food group ingested 8 tablets (total 4.8 g) of the placebo food daily. The placebo control food was devoid of lactic acid bacteria-derived ingredients and theanine (0.0 g of proteins, 0.1 g of lipids, 4.5 g of carbohydrates, 0.04 mg of sodium, and calorific value of 13 kcal).

The subjects ingested the tablets with or without chewing in the month within 30 minutes after dinner (or the first meal of the day if the subject did not eat dinner).

EXAMINATIONS

Physical examination and laboratory test items

Body height; body weight; systolic and diastolic blood pressure; pulse; blood levels of uric acid, urea nitrogen, AST (GOT), ALT (GPT), γ-GTP, ALP, LDH, total bilirubin, total protein, albumin, creatinine (Cre), CPK, serum amylase, total-Cho, HDL-Cho, LDL-Cho, triglycerides, glucose, Na, Cl, K, Mg, Ca, and Fe; hematologic parameters; qualitative urine tests for protein, glucose, urobilinogen, bilirubin, occult blood, and ketone body; and urine specific gravity and pH were measured.

Sleep electroencephalography

On VISIT 1 examinations, each subject received a sleep electroencephalograph (Brain wave sensor ZA, Proassist Ltd., Osaka) and an explanation of how to use it. Subjects measured sleep EEGs at home for 3 days after VISIT 1 examinations. Subjects also measured sleep EEGs for 3 days before VISIT 2 examinations. Subjects consumed the test food for 4 weeks from 3 days after VISIT 1 examinations. After a 6-week washout period after VISIT 2 examinations, subjects underwent VISIT 3 examinations. Similar to VISIT 1 examinations, VISIT 3 examinations

included specified physical examinations/laboratory tests, test food supply, and sleep EEG measurement. Subjects consumed the test food for 4 weeks from 3 days after VISIT 3 examinations. Subjects measured sleep EEGs for 3 days before VISIT 4 examinations.

A portable recording system (ZA-9, Proassist, Ltd, Osaka, Japan) was used for recording Sleep EEG as previously reported by Nonoue et al. (2017). The recorded data were manually scored by one scorer, using the American Academy of Sleep Medicine Manual for Scoring Sleep 2007. Scorer was kept blinded to any background data for the subject of recorded data. The scoring software is SleepSign ver.3.3 (Kissei Comtec, Nagano, Japan). After sleep stage scoring was done, the sleep-related parameters were calculated as shown in Table 2.

Table 2. Assessed sleep-related parameters from EEG measurement.

Sleep parameter	Method of calculation
Sleep Period Time (SPT)	Duration from sleep onset to final awakening.
Total Sleep Time (TST)	Total time of REM and NREM stages in (SPT)
Sleep efficiency	(TST) / (SPT)
Wake time After Sleep Onset (WASO)	Wake time in (SPT)
Rate of wake time after sleep onset	(WASO) / (SPT)
Sleep latency	Duration from recording start time to sleep onset.
REM latency	Duration from sleep onset to the first REM stage.
Time in bed	Duration from recording start time to end time.

Subjective evaluation

In total, subjects were administered four surveys on the following items in OSA sleep questionnaire, PSQI, and VAS questionnaire on VISITS 1, 2, 3, and 4.

The OSA sleep survey (MA version) [7] was consists of sixteen adjectives with responses rated on a 0-4 scale which can be consolidated into five factors as the following: (1) sleepiness on rising; (2) initiation and maintenance of sleep; (3) frequent dreaming; (4) refreshing; (5) sleep length. The OSA-MA score was calculated using an MS-Excel sheet.

The PSQI (Japanese version version) sleep difficulty survey [8, 9] was conducted on the 7 items: (1) quality of sleep; (2) sleep-onset time; (3) sleep duration; (4) sleep efficiency; (5) sleep disturbance; (6) use of sleep medications; and (7) daytime dysfunction.

The VAS questionnaire survey was conducted on the following 12 items: (1) feeling of physical fatigue; (2) feeling of mental fatigue; (3) speed of thinking (confusion); (4) healing of tiredness; (5) ability to concentrate; (6) motivation (liveliness); (7) lightness of the body; (8) mood; (9) calmness; (10) awakening; (11) onset of sleep; and (12) subjective sleep duration.

Evaluation of adverse events

Adverse events included any undesirable or unintended injuries and diseases or signs thereof (including abnormal laboratory test results) that occurred in subjects regardless of whether there is a causal relationship with the study. The investigators made distinctions between subjective findings (i.e., subjective symptoms of the subjects) and objective findings (i.e., adverse events). Abnormal laboratory tests results were identified as adverse events by the investigators based on reference values of the study site.

Statistical analysis

All subjects who completed all the prescribed examinations and surveys as scheduled and did not meet any of the following exclusion criteria for the analysis set were included in efficacy analyses: (1) those who conspicuously engaged in behavior negatively impacting the reliability of test results, such as missing information in the diary; (2) those with less than 70% adherence to the test food regimen; and (3) those who did not satisfy the eligibility criteria for participation or were not capable of complying with the restrictions after enrollment.

Changes from data obtained before the consumption of the test food were compared between the test food group and the control food group using paired t-test for results of sleep encephalography and OSA sleep questionnaire survey, in addition to the Wilcoxon signed rank test for results of PSQI and VAS questionnaire surveys.

RESULTS

Inclusion and drop-out

Of 78 volunteers who participated in the preliminary examination, 38 were excluded for reasons such as low PSQI scores. Finally, 40 were included in this study. The selected subjects were 20 men and 20 women (mean age, 51.5 ± 9.0 years). Afterwards, one subject withdrew at week 0 examination. One subject dropped out for personal reasons after the start of the study. Out of the 38 subjects who completed the study, 19 were excluded from statistical analyses for reasons such as difficulties in measuring EEGs and failure of data recording. As a result, for 21 subjects who could measure sleep EEGs were included in analyses for all items (11 men and 10 women; mean age, 53.6 ± 6.9 years). (Fig 2)

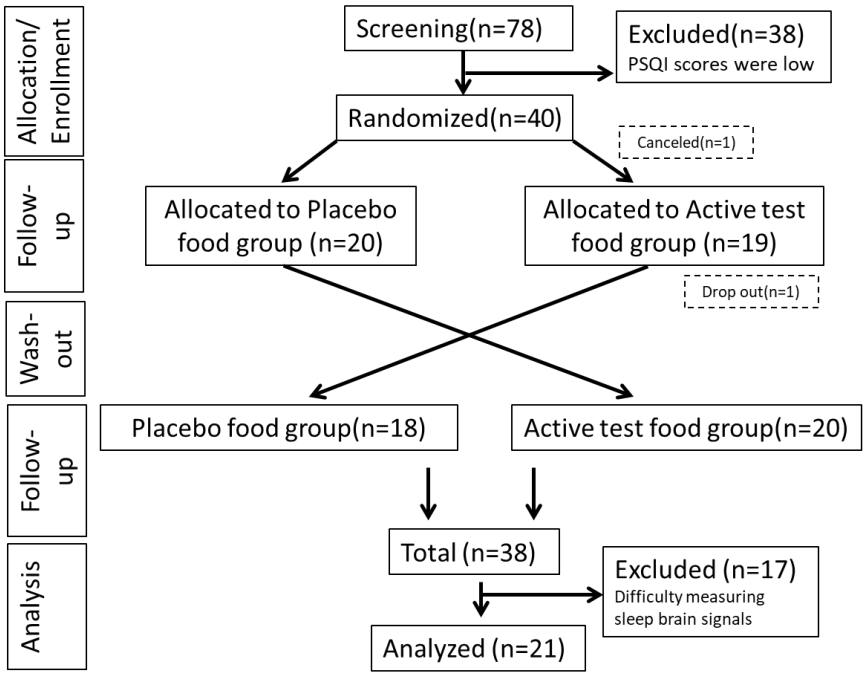

Figure 2. Flow diagram of the subjects who participated in this trial.

Sleep electroencephalography

Table 3 shows the results of sleep electroencephalography. The intergroup comparison in changes of the sleep efficiency (SE) until the 4th week showed a significant increase (improvement): control food group, −0.70 ± 5.69%; test food group, 2.13 ± 4.34% (p = 0.0342). Additionally, the SE was compared within the group. In the test food group, the SE increased (improved) significantly in 4 weeks: week 0, 93.29 ± 6.90%; week 4, 95.41 ± 4.16% (p = 0.0360).

Table 3. Change in sleep-related parameters calculated from EEG measurement.

			week 0 (baseline)	**week 4**	**⊿week 4**
Sleep period time	(min)	Active	339.12 ± 77.32	340.38 ± 87.73	1.26 ± 71.11
		Placebo	357.33 ± 57.20	338.57 ± 87.65	−18.76 ± 77.23
		p	0.1493	0.9294	0.4113
Total sleep time	(min)	Active	316.21 ± 75.45	323.93 ± 81.44	7.71 ± 70.60
		Placebo	338.93 ± 52.10	317.67 ± 77.26	−21.26 ± 70.69
		p	0.0905	0.7473	0.2335
Sleep efficiency	(%)	Active	93.29 ± 6.90	95.41 ± 4.16*	2.13 ± 4.34
		Placebo	95.06 ± 4.48	94.36 ± 4.85	−0.70 ± 5.69
		p	0.1660	0.4695	0.0342†
Wake time after sleep onset	(min)	Active	17.55 ± 27.61	13.55 ± 17.64	−4.00 ± 13.26
		Placebo	14.24 ± 18.48	17.40 ± 20.05	3.17 ± 23.52
		p	0.4507	0.5095	0.0993
Rate of wake time after sleep onset	(%)	Active	4.95 ± 6.90	3.64 ± 4.24	−1.31 ± 3.52
		Placebo	3.74 ± 4.64	4.57 ± 4.85	0.82 ± 5.97
		p	0.3167	0.5288	0.0886
Sleep latency	(min)	Active	15.50 ± 18.89	18.14 ± 15.98	2.64 ± 20.67
		Placebo	16.29 ± 20.49	15.86 ± 12.30	−0.43 ± 20.48
		p	0.7044	0.5079	0.5030
REM latency	(min)	Active	65.14 ± 28.53	58.57 ± 25.59	−6.57 ± 32.95
		Placebo	61.95 ± 15.04	72.98 ± 36.86	11.02 ± 33.50
		p	0.5734	0.0514	0.0702
Time in bed	(min)	Active	361.21 ± 78.81	361.90 ± 89.89	0.69 ± 65.80
		Placebo	376.93 ± 63.28	358.76 ± 89.39	−18.17 ± 77.14
		p	0.2399	0.8794	0.4459

Each value is shown as the mean ± SD. Statistical differences were evaluated by paired t-test. † $p < 0.05$ among the groups, * $p < 0.05$ versus baseline (n=21).

OSA sleep questionnaire

Table 4 shows the results of standardized scores of the OSA sleep survey. No significant differences were found between the groups. In intragroup analyses between week 0 and week 4, significant increases (improvements) were found in the test food group in terms of four out of the following five factors: factor I, sleepiness on awakening; factor II, onset and maintenance of

sleep; factor III, dreaming; and factor IV, recovery from fatigue ($p < 0.05$). In the control food group, a significant increase (improvement) was noted only for factor IV ($p = 0.0156$).

Table 4. Change in standardized scores of the OSA sleep questionnaire.

		week 0 (baseline)	**week 4**	**⊿week 4**
Factor I	Active	44.7 ± 6.4	48.4 ± 7.8*	3.8 ± 8.1
Sleepiness on awakening	Placebo	46.1 ± 8.6	47.8 ± 6.5	1.7 ± 7.7
	p	0.4609	0.6081	0.3113
Factor II	Active	39.1 ± 5.4	44.4 ± 7.1**	5.3 ± 7.4
Onset and maintenance of sleep	Placebo	38.8 ± 6.8	41.8 ± 10.0	3.0 ± 10.3
	p	0.8494	0.2780	0.3878
Factor III	Active	48.9 ± 11.3	53.6 ± 8.3*	4.6 ± 8.5
Dreaming	Placebo	49.1 ± 11.6	51.8 ± 8.5	2.6 ± 8.7
	p	0.8641	0.2554	0.3748
Factor IV	Active	42.3 ± 6.6	48.2 ± 6.2**	5.8 ± 6.4
Recovery from fatigue	Placebo	43.3 ± 6.0	46.8 ± 7.0*	3.5 ± 6.1
	p	0.4996	0.2927	0.1641
Factor V	Active	46.6 ± 7.3	46.3 ± 9.1	-0.4 ± 9.8
Sleep duration	Placebo	46.7 ± 8.1	45.7 ± 8.4	-1.0 ± 9.8
	p	0.9793	0.8207	0.8482

Each value is shown as the mean ± SD. Statistical differences were evaluated by Wilcoxon signed-rank test. ** $p < 0.01$, * $p < 0.05$ versus baseline (n=21).

PSQI

Table 5 shows the results of PSQI scores. No significant differences were found between the groups. In intragroup comparisons between week 0 and week 4, significant decreases (improvements) were found in the test food group for the following factors: C1, quality of sleep ($p = 0.0020$) and total score ($p = 0.0046$). In the control food group, significant decreases (improvements) were noted for the following factors: C1 ($p = 0.0313$) and C7, daytime awakening difficulty ($p = 0.0059$).

VAS questionnaire

Table 6 shows the results of VAS questionnaire scores. The intergroup comparison in changes of the Feeling of physical fatigue, Motivation (liveliness), and Calmness until the 4th week showed significant decreases (improvement): control food group, -0.22 ± 1.80, -0.30 the results of VAS questionnaire scores. The intergroup comparison in c± 1.95 ($p = 0.0317$), -1.09 ± 1.69 ($p = 0.0444$).

In intragroup comparisons between week 0 and week 4, significant decreases were found in the test food group in terms of Feeling of physical fatigue ($p = 0.0035$), Speed of thinking (confusion) ($p = 0.0080$), Motivation (liveliness) ($p = 0.0032$), Lightness of the body ($p = 0.0002$), Mood ($p = 0.0014$), Calmness ($p = 0.0081$), Awakening ($p < 0.0001$), and Subjective

sleep duration (p = 0.0410). In the control food group, significant decreases were noted for Speed of thinking (confusion) (p = 0.0423), Lightness of the body (p = 0.0166), Awakening (p = 0.0012), and Subjective sleep duration (p = 0.0073).

Table 5. Change in PSQI scores of subjects.

		week 0 (baseline)	**week 4**	**⊿week 4**
C1: Quality of sleep	Active	1.9 ± 0.4	1.4 ± 0.6**	−0.5 ± 0.5
	Placebo	1.7 ± 0.5	1.4 ± 0.5*	−0.3 ± 0.5
	p	0.2891	1.0000	0.3438
C2: Sleep onset time	Active	1.7 ± 0.8	1.5 ± 1.0	−0.2 ± 0.8
	Placebo	1.5 ± 0.8	1.3 ± 1.0	−0.2 ± 0.8
	p	0.3667	0.3535	1.0000
C3: Sleep duration	Active	1.5 ± 0.7	1.7 ± 0.9	0.2 ± 0.5
	Placebo	1.5 ± 0.7	1.6 ± 0.9	0.1 ± 0.8
	p	1.0000	0.5898	0.5898
C4: Sleep efficiency	Active	0.4 ± 0.5	0.2 ± 0.4	−0.2 ± 0.4
	Placebo	0.2 ± 0.4	0.2 ± 0.4	0.0 ± 0.6
	p	0.1250	1.0000	0.3984
C5: Sleeping difficulty	Active	1.0 ± 0.0	1.0 ± 0.2	0.0 ± 0.2
	Placebo	1.0 ± 0.2	0.9 ± 0.3	0.0 ± 0.2
	p	1.0000	1.0000	1.0000
C6: Hypnotics use	Active	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Placebo	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	p	1.0000	1.0000	1.0000
C7: Daytime awakening difficulty	Active	1.2 ± 0.9	1.0 ± 0.8	−0.3 ± 0.6
	Placebo	1.4 ± 0.9	0.9 ± 0.6**	−0.5 ± 0.7
	p	0.3877	1.0000	0.3367
Total Score (C1~C7)	Active	7.8 ± 2.0	6.8 ± 2.4**	−1.0 ± 1.5
	Placebo	7.2 ± 2.1	6.3 ± 2.1	−1.0 ± 2.4
	p	0.9793	0.3096	0.9700

Each value is shown as the mean ± SD. Statistical differences were evaluated by Wilcoxon signed-rank test. ** $p < 0.01$, * $p < 0.05$ versus baseline (n=21).

Evaluation of adverse events

Changes in the physical examination results were within the range of physiological fluctuations of the subjects, and the investigators considered that there was no problem. Additionally, changes in laboratory test results over time were within the range of the subjects' characteristic values or physiological fluctuations and were considered no clinical problem.

However, some subjects demonstrated changes beyond the range of reference values. Subjects reported some subjective symptoms, which emerged the day after ingestion of the test food and could not be attributed to any specific cause.

Although their causal relationships with the test foods could not be ruled out, all symptoms were mild and disappeared after the completion of ingestion. As a result, the investigators did not consider these incidents as adverse events (Tables 7-9).

Table 6. Change in VAS questionnaire scores of subjects.

		week 0 (baseline)	week 4	⊿week 4
Feeling of physical fatigue	Active	6.74 ± 1.34	4.79 ± 2.28**	−1.95 ± 2.57
	Placebo	5.76 ± 2.17	5.54 ± 2.17	−0.22 ± 1.80
	p	0.0429†	0.1432	0.0350†
Feeling of mental fatigue	Active	5.80 ± 1.96	4.91 ± 2.12	−0.89 ± 2.21
	Placebo	5.33 ± 2.55	4.71 ± 2.76	−0.62 ± 2.55
	p	0.5002	0.9599	0.6502
Speed of thinking	Active	5.34 ± 1.85	3.89 ± 2.26**	−1.45 ± 2.28
(confusion)	Placebo	4.70 ± 1.94	4.12 ± 2.00*	−0.58 ± 1.99
	p	0.0608	0.8933	0.1861
Healing of tiredness	Active	4.80 ± 2.01	3.96 ± 2.26	−0.84 ± 2.64
	Placebo	4.62 ± 2.41	4.15 ± 2.34	−0.48 ± 1.91
	p	0.5788	0.8669	0.8337
Ability to concentrate	Active	4.62 ± 2.10	3.98 ± 2.20	−0.64 ± 2.17
	Placebo	4.39 ± 2.20	3.93 ± 1.79	−0.46 ± 2.23
	p	0.3603	0.7627	0.6746
Motivation (liveliness)	Active	4.94 ± 2.36	3.60 ± 1.95**	−1.34 ± 1.95
	Placebo	4.60 ± 2.17	4.29 ± 2.02	−0.30 ± 1.49
	p	0.3335	0.2565	0.0317†
Lightness of body	Active	5.99 ± 1.95	4.52 ± 2.17**	−1.47 ± 1.60
	Placebo	5.84 ± 2.19	4.69 ± 2.23*	−1.16 ± 2.08
	p	0.6439	0.9332	0.2999
Mood	Active	4.61 ± 1.89	3.33 ± 1.68**	−1.29 ± 1.62
	Placebo	4.51 ± 2.17	4.00 ± 2.02	−0.51 ± 1.81
	p	0.7415	0.1697	0.1336
Calmness	Active	4.03 ± 1.61	2.95 ± 1.71**	−1.09 ± 1.69
	Placebo	3.64 ± 1.99	3.39 ± 2.06	−0.25 ± 1.46
	p	0.0692	0.1308	0.0444†
Awakening	Active	5.61 ± 2.28	3.54 ± 2.14**	−2.08 ± 2.10
	Placebo	5.24 ± 2.52	4.12 ± 2.58**	−1.12 ± 1.54
	p	0.5768	0.0807	0.0680
Onset of sleep	Active	5.63 ± 2.31	4.57 ± 2.77	−1.07 ± 2.16
	Placebo	5.50 ± 2.03	4.62 ± 2.75	−0.88 ± 2.76
	p	0.5903	0.9733	0.5906
Subjective sleep duration	Active	5.93 ± 1.79	4.76 ± 2.52*	−1.17 ± 2.20
	Placebo	5.95 ± 2.41	4.66 ± 2.72**	−1.29 ± 2.39
	p	0.9866	0.7352	0.8669

Each value is shown as the mean ± SD. Statistical differences were evaluated by Wilcoxon signed-rank test. † $p < 0.05$ among the groups, ** $p < 0.01$, * $p < 0.05$ versus baseline (n=21).

Table 7. Change in serum biochemical parameters of subject.

		Reference value			week 0 (baseline)	week 4
Total bilirubin		0.2–1.2	mg/dL	Active	0.81 ± 0.22	0.92 ± 0.34*
				Placebo	0.89 ± 0.33	0.92 ± 0.26
AST (GOT)		10–40	U/L	Active	24.0 ± 6.7	25.7 ± 8.7*
				Placebo	31.3 ± 44.4	25.4 ± 7.3
ALT (GPT)		5–45	U/L	Active	20.8 ± 9.7	22.3 ± 11.0
				Placebo	31.4 ± 64.5	22.2 ± 10.0
ALP		100–323	U/L	Active	210.0 ± 65.8	204.8 ± 58.8
				Placebo	213.9 ± 66.5	207.4 ± 59.0
LDH		120–240	U/L	Active	187.6 ± 24.4	187.4 ± 27.4
				Placebo	193.7 ± 43.7	189.2 ± 32.8
γ-GTP	Male	< 80	U/L	Active	35.0 ± 22.8	32.3 ± 18.7
	Female	< 30		Placebo	35.3 ± 33.6	33.4 ± 18.4
Total protein		6.7–8.3	g/dL	Active	7.54 ± 0.27	7.43 ± 0.33*
				Placebo	7.57 ± 0.40	7.48 ± 0.38
Albumin		3.8–5.2	g/dL	Active	4.51 ± 0.23	4.45 ± 0.27
				Placebo	4.52 ± 0.28	4.51 ± 0.22
Creatinine	Male	0.61–1.04	mg/dL	Active	0.760 ± 0.171	0.749 ± 0.152
	Female	0.47–0.79		Placebo	0.754 ± 0.154	0.750 ± 0.164
Urea Nitrogen		8.0–20.0	mg/dL	Active	12.55 ± 2.75	12.41 ± 3.19
				Placebo	12.97 ± 3.18	12.95 ± 3.29
Uric Acid	Male	3.8–7.0	mg/dL	Active	5.38 ± 1.59	5.28 ± 1.32
	Female	2.5–7.0		Placebo	5.21 ± 1.25	5.41 ± 1.63
Serum amylase		40–122	U/L	Active	77.1 ± 19.7	76.0 ± 20.7
				Placebo	77.4 ± 19.9	77.2 ± 20.2
CPK	Male	60-270	U/L	Active	112.5 ± 54.8	107.9 ± 43.2
	Female	40-150		Placebo	114.3 ± 47.1	117.0 ± 58.4
Sodium		137–147	mEq/L	Active	142.2 ± 2.0	141.2±2.0**
				Placebo	142.2 ± 1.8	141.2±1.8**
Potassium		3.5–5.0	mEq/L	Active	4.24 ± 0.22	4.22 ± 0.23
				Placebo	4.24 ± 0.29	4.27 ± 0.27
Chlorine		98–108	mEq/L	Active	104.1 ± 2.5	103.9 ± 2.0
				Placebo	104.2 ± 2.3	104.1 ± 2.3
Calcium		8.4–10.4	mg/dL	Active	9.54 ± 0.27	9.42 ± 0.31*
				Placebo	9.56 ± 0.32	9.51 ± 0.33
Magnesium		1.9–2.5	mg/dL	Active	2.16 ± 0.13	2.18 ± 0.13
				Placebo	2.18 ± 0.14	2.19 ± 0.13
Iron	Male	50–200	μg/dL	Active	108.7 ± 40.8	113.1 ± 42.8
	Female	40–180		Placebo	112.2 ± 41.8	118.4 ± 40.4
Glucose		70–109	mg/dL	Active	82.97 ± 8.86	84.21 ± 10.08
				Placebo	82.32 ± 9.38	83.21 ± 8.06
Total cholesterol		120–219	mg/dL	Active	222.1 ± 34.5	214.6 ± 36.4
				Placebo	216.1 ± 32.9	219.9 ± 35.4
LDL cholesterol		65–139	mg/dL	Active	132.3 ± 30.1	125.6 ± 29.2*
				Placebo	125.3 ± 26.9	130.9 ± 33.1*
HDL cholesterol	Male	40–85	mg/dL	Active	68.2 ± 17.1	66.0 ± 19.8
	Female	40–95		Placebo	68.4 ± 18.9	66.4 ± 17.2*
Triglyceride		30–149	mg/dL	Active	98.8 ± 61.3	94.3 ± 57.6
				Placebo	105.8 ± 62.1	94.4 ± 57.2*

Each value is shown as the mean ± SD. Statistical differences were evaluated by paired t-test. ** $p < 0.01$, * $p < 0.05$ versus baseline (n=38).

Table 8. Change in hematologic parameters of subjects during test period.

	Reference value				week 0 (baseline)	week 4
Leukocyte content		3300–9000	/μL	Active	5250.0 ± 1297.3	5407.9 ± 1529.1
				Placebo	5605.3 ± 1515.5	5457.9 ± 1221.8
Erythrocyte content	Male	430–570	$\times 10^4$/μL	Active	463.6 ± 41.1	466.4 ± 37.5
	Female	380–500		Placebo	460.2 ± 43.9	466.2 ± 42.7*
Hemoglobin	Male	13.5–17.5	g/dL	Active	14.13 ± 1.70	14.13 ± 1.70
	Female	11.5–15.0		Placebo	13.99 ± 1.70	13.97 ± 1.52
Hematocrit	Male	39.7–52.4	%	Active	43.40 ± 4.35	42.94 ± 3.67
	Female	34.8–45.0		Placebo	43.12 ± 4.18	42.85 ± 4.24
MCV		85–102	fL	Active	93.7 ± 6.7	92.2 ± 6.4**
				Placebo	94.0 ± 6.9	92.1 ± 6.6**
MCH		28.0–34.0	pg	Active	30.48 ± 2.73	29.98 ± 2.69**
				Placebo	30.48 ± 3.04	30.05 ± 2.93**
MCHC		30.2–35.1	%	Active	32.47 ± 1.07	32.47 ± 1.17
				Placebo	32.38 ± 1.34	32.58 ± 1.32
Platelet content		14.0–34.0	$\times 10^4$/μL	Active	26.69 ± 7.97	25.98 ± 6.45
				Placebo	26.15 ± 7.03	25.75 ± 6.71

Each value is shown as the mean ± SD. Statistical differences were evaluated by paired t-test. ** $p < 0.01$, * $p < 0.05$ versus baseline (n=38).

Table 9. Change in urinary parameters of subjects during the test period.

	Reference value		week 0 (baseline)	week 4
pH	5.0–7.5	Active	6.22 ± 0.65	6.29 ± 0.84
		Placebo	6.14 ± 0.73	6.14 ± 0.69
Gravity	1.006–1.030	Active	1.0159 ± 0.0074	1.0152 ± 0.0079
		Placebo	1.0151 ± 0.0079	1.0171 ± 0.0087
Protein	(-)	Active	0.1 ± 0.4	0.1 ± 0.3
		Placebo	0.1 ± 0.2	0.1 ± 0.2
Glucose	(-)	Active	0.0 ± 0.0	0.0 ± 0.0
		Placebo	0.0 ± 0.0	0.0 ± 0.0
Urobilinogen	(±)	Active	1.0 ± 0.0	1.0 ± 0.0
		Placebo	1.0 ± 0.0	1.0 ± 0.2
Bilirubin	(-)	Active	0.0 ± 0.0	0.0 ± 0.0
		Placebo	0.0 ± 0.0	0.0 ± 0.0
Occult blood	(-)	Active	0.3 ± 0.9	0.2 ± 0.7
		Placebo	0.3 ± 0.9	0.3 ± 0.9
Keton bodies	(-)	Active	0.0 ± 0.0	0.0 ± 0.0
		Placebo	0.0 ± 0.0	0.0 ± 0.0

Each value is shown as the mean ± SD. Statistical differences were evaluated using paired t-test (n=38).

DISCUSSION

Recently, lactic acid bacteria have been reported to have useful biological functions, such as improvement of intestinal flora [10-12] as well as positive effects on lipid metabolism [13, 14], gut–brain interaction [15, 16], and circadian rhythm [17].

Based on various functions of lactic acid bacteria, we studied their effects on the quality of sleep using subjective evaluations (i.e. questionnaires) and objective evaluations (i.e. electroencephalography) and revealed improvement of some of the indices of sleep and fatigue. Specifically, in addition to the quality of sleep, Feeling of physical fatigue, Motivation (liveliness), and Calmness improved after ingesting a test lactic acid bacteria-containing food. The SE also improved in the test food group, although no intergroup differences were seen in the sleep duration. The SE is a measure indicating a ratio of actual sleeping time to time in bed. Both the ratio and length of arousal during sleep tended to improve in the test food group. Therefore, the findings of the study suggest that the consumption of LBH MIKI-020 improved the quality of sleep, thereby improving the feeling of physical fatigue and motivation. The portable recording system (ZA) used for objective sleep evaluation is a highly reliable system to accurately understand the sleep state. The portable recording system (ZA) is made up of a wireless transmitter and a receiver. Electrodes attached to the forehead and the rims of eyes on both sides transmit digitally converted signals to the receiver placed on the bedside, which stores the sleep-related EEG information [18].

LBH MIKI-020, the lactic acid bacterium strain used in this study, was isolated from a newborn baby and is used in a drink product for health promotion and nutritional supplementation in children [19]. The lactic acid bacteria-containing food prepared as the test food contained a mixture of fermentation products, living bacteria, and ground bacteria of LBH MIKI-020. Many studies have shown that useful bacteria, such as lactic acid bacteria, generally produce some activity upon ingestion whether they are live or killed bacteria or simply their metabolites. Examples of such activities include intestinal regulation action based on the improvement of the intestinal environment and antiallergic action via immunomodulation [20, 21]. The test food contained theanine (200 mg/day) in addition to the LBH MIKI-020; theanine reportedly has a relaxation effect and improves the quality of sleep [6, 22]. However, previous studies have not addressed the improvement of subjectively assessed the quality of sleep by theanine. Under these circumstances, the test food was prepared to test if theanine and lactic acid bacteria work synergistically.

We consider that the lactic acid bacteria-containing food improved the quality of sleep in this study because of the following reasons. Recent studies have shown that body temperature changes represent an important factor in the circadian rhythm [23, 24]. Deep body temperature increased in the day and decreased in the night, while peripheral cutaneous temperature was elevated in the night. These diurnal changes in body temperature are reportedly necessary for balanced heat production and heat dissipation in the body and are deeply involved in the emergence of REM/non-REM sleep [25, 26]. As a possible link between this phenomenon and the mechanism of sleeping, peroxisome proliferator-activated receptor alpha (PPARα) agonists and other drugs that accelerate lipid metabolism have been reported to be involved in the regulation of the biological clock and improvement of the circadian rhythm in sleep disorders

[27]. PPARα agonists induce fibroblast growth factor 21 (FGF21) expression in the liver, which reaches the brain and induces the expression of neuropeptide Y (NPY), thereby resulting in hypothermia [28-31]. Furthermore, levels of serotonin in the brain were reduced by decreasing enteric bacteria in experiments in mice [32]. Killed lactic acid bacteria have been reported to improve not only the intestinal environment but also lipid metabolism via PPARα in a mouse model [33], suggesting that they regulate body temperature through FGF21 production.

On the basis of these findings, to clarify its mechanism of action, we plan to study the effects of LBH_MIKI-020 alone on the PPARα ligand action, the deep body temperature lowering action via liver FGF21 production, and effects on the endocrines involved in the circadian rhythm in the future.

CONCLUSION

The results of the present study suggest that SE improved by continuous consumption of lactic acid bacteria-containing foods. Among various sleep disorders, a large population in Japan has trouble with the quality of sleep. Consumption of lactic acid bacteria-containing foods can be a useful means to safely improve the quality of sleep from the viewpoint of self-medication as well. Future tasks include further clarification of active ingredients and mechanism of action.

List of Abbreviations: OSA sleep questionnaire, Obstructive Sleep Apnea Syndromes Oguri-Shirakawa-Azumi sleep questionnaire; PSQI, Pittsburgh Sleep Quality Index; VAS, Visual Analogue Scale; EEG electroencephalogram.

Authors' Contributions: MN, MK, and HY designed the research protocol. MK provided test and placebo foods tablet. N.M. is investigator of this study and analyzed the physical data for evaluation of adverse events. MK performed statistical analysis. KW and HY wrote the manuscript. HY and TE reviewed and edited the manuscript. TE had primary responsibility for the final content. All authors read and approved the final version of the manuscript.

Competing Interests: Masafumi Nakagawa, Miyuki Kawaji, and Hirotaka Yamamoto are employees of MIKI Corporation. MIKI Corporation has a food product containing LBH MIKI-020 and theanine.

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