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Effect of combining sleep-promoting food intake and electric field application on sleep in healthy participants: A pilot study

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ABSTRACT

Background: Functional foods and electric fields (EFs) have been previously reported as interventions for insomnia other than medications. As for functional foods, gamma-aminobutyric acid (GABA) and lafma have been reported to be related to sleep. EFs have also been reported to have a sleep-related, anti-stress effect in mouse model experiments. However, the effects of combining these two methods on the human body remain poorly studied.

Objective: Thus, this study aimed to investigate the cointervention effect of sleep-promoting functional food intake and EF application on sleep quality in healthy participants.

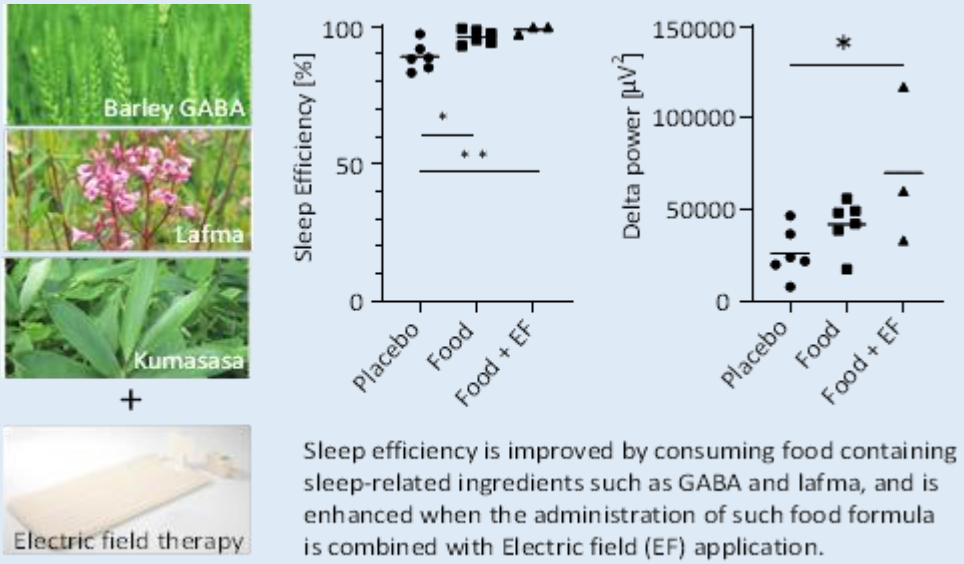
Methods: Fifteen healthy participants were divided into three groups. The Food and Placebo groups were given active tablets containing food mixture of GABA and lafma, and placebo tablets, respectively, for 4 weeks. Meanwhile, the Food plus EF group used an EF therapy device during sleep in addition to the active food tablets. Sleep quality was evaluated using electroencephalography and sleep questionnaires.

Results: Sleep efficiency (SE) was significantly higher in the Food group and the Food plus EF group than the Placebo group at 4 weeks. The Food plus EF group also had a significantly higher SE involving sleep latency.

Conclusions: Food mixture containing known sleep-promoting ingredients such as GABA and lafma can improve sleep quality, and the improvement effect can be enhanced when administered in combination with an EF.

Keywords: electric field therapy, Kumasasa (*Sasa senanensis*), electric fields, sleep quality, electroencephalography

Clinical trial registration: Approval No.: R1812; Approval date: 21 Feb. 2019



INTRODUCTION

In today's urban living, mental health problems and reduced sleep quality are becoming a problem because of changes in the environment and various lifestyles. For example, an epidemiological study involving Chinese university students revealed problematic mobile phone use is associated with mental health and reduced sleep quality [1]. A Japanese study also reported that sleep duration depends on seasonal changes; in other words, sleep duration is longest in the winter and shortest in the summer [2]. This relationship between season and sleep is more prevalent in middle-aged people than in younger people [3]. In addition, a diet high in carbohydrates, such as sweets and noodles, and low in vegetables was associated with a lower sleep quality among Japanese female workers. In elderly population living in urban areas, dietary content and appetite stimulation are important to maintain sleep quality. Thus, sleep quality varies depending on lifestyle, age group, season, and dietary habits [4].

Given these health problems experienced by modern people, the Ministry of Health, Labor, and Welfare has approved the use of electric field (EF) therapy devices for insomnia [7]. This type of device treats the human body with EFs at an extremely low frequency to modulate the blood flow, nervous system, and endocrine system, for prevention and treatment of

various symptoms such as insomnia, headache, shoulder stiffness, and chronic constipation. For example, animal experiments showed that EFs could suppress stress hormones in immobilization-induced mice; hence, EFs have an anti-stress effect, considering that the EF effects depend on the strength, duration, and area of EF exposure [5-6]. Clinically, EF improves sleep [7] and increases the electroencephalographic (EEG) theta wave during daytime [8]. The effects of EF on insomnia can be explained by the anti-stress effect.

Meanwhile, functional food ingredients for sleep such as glycine [9], L-serine [10], L-theanine [11], ornithine [12], gamma-aminobutyric acid (GABA) [13], lactoferrin [14], vitamin B [15], asparagus [16], and lafma [17], are related to sleep and relaxation. These ingredients are grouped into amino acids and their analogs, herbal extracts, and probiotics, which all reportedly enhance sleep quality and recovery from fatigue. Some of the previous reports suggested improvement in sleep was associated with stress-relieving effect [12-13, 16].

Although solitary administration of EFs or functional foods has been reported to improve health problems and sleep quality in humans, the effectiveness is not enough. Moreover, the effect of combining these two approaches on sleep remains unreported. Thus, this study aimed to investigate the cointervention effect of a food mixture containing sleep-enhancing ingredients and EF on sleep.

METHODS

A randomized, double-blind, placebo-controlled, parallel intergroup comparison study was conducted as a human trial. The investigational review board of Miura Clinic approved this study (Approval No.: R1812; Approval date: 21 Feb. 2019)
(UMIN Trial ID: UMIN000035977)

Participants: Healthy middle-aged females were recruited.

A. The inclusion criteria were:
   a. healthy females over 40-64 years of age
   b. after menopause
   c. with lower sleep quality
   d. without sleep medication
   e. well-informed about the aim and content of the study, competent to consent, voluntarily applied for participation, and gave a written consent.

B. The exclusion criteria were:
   a. volunteers with medical history of liver, kidney, digestive system, heart, and/or any other disease which can interfere the study results
   b. diagnosed with heart disease limiting strenuous exercise
   c. with implantable medical electronic devices vulnerable to electromagnetic interference
   d. with wearable medical electronic devices (ex. electrocardiographs, pacemakers)
   e. with abnormal liver and kidney function tests
   f. under treatment
   g. with food and/or drug allergies
   h. playing hard sports and/or on a diet
   i. unable to refrain from taking health food and/or quasi-drug
   j. taking continuously over-the-counter drug
   k. taking health food and/or medication for sleep improvement
   l. using sleep-promoting bedding and/or devices
   m. being or having been a user of EF therapy devices
   n. drinking excessive alcohol or being unable to refrain from drinking during the EEG measurement and medical checkups
   o. during pregnancy or having a plan to be pregnant and breastfeeding
   p. currently participating in, or having a plan to participate in another clinical study
   q. being judged inappropriate by the attending doctor

Test samples and the administration method: The ingredients of the three tablets for our food sample are listed in Table 1. Main functional ingredients for sleep were lactic-fermented barley GABA (120 mg), lafma extract (50 mg), 0.1% vitamin B12 (3 mg), and vitamin B6 (1.5 mg) (Table 1). For the placebo sample, the main ingredients of the three tablets were dexitrin (264 mg) and maltitol (264 mg). Of note, the placebo tablets resembled the food sample in appearance.

In both samples, one package (containing 3 tablets) was administered daily, orally with water, 30-60 minutes before sleep, with no chewing, for 4 weeks.

Table 1. Ingredient list of the food samples.

Ingredients per 3 tablets	Investigational food	Placebo food
Lactic-fermented barley GABA	120.0 mg	-
Lafma extract	50.0 mg	-
Vitamin B12 (0.1 %)	3.0 mg	-
Vitamin B6	1.5 mg	-
Kumasasa powder	50.0 mg	-
Dexitrin	-	264.0 mg
Maltitol	-	264.0 mg
Cellulose	400.0 mg	112.2 mg
Granular silicon oxide	13.0 mg	6.6 mg
Calcium stearate	22.5 mg	13.2 mg
Methyl cellulose	30.0 mg	30.0 mg
HPMC		
Titanium oxide		
Glycerine		
Total	690.0 mg	690.0 mg

GABA, gamma-aminobutyric acid; HPMC, hydroxypropyl methylcellulose.

EF exposure system: We used an EF therapy device for home use (Healthtron HEF-N6000WG; Approval No.: 228AKBZX00085000) (Fig. 1). Antiphase voltages up to 5000 V were applied to the upper and lower electrodes, causing 60 Hz, sinusoidal EF generation between the electrodes. While sleeping up to 8 hours, participants were treated with the EFs on the pad.

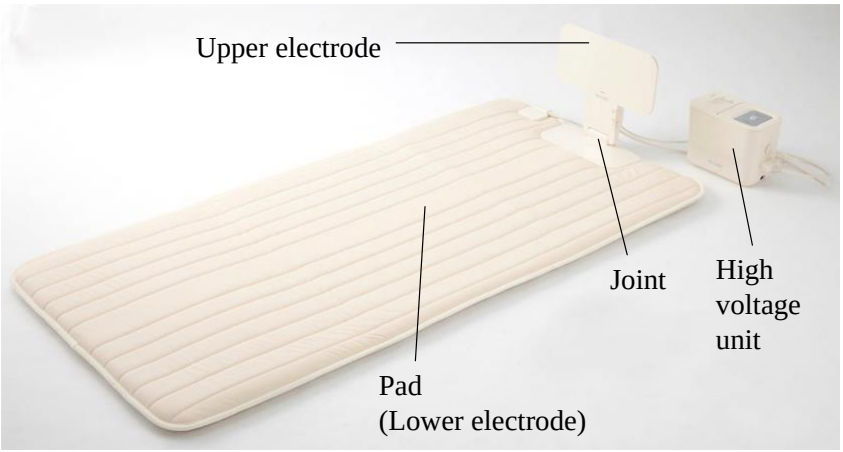


Figure 1. EF therapy device. Antiphase voltages were applied to the upper and lower electrodes, leading to sinusoidal EF generation between the electrodes. Participants were treated with the EFs during sleep on the pad. EF, electric field.

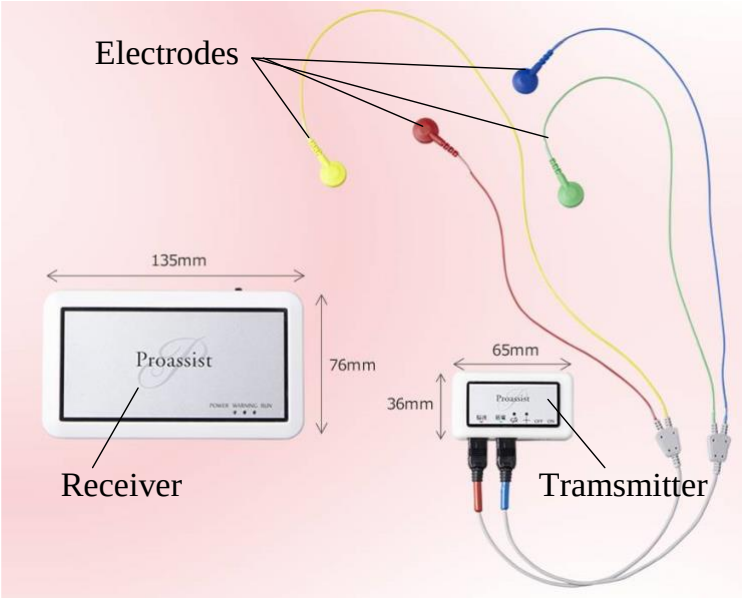


Figure 2. Sleep measuring device: "Brain wave sensor ZA-X (Ten)." Electroencephalogram and electromyogram were derived from the electrodes. Signals were wirelessly transmitted and received for sleep analysis.

Sleep measurement device and sleep parameters: Sleep EEG was measured by the EEG sensor ZA-X (Proassist, Ltd., Osaka, Japan) (Fig. 2).

The changes in the stages of sleep during night are represented as hypnograms (Fig. 3). Rapid eye movement (REM) and non-REM sleep alternate in the sleep cycle during night. Sleep is classified in four stages, non-REM sleep stage N1, N2, N3, and REM sleep stage. Non-REM sleep becomes deeper from N1 to N3.

Sleep parameters were defined as below.

Sleep Period Time (SPT) [min]: The duration of time from sleep onset to final awakening. Sleep Total Sleep Time (TST) [min]: The actual sleep time except for intermittent awakening in SPT.

Sleep Efficiency (SE) [%]: (TST/SPT) × 100.

Sleep Latency (SL) [min]: The duration of time from bedtime to the onset of sleep.

SE including SL [%]: (TST/(SPT + SL)) × 100.

Stage N3: Twenty percent or more of an epoch consists of slow wave activity, or EEG delta wave of 0.5 Hz to 2 Hz with peak-to-peak amplitude of more than 75 μV.

Delta power: The power of EEG delta wave expressed in μV^2.

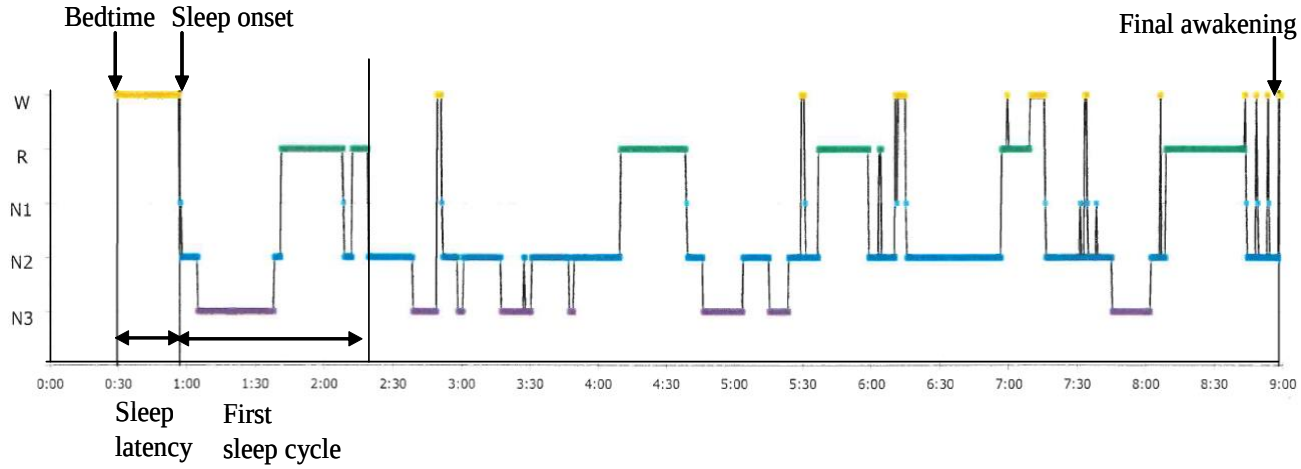


Figure 3. Hypnogram. Rapid eye movement (REM) and non-REM sleep alternate in the sleep cycle during night. Sleep is classified in four stages, non-REM sleep stage N1, N2, N3, and REM sleep stage. Non-REM sleep becomes deeper from N1 to N3. W, stage wake; R, stage REM; N1, non-REM sleep stage N1; N2, non-REM sleep stage N2; N3, non-REM sleep stage N3.

Study design: Healthy females who fulfilled the inclusion criteria were selected and assigned to the following groups: Placebo group, administered with the placebo food; Food group, administered with the investigational food; and Food plus EF group, cotreated with the investigational food and the EF therapy device.

Study schedules and design are indicated in Fig. 4. All participants visited the medical site and underwent intake and inquiry, physical tests, and questionnaire survey. After the measurement method was explained, they were offered investigational or placebo food and the EEG sensor was applied. Investigational devices were then sent to participants' home.

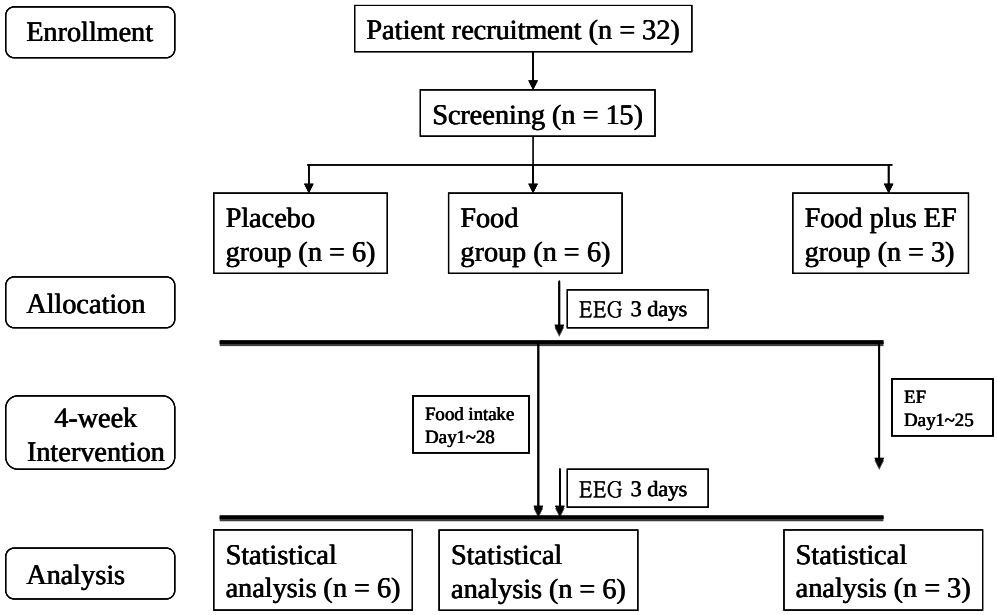


Figure 4. Flowchart of the trial. Out of 32 potential candidates recruited, 15 were selected after screening, of which 6, 6, and 3 were assigned to the Placebo, Food, and Food plus EF groups, respectively. All participants were eligible.

For the Placebo and Food groups, sleep EEG was measured for 3 days before intake according to the user's manual at participants' home. Similarly, sleep EEG was measured for 3 days before the checkup at week 4. Study food was administered from day 1 to day 28.

For the Food plus EF group, the investigational device was used every night at bedtime according to the user's manual from day 1 to day 25, whereas the investigational food was administered from day 1 to day 28. During the sleep EEG measurement period, the investigational device was not applied to prevent electric interference.

From day 1 to day 28, the participants were asked to record their placebo or investigational food intake, alcohol intake, and exercise in a sleep diary. They were also asked to record their activity during the sleep EEG measurement period. When they could not undergo checkups during the designated time, they were allowed to change the schedule before or after a 7-day period.

Subsequently, the participants were asked to revisit the site immediately after the 4-week intervention to conduct the week 4 (day 28) checkups, physical tests, and questionnaires.

Physical examination was conducted before and after the 4-week period. Likewise, sleep quality was evaluated using the Oguri-Shirakawa-Azumi (OSA) sleep inventory and visual analog scale (VAS) questionnaire for sleep and fatigue before and after the intervention. In addition, sleep EEG was measured by the EEG sensor ZA-X (Proassist, Ltd., Osaka, Japan) (Fig. 2). VAS questionnaire for physical condition was applied to the Food plus EF group only.

Statistical analysis: Intergroup differences were evaluated at weeks 0 and 4, where we used the Tukey-Kramer test for the sleep EEG and OSA sleep inventory and the Steel-Dwass test for the VAS questionnaire. Conversely, intragroup differences from week 0 to 4 were evaluated by paired sample *t*-test for clinical characteristics, sleep EEG, and OSA sleep inventory, and Wilcoxon signed rank test for the VAS questionnaire. We considered $p < 0.05$ to be statistically significant.

RESULTS

Table 2 summarizes the participants' physical examination data. The Placebo, Food, and Food plus EF groups consisted of 6, 6, and 3 eligible participants, with a mean age of 55.7 ± 4.9, 55.8 ± 4.2, and 54.7 ± 5.8 years, respectively, indicating no statistical difference (Table 2). Other data also showed no statistical difference, except the reduced systolic blood pressure in the Placebo group after the 4-week intervention (Table 2).

Table 2. Physical characteristics of the participants.

	Group	n	0 week			4 week			p value
Age(years)	Placebo	6	55.7	±	4.9		-		-
	Food	6	55.8	±	4.2		-		-
	Food + EF	3	54.7	±	5.8		-		-
Height (cm)	Placebo	6	156.52	±	3.49		-		-
	Food	6	160.98	±	5.42		-		-
	Food + EF	3	160.17	±	1.02		-		-
Weight (kg)	Placebo	6	55.13	±	5.20	54.85	±	6.71	0.7036
	Food	6	55.13	±	5.70	54.22	±	5.36	0.1331
	Food + EF	3	49.50	±	4.19	50.07	±	3.41	0.4145
BMI (kg/m2)	Placebo	6	22.55	±	2.57	22.45	±	3.20	0.7322
	Food	6	21.28	±	2.05	20.91	±	1.65	0.1402
	Food + EF	3	19.29	±	1.46	19.51	±	1.19	0.4137
Systric BP (mmHg)	Placebo	6	123.7	±	18.5	118.7	±	19.3	0.0449*
	Food	6	112.8	±	14.4	113.5	±	11.6	0.7799
	Food + EF	3	115.0	±	10.4	108.3	±	6.0	0.4532
Diastric BP (mmHg)	Placebo	6	79.0	±	13.5	76.3	±	13.2	0.4600
	Food	6	70.3	±	15.0	71.7	±	10.2	0.5880
	Food + EF	3	62.7	±	1.5	59.7	±	9.8	0.6440
Pulse (/min)	Placebo	6	85.2	±	7.7	82.7	±	3.7	0.5177
	Food	6	74.7	±	13.8	80.3	±	14.2	0.0848
	Food + EF	3	75.3	±	10.1	74.7	±	7.6	0.9534

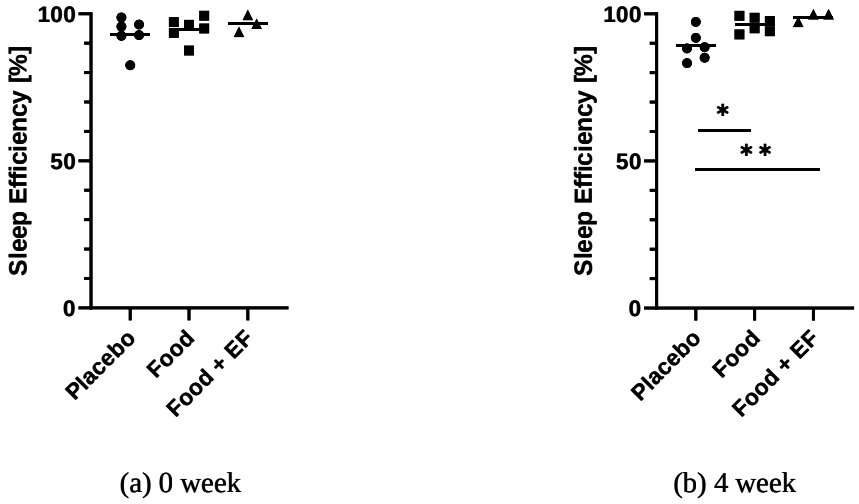
Intragroup changes were analyzed using paired sample *t*-test. *: $p < 0.05$ BMI, body mass index; BP, blood pressure.

The food intake rate was 100.0% ± 0.0%, 99.4% ± 1.5%, and 99.0% ± 1.8% in the Placebo-, Food-, and Food plus EF group, respectively, with no statistical difference.

At 4 weeks, sleep efficiency (SE) was significantly higher in the Food group and the Food plus EF group than the Placebo group (Fig. 5); when sleep latency was involved in the SE, the Food plus EF group showed a significantly higher value than the Placebo group (Fig. 6).

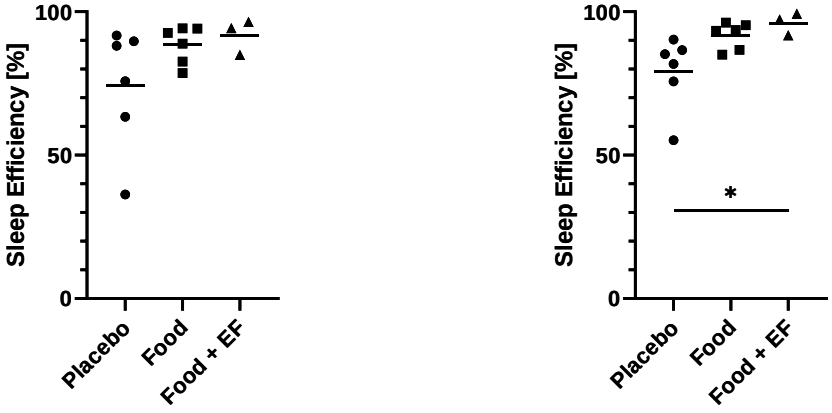
Sleep latency and the amount of stage N3 were not significantly different between the three groups at week 4 (Fig. 7).

In contrast, power spectrum analysis of EEG revealed that the EEG delta power in the first sleep cycle was significantly higher in the Food plus EF group than in the Placebo group at week 4 (Fig. 8).



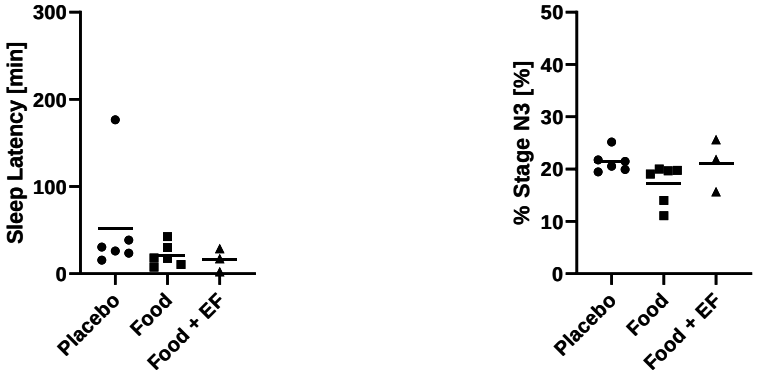
(a) 0 week (b) 4 week

Figure 5. Sleep efficiency at weeks (a) 0 and (b) 4. Intergroup differences were analyzed using the Tukey-Kramer test. Sleep efficiency was significantly higher in the Food group and Food plus EF group than in the Placebo group at 4 weeks. **: $p < 0.01$, *: $p < 0.05$. EF, electric field.



(a) 0 week (b) 4 week

Figure 6. Sleep efficiency including sleep latency at weeks (a) 0 and (b) 4. Intergroup differences were analyzed using the Tukey-Kramer test. The Food plus EF group showed a significantly higher value than the Placebo group at 4 weeks. *: $p < 0.0$



(a) Sleep latency (b) Percentage of sleep N3

Figure 7. (a) Sleep latency and (b) percentage of sleep N3 at week 4. Intergroup differences were analyzed using the Tukey-Kramer test. Sleep latency and the amount of stage N3 showed no statistically significant difference between the three groups.

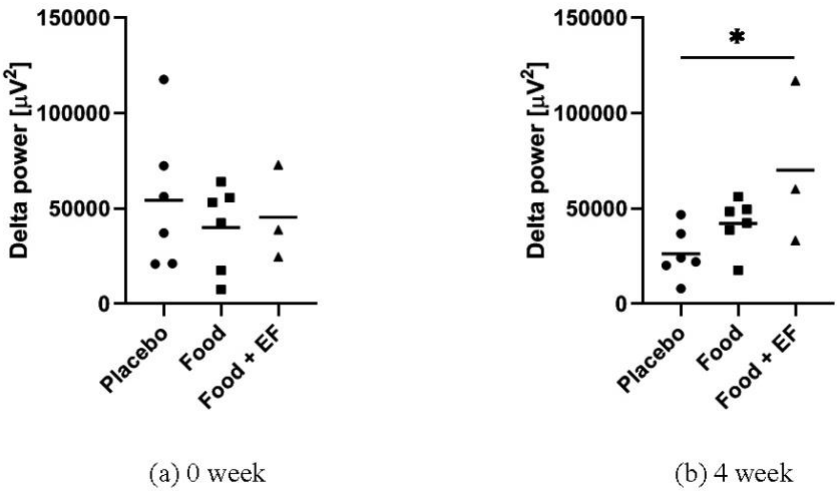


Figure 8. Delta power at (a) 0 week and (b) 4 weeks. Inter-group differences were analyzed using Tukey-Kramer test. Food plus EF group showed significantly higher value than that of Placebo group at 4 weeks. *: $p < 0.05$

The OSA sleep inventory showed that intragroup changes were significant in Factor II (Initiation and maintenance of sleep) of the Food plus EF group and Factor V (Sleep time) of the Food group (Table 3).

Results of the VAS questionnaire for sleep and fatigue revealed that intragroup changes were significant in "Subjective sleep time" of the Food group and "Waking up" of the Placebo group and Food group (Table 4).

The VAS questionnaire for physical condition was not significantly different between the pre and post intervention in the Food plus EF group (Table 5). Meanwhile, one participant in the Food plus EF group filled in the free comment section, stating that she felt relieved from neck and shoulder stiffness after using the EF therapy device but felt more tired than before.

Table 3. OSA sleep inventory.

	Group	n	0 week			4 week			⊿4 week		
Factor I	Placebo	6	40.9	±	4.5	44.3	±	8.9	3.3	±	5.8
Sleepiness on rising	Food	6	42.8	±	9.2	51.8	±	12.6	9.0	±	12.5
	Food + EF	3	34.9	±	5.0	43.7	±	6.6	8.8	±	8.2
Factor II	Placebo	6	34.9	±	5.9	34.4	±	13.1	-0.5	±	9.6
Initiation and	Food	6	32.9	±	7.6	44.9	±	7.6	12.0	±	14.6
maintenance of sleep	Food + EF	3	26.1	±	4.3	44.9	±	6.8	18.7	±	4.8 *
Factor III	Placebo	6	43.0	±	10.3	41.8	±	9.0	-1.1	±	14.8
Dream	Food	6	45.8	±	17.1	39.7	±	13.4	-6.1	±	20.3
	Food + EF	3	38.8	±	7.3	53.9	±	7.8	15.1	±	14.0
Factor IV	Placebo	6	38.7	±	7.4	42.7	±	14.0	3.9	±	10.5
Recovery from Fatigue	Food	6	37.8	±	10.9	54.8	±	10.3	17.0	±	16.5
	Food + EF	3	33.2	±	9.1	39.9	±	2.9	6.7	±	12.0
Factor V	Placebo	6	40.0	±	4.2	43.9	±	9.6	3.9	±	8.6
Sleep time	Food	6	38.6	±	4.3	50.9	±	9.1	12.4	±	10.2 *
	Food + EF	3	40.0	±	4.4	42.7	±	8.7	2.8	±	9.6

Intragroup changes were analyzed using paired sample *t*-test. *: $p < 0.05$

Table 4. Visual analog scale questionnaire for sleep and fatigue.

	Group	n	0 week			4 week			⊿4 week		
Subjective	Placebo	6	7.28	±	1.07	6.07	±	2.47	-1.22	±	2.12
sleep time	Food	6	6.85	±	1.48	3.20	±	2.20	-3.65	±	2.33 *
	Food + EF	3	8.13	±	0.42	4.17	±	1.89	-3.97	±	1.53
Sleep latency	Placebo	6	7.45	±	1.96	7.25	±	2.38	-0.20	±	2.09
	Food	6	7.13	±	1.25	4.33	±	3.46	-2.80	±	3.16
	Food + EF	3	8.00	±	1.30	4.40	±	2.07	-3.60	±	1.97
Waking up	Placebo	6	6.60	±	2.34	3.70	±	3.20	-2.90	±	2.75 *
	Food	6	6.00	±	2.52	2.65	±	2.30	-3.35	±	1.94 *
	Food + EF	3	7.90	±	1.65	5.37	±	1.63	-2.53	±	3.24
Physical fatigue	Placebo	6	6.52	±	2.59	5.38	±	3.37	-1.13	±	2.37
	Food	6	6.63	±	2.28	3.15	±	2.44	-3.48	±	3.83
	Food + EF	3	7.37	±	1.63	5.63	±	1.00	-1.73	±	2.48
Mental fatigue	Placebo	6	5.95	±	2.25	4.48	±	4.01	-1.47	±	2.46
	Food	6	6.13	±	2.22	2.13	±	1.45	-4.00	±	3.03
	Food + EF	3	8.00	±	0.46	4.93	±	1.56	-3.07	±	1.93

Intragroup changes were analyzed using Wilcoxon signed rank test. *: $p < 0.05$

Table 5. Visual analog scale questionnaire for physical condition.

	Group	n	0 week			4 week			⊿4 week		
Pain in the back or waist	Food + EF	3	7.63	±	1.68	5.17	±	2.40	-2.47	±	1.01
Shoulder stiffness	Food + EF	3	9.23	±	0.12	5.43	±	3.17	-3.80	±	3.24
Appetite	Food + EF	3	4.97	±	1.32	2.57	±	1.88	-2.40	±	1.11
Pain in the hands, feet, or joints	Food + EF	3	6.23	±	3.38	4.80	±	3.65	-1.43	±	0.75
Palpitation and breath shortness	Food + EF	3	5.83	±	1.03	3.13	±	2.17	-2.70	±	2.36
Blurred vision or eyestrain	Food + EF	3	8.27	±	1.72	7.63	±	1.14	-0.63	±	1.05
Constipation	Food + EF	3	4.87	±	3.76	3.77	±	2.80	-1.10	±	1.64
Upset stomach or stomachache	Food + EF	3	6.33	±	1.50	2.83	±	2.69	-3.50	±	1.44
Irritation	Food + EF	3	7.40	±	1.01	4.93	±	2.15	-2.47	±	1.72
Blood pressure abnormality	Food + EF	3	3.90	±	2.77	2.10	±	0.87	-1.80	±	3.35
Headache or heaviness of the head	Food + EF	3	7.90	±	1.82	5.23	±	2.34	-2.67	±	3.09

Intragroup changes were analyzed using Wilcoxon signed rank test.

DISCUSSION

This study investigated the effect of administering functional food, EF therapy device, and both on sleep quality for 4 weeks by measuring the sleep EEG and assessing the subjective symptoms. The intra- and intergroup differences of the three groups were also assessed.

First, SE showed no significant differences in intragroup comparisons nor in intergroup comparisons of pre-post differences (Data not shown). In contrast, SE at week 4 was significantly higher in the Food group and Food plus EF group than in the Placebo group (Fig. 5). This change probably reflected the intervention effects

because no significant difference was noted at week 0. In fact, pre-post improvements were significant for "Sleep time" in OSA sleep inventory (Table 3) and "Subjective sleep time" in VAS questionnaire (Table 4) for the Food group, and "Initiation and maintenance of sleep" in OSA sleep inventory (Table 3) for the Food plus EF group; thus, subjective improvement also occur. Accordingly, SE improved in the Food group and Food plus EF group.

Conversely, sleep latency (Fig. 7-a) and stage N3 (Fig. 7-b) was not significantly different between the groups. Hence, the intervention had an effect to elevate SE, rather than to induce sleep or increase the total amount of deep sleep stage.

Sleep is scored as stage N3 when 20% or more of an epoch consists of slow wave activity, or delta wave of 0.5 Hz to 2 Hz with peak-to-peak amplitude of more than 75 μV. Thus, the delta wave content can be different in the same stage N3. In fact, the EEG delta power in the first sleep cycle was higher at week 4 in the Food plus EF group than the Placebo group (Fig. 8-b), suggesting that sleep became deeper by the intervention. In addition, SE including sleep latency significantly improved at week 4 in the Food plus EF group as compared to the Placebo group (Fig. 6-b). Taken together, EF treatment exhibited a synergetic effect on sleep quality.

Currently available sleep studies using the EF therapy device were conducted mainly by subjective questionnaire survey, and objective measurements were mostly performed using an actigram and body movements. Sleep structure, such as the discrimination of sleep stage N1 and stage N2, cannot be analyzed without EEG measurement. To our knowledge, our study is the first to measure sleep with EEG for the study of EF therapy device. Additionally, no previous studies have reported improvements in SE.

This study is also the first to evaluate the combined effect of functional food intake and EF therapy device use. The food sample used in this study was composed of ingredients such as lactic-fermented barley GABA (120 mg), lafma extract (50 mg), 0.1% vitamin B12 (3 mg), vitamin B6 (1.5 mg). GABA reportedly has an effect on sleep quality. In a previous review, benefits of GABA consumption at the early sleep stage could be associated with GABA's stress reduction properties rather than direct sleep-inducing and/or -maintaining benefits per se [13]. Lafma (*Apocynum venetum*) has anti-platelet aggregation [27], antioxidant [28-29], antihypertensive, antihyperlipidemic, antidepressant, and antidiabetic effects [30] because of its various flavonoid components. The combination of lafma and GABA improves sleep quality in a human clinical trial [31]. Moreover, vitamin B12 deficiency has been linked to depression and insomnia. And then, the combination of vitamin B complex, melatonin, and magnesium effectively improved sleep quality in 60 people diagnosed with insomnia [15]. Sleep-promoting effect of these ingredients was observed in our study.

Physical examination revealed the systolic blood pressure was significantly lowered in the Placebo group. This can be attributed to the higher initial value for the Placebo group than that for the other groups.

We have developed a functional food using Kumasasa (*Sasa senanensis*) leaf, which contains amino acids, vitamin K, polysaccharides, and low-molecule compounds, such as *p*-coumaric acid, ferulic acid, vanillin, apigenin, luteolin, tricin, syringaresinol, phenylpropanoid, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, and 3,4-dihydroxybenzaldehyde [18-19]. Kumasasa demonstrated antiultraviolet [20], antiviral [21], and antioxidation effects in a rat mesenchymal ischemia-reperfusion model [22], an antitumor effect in mice K562 cells (human chronic myeloid leukemia) and YAC1 cells (murine lymphoma) [23], and an improvement effect for oral environments [24]. It can also inhibit nervous injury by amyloid beta

[25]; nitric oxide and prostaglandin E2 from macrophages; and histamine release from rat peritoneal cells [26]. Although relaxation and sleep-inducing effects of Kumasasa are not reported, antioxidation and immune modulation effects are expected. Thus, Kumasasa powder was used as an additional ingredient.

According to clinical studies of the EF therapy device, EF enhances EEG theta wave [8] and sleep quality [7]. In an animal study, EF suppressed stress hormone [5-6] and inflammatory compounds [5]. Similar to GABA intake, EF may elicit sleep-improving effect via stress reduction.

Although an ELF-EF is higher on the body surface, it is significantly attenuated inside the human body because of its shielding effect. Internal EFs were in the order of magnitude of mV/m
[8]. The insight into whether the internal EFs elicit the physiological response, or the body surface EF is transduced to the physiological system remains uncertain.

Additionally, the food samples used in this experiment were formulated with ingredients that have already been reported for their effects on sleep [32]. This study hopes that the combined use of the healthy food formula and the EF therapy device will improve the sleep-related problems of modern people.

Limitation of this study is that the number of participants was small, especially in the Food plus EF group. In addition, solitary effect of EF application remains unknown due to combination with the functional food. Further research with more participants and with “EF only group” is needed.

CONCLUSIONS

In conclusion, sleep efficiency is improved by consuming food containing sleep-related ingredients such as GABA and lafma and is enhanced when the administration of such food formula is combined with EF application.

List of Abbreviations: EEG: electroencephalography, EF: electric field, GABA: gamma-aminobutyric acid, OSA: Oguri-Shirakawa-Azumi, REM: Rapid eye movement, SE: sleep efficiency, SL: sleep latency, SPT: Sleep period time, TST: Total sleep time, VAS: visual analog scale.

Competing Interests: The human trial was supported by funding from Hakuju Institute for Health Science Co., Ltd. and was outsourced to Oneness Support Inc. Although T.N., K. H. and S. H. are employees of Hakuju Institute for Health Science Co., Ltd., these authors are not involved in the interpretation of the results of human experiments, and the independence of the investigators and the study is ensured. The other authors have no conflict of interests to declare.

Authors’ Contributions: T.N., K. H. and S. H. designed the research protocol. K. H. provided test and placebo foods tablet. N.M. is investigator of this study and analyzed the physical data for evaluation of adverse events. K.W. performed statistical analysis. T.N., S.H. and K.W. wrote the manuscript. T.N. and K.W. reviewed and edited the manuscript. K.W. had primary responsibility for the final content. All authors read and approved the final version of the manuscript.

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