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An Exploratory Evaluation of a Low Dose Theanine Consumption on Improving Sleep in Middle-Aged and Older Males

Takeichiro Tominaga[1], Daisuke Furushima[1*], Keiko Unno[2], Ibuki Sugiyama[1], Makoto Ozeki[3], Yoriyuki Nakamura[2] and Hiroshi Yamada[1]

[1]*Department of Drug Evaluation & Informatics Graduate School of Pharmaceutical Science, University of Shizuoka, Japan.*

[2]*Tea Science Center, University of Shizuoka, Japan.*

[3]*Department of Research and Development, Nutrition Division, Taiyo Kagaku Co. Ltd, Japan.*

[*]Correspondence:
Daisuke Furushima, PhD, Department of Drug Evaluation & Informatics, Graduate School of Pharmaceutical Science, University of Shizuoka, 52-1 Yada, Suruga-Ku, Shizuoka, 422-8526, Japan, Tel/ Fax: +81-054-264-5591.

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ABSTRACT

L-theanine (theanine), one of the ingredients of green tea, had been proven to have anti-anxiety, anti-stress, memory, cognitive, and sleep improvement effects in most clinical trials. However, these effects have shown efficacy at a dose of 200–400 mg of theanine a day. In this study, we explored the sleep-improving effects of a lower dose (100 mg/day) of theanine.

A double-blind, randomized crossover study was conducted on 25 middle-aged and older males. Participants were randomly assigned to Groups A and B. Group A took theanine first, and Group B took theanine later in the intervention period. The participants took theanine/placebo for one week. Electroencephalogram (EEG) parameters were measured with a monitoring device during sleep, and subjective sleep quality after waking up was evaluated using a questionnaire. Following the statistical analysis between interventions, subgroup analyses according to the participants' background information were conducted on the representative sleep parameters of non-REM sleep, REM sleep, and wakefulness.

Of the 25 participants, three withdrew from this study. In the sleep analysis, there was no significant difference in all sleep parameters between the theanine and placebo periods. In particular, the difference in the percentage of non-REM sleep in stage 2 per sleep duration was not significant ($p = 0.053$). However, subgroup analysis of the results showed that the percentage of non-REM sleep in stage 2 in groups aged <50 years was higher in theanine than that of the placebo, and in groups with green tea consumption habit of <3 to 4 days per week ($p = 0.018, 0.048$).

The intake of 100 mg/day of theanine does not have an effect on sleep improvement. However, subgroup analyses showed that theanine had an improved effect in stage 2 non-REM sleep according to age and green tea consumption habit.

Keywords

Crossover study, Electrtoencephalogram, Non-Rem sleep, Theanine.

Abbreviations

EEG: Electroencephalogram; SD: Standard Deviations.

Introduction

Sleep is an important foundation for a healthy life. However, the number of people suffering from sleep disorders has been increasing. In an international survey of sleeping problems, 56% of people in the United States, 31% of people in Europe, and 23% of people in Japan complained about not getting enough and proper sleep [1]. These results showed half of people are not satisfied with

their sleep. Sleeping changes may be caused by internal factors such as illness and stress and external factors such as lifestyle and sleep environment. Psychological stress is one of the main causes of sleep disorders [2]. If psychological stress can be controlled, it is thought that sleep disorders may improve, and people can get comfortable sleep. Therefore, it is important to search for methods that reduce stress before bedtime and improve sleep disorders.

Theanine is a free amino acid that is abundant in green tea and is known as one of the tasty ingredients of tea [3]. The chemical structure of theanine is similar to that of glutamate and glutamine, and theanine can easily cross the blood-brain barrier [4]. In the central nervous system, it is believed that theanine competitively antagonizes glutamine and inhibits glutamine transporters, thereby suppressing excitatory neurotransmission of glutamate [5-6]. Through this mechanism, theanine is thought to exert physiological effects. Previous clinical trials have reported that theanine has psychological effects such as anxiolytic and anti-stress, as well as improving memory and cognitive functions [7-10].

Recent animal research and clinical trials have shown that theanine may affect sleep improvement. In an animal experiment that examined the effects of theanine intake on sleep, rats were found to suppress the sleep-inhibiting effects of caffeine by theanine administration [11]. Kim et al. [12] reported that the administration of theanine to mice improved their brain waves, including REM sleep and waking during sleep. In contrast, in clinical trials in humans, theanine intake in healthy adult males and postmenopausal middle-aged and older women improved the feeling of falling asleep, waking up, and sleep efficiency [13-14]. They also examined whether theanine has a strong drowsiness effect during daytime [15]. Together with the previous results, the authors considered that theanine does not have a strong drowsiness effect or a strong hypnotic effect like sleeping drugs but has a very mild effect on improving sleep quality. Additionally, a randomized controlled trial of children with attention-deficit/hyperactivity disorder (ADHD) reported that theanine intake improved sleep efficiency [16].

Previous clinical trials examining the effects of theanine on sleep have commonly used theanine doses of 200-400 mg/day [13-16]. However, low-caffeine green tea with a theanine content of 50-100 mg tends to improve sleep in middle-aged and older adults [17-18]. These results suggested that even a low dose of theanine improves sleep quality. Theirs is the only report on the psychological improvement effects of a low dose of theanine. Nobre et al. [19] reported that 50 mg/day of theanine produced a relaxation effect in the resting state.

In line with these studies, we hypothesized that theanine might have a sleep-improving effect even when theanine doses are low. In this study, we examined whether theanine intake of 100 mg/day would improve sleep quality in middle-aged and older men.

Methods

Participants

Twenty-five middle-aged and older male workers living in Kakegawa City, Shizuoka Prefecture, Japan, were enrolled in this study. The inclusion criteria were as follows: 40-65 years old men, working from Monday to Friday with Saturday and Sunday as holidays, and working from approximately 9:00 am to 5:00 pm. The exclusion criteria were as follows: participants taking medications such as tranquilizers and sleep inducers that affect sleep, cigarette smokers, those who consumed alcohol during the study period, and those who were not eligible to participate as advised by a physician. The participants were instructed to refrain from consuming caffeine-containing beverages, such as green tea and coffee, during the study period. The sample size per group was determined by considering the number of participants in the previous study and dropouts. Written informed consent was obtained from all participants before participating in the study, and participants were screened for eligibility based on the selection and exclusion criteria. This study was conducted in accordance with the Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects. The study protocol was approved by the Research Ethics Committee of the University of Shizuoka (No.1-39).

Study design

The study design was a randomized, placebo-controlled, double-blinded, and crossover study. Randomization was performed by generating a pseudo-random number on a computer and creating an allocation table (*Figure 1*). Participants were randomly assigned to groups A and B in a 1:1 ratio. The study period was divided into

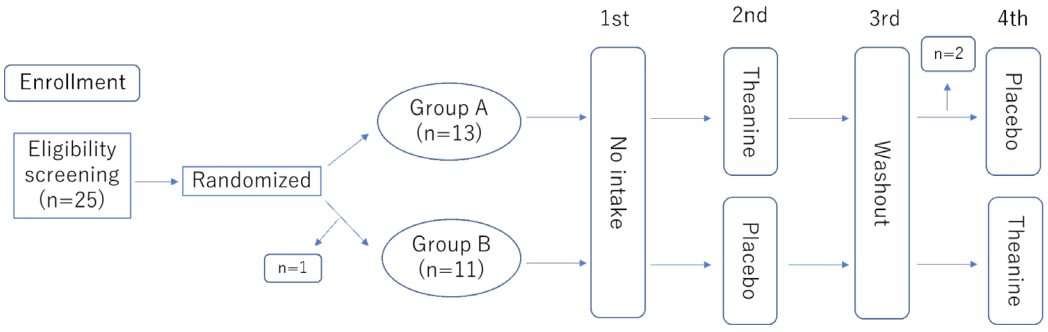

Figure 1: A flowchart of the cross-over study design.

One subject withdrew after randomization. Two subjects withdrew before the 4th period.

four, and one period was equivalent to 1 week. The first period was the no-sample intake period. Group A took theanine in the second period and a placebo in the fourth period, and Group B took a placebo in the second period and theanine in the fourth period. The third period was the washout period. The study was performed from February 1 to 28, 2020.

The theanine used in this study was a commercially available product, "Suntheanine" (Taiyo Kagaku Co., Ltd. Mie, Japan). The placebo was lactose, which is almost identical in appearance, taste, and aroma to theanine. The participants took 100 mg tablets of either theanine or placebo once a day, with water or barley tea for one week. The tablets were taken approximately two hours before bedtime. After taking the tablets, the participants were instructed to refrain from eating, exercising, and using blue light devices such as computers and smartphones as much as possible. The participants' background information and other lifestyle habits including, green tea and coffee drinking habits, medication use, and healthy food intake, were obtained from a survey questionnaire.

Measurement of EEG data and subjective sleep quality

The outcome was the electroencephalogram (EEG) data during sleep. EEG measurements were performed according to the criteria of the American Academy of Sleep Medicine [20]. A portable single-channel EEG monitoring device (Sleep Score, SleepWell Co., Osaka, Japan) was used for the EEG data [21].

Participants attached electrodes to their forehead and mastoid before sleeping to collect electrophysiological signals. Measurements started when the participants slept and finished when they woke up. The measurements were recorded for three days, from Wednesday to Friday (*Figure 2*). The raw EEG data were analyzed by Sleep Well Co. (Japan), and the most suitable day for analysis were selected, and unanalyzable days due to missing data were excluded from the analysis. The EEG data measurement items were as follows: sleep time (min), sleep latency (min), sleep efficiency (%), percentage of non-REM sleep, which is classified into stage 1 (N1), stage 2 (N2), and stage 3 (N3) per sleep time (%), percentage of REM sleep per sleep time (%), percentage of awakening per sleep time (%), total awakening time 2 hours before the final awakening (min), the number of awakenings per 1hr (counts/ hour), and the delta power of non-REM sleep in the 1st cycle per time (μV^2/ min). In the EEG analysis, sleep time was an indicator of sleep quantity, but the other parameters were indicators of sleep quality. Since the length of REM sleep, non-REM sleep, and the awakening state depends on the sleep time of each person, the length was normalized by the sleep time and calculated as a percentage. The values of delta power reflect the sleep depth.

Subjective sleep quality was measured for five days from Monday to Friday. Participants evaluated their sleep quality after waking up based on a 3-point score (1 = bad, 2 = normal, 3 = good). The mean value of subjective sleep quality for five days was used as the representative value.

Statistical analysis

The full analysis set was used for all analyses. The qualitative variables of the background factors were calculated as frequencies and proportions. Means and standard deviations (SD) were calculated for the continuous variables. The distribution of the data was checked using the Shapiro–Wilk test for normality. A paired t-test was performed to compare the differences between interventions for normality data, and a Wilcoxon signed-rank test was performed for non-normality data. A p-value of <0.050 was considered statistically significant. Normality analysis was carried out as a one-tailed test, while other analyses were carried out as a two-tailed test. The participants were divided into subgroups according to background factors such as age, green tea drinking habit, and coffee drinking habit. Then, sub-group analysis was performed on sleep parameters, the percentage of REM sleep, non-REM, and awakening, which are typical indicators of sleep quality. All statistical analyses were performed using the statistical analysis software SAS.9.4.ver (SAS Institute Inc., Cary, NC, USA).

Results

Background of the participants

Of the 25 participants, one withdrew from the study after a randomized assignment (*Figure 1*). Therefore, the study was conducted with 13 participants in Group A and 11 in Group B. Two participants withdrew before the 4th period of the study. The background information of the participants is presented in *Table 1*. The mean age was 46.2 years (SD = 5.86) in Group A and 47.1 years (SD = 6.16) in Group B. The tendency of green tea and coffee drinking habits was similar between groups A and B. In

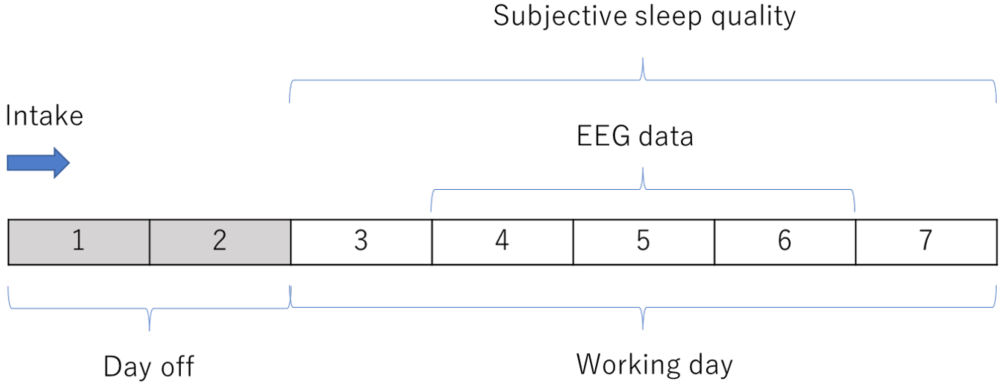

Figure 2: One period schedule for measurement.

green tea and coffee drinking habits, the number of respondents who answered "every day" and "5 or 6 days a week" accounted for more than half of the total participants. The numbers of respondents taking medicines and health food products were also almost the same, the numbers of Group A was 1,2 and the numbers of Group B was 1,3. The medication was mainly antihypertensive and diabetes drugs. Although one patient in Group B was taking olopatadine, an allergic rhinitis medicine with drowsiness as a side effect, this was included in the analysis due to the full analysis set. The type of health food products is mainly vitamins and probiotics.

Table 1: The background of the subjects.

Factor	Group A (N=13)	Group B (N=11)
Age, Mean (SD)	46.2 (5.86)	47.1 (6.16)
Green tea drinking habit, N (%)		
Everyday	8 (62)	8 (73)
5 or 6 days per week	1 (8)	1 (9)
3 or 4 days per week	1 (8)	1 (9)
1 or 2 days per week	2 (15)	1 (9)
1 or 2 days per month	1 (8)	0 (0)
No consumption	0 (0)	0 (0)
Coffee drinking habit, N (%)		
Everyday	7 (54)	4 (36)
5 or 6 days per week	1 (8)	1 (8)
3 or 4 days per week	2 (15)	2 (18)
1 or 2 days per week	2 (15)	1 (9)
1 or 2 days per month	0 (0)	0 (0)
No consumption	1 (8)	4 (36)
Medication, N (%)	1 (8)	1 (9)
Health food product, N (%)	2 (15)	3 (27)

Effects of low dose theanine on sleep

The EEG results for sleep and subjective sleep quality are shown in *Table 2*. Participants who could not properly measure EEG data were excluded, and 19 participants were analyzed. The present study results showed that the difference in all sleep parameters between the intervention periods was not statistically significant. In N2%, which indicates sleep quality, the values of the theanine group (SD = 51.46) tended to be higher than that of the placebo group (SD = 48.64), but the difference was not statistically significant ($p = 0.053$).

Table 2: Sleep parameter; All parameters are expressed as the mean (SD). n=19, α=0.05, a; paired t-test, b; Wilcoxon signed-rank test.

Parameter	Placebo	Theanine	p- value
Sleep time (min)	348.00 (67.97)	335.53 (86.46)	0.282[a]
Sleep latency (min)	9.39 (6.46)	14.34 (16.73)	0.113[b]
Sleep efficiency (%)	87.62 (6.28)	87.06 (6.83)	0.712[a]
N1 non-REM (%)	20.32 (9.01)	19.42 (6.31)	0.506[a]
N2 non-REM (%)	48.64 (10.51)	51.46 (7.91)	0.053[a]
N3 non-REM (%)	2.82 (3.62)	2.58 (3.89)	0.599[b]
N1+N2+N3 non-REM (%)	71.78 (4.96)	73.47 (5.59)	0.268[a]
REM (%)	19.43 (5.37)	18.46 (4.60)	0.383[a]
Awakening (%)	8.80 (5.23)	8.08 (5.27)	0.560[a]
Total awakening time 2 hours before the final awakening (min)	15.24 (14.45)	13.32 (8.17)	0.775[b]
The number of awakening (count/hr)	10.56 (6.27)	9.69 (6.3)	0.560[a]
δ power in the 1st sleep (μV^2/min)	1387.08 (566.88)	1431.53 (1155.18)	0.622[b]
Subject sleep quality score	2.11 (0.11)	2.07 (0.13)	0.484[a]

Sub-group analysis

Following the overall analysis, subgroup analysis according to the participants' backgrounds was conducted. Nobre et al. [19] and Kobayashi et al. [22] examined the relaxation effects of 50 mg theanine; the former observed the participants relaxing, but the latter did not. A clear difference between these studies is the subject background. The participants of the former study were young, healthy human volunteers; in contrast, the participants of the latter study were highly anxious volunteers. We hypothesized that the effect of low dose theanine might vary depending on the background factors of the participants; thus, we performed a subgroup analysis (*Table 3, Figures 3a, 3b*).

Table 3: Sub-group analysis; All values are expressed as the mean (SD). p <0.05 are indicated in bold digits and underlined (a; paired t-test, b; Wilcoxon signed-rank test).

	Placebo	Theanine	p-value
<50 years old (n=15)			
N1 (%)	19.62 (9.47)	18.46 (5.25)	0.461[a]
N2 (%)	48.48 (11.59)	52.33 (8.49)	**0.018[a]**
N3 (%)	3.56 (3.75)	3.26 (4.14)	0.636[b]
N1+N2+N3 (%)	71.66 (5.08)	74.05 (3.98)	0.118[a]
REM (%)	19.86 (5.46)	19.01 (4.30)	0.546[a]
Awakening (%)	8.49 (5.39)	6.96 (3.05)	0.145[a]
>50 years old (n=4)			
N1 (%)	22.95 (7.53)	23.04 (9.38)	0.981[a]
N2 (%)	49.25 (5.95)	48.2 (4.54)	0.765[a]
N3 (%)	0.03 (0.05)	0.05 (0.06)	1.000[b]
N1+N2+N3 (%)	72.23 (5.18)	71.28 (10.26)	0.856[a]
REM (%)	17.83 (5.45)	16.4 (5.79)	0.147[a]
Awakening (%)	9.95 (5.16)	12.28 (9.66)	0.646[a]
Green tea consumption habits			
<3 or 4 days per week in green tea drinking habits (n=4)			
N1 (%)	23.53 (16.47)	18.98 (8.57)	0.341[a]
N2 (%)	45.1 (17.68)	52.53 (13.13)	**0.048[a]**
N3 (%)	1.58 (1.88)	2.15 (3.97)	0.641[a]
N1+N2+N3 (%)	70.2 (3.19)	73.66 (5.59)	0.373[a]
REM (%)	22.6 (0.77)	20.18 (5.16)	0.386[a]
Awakening (%)	7.2 (3.08)	6.23 (2.83)	0.413[a]
>5 or 6 days per week in green tea drinking habits (n=15)			
N1 (%)	19.47 (6.52)	19.54 (5.94)	0.955[a]
N2 (%)	49.59 (8.4)	51.18 (6.56)	0.303[a]
N3 (%)	3.15 (3.94)	2.7 (4.00)	0.447[b]
N1+N2+N3 (%)	72.2 (5.34)	73.42 (5.78)	0.482[a]
REM (%)	18.59 (5.77)	18 (4.51)	0.645[a]
Awakening (%)	9.23 (5.68)	8.57 (5.72)	0.676[a]
Coffee consumption habits			
<3 or 4 days per week in coffee drinking habits (n=9)			
N1 (%)	20.46 (12.04)	18.38 (5.85)	0.408[a]
N2 (%)	48.71 (12.72)	53.42 (8.30)	0.057[a]
N3 (%)	3.39 (3.27)	2.64 (3.45)	0.553[a]
N1+N2+N3 (%)	72.56 (6.04)	74.44 (4.60)	0.393[a]
REM (%)	19.68 (5.60)	19.24 (4.48)	0.845[a]
Awakening (%)	7.78 (2.98)	6.32 (2.37)	0.052[a]
>5 or 6 days per week in coffee drinking habits (n=10)			
N1 (%)	20.2 (5.78)	20.36 (6.86)	0.910[a]
N2 (%)	48.58 (8.79)	49.7 (7.52)	0.520[a]
N3 (%)	2.3 (4.02)	2.53 (4.43)	0.750[b]
N1+N2+N3 (%)	71.08 (3.94)	72.59 (6.47)	0.507[a]
REM (%)	19.21 (5.46)	17.75 (4.82)	0.135[a]
Awakening (%)	9.72 (6.70)	9.66 (6.68)	0.977[a]

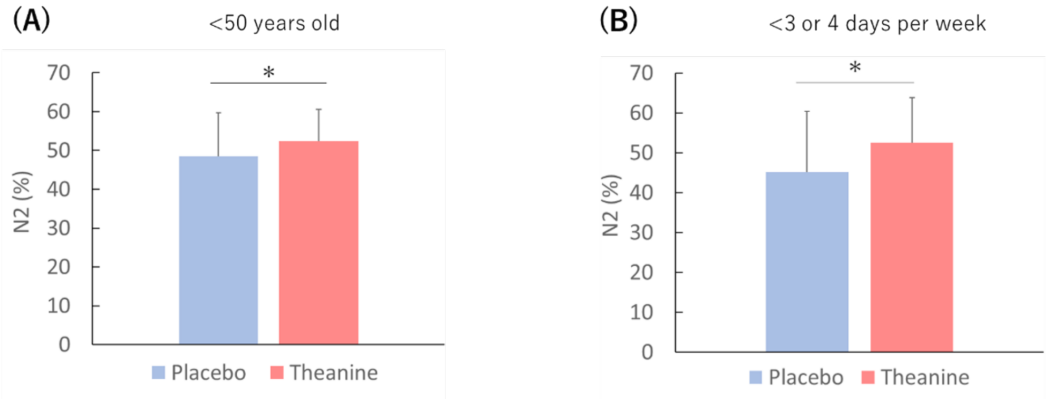

Figure 3: Sub-group analysis of N2.

In participants aged <50 years, theanine intake significantly increased N2 (%) (A). In participants who drank less than 3 or 4 days a week, N2 (%) was significantly higher in the theanine group than in the placebo group (*, p <0.050).

Based on an age-group analysis, the value of N2% of the <50 years old group was 48.48 (11.59) in the placebo period and 52.33 (8.49) in the theanine period and N2% of the theanine period was significantly higher than that of the placebo period (p=0.018). The results of the comparison of green tea consumption habit also showed that the value N2% was 45.1 (17.68) in the placebo period and 52.33 (8.49) in the theanine period, and it's significantly higher in the group consuming tea <3 to 4 days per week (p = 0.048). Although age and the group analysis of green tea consumption habits showed a difference in N2% between the theanine and placebo periods, the results of the comparison by coffee consumption habits did not show any difference. In the <3 to 4 days per week group, the values of N2% were 53.42 (8.30) in the theanine group and 48.71 (12.72) in the placebo group. There was a high tendency for theanine, but the difference was slightly less than significant (p = 0.057). In the same group, the value of awakening % was 6.32 (2.37) in the theanine period and 7.78 (2.98) in the placebo period. Its tendency was lower in the theanine period, but this difference was not significant (p = 0.052). Another group or any other values showed no differences between the theanine and placebo periods.

Discussion

In the present study, we conducted an exploratory study to evaluate the effect of theanine dose (100 mg/day) on sleep improvement. The results of this study showed no significant differences in sleep parameters between the theanine and placebo periods. However, the value of N2 % showed a higher tendency during the theanine period. N2 is one of the stages of non-REM sleep. The non-REM sleep stage was divided into a light sleep stage and a deep sleep stage. The light sleep stages are N1 and N2, and the deep sleep stage is N3. Although the physiological role of the non-REM sleep stage is still unclear, the role of N2 is to decrease the heart rate and body temperature [23]. This represents the resting state of the brain [24]. Therefore, it is said that getting longer non-REM sleep is getting a more refresh effect, and thus a high N2 % reflects a good quality of sleep.

We then examined the influence of age, green tea, and coffee consumption habits on sleep parameters, including N2, by subgroup analysis. The results showed that N2 % in the theanine period was significantly higher than that in the placebo period in the group of people who were <50 years old and in the group of people who consumed green tea <3 to 4 days per week (p=0.018, 0.048). However, in the group that consumed coffee <3 to 4 days per week, N2 % in the theanine period showed a high tendency, and the awakening % in the theanine period showed a low tendency, but these differences were not significant (p = 0.057, 0.052). These results suggest that the effects of a low dose (100 mg) of theanine on non-REM sleep stage 2 were affected by age and green tea consumption habits. This may be because age and green tea components such as theanine and caffeine are related to the sleep state [25-27]. In other words, these results may lead to the hypothesis that the effects of low dose theanine were dependent on the participants' background. Additionally, the N2 stage is considered important in the memory process [28], and memory improvement effects of theanine in humans have been reported [10,29]. Therefore, the results of this study may support the beneficial effects of theanine on memory.

This study had some limitations. First, there was gender and age bias. In this study, only middle-aged and older men were included. Therefore, it is difficult to generalize the effects of low-dose theanine. Hence, women and younger generations need to be included in the study for further investigation. Second, the effect of the regular dose (200-400 mg) of theanine was not confirmed in this study, and the dif ference of the effects between low (100 mg) and regular (200–400 mg) doses is yet to be elucidated. Third, the participants were asked to refrain from drinking caffeine-containing beverages such as green tea and coffee during the study period but were not prohibited from drinking them. Therefore, the participants might consume caffeinated beverages, and the effects of theanine might have been attenuated by caffeine consumption. Caffeine has awakening effect on sleep [27]. However, the consumption of caffeinated beverages in daily life is free, and the practical use of theanine can be evaluated. Further research is needed to elucidate the effects of low doses (100 mg) of theanine on sleep.

Conclusions

In the present study, consumption of 100 mg/day of theanine has no significant effect on sleep improvement. However, analysis of background factors such as age and green tea drinking habits revealed that low-dose theanine intake affected non-REM sleep, suggesting that the effects of low-dose theanine may vary depending on the characteristics of the participants.

Conflicts of Interest

Ozeki M. is employee of Taiyo Kagaku Co., Ltd. which supplied the L- theanine and placebo tablets used in this trial. This research was funded by the University of Shizuoka and partly by Taiyo Kagaku Co., Ltd., Mie, Japan.

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