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An extract from asparagus improves sleep quality and reduces sleepiness and fatigue on awakening: A pilot randomized controlled trial

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ABSTRACT

Background: Good sleep is essential for a healthy, active life that supports disease prevention. Sleep debts caused by sleep deprivation, irregular sleep, and insomnia increase the risk of metabolic syndrome, mood disorders, dementia, and cancer. Today's lifestyles in developed countries are prone to sleep debts, which has led to social interventions that aim to reduce sleep-related health problems. ETAS®50 is a standardized extract of *Asparagus officinalis* stem, and has been reported to improve sleep quality, maintain sleep rhythms, and relieve mental stress.

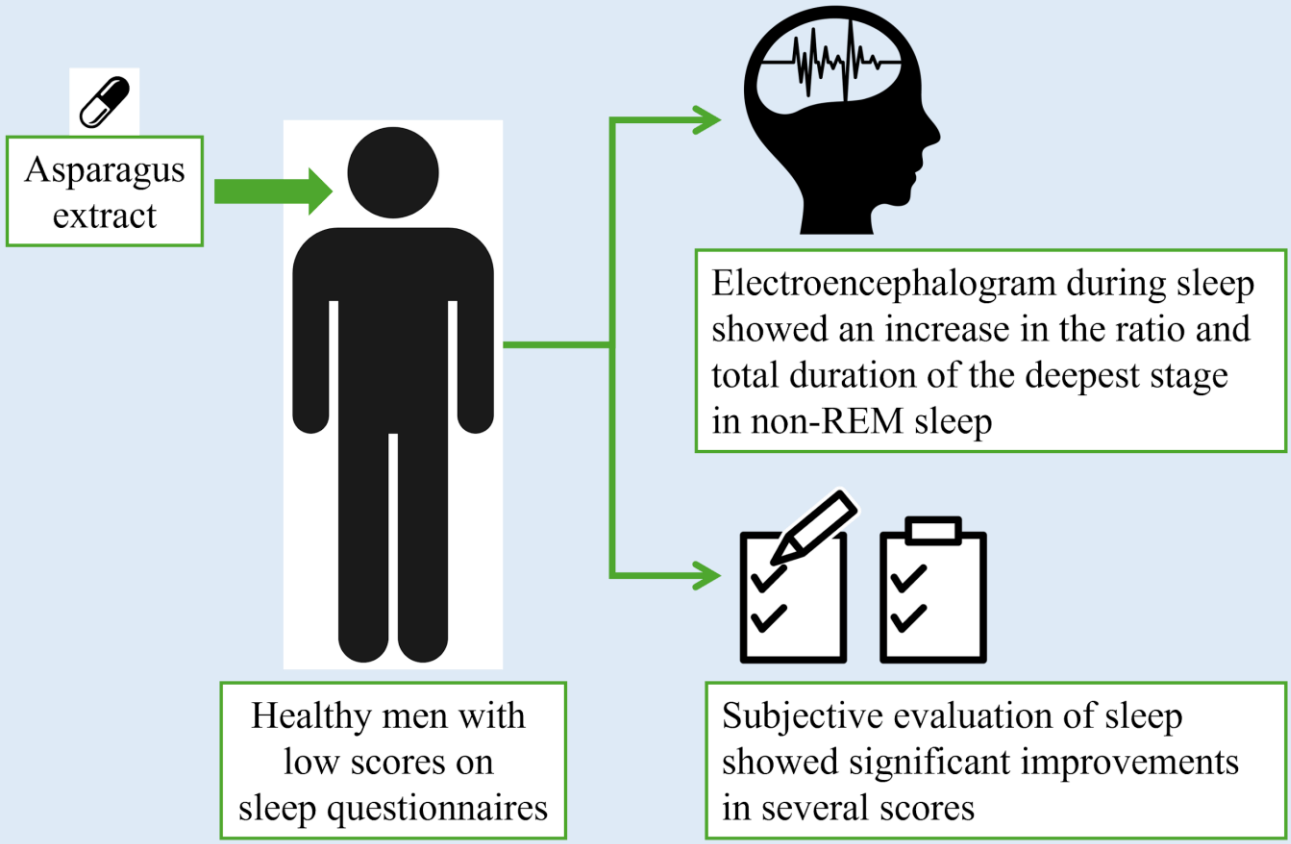
Methods: This pilot randomized, double-blind, placebo-controlled, crossover study was conducted in healthy adult males with sleep problems to evaluate the effects of ETAS®50 on sleep quality. Participants took ETAS®50 (300 mg/day) or placebo, and underwent an electroencephalogram, measured during sleep with a device known as the InSomnograf K2®. They were given a subjective assessment of sleep through a questionnaire known as the Oguri-Shirakawa-Azumi sleep inventory, specifically the Middle-Aged and Aged version (OSA-MA). Trial registration: the University Hospital Medical Information Network Clinical Trial Registry UMIN000052913.

Results: ETAS®50 showed a tendency to increase the ratio and total duration of Stage N3, known as the deepest stage in non-REM sleep (NREM). In addition, the subjective evaluation of sleep showed significant improvements in scores corresponding to tiredness upon rising, initiation and maintenance of sleep, refreshment after waking, and total scores.

The total duration and ratio of Stage N3 are important indicators for assessing sleep quality. The favorable results suggested that dietary intake of ETAS®50 may improve sleep quality, enhancing subjective sleep assessment the next morning.

Conclusion: Supplementation of ETAS®50 may offer a practical approach to improving sleep quality.

Keywords: Sleep debts, electroencephalogram, ETAS®50, InSomnograf K2®, diketopiperazine



INTRODUCTION

Sleep is necessary in the daily life of animals, including humans, accounting for about one-third of the day. However, due to urban lifestyles such as long-distance commuting, increased nocturnal states caused by prolonged/shift work, and overuse of electronic devices at night, many people suffer from sleep problems through disrupted circadian rhythm and/or shortened sleeping hours. Regarding the evaluation of sleep, there are three components: "quantity," or duration of sleep, "phase," or the regularity of sleep habits, and "quality," which is the onset and maintenance of sleep [1-2]. Many epidemiological studies have found that the sleep debt caused by sleep deprivation, irregular sleep, or poor quality of sleep promotes metabolic syndrome [3], increases the risk of cardiovascular disease [4], and dysfunctional emotional control [5]. In addition, it has been reported that sleep phase shifts, such as fluctuating sleeping and nocturnal lifestyles, affect the body's physiological functions, including the autonomic nervous

system, endocrine system, and immune system [6-9]. Some symptoms attributable to poor sleep quality show difficulty in falling asleep, a type of depressive symptom, and an increase in mid-night and early morning awakenings [5].

ETAS®50 is a standardized extract of the stem of *Asparagus officinalis*, and it has been reported that its supplementation induces the expression of heat shock protein 70 (HSP70) [10-11]. On the other hand, it has been known that induction of HSP70 improves sleep [11-13]. In a randomized, double-blind, placebo-controlled, crossover study of 17 healthy men with nocturnal lifestyles, it was reported that ETAS®50 supplementation showed significant improvement in the Athens Insomnia Scale, which is generally used to assess the severity of insomnia symptoms and daytime work efficiency [12]. Another study of 50 healthy dayshift workers with different sleep phases on weekdays and holidays indicated significant improvements in wakefulness, subjective sleep quality, and health-related quality of life evaluated by questionnaires with ETAS®50 supplementation [13]. In addition, a study of 18 healthy adult males with sleep concerns showed that in subgroup analysis, ETAS®50 supplementation modulated the sleep time among those with short or excess sleep time and ameliorated subjective sleep assessment compared to placebo [11]. Consequently, ETAS®50 supplementation has been reported to improve sleep using subjective evaluations in studies conducted.

However, there is a limitation in the subjective evaluation of sleep using questionnaires, as the subjective sleep assessment is affected by sleep stage [14-15]. The objective evaluation of sleep through the measurements of electroencephalogram (EEG) and other bioelectric potentials such as electromyogram (EMG) and electrooculogram (EOG), and the sleep staging in accordance with the American Academy of Sleep Medicine (AASM) scoring manual [16], offers a reliable method to quantitatively determine the three components of sleep evaluation, *i.e.*, quantity, phase, and quality. For this reason, evaluation by an objective index represented by EEG during sleep is one of the approaches to clarifying the mode of action of ETAS®50 supplementation on sleep.

In this study, to evaluate the overall sleep and sleep quality improvement by ETAS®50 supplementation using the objective indices, various sleep parameters (total sleep time, sleep onset latency, wake after sleep onset, and ratio and total duration of sleep stages) were calculated from hypnograms, which were drawn from the results of sleep scoring of EEG, EOG, and EMG collected overnight. A subjective sleep evaluation was also conducted using the comprehensive questionnaire, OSA-MA. In addition, HSP70 expression during ETAS®50 supplementation was measured, and its relevance to the change of the sleep parameters was discussed.

MATERIALS AND METHODS

Study design: This pilot randomized, double-blind, placebo-controlled crossover trial investigated changes in sleep by measuring sleep EEG with ETAS®50 or placebo supplementation. The dose for ETAS group was set at 300 mg of ETAS®50/day (100 mg of ETAS®50 plus 100 mg dextrin per capsule, 3 capsules/day) and the dose for placebo group was set at 600 mg of dextrin/day (200 mg of dextrin per capsule, 3 capsules/day). The overall study design is shown in Figure 1. This study consisted of the first and second cycles separated by a 14-day washout period. In each cycle, 14 consecutive days, including seven days before the start of sleep EEG measurements, were subject to the intervention period for intake of the intervention, ETAS®50 or placebo, ingested after dinner. The EEG during sleep was measured using InSomnograf K2® (S'UIMIN, Inc.), which is an approved medical device (license number: 304AFBZX00117000), for seven consecutive days, beginning on the seventh day after the start of administration of the intervention. In addition, a subjective sleep evaluation using the OSA-MA

questionnaire was conducted upon waking in the morning after the sleep EEG measurement [17]. Peripheral blood samples were collected on days 0 and 7 to measure HSP70 mRNA expression. Day 0 was before the start of the intervention, and day 1 was the first day of supplementation. Participants kept a logbook during the intervention and were monitored for adverse events. The ten final participants were randomly assigned to two groups using computer-generated random numbers. The allocation to ETAS®50 or placebo was performed by personnel independent of this study. The allocation information was concealed from the participants, doctors, administrators, and analysts until the analysis data was finalized. The study period was from April 27th, 2021, to October 26, 2021.

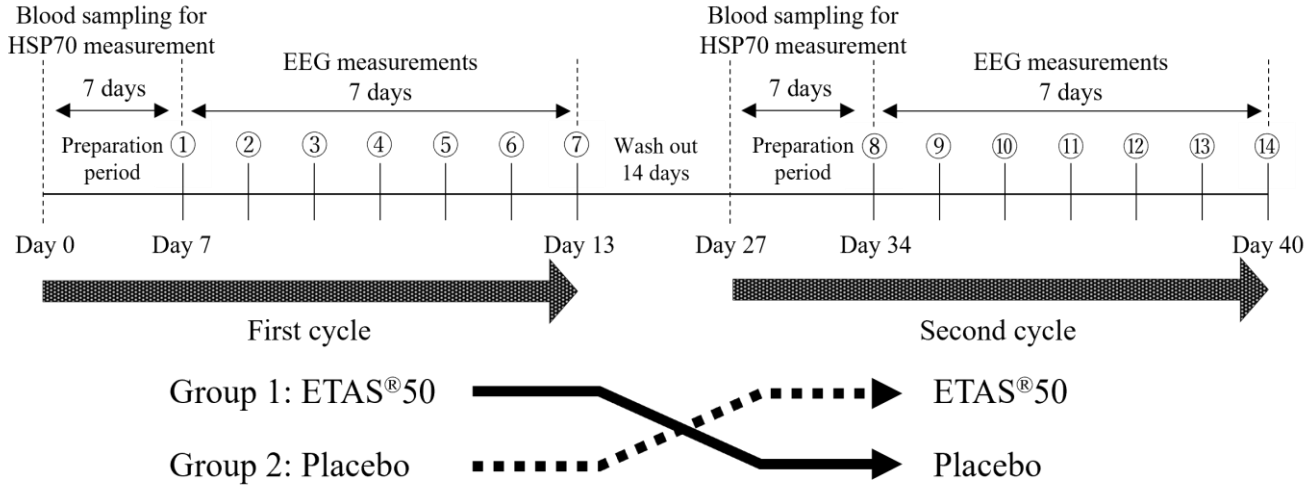


Figure 1. The study consisted of the first and second cycles, with a 14-day washout period between each cycle. Participants were randomly divided into groups 1 and 2, and the intervention was exchanged in the first and second cycles. Each cycle consisted of a 7-day pretreatment period and a 7-day EEG measurement period. The participants took the intervention pill every night after the meal. Blood samples for HSP70 measurement were collected on day 0, 7, 27, and 34 before and after the pretreatment period.

Test food preparation: The test sample ETAS®50, a standardized extract of *Asparagus officinalis* stem (Amino Up Co., Ltd., Sapporo, Japan), was manufactured under Good Manufacturing Practice for dietary supplements, ISO 9001: 2015, and ISO 22000: 2018. ETAS®50 was prepared according to the production method described in the previous reports [10-11, 18-19]. ETAS®50 contains 50% of asparagus extract, which contains more than 50 μg/g of three diketopiperazines: cyclo(L-Phe-L-Pro), cyclo(L-Tyr-L-Pro), and cyclo(L-Leu-L-Pro) as functional ingredients [19].

Participant screening: Healthy adult males with sleep problems without regular hospital visits or medication were selected. Preliminarily, candidates were selected from men who worked weekdays and scored between 31 to 69 on the Center for Environmental Therapeutics, considering the effects of social jet lag on sleep. Candidates who did not meet this criterion were excluded by checking for frequency and amount of daily caffeine intake, smoking, and alcohol consumption. In addition, candidates were limited to those with the Japanese version of the Epworth Sleepiness Scale (JESS) < 11 points or BMI < 25 to exclude pathological sleep disorders such as sleep apnea. Lastly, a sleep survey was carried out in the candidates. Specifically, one or more indices of sleep phase, quality, and quantity on the 3-Dimensional Sleep Scale (3DSS) were matched with caution/attention (phase: 0-8 points, quality: 0-10 points,

and quantity: 0-8 points), and the Pittsburgh Sleep Quality Index (PSQI) corresponded to a score of 6 or higher was considered as healthy participants.

Ethical considerations: This study was conducted under the Declaration of Helsinki and approved by the Ethical Committee of the Non-profit organization Hokkaido Activation Center, TACTICS (Accession no: 2020-155). Participant recruitment began after ethical approval was obtained. While recruiting participants, candidates were enrolled upon written consent after providing a verbal and documentary explanation of the significance, purpose, methods, potential adverse effects, risks, and safety study.

Trial registration: This study's protocol was registered at the University Hospital Medical Information Network Clinical Trial Registry (UMIN000052913).

Sleep evaluation: In this study, the sleep EEG, EOG, and EMG were measured using sleep stage at each period (30 sec.) scored according to the AASM manual. This resulted in a hypnogram, from which the following indices were calculated [16]: sleep onset latency, total sleep time, wake after sleep onset, sleep efficiency, ratios and durations of Stage N1, Stage N2, and Stage N3 in NREM, ratio and duration of REM, and REM latency.

As a secondary endpoint, the OSA-MA questionnaire consisted of 16 components, each graded as four levels (1-4 score) and divided into five categories: sleepiness on rising, initiation and maintenance of sleep, frequent dreaming, refreshing, and subjective sleep length. The scores for each category were added together to yield a total score [17].

Peripheral blood collection and measurement of hsp70 expression: Expression levels of *hsp70* and an endogenous control *β2-microglobulin* (*b2m*) in peripheral blood cells were measured using the RT-PCR method. Participants' blood (treated with EDTA-2K) samples were collected on days 0 and 7, in the presence of a medical doctor. Primers used for amplification of *hsp70* and *b2m* mRNAs, RT-PCR conditions, and analysis methods were described in the previous report [20].

Statistical analysis: Of the seven consecutive days of sleep EEG measurements, data from 6 days of measurements (except for the first day) were used in the analysis to exclude the first night effect. We calculated averages of 6 days of sleep objective variables for each ETAS®50 or placebo supplementation intake period, and their comparisons were made using one-way repeated analysis of variance. All analyses were performed using SPSS for Windows, version 28.0 (IBM Corp., Armonk, NY, USA). Statistical significance was set at $p < .05$ (two-tailed). The effect size of the sleep parameters was determined using Cohen's d between placebo and ETAS®50 supplementation [21].

RESULTS

Background and dropout cases: A sleep survey was performed on healthy adult male participants in this study. Of those who answered the sleep survey, ten participants (age: 47.5±11.4(SD), 3DSS phase: 10.5±3.69, quality: 10.2±1.93, quantity: 7.6±3.53, PSQI: 4.4±2.01, BMI: less than 25) who met the participant inclusion criteria were selected as participants (Figure 2). Participants were randomly assigned to two groups. The groups were allocated: one group received ETAS®50 for the first cycle and then a placebo for the second cycle, and another group received a placebo first, followed by ETAS®50.

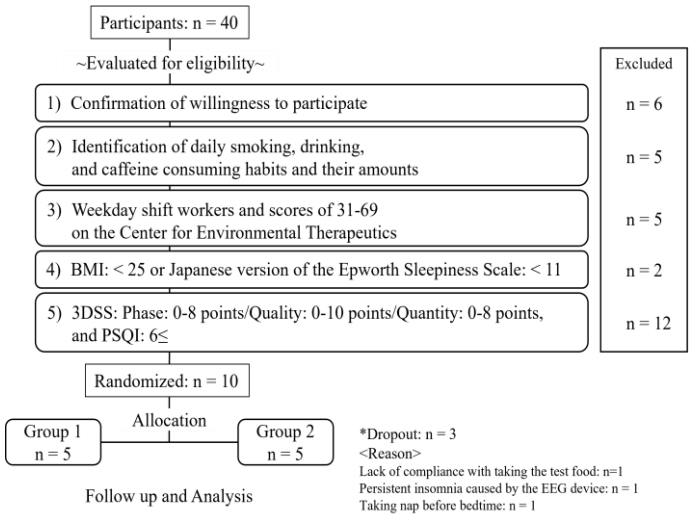


Figure 2. Among the 40 candidates, 10 participants were selected based on their scores on the 3DSS and PSQI. Of the remaining 22 participants, 18 who met the exclusion criteria were eliminated from the study. 3DSS: 3-Dimensional Sleep Scale. PSQI: Pittsburgh Sleep Quality Index.

A total of two participants dropped out of the study during the intervention period: one for lack of compliance with taking the intervention supplement, and the other experienced persistent insomnia caused by wearing the EEG device. In addition, one individual was found to nap before bedtime, which biased the groups via a record on the logbook, and was excluded from the analysis. Thus, seven participants' baseline and end values were calculated for the protocol per set. There were no adverse events related to the supplementation of the test food during the intervention and pre- and post periods.

Primary endpoint: EEG measurement: Sleep was evaluated objectively by the following indices, which were calculated from hypnograms obtained by sleep scoring based on sleep EEG, sleep onset latency, total sleep time, wake after sleep onset, sleep efficiency, total time and ratio of Stage N1, Stage N2, and Stage N3 in NREM sleep, total time and ratio of REM sleep, and REM latency (Figure 3).

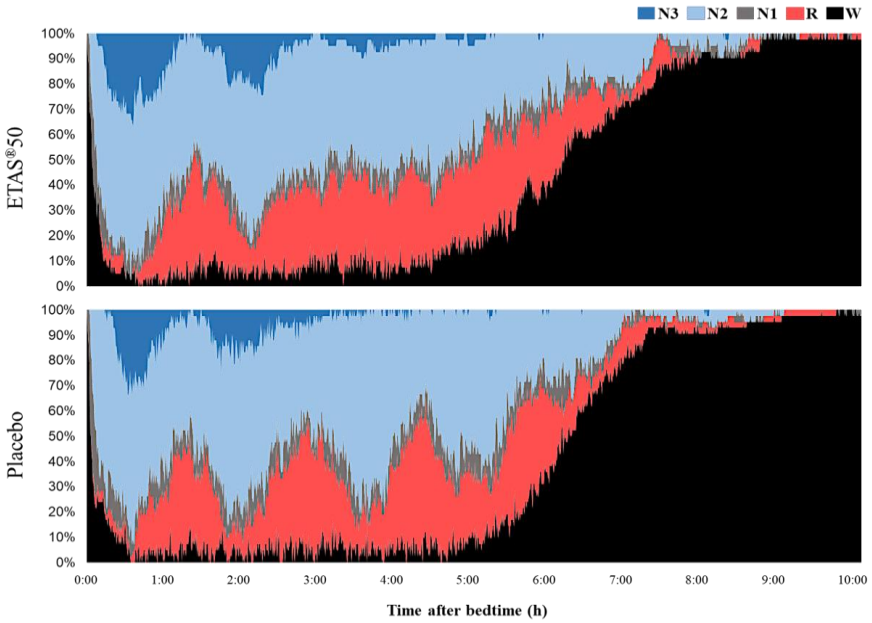


Figure 3. Figure 3 offers a cumulative display of sleep architecture in all 7 participants. The percentage of participants in each sleep stage is shown for stages W (black), N1 (gray), N2 (light blue), N3 (blue), and R (red). W, awake; R, rapid eye movement sleep; N, non-rapid eye movement sleep.

There are no significant differences between ETAS®50 and placebo in EEG measurements (Figure 4-6). However, the effect size of ETAS®50 supplements in total time of stage N2 (d = 0.79) and ratio of stage N3 (d = 0.59) was moderate (Table 1).

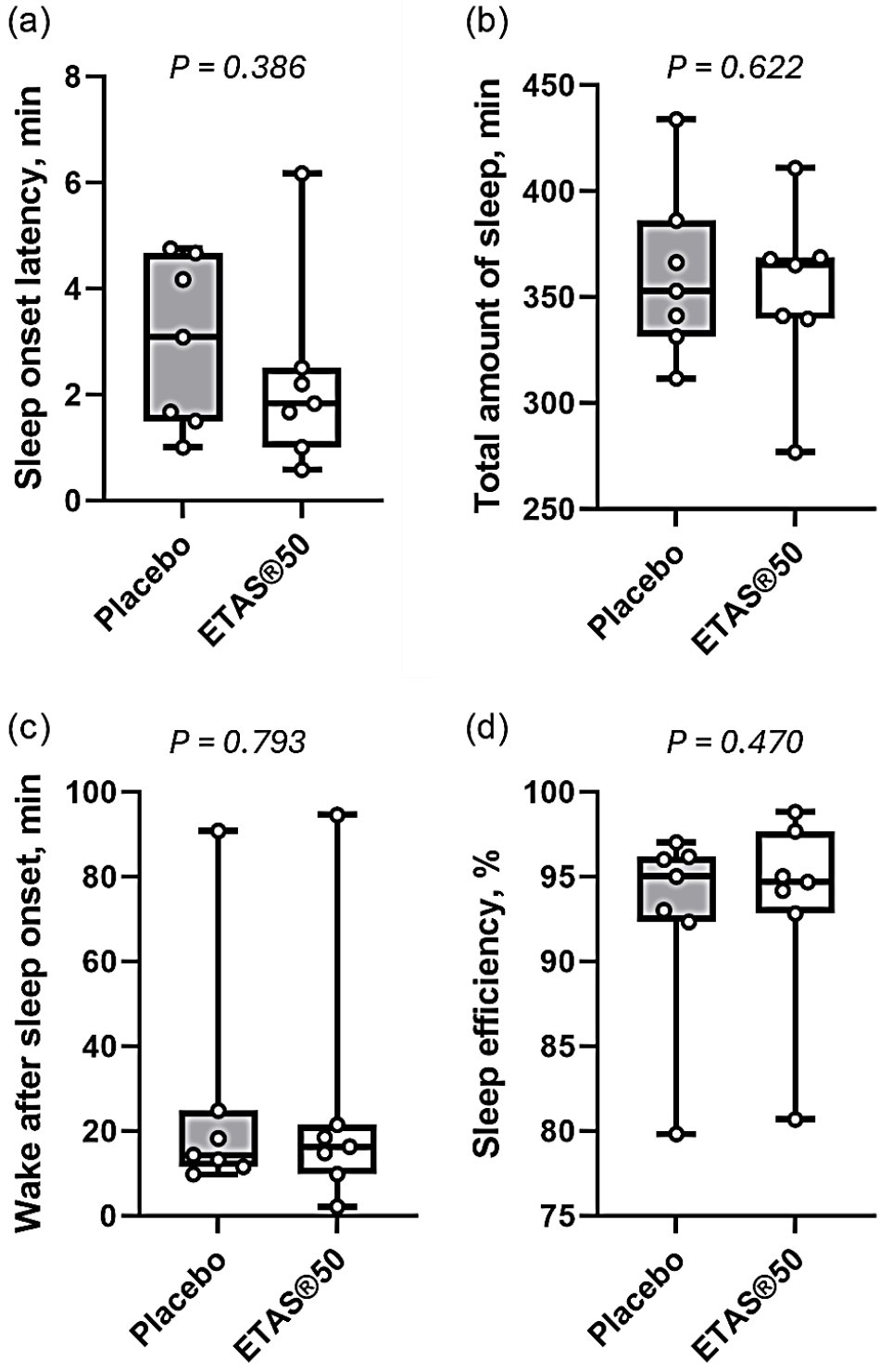


Figure 4. Figure 4 depicts the EEG measurements data during sleep. The box and whisker plots below show: Sleep onset latency (a), Total amount of sleep (b), Wake after sleep onset (c), and Sleep efficiency (d). Error bar: ± standard deviation.

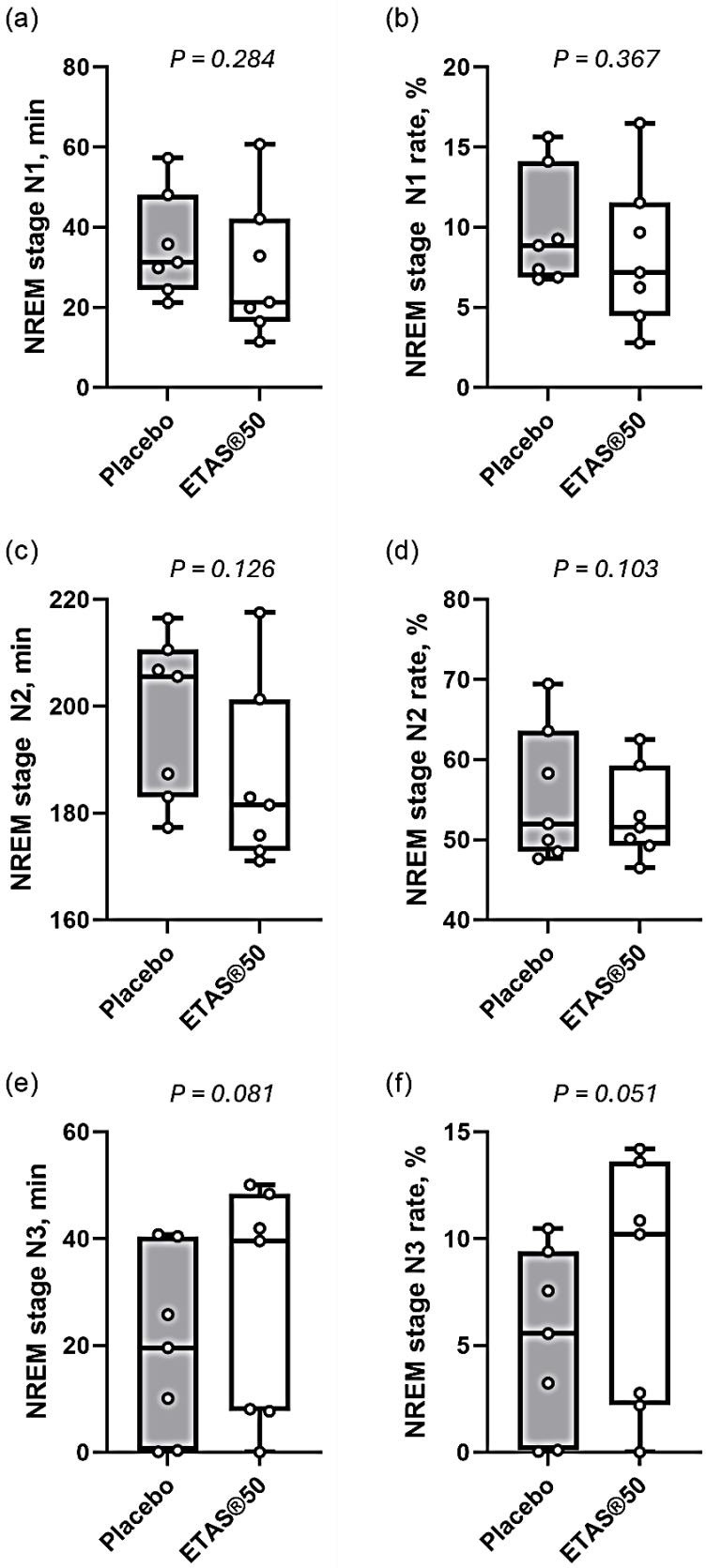


Figure 5. Figure 5 depicts EEG measurements data of NREM sleep. The box and whisker plots below show: NREM stage N1 (a), NREM stage N1 rate (b), NREM stage N2 (c), NREM stage N2 rate (d), NREM stage N3 (e), and NREM stage N3 rate (f). Error bar: ± standard deviation.

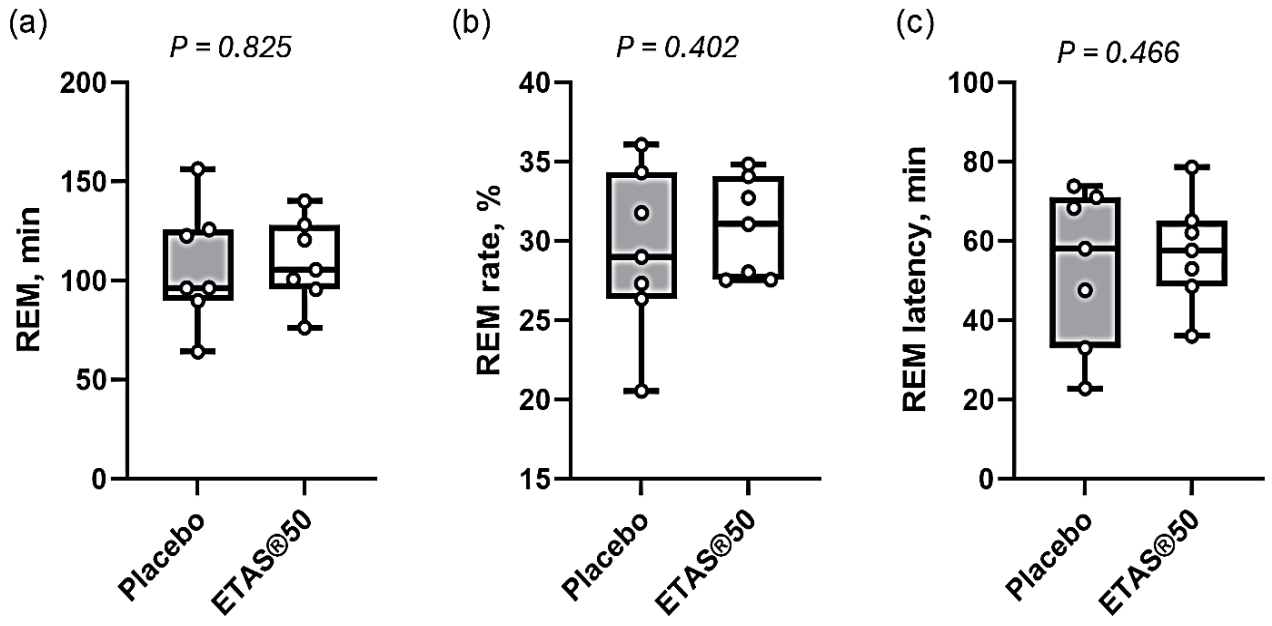


Figure 6. Figure 6 displays the EEG measurement data of REM sleep. The data explains the total amount of REM (a), REM rate (b), and REM latency (c). Error bar: ± standard deviation.

Table 1. Effect size of ETAS®50 supplementation in objective and subjective sleep variables.

Variables	Effect size (Cohen's *d*)
EEG measurements	
Sleep onset latency	0.44
Total sleep time	0.19
Wake after sleep onset	0.03
Sleep efficiency	0.11
Duration of Stage N1	0.48
Duration of Stage N2	0.79
Duration of Stage N3	0.49
Duration of Stage REM	0.08
Ratio of Stage N1	0.42
Ratio of Stage N2	0.30
Ratio of Stage N3	0.59
Ratio of Stage REM	0.29
REM latency	0.19
Subjective Sleep Assessment (OSA-MA)	
Sleepiness on rising	0.63
Initiation and maintenance of sleep	0.85
Frequent dreaming	0.09
Refreshing	0.55
Sleep length	0.19
Total score	0.52

Note: Cohen's *d*: 0.2 = small; *d*: 0.5 = moderate; *d*: 0.8 = large

Secondary endpoint: Subjective sleep assessment (OSA-MA): The comparison between the adjusted standard deviation scores of the placebo and ETAS®50 supplementation showed significant differences in the following three parameters (Figure 7a, b, and d). The scores of sleepiness on rising with placebo and ETAS®50 supplementation were 46.23 ± 6.06 and 50.07 ± 6.45 (p = 0.001), respectively. The scores of initiation and maintenance of sleep were 46.91 ± 5.25 and 51.38 ± 5.24 (p = 0.032). The refreshing scores were 46.86 ± 6.21 and 50.28 ± 5.11 (p = 0.009). Furthermore, the total score of all five factors showed a significant improvement with ETAS®50 (248.94 ± 22.85, p = 0.001) compared to the placebo (235.45 ± 25.80) (Figure 7f). There were no significant differences between placebo and ETAS®50 supplementation in the scores of frequent dreaming and objective sleep length (Figure 7c and e).

The effective size of the ETAS®50 supplement on sleepiness on rising (*d* = 0.63), refreshing (*d* = 0.55), and the total score (*d* = 0.52) were moderate, and initiation and maintenance of sleep (*d* = 0.85) concluded a high effect size (Table 1).

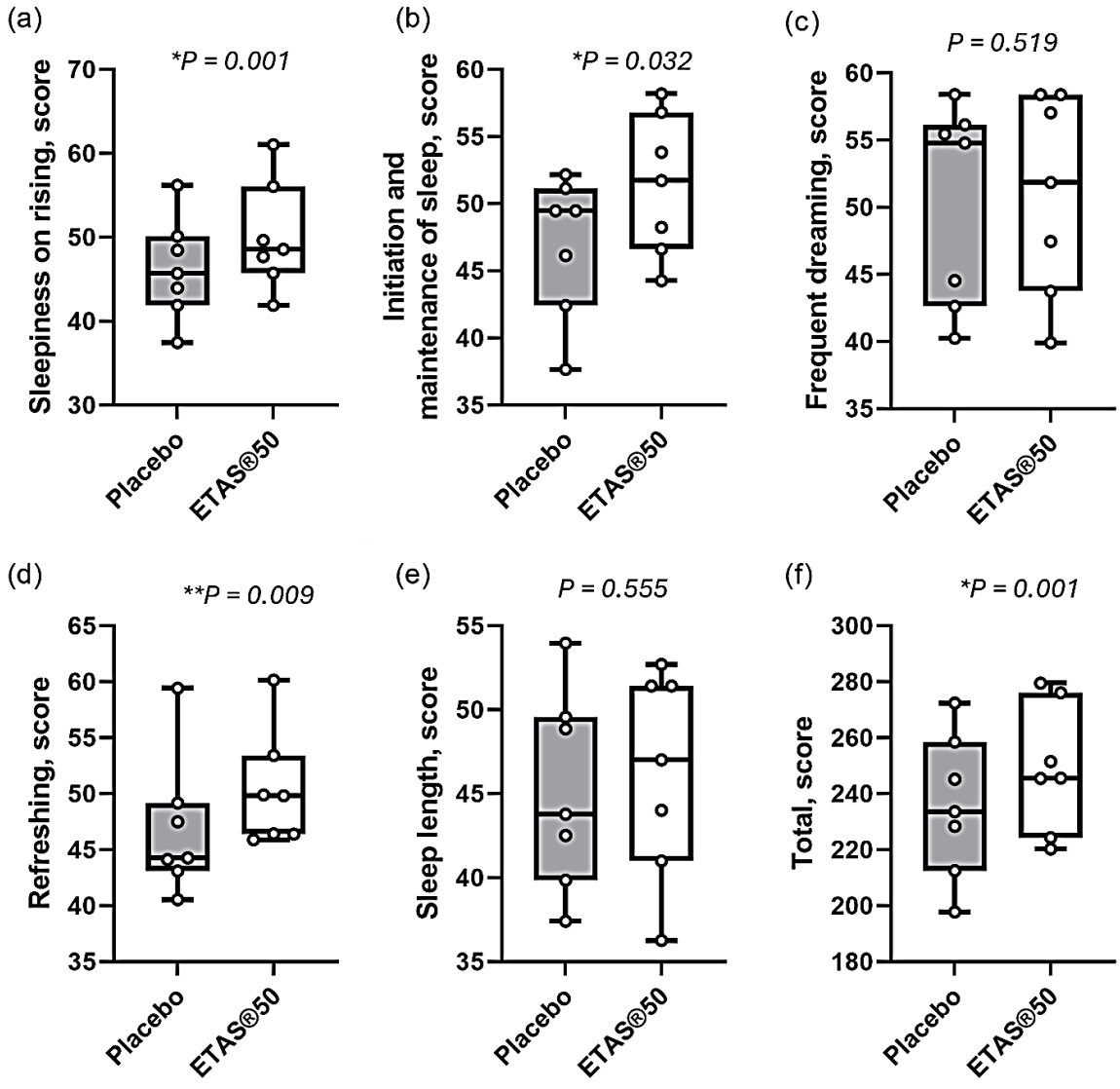


Figure 7. Figure 7 displays the subjective evaluation score of sleep introspection. The box and whisker plots show: Sleepiness on rising (a), Initiation and maintenance of sleep (b), Frequent dreaming (c), Refreshing (d), Sleep length (e), and the total (f). Error bar: ±SD.

Hsp70 expression in peripheral blood cells: The expression of *hsp70* mRNA in peripheral blood cells before day 0 and after day 7, placebo, and ETAS®50 supplementation showed no significant changes (data not shown).

DISCUSSION

One of the significant mechanisms of sleep appears to be recovery from mental and physical fatigue. Overcoming sleep debt is a crucial issue to be solved for many people living in sleep-deprived environments and for older adults with a marked decline in sleep quality. In a deep sleep, NREM is characterized by sleep spindles (transient thalamocortical oscillations of 11-16 Hz), κ-complexes (a brief negative high-voltage peak followed by a slower positive complex), and slow waves in the sleep EEG [22]. Slow waves are characterized by high amplitude and low frequency (e.g., < 4 Hz) and are known to increase in amplitude and duration as sleep deepens. The transition from the second stage of NREM (Stage N2) to the deepest sleep stage (Stage N3) is characterized by slow waves for more than 20% of the NREM time [23]. Stage N3 has been reported to promote the secretion of growth hormone necessary for tissue repair and metabolism [2]. This directly affects the balance of the autonomic nervous system, which is related to stress tolerance and recovery from stress [24]. Changes in autonomic activity are also thought to affect several physiological processes, including glucose metabolism [25], immunity [26], and cognitive function [27]. Thus, the functions of Stage N3 sleep have drawn substantial attention, as it is deeply involved in mental and physical health. Meanwhile, our past sleep studies with ETAS®50 supplementation had been limited to subjective wakefulness and sleep quality assessments. We hypothesized that the improved subjective sleep evaluation upon waking by ETAS®50 supplementation may be closely associated with increased deep sleep, mainly Stage N3. This pilot study was designed to objectively evaluate sleep by measuring sleep EEG, to elucidate the mode of action of ETAS®50. Unfortunately, there are no significant differences between ETAS®50 and placebo. Nevertheless, our results demonstrate a trend towards an increase in the ratio (p = 0.051; Figure 5f) and the total time of stage N3 (p = 0.081; Figure 5e). This data supports the hypothesis of the mode of action. Furthermore, the moderate effect size in the N3 stage (Cohen's d: ratio = 0.59, duration = 0.4; Table 1) promises that increasing the number of participants leads to significantly different results in the ratio and duration of the N3 stage.

When comparing mean values of the sleep parameters between the placebo and ETAS®50 groups, the ratio and total time of stage N1 were reduced. However, there were no significant changes in the quantitative indices such as total sleep time, wake after sleep onset, and sleep efficiency. We interpret that the decrease in Stage N1 may be due to the cost of an increase in Stage N3. Autonomic regulation and sleep are closely associated through the physiological and anatomical levels [28], while the transition between sleep stages occurs through coincidental fluctuations in sympathetic activity [29]. In Stage N2 and Stage N3, the parasympathetic nervous system is activated and compared to wakefulness [30], while the sympathetic nervous system is downregulated [31]. NREM sleep is parasympathetically predominant, whereas sympathetic activity is predominant during REM sleep only [31]. In a clinical study to elucidate the relationship between ETAS®50 and the autonomic nervous system, J Takanari *et al*. reported [32] that 300 mg of ETAS®50 daily supplementation in healthy participants with psychological stress showed positive effects on modulating the autonomic nervous system balance, reducing discomfort and fatigue, and improving sleep quality. ETAS®50 has also been reported to increase the number of neurons in the suprachiasmatic nucleus (SCN) of Senescence-Accelerated Mouse Prone 8 and normalize the expression levels of melatonin receptors MT1 and MT2 [33]. SCN, an internal clock, also acts on the pineal gland, which secretes melatonin, a sleep-inducing hormone closely related to the autonomic nervous system [34]. The increasing tendency in duration and ratio of Stage N3 sleep induced by ETAS®50 supplementation may contribute to the normalization of

circadian rhythm through the SCN and its downstream neural networks. The changes in autonomic balance may have resulted in significant improvements in the subjective sleep assessment, such as sleepiness on awakening, onset and maintenance of sleep, refreshment after sleep, and the total score. These results and considerations on the mode of action are consistent with the previous findings of ETAS®50 studies demonstrating the effects of improving sleep.

T Ito *et al.* reported that *hsp70* mRNA expression in peripheral blood cells tended to increase in 20 healthy men after taking 300 mg of ETAS®50 for 7 days [10]. The study placed participants under strict conditions to avoid warm baths above 40°C and asparagus consumption. We hypothesized that sleep improvement could be attributable to the *hsp70* expression increased by ETAS®50 supplementation. However, there were no significant changes in the *hsp70* expression in peripheral blood cells during the intervention supplementation in the current study. This study was primarily conducted to evaluate the changes in sleep induced by ETAS®50 supplementation using EEG monitoring to allow the participants to keep their daily routines. While the current study had no strong restrictions, other factors may have contributed to the effect on the expression of *hsp70*. For *hsp70* expression analysis, T Ito *et al.* used a determinant PCR method, whereas this study used a real-time PCR method. Therefore, it is possible that this study increased the accuracy of the measurement, and thus no significant difference was observed [9]. These differences may explain why no correlation between ETAS®50 supplementation and hsp70 expression in peripheral blood cells was detected.

As a pilot study, the sample size of this study is small. This study was limited to a short period and only conducted the test among male participants. Hence, for a more advanced evaluation, a randomized, double-blind, placebo-controlled trial with larger and longer studies in diverse populations, including women and the elderly, should be conducted. Consequently, the trial could prove that ETAS®50 improves sleep quality through EGG data, demonstrating a proactive intervention.

CONCLUSIONS

This study found that ETAS®50 supplementation increased the total duration of the deepest stage of NREM and improved scores on the Sleep Assessment Questionnaire in participants with sleep problems evaluated in this study. The use of ETAS®50 may enhance sleep quality and provide an approach to resolving sleep debt problems.

Abbreviations: OSA-MA: Oguri-Shirakawa-Azumi Sleep Inventory, Middle-age and Aged version; NREM: non-REM sleep; HSP70: heat shock protein 70; EEG: electroencephalogram; EMG: electromyogram; EOG: electrooculogram; AASM: American Academy of Sleep Medicine; JESS: Japanese version of the Epworth Sleepiness Scale; 3DSS: 3-Dimensional Sleep Scale; PSQI: Pittsburgh Sleep Quality Index; *b2m*: endogenous control *β2-microglobulin*; SCN: suprachiasmatic nucleus.

Author contributions: Conceptualization: T.T. and J.T. Data curation: T.T., J.S., and T.K. Formal analysis: T.T., J.S., and T.K. Investigation: T.T., J.T., and K.G. Project administration: J.T. and T.K. Writing-original draft: T.T. Writing-review and editing: T.K. and K.G.

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