Review

In Search of a Safe Natural Sleep Aid

Theertham P. Rao, PhD, Motoko Ozeki, PhD, and Lekh R. Juneja, PhD

Taiyo Kagaku Co. Ltd., Yokkaichi, Mie, JAPAN

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Sleep deprivation is associated with an elevated risk of various diseases and leads to a poor quality of life and negative socioeconomic consequences. Sleep inducers such as drugs and herbal medicines may often lead to dependence and other side effects. L-Theanine (γ-glutamylethylamide), an amino acid naturally found abundant in tea leaves, has anxiolytic effects via the induction of α brain waves without additive and other side effects associated with conventional sleep inducers. Anxiolysis is required for the initiation of high-quality sleep. In this study, we review the mechanism(s), safety, and efficacy of L-theanine. Collectively, sleep studies based on an actigraph, the obstructive sleep apnea (OSA) sleep inventory questionnaire, wakeup after sleep onset (WASO) and automatic nervous system (ANS) assessment, sympathetic and parasympathetic nerve activities, and a pediatric sleep questionnaire (PSQ) suggest that the administration of 200 mg of L-theanine before bed may support improved sleep quality not by sedation but through anxiolysis. Because L-theanine does not induce daytime drowsiness, it may be useful at any time of the day. The no observable adverse effect level (NOAEL) for the oral administration of L-theanine was determined to be above 2000 mg/kg bw/day.

Key teaching points:

- Sleep deprivation–associated morbidity is an increasing public health concern posing a substantial socioeconomic burden.
- Chronic sleep disorders may seriously affect quality of life and may be etiological factors in a number of chronic diseases such as depression, obesity, diabetes, and cardiovascular diseases.
- Most sleep inducers are sedatives and are often associated with addiction and other side effects.
- L-Theanine promotes relaxation without drowsiness.
- Unlike conventional sleep inducers, L-theanine is not a sedative but promotes good quality of sleep through anxiolysis.
- This review suggests that L-theanine is a safe natural sleep aid.

INTRODUCTION

Sleep is necessary to maintain overall health, yet many are deprived of it. Sleep has been recognized as essential for rejuvenating the body and promoting good health and performance [1–9]. Humans, like most animals, have circadian rhythms that are controlled by an endogenous biological clock that dictates sleep time and length as a natural process [10,11]. However, physical, physiological, psychological, and environmental factors can greatly influence the onset and quality of sleep.

Previous research suggests that sleep deprivation is related to chronic neurological and other physiological deficits [12–14], which may lead to the development of diabetes [15], cardiovascular diseases [16], obesity [17], and depression [18,19]. A lack of sleep is also responsible for compromised social behaviors, which can lead to major socioeconomic consequences. In the United States, sleep deprivation and related disorders affect between 50 and 70 million people. Approximately 20% of all road accidents/injuries are due to a lack of sleep, accounting for hundreds of billions of dollars in medical costs [20, 21].

A number of sleep inducers and sedatives, such as the "Z drugs" (non-benzo- or benzo-diazepines), antihistamines, alcohol, and hormones (melatonin) are commonly employed for sleep; however, these often lead to addiction. Their prolonged use has been linked to numerous side effects, including chronic sedation and drowsiness, decreased alertness and concentration, as well as depression and loneliness [22, 23]. A number of natural-based sleep inducers are available as well, including cedrol, a cedar oil sesquiterpene alcohol [24, 25]; valerian (*Valeriana officinalis* L.) root extract [26, 27]; kava kava (*Piper methysticum*) root extract [28]; and *Panax ginseng* root extract [29, 30], all of which have been reported to improve sleep patterns. These have also been shown to have serious side effects that would warrant caution in their use [31–34].

Sleep can be categorized by multiple cycles of rapid eye movement (REM) to non-rapid eye movement (NREM), where one cycle can last for approximately 90 minutes on average [35]. The sleep cycle proceeds from REM to NREM in 4 stages, namely, N1 to N4, in which the last stage refers to deep sleep. The autonomic nervous system regulates sleep; the intensity of sleep is induced by a progressive increase in parasympathetic activity and a corresponding decrease in sympathetic activity [36]. Sleep disturbances and problems with the onset of sleep are clearly related to physical and mental status including tiredness, stress, anxiety, and excitement. Therefore, an approach to inducing high-quality sleep that is composed of an anxiolytic approach that (1) induces somnolence that calms the body and mind before bed and (2) decreases the incidence of waking and dreams with proper modulation of the autonervous system without untoward side effects is needed.

Though coffee and tea have almost equal amounts of caffeine, most coffee has a stimulant effect, whereas tea often has a calming effect. L-Theanine (γ-glutamylethylamide), an amino acid abundantly and exclusively found in tea leaves, is known to be the active ingredient that induces this calming effect. L-Theanine, produced via an enzymatic fermentation process, induces relaxation within 30 minutes after oral intake by generating α-brain waves, an indication of a relaxed yet alert state of mind [37]. A convincing body of evidence supports that this substance has several pharmacological functions, including reducing blood pressure in spontaneously hypertensive rats [38] and antagonizing caffeine-induced convulsions [39–41] and sleep disturbances [42]. Moreover, this compound has been shown to decrease physical stress in men [43, 44] and to suppress anxiety and stress during the premenstrual period in women [45] and anxiety levels in both men and women [46–49]. The structural similarity of L-theanine to glutamic acid, a neurotransmitter in the brain, has also prompted researchers to investigate its competitive action on the nervous system as a glutaminergic antagonist [50]. L-Theanine rapidly induces changes in serotonergic and dopaminergic transmission [51–53]. Upon oral administration, L-theanine is absorbed quickly in the intestinal tract [54] and crosses the blood–brain barrier via the L-system, reaching a peak concentration within 30–40 minutes after intake [55] in animals. The rapid modulation of neurotransmitters and neuronal function, promotion of relaxation without drowsiness, relief from stress (including physical stress), and excitement by L-theanine has prompted scientists to test its use for the improvement of sleep quality and recovery from exhaustion in adult men and women and in children. All of these studies used 99% pure L-theanine (Suntheanine, Taiyo Kagaku Co Ltd., Yokkaichi, Japan), which was produced via an enzymatic fermentation process. In this review, the effects of L-theanine on sleep and its safety for daily use are summarized.

IMPROVEMENT OF SLEEP QUALITY

Sleep Efficiency in Men

A study at the National Institute of Mental Health and the National Center of Neurology and Psychiatry in Japan suggests that the ingestion of 200 mg of L-theanine by men 1 hour before bed improved sleep quality [56]. Twenty-two healthy male subjects (mean age of 27.5 ± 0.9 years) consisting of daytime workers ($n = 12$; mean age of 28.0 ± 1.0 years) and university students ($n = 10$; mean age of 27.0 ± 1.5 years old) were selected for the study after screening 26 subjects for their personal, psychiatric, and sleeping conditions with a Maudsley personality test, the Cornell Medical Index (CMI), and a sleep health questionnaire. Additionally, subjects with a past and present medical history and the use of any medication that could affect sleep were identified and excluded from the study. The remaining 20 healthy subjects were examined for their sleep quality with the Obstructive Sleep Apnea (OSA) sleep inventory questionnaire [57] and actigraphic [58] methods. The study was a randomized, double-blind, placebo-controlled crossover study, in which the baseline period without interfering medication lasted 3 days and the 2 treatment periods lasted for 6 consecutive days with a 1-day gap between the 2 test periods. One hour before going to bed, the subjects were given 4 tablets, each containing either 50 mg of L-theanine or a placebo (without L-theanine). All of the subjects were asked to fill out the OSA sleep inventory, which consisted of 5 sleep quality factors; that is, refreshed awakening, improved quality of sleep (naturally falling asleep and sleep maintenance), dream quality (few dreams, fewer nightmares), recovery from exhaustion (fatigue) and feeling about sleep time, and 3 mood factors, namely, motivation, good mood, and self-confidence upon awaking in the morning during the study period. Additionally, a wrist activity monitor (Actigraph, Ambulatory Monitoring, Inc., Ardsley, NY) was strapped to the nondominant wrist of the 12 subjects, and the sleep/waking patterns and time during the night were estimated on the third and fifth days of the test period.

In all subjects L-theanine improved sleep by 5 OSA sleep quality factors (Fig. 1). No significant difference was found
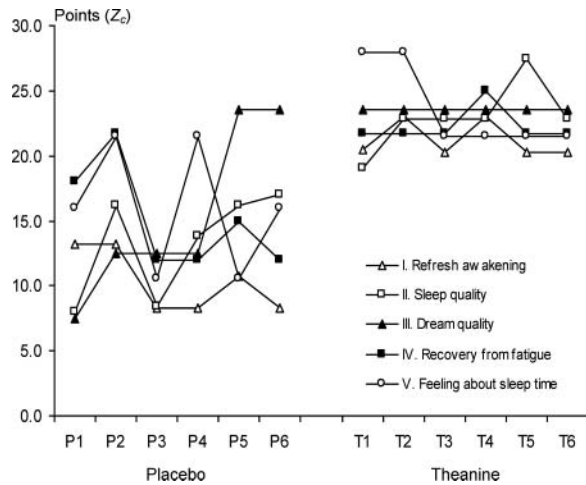
Fig. 1. Everyday sleep inventory OSA-MA assessment on the feelings about sleep and exhaustion upon awake during the test period with or without administration of 200 mg of L-theanine in male subjects ($n = 22$).

between the L-theanine and placebo treatment for the sleep length factor, but L-theanine improved the quality of sleep ($p < 0.055$) and dream quality ($p < 0.072$) by decreasing both dream recall and nightmares. Compared with the placebo, the L-theanine treatment also significantly ($p < 0.042$) improved the feeling of recovery from exhaustion or fatigue. Subjects also reported a significant improvement in a refreshed feeling upon awakening ($p < 0.014$) with the L-theanine treatment. On the other hand, the mood condition on motivation was not significantly different between the treatments. However, the states of good mood ($p < 0.088$) and self-confidence ($p < 0.075$) improved with L-theanine.

Sleep-log data from the actigraph indicate the state of wake and sleep times and indirectly infer the motor activities of the brain. The actigraph data from 10 subjects (the data from 2 subjects who deviated from the requirements of the study protocol were not assessed) showed that motor activities during the sleep

period were slightly higher in the placebo compared with the L-theanine treatment. Additionally, intermittent awakening was not observed within the L-theanine treatment group. Sleep-log data were also collected for bedtime, sleep offset, and sleep-log sleep interval. The sleep interval was defined as the actigraphic sleep onset minus the sleep offset, and the total sleep was defined as the total actual time of computer-inferred sleep between the actigraphic sleep onset and sleep offset. The actigraphic wake after sleep onset (WASO) was defined as the time spent awake over the same interval (intermittent awakening). The actigraphic sleep efficiency was defined as the actigraphic total sleep divided by the actigraphic sleep interval. The sleep length, sleep efficiency, and WASO data are presented in Fig. 2. There were no significant differences between the placebo (6:22 $\pm$ 0:10 hr) and L-theanine (6:20 $\pm$ 0:11 hr) groups in the time spent asleep during the computer-inferred actigraphic sleep interval. However, the sleep efficiency measured by the actigraph revealed a significant increase ($p < 0.047$) in the percentage of sleep with L-theanine (96.6% $\pm$ 1.3%) compared with the placebo treatment (93.8% $\pm$ 3.0%) during the treatment periods. The WASO results, in which the length of the appearance of slow wave sleep and the appearance of REM during sleep was interpreted as intermittent awaking and was significantly ($p < 0.044$) shorter in the L-theanine treatment group (12.6 $\pm$ 4.5 min) than in the placebo treatment group (19.8 $\pm$ 7.6 min). The actigraph results concur with the OSA sleep inventory questionnaire results, in which a significant improvement in sleep efficiency was noticed with L-theanine.

Both the OSA data and actigraph results have confirmed that the L-theanine does not induce sleep. However, both results suggest that the L-theanine was effective in improving the sleep quality by reducing the WASO, which was reflected in the OSA data with reduced fatigue and refreshed awakening in the morning in young male volunteers. It is further suggested that subjects experienced better or deeper sleep than usual. A high quality of sleep due to the consumption of L-theanine
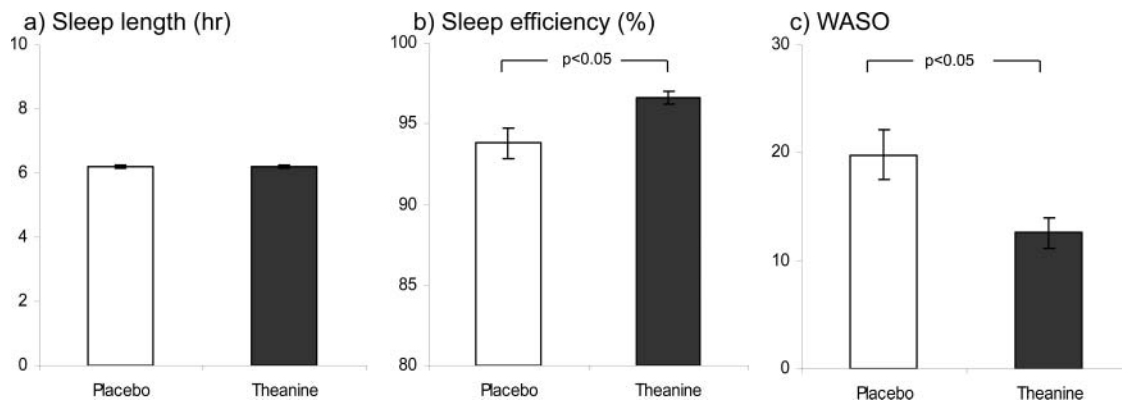

Fig. 2. (a) Sleep length, (b) sleep efficiency, and (c) intermittent awakening WASO data interpreted from actigraph measurements in 10 male subjects. The values represent mean $\pm$ standard error. Statistical significance was measured using Student's paired *t*-test.

could be presumed to be due to its effect on relaxation and the modulation of neurotransmitters. A previous human study indicated that the intake of L-theanine resulted in the generation of α-waves (8–13 Hz), the brain waves which represent relax and alert state of mind, within 30–40 minutes in the occipital and parietal regions of the brain [37]. Furthermore, it was found that the ingestion of L-theanine decreased β-waves (higher than 14 Hz), an indication of hyperarousal, and increased α-waves (8–13 Hz), thereby confirming a relaxed state [59]. It is generally perceived that one can enter into sleep easily and can experience good or deep sleep if one is in a relaxed state prior to going to bed. Second, sleep is affected by neurotransmitters, which have corresponding effects on suppressive or excitatory neurons in the brain. It is known that γ-aminobutyric acidergic (GABAergic) neurons are inhibitory neurons and that glutamatergic neurons are excitatory neurons, which correspond with the promotion and inhibition of sleep, respectively. L-Theanine administration was found to increase GABA levels in the brains of mice [39]. Antagonists of excitatory neurons are also substances known to promote sleep. Oxidized glutathione binds to the receptors of glutamatergic neurons and suppresses their activity. Oxidized glutathione was also identified within the brain stem of sleep-deprived rats as a sleep-promoting substance that suppressed the binding of glutamate to the glutamatergic neuron receptors located on the synaptic membrane of neurons [60]. However, L-theanine showed a weak inhibiting effect on the 3 glutamate receptor subtypes, (RS)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), kainite, and N-methyl-D-aspartate (NMDA) suggesting an antagonistic effect of L-theanine on the glutamatergic neuron receptors [61]. However, the binding activity of L-theanine on the glutamate receptors was lower than that of L-glutamate. Therefore, L-theanine not only promotes the activity of the inhibitory neurons but inhibits the activity of the excitatory neurons in the brain to improve sleep quality. According to this information, L-theanine can be considered an excellent aid for improving the sleep state. Sleep is also further related to the automatic nervous system (ANS), so the effects of L-theanine on the modulation of the ANS were further investigated to confirm its effect on sleep conditions.

Sleep Quality in Women

Postmenopausal elderly women often experience a lack of good-quality sleep due to intermittent awakening. Therefore, another study conducted at the National Institute of Mental Health and the National Center of Neurology and Psychiatry in Japan [62] examined the effect of L-theanine on sleep quality in postmenopausal elderly women (>50 years). Initially, 53 subjects (mean age of 58.1 ± 4.0 years) were recruited and screened with the Maudsley personality test, the CMI, a sleep health questionnaire, a past and present disease history, and medication use for any disease or sleep disorder, resulting in

the removal of 33 subjects, who were not sure of their menopausal status. Those who often experienced an extreme lack of sleep with <5 hours on workdays and those who were not sure about their health condition were also excluded. Finally, 20 subjects in the 50–65 age group with an average age of 57.3 ± 3.9 years, a body weight of 52.2 ± 6.4 kg, and a body mass index of 21.5 ± 2.2 were selected for the study. The menopausal status of the subjects was confirmed using the average simple menopause index (33.9 ± 15.0), which consists of vasomotor nervous system activity (14.5 ± 8.2) and malaise symptoms (19.5 ± 9.1). The subjects were otherwise physically and mentally healthy. The study was a randomized, double-blind crossover with comparisons between the L-theanine and placebo groups. A 3-day nonmedicated treatment followed by 6 consecutive days of ingestion of either 200 mg of L-theanine or placebo 1 hour before bed was adopted. Subsequently, with a 1-day gap, the treatments were crossed over and the effects on the subjects were examined for another 6 consecutive days. The effect of the L-theanine on sleep conditions was examined using the modified sleep inventory Orgi-Shirakawa-Azumi sleep inventory MA version (OSA-MA) OSA-MA questionnaire for the middle-aged [57], and the automatic nervous system was examined during sleep using a pulse frequency demolition method [63–65].

Sleep conditions, categorized into 5 factors (refreshed awakening, quality of sleep, dream quality, recovery from exhaustion, and perceived length of sleep), were assessed using the OSA-MA. Both sympathetic and parasympathetic nerve activities were assessed via a heart rate spectral analysis. The pulse rate (PR) at low-frequency heart beat (LF: 0.04–0.15 Hz) and high-frequency heart beat (HF: 0.15–0.4 Hz) were recorded with a complex demodulation. The sympathetic nerve activity represented as %LF was measured as the ratio of LF/(LF + HF), and the parasympathetic nerve activity was measured as the interbeat interval (IBI) of the HF.

The sleep inventory OSA-MA data (Table 1) suggest an improvement in refreshed awakening, quality of sleep, dream quality, and recovery from exhaustion with L-theanine. The pulse rate (Table 2) was lowered from 61.2 bpm in the placebo group to 58.8 bpm in the L-theanine group subjects in the first half of sleep. The pulse rate in the latter half of sleep was low (~58 bpm) and almost the same in both groups. The sympathetic nerve activity (%LF) was significantly lower in the latter half ($p < 0.05$) and in total sleep ($p < 0.05$) in the L-theanine group compared with the placebo group (Table 2). The parasympathetic nerve activity (IBI) was significantly higher in the L-theanine group in the first half ($p < 0.05$) and total sleep ($p < 0.05$) compared with the placebo group (Table 2).

In this study, menopausal women were chosen because such subjects have minimal mood swings and agitations compared to many younger women during their menstrual periods, as well as having a greater difficulty with sleep and early awakening with little relief from exhaustion. Therefore, the effect of

Table 1. OSA-MA Assessment Data on the Last Night Sleep Conditions in Postmenopausal Women ($n = 20$) With or Without Administration of 200 mg of L-Theanine. The Data Represent Mean $\pm$ Standard Error. Statistical Significance Was Measured Using Student's Paired t-Test

Factor	OSA-MA Parameters	Score (Z_c) Placebo	Theanine	p Value
1	Refresh awakening	17.9 ± 0.32	19.0 ± 0.30	ns
2	Sleep quality	14.3 ± 0.26	14.7 ± 0.22	ns
3	Dream quality	21.0 ± 0.31	21.7 ± 0.40	ns
4	Recovery from exhaustion	17.8 ± 0.27	19.4 ± 0.29	0.087
5	Feelings on sleep time	17.9 ± 0.25	17.2 ± 0.20	ns

OSA-MA = Orgi-Shirakawa-Azumi sleep inventory MA version, ns = nonsignificant.

the L-theanine on their sleep quality was measured without the interference of the above factors. The modulation of the auto-nervous activities is closely related to motor activity and sleep [36]. The changes in the parasympathetic (PNA) and sympathetic nerve activity (SNA) were closely related to relaxation, sleep, and stress [66], where the sleep stage proceeds to deep sleep, PNA increases, and SNA decreases [67–69]. Moreover, the pulse rate decreases with sleep. The significant reduction in the pulse rate and the increase in the PNA with ingestion of L-theanine support its effect on sleep. The ingestion of L-theanine significantly increased PNA in the first half of sleep and decreased the SNA in the second half of sleep, suggesting that elderly women, who normally wake up early, might have had good and deep sleep throughout the night, which was reflected in the significant improvement in the recovery from exhaustion in the Visual Analog Scale (VAS) results. The improvement in sleep quality with the administration of L-theanine was proven in men and women. The effect of L-theanine on sleep was further investigated in children with attention deficit hyperactivity disorder (ADHD) whose quality of life is poor due to deprived sleep and hyperactivity.

Sleep Efficiency in Children

ADHD is the most frequently diagnosed pediatric disorder, affecting 3%–5% of children in the United States and Europe [70]. Approximately 50%–60% of children with ADHD have sleep problems [71] along with their recognized learning disabilities (10%–30%), language disabilities (30%–50%), and oppositional behaviors (30%–80%). In addition to the L-theanine research confirming the relaxation effect [37,71], memory and learning performance [51, 72], neuroprotection [61, 73, 74], and sleep [56, 62, 75], a study was conducted at the University of British Colombia in collaboration with The Canadian Center for Functional Medicine in Canada to examine the effect of L-theanine on sleep and brain functions in children with ADHD [76].

The study was a 10-week randomized double-blind, placebo-controlled trial with 93 boys aged 8–12 years with ADHD. The diagnosis was confirmed using the guidelines set forth by the American Academy of Pediatrics. The subjects were given 6 weeks of treatment consisting of 200 mg L-theanine or placebo per serving. The treatment was given twice per day as chewable tablets in the morning and after school. Prior to the treatment, the children were assessed using a Conners Parent Questionnaire [77]. The subjects were randomized such that 46 subjects received the treatment (mean age of 9.45 years) and 47 received the placebo (mean age of 9.74 years). A total of 27 of the subjects were on stimulant medication during the study; 13 subjects were in the treatment group and 14 subjects were in the placebo group. The remaining 64 subjects were not on any stimulant medication. Sleep conditions such as the sleep percentage (%) and sleep efficiency were assessed by a parental assessment of the child's sleep problems using the Pediatric Sleep Questionnaire (PSQ) [78], and

Table 2. Pulse Rate (bpm), Sympathetic Nervous Activity (%LF), and Parasympathetic Nerve Activity (IBI, ms) Was Measured in Postmenopausal Women ($n = 20$) With or Without Administration of 200 mg of L-theanine. The Data Represent Mean $\pm$ Standard Error. Statistical Significance Was Measured Using Student's Paired t-Test

Items	Score Placebo	Theanine	p Value
Pulse rate (bpm)			
Total sleep time	61.0 ± 1.59	58.8 ± 1.48	0.098
First half of sleep	62.5 ± 1.61	58.8 ± 1.83	0.068
Second half of sleep	58.6 ± 1.25	58.7 ± 1.50	0.900
Sympathetic nervous system (%LF)			
Total sleep time	32.5 ± 2.01	28.2 ± 1.57	0.033
First half of sleep	30.0 ± 1.95	26.5 ± 1.54	0.088
Second half of sleep	35.5 ± 2.39	31.8 ± 1.61	0.037
Parasympathetic nervous system (IBI, ms)			
Total sleep time	522.1 ± 86.5	629.3 ± 86.4	0.052
First half of sleep	546.8 ± 98.3	668.6 ± 104.7	0.038
Second half of sleep	509.6 ± 77.9	588.6 ± 72.6	0.222

%LF = percentage low-frequency heart beat, IBI = interbeat interval.

waking bouts were assessed using an actigraph. Additionally, a Das-Naglieri Cognitive Assessment (CAS) [79] on the improvement of cognitive functions such as planning, attention, simultaneous and successive processing and a Rey Auditory Verbal Learning Test (RAVLT) [80] on the improvement in memory function, principally short-term working memory, were assessed.

Parental assessment through PSQ suggested that the intake of L-theanine significantly (correlation coefficient 0.2–0.3) improved both the sleep percentage and sleep efficiency in the children with ADHD. The actigraph results (Fig. 3) further confirmed that L-theanine did have consistent effects on the sleep percentage ($p < 0.05$), sleep efficiency ($p < 0.05$), and WASO ($p < 0.05$). The subjects had experienced a 22.8% lower incidence of waking bouts (WASO) during sleep compared with the placebo group, suggesting that the L-theanine improved sleep conditions in the children with ADHD. These effects were independent of the stimulant effect. The CAS and RAVLT assessments further confirmed an improvement in their attention, cognitive processing, and short-term working memory (data not shown). The parents also noticed a substantial improvement in behavior, such as anger and oppositional behavior, in the L-theanine-treated subjects.

Sleep disturbances in children with ADHD are attributed to hyperactive and impulsive behaviors. The abnormal brain functions in children with ADHD have been interpreted to signify reduced dopamine neurotransmitter activity. Thus, dopamine is known to play a critical role in learning, motivation, and emotional memory [81, 82]. The sleep improvement effect with L-theanine could be attributed to its significant effect on the release and reduction of the neurotransmitters dopamine and serotonin [51–53, 83] and its relaxation power to induce alpha brain wave activity [37, 45, 47, 84].
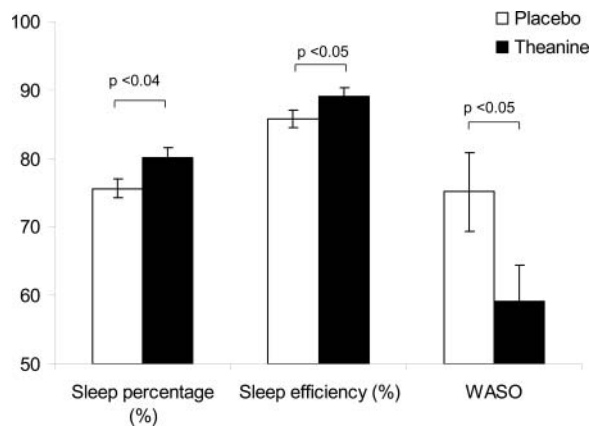

Fig. 3. Sleep percentage, sleep efficiency (%), and intermittent awakening time (WASO) was interpreted from actigraph data of children with ADHD. The subjects fed with or without 400 mg of theanine as chewable tablets. Statistical significance between the treatments was measured with multivariate analysis of variance.

SAFETY

Inadequate sleep at night adversely affects quality of life during the daytime and subsequently affects the economy of the country. In America alone, sleep-related or daytime drowsiness accidents account for $471 billion per year [20, 21]. In previously discussed studies, L-theanine has been shown to provide a significant improvement in sleep quality and efficiency. L-Theanine is also recommended for use during the daytime due to its various beneficial effects including relaxation, suppression of stress and anxiety, improvement of focus and concentration, and recovery from fatigue and physical stress. These studies suggest that L-theanine does not induce sleep but rather prepares the body and mind to enter sleep efficiently and that it improves the quality of sleep, which could be interpreted as effectively anxiolytic. Thus, unlike sedatives that often lead to daytime drowsiness, the use of L-theanine is not expected to induce sedative effects or daytime drowsiness. However, it is important to fully understand any potential consequences of L-theanine use during the daytime and its effect on daytime drowsiness for the safety of its usage.

It is also important to know the safety limits of L-theanine for the recommendation of its safe dosage limits for daily consumption. Therefore, this review further focuses on the consolidation of studies conducted on the safety and toxicological aspects of L-theanine and its effects on daytime drowsiness.

Safety of Usage During Daytime

The effects of L-theanine on daytime drowsiness were investigated in 27 healthy male daytime workers at the National Institute of Mental Health and the National Center of Neurology and Psychiatry in Japan [75]. Daytime drowsiness with ingestion of 200 mg L-theanine was assessed in the morning (10 AM to 11 AM) with 13 subjects (36.4 ± 4.5 years) and in the afternoon (2 PM to 5 PM) with 14 subjects (30.8 ± 7.1 years). The study was a randomized, double-blind, placebo-controlled crossover design. L-Theanine was administered 1 hour before the test, and drowsiness was assessed according to the methods described elsewhere [85], where a Psychomotor Vigilance Task (PVT) test [86, 87] was used as an objective evaluation and the VAS method [88–90] was adopted as a subjective evaluation. In the PVT test, the ratio of correct answers, reaction time, and variance in reaction time were assessed, where any decrease in the above parameters was considered as less vigilant. In the VAS method, the subjects were asked to fill in their responses on 22 parameters (Table 3) related to their mental and sleep status. Furthermore, the previous night's sleep conditions (sleep onset, wake-up time, and sleep time) were assessed with the Multiple Sleep Latency Test [91, 92]. The subjects were asked to avoid any physical hard work or smoking from the morning until the test was completed.

In the pre-assessment, the sleep onset at around 12:00 AM, wake-up time at around 6:30 AM, and sleep time of approximately 6 hours the previous night were similar between the L-theanine and placebo groups and did not differ from normal sleep patterns. The number of correct responses (Fig. 4a) and reaction times (Fig. 4b) were not significantly different between the L-theanine and placebo group subjects in both the morning and afternoon. Additionally, the variance in the reaction time was not significantly different (data not shown). The results of the VAS evaluation are summarized in Table 3. The data for the 22 VAS parameters were broadly divided into 3 groups, namely, (1) good physical and mental conditions (1–8), (2) relaxed and refreshed mood conditions (9–13), and (3) sleep conditions (14–22). In the morning evaluation, few to no significant differences were observed between the treatments for almost all the parameters in groups 1 and 2 except for alertness (6), which was significantly higher ($p < 0.05$) in the L-theanine group compared to placebo. However, most of the parameters in group 3 (14, 15, 17, 18, 20, and 22) were significantly different between L-theanine and placebo. These results suggest that the subjects who consumed L-theanine were more alert and less drowsy than the placebo group subjects. In the afternoon evaluation, most of the parameters in all 3 groups were equal between the subjects in the L-theanine and placebo groups. The L-theanine subjects reported significantly better physical status (4, 5, and 8), a tension-free mood (9), and greater alertness (22) than the placebo subjects. The afternoon evaluation also suggests that the L-theanine subjects felt better physical, mood, and arousal conditions than the placebo subjects.

Sleep inducers and sedatives normally induce drowsiness or sleep within an hour [93], whereas L-theanine, which achieves its maximum uptake in the brain within 30–40 minutes [53], did not induce any sleep or drowsiness in the subjects. Moreover, most of the parameters related to alertness were much better with the L-theanine, especially in the morning with subjects who are in the habit of going to sleep after midnight. The PVT results further confirmed that the L-theanine subjects were equally alert in terms of responding with correct answers and reaction times. Additionally, the variation in the reaction times confirmed that the L-theanine subjects were as reactive and alert as normal subjects. Previous studies suggest a significant improvement in the reaction time and the occurrence of correct answers in young male subjects with the ingestion of 200 mg of L-theanine [46]. According to an earlier study, the PVT and multiple sleep latency test (MSLT) were most comparable for a sleep and drowsiness assessment [94]. The results of this study confirm that L-theanine does not induce sleep or drowsiness during the daytime.

Table 3. Visual Analog Scale Evaluation Data on Daytime Drowsiness Measured 1 Hour After Administration of 200 mg of L-theanine or Placebo to Healthy Subjects in the Morning ($n = 13$) and Afternoon ($n = 14$)

Group	No.	VAS Parameters	Morning		Afternoon	
			Theanine	Placebo	Theanine	Placebo
1	1	Energetic	56.2 ± 4.47	53.8 ± 5.82	52.9 ± 5.31	45.8 ± 6.36
	2	Enriched willpower	60.6 ± 5.55	53.4 ± 5.77	56.1 ± 5.37	45.3 ± 6.52
	3	Efficient	55.2 ± 4.77	52.8 ± 6.13	45.7 ± 5.05	43.1 ± 5.02
	4	Light foot	53.7 ± 5.27	49.7 ± 6.02	57.7 ± 5.53	43.3 ± 5.77
	5	Perspective	51.7 ± 4.74	50.2 ± 5.91	50.6 ± 3.37	36.6 ± 3.85
	6	Good thinking power	56.7 ± 6.60	47.0 ± 6.21	61.3 ± 5.0	50.1 ± 6.47
	7	Good alertness	52.7 ± 6.38	49.5 ± 5.57	48.4 ± 5.56	43.3 ± 6.44
	8	Body is not wavy	51.8 ± 5.74	49.3 ± 6.82	60.2 ± 7.30	40.6 ± 6.28
2	9	Tension-free mood	46.0 ± 6.35	46.2 ± 5.27	73.4 ± 3.61	56.4 ± 6.20
	10	Neither weary nor refreshed	53.4 ± 7.29	57.2 ± 6.60	58.9 ± 4.92	54.6 ± 6.04
	11	Firm feelings	67.3 ± 5.74	60.3 ± 6.46	68.8 ± 4.62	62.4 ± 7.91
	12	Mood distraction	56.2 ± 6.63	46.8 ± 6.21	62.5 ± 4.22	64.0 ± 5.56
	13	Feel bit lousy but not when in action	61.2 ± 7.27	48.2 ± 7.18	37.3 ± 7.11	34.5 ± 6.25
3	14	Not clear thinking	59.4 ± 6.18	43.2 ± 6.21	54.5 ± 6.98	52.6 ± 6.20
	15	Thoughts are dull	59.0 ± 5.91	45.5 ± 6.77	51.6 ± 6.28	57.8 ± 6.33
	16	Absentminded	57.5 ± 5.46	48.6 ± 6.80	47.5 ± 7.22	49.7 ± 7.32
	17	Eyes are bleary	62.2 ± 5.96	45.5 ± 7.60	67.0 ± 6.31	54.9 ± 8.66
	18	Eyelids are heavy	68.5 ± 6.43	50.6 ± 7.52	62.9 ± 7.48	58.6 ± 7.48
	19	Feel like going to bed	66.0 ± 6.93	51.4 ± 8.46	49.8 ± 7.54	52.4 ± 7.24
	20	Drowsy	72.5 ± 4.43	58.5 ± 7.82	71.5 ± 6.44	60.4 ± 7.48
	21	Eyelids closing without knowledge	81.6 ± 4.16	73.8 ± 5.21	77.6 ± 4.84	73.0 ± 6.47
	22	Sleepy	86.5 ± 3.86	74.8 ± 5.19	85.6 ± 3.69	75.0 ± 4.49

VAS = Visual analog scale.

Groups (1) good physical and mental conditions, (2) mood on relax and refresh conditions and (3) sleep conditions, wherein 100 points represent positive response for groups 1 and 2 parameters and not negative for group 3 parameters. Data are represented as mean ± standard error and significance as *$p < 0.05$ and **$p < 0.01$.
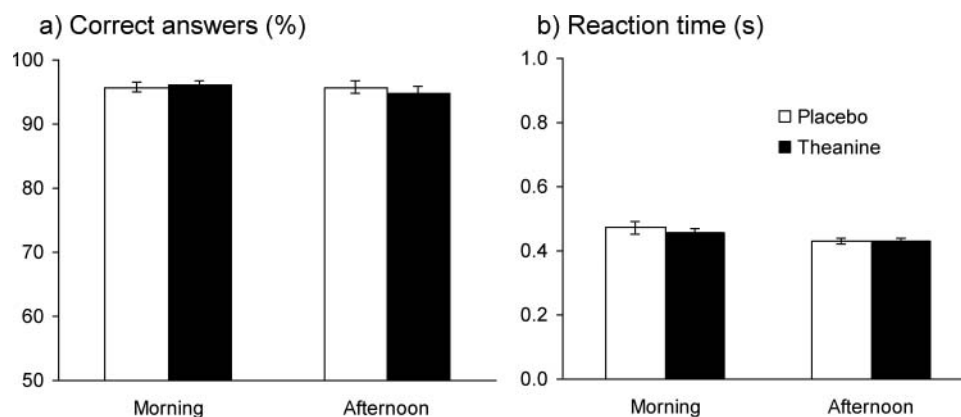
Fig. 4. PVT response on (a) percentage of correct answers and (b) reaction time of healthy male subjects with either administration of 200 mg of L-theanine or placebo in the morning ($n = 13$) and afternoon ($n = 14$). The data represent mean $\pm$ standard error. Statistical significance was measured using Student's paired t-test.

Safety Limits for Consumption

Tea is the only major source of naturally occurring L-theanine. After water, tea is the most widely consumed beverage in the world in all age groups. In the United Kingdom and the United States, the estimates indicate the consumption of 165 and 178 million servings of tea per day, respectively. This is equal to approximately 1 to 3 servings of tea per person per day. Around the world, depending on the country and type of tea, tea consumption among tea drinkers appears to be anywhere between 1 and 10 servings per day. The theanine content in tea leaves is generally in the range of 0.5%–2.5%; in general, 3 to 5 g of tea leaves is consumed for one serving of tea. Because theanine is highly soluble in water, nearly all of the theanine in the leaves is likely to dissolve in the water when a tea beverage is prepared. Therefore, in general, the estimated intake of theanine, nearly all of it as L-theanine by tea drinkers, could be from as low as 15 mg to as high as 375 mg/day for 1 to 3 servings per day, respectively. At the high end, with the intake of 10 servings a day, the consumption of L-theanine could be approximately 1.25 g/day, providing a high safety margin for the use as a sleep aid at 200 mg.

Metabolic and toxicokinetic studies in animals have shown that the intestinal absorption of L-theanine is mediated through an Na^+-coupled co-transporter in the brush-border membrane [95]. After absorption, theanine is rapidly incorporated into the blood and many tissues, such as the liver and brain, the latter via a leucine-preferring transport system [96]. Theanine is hydrolyzed to glutamine and ethylamine, primarily in the kidneys but possibly also in the liver [97]. Much of the metabolite is immediately excreted, but some returns to the plasma. Both theanine and its metabolites reach peak concentrations in the plasma and in tissues within a few hours and then rapidly decrease, along with a concomitant increase in the urinary concentrations [98]. In a recent human pharmacokinetic study [99], the maximum plasma concentration adjusted to a body weight of 70 kg (C_{max70}) was approximately 4.4 mg of L-theanine/L after 40 minutes (t_{max}) with intake of 100 mg of L-theanine. The half-life of absorption and elimination was observed at 15 and 65 minutes, respectively. The C_{max} values were dose dependent ($R^2 = 0.89$). Another study examined the kinetics of L-theanine absorption and its metabolites ethylamine and glutamic acid in healthy human subjects [100]. The study results suggest that a majority of the L-theanine was hydrolyzed to ethylamine and glutamic acid, most of which were excreted in the urine within 24 hours. The available evidence shows that theanine does not accumulate in the plasma but is rapidly excreted.

L-Theanine, and specifically Suntheanine, which was used in all of the above sleep studies, has been investigated in several toxicity studies [72, 101, 102]. Genetic assays have shown that it is not mutagenic, and a 78-week chronic study in mice found no evidence of carcinogenicity. The oral LD_{50} of L-theanine is >5000 mg/kg, showing a low order of oral toxicity. A subacute (28-day) study obtained the no observable adverse effect level (NOAEL) of an oral dose as 2000 mg/kg bw/day. In a 13-week subchronic dietary study, rats were dosed with 1500, 3000, or 4000 mg L-theanine/kg bw/day. No toxicity was found at any tested dose, and the NOAEL was the highest dose tested, 4000 mg/kg bw/day. As per the above sleep studies, the recommended dosage of 200 mg of L-theanine for a good quality of sleep is considered safe because it is well within the limits of daily consumption of tea drinkers and far below the NOAEL limits.

CONCLUSION

Sleep is an important phenomenon in human life and is necessary for both health and performance. A lack of sleep and sleep disorders are a common concern that seriously affects the quality of life and indirectly affects society as a whole. The reviewed literature supports the notion that the quality of sleep

is more important than the duration of sleep (sleep length), because poor sleep quality does not provide complete recovery from exhaustion, fatigue, and mental and physical stress. Moreover, poor quality of sleep during the night often leads to daytime drowsiness, which affects work performance and even leads to driving and work-related accidents. Sedatives are often used for the treatment of sleep disorders; however, they have the tendency to lead to addiction and have a number of side effects requiring warnings for safe use.

L-Theanine was found to improve relaxation, modulate neurotransmitters, and inhibit excitatory neurons by improving the quality of sleep in men, women, and children. Actigraphic and OSA inventory sleep assessments have shown an improved quality of sleep with the administration of 200 mg of L-theanine by reducing intermittent awakening (WASO) and thereby improving the sleep percentage and sleep efficiency. The improvement in the quality of sleep was further reflected in the recovery from exhaustion and refreshed awakening. On the other hand, the modulation of the automatic nervous system, namely, the sympathetic and parasympathetic nervous system, during sleep determines the quality of sleep. The administration of L-theanine simulated increased parasympathetic nerve system responses and decreased sympathetic nerve system responses. These results not only confirm the improvement in sleep quality but also validate the OSA sleep inventory questionnaire and actigraphic data. In none of the studies did L-theanine show an induction of sleep. Therefore, the mechanism of action of L-theanine in the improvement of sleep quality could be different from sedatives and sleep inducers.

The L-theanine as Suntheanine used in the above studies was produced from a natural fermentation process and was thoroughly investigated for its safety via toxicological studies, namely, an Ames mutagenicity test; 28-day, 78-day, and 90-day subacute toxicity tests; and a single dose (5 g) or a 2-week, 6.5-g feeding acute toxicity test. In Japan, the Ministry of Health and welfare approved L-theanine for its unlimited use (except for infants). In the United States, L-theanine as Suntheanine was affirmed as generally recognized as safe and further received a Letter of No Objection from the Food and Drug Administration. L-Theanine prepared through an enzymatic fermentation process has been thoroughly investigated for various physiological functions, including relaxation, relief from stress and anxiety, relief from premenstrual symptoms, recovery from physical stress, improvement of cognitive performances, neuroprotection, and an improvement in immunity against flu and common cold symptoms.

The preponderance of clinical and safety studies summarized in this review supports the use of L-theanine, prepared via an enzymatic fermentation process, as a safe natural aid for the improvement in sleep quality in all age groups. However, because the Japanese studies were underpowered due to a small number of subjects, and considering the effect of diverse factors on sleep quality among people, further studies with more participants would be warranted to clarify the role of L-theanine in sleep quality.

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