

The effect of green tea amino acid L-theanine formulation on markers of stress and quality of sleep (THESleep project) – a study protocol for a pilot randomised control trial

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ABSTRACT

L-theanine (L-THE) is a non-proteinogenic amino acid predominantly found in the leaves of the tea plant, *Camelia sinensis*, whose varieties produce green, white, oolong, black, aged Pu-erh, and ripened Pu-erh teas. The potential nootropic effects of L-THE, particularly for alleviating stress and anxiety and improving sleep quality, have seen an increase in its commercial application in products such as functional beverages and supplements despite the lack of conclusive evidence in the literature. The aim of this research project was to evaluate the effects of different concentrations of L-THE, in the form of a functional beverage sachet, on sleep and stress markers in individuals with mild to moderate sleep disturbances and determine an optimal concentration.

In this randomised, double-blind, placebo-controlled, parallel-group, four-arm, dose response intervention trial, sixty healthy participants aged 18–65 with mild to moderate sleep disturbances were recruited to the study (UCHREC Approval Number: 2023–13247 and ACTRN12623000776606; Universal Trial Number (UTN) is U1111–1292–0328).

Enrolled participants were randomly allocated to one of four treatment arms (100mg L-THE, 200mg L-THE, 400mg L-THE or placebo) in a 1:1:1:1 ratio, where they consumed a functional beverage for 14-days following a 7-day baseline period. The primary outcomes of this study were changes from baseline in objective and subjective sleep quality parameters alongside changes in secondary outcome measures of biochemical, physiological, and subjective stress markers relative to baseline.

This clinical trial is the first to comprehensively evaluate the effectiveness (optimal dose) of L-THE at improving sleep quality and stress markers using a suite of objective, subjective, physiological, and biochemical tools.

1. Introduction

Sleep is essential for health and wellbeing. However, nearly one third of adults report suffering from poor sleep despite the increase in scientific research and public health campaigns [5,33]. For many people, insufficient sleep is a consequence of “modern life” characterised by

increased artificial light exposure, screen time, emotional stressors (work, relationships, financial), access to energy dense foods, and sedentary lifestyles [16,47]. A commonly used strategy to improve sleep quality long term focuses on addressing sleep habits such as nighttime routines, bedroom environment (noise, temperature, light), meal timing, caffeine and alcohol intake, exercise, and mindfulness [5].

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Another common strategy involves the use of prescription or over-the-counter sleep aids such as the Benzodiazepines, Benzodiazepine-like drugs (Z class drugs), and other classes including antidepressants and antihistamines [64]. These can be effective treatment options due to their fast action, but are primarily used for acute relief because of the risk of serious negative side effects (fatigue, daytime sleepiness, dependency, resistance) associated with their use [23,44]. The search for safe, effective alternatives to pharmacological treatment has seen a rise in research interest in bioactive compounds derived from herbs and plants that are typically used to reduce stress and anxiety and manage sleep disturbances in traditional medicine practices [7]. More recently, research focus has shifted towards assessing bioactive molecule bioavailability and potential supply via alternative delivery methods such as functional foods and beverages [46]. These products contain bioactive compounds naturally occurring or fortified during manufacturing with specific health benefits [65]. They also offer several advantages including the ability to deliver one or several compounds in the one product, convenience, storage capabilities, appealing colour, size and flavour variabilities, and relatively low costs associated with their production [17].

L-theanine (L-THE) is a naturally occurring, non-essential/non-proteinogenic amino acid predominantly found in the leaves of the tea plant, *Camelia sinensis*, of which green, white, black, aged Pu-erh, and ripened Pu-erh teas are enriched [32,40]. It accounts for 1–2% of the dry weight of tea leaves with the amount depending on geographical region, sunlight exposure, time of harvest in the season, processing techniques, and even infusion preparation of the tea [32,51,55]. Additionally, L-THE has several properties that make it an ideal candidate for commercial food and beverage applications including being colourless, soluble in water, stable up to 90 °C and in acidic conditions (pH < 4) [58]. In humans, L-THE is absorbed relatively quickly (within 30–40 min) in the small intestine and can cross the blood brain barrier [48,56]. Similar in structure to the excitatory neurotransmitter, glutamate, animal studies have shown that L-THE in the central nervous system may affect the transmission of important neurotransmitters involved in the sleep-wake cycle. In particular, L-THE may affect the transmission of glutamate either directly, by binding glutamate receptors [30], or indirectly by inhibiting transport of its precursor, glutamine [29]. It has also been shown to enhance the inhibitory neurotransmitter gamma aminobutyric acid (GABA) by increasing expression of its receptors [19]. Research on the health benefits of L-THE consumption in humans is continuing to grow with these studies now being the subject of recent systematic reviews and meta-analyses. A systematic review summarising the effects of L-THE consumption on stress and anxiety in humans found that daily consumption of L-THE between 200 and 400 mg may exert physiological and psychological benefits associated with the reduction of stress and anxiety [60]. Furthermore, a relatively recent systematic review and meta-analysis of 19 studies by our team [8] proposed that L-THE consumption significantly improved subjective sleep onset latency (SOL), daytime dysfunction, and overall sleep quality. However, heterogeneity in formulation (e.g., combined ingredients), dosage, and intervention duration were key limitations [8]. A similar finding was observed in a systematic review of 15 studies conducted by Cotter et al. [18] summarising the effects L-THE supplementation alone on sleep. In this review the authors suggest that 200–450mg/day L-THE may be beneficial for improving sleep onset latency, sleep maintenance, and sleep efficiency [18]. Finally, a systematic review and meta-analysis conducted by Payne et al. [45] found that L-THE in combination with caffeine may improve attention tasks, whereas L-THE alone may be beneficial for cognition and overall mood [45]. In this systematic literature review, 49 studies were included and 15 of these were included in a meta-analysis. No effect of L-THE consumption was found on sleep, although only eight studies reported sleep outcomes. The authors of this review highlighted that were unable to draw any definitive conclusions of the true effects of L-THE consumption on cognition, mood, and sleep as a result of the large prediction intervals [45]. The aforementioned

reviews highlight the inability to conclusively state the effectiveness of L-THE consumption on sleep and that further research is required.

To fill this gap, the aim of this research project was to comprehensively evaluate, using objective and subjective assessment tools, the effects of different concentrations of L-THE incorporated within the functional beverage sachet, on sleep and stress markers in individuals with mild to moderate sleep disturbances. We hypothesize that there is an optimal concentration that is beneficial for improving sleep quality in this population.

2. Method

The protocol for this study has been developed in accordance with the Standard Protocol Items: recommendations for Interventional Trials (SPIRIT) 2025 statement: updated guideline for protocols of randomised trials [15]; Consolidated Standards of Reporting Trials (CONSORT) 2025 statement: updated guideline for reporting randomised trials [26]; and the Reporting of Multi-arm, Parallel-Group Randomised Trials Extension of the CONSORT 2010 statement [28]. Human ethics for this study has been approved by the University of Canberra Human Research Ethics Committee (UCHREC Approval Number: 2023–13247) and this project is registered with the Australian and New Zealand Clinical Trials Register (ACTRN12623000776606; Universal Trial Number U1111–1292-0328).

2.1. Study design

This study was designed as a randomised, double-blind, placebo-controlled, four-arm, parallel-group, dose response intervention trial (Fig. 1). Participants were required to attend four in-person clinics at the University of Canberra; a screening clinic and three additional testing clinics where a range of physiological and anthropometric measures were conducted (blood pressure (BP), heart rate (HR), heart rate variability (HRV), height (cm), weight (kg), and waist circumference (cm)). As the physiological measures were all at resting, participants refrained from consuming caffeine and food at least two hours prior to the clinic and refrained from moderate to vigorous activity at least 24 h prior to the clinic. All other assessments were conducted in the participants own home. The trial commenced with a 7-day baseline period (without supplementation). During this period, data relating to the participants sleep, physical activity, stress and dietary intake were collected using an actigraphy device (sleep and physical activity), self-assessment questionnaires (sleep, stress and dietary intake), and saliva collections tubes (biochemical stress markers). Following this, participants then consumed a functional beverage daily, at home, containing one of three different concentrations of L-THE (100mg, 200mg or 400mg) or placebo for fourteen days. Data was collected as per the baseline period for each week of the intervention period. Additionally, participants also monitored for any adverse events. A two-week follow-up of adverse events and overall sleep quality was conducted to determine any long-lasting effects post cessation of the intervention. Table 1. outlines the participant study schedule.

Following completion of the study, participants were provided with a \$50 gift card.

2.2. Informed consent

Prior to commencing the study, participants were provided with the study information sheet and given a verbal explanation of the study protocol at an in-person clinic. Participants were required to provide signed, written consent before eligibility was determined. Once informed consent was provided, participants completed a sleep quality questionnaire and general health questionnaire to determine if they met the inclusion criteria.

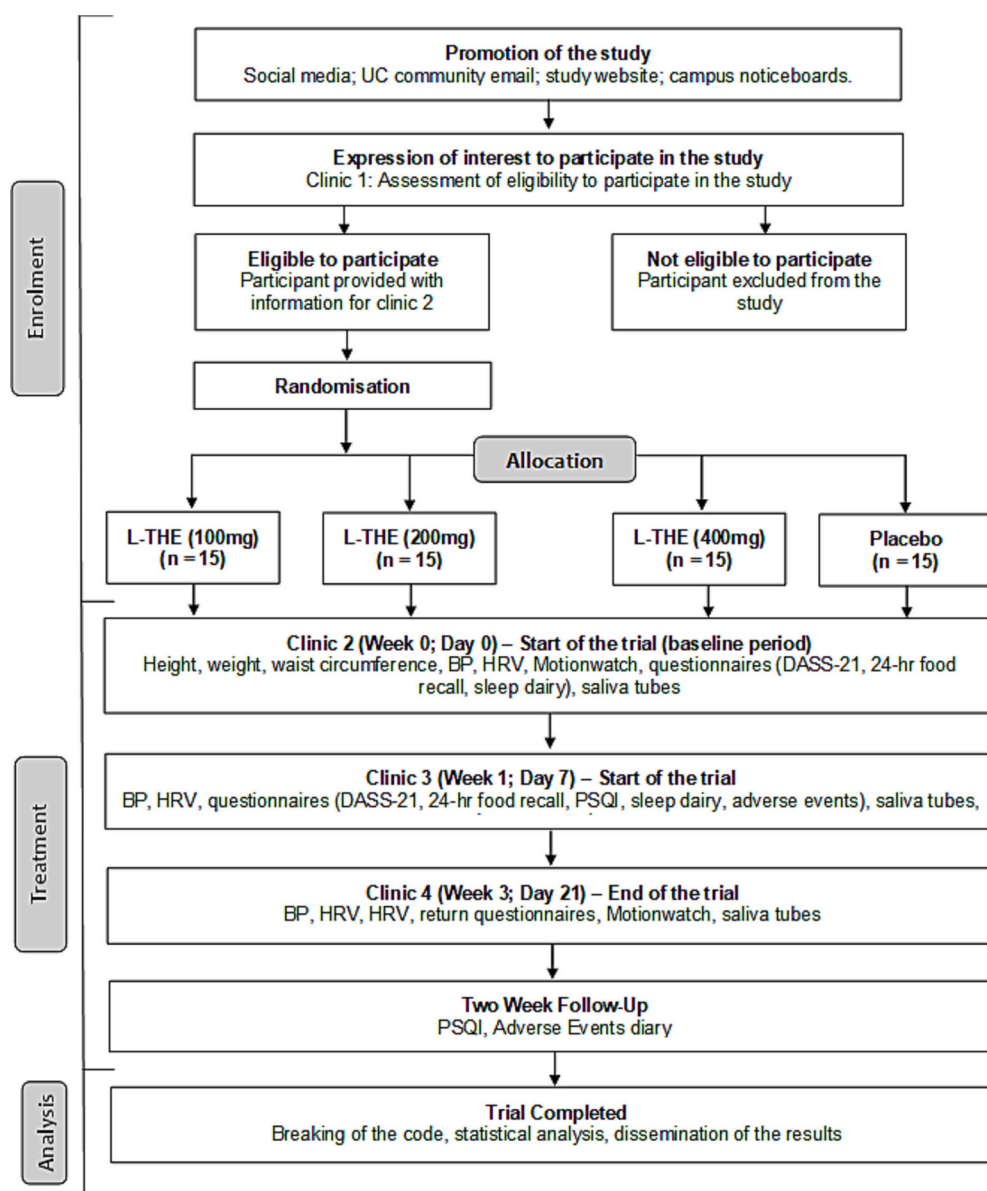


Fig. 1. THESleep study project design.

2.3. Inclusion criteria

To be included in the study, eligible participants were male and female participants aged between 18 and 65 years with mild to moderate sleep disturbances, scoring ≥ 5 on the Pittsburgh Sleep Quality Index (PSQI) questionnaire (which evaluates sleep quality over the previous month) [10] and were otherwise healthy.

2.4. Exclusion criteria

Eligible participants were excluded from participating if they were smokers, had any current or past known health issues such as cardiovascular, hepatic, gastrointestinal, renal, premalignant, neurological, degenerative illnesses, suffered from diabetes mellitus (type 1 or type 2), were pregnant, breastfeeding, or lactating [59]. Participants were also excluded if they lived with a clinical sleep disorder (obstructive sleep apnoea), were shift workers or completed trans-meridian travel within the previous month. Additionally, participants who were taking medication or herbal supplements for sleep or taking antipsychotic or hypnotic medication were also excluded.

2.5. Recruitment

Participants were recruited from individuals residing in the greater Australian Capital Territory region (Australia), responding to an advertisement poster placed on University of Canberra noticeboards on campus, via university community email, social media (X™, LinkedIn®) and THESleep project dedicated website (www.ffnrlaboratory.org/thesleep). Each responder received an information statement letter detailing the procedures, protocols and possible associated risks of participating in the study.

2.6. Sample size and power calculation

The total number of participants required for this study was 60 (15 in each group). This number was calculated based on the sample size for a pilot study to explore a 30–40% primary outcome effectiveness in 10–15 participants per arm following the previously proposed sample sizes for pilot randomised trials [57]. Power was set to 0.8 and a type 1 error rate $\alpha = 0.05$. The sample size also accounted for a relatively modest participant dropout (20%) and incomplete sleep data.

Table 1
Participant study schedule.

	Study Schedule					
	Enrolment	Allocation	Post allocation			Closeout
Timepoint	T ₋₇	T ₀	T ₀	T ₊₇	T ₊₁₄	T ₊₁₄
Enrolment						
Informed consent	X					
General Health Questionnaire	X					
PSQI	X					
Allocation		X				
Intervention						
L-THE (100mg)				◆	◆	
L-THE (200mg)				◆	◆	
L-THE (400mg)				◆	◆	
Placebo				◆	◆	
Assessments						
Height (cm)			X			
Weight (kg)			X			
Waist Circumference (cm)			X			
BP			X	X	X	
Resting HRV			X	X	X	
PSQI			X	X	X	X
24-hour Food Recall			X	X	X	
DASS-21			X	X	X	
CSD-M			X	X	X	
Saliva Biomarkers			X		X	
Compliance			X	X	X	
Adverse events				X	X	X

2.7. Concealment, randomisation, and blinding

Upon enrolment, participants were allocated a unique identification number, followed by block randomised (1:1:1:1) allocation into one of the four treatment arms (<https://www.randomizer.org/>). Participants in each group were not matched for age, sex or BMI. Randomisation was conducted by a researcher not involved in the coordination of the study. This researcher stored the list of participant names, their associated unique identification number and their intervention allocation in a sealed envelope in a secure location. The intervention sachets were allocated a unique identification number determined using the random number generator function in Microsoft Excel (Microsoft® Excel® for Microsoft 365 MSO (Version 2504 Build 16.0.18730.20226) 64-bit). The research investigators and participants were blinded to the intervention allocation sequence. Participants were also blinded to the study hypotheses and outcomes. Enrolment of participants and allocation of the unique identification number and intervention sachets was conducted by one researcher (AB). Once data from all participants was collected and entered into an electronic database, and prior to statistical analysis,

the research investigators directly involved in the study were provided access to the allocation concealment envelope.

2.8. Intervention

The beverage product used for the intervention was in the form of an effervescent powder sachet containing a selection of ingredients and either placebo (no L-THE), 100mg, 200mg, or 400mg L-THE. L-THE (MyProtein; Manchester; United Kingdom) was sourced from a commercial supplier with the purity greater than 98% (based on the certificate of purity provided by manufacturer). The taste and appearance of the formulation for the placebo was identical to the treatment formulation containing L-THE.

The product formulation was prepared and manually dispensed into foil sachets and sealed using a sealing machine in the Functional Foods and Nutrition Research Laboratory at the UC [59]. The sealed sachets were vacuum packed and stored in individually labelled and numbered cardboard containers in a dry, dark environment.

Participants were required to dissolve one sachet/evening into

125mL cold water (not hot or boiling), and to consume it immediately 30–45 min before going to bed. Any unused sachets were returned at the end of the treatment period for compliance.

2.9. Safety

Consumption of L-THE is generally well tolerated, with animal toxicity studies revealing no significant adverse events across a range of measures for morbidity, mortality, body weight, food consumption, clinical chemistry, haematology, or gross pathology at a maximum dosage of 4000mg/kg body weight/day for 13 weeks [6]. Gastric intubation of 10mmol L-THE/kg body weight/day for two weeks in rats decreased lipid peroxidation in the brain [41]. In humans, a systematic review of 19 studies containing L-THE in the intervention found the majority of adverse events reported were considered minor, with headaches and gastrointestinal discomfort the most commonly reported symptoms [8]. One study reported symptoms such as sleep disturbance, drowsiness, weakness, fatigue, irritability, trouble concentrating and gastrointestinal discomfort [49]. In this study, participants were consuming between 450mg - 900mg L-THE daily (225mg - 450mg twice/day) and were also taking medication for generalised anxiety disorder. Additionally, these symptoms were also reported in the placebo group [49]. The highest concentration of L-THE used in this study was 400mg and no adverse events were expected to occur, however, sensitivity to the effects of L-THE could not be predicted. Participants were required to monitor any adverse events daily during the intervention period. They were required to record their symptoms and contact the research team in the event a serious adverse event occurred. In this case participants ceased taking the intervention product immediately and were withdrawn from the study. Additional safety measures include the measurement of participants blood pressure and heart rate at each week of the study, and consumption of the beverage was before bedtime reducing the risk of potential daytime fatigue.

2.10. Clinical outcomes

The primary outcome of this study was changes from baseline in objective and subjective sleep quality parameters following consumption of different concentrations of L-THE or a placebo for fourteen days. Sleep quality parameters included sleep onset latency (SOL), wake after sleep onset (WASO), total time in bed (TIB), total sleep time (TST), and sleep efficiency (SE%).

Secondary outcome measures for this study were changes from baseline of biochemical, physiological, and subjective stress markers. The biochemical markers of stress were changes in morning-evening delta of salivary cortisol and salivary alpha-amylase (sAA). Physiological markers of stress included the measurement of resting blood pressure, heart rate, and heart rate variability (HRV) for assessment of the autonomic nervous system. Subjective stress was determined using a validated questionnaire.

Additional factors that were measured included dietary information (macronutrient intake, alcohol, caffeine intake, and timing of meals), and daily physical activity levels (intensity, duration, and frequency).

2.11. Data collection methods

2.11.1. Anthropometry

Participants height was measured using a stadiometer (ADE, Sydney, Australia) and weight was measured using the previously calibrated portable Multi-Frequency Total Body Composition Analyser (DC-430U, Tanita, Arlington Heights, IL, USA). This instrument provides additional information related to body composition (fat percentage, fat mass, fat free mass, muscle mass, total body water, total body water percentage, bone mass, basal metabolic rate, body mass index, and visceral fat rating) [42]. Waist circumference was measured using a tape measure following CDC guidelines [13].

2.11.2. Sleep quality

Objective sleep quality was recorded using the MotionWatch 8 (CamNtech Ltd.; Cambridgeshire; UK) actigraphy device. The device contains a triaxial accelerometer that detects physical movement in the form of activity counts over set time intervals. These activity counts were then converted into sleep and wake periods through a validated software algorithm for the determination of sleep quality and physical activity levels (The MotionWatch User Guide, [11]). The Motionwatch 8 device was set to high sensitivity threshold of ≥ 20 activity counts collected over 30 s intervals. This setting has been previously undergone validity and reliability testing against polysomnography [21]. Participants wore the device continuously for a total of 21 days on their nondominant wrist and were instructed to press the event marker button each night in bed when they intended to go to sleep, and again in the morning at their final awakening when they intended to get out of bed for the day. Participants were also instructed to only remove the device for certain activities (showering, entering a sauna, playing contact sports, snorkelling or scuba diving) as per the manufacturer's instructions [11].

Data downloaded from the activity monitor was analysed using MotionWare software (version 1.4.10; CamNtech Ltd.; Cambridgeshire; United Kingdom) and the Society of Behavioural Sleep Medicine (SBSM) Guide to Actigraphy Monitoring: Clinical and Research Applications manual to determine the main sleep period according to the manuals scoring hierarchy [2].

Subjective sleep quality was recorded using the Consensus Sleep Diary-M (CSD-M) [12] and the Pittsburgh Sleep Quality Index (PSQI) questionnaire [10]. The CSD-M is a 15-item standardised tool comprising of 10 items relating to sleep quality (SOL, WASO, TST, TIB, and SE%). It also has an additional five items that are related to alcohol and caffeine intake, daytime napping, and sleep medication intake [12]. Participants completed the CSD-M daily upon waking for the duration of the study. The diary was used to correlate the sleep onset and offset in the actigraphy data. The PSQI is a validated 19-item questionnaire assessing sleep quality in the previous month and has been validated in several clinical populations [4,38]. It comprises several sleep quality measures including, sleep onset latency, sleep duration, sleep efficiency, sleep medication use, and next-day dysfunction [9].

2.11.3. Subjective stress

Subjective stress was measured using the short form of the Depression, Anxiety, Stress Scale (DASS-21) [35]. This is a modified version of the original 42-item questionnaire and comprises a 21-item questionnaire divided into three scales of seven items each that assesses depression, anxiety, and stress in the past week. A score for each scale is determined by the addition of each of the seven items which are then multiplied by two, with higher scores indicating a higher level of depression, anxiety, and/or stress [35].

2.11.4. Physical activity

Physical activity levels were determined from the activity data collected from the Motionwatch 8 (CamNtech LTD, United Kingdom) device. The MotionWare software (version 1.4.10; CamNtech Ltd.; Cambridgeshire; United Kingdom) automatically classified vigorous, moderate, low daily physical activity as well as sedentary intervals after excluding the sleep periods.

2.11.5. Saliva collection

Participants collected saliva at three time points during the baseline period and post intervention period- in the evening (8.00PM; days 7 and 21), immediately upon awakening, and 30–45 min following the initial morning collection (Days 8 and 22). The evening sample collection was performed at least one hour following their evening meal and after consumption of the sachet, before going to bed. They also refrained from consuming any dairy or brushing their teeth prior to this sample. Participants were also required to refrain from brushing their teeth,

drinking tea, coffee or water, or consuming food before and between the two morning samples. Saliva was collected into tubes using the passive drool collection method following a previously described protocol [37]. Samples were stored in the participants freezer until their next clinic visit. Cortisol and sAA were measured using the Salivary Cortisol ELISA kit and sAA Kinetic/Enzymatic kit (Salimetrics, USA), respectively, according to the manufacturer's instructions.

2.11.6. Blood pressure

Resting blood pressure was measured using a digital blood pressure monitor (Omron, Florida, USA) after at least 10 min in a sedentary position based on the guidelines used for the 2nd Australian National Blood Pressure study [62].

2.11.7. Heart Rate Variability (HRV)

Resting HRV was measured using the heart rate chest strap and Firstbeat data acquisition software (version 4.7.3.1 (3.3.8.3), 2016, Firstbeat Technologies Ltd., Finland). R-R intervals for HRV calculation were collected while participants are in a supine position for a period of 10 min [59]. Data was exported as a text file to the HRV analysis software (Kubios Heart Rate Variability Software, version 2.0; Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland) and manually corrected for ectopic/missing beats according to a previously published method [52]. HRV parameters were automatically determined by the software and included time domain, resting heart rate, R-R intervals (IBI), standard deviation of normal-normal intervals (SDNN), mean square root differences of the standard deviation (RMSSD), low frequency normalised units (LF), high frequency normalised units (HF), and low frequency to high frequency normalised units ratio (LF/HF).

2.11.8. Dietary intake

Dietary intake was determined using a self-administered 24-h dietary questionnaire completed on days 7, 14 and 21 of the study periods. Data was analysed using Foodworks v10 (Xyris Software, QLD, Australia). Participants did not restrict their caffeine consumption throughout the study and were encouraged to continue their normal daily routine. The influence of caffeine intake, alcohol intake, macro- and micro-nutrient intake on sleep and stress will be included in the data analysis.

2.11.9. Participant privacy and confidentiality

All data was coded without the link to any of the participant personal details. Only deidentified data will be used in the analysis of the results and in any journal publications. Personal information and data collected during the study is stored in a secure location and on a password protected computer at the University of Canberra. Data files will be kept for a period of 15 years after which they will be destroyed according to the University protocols. Data collected from any participants that withdraw from the study had all their personal information and data destroyed as per University of Canberra protocols.

2.11.10. Data analysis

All data will be exported and analysed using IBM SPSS Statistics for Windows (v30, IBM Corp, Armonk, NY, US). Statistical analysis will comprise baseline to post-intervention within-group and between-group effects using paired Student's *t*-test and repeated measures ANOVA for within-between group analysis with Bonferroni post-hoc correction. A general linear model adjusted for confounders (diet quality, age, gender, stress markers, physical activity) will be attempted depending on data completeness. Mediation analysis is not feasible at this stage due to the nature of the pilot trial and small sample size. Stress measures (HRV, DASS-21, diurnal salivary cortisol and sAA) will be approached as potential confounders in the analysis and mediation models might be considered should the trial be extended beyond pilot stage.

3. Discussion

To the best of our knowledge, this study will be the first to comprehensively evaluate the dose-response effects of L-THE consumption on sleep and stress markers in healthy participants with mild to moderate sleep disturbances and determine an optimal dose. Designed and powered as a pilot study, the outcomes will provide a framework for future, larger intervention studies. This study has a number of major strengths. Firstly, this study uses objective (actigraphy) and subjective (sleep diary) measures of sleep quality. Actigraphy is a non-invasive device that has been validated against polysomnography (PSG), considered the gold standard of sleep assessment tools [63,66]. The sleep parameters that can be determined from this device include, SOL, TST, TIB, WASO, and sleep efficiency (%). Actigraphy has an excellent sensitivity for sleep, although, one of its drawbacks is that it has a lower specificity for wakefulness [20]. However, when combined with the use of a validated sleep diary, such as the CSD-M, and at least 7 days of continuous sleep data, as we have done in this study, its reliability is significantly improved [2,66]. Secondly, the study design allowed participants sleep to be measured under normal conditions in their own environment, maintaining their normal daily routines. A third major strength is the duration of the intervention. According to previous studies [3,24,25,27,31,36,39,43,49,53,54] (including the work from our team [8,60]) the 14 day intervention was deemed to be sufficient to observe a measurable effect of L-THE supplementation. This timeframe allows for participant adjustment to consuming a beverage before bed, particularly for those who typically do not. Furthermore, this timeframe also aligns with the recommendations of the scoring criteria for actigraphy data [2]. Finally, the study setup will allow us to assess the hypothalamic pituitary axis (HPA) and the autonomic nervous system which are both affected by stressors such as psychosocial stress and insufficient sleep [1,14,22].

While this study has many strengths, it does come without some limitations. Firstly, as mentioned previously, sleep was assessed using actigraphy instead of PSG. Actigraphy provides several informative sleep parameters but is unable to measure sleep architecture. Sleep architecture is comprised of three stages of non-rapid eye movement sleep (NREM) that moves from light sleep (stage 1 and 2) into deeper, slow wave sleep (stage 3) followed by one stage of rapid eye movement (REM) sleep where dreaming and memory consolidation occur [5]. PSG utilises several tools to measure sleep architecture including electroencephalography (EEG), electrooculography (EOG), electromyography (EMG), and electrocardiography (ECG), however, it can be costly and is performed in a dedicated sleep laboratory meaning subjects are outside their normal sleeping environment [61]. Secondly, eligibility to participate in the study was determined by scoring ≥ 5 on the PSQI. This questionnaire assesses sleep quality in the previous month; it does not capture the duration of sleep disturbances. A third limitation was the small sample size in each arm of the study. This study was a parallel, dose-response study with four treatment arms. A much larger sample size in each arm would be expensive and more time consuming to complete. As this is a pilot study, it allows us to extrapolate the findings to design much larger studies using the L-THE dose which emerged as effective in modulating sleep quality. The final limitation was participant compliance. Participants were only required to return any unused sachets, however, as they did not consume the beverage in front of researchers, we cannot be entirely sure of complete compliance. For future studies it may be beneficial for participants to return all opened sachets, although this may be difficult in studies of longer duration. Participants could also be asked to provide a count of the remaining intervention, similar to that done by Lopresti et al. [34] in their study on the effect of a saffron extract on sleep quality, cortisol, and melatonin concentrations in adults [34]. Alternatively, it may be beneficial to assess ethylamine and glutamic acid (L-THE metabolites) in urine as potential markers of L-THE intake [50].

4. Conclusion

The outcomes of THESleep Project will provide valuable information on the true effectiveness of L-THE and its optimal dose for the improvements of sleep quality and stress. It will also provide a framework for future studies evaluating L-THE in a healthy and clinical population.

CRediT authorship contribution statement

Amanda Bulman: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Andrew McKune:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Ekavi Georgousopoulou:** Writing – review & editing, Data curation. **Domenico Sergi:** Writing – review & editing, Conceptualization. **Nenad Naumovski:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare no conflicts of interest.

Data availability

No data was used in the development of this article.

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