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Assessment of a multi-ingredient dietary supplement on sleep quality: a randomized, double-blinded, placebo-controlled crossover clinical study

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Abstract

Background Sleep disorders, specifically insomnia, are recognized as global public health concerns. The present study is to evaluate the potential effects of a multi-ingredient formula through a multimodal approach on sleep quality in people with insomnia. The study supplement contained bovine colostrum and egg yolk extracts, ashwagandha root extract, lavender oil, pyridoxal-5-phosphate, melatonin, GABA, and magnesium. Colostrum and egg yolk extracts have been shown to support immune system function and thus likely support sleep health. Ashwagandha root extract, lavender oil, pyridoxal-5-phosphate, melatonin, GABA, and magnesium have been shown to help sleep health and quality via different mechanisms of action.

Methods A total of 30 healthy adults with self-reported symptoms of insomnia, divided into placebo and treatment groups, completed a 6-week randomized, double-blinded, placebo-controlled crossover study (1-week washout, 2-week intervention, 1-week washout, and 2-week intervention, sequentially). Participants were instructed to fill out Insomnia Severity Index (ISI) and the Leeds Sleep Evaluation Questionnaire (LSEQ) survey weekly, and keep a sleep diary daily, which were used to assess their sleep quality. Saliva samples were taken from participants before and after each 2-week treatment period to measure salivary melatonin level. Participants were instructed to wear a Fitbit Inspire 2 device to track their sleep and wake data. Linear mixed model ANOVA was used to evaluate statistics for the study.

Results Study supplement significantly improved sleep quality of participants compared to placebo group, specifically difficulty falling asleep ($p < 0.01$). The study supplement also significantly improved the number of awakenings during the night compared to baseline ($p < 0.01$). Overall sleep health was improved, specifically sleep quality (wakefulness) ($p < 0.01$). Study supplement also significantly improved salivary melatonin levels compared to placebo group ($p < 0.01$). Some limitations of the study include a lack of controlled environment and a heterogeneous population, among others.

Conclusions The preliminary findings observed in the present study showed that the study supplement improved sleep health and quality for people with insomnia. The importance of finding effective and safe solutions for poor sleep and insomnia cannot be overstated.

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Study registration ClinicalTrials.gov NCT05368909 "Clinical Study to Assess a Dietary Supplement on Sleep Health and Quality", registered May 10, 2022. https://clinicaltrials.gov/study/NCT05368909.

Keywords Bovine colostrum, Insomnia, Sleep, Sleep quality, Sleep health, Melatonin

Introduction

The purpose of the present study is to evaluate the effects of a proprietary, multi-ingredient formula through a multimodal approach on sleep quality in people with subclinical insomnia. Sleep health is increasingly recognized as a key contributor to many domains of health, including cardiovascular, immunologic, metabolic, cognitive, behavioral, and mental health (Grandner and Fernandez 2021). Additionally, sleep health has been recognized as a national public health goal in Healthy People 2030 (Healthy People 2030) and has been recently included in the American Heart Association's "Life's Essential 8" components of heart health (Lloyd-Jones et al. 2022) alongside blood pressure, lipids, fasting glucose, weight, diet, physical activity, and smoking. Decades of data show how sleep health is associated with health and longevity (Girardin et al. 2021), yet sleep disorders are alarmingly common. Insomnia is the most prevalent sleep disorder (Dopheide 2020), and insomnia symptoms impact approximately one third of US adults (Grandner et al. 2013). Difficulty falling asleep and staying asleep adversely impacts sleep quality, which can result in worse overall sleep health and, consequently, other health outcomes (Buysse 2014). Real-world outcomes associated with worse sleep health include increased risk for cardiometabolic disease (e.g., hypertension (Li et al. 2023), diabetes, and obesity (Liu et al. 2022)), worse mental health (e.g., anxiety, depression, and suicide (Tubbs et al. 2020)), impaired daytime function (e.g., impaired attention, memory problems, decision-making deficits) (Zamore and Veasey 2022), and more.

Previous studies have documented associations between sleep health and immune function (Irwin et al. 2016). The relationship between sleep and the immune system is reciprocal. Poor sleep quality can lead to a weakened immune system, and a weakened immune system (often manifested as illness) can lead to poor sleep (Besedovsky et al. 2019). Disrupted sleep has been shown to cause inflammation in the immune system, which increases susceptibility to chronic and acute disease (Lueke and Assar 2022).

Pharmacologic interventions have demonstrated efficacy for treating insomnia (Sateia et al. 2017) but are typically not preferred due to limited efficacy in the context of side effects of medical sedation. Despite these options, interventions for sub-clinical sleep difficulties are less well studied. Nutritional supplements, including certain vitamins, minerals, and botanical extracts, have emerged as an accessible option for improving sleep health (Chan and Lo 2022). The ingredients included in the study supplement have been chosen based on availability, price, and literature evidence of their efficacy. Studies of this nature have been performed on multi-ingredient formulas in the past (Stevens et al. 2017).

Ashwagandha has been shown to improve sleep parameters such as sleep onset latency, sleep time, and sleep efficiency (Langade et al. 2021). Evidence shows that lavender oil can be used to promote relaxation and increase drowsiness (Diego et al. 1998) and can have a positive effect on sleep disturbances (Kasper et al. 2017). Peters et. al demonstrated an association between vitamin B6 and higher sleep quality and decreased insomnia (Peters et al. 2015). Melatonin has been shown to reduce sleep onset latency and increase sleep efficiency and duration (Brzezinski et al. 2005). Research shows that although GABA (gamma-amino butyric acid) does not cross the blood–brain barrier, it may indirectly improve sleep and decreases morning drowsiness (Hepsomali et al. 2020). Magnesium has been shown to decrease ISI (Insomnia Severity Index) (Bastien et al. 2001) scores and decrease sleep onset latency (Abbasi et al. 2012).

Colostrum and egg may contain appreciable levels of melatonin (Meng et al. 2017) and other sleep-benefiting ingredients (Yang et al. 2011). Moreover, colostrum and egg extracts have been studied extensively for their immune benefits (Vetvicka and Vetvickova 2020), which may aid in recovery during sleep (Besedovsky et al. 2019). Additionally, an immune system responding to threats can disrupt sleep through disruptions of the levels of body neurotransmitters and cytokines, among others, as sleep and the immune system are closely related ((Bland 2022; Imeri and Opp 2009)). Therefore, it is plausible to suggest that these ingredients may improve sleep quality. However, no extant research has explored these ingredients relative to sleep quality.

The combination of several dietary ingredients allows us to approach the problem of insomnia from several angles, with each ingredient improving sleep in at least one way. The goal is to create synergy between all the ingredients in the formula, as this study aims to demonstrate.

The use of dietary supplements as sleep aids represents a potential solution for individuals who experience sleep symptoms but do not meet criteria for a sleep disorder. This is because dietary supplements are widely available to the general public and do not require a prescription; on the other hand, they are specifically not marketed to treat or cure any medical condition (Facts about dietary supplements 2023]). Rather, their role, as defined by the US Food and Drug Association, is to support healthy function within normal (e.g., sub-clinical) ranges. For example, a dietary supplement is not intended to treat insomnia disorder; rather, it may be intended to support healthy sleep, improve minor sleep complaints, and improve aspects of sleep. This distinction (supporting sleep health versus treating sleep disorders) reveals a role for empirically-supported dietary supplements as potential tools to address sub-clinical sleep problems for which there are few empirically-supported strategies, despite high prevalence (Sleep difficulties in adults 2020).

By evaluating the efficacy of the study supplement, we aim to demonstrate that targeting negative contributors to sleep quality, such as anxiety, stress, and the immune system, may lead to improved outcomes for this population.

Materials

Materials

The study supplement contained a proprietary blend of colostrum and egg yolk extracts, ashwagandha root extract, lavender oil, pyridoxal-5-phosphate, a proprietary slow-release melatonin, GABA, and magnesium in a slightly translucent white gelatin capsule (4Life Transfer Factor® SleepRite®). The placebo used in the study consisted of maltodextrin in an identical opaque gelatin capsule. The two products had a similar appearance but had a subtle color difference. The study supplement had a slight lavender aroma not exhibited by the placebo. The study subjects were not informed of this difference before and during the entire study

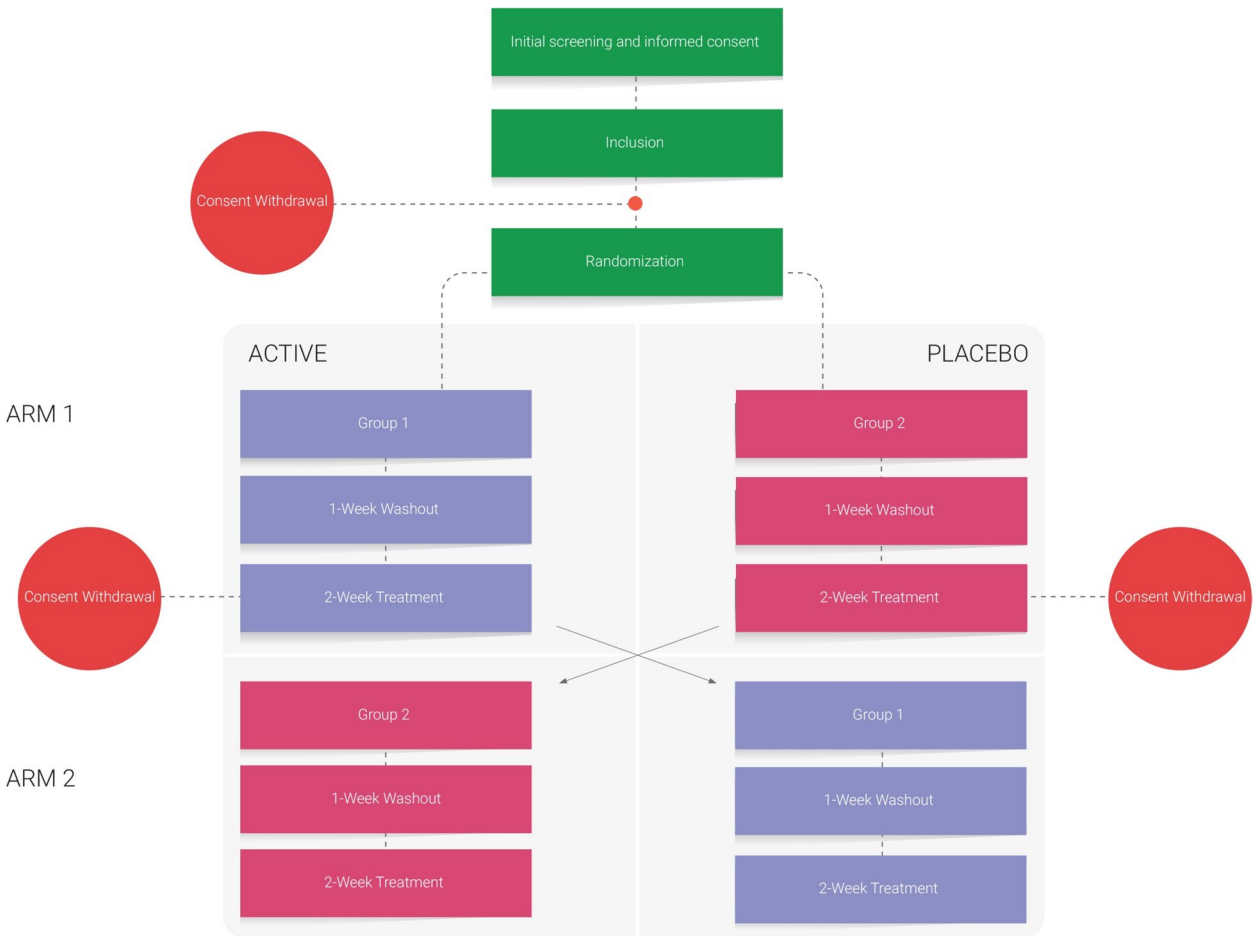

Fig. 1 Study design. Format of the 6 week, 2-arm crossover design

Methods

This study was a double-blind, randomized, placebo-controlled crossover study with 2 study arms, each 3 weeks long (6 weeks total). During each arm, participants took no sleep aids for a one-week washout period, then took either the study supplement or a placebo for two weeks. During the next arm, participants switched from treatment to placebo and vice versa (Fig. 1).

Participants were recruited through posters and emails to employees of 4Life Research and their friends and families. Participants were rewarded with a $50 Amazon gift card and were allowed to keep the provided Fitbit. Employees who referred participants also received an additional gift card for any participants who completed the study.

Those interested in the study took the ISI (Insomnia Severity Index) and a self-reported screening survey asking about medical history (including insomnia) and current medications. A power calculation suggested at least 40 participants were needed for this study. 62 people completed the screening surveys, out of which 39 were accepted into the study. Participants were accepted into the study if they met all the following criteria: between 18 and 60 in age, non-smoking, not pregnant or breastfeeding, in general good health, having no health conditions that affect sleep (see below), and an ISI score of 8 or higher. Candidates were excluded if they were clinically diagnosed with any sleep disorder, on any prescribed medications that affect sleep, on any medications or supplements that have a sedative or stimulant effect, or if they were unwilling or unable to discontinue use of any OTC or herbal sleep remedies for the duration of the study. Candidates were excluded who habitually consumed more than two alcoholic beverages per day or who were unwilling to discontinue consumption of caffeine after 2:00 p.m. Also excluded were shift workers or candidates with other non-traditional sleep habits. Participants were asked to maintain the same dietary, sleep, and exercise routines throughout the study. Demographics of the study participants are shown in Table 1.

31 participants attended the orientation and received all study materials. 30 participants completed the entire study. One subject dropped out of the study for unknown reasons before the first washout period. Participants were randomized to each participant group and randomization information was kept confidential from study staff and participants, and unblinding took place once data was ready to be analyzed. This study was reviewed and approved by an Independent Review Board (IRB) before the start of the study and after the completion of the study (Solutions IRB, Yarnell, AZ). This study was registered on ClinicalTrials.gov (NCT05368909). Informed consent was obtained from all participants.

Table 1 Demographics

Demographic Information of Study Participants ($n = 30$)	
Age (years) (mean, range)	42.1 (24–59)
Body weight (Self-report) (pounds) (mean, range)	174.5 (122–250)
BMI (kg/m^2) (mean, range)	27.3 (16.9–44.8)
Race/Ethnicity	
White	19 (63.3%)
Asian	2 (6.7%)
Hispanic/Latino	9 (30%)
Gender	
Female	15 (50.0%)
Male	15 (50.0%)
Medication Use (non-sleep related)	
Prescription medications	8 (26.7%)
Over-the-counter drugs	3 (10%)
Natural Supplements	20 (66.7%)
Essential Oils	3 (10%)
None reported	1 (3.3%)
Insomnia Severity Index Initial Score	
8–14 (minor insomnia)	17 (56.7%)
15–21 (moderate insomnia)	10 (33.3%)
22–28 (severe insomnia)	3 (10%)

Demographic information of the 30 participants who completed the study

Measurements

Sleep quality was assessed using the Insomnia Severity Index (ISI) and the Leeds Sleep Evaluation Questionnaire (LSEQ). The ISI is a 7-item questionnaire that is well-validated for the assessment of the severity of insomnia-related symptoms (Manzar et al. 2021). Scores range from 0–28, with 0–7 indicating none-minimal severity, 8–14 indicating mild severity, 15–21 indicating moderate severity, and 22–28 indicating severe symptoms (Bastien et al. 2001). The LSEQ is also a well-validated questionnaire (Tarrasch et al. 2003) that assesses general sleep quality. It assesses 10 items along a 100 mm visual analog scale. Previous studies of sleep interventions have utilized the ISI (Guthrie et al. 2018) and LSEQ (Zisapel and Laudon 2003) as outcome measures. Both questionnaires were adapted to online survey format using Survey Monkey.

Prospective sleep data were collected using a sleep diary and wearable device. The sleep diary was based on the Consensus Sleep Diary (Carney et al. 2012) and was the same as the one used in a previous clinical trial of a dietary supplement intervention (Tubbs et al. 2021). It included daily measures of time in and out of bed, sleep latency (SL), wake after sleep onset (WASO), and computed values for time in bed (TIB), total sleep time (TST), and sleep efficiency (SE%). These were calculated using the standard approach (Carney et al. 2012). Participants

also wore a Fitbit Inspire 2 device. These devices have been validated relative to polysomnography in the lab(Souza et al. 2003) and at home (Sánchez-Ortuño et al. 2010), and relative to sleep diary measurements at home (Klier and Wagner 2022). Note that different Fitbit devices use essentially identical scoring for sleep (Website 2023) and therefore are comparable across studies. Variables collected from the Fitbit devices included total sleep time and total wake time during the sleep period, time in bed, and number of awakenings.

Melatonin was assessed by a competitive immunoassay kit at Salimetrics (State College, PA) using their proprietary melatonin test kit. Saliva samples were collected using a flow-through device collecting passive drool into a small vial. Participants did not eat or drink other than water or brush their teeth for one hour before saliva collection and rinsed their mouths with water ten minutes prior to saliva collection. Samples were frozen and kept on ice until analysis. In the assay, melatonin in samples interact with antibody binding sites on a microplate. Samples are incubated, then washed to remove unbound components. Bound melatonin enzyme conjugate is measured when horseradish peroxidase enzyme reacts with tetramethylbenzidine (TMB), which produces a blue color. The reaction is stopped, yielding a yellow color, and then the plate is read at 450 nm. The optical density is inversely proportional to the concentration of melatonin in the original saliva sample and is calculated using a simple equation or through the plate reader software (Salimetrics Website 2018).

Procedures

Participants were given either the study supplement or a placebo. Participants were instructed to take 2 capsules about 30 min before bedtime, and were instructed to take the capsules whole. Used bottles were collected and unused capsules were counted to ensure compliance. Participants were considered "in compliance" if they had missed fewer than 20% of doses (three or fewer) during the two-week arm. Participants were given a calendar outlining which tasks were to be completed each day. Participants completed the ISI before beginning the study and after each arm of the study. Participants completed the LSEQ at the end of each week including the washout weeks. Participants completed the Sleep Diary daily upon waking. Saliva samples were collected at the end of each washout week and at the end of each two-week treatment period. One sample was collected immediately before bed (30 min after taking the study supplement or placebo), and another the next morning 2 h after waking. Participants were asked to freeze saliva samples immediately after collection. Participants wore a Fitbit (Inspire 2 model) nightly.

Statistical analyses

All variables were assessed using descriptive statistics (mean and standard deviation for continuous variables and percents for categorical variables) and histograms to characterize the sample. To assess group differences, a set of linear mixed model analysis of variance (ANOVA) analyses were completed, with blocking on subject. The independent variables in the model were the order in which the treatments were administered, treatment (placebo or treatment), week of the measures in the study, and the interaction between week and treatment. Post-hoc Tukey-adjusted pairwise tests were performed for the variables in the model. The tests of interest were looking at differences in treatments for each week (1, 2, or 3), differences in the weeks for the placebo, and differences in the weeks for the treatment. ANOVA allows analysis of repeated measurements and accounts for the crossover design, where each participant acts as their own control. All analyses were performed in SAS version 9.4 (SAS, Inc.). Differences were considered statistically significant if $p < 0.01$, due to low number of participants; however, measurements with $p < 0.05$ have also been indicated in the tables, as they would have been significant had the study included enough participants based on a power calculation.

Results

Primary endpoints

Insomnia severity index (ISI)

The overall ISI score improved in both treatment and placebo groups. Difficulty Falling Asleep improved significantly in the treatment group, and Difficulty Staying Asleep improved significantly in the placebo group, compared to baseline. All other results were nonsignificant (ns) (Table 2).

Melatonin

Due to wide variations of individual saliva melatonin levels among participants, values were converted to a logarithmic scale. Despite the variations, significant differences were present between the morning and night measurements in both the baseline and treatment measurements, and the treatment group showed a significant increase in salivary melatonin between the baseline and treatment period (Table 3).

Secondary endpoints

Sleep diary

In the secondary endpoints measured with the sleep diary, only Number of Awakenings showed an improvement in both placebo and treatment groups compared to the baseline of the treatment group, with all other results non-significant (Table 4).

Table 2 Insomnia Severity Index (ISI) results

	Baseline	Placebo	Treatment	p
Overall	13.80 ± 1.77[ab]	10.80 ± 1.77[a]	11.00 ± 1.77[b]	[a] 0.0024 [b] 0.0049
Difficulty Falling Asleep	1.70 ± 0.39[a]	1.27 ± 0.39	1.13 ± 0.39[a]	[a] 0.0087
Difficulty Staying Asleep	2.16 ± 0.41[a]	1.52 ± 0.41[a]	1.76 ± 0.41	[a] 0.0099
Problem Waking Up Too Early	1.91 ± 0.41[a]	1.18 ± 0.41[a]	1.41 ± 0.41	[a] 0.0136
How Satisfied With Your Sleep	2.46 ± 0.36	2.13 ± 0.36	2.16 ± 0.36	ns
Noticeable Impairment to Quality of Life	1.45 ± 0.30	1.35 ± 0.30	1.25 ± 0.30	ns
Worried/Distressed about Sleep Problems	2.07 ± 0.37[a]	1.67 ± 0.37	1.61 ± 0.37[a]	[a] 0.0294
Extent of Interference with Daily Functioning	2.04 ± 0.36	1.67 ± 0.36	1.67 ± 0.36	ns

[a b] significant difference between the groups with the same letter of annotation. *P* values noted on the right side of table. Significance is indicated by a *p* value < 0.01. ns, nonsignificant. Overall score is on a scale from 0 to 28, a lower score meaning better sleep. All other metrics are on a scale from 0 to 4, with 0 being best. ± 95% confidence intervals

Table 3 Salivary melatonin results

	Baseline	Placebo	Treatment	
Morning	0.749 ± 0.558[ab]	0.811 ± 0.630	3.536 ± 0.630[bc]	[a] 0.0025 [b] < 0.0001
Night	1.638 ± 0.553[ad]	1.418 ± 0.630	5.009 ± 0.630 [cd]	[c] < 0.0001 [d] < 0.0001

Due to wide variation in salivary melatonin measurements, values are reported in $\log_{10}$ scale. [a b c d] significant difference between the groups with the same letter of annotation. Significance is indicated by a *p* value < 0.01. ± 95% confidence intervals

Table 4 Sleep diary results

		placebo	treatment	
Sleep Onset latency (minutes)	Washout	20.9 ± 5.7	21.9 ± 5.7	ns
	Week 2	20.4 ± 5.7	21.7 ± 5.7	
	Week 3	19.8 ± 5.7	22.2 ± 5.7	
		placebo	treatment	
Sleep Time (minutes)	Washout	460.5 ± 25.7	456.6 ± 25.7	ns
	Week 2	461.4 ± 25.7	485.1 ± 25.7	
	Week 3	459.1 ± 25.7	474.1 ± 25.7	
		placebo	treatment	
Sleep Efficiency (sleep time/time in bed)	Washout	95.4 ± 1.3%	95.0 ± 1.3%	ns
	Week 2	95.5 ± 1.3%	95.5 ± 1.3%	
	Week 3	95.7 ± 1.3%	95.0 ± 1.3%	
		placebo	treatment	
Number of awakenings	Washout	2.29 ± 0.60	2.66 ± 0.60[abc]	[a] 0.0086
	Week 2	2.22 ± 0.60[c]	2.39 ± 0.60	[b] 0.0098
	Week 3	2.18 ± 0.60[a]	2.18 ± 0.60[b]	[c] 0.0241
Morning sleepiness (scale of 1 to 9, 1 is best)		placebo	treatment	
	Washout	5.11 ± 0.53	5.21 ± 0.53	ns
	Week 2	5.14 ± 0.53	5.13 ± 0.53	
	Week 3	4.93 ± 0.53	5.08 ± 0.53	
		placebo	treatment	
Awake during the night (minutes)	Washout	15.4 ± 5.4	15.2 ± 5.4	ns
	Week 2	15.7 ± 5.4	15.1 ± 5.4	
	Week 3	16.8 ± 5.4	15.9 ± 5.4	

[a b c] significant difference between groups with the same letter of annotation. *P* values noted on the right side of table. Significance is indicated by a *p* value < 0.01. ns, nonsignificant. ± 95% confidence intervals

Leeds sleep evaluation questionnaire (LSEQ)

Of the secondary endpoints measured with the LSEQ, the overall score for the washout period vs the placebo and treatment periods approached significance with a $p < 0.05$. Sleep quality (wakefulness) showed a significant difference between the washout period and the second week of the treatment period, but the placebo also showed improvement. All other metrics did not show a significant difference between washout and treatment or placebo (Table 5).

FitBit

Secondary endpoints were measured with FitBit devices. There were no significant differences between baseline, placebo, and treatment results (Table 6).

Adverse events

Adverse events during the study are presented in Table 7. There were no serious adverse events throughout the study or 8 weeks after completion. The adverse events observed in this study can likely all be attributed to melatonin, which has side effects of stomach ache and nausea, headache, dizziness, irritability and restlessness, and night sweats(Side effects of melatonin, NHS website. 2023).

Table 5 The Leeds Sleep Evaluation Questionnaire (LSEQ) Results

		placebo	treatment	
Overall Score	Washout	2.91 ± 0.13	2.84 ± 0.13[abc]	[a] 0.0129
	Week 2	3.02 ± 0.13	3.08 ± 0.13[b]	[b] 0.0280
	Week 3	3.09 ± 0.13[a]	3.10 ± 0.13[c]	[c] 0.0129
		placebo	treatment	
Sleep Quality (wakefulness)	Washout	2.86 ± 0.20	2.73 ± 0.20[abc]	[a] 0.0054
	Week 2	3.03 ± 0.20	3.16 ± 0.20[c]	[b] 0.0249
	Week 3	3.16 ± 0.20[b]	3.23 ± 0.20[a]	[c] 0.0249
		placebo	treatment	
Difficulty Falling Asleep	Washout	2.90 ± 0.24[a]	2.83 ± 0.24[bc]	[a] 0.0469
	Week 2	3.20 ± 0.24	3.30 ± 0.24[b]	[b] 0.0469
	Week 3	3.16 ± 0.24	3.36 ± 0.24[ac]	[c] 0.0139
		placebo	treatment	
How Quickly Fall Asleep	Washout	2.87 ± 0.24	2.84 ± 0.24	ns
	Week 2	3.14 ± 0.24	3.27 ± 0.24	
	Week 3	3.24 ± 0.24	3.27 ± 0.24	
		placebo	treatment	
Sleepy Before Bed	Washout	3.15 ± 0.22	2.92 ± 0.22[a]	[a] 0.0237
	Week 2	3.15 ± 0.22	3.29 ± 0.22	
	Week 3	3.25 ± 0.22	3.39 ± 0.22[a]	
		placebo	treatment	
Restless During Sleep	Washout	2.80 ± 0.23[a]	2.83 ± 0.23[b]	[a] 0.0190
	Week 2	3.00 ± 0.23	2.93 ± 0.23	[b] 0.0357
	Week 3	3.30 ± 0.23[ab]	3.13 ± 0.23	
		placebo	treatment	
How Difficult to Wake Up	Washout	2.93 ± 0.22	2.86 ± 0.22	ns
	Week 2	3.03 ± 0.22	2.90 ± 0.22	
	Week 3	2.96 ± 0.22	2.90 ± 0.22	
		placebo	treatment	
How You Feel When You Wake Up	Washout	2.78 ± 0.24	2.72 ± 0.24	ns
	Week 2	2.98 ± 0.24	3.02 ± 0.24	
	Week 3	2.92 ± 0.24	2.95 ± 0.24	
		placebo	treatment	
How Long to Wake Up	Washout	2.97 ± 0.16	2.90 ± 0.16	ns
	Week 2	2.83 ± 0.16	3.03 ± 0.16	
	Week 3	2.83 ± 0.16	2.97 ± 0.16	
		placebo	treatment	
Balance/Coordination Upon Waking	Washout	3.00 ± 0.12	2.93 ± 0.12	ns
	Week 2	3.03 ± 0.12	3.03 ± 0.12	
	Week 3	3.13 ± 0.12	3.00 ± 0.12	

[a b c] significant difference between groups with the same letter of annotation. *P* values noted on the right side of table. Significance is indicated by a *p* value < 0.01. ns, nonsignificant. The LSEQ has each parameter on a scale of 1 to 5, with 5 being best. ± 95% confidence intervals

Discussion

The purpose of this study is to evaluate the effects of a proprietary, multi-ingredient formula on sleep quality in people with insomnia. A previous study with a similar design that was performed on a multi-ingredient supplement showed improvements in some aspects of sleep health but not others (Stevens et al. 2017), which is what was expected of this supplement, and is what our results indicate. One of the primary endpoints investigated was salivary melatonin. Measurements in the placebo and treatment groups indicated that the study supplement significantly increased basal salivary melatonin levels prior to falling asleep and after waking up. These results were consistent with an earlier study showing an increase

Table 6 FitBit results

		placebo	treatment
Minutes Asleep	Washout	404.3 ± 19.8	398.6 ± 20.0
	Week 2	400.4 ± 19.7	409.6 ± 20.0
	Week 3	393.3 ± 19.7	396.5 ± 19.8
		placebo	treatment
Minutes Awake	Washout	54.2 ± 5.4	53.7 ± 5.4
	Week 2	54.5 ± 5.3	54.5 ± 5.4
	Week 3	54.5 ± 5.3	52.8 ± 5.4
		placebo	treatment
Number of Awakenings	Washout	23.0 ± 3.1	23.2 ± 3.1
	Week 2	23.4 ± 3.1	23.5 ± 3.1
	Week 3	22.5 ± 3.1	22.4 ± 3.1
		placebo	treatment
Time in Bed	Washout	459.6 ± 21.7	453.3 ± 21.9
(minutes)	Week 2	455.4 ± 21.6	464.8 ± 21.9
	Week 3	448.9 ± 21.6	449.9 ± 21.7

No significant differences. ± 95% confidence intervals

Table 7 Adverse Events

Symptom	Treatment	Placebo
Heartburn	1 (3.3%)	0
Headache	2 (6.7%)	0
Anxiety/restlessness	2 (6.7%)	0
Night sweats	1 (3.3%)	0
Dizziness	1 (3.3%)	0
Dry Mouth	0	1 (3.3%)

No serious adverse events over the course of the six-week trial

in salivary levels with melatonin supplementation (Shirikawa et al. 1998). In that study, peak salivary levels corresponded with peak serum levels at about 60 min after ingestion and suggested it could serve as a suitable marker of circulating melatonin levels. A more recent study, however, indicates that physiological levels of melatonin do not reflect its real concentration in tissues or in other body fluids, and may underestimate the true in vivo levels (Tan et al. 2010). However, the study design was limited to salivary measurements due to the complexity and cost of collecting and measuring blood samples.

Elevated circulating melatonin levels may help explain the supplement's effectiveness in reducing difficulty falling asleep and reducing the number of awakenings (Lakhan and Finesmith 2013). These effects and wakefulness could also be attributed to the slow-release profile of melatonin ingredient contained in this supplement. These benefits were also observed with middle-aged people with insomnia (Lemoine et al. 2012) in a previous study. In that study, improvements in sleep quality and morning alertness were demonstrated using only slow-release melatonin, whereas the study supplement here included other ingredients such as magnesium, which also has been associated with better sleep quality (Jahrami et al. 2021).

The overall score of the ISI improved in both the treatment and placebo groups. This, and a few other improvements in the placebo group, indicate a potential overall placebo effect. This could be substantiated with a larger group of participants.

Difficulty falling asleep, measured by the ISI, improved in the treatment group. This is consistent with earlier studies with ashwagandha (Cheah et al. 2021), melatonin (Bueno et al. 2021), and lavender oil (Diego et al. 1998), all of which have been shown to decrease sleep onset latency and promote relaxation and drowsiness. GABA has also been shown to reduce sleep latency, through rapid absorption into the bloodstream and likely distribution through the peripheral nervous system to reduce anxiety and psychological stress and decrease core body temperature prior to falling asleep (Yamatsu et al. 2016).

Lavender is a unique ingredient in that it has benefits via oral ingestion (Greenberg and Slyer 2018), and more so as an essential oil. Lavender aromatherapy has been well-studied and reviewed as a sleep intervention (Her and Cho 2021). From the combined results of 30 different clinical studies, an improvement in sleep quality was found to be consistent and statistically significant, with additional benefits of reducing stress and anxiety (Her and Cho 2021). Improvement in stress and anxiety prior to sleep has been shown to decrease difficulty in falling asleep (Staner 2003). This last point on improvements in stress and anxiety is partially consistent with this study's ISI measure of "worry/distressed about sleep problems" approaching significance when comparing baseline to treatment, though the question's context is not the same.

Number of awakenings, measured by both the Fitbit and the sleep diary, gave very different measurements between the two methods, with the Fitbit measuring roughly ten times the number of awakenings as the sleep diary. This difference can be explained by the method of measurement; the Fitbit measures awakenings based on movement and the diary bases its measurement on participant recollection. The number of awakenings based on the sleep diary was significantly improved in both the treatment and placebo groups, but only compared to the baseline of the treatment group. The baselines of the treatment group and the placebo group were quite different, though not statistically so. Though the difference is not significant, it may have muddled the results as neither group was statistically different than the baseline of the placebo group.

The melatonin level results were quite conclusive and clinically relevant. Despite the statistical differences of

the other mentioned improvements, it is questionable whether these small differences are clinically relevant. For example, the ISI-measured improvement in "Difficulty falling asleep" in the treatment group comprised of a decrease of < 0.6 points on a 5 point scale. Whether this is enough of a decrease to make a difference to a patient experiencing sub-clinical insomnia is unsure.

Despite some positive improvements in sleep quality, there were several domains of sleep that did not improve with the study supplement such as difficulty staying asleep, problem of waking up too early, and extent of interference with daily functioning, to name a few. Some of these measurements trended in a positive direction from baseline but were not different enough from the placebo treatment to attain statistical differentiation. This could be attributed to insufficient number of study subjects, which was calculated based on power measurements of only a few primary outcome measures, or it could be attributed to inefficacy of the study supplement.

The FitBit measurements showed no statistical differences comparing placebo to the treatment group, or any before and after measurements. These results are consistent with a similar study evaluating a sleep supplement, which demonstrated some improvements in sleep surveys, but not in the objective FitBit measurements (Grandner and Fernandez 2021). This suggests that the dietary supplement's effects on sleep are captured in general subjective experiences of sleep rather than physiological changes in sleep continuity. This is clinically meaningful, since subjective perceptions of poor sleep quality are prevalent and associated with adverse outcomes (Harvey et al. 2008).

Placebo effects were observed in some of the measurements. The placebo effect is well-known among scientists and non-scientists alike, and demonstrates how expectancies on the participants' part can influence actual measurements, whether objective or subjective. In this study, the participants were expecting improvements in their sleep health, and in some cases those improvements did occur in both treatment groups. Many aspects of sleep health are influenced by psychological factors and this has been demonstrated in this study. The observed placebo effect calls into question the validity of some of the results: do these combined ingredients actually improve sleep health, or are they just perceived as improving sleep health?

Although this clinical study was rigorous in design with well-validated endpoints, there were several limitations primarily related to the lack of a controlled environment and calibrated equipment, a slightly differentiating feature in the placebo and active products, and a heterogenous study population.

While the salivary melatonin measurements were objective, the rest of the measures used in this study were subjective. The ISI and LSEQ surveys, while validated, are taken by the participants based on their recollections and perceptions of the sleep over the previous 7 (LSEQ) or 14 (ISI) days. There is therefore room for human error and bias based on the subjective nature of the questions and the long time frame between the beginning of the phase and the survey completion. Furthermore, this encourages scrutiny of whether the results of the surveys translate to real-world benefits to sleep health, especially considering the lack of Fitbit data to back up these results.

While the Fitbit device offers convenience of at-home measurements for studies such as this one, it may still not be accurate or precise enough to take the place of the gold standard polysomnography, which is typically only performed in sleep labs (Zambotti et al. 2018). Fitbit devices measure few and limited aspects of sleep compared to polysomnography, and they are not useful for detecting small changes in different aspects of sleep. Additionally, at-home Fitbit devices cannot be calibrated for use in objective studies.

There was a certain lack of environmental control during this study. Daily sleep surveys were monitored online for completion and text and email reminders were sent, but several were not finished immediately upon waking as instructed, potentially leading to inaccurate recollections. Saliva samples were collected at-home, and while participants were instructed as to the timing of the collection, there was sure to be variance. This and the fact that salivary melatonin measurements have their own wide variability even between samples from the same person on the same day, leads to the conclusion that while we got statistically significant differences between groups there may be more variability than would be optimal.

Having only 30 participants finishing the study, the results of the study are not necessarily generalizable to the entire population. Our participants were a heterogeneous mix of ages, BMIs, and severity of sub-clinical insomnia. Enrolling more participants would have given the opportunity to do sub-group analyses to show more specifically what population the results could be generalized to.

There was also the possibility for unintentional unblinding as the two treatment supplements were not identical; there were slight variations in color and a lavender aroma in the treatment supplement, but not the placebo supplement. Moreover, the lavender aroma strength in the study supplement slowly dissipates over time, perhaps impacting its anxiolytic effect in the subject pool receiving the supplement later in the study. Since this

study had a crossover design, the participants were split into two groups: those who took the study supplement first, and those who took the placebo first. Those who took the placebo first would not have known whether they were taking a placebo or the study supplement, but may have realized upon switching that the second phase was the actual supplement. Those who took the supplement first may have realized upon switching that the placebo did not have a lavender aroma and was more white in color. Thus unintentional unblinding was a real possibility during this study. The statistical methods of using each person as their own control helps in this respect, but the limitation remains.

The subject pool has a wide range of pre-study ISI scores, covering three different levels of insomnia. This broad variation created a heterogenous study population, so the mean changes in the sleep quality parameters between the different populations may be less statistical and clinically significant for one level of insomnia compared to another. The low number of participants in the study also precluded any sub-population analysis based on insomnia severity, age, BMI, or demographics.

Another limitation of the study was the conflict of interest. The study supplement is produced by 4Life Research, LLC., and 4 of the six authors are employed by such. In order to decrease bias, saliva samples were self-collected by the participants, measurements of salivary melatonin were completed by an outside lab, surveys were self-reported by participants themselves, and all data was analyzed by an outside statistician with no connection to 4Life Research, LLC. All researchers at 4Life Research were blinded throughout the study until data analysis.

Conclusion

Despite the high prevalence of sleep disturbances and the multitude of single-ingredient pharmaceutical and nutraceutical choices, there is still a lack of empirically-supported options that provide a multimodal approach to improving sleep quality via a nutritional supplement. In this study, the potential of such a supplement is moderately exhibited through improvements in sleep quality, namely the ability to fall asleep and wakefulness during sleep. These improvements and the low rate of side-effects indicate that this supplement could be used alone or in conjunction with other methods of sleep improvement in a safe manner. Several other measures were more ambiguous or not available, such as immune system parameters, and deserve further research with larger cohorts, more controlled environments, and better sleep testing capabilities. Future research could include a larger number of participants in order to allow for sub-group analysis for parameters such as demographics, age, BMI, and severity of sub-clinical insomnia. New experiments could include comparisons between the individual ingredients of the supplement to explore potential synergy, or direct comparisons of the study supplement with other known sleep aids. Future experiments could also test the immune-sleep connection with measurements of immune markers and further justify the use of egg yolk and colostrum extracts to improve sleep quality.

Abbreviations

GABA	Gamma aminobutyric acid
ISI	Insomnia Severity Index
LSEQ	Leeds Sleep Evaluation Questionnaire
IRB	Institutional Review Board
OTC	Over-the-counter drugs
SL	Sleep Latency
WASO	Wake After Sleep Onset
TIB	Time in Bed
TST	Total Sleep Time
SE%	Sleep Efficiency Percentage
TMB	Tetramethylbenzidine
ANOVA	Analysis of Variance
SAS	Previously "Statistical Analysis System"
Ns	Non-significant
BMI	Body Mass Index

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Authors' contributions
AA wrote and edited the manuscript, helped design the study, supervised the acquisition of data, and interpreted data. DV substantially revised the manuscript. BV interpreted data. DE performed the analysis of data. MG provided consultation on the study design and substantially revised the manuscript. XH designed the study and helped draft the manuscript.

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Data availability
The datasets used and analyzed during this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by an IRB (Solutions IRB, Yarnell, AZ) and consent was given from every participant.

Consent for publication
Not applicable.

Competing interests
AA, DV, and BV are all current employees of 4Life Research LLC, the manufacturer of the study supplement, and therefore draw salary from such. XH was employed by 4Life Research LLC at the time the study was conducted, and therefore drew salary from such. The study was funded by 4Life Research LLC. DE is employed by the BYU Statistics department and therefore declares no conflict of interest. MG is employed by the University of Arizona College of Medicine and therefore declares no conflict of interest.

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