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Research Article

Participant Experiences from Chronic Administration of Sustained-Release GABA and Alpha-s1 Casein Hydrolysate compound Versus Melatonin on Sleep Quality: An Exploratory Analysis of a Single-Blind Clinical Trial

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Abstract:

Sleep difficulty is relatively common among people, and those affected often resort to short-term use of herbal supplements to ease the symptoms. Here we introduce and investigate the effects of a novel composite product, NatureU Sleep, that contains sustained-release gamma-aminobutyric acid (GABA) and alpha-s1 casein hydrolysate, on sleep quality compared to the commercially available drug-melatonin. The exploratory clinical trial was designed to assess the deep sleep conditions including 17 adult participants, with an expectation that 15 would complete the trial. Utilizing wearable monitoring devices, the study evaluates the impact of the test product on deep sleep duration and proportion. The results indicated a significant increase in deep sleep duration and proportion for participants consuming sustained-release GABA and alpha-s1 casein hydrolysate, suggesting its potential as an effective sleep aid without any observed adverse events. This study aimed to compare the acute effects of a single dose of NatureU Sleep versus a single dose of commercial melatonin on objective measures of deep sleep.

Key words: sleep quality; clinical trial; sustained-release gaba; alpha-s1 casein hydrolysate

Abbreviations

GABA: gamma-aminobutyric acid

Introduction

Insomnia is characterized by disturbances in sleep quality accompanied by impaired daytime functioning, such as fatigue and low mood1. Sleep quality disturbances include difficulty falling asleep, staying asleep, or experiencing non-restorative sleep despite having adequate opportunities for rest[1]. Insomnia is a prevalent sleep disorder affecting individuals globally, with reported prevalence rates ranging from 10% to 40% across different regions [2,3]. This widespread issue disrupts daily life, work productivity, and is associated with a variety of health concerns, including cardiovascular diseases, diabetes, and mental health conditions. As such, seeking effective remedies to improve sleep quality has become a significant public health concern.

In this context, sleep aids in the form of dietary supplements play a crucial role[4]. Melatonin supplements[5], gamma-aminobutyric acid (GABA)-containing products[6,7], and a variety of botanical sleep aids have been offering alternatives to those who prefer non-pharmacological interventions for insomnia. They work through different mechanisms, such as regulating melatonin levels[5], reducing stress responses, or promoting relaxation and sleep by activating inhibitory neurotransmitter receptors[6,7]. GABA is an important inhibitory neurotransmitter in the central nervous system of mammals[6]. Allosteric sites on the GABAa receptors enable precise regulation of neuronal inhibition in specific brain regions. These sites serve as molecular targets for anxiolytic and hypnotic drugs[8]. Activation of the GABAa receptor (also known as the gamma-aminobutyric acid type A receptor) promotes sleep[7]. A lack of GABA

in the human body can lead to symptoms such as anxiety, insomnia, and fatigue, which can be improved by timely supplementation of GABA[6,9]. In another side, GABA has been widely accepted as a safe food substance[10–12], guaranteeing its daily use to promote sleeping quality.

Alpha-s1 casein hydrolysate is another supplement reported effective in aiding sleep[13]. It a patented hydrolysate of milk protein, which is less likely to cause allergies compared to regular proteins and does not lead to lactose-induced indigestion. Additionally, it can increase the activity of gamma-aminobutyric acid (GABA)[4], effectively relieving stress and improving symptoms of anxiety and sleep disorders caused by stress through mechanisms such as reducing cortisol and lowering blood pressure.

NatureU Sleep is a new combined formulation for better sleep quality. Although the individual components in NatureU Sleep have been studied for their effects on sleep and/or stress (a common cause of sleeping difficulties), the efficacy of the combined formulation as a treatment for sleep disturbances has not yet been investigated. Here, we report the results of a clinical trial with the primary objective of examining the short-term effect of a single dose of NatureU Sleep on sleep quality compared to a single dose of commercial melatonin, specifically focusing on the duration and proportion of deep sleep as measured by wearable technology. We hypothesized that the sustained-release GABA formulation in NatureU Sleep would lead to a significant improvement in deep sleep parameters compared to immediate-release melatonin.

Materials and Methods

This study is an exploratory research based on wearable monitoring devices. It compares the deep sleep conditions of adults consuming a new composite product containing sustained-release GABA and alpha-s1 casein hydrolysate—NatureU Sleep—with those consuming commercially available melatonin.

This trial is a single-center, single-blind exploratory clinical study. It aims to recruit 17 participants, with at least 15 participants expected to complete the trial. The study was executed by CTI AiPu Medical Laboratory (Shanghai) Co., Ltd.

Inclusion Criteria:

1. Adults aged 18-50 years. No restrictions on gender.
2. Mild to moderate sleep disturbances in the past month, with a Self-Rating Scale of Sleep (SRSS) score between 15-35.
3. Willingness to comply with the study protocol, Self-Rating Scale of Sleep score ranges
4. Ability to provide informed consent.

Exclusion Criteria:

1. Special population including pregnant or breastfeeding women and individuals engaged in severe physical labor or high-intensity exercise.
2. Individuals with a history of severe psychiatric or neurological disorders.
3. Individuals with a history of alcohol or substance abuse.
4. Individuals who smoke more than 10 cigarettes per day.
5. Participants have been taking other sleep aids or medications that affect sleep in the past month.
6. Individuals with known allergies to NatureU Sleep or commercial melatonin components.
7. Individuals with severe medical conditions that could interfere with the study (e.g., uncontrolled diabetes, severe cardiovascular diseases).
8. Individuals currently undergoing non-pharmacological treatments for insomnia (e.g., cognitive behavioral therapy, relaxation therapy, etc.).
9. Individuals who are unable or unwilling to complete the entire experiment.

Criteria For Withdrawal

1. All participants have the right to withdraw from the study at any time, regardless of the reason.
2. Failure to adhere to the study protocol, including not taking the test products as instructed, developing allergies, failing to show tolerance to the test food, or exhibiting other factors that severely impact the experiment result.
3. The investigator may decide to withdraw a participant if it is in their best interest, for reasons including but not limited to new medical findings or conditions that arise during the study period.

The workflow of the trial

The participants subjects participated in two independent tests. As shown in Table 1, NatureU Sleep Group (called NatureU group in the following situations) is a combination of sustained-release GABA and alpha-s1 casein hydrolysate, and the control group is a commercially available melatonin.

	NatureU Group	Melatonin Group
Ingredients Information	Alpha-s1 casein hydrolysate, Sustained-release GABA, Maltodextrin	Melatonin, Vitamin B6, Edible Corn Starch, Silicon Dioxide, Magnesium Stearate, Gelatin Capsules.
Product Name	NatureU Sleep	Notrand Melatonin Lifeism Melatonin Capsules
Serial Number	20230501	20230322
Shelf Li	24 months	24 months
Storage Conditions	Room temperature	Room temperature
Precaution	Pregnant and lactating women, minors, and individuals allergic to milk are contraindicated.	This product cannot replace medication. It is not recommended for individuals outside the suitable population to consume this product. Do not exceed the recommended dosage or consume it simultaneously with similar nutrients. Those engaged in driving, machinery operation, or hazardous tasks should not consume it before or during these activities. Individuals with autoimmune diseases (such as rheumatoid arthritis) and hyperthyroidism should use this product with caution.
Specifications	0.4 gram/capsule	0.4 gram/capsule

PS: All the test food is provided by OmniSolutions Laboratory Holdings Limited

Table 1: Food Information

On each test day, each participant will receive a pre-packaged product (one capsule of NatureU Sleep or one capsule of commercial melatonin). The test product should be taken with warm water one hour before bedtime. The deep sleep duration and proportion are collected by wearable bands and analyzed as evaluation metrics.

Note: During the test period, there are no dietary restrictions, but participants should avoid alcohol.

The study implementation process consists of two phases: the screening phase and the trial phase as shown in Figure 1

In the screening phase, research staff involved in this study are responsible for selecting suitable participants for enrollment. They will explain the significance and process of the study to the participants, as well as provide instructions on the method of consumption of the products and the use of the wearable monitoring device (Huawei Band 8). When participants indicate no objections to the study process, voluntarily sign the informed consent form, and the research staff determine that the participants meet the inclusion and exclusion criteria, fully understand the project, and are willing to participate, they are formally enrolled in the study.

Once all participants are enrolled, the research staff will arrange the trial according to the following testing procedures as shown in Figure 2.

1. Assign numbers to the qualified voluntary participants and distribute the Huawei Band 8 and test products. Before and after the test, participants should maintain a normal diet and regular routine, and avoid alcohol consumption.
2. For two consecutive nights before the test, participants should wear the wristband to sleep and manually record the sleep and wake times to calibrate with the wristband's recordings.
3. First Test: Participants should wear the wristband, ensuring it is fully charged. They should avoid overly stimulating activities for 2 hours before sleep. One hour before sleep, they should take the commercially available melatonin with warm water. All light sources should be turned off before going to bed. The next morning, after waking up, they should use the Huawei Health App on their phones to export the sleep monitoring data, specifically the deep sleep duration and proportion.
4. The second test will be conducted 3 days after the first test. Repeat step 3, consuming NatureU Sleep.
5. The trial reaches the endpoint after all participants complete two independent test sessions according to the trial protocol and fill out the questionnaire for each session.

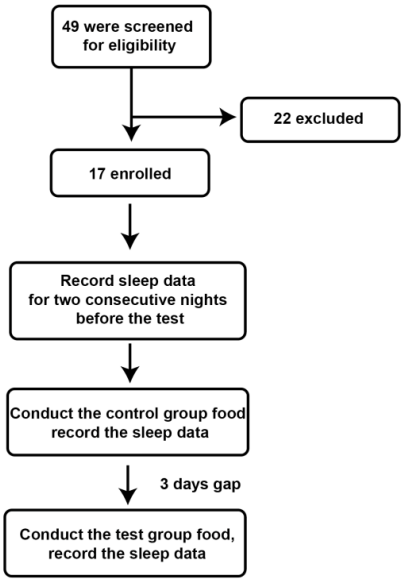

Figure 1: Participant Fl

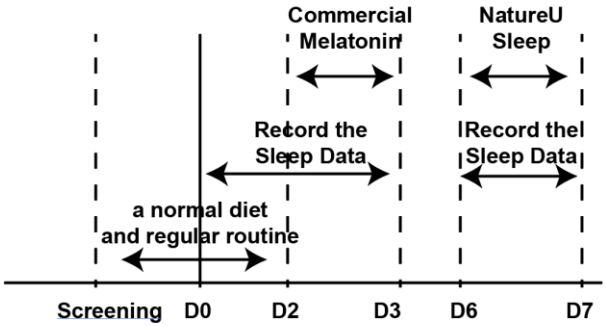

Figure 2: Study Design. D represents Study Day

Data Statistics and Analysis

Data statistical analysis will be performed using the statistical analysis software SPSS 27.0. Data will be expressed as mean ± standard deviation ($x \pm s$). A paired-samples t-test was used to analyze the differences within participants between the two intervention nights (Melatonin vs. NatureU Sleep) with the significance level set at $P < 0.05$.

Calculate the mean ± standard deviation and the rate of change for each parameter at various time points.

The formula used for calculation：

Rate of change = (NatureU group Sleep Value – Melatonin Group Sleep Value)/Melatonin Group Sleep Value × 100%

Participant Information

From April 19, 2024, 49 volunteers were screened. After verifying the inclusion and exclusion criteria by the researchers, 17 participants were enrolled in the project on April 20, 2024. Two participants did not complete the trial, resulting in valid data from 15 participants, meeting the project's requirements for valid data. The demography and baseline characteristics are demonstrated in Table 2 and Table 3.

Item	Information
Gende	Male and Female
Age	18~44
Recruit Number	45
Number of Participants Screened and Enrolled	17
Number of Withdrawal Participants	2
Validate participant	15

Table 2: Participant Demography

Series Number	Gender	Age	SRSS Grade
1	Female	24	26
2	Female	36	31
3	Female	29	29
4	Female	33	31
5	Male	29	27
6	Female	39	34
7	Female	38	28
8	Female	35	30
9	Female	24	26
10	Male	36	25
11	Female	40	29
12	Female	27	30
13	Female	35	31
14	Female	44	33
15	Male	29	31

Table 3: Participant Baseline Characteristics

Test Results

Based on the monitoring data from the Huawei Health App, the deep sleep duration results for 15 participants from two independent test sessions were obtained (Table 4 Deep Sleep Duration Result of the Participants). A bar chart showing the results of the two tests was also created (Figure 3A).

Series Number	NatureU Group (min)	Melatonin Group (min)
1	106	72
2	165	112
3	184	98
4	149	110
5	137	156
6	158	132
7	120	81
8	87	109
9	203	135
10	135	91
11	122	53
12	117	75
13	94	65
14	67	65
15	107	99
Mean	130.07	96.87
Standard Deviation	37.07	29.37

Table 4: Deep Sleep Duration Result of the Participants

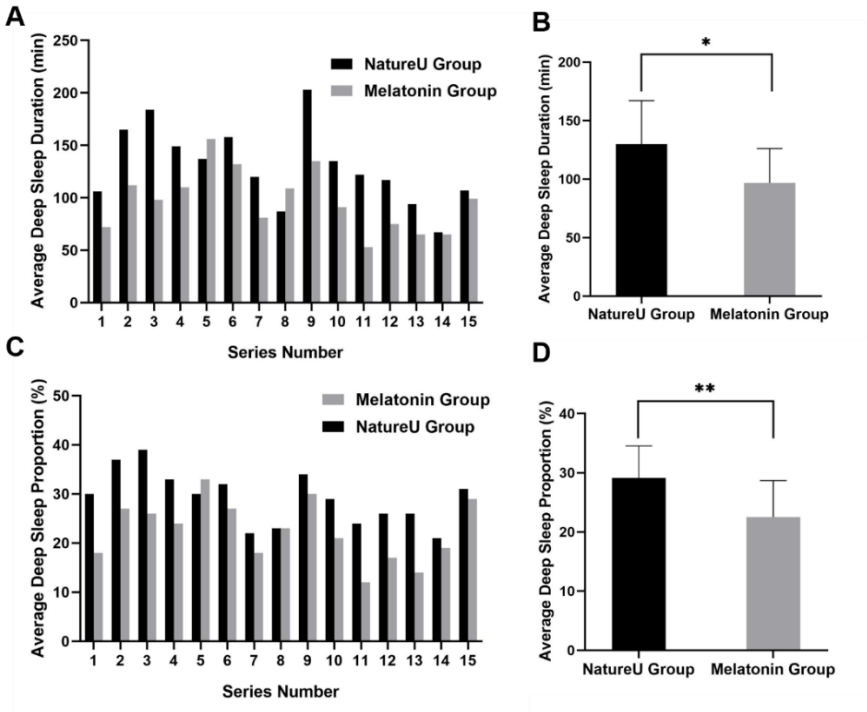

Figure 3: Test Result. A), Deep sleep duration for every participant after using NatureU Sleep and commercial melatonin. B), Summarized deep sleep duration change after using NatureU Sleep and commercial melatonin. C), Deep sleep proportion for every participant after using NatureU Sleep and commercial melatonin. D), Summarized deep sleep proportion change after using NatureU Sleep and commercial melatonin. PS. *means statistical significance.

Statistical analysis using the paired-samples t-test confirmed the significance of the improvements observed. The mean deep sleep duration for the commercial melatonin was 96.87 minutes with a standard deviation of 29.37 minutes. The mean deep sleep duration for NatureU Sleep was 130.07 minutes with a standard deviation of 37.07 minutes. (Figure 3B). A significant increase in deep sleep duration took place when the participants conducted NatureU Sleep, compared with commercial melatonin.

Based on the deep sleep duration results from the two independent test sessions, the difference in deep sleep duration and the rate of change for the NatureU Sleep group and commercial melatonin group was calculated. A comparative analysis of the differences between the two groups was also conducted (Table 5).

	NatureU Group	**Melatonin Group**
Mean	130.07	96.87
Standard Deviation	37.07	29.37
Mean Difference	33.2	/
Rate of change	34%	/
P Value	0.01*	/

PS. *means statistical significance

Table 5: Deep Sleep Duration Difference Summary

It was found that compared to the commercial melatonin, the average deep sleep duration of the 15 participants increased by 34% (a difference of 33.20 minutes) after consuming NatureU Sleep. The data from the two test sessions showed a normal distribution and were statistically significant ($p < 0.05$).

Based on the monitoring data from the Huawei Health App, the deep sleep proportion results for 15 participants from two independent test sessions were obtained (Table 6 Deep Sleep Proportion Result of the Participants). A bar chart showing the results of the two tests was also created (Figure 3C).

It was found that the mean deep sleep proportion for the commercial melatonin was 22.53%, with a standard deviation of 6.16%. For the test food (NatureU Sleep), the mean deep sleep proportion was 29.13%, with a standard deviation of 5.42%

Series Number	NatureU Group	Melatonin Group
1	30	18
2	37	27
3	39	26
4	33	24
5	30	33
6	32	27
7	22	18
8	23	23
9	34	30
10	29	21
11	24	12
12	26	17
13	26	14
14	21	19
15	31	29
Mean	29.13	22.53
Standard Deviation	5.42	6.16

Table 6: Deep Sleep Proportion Result of the Participants

Based on the deep sleep proportion results from the two independent test sessions, the difference in deep sleep proportion and the rate of change for the NatureU Sleep group and the commercial melatonin group was calculated. A comparative analysis of the differences between the two groups was also conducted (Table 7).

	NatureU Group	Melatonin Group
Mean	29.13	22.53
Standard Deviation	5.42	6.16
Mean Difference	6.6	/
Rate of change	29%	/
P Value	0.01*	/

PS. *means statistical significance

Table 7: Deep Sleep Proportion Change Summary

It was found that compared to the commercial melatonin, the average deep sleep proportion of the 15 participants increased by 29% (a difference of 6.6%) after consuming NatureU Sleep. The data from the two test sessions showed a normal distribution and were statistically significant ($p < 0.05$).

Discussion

The findings from this exploratory clinical trial suggest that the novel composite product, NatureU Sleep, which contains sustained-release GABA and alpha-s1 casein hydrolysate, significantly impacts sleep quality, particularly deep sleep duration and proportion. The observed improvements in sleep parameters offer compelling evidence for the potential of this formulation as an effective sleep aid.

One of the key factors contributing to the effectiveness of NatureU Sleep may lie in its unique composition. GABA, as an essential inhibitory neurotransmitter in the central nervous system, is well-recognized for its anxiolytic and hypnotic properties.[14] The activation of GABAa receptors by GABA is known to promote sleep[7], and timely supplementation can alleviate symptoms associated with GABA deficiency[15], such as anxiety and insomnia. Our findings align with emerging research on Sirtuin 1 activation by GABAergic compounds, suggesting a potential dual mechanism of action through both

neurotransmitter modulation and anti-aging pathways. However, further studies measuring Sirtuin 1 expression levels are needed to confirm this hypothesis. Alpha-s1 casein hydrolysate, a milk protein hydrolysate, is also noted for providing additional stress relief and sleep improvement[16].

Nevertheless, the innovative aspect of this study lies in the use of sustained-release GABA, which differentiates NatureU Sleep from traditional GABA formulations. Different from traditional GABA supplementary which reaches peak concentration within 30 minutes, sustained-release formulations are designed to release the active ingredient gradually over 6 hours. This maintains a more consistent level of the compound within the body, which is potentially beneficial for addressing sleep issues, as it may provide a more stable and prolonged effect throughout the night, similar to the advantages observed with sustained-release melatonin formulations[17–19]. The sustained-release mechanism could ensure that GABA levels are maintained at an optimal therapeutic window, enhancing the compound's efficacy in promoting sleep without causing an abrupt peak that might be associated with faster-release formulations. This gradual release may also contribute to a smoother transition through the sleep stages, leading to the significant increase in deep sleep duration and proportion observed in the study participants.

Moreover, the controlled clinical trial design, with its randomized, single-blind approach, strengthens the validity of the findings by minimizing bias and ensuring that the observed effects can be attributed to the test product itself. The lack of adverse events throughout the trial further supports the safety and tolerability of NatureU Sleep.

However, this study has several limitations that should be considered. The single-blind design could introduce potential bias, although the use of objective wearable metrics mitigates this concern somewhat. The sample size, while sufficient for this exploratory analysis, was relatively small, which may affect the generalizability of the findings. The washout period, though intended to minimize carryover effects, was relatively short (3 days); however, given the acute, single-dose nature of both interventions and the relatively short half-lives of the components, this was deemed appropriate for this initial comparison. Furthermore, the study assessed only the acute effects of a single dose; the long-term efficacy and safety of chronic administration remain to be investigated. Future research should prioritize larger sample sizes, double-blind placebo-controlled designs, longer intervention periods, and validation against PSG where feasible.

In conclusion, the positive outcomes observed in this study may be attributed to the synergistic effects of the compound ingredients and the innovative sustained-release formulation of GABA. The results encourage further research into the benefits of sustained-release GABA in sleep management and warrant exploration into its potential advantages over traditional GABA formulations in providing a more effective and safer sleep aid for individuals experiencing sleep disturbances.

Conclusion

NatureU Sleep is a functional food with sustained-release GABA and alpha-s1 casein hydrolysate as its main active ingredients. This exploratory self-controlled trial included 15 validated participants. The data were processed following statistical requirements, ensuring authenticity and reliability. The sample size met the design requirements. Compared to the commercial melatonin, the 15 participants who consumed NatureU Sleep experienced an average increase in deep sleep duration of 26% (33.20 minutes) and an average increase in deep sleep proportion of 23% (6.6%). The differences between the two groups were statistically significant ($P < 0.05$), indicating the effectiveness of NatureU Sleep. No adverse events were observed during the entire trial.

In summary, experimental verification performed by CTI AiPu Medical Laboratory (101 & 201, No. 309 Jiangchang 3rd Road, Jing'an District, Shanghai, China) Co., Ltd. indicates that NatureU Sleep, developed by OmniSolutions Laboratory Holdings Limited, can significantly increase deep sleep duration and deep sleep proportion, thereby helping to improve sleep quality.

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Author contributions: Lorry Luo and Luke Law conceived and designed the work that led to the submission, acquired data. Naining Zhang experimented and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Miexin Yang drafted, revised the manuscript, and approved the final version.

Declaration of Interests: Lorry Luo, Luke Law and Miexin Yang are OmniSolutions Laboratory Holdings Limited employees. The other authors declare none.

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Ethical Approval

This study was conducted according to the guidelines in the Declaration of Helsinki. All procedures involving human subjects/patients were approved by the [Shanghai Ethics Committee for Clinical Research; CTI-2024-2]. Written informed consent was obtained from all subjects/patients.

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