

The effects of a sleep/recovery supplement: 'Night Time Recharge' on sleep parameters in young adults.

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The effects of a sleep/recovery supplement: 'Night Time Recharge' on sleep parameters in young adults

Abstract:

Background: Concentrated cherry juice reportedly contains melatonin which in turn has been highlighted as an important regulator in initiating sleep. **Aim:** The present investigation aims to clarify whether Night Time Recharge (NTR), a marketed sleep aid, containing cherry extract improves key sleep parameters in young, active adults. **Methods:** A double-blind, randomized, placebo-controlled, cross-over study design was employed. 20 participants (9 female) consumed either NTR or a placebo for 7 days. Accelerometers were used to assess sleep quality and physical activity levels. Urinary levels of 6-sulfatoxymelatonin (6-SMT) a marker of melatonin synthesis was assessed via ELISA. **Results:** 6-SMT levels increased following NTR treatment (28.95 ng/ml) compared to a placebo (4.0 ng/ml), ($p < 0.001$). There was also a significant difference ($p = 0.047$) in dietary tryptophan consumption during the NTR treatment (1236 mg) vs placebo (1149 mg). No trace of melatonin was detected from our NMR analysis. NTR had no effect on any sleep parameters with the exception of sleep latency ($P = 0.001$). **Conclusions:** As chemical analysis of NTR by Nuclear Magnetic Resonance imaging (NMR) identified no detectable melatonin tryptophan intake is a likely reason for this. These results are in contrast to previous studies which have found a positive effect on sleep following cherry supplementation. Future work should focus on sleep latency and investigating whether cherry juice is effective in participants with problems initiating sleep.

Introduction

Sleep deprivation has an effect on: mood, glucose metabolism, appetite regulation and immune function (Halson, 2014; Williamson and Friswell, 2011; Poh et al., 2016). Sleep deprivation may also be an important factor in predicting disease risk (Rafalson et al., 2010; Chandola et al., 2010). Current guidelines recommend adults should aim to sleep 7-8 hours per night (Center for Disease Control and Prevention, 2018). Despite this recommendation over 50 % of young adults report a lack of sleep and poor sleep quality (Gaultney, 2010; Sato et al., 2016). College aged adults are thought to be particularly poor at meeting the CDC guidelines, with some data indicating that 27% of young adults sleep less than 7 hours per night and regularly report having poor quality sleep (Center for Disease Control and Prevention, 2018). Moreover, research has identified that short (< 6 h) and interrupted sleep is an independent risk factors for type 2 diabetes mellitus and coronary heart disease (Rafalson et al., 2010; Chandola et al., 2010). Additionally, a lack of quality sleep has also been linked to lower grade point averages in college students (Gaultney, 2010). Physical activity is thought to influence sleep in a positive manner, with the exception of exercise close to bedtime which may interrupt or delay sleep (Passos., et al 2010; Fairbrother., et al, 2014). This suggests an importance in recording and comparing the physical activity levels of participants during sleep trials. Moreover, sleep medications and dietary supplements may also improve sleep quality and duration.

Commonly used sleep medications such as benzodiazepine receptor agonists, are effective for improving sleep but have adverse effects, such as impaired driving performance (Miyata et al., 2015), increased drowsiness and cognitive impairment (Boockvar, 2016). Dietary supplements rather than pharmaceuticals which enhance sleep without adverse side effect are therefore of

interest. 'Night Time Recharge' (NTR) is one such dietary supplement, containing, ingredients commonly claimed to improve sleep: tryptophan and cherry active. NTR purports to improve sleep quality, although no studies on this specific supplement to date have tested this claim. Tart cherry juice has been shown to increase 6-sulfatoxymelatonin (6-SMT) concentrations, consequently increase melatonin, an important regulator of sleep (Nédélec et al., 2015). Melatonin, a hormone secreted by the pineal gland, plays a crucial role in regulating the sleep-wake cycle (Appleton and Gringras, 2013). It does this by promoting sleep onset (sleep latency) and continuity via increasing the homeostatic drive to sleep (Khullar, 2012). Exogenous melatonin and melatonin agonists have also been highlighted for their potential beneficial effects on improving sleep quality (sleep efficiency) in people suffering from sleeping disorders e.g. the number of waking episodes a person experiences once they fall asleep (Van Maanen et al., 2011; Goldman et al., 2014; Wade et al., 2011; Van Geijlswijk et al., 2010; Van Maanen et al., 2017; Gringras et al., 2012). Furthermore, a double-blind placebo-controlled trial by Howatson et al. (2012) found that one week of tart cherry juice (high in melatonin) consumption had positive effects on key sleep parameters.

The essential amino acid tryptophan (present in NTR) is thought to play a role in the regulation of sleep and circadian rhythms by acting as a precursor for endogenous melatonin synthesis (Lieberman et al., 2016). Increased dietary consumption of tryptophan has been shown to increase levels of melatonin (Halson, 2014) and interventions using tryptophan have resulted in improved sleep efficiency (Wada et al., 2013; Bravo et al., 2013; Galán et al., 2017). The present investigation aims to test the efficacy of NTR on sleep efficiency in healthy, young, active adults. It was hypothesized that the consumption of NTR in young, healthy adults, over a 7-day treatment period, would improve participants' overall sleep efficiency.

Methods

Participants

A total of 20 (11 male) undergraduate university students (age 21.0 yrs \pm 1.0; height, 1.75 m \pm 0.12; body fat 22.32 % \pm 7.25; muscle mass 56.78 kg \pm 12.17) volunteered to take part in the study, one participant dropped out citing health reasons, not related to the present investigation. Participants were all healthy, and free from any physical illness or allergies. All participants provided written informed consent and the study was approved by the university ethics board. Sleep quality was assessed at baseline via the PSQI and mean scores of 6.51 \pm 1.51 indicated 'poor' sleep in our participants; this is of practical significance as 15 out of our 20 participants had scores over 5, with a score of 5 or more indicating a 'poor' quality sleeper.

Study design and procedure

A double-blind, randomised, placebo-controlled, counterbalanced, crossover design was employed to assess the effects of NTR on sleep efficiency and latency. This study was carried out between February and March in 2018, in the North of England. The following outcome measures were recorded: sleep patterns and physical activity via GENEActiv accelerometers, dietary inventory and urinary-6-sulfatoxymelatonin. There were two treatment periods, each lasting one week each separated by a week-long wash-out period. Participants visit the laboratory a total of four times. On visit 1: participants reported to the lab fasted and voided their bladder for urine analysis, and were fitted with a GENEActiv triaxial accelerometer. They were given a 7-day diet diary and instructions

on how to complete it. Participants were then given either 7 days of the supplement or a placebo. Participants drank the mixture provided with 200 ml of water every evening approximately one hour before going to sleep. Visit 2: Participants reported back to the lab, returned the accelerometers for analysis and provided a urine sample before beginning a 1 week washout period before returning for the laboratory to repeat the process for the alternative arm of the trial on visits 3 and 4. On the first and last visit, basic anthropometric measures were taken, including height, weight and body composition.. At all four of the visits they provided a urine sample. Immediately following collection, urine samples were spun in a centrifuge at 4000 rpm for 10 minutes and placed in a -80 °C freezer for later analysis. Diet diaries, accelerometers and 7 drinks of either the placebo or NTR packaged in tinted bottles were given to participants at visits 1 and 3 and the empty bottles collected at visits 2 and 4.

Anthropometric measures

Height (without shoes) and body mass, wearing light clothing, and body composition were recorded to the nearest 0.1 cm and 0.1 kg respectively using a SECA 220 telescopic stadiometer (SECA, Birmingham, United Kingdom) and multi-frequency bioelectrical impedance scale (MC980MA, Tanita, Amsterdam Holland). For consistency participants were asked to wear the same clothing for each visit. Height was recorded at the point of normal breath inspiration with the head orientated in the Frankfurt plane. Body mass was recorded in kilograms and body mass index (BMI) calculated by dividing body mass by height meters squared and rounded to the nearest kg/m².

Sleep Efficiency and Physical Activity

GENEActiv triaxial accelerometers were used during treatment periods to provide an objective assessment of sleep quality and physical activity. Accelerometers were fitted to the non-dominant wrist of each participant. Data recorded 7-days' capturing sleep and physical activity GENEActiv watches have been previously validated (de Souza et al., 2003) against polysomnography for assessing sleep. Sleep data including sleep efficiency, sleep latency, time in bed and total duration of sleep was recorded. Time in bed was corroborated with sleep diaries, and changes in light and temperature of participants. Physical activity intensity (expressed as METs; 1.0 MET = sedentary, > 1.0 < 3.0 = light, 3.6 METs = moderate and > 6.0 = vigorous intensity activity) and duration during the trial were downloaded and recorded. . Complete accelerometer data was collected for 18 of the 19 participants (1 lost due to technical error). Participants were advised to follow their regular exercise and sleep regimes throughout the duration of the study, and to avoid starting any new exercise programmes or sleep patterns.

Pittsburgh Sleep Quality Index (PSQI) and Questionnaire

The PSQI is a validated subjective questionnaire assessing sleep quality, measuring sleep across seven separate domains: sleep efficiency, subjective sleep quality, sleep latency, sleep duration, sleep disturbances, the use of sleep medication, and daytime 'dysfunction'. The participant self rates each domain. Scoring is carried out via a 0 to 3 likert, with 3 reflecting the negative extreme. Essentially a global score of 5 or more indicates a "poor" sleeper. Participants completed the PSQI at baseline to assess sleep status. Participants were also asked about their feelings towards the

supplement, following the two treatment periods. This was done using a yes/no response to the following three questions: Did the supplement help increase sleep? Could you tell which one was the supplement? and, would you take the supplement if you were struggling to sleep?

Diet

Tryptophan, caffeine, alcohol, melatonin, macronutrient and total energy intake were assessed via a 7 day diet diary and nutritional analysis software (Nutritics research edition version 5.0 Dublin Ireland). Each subject completed the diet diary during both treatment weeks of the study (a total of 14 days). These were collected at the end of each treatment week 2 and week 4. The data was inputted into Nutritics software which was then analyzed to produce a diet report. All data was analysed twice by separate researchers and the coefficient of variance calculated for this data. Data with a CV of less than 10% was considered as reasonably consistent based on repeat analysis studies in other fields (Cui 1989, Atkinson and Nevill 1998). Finally, participants were advised to follow their regular dietary patterns throughout the trial period and not to start any new supplementation or dietary regimes.

Urine samples

Participants were asked to collect a urine sample on the morning of the visit to the laboratory. The urine sample was the first morning void, and spot urine samples are known to be reflective 24 hr 6-SMT concentrations (Schernhammer et al. 2004). Upon collection, total urine volume was measured, and all urine samples were collected then frozen at -80 for later analysis. Analysis was carried out using an Enzyme-linked Immunosorbent assay (ELISA) test (Buhlman Germany) to detect 6-sulfatoxymelatonin, the principal metabolite of melatonin.

Figure 1 here

Supplement ingredients and supplement quality

Each supplement serving (15.6 g) was reported by the manufacturer to contain 54 kcal which is made up of 13 g of protein (L-tryptophan 3 g, L-Leucine 3 g, Glycine 2 g, Taurine 2 g, Isoleucine 2 g, Valine 1.5 g), 0.5 g carbohydrate, 1000 mg of Cherry active and 200 mg of magnesium. The placebo contained 54 kcal and was made up of 50 μ L artificial cherry flavouring (containing no polyphenols), 15 g of glucose powder, 0.65 g sucralose and 0.06 g of salt. The placebo was a cherry flavoured cordial containing no actual cherries.

Analysis of the amino acid composition of the supplement was carried out by dissolving 100 mg of the supplement in 1 mL of 1M LiOH in D₂O. Full characterisation of the composite amino acids was performed using 1D and 2D NMR experiments (1H, 13C, DEPT 135, HSQC and HMBC). 0.1 mmol of 4-nitrobenzoic acid was added to the sample as an internal standard and the 1H integrations from each amino acid relative to the standard were used to calculate component concentration in

solution (and thereby the composition in the supplement). Each measurement was carried out in triplicate.

Analysis of the melatonin content of the supplement was carried out by dissolving 10 mg of the supplement in 1 mL of 1:1 MeCN:H₂O with 0.4% formic acid. The sample was analysed on a Waters Xevo LC MS system using positive mode electrospray ionisation and time-of-flight mass analysis on a solvent gradient from 100% H₂O (+0.4% formic acid) to 100% MeCN (+0.4% formic acid) over 60 minutes. A standard of pure melatonin was run under the same conditions for comparison.

Statistical Analysis

Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS, version 24, Chicago Illinois). Data was assessed for normality via histograms and Shapiro-Wilk's values. Where data met the requirements for parametric testing these were used. The mean values for sleep efficiency, total sleep and sleep latency and dietary intake were compared using a two tailed paired samples t-test or Wilcoxon signed-rank test as appropriate. Statistical significance was set at alpha 95%. Cohen's *d* to assess practical significance was calculated for the effect of supplement on sleep efficiency, latency, total sleep, dietary intake and physical activity. Pooled standard deviations were used to calculate Cohen's *d*. Effect size (ES) was multiplied by an adjustment factor of 0.975 to correct for bias to produce *d* and confidence intervals (CI) calculated thereafter for all normally distributed data. Where CI for *d* did not include 0.0 or negative values it was assumed some effect had taken place (Ivarsson et al., 2013). Effect size cut-offs for *d* were defined based on Hopkins (2002) guidelines for sports science as 0.2 to 0.6, 0.6 to 1.2, 1.2 to 2.0, 2.0 to 4.0 and >4.0 for small, moderate, large, very large and near perfect effect sizes respectively. Effect size estimates for data that violated the assumptions of normality were calculated and expressed as *r*. Effect size cut offs for *r* were defined based on Cohens (2013) suggested categories for non-parametric data > 0.10, > 0.24 and > 0.34 for small, medium and large.

Results

Sleep efficiency/Sleep latency/total sleep

There was no statistically significant difference in the main outcome measure of mean sleep efficiency between placebo (65.96 ± 13.53 %) and supplement (71.83 ± 8.85 %), *t* (17) = 1.862, *P* = 0.080 (two-tailed). An ES for sleep efficiency was also calculated (*d* = 0.50, 95% CI [-0.16, 1.17]); therefore confidence intervals indicated no effect size on sleep efficiency. There was also no statistically significant difference in total amount of sleep between placebo (6.43 ± 0.08 h) and supplement (6.80 ± 0.74 h) (*t* (11) 1.198 *P* = 0.244); although the ES for total sleep suggest there may be a small effect: (*d* = 0.47, 95% CI -0.33, 1.30]). A Wilcoxon Signed Rank Test revealed a statistically significant reduction (*Z* = 3.277 *P* = 0.001) and medium ES (*r* = 0.25) in time to fall asleep (latency) between placebo (Md 19 IQR 12 to 30 mins) and supplement (Md 9 IQR 5 to 23 mins) treatments. Table 1 summarizes the difference between groups in sleep parameters.

Table 1 here

Physical activity

The GENEActiv units collect four types of physical activity data, collating the percentage of time participants spent being sedentary or doing light, moderate and vigorous activity. The mean time spent performing each type of activity as percentages is displayed in table 3. No significant differences ($P > 0.05$) were identified between any of the groups, suggesting participants activity level did not differ between supplement and placebo weeks.

Urine analysis

The cohort's urine samples were analysed from placebo and supplement treatments. The mean average 6-SMT for both supplement and placebo are displayed in figure 1. There was a statistically significant difference ($t(10) 4.949 P= 0.001$) in mean 6-SMT urine output between the placebo and supplement group.

Table 2 here

Diet

The mean nutrient values for key dietary components are displayed in Table 2. The scores of which were analysed in a paired samples t-test and no significant differences ($P > 0.05$) were found between either treatment groups with the exception of tryptophan.

Table 3 here

Analysis of Supplement Quality

Results of the NMR analysis compared to the manufacturer's specification are provided in figure 2. Analysis of the amino acid content of the supplement indicated similar levels of amino acids to those advertised.

Figure 2 here

Figure 3 here

Analysis of the amino acid content of the supplement (see table 4) indicated similar levels of amino acids to those advertised. Analysis of the melatonin content of the supplement indicated no detectable melatonin within the supplement.

Table 4 here

Qualitative Data

In order to briefly explore the participants feelings towards the supplement, following the two treatment periods- they were asked for a yes/no response to the following three questions: Did the supplement help increase sleep? (Yes = 60%) Could you tell which one was the supplement? (Yes = 70%) and, would you take the supplement if you were struggling to sleep? (Yes = 50%).

Discussion

The primary aim of this study was to test whether the supplement, NTR, improved young, active people's sleep. Supplementation with NTR resulted in an increase in urine levels of 6-SMT, a surrogate marker of melatonin production, indicating that the compliance of the study was high and that the supplement increased melatonin production. Dietary intake except for tryptophan was also similar between the placebo and supplement groups throughout the trial, although differences in tryptophan intake were not clinically significant (< 87 mg). Moreover, there was also no difference and caffeine or alcohol intake between treatment phases, two compounds known to influence sleep. Furthermore, no statistical difference between supplement and placebo was found for total physical activity levels. The results of data analysis for sleep latency found a statistically significant change between supplement and placebo groups. Statistical analysis also indicated a trend for improved sleep efficiency. Effect size testing CI indicated that improvements in sleep efficiency and latency were likely null to small. Taken together, these results suggest this supplement may have some small effect on sleep latency and perhaps a small effect on efficiency in this group of young active men and women, with moderately poor sleep, further research is needed to confirm these findings.

To our knowledge no studies have been carried out on this specific supplement (NTR) or the combination of ingredients in the product, however previous studies have investigated individual constituents of the supplement: cherry active (Howatson et al., 2012); melatonin (Goldman et al., 2014; Howatson et al., 2012; Walecka-Kapica et al., 2014); and tryptophan (Mohajeri et al., 2015; Lieberman et al., 2016) with contrasting results. Studies on melatonin suggest that the presence of this hormone in a supplement has beneficial effects on sleep (Goldman et al., 2014; Van Maanen et al., 2017; Walecka-Kapica et al., 2014). In sleep studies of melatonin supplementation in adults with and without sleep disorders, a dosage between 2 to 5 mg has produced positive results (Wade et al., 2011; Van Geijlswijk et al., 2010; Van Maanen et al., 2017; Walecka-Kapica et al., 2014; Kunz and Mahlberg, 2010; Garrido et al., 2010). Furthermore, Low dosages of melatonin (1 mg) have also been found to have a positive effect on sleep in children with existing sleep problems (Gringras et al., 2012). Analysis of the melatonin content of different cherry varieties indicates cherries contain between 2.06 to 13.46 ng/ g dry weight, or 0.01 to 20 ng / g frozen weight (Meng et al., 2017). The cherries used in the NTR supplement are Montmorency cherries. Montmorency cherries given at a dose of 83 ug/day in a cherry juice over a 7 day has been shown to improvement sleep quality (Howatson et al., 2012). Garrido et al. (2010) also saw an improvement in sleep quality when participants were provided with tart cherry juice from cherries grown in the Jerte Valley (Spain) which produces several cherry cultivars containing high levels of melatonin. It is of concern that our chemical analysis of the melatonin content in the present investigation by NMR indicated that supplement contain no melatonin, This is despite the fact the supplement contains the ingredient "Cherry bomb", which is purported to be a concentrated form of tart cherries and described as high in melatonin by the manufacturer.

It is possible that processing may have influenced the melatonin content of the supplement. By way of illustration, previous studies have utilized different delivery methods for cherries to influence sleep; Garrido et al. (2010) provided participants with 200 g of whole cherries, and Howatson et al. (2012) used a concentrated tart cherry juice. By comparison NTR is a powder supplement designed to be stored and mixed into a drink with water. Processing and storage of cherries to concentrated juices has been demonstrated to diminish in some cases all the melatonin content of commercially available products (Özen and Ekşi, 2016), so these findings are not without precedent. Furthermore, the ripening stage when the cherries are picked is known to influence melatonin content (unripe vs ripe) (González-Gómez et al., 2009). It therefore seems likely that the increase in 6-SMT levels seen in the urine samples was the result of the large dose of tryptophan delivered from the dietary supplement rather than any cherry derived melatonin. Tryptophan is known to be an important precursor to melatonin, via a short metabolic pathway to serotonin, while urinary 6-SMT reflects plasma melatonin status (Peuhkuri et al., 2012). It therefore seems likely that any improvement in sleep time and overall quality was likely driven by the tryptophan content of this supplement in the present investigation.

Protein intake studies tend to show a relationship between high protein intake and better sleep (Dashti et al., 2014; Zhou et al., 2016). The exact mechanism behind this is again unclear; however, it is potentially linked to tryptophan. Tryptophan studies have been equivocal with mixed effects; Mohajeri et al. (2015) compared women consuming 70 mg of tryptophan per day with a control group finding no significant effects on sleep efficiency or sleep latency. Bravo et al. (2013) and Galán et al. (2017) by contrast found that tryptophan did improve sleep efficiency, using accelerometers to monitor sleep, increasing sleep time and reducing sleep latency. Compared to the present investigation there was a trend and a significant increase in both sleep efficiency and latency respectively, what's more a small effect size was noted for sleep latency. Thus more research is warranted to tease out the effects of tryptophan supplementation on sleep latency.

When dietary intake was analyzed we found, no significant difference in any of the dietary constituents commonly associated with affecting sleep-i.e. no alcohol was consumed and caffeine did not differ between treatments neither did overall energy intake or macronutrients. We did however; identify a significantly higher dietary tryptophan intake in the supplement group, driven by a trend towards a higher protein intake. The difference in the dietary tryptophan intake between the groups equated to less than 100 mg (NTR 1236 mg vs placebo 1149mg). By comparison the supplement contained a 3 g serving of tryptophan, so it seems unlikely the additional dietary tryptophan would make any clinically significant difference. Moreover, the greater mean intake of tryptophan was observed in the supplement arm of the trial and so theoretically this would have only strengthened the trend towards greater sleep quality/quantity rather than obfuscating the difference between supplement and placebo.

Limitations

The gold standard method for measuring sleep and sleep quality is polysomnography rather than accelerometers (Water et al., 2011). Polysomnography allows researchers to measuring additional sleep variables and potentially allows a more detailed look at participants' sleep-wake cycles. Sleep research however requires a practical approach; polysomnography requires participants to visit a sleep laboratory for monitoring and would not as readily capture the natural patterns of free-living

individuals compared to the relatively non-invasive wearing of an accelerometer. Moreover, we investigated NTR as a novel sleep supplement based on the purported melatonin and tryptophan content, analysis indicated however that the supplement contained no melatonin. This fact may have influenced our ability to find a significant results, as supplementation with melatonin has been demonstrated to positively impact sleep (Goldman et al., 2014; Howatson et al., 2012; Walecka-Kapica et al., 2014)). The chemical analysis of the supplement should therefore be regarded as a strength, and researchers encouraged to ascertain the profile of ingredients in investigations involving commercially available dietary supplements. Under reporting of habitual dietary intake from food diaries is a well-known limitation, however urinary levels of 6-SMT did increase in the supplement group suggesting good compliance and reflecting the high tryptophan content of the supplement. Analysis of the PSQI indicated the cohort suffered from poor sleep quality, consistent with previous reports of college aged students (Gaultney, 2010; Sato et al., 2016). Supplementing with NTR (effectively an amino acid supplement), had a small effect on sleep latency and efficiency in this population. Although these findings may not be readily extrapolated to other populations with higher quality sleep at baseline. Moreover, although we blinded participants to the treatment regimes, and observed good compliance, indicating participants consumed the supplement. 70% of participants were able to distinguish between the placebo and correctly identify the supplement. Indicating that our blinding may not have been successful. Furthermore, a limitation of the supplement, rather than study was the fact that only 50 % of the participants would be willing to take the supplement after the trial because of its poor taste. A capsule delivery may therefore resolve any issues around blinding and palatability. Finally, there are potentially multiple confounding factors that affect a person's sleep which were not accounted for e.g. participants' stress levels, although this would, to some extent, be mitigated against by the RCT-crossover design employed.

Conclusions

We conclude that NTR may have produced a small improvement in sleep latency in young healthy adults without diagnosed sleep problems. This supplement did not significantly alter the other sleep parameters measured. The suggested mechanism for this finding is an increase in the endogenous production of melatonin via increased tryptophan intake. Further research is needed on larger sample sizes to identify if the combination of melatonin containing cherries and tryptophan can positively affect sleep in healthy individuals without sleep disorders. Currently our thinking is that the supplement decreases the time it takes to fall asleep which may drive an effect on overall sleep efficiency. Of note however, only 50 % of participants would be willing to take the supplement after the trial despite some participants experiencing positive effects largely, owing to the supplements unpalatable taste. Supplement research should investigate and report the supplement ingredients to improve the interpretation of results. Finally, researchers should seek to compare the effects of dietary supplementation to guidelines for improved sleep hygiene e.g. regular sleeping patterns, reduced screen time and reduced caffeine and alcohol intake around bed times.

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Declaration of conflicting Interests

"On behalf of all authors, the corresponding author states that there is no conflict of interest."

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Consent for publication and ethical approval

This project was approved by the university Food Research Ethics Committee (review ref:ER5215839) therefore this study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Availability of data and materials

The data is available on request from the corresponding author

Author's contributions

T.S conceived and designed the experiments; T.S and M.G. performed the experiments; T.S., A.C and M.G analyzed the data; L.T. contributed to the analysis T.S, A.C. M.G and L.T wrote the manuscript. DA carried out the analysis of the supplement and contributed to the analysis and the manuscript.

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