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Annals of Clinical Nutrition

Open Access | Review Article

# Tryptophan and hops: Chrononutrition tools to improve sleep/wake circadian rythms

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Received: Mar 02, 2018
Accepted: Apr 15, 2018
Published Online: Apr 24, 2018
Journal: Annals of Clinical Nutrition
Publisher: MedDocs Publishers LLC
Online edition: http://meddocsonline.org/


**Abstract**

Chrononutrition is a field of chronobiology that establishes the principle of consuming foodstuffs at times of the day when they are more useful for health to improve biological rhythms. Tryptophan is an essential amino acid both serotonin and melatonin precursor which are involved in sleep/wake circadian rhythm, moreover melatonin synchronizes most of biological circadian rhythms and has direct scavenging actions against free radicals. In this way, through tryptophan-enriched diets our research group has demonstrated that sleep problems in babies and in elderly people can be enhanced increasing melatonin levels. On the other hand, the hop, a component of beer, can be used to improve circadian rhythms impairment because of its sedative effects due to GABA a receptors modulation. In this case, birds and a work-stressed population improved their sleep conditions with the equivalent dose of a non-alcoholic beer. In conclusion, chrononutrition can be used to become better sleep conditions and consequently increased quality of life, even delaying ageing effects.

**Keywords:** Chrononutrition; Tryptophan; Hops; Sleep; Actigraphy

## Introduction

### Tryptophan: Serotonin and melatonin precursor

Tryptophan (Trp) is an essential amino acid which has to be taken from diet [1,2]. After Trp is ingested albumin joins to it in the blood and it is transported across the blood-brain barrier by a specific carrier for neutral aminoacids in competition with rest of Large Neutral Amino Acids (tryptophan, tyrosine, phenylalanine, leucine, isoleucine and valine) [3,4].

After Trp reaches Central Nervous System (CNS) it is converted into neurotransmitter serotonin (5-hydroxitryptamine, 5-HT) through a two-step synthesis process. These reactions are limited by tryptophan availability in serum [5]. Most of the synthesis of 5-HT takes place in the Raphe Nucleus whose neurons innervate widespread areas of the brain like striatum, cerebellum, hippocampus and pineal gland [6]. Serotonin modulates some developmental events such as neuron migration, cell differentiation, cell division or synaptogenesis. Also it is involved in a wide variety of CNS functions including the control of appetite, sleep, memory and learning, temperature regulation, mood, behavior (including sexual), cognition or mental disorders among others [7,8].

Suprachiasmatic Nucleus (SCN) of the hypothalamus is the director of the circadian rhythms orchestra in mammalian. Through the SCN many physiological and behavioural functions and rhythms are coordinated and put in hour [9]. In this way,

**Cite this article:** Bravo R, Franco L, Rodríguez AB, Ugartemendi L, Barriga C, et al. Tryptophan and hops: Chrononutrition tools to improve sleep/Wake circadian rythms. Ann Clin Nutr. 2018; 1: 1004.

SCN gives the information to synthesize indol melatonin during dark hours through specialized photoreceptive cells in the retina, which detect light [10].

In the pineal gland 5-HT is transformed into melatonin – a lipid soluble indolamine - during dark hours through NAAT witch is activated in the nocturnal period and inhibited by light [11,12]. Melatonin signaling presumably influences all cells in the organism and reports them information about environmental photoperiod – the onset of night - and seasonal information [10,13]. In this way, melatonin is considerate as an internal sleep "facilitator" and its administration induces sleep and improves sleep problems [14].In addition, this indol have immunoregulatory properties and is a potent free radical scavenger [15] (Figure 1).

**Tryptophan-enriched diets and sleep**

In a previous study our research group reported that Trp-present in breast milk has direct consequences on sleep quality and, on the other hand, formula-fed babies sleep worse than breast-fed babies (Tables 1 and 2) [16] through actimetry: a well-validated and non-invasive technique to study nocturnal sleep [17]. Parting from these results, through actimetry, we decided to perform a double-blind assay with babies less than 5 months who suffered from sleep problems (more than three nocturnal arousals) and fed formula milks. In this study infants (n=30) consumed different formula milk combinations over a period of three weeks. Three diets were considered in this study: Diet A, consisting of standard milk (Blemil plus 1 Forte, Ordesa S.L.) both day and night for a week; Diet B, consisting of standard milk Blemil Plus 1 Forte during the day (06:00-18:00) and Blemil Plus 1 Night (formula dissociated into its nutritional components to consolidate sleep) during the night (18:00-6:00) for another week; Diet C, infants were given Blemil Plus 1 Day (formula dissociated into its nutritional components that consolidate wakefulness) during the day (06:00-18:00), and Blemil Plus 1 Night (Ordesa S.L.) during the night (18:00-06:00) (Table 3). Every participant had a different diet combination through the 3 weeks of the study.

The analysis of the actimetry data indicated and improvement in the infants´ nocturnal sleep in both the week with Diet B (Control/Night) and that with Diet C (dissociated Night/Day milk). In particular, Diet C showed increased hours of *Actual Sleep* (7.68±0.54h vs. 6.77±0.12h for the Diet A control; $p<0.05$) and improved *Sleep Latency* (0.44±0.04h vs. 0.60±0.08h for the Diet A control; $p<0.05$). The same infants receiving the Diet B in another different week showed an improvement in *Sleep Efficiency* (76.43±3.4% vs the Diet A control 69.86±0.94%; $p<0.05$) and *Sleep Latency* (0.45±0.04h vs. the Diet A control 0.60±0.08h; $p<0.05$). So, the use of infant milk formulas with chronobiologically-adapted nutritional components improved the consolidation of the sleep/wake cycle in bottle-fed infants (Figure 2).

After that, knowing that about 30 % of people over 50 years suffer from sleep problems [18], the next step was to evaluate in a single-blind study if Trp-enriched cereals could improve sleep problems in elderly people using actigraphy. It is well known that elderly people tend to suffer from alterations in the circadian rest-activity rhythm due to impairments in their circadian system [19,20] and quantity and quality of sleep are decreased [2]. This study was carried out in 35 elderly volunteers aged 55-75 years old who suffered from sleep problems (mainly sleep onset and sleep fragmentation problems) and non-reported sleep pathologies or depression during the assay. Three experimental weeks with a different diet per week were considered: First week (control week), participants consumed control cereals (22.5 mg tryptophan in 30 g cereals per dose) at breakfast and dinner; second week (treatment week), volunteers consumed 30 g of tryptophan-enriched cereals (containing 60 mg tryptophan) both at breakfast and dinner; the third week (post-treatment week), volunteers consumed their habitual diet. Figure 2 shows that during the treatment week most of sleep parameters enhanced as compared to both the control week and the post-treatment week. In fact, *Actual Sleep Time* (3.5%; $p<0.01$), *Sleep Efficiency* (5.7%, $p<0.001$) and *Immobile Time* (2.8%, $p<0.01$) increased with respect to control levels. On the other hand, *Sleep Latency* (31.6%, $p<0.05$), number of *Wake Bouts* (11.1%;$p<0.5$), *Nocturnal Total Activity* (16.2%, $p<0.01$) and *Sleep Fragmentation* (7.8%, $p<0.001$) decreased. Also, quantification of serotonin and melatonin urinary metabolites showed that serotonin and melatonin became higher during the treatment week (Table 4) (Figure 3).

Tryptophan have been previously shown to have direct effects on sleep regulation by increasing the availability of brain serotonin, which is involved in *Sleep Latency* [21,22], presumably to its direct consequences on GABAergic neurons [23,26]; on the other hand melatonin, which is produced from serotonin is more involved in sleep quality than in sleep onset [24].

To summarize, tryptophan-enriched diets are useful tools to improve sleep conditions increasing serotonin and melatonin levels in people who suffer from sleep difficulties. These kinds of diets are able to reduce sleep/wake cycle problems due to early ages and aging.

**Hops, beer and its sedative effects**

Nowadays many plants are used to prevent sleep problems or anxiety disorders like common valerian, passionflower, chamomile, linden or hop because of their sedative effects [6,3].

The hop (*Humulus lupulus*, fam. Cannabaceae) is a plant used in the brewery industry for its aromatic characteristics to add bitterness and flavour to beer. It has also been traditionally used for its soothing properties. Its sedative activity lies mainly in its bitter resins, and in particular in the products of oxidative degradation, such as those resulting from the degradation of α-acids, a major example being 2-methyl-3-buten-2-ol, compound generated during the storage in the presence of oxygen [27,28]. In addition, to these bitter degradation products, there are other active components such as xanthohumol, one of the mayor flavonoids present in beer, and myrcenol [29,30]. The main mechanism of the soothing action of hops is by modulating brain GABA A receptors, thus inhibiting the CNS [30].

Basic research on hops has found effective applications in healthy human populations as an aid to sleep, among others health disorders, like obesity, anti-inflammatory or antithrombotic effects [31,32]. In addition to its use in people with sleep problems, the sedative action associated with the components of hops has been used to correct temporary sleep latency and sleep fragmentation disorders in human populations [33].

The first of our experiments with hops was in common quails (*Coturnix coturnix*) similar to humans in the sleep/wake circadian rhythm. Two groups were considered: a basal group without treatment, a control group which only ingested methylcellulose excipient, and a treatment group that consumed 1, 2 and 11 mg extract of hop as one capsule per day one hour before lights turn off for one week per treatment. Each treatment week was inter-

calated with a wash-up week and performed by duplicated.

Our research show that with the dose of 2 mg, there was a statistically significant ($p<0.05$) reduction of the arithmetic mean nocturnal activity (23±3.0) with respect to the basal (38.56±2.79), control (38.1±2.8), and other doses groups 1 mg (54.04±3.65) and 11 mg (47.47±5.88) (Figure 4).These results support previous studies performed in mice [34]. On the other hand, 1 and 11 mg extract of hop did not show results as positive as 2 mg extract, which is the dose similar to the concentration in beer.

Our next objective was to analyze the sedative effect of hops as a component of non-alcoholic beer on the sleep/wake rhythm in a work-stressed population. The experiment was conducted with healthy female nurses (n=17) working at rotating shifts. Overnight sleep and chronobiological parameters were assessed by actimetry (Actiwatch©) after ingestion of non-alcoholic beer (333 ml with 0.0% alcohol) with supper for 14 days (treatment).

The actigraphy results for the study indicated that the quality of sleep improved relative to the control group during the two weeks of work stress with ingestion of non-alcoholic beer (Figure 5). Although there was no difference in the amount of night-time sleep, the quality of sleep improved as shown by improvement in parameter of night-time sleep. In particular, treatment sleep latency (12.01±1.19 min) decreased ($p<0.05$) with respect to control (20.50±4.21) and the same happened with total nocturnal activity (control=7258.78±898.89 activity pulses vs. treatment=5284.78±836.99 activity pulses; $p<0.05$).

From these results our research group supports that ingestion of non-alcoholic beer increase the quality of night-time sleep. The mechanism would principally be modulating the GABA ergic response through the effects of the hop components myrcenol, xantohumol, and such α-acid derivates as 2-methyl-3-buten-2-ol.

## Tables

**Table 1:** Chronobiological parameters of urinary 6-sulfatoxymelatonin in breast-fed babies (n=8), their breast-feeding mothers (n=8), and formula-fed babies (n=8), and of tryptophan in breast milk (n=8) [17].

| Parameters | MESOR | Acrophase | Nadir |
|---|---|---|---|
| Tryptophan (breast milk) | 67.95 µmol/L | 03:00 h | 15:00h |
| 6-sulfatoxymelatonin (natural) | 11.45 ng/mL | 06:00 h | 18:00 h |
| 6-sulfatoxymelatonin (mothers) | 9.72 ng/ mL | 12:00 h | 20:00 h |
| 6-sulfatoxymelatonin (formula) | 9.82 ng/ mL | 11:00 h | 00:00 h |

**Table 2:** Comparison of the sleep parameters of breast-fed (n=8) and formula-fed (n=8) babies.
[a] $p<0.05$ with respect to the formula milk [17].

| Sleep variables | Formula | Breast milk |
|---|---|---|
| Time in crib | 10 h 2 min+1 h 10 min | 9h 5 min+2 h 36 min |
| Assumed sleep | 8h 53 min+52 min | 9h 35 min+41 min [a] |
| Actual sleep | 7h 7 min+43 min | 8h 30 min+49 min [a] |
| Sleep efficiency | 70.512+5.78 % | 81.45+5.48 % [a] |
| Sleep latency | 1h+45 min | 30 min+17 min |

**Table 3:** Nutrient composition of the Blemil Plus 1 Night and Blemil Plus 1 Day, both of which satisfy the 1996/49/CE and 2003/14/CE directives for infant milk formulas [13].

| **NUTRIENTS** (per 100 g milk powder) | **BLEMIL 1 PLUS NIGHT** (3.4 g tryptophan/ 100 g protein) | **BLEMIL 1 PLUS DAY** (1.5 g tryptophan/ 100 g protein) |
|---|---|---|
| **Macronutrients** | | |
| Proteins | 10.7 g | 12 g |
| Tryptophan total | 0.40 g | 0.18 g |
| Fats | 26 g | 26 g |
| *Vegetable* | 16.4 g (63%) | 25.7 g (98.75%) |
| *MCT* | 9.6 g (37%) | |
| *Formulaid* | | 0.3 g (1.25%) |
| Carbohydrates | 59.3 g | 58 g |
| Lactose | 44.8 g | 44.8 g |
| Maltodextrin | 14.5 g | 13.2 g |
| Taurine | 32 mg | 32 mg |
| L-carnitine | 17 mg | 17 mg |
| **Minerals** | | |
| Minerals | 2.5 g | 2.5 g |
| Sodium | 175 mg | 175 mg |
| Potassium | 535 mg | 535 mg |
| Chlorine | 290 mg | 290 mg |
| Calcium | 420 mg | 420 mg |
| Phosphorus | 230 mg | 230 mg |
| Iron | 6 mg | 6 mg |
| Magnesium | 42 mg | 42 mg |
| Zinc | 4.4 mg | 4.4 mg |
| Copper | 300 mcg | 300 mcg |
| Iodine | 70 mcg | 70 mcg |
| Manganese | 50 mcg | 50 mcg |
| Selenium | 10.7 mcg | 10.7 mcg |
| Ca/P ratio | 1.8 | 1.8 |
| **Vitamins** | | |
| Vitamin A | 500 mcg/1756 IU | 640 mcg/2133 IU |
| Vitamin D | 10.3 mcg/412 IU | 10.3 mcg/412 IU |
| Vitamin E | 12.2 mg | 25 mg |
| Vitamin K | 42 mcg | 42 mcg |
| Vitamin B1 | 520 mcg | 520 mcg |
| Vitamin B2 | 620 mcg | 620 mcg |
| Vitamin B6 | 825 mcg | 825 mcg |
| Vitamin B8 | 16 mcg | 16 mcg |
| Vitamin B12 | 2 mcg | 2.5 mcg |
| Vitamin 9 | 42 mcg | 42 mcg |
| Vitamin C | 50 mg | 60 mg |

| | | |
|---|---|---|
| Calcium pantothenate | 3.2 mg | 3.2 mg |
| Nicotinamide | 6 mg | 6 mg |
| **Nucleotides** | | |
| Cytidine 5´monophosphate | | 7.9 mg |
| Uridine 5´monophosphate | 5.3 mg | |
| Adenosine 5´monophosphate | 2.7 mg | |
| Guanosine 5´monophosphate | | 1.6 mg |
| Inosine 5´monophosphate | | 1.6 mg |

**Table 4:** Effects of tryptophan-enriched cereals intake in elderly people on 6-sulfatoxymelatonin (6-MTS) and 5 Hidroxiindolacetic acid (5-HIAA). Results are expressed as fold-increase over control levels (normalized and expressed as 1). Each value represents mean±SEM. Control: first week (22,5 mg Tryptophan was consumed in breakfast and dinner); Treatment: second week (60 mg Tryptophan was ingested in breakfast and dinner); Post-treatment: third week (habitual diet). *p<0.05 with respect to control values [2].

| | Control | Treatment | | Post-Treatment |
|---|---|---|---|---|
| | | 1st Treatment Day | 7th Treatment Day | |
| 6-MTS | 1 | 1.04±0.11 | 1.22±0.06* | 0.81±0.10 |
| 5-HIAA | 1 | 1.46±0.27 | 1.90±0.31* | 1.26±0.23 |

## Figures

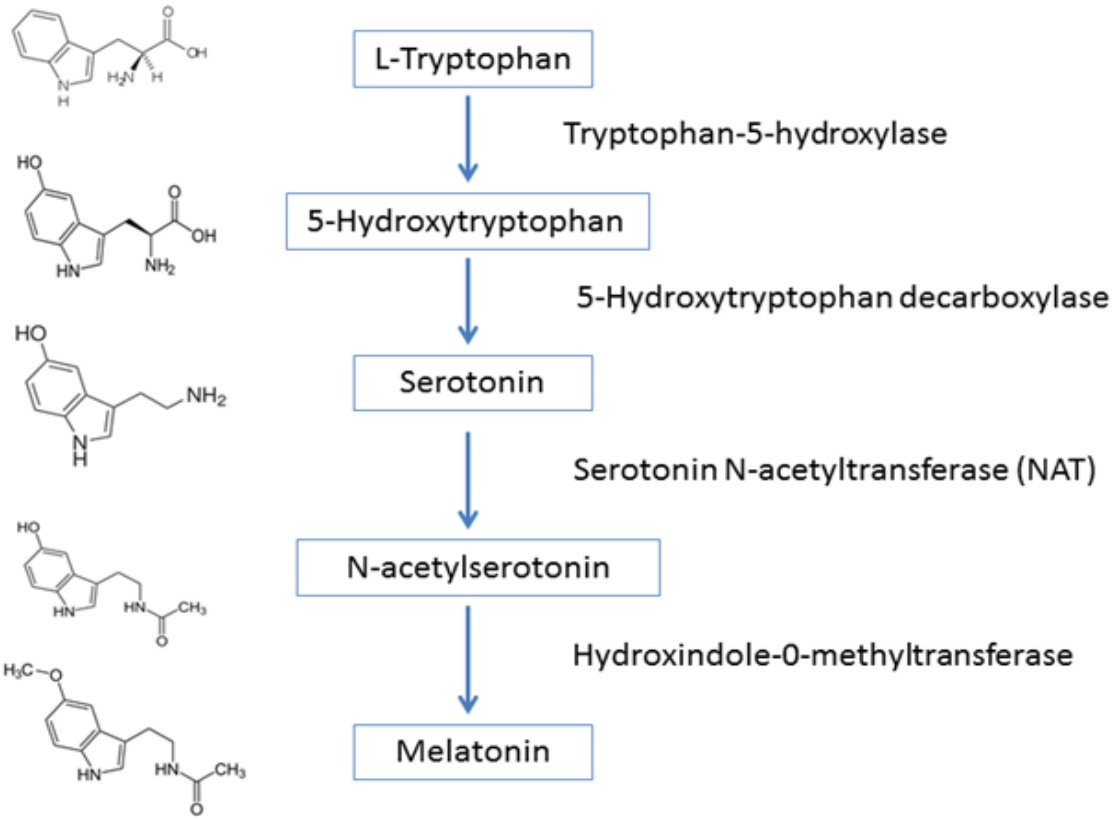


**Figure 1:** Tryptophan metabolism (Indolemines pathway).

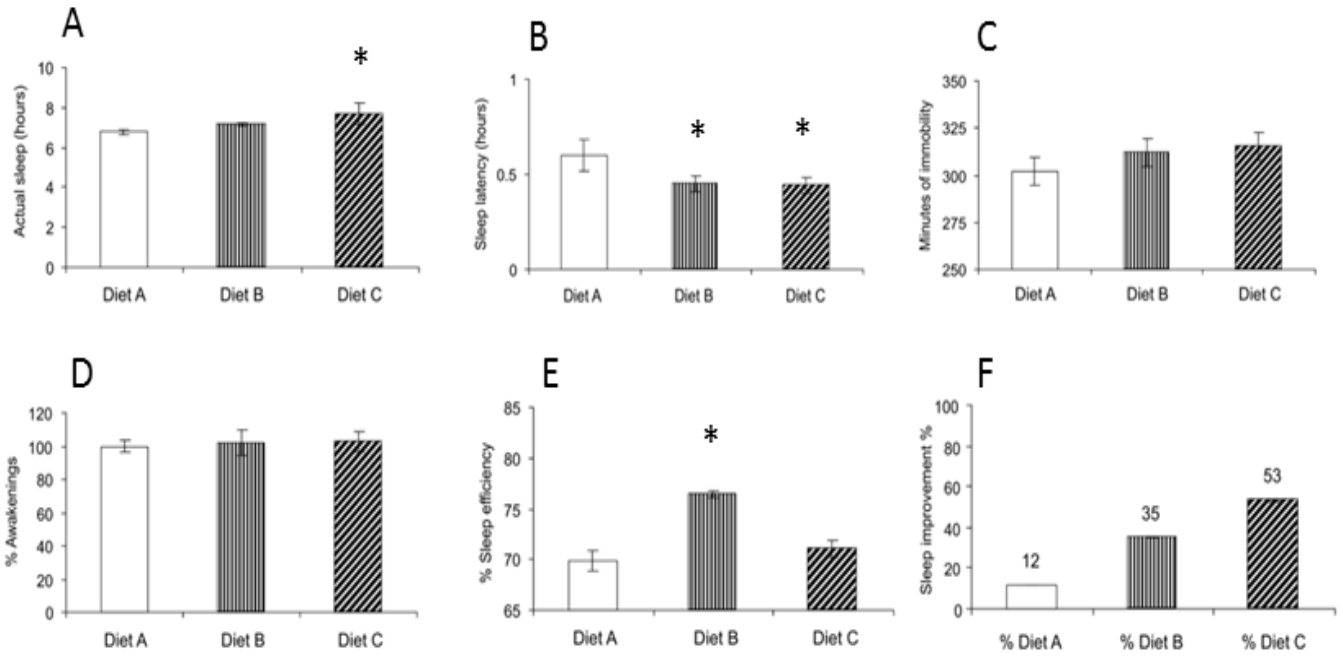


**Figure 2:** Different sleep parameters (Mean±SD) of infants 4-20 weeks of age, fed for 3 weeks with three different formula diets: Diet A (control): Normal Initation Milk; Diet B: of 06:00-18:00 Normal Initation Milk and 18:00-06:00 Night; Diet C: Day/Night formulas in the scheduled time previosuly. A: Actual sleep time; B: Sleep latency; C: Minutes of immobility; D: Percentage of nocturnal arousals; E: Sleep Effiiciency; F: Percentage of sleep improvement. *: statistically significant with respect to the week with the diet A (p<0,05) [17].

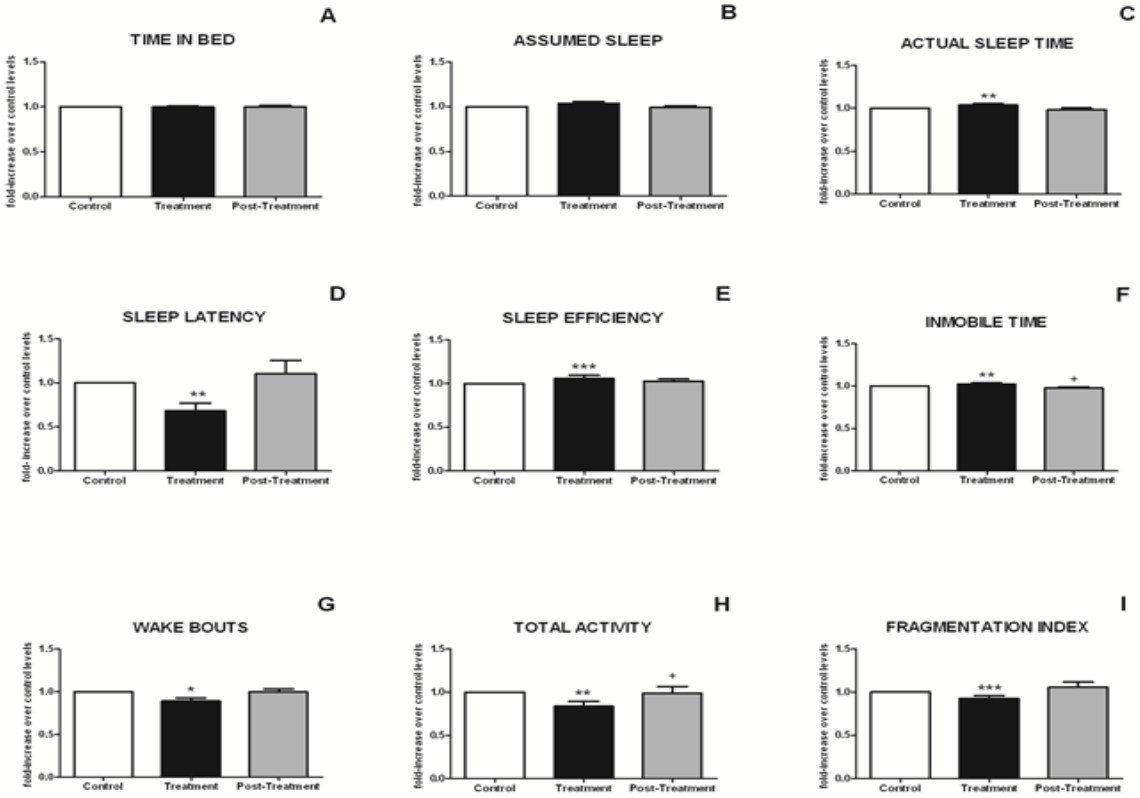


**Figure 3:** Effect of tryptophan-enriched cereal intake in elderly people on time in bed (a), assumed sleep (b), actual sleep time (c), sleep latency (d), sleep efficiency (e), immobile time (f), number of wake bouts (g), total activity score (h), and fragmentation index (i). Results are expressed as fold-increase over control levels (normalized and expressed as 1). Each value represents the mean ± SEM. Control: first week (22.5 mg of tryptophan was consumed in breakfast and dinner); treatment: second week (60 mg of tryptophan was ingested in breakfast and dinner); posttreatment week: habitual diet. *p<0.05 control vs. treatment; **p<0.01 control vs. treatment; ***p<0.001 control vs. treatment; +p<0.05 treatment vs. posttreatment; Kruskal–Wallis test and Dunn's multiple comparison test (posttest) were performed. N=35 [2]

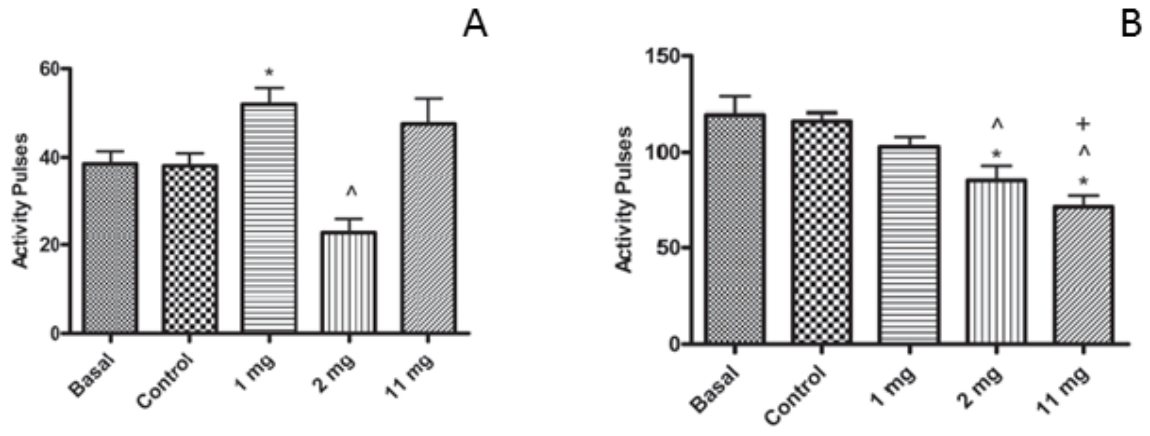


**Figure 4:** Nocturnal (A: 19:00–07:00 h) and diurnal(B: 07:00-19:00) activity pulses (mean ± SE) recorded in each treatment group (1 mg, 2 mg, 11 mg, respectively, to 3.80, 7.60, 41.8 mg/kg b. w.) during a week. * $p < 0.05$ with respect to the basal value; ^ $p < 0.05$ with respect to the control value; + $p < 0.05$ with respect to the 1 mg treatment (n = 5) [28]

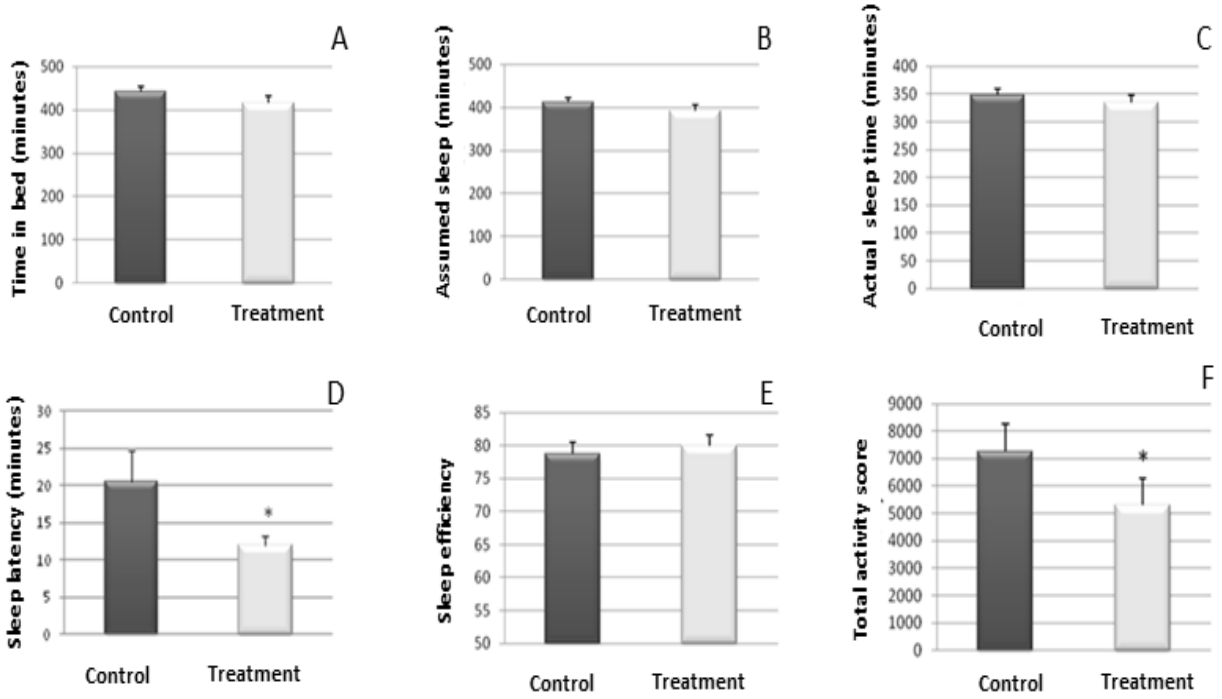


**Figure 5:** Sleep parameters during the night-time period of eache of the weeks, recorded for 17 work-stressed nurses (Mean±SE). A: Time (minutes) in bed; B: Assumed sleep time (minutes); C: Actual sleep time (minutes); D: Sleep onset time (minutes); E: Sleep efficiency (%); F: Total nocturnal activity (activity pulses). *p<0,05 [33]

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