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ROLE OF 10-DAY ORAL PROTEIN SUPPLEMENTATION ON SERUM SEROTONIN LEVELS AND SLEEP PARAMETERS IN YOUNG ADULTS

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ABSTRACT

Background: One of the serotonergic neurobiological mechanisms underlying frequently disrupted and restricted sleep is the disruption of the brain's serotonergic neurotransmission caused by reduced serotonin levels and desensitisation of the serotonergic receptors. This study evaluates the effect of oral protein supplementation on serum serotonin levels and sleep parameters among healthcare students.

Materials and Methods: Male and female health care students (n=100) aged 18 to 25 years were enrolled after obtaining informed written consent. The study documents were approved by the institutional ethics committee. The participants were free of any pre-morbidities and not receiving any other medications. The participants received 30 gm of protein powder per day orally for 10 days. Study assessments included serum serotonin and sleep quality, assessed using the Pittsburgh Sleep Quality Index (PSQI) at baseline and after 10 days.

Results: Improvements were seen with protein supplementation for various parameters on the PSQI (time to bed, difficulty in sleeping, time of sleep, sleep planning, thoughts keep awake, difficulty in staying asleep, waking up at night, falling back to sleep, waking up time, and needing an alarm to wake up). Serum serotonin levels increased with 10-day oral protein supplementation ($P < 0.05$).

Conclusion: This study highlights the role of protein supplementation in sleep quality.

Keywords: Protein, Serotonin, sleep, Tryptophan.

INTRODUCTION

In 1948, Maurice M Rapport and team discovered a substance having vasoconstrictor activity in serum, which they named serotonin.[1] Serotonin, or 5-hydroxy-tryptophan (5-HT), has been associated with motor system functions, sleep-wake cycles,[2,3] circadian rhythms, respiratory stability,[4,5] embryonic development, and reward processing.[6] 5-HT neurons in the nuclei originally classified by Dalhstrom and Fuxe project to a wide variety of brain regions, and a bewildering array of 5-HT receptors has been identified. Serotonin is synthesized in serotonergic terminals from tryptophan, which competes with tyrosine and branched-chain amino acids for transport across the blood-brain barrier.[7] Autoreceptors play a significant role in regulating 5-HT in the brain and continuously regulate receptor expression. Serotonin acts both in one-to-one neural signalling and as a neuromodulator, via volume transmission, of the effects of other neurotransmitters.[8] Each of these facts plays a key role in neuropharmacology and neuropsychiatry.

Serotonin is associated with sleep initiation and maintenance, and the complex effects of 5-HT on sleep regulation arise from its actions across different brain regions, as evidenced by recordings of raphe neurons in naturally sleeping animals. Studies of serotonin and sleep have recorded biphasic responses and arousal followed by synchrony following serotonergic manipulations.[9]

Serotonin promotes the waking state and inhibits REM sleep. The parasympathetic neurons in the pons are regulated by the brainstem serotonergic and

noradrenergic neurons, and they trigger REM sleep by activating the glutamatergic sublaterodorsal nucleus.[10,11] The glutamatergic pathway activates glycinergic and GABAergic neurons, inhibiting motor neurons, brainstem serotonergic and noradrenergic neurons.[11] Thus, the physiological reduction in serotonin during REM sleep reinforces REM atonia by attenuating motor neuron activation,[12] whereas an abnormal increase in serotonergic tone induces REM sleep without atonia. Serotonin is involved in physiological processes regulating sleep and wakefulness, depending on its localisation in the brain, receptor type, and the individual's current state.[13] Serotonin also has a variety of functions, such as thermoregulation, mood, memory, appetite, serving as a precursor for melatonin, and regulating the body clock.[14] Within the sleep cycle, serotonin promotes wakefulness and also has a role in NREM deep sleep.[15] The body produces serotonin in the gut endocrine cells and in the brain via raphe neurons. Melatonin, the sleep hormone that helps initiate sleep, is secreted by the pineal gland via the methylation of serotonin in the brain.[16,14] The highest secretion occurs in the middle of the night and decreases towards morning. Melatonin stimulates the urge to sleep and also resets the circadian rhythm.

Effects of the 5-HT2A and 5-HT2C serotonin receptor subtypes contribute to sedation. At low concentration, 5-HT blocks the histamine receptor due to a higher receptor affinity. The build-up of serotonin in certain parts of the brain during periods of wakefulness contributes to the onset of sleep.[17,18] People with low serotonin levels tend to spend less time in restorative non-REM sleep over the course of the night.[18,19] That is why problems such as depression and anxiety often lead to sleep disorders. Sleep is triggered by two interrelated processes: the cyclical production and buildup of hypnogenic substances in the body.[20] Melatonin plays a fundamental role in chronobiology, regulating the biological clock and governing the entire sleep-wake cycle, whereas serotonin is involved more specifically in wakefulness, sleep triggering, and REM sleep.[21,22] Sedation is an expected effect of the serotonergic agent. It is usually most noticeable in the first few weeks of therapy and develops 7-10 days after beginning treatment. Increased duration of sleep at low plasma concentration and improved sleep quality at increased concentration. Studies show that serotonin consistently improves sleep efficiency, total sleep time, and sleep quality, with specific improvements in sleep among participants. In this study, we assessed the serotonin level and sleep quality after oral protein supplementation.

MATERIALS AND METHODS

Study Design and Participants: The present cross-sectional study included 100 first-year medicine students (51 male and 49 female), aged 18–25 years, from D Y Patil Deemed to be University School of Medicine, Nerul, Navi Mumbai, Maharashtra, India. The study protocol was reviewed and approved by the Institutional Ethics Committee (DYP/IEC/2018). Participants were informed about the study protocol, and written informed consent was obtained before any study-related procedures.

Participants with acute or chronic illness, a history of smoking or alcoholism, diminished hearing or vision, colour blindness, psychiatric disorders affecting psychomotor abilities, or current medication use were excluded. Individuals unwilling to provide written informed consent were also excluded.

Protein Supplementation: Each participant received 30 g of protein powder (Protein Hydrolysate 20%, Intas Pharmaceuticals Ltd., India) orally once daily with water between 10:00 AM and 1:00 PM for 12 days.

Assessment of Sleep Parameters: Anthropometric parameters were recorded on the first day of the study. Sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI). The PSQI was administered at baseline (day 1) and after supplementation (day 11).

Serum Serotonin Estimation: Venous blood samples were collected in plain activator tubes on day 1, before protein supplementation, and on day 11. Serum serotonin levels were estimated using an EIA (ELISA immunoassay) method within 12 hours of sample collection.

Statistical Analysis: Statistical analysis was performed using MedCalc Statistical Software version 19.0.1 (MedCalc Software bvba, Ostend, Belgium). Changes in mean serum serotonin levels between day 1 and day 11 were assessed using a paired t-test. One-way analysis of variance (ANOVA) was used to compare serotonin levels and changes in serotonin levels across gender subgroups. Changes in PSQI parameters between baseline and day 11 were assessed using the chi-square test. All analyses were two-sided, with an alpha level of 0.05 corresponding to a 95% confidence level.

RESULTS

The demographic profile of participants is presented in [Table 1]. The males and females were equally distributed. [Table 2] presents the blood serotonin levels on day 1, day 11 and the change (mean change and per cent change) in serotonin levels on day 11 in males and females. No statistically significant differences were observed in the serotonin levels among males and females in terms of serotonin levels on day 1 and day 11 (post protein supplementation). After protein supplementation for 10 days (day 11), there is a significant increase in the blood serotonin levels in males and females ($p<0.0001$).

Responses to PSQI items by the responders on day 1 and day 11 are presented in [Table 3]. Significant changes were observed on day 11 from day 1 for the items ‘thoughts’ ($p<0.0001$), ‘thoughts keep awake’

(p=0.018), and 'difficult to stay asleep' (p=0.022). No changes were observed in other PSQI items.

Table 1: Profile of participants

	No.	%	
Total	100		
Male	51	51.0%	
Female	49	49.0%	
Male / Female	5.1/4.9		
			Male Vs Female
Age (yrs.)	**Mean**	**SD**	**p (t-test)**
Male	18.53	0.86	0.391
Female	18.41	0.50	
Total	18.47	0.70	

Table 2: Mean Serotonin in males and females

	Serotonin levels		Change in serotonin levels from Pre		Pre Vs Post
	Pre	**Post**	**Mean change**	**% Change**	**p (t-test)**
Male (n=51)	102.53 (29.57)	109.02 (31.65)	6.49 (8.85)	6.57 (7.83)	<0.0001
Female (n=49)	112.95 (35.18)	119.86 (37.06)	6.91 (5.37)	6.36 (6.47)	<0.0001
Total (n=100)	107.64 (32.70)	114.33 (34.66)	6.70 (7.32)	6.47 (6.47)	<0.0001
Males Vs Females					-
p (t-test)	0.112	0.119	0.777	0.872	

Table 3: Sleep parameters (PSQI) observation and statistics

Sleep Parameters	**Day 1 (N=100) (Pre-supplementation)**		**Day 11(N=100) (Post-supplementation)**		**Chi-square test**
	No.	**%**	**No.**	**%**	**p**
Bedtime					
Regular	5	5%	4	4%	0.739
Late	95	95%	96	96%	
Difficulty in sleep					
Yes	27	27%	23	23%	0.493
No	73	73%	77	77%	
Time to sleep					
0 minute	73	73%	77	77%	0.072
< 30 minutes	9	9%	16	16%	
>30 minutes	18	18%	7	7%	
Planning					
Yes	73	73%	65	65%	0.206
No	27	27%	35	35%	
Thoughts					
Yes	58	58%	29	29%	<0.0001
No	42	42%	71	71%	
Thoughts keep awake					
Yes	39	39%	23	23%	0.018
No	61	61%	77	77%	
Difficult to stay asleep					
Yes	8	8%	19	19%	0.022
No	92	92%	81	81%	
Wake up at night					
No night waking	92	92%	81	81%	0.127
1-2 Times	4	4%	15	15%	
>2 Times	4	4%	4	4%	
Fall back to sleep					
None	50	50%	57	57%	0.434
< 15 minutes	38	38%	32	32%	
>15 minutes	12	12%	11	11%	
Before 7am	43	43%	36	36%	0.317
After 7 am	57	57%	64	64%	
Need alarm					
Yes	80	80%	82	82%	0.705
No	20	20%	18	18%	

DISCUSSION

Numerous studies show that raphe nucleus activity and serotonin is responsible for waking from sleep. The activity of serotonergic neurons is strongly related to spontaneous changes in behaviour, and is highest during inactive waking and lowest during REM sleep.[9] Protein supplementation increases serotonin levels and is linked to wakefulness.[10] L-tryptophan is the primary source of serotonin in the CNS, whereas in peripheral tissues, dietary

tryptophan is the primary source of serotonin. The normal plasma serotonin levels in healthy adults are between 70 and 270 ng/ml.[23,24] L-tryptophan is converted to 5-hydroxytryptophan (5-HTP) by the addition of a hydroxyl group with the help of the enzyme tryptophan hydroxylase.[1] The rate-limiting step in the synthesis of serotonin is the conversion of L-tryptophan to 5-HTP, and the 5-HTP is finally converted to serotonin through decarboxylation by aromatic amino acid decarboxylase.[1]

Protein supplementation in a diet containing tryptophan causes increased 5-HT biosynthesis in the brain and peripheral tissues, and a deficiency of dietary tryptophan can cause a substantial decline in serotonin synthesis and turnover.[25,26] A daily dose of 6 g of protein is sufficient to saturate tryptophan hydroxylase and double the rate of serotonin synthesis.[27,28] Central serotonin or its precursor 5-HT causes hypophagia and increases metabolic rate.

WHO recommends a dietary tryptophan intake of 0.36g⁄kg/day for adults.[29] An average Indian daily diet contains about 0.8g/kg/day of tryptophan, of which only 1–2% is utilised for serotonin synthesis in the CNS.[30-32] The entry of tryptophan to the brain depends on two major factors: i) the proportion of free to bound tryptophan in the plasma, and ii) the concentration of other large neutral amino acids in the plasma. Serotonin is an important precursor of melatonin which is synthesized by methylation of serotonin in the pineal gland. Thus, protein supplementation could also affect Melatonin secretion. Melatonin is a known regulator of chronobiology and the sleep cycle.[33]

Serotonergic neurons play an important role in inhibiting sleep, and the complex effects of 5-HT on sleep regulation are partly due to its actions in different brain regions.[34] We observed significant changes in the PSQI items like ‘thoughts’, ‘thoughts keep awake’, and ‘difficult to stay asleep’ with protein supplementation for 10 days. Serotonin in the body modulates various sleep parameters to promote wakefulness and inhibit rapid eye movement sleep. Manipulation of serotonin level causes serotonergic activation through selective serotonergic innervation of the cerebral cortex, amygdala, basal ganglion, forebrains, thalamus, preoptic and hypothalamic areas, raphe nuclei, locus coeruleus and pontine reticular formation comes from the dorsal raphe nucleus make changes in sleeping behavior and various parameters of sleep like bed time, difficulty in sleep, time of sleep, sleep planning, thoughts keep awake, difficult to stay asleep, wakeup at night, fall back to sleep, wakeup time, need alarm.[35]

Sleep structure is altered with age; hence, the geriatric population is susceptible to various external and internal factors that lead to sleep disturbances. Sleep disruption can alter various functions, ranging from thermoregulation to learning and memory, in the awake state.[9,20] Although sleep, which appears to be a body and mind at rest, is in fact a dynamic and complex physiologic state that is necessary for survival.[21] Determinants of sleep regulation include homeostatic and circadian processes. Despite being highly regulated, sleep is fragile, its stages and duration affected by multiple factors, such as age, drugs, temperature, and medical and psychiatric disease. Variations in nighttime sleep impact subsequent sleep periods as well as daytime function.[22,35]

Our study has certain limitations, such as the fact that it involves a small number of young, healthy adults; hence, the results cannot be extrapolated to the population level. Also, the study duration was only 10 days; the effects of long-term administration and of bolus administration over the long term could not be ascertained. The advantages of this study being a well-defined study cohort in a controlled environment.

CONCLUSION

The present study revealed the importance of appropriate daily protein intake, the relationship between protein administration and serum serotonin, and the role of tryptophan in sleep and sleep behaviour in healthy subjects.

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