

## The effect of pyridoxine administration on melatonin secretion in normal men

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### Abstract

**OBJECTIVES:** To determine pineal response to pyridoxine in normal men.

**MATERIAL AND METHODS:** Twelve healthy men were given orally pyridoxine (100 mg) or placebo at 1700h. Serum melatonin levels were determined every 30 minutes with simultaneous measurement of core body temperature between 1700h to 0300h. Polysomnographic sleep recordings were performed between 1800h to 2000h.

**RESULTS:** Serum melatonin levels after both placebo and pyridoxine showed a nocturnal rise occurring at 22:10±1:22h and 22:24±1:09h, respectively. The melatonin onset, peak, mean and area under the curve (AUC) values after pyridoxine (3.2±1.6 pg/ml, 47.2±22.6 pg/ml, 31.5±11.0 pg/ml and 173.5±138.4 pg/ml x min, respectively) were similar to the values after placebo administration (4.7±1.6 pg/ml, 53.9±26.0 pg/ml, 37.2±2.8 pg/ml and 205.3±137.8 pg/ml x min, respectively). CBT revealed a significant nocturnal decline but without significant difference between pyridoxine and placebo. Sleep amount and architecture were similar after the two treatments.

**CONCLUSIONS:** In adult man, the oral administration of 100 mg-pyridoxine during the evening hours has no effect on melatonin secretion nor does it alter CBT or sleep quality.

## Introduction

Melatonin, the main hormone produced by the pineal gland, displays a circadian rhythm peaking at night [1]. Pinealocytes use tryptophan as substrate for melatonin synthesis, and melatonin levels change as a function of tryptophan availability [2]. Pyridoxine is converted to its active coenzyme form, pyridoxal phosphate (PLP). More than 60 PLP-dependent enzymes are known, including enzymes that participate in decarboxylation reactions such as the decarboxylation of DOPA to dopamine and 5-hydroxytryptophan to serotonin [3–4]. The activity of pyridoxine as a coenzyme in the tryptophan metabolism was described in the kinurenine and methoxyindole pathways [5]. Pyridoxine acts as a coenzyme of 5-hydroxytryptophan decarboxylase. The enzyme carboxylates 5-hydroxytryptophan to serotonin, the immediate precursor of melatonin [5]. The effect of pyridoxine on aromatic amino acid decarboxylase activity supports a regulatory role of pyridoxine on the synthesis of neurotransmitters [6–7]. Melatonin was shown to increase brain pyridoxal phosphokinase activity, inhibition of glutaminergic neurotransmission, resulting in inhibitory effects on central nervous system activity [8]. The participation of endogenous melatonin in the normal sleep-wake cycle regulation has been inferred from the temporal relationships between melatonin cycle and the 24-hour cycle in sleep propensity, and particularly between the nocturnal melatonin onset and the nocturnal sleep gate [9–11]. The typical 24-hour sleep propensity pattern reveals a midafternoon sleepiness peak followed by a forbidden zone for sleep, which is characterized by very low sleep propensity in the early evening hours and then followed by the nocturnal sleep gate. This term refers to a steep rise in sleepiness occurring in the late evening hours [12]. Exogenous melatonin given prior to an early evening nap, during the forbidden zone for sleep at 1800–2000h, significantly shortened sleep latency and increased total sleep time. These data suggested that timed administered melatonin can modify sleep propensity [13].

Exogenous melatonin administration has been shown to lower core body temperature (CBT) by 0.2–0.4°C [14–17]. Acute exposure to bright light at night elevated the nocturnal CBT and inhibits melatonin secretion [18]. This change in CBT was reversed by a constant infusion of melatonin [19]. The time taken to reach the maximum drop in CBT following melatonin administration was about 3 hours [17].

We hypothesized that pyridoxine may participate in the nocturnal melatonin secretion and therefore can modify other circadian rhythms as sleep and temperature when administered in the late afternoon hours. To examine this hypothesis, we determined serum melatonin levels, CBT and sleep quality in healthy young adult men given a single oral dose of pyridoxine or placebo in the evening hours.

## Material and Methods

### *Subjects and protocol*

The study was approved by the institutional review board (Helsinki committee) and all participants gave their informed consent before the start of the study. Twelve healthy males (aged: 22–26 years) participated in the study. Participants were receiving no medications and during the study they were instructed to refrain from smoking, coffee and alcohol, and to have 7–8 hours of sleep per night, one week prior to the study. The study comprised of two sessions, two weeks apart.

On each experimental night, an IV catheter was inserted in an antecubital vein, kept patent by a slow infusion of 0.9% NaCl. Blood samples (2ml) were collected every 30 minutes from 1700h to 0300h for the determination of serum melatonin levels. Rectal thermistor was inserted to record core body temperature (CBT) between 1700h to 0300h. Subjects were awake between 1700–1800h and between 2000–0300h, with lights on (50 Lux at eye level) and assumed the upright posture. Between 1800–2000h subjects were lying in bed attempting to fall asleep. Conventional sleep recordings were obtained to verify sleep quality.

### *Melatonin measurements*

Blood was centrifuged, immediately separated and stored at –20°C until assayed. Serum melatonin levels were determined by radioimmunoassay (Buhlman Lab., Albschwill, Switzerland). The assay sensitivity was 0.3 pg/ml. The intra-assay coefficients of variation (CV) were 4.9% and 5.8% for low (0.9–2.6 pg/ml), and high (9.0–23.0 pg/ml), respectively. The interassay CVs were 7.8% and 6.7%, respectively.

### *Core body temperature*

CBT was monitored by rectal thermometer at one-minute intervals between 1700–0300h, using the Minimeter series 2000 YSI, Yellow-Spring, USA.

### *Analysis of sleep stages*

Electrodes were attached for the following electrophysiological recordings: two electroencephalograms (EEG levels C3-A2, C4-A1), two electrooculograms and one electromyogram of the mentalis. Sleep stages were scored in 30 seconds epochs according to conventional criteria [20]. The following parameters were determined: total recording time (TRT), sleep latency (time from lights off until 3 consecutive minutes of stage 2), actual sleep time (AST=TRT– sleep latency+waking periods), rapid eye movement (REM) latency (time from beginning of sleep to the first REM episode), sleep efficiency (AST/TRT) and percentages of sleep stage 2,3/4, and REM.

### *Medications*

Subjects were given an oral dose of 100 mg- pyridoxine (vitamin B6, Pyridoxine HCL, Tarima, Maabarot, Israel) or a look-alike placebo (starch) at 1700h, in a double blind Latin square design.

**Table 1.** Nocturnal serum melatonin levels after an oral dose of pyridoxine (100 mg) or placebo given at 1700h in normal men (Data are mean ± SD)

Melatonin data	Pyridoxine	Placebo	P value
Onset time (clock h)	22:24±1:09	22:10±1:22	NS
Onset level (pg/ml)	3.2±1.6	4.7±1.6	0.09
Peak level (pg/ml)	47.2±22.6	53.9±26.0	NS
Mean nocturnal rise (onset to 0300h (pg/ml)	31.5±11.0	37.2±12.8	NS
AUC (onset to 0300h) (pg/ml x h)	173.5±138.4	205.3±137.8	0.15

NS: Not significant; AUC: Area under the curve

**Table 2.** Sleep recording data after pyridoxine or placebo administration in normal men (Data are mean ± SD)

Sleep parameters	Pyridoxine	Placebo	P value
Total recording time (h:min)	1:50±0:03	1:44±0:07	0.08
Actual sleep time (h: min)	1:23±0:11	1:19±0:16	NS
Sleep efficiency (%)	69.4±9.7	66.0±12.5	NS
Sleep latency (minutes)	10±3	15±8	0.09
REM latency (minutes)	37±30	49±8	NS
Stage 1 (%)	4.5±1.7	3.5±2.9	NS
Stage 2 (%)	53.6±9.4	46.3±7.5	NS
Stage 3/4 (%)	8.9±7.7	16.7±14.4	NS
REM (%)	12.4±7.7	9.3±10.7	NS

NS: not significant

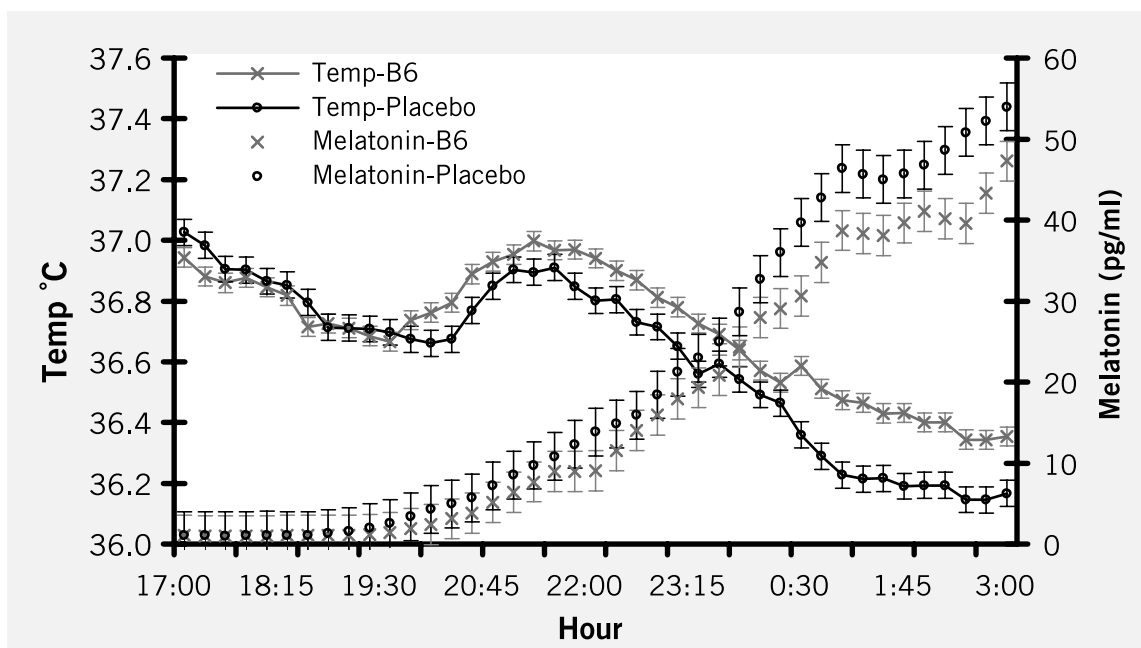
**Statistical analysis**

The onset of the nocturnal melatonin rise was defined as the time at which the first of three consecutive samples exceeded the mean levels of the day-time values (1700–2000h) by more than 1 SD. The integrated melatonin values were determined as the area under the curve (AUC) from the time of melatonin onset to 0300h. Independent *t* test were used to test the differences in serum melatonin levels, CBT and polysomnographic data between pyridoxine and placebo treatments. Data are expressed as mean ± SD.

**Results**

In this study, we determined serum melatonin levels, core body temperature and sleep quality in 12 men, aged 23.4±2.6 years, after a single oral dose of 100 mg pyridoxine or placebo given at 17:00h. Serum melatonin onset levels after pyridoxine (3.2 ±1.6 pg/ml) were lower than the values after placebo (4.7±1.6 pg/ml),

occurring at 22:24±1:09h and 22:10±1:22h, respectively (Table 1). Likewise, peak levels after pyridoxine (47.2±22.6 pg/ml) were lower than the values after placebo (53.9±26.0 pg/ml). The integrated amount of melatonin secreted (area under the curve between 1700–0300h) after pyridoxine (173.5±138.4 pg/ml x h) was lower than the value after placebo (205.3±137.8 pg/ml x h). The melatonin values after pyridoxine were statistically not significantly different from the values after placebo. Core body temperature (CBT) curves after both treatments were statistically not significantly different. The peak CBT values after pyridoxine and placebo were 37°C±0.27 and 36.9±0.26, respectively; occurring at 02:30h ±0:45 and 02:30h ±0:50, respectively (Figure 1). Quality of sleep was assessed by actual sleep time and by sleep efficiency. Sleep efficiency was similar after both treatments and was relatively low (66–69%). This is explained by the fact that subjects had disturbed sleep, probably due to frequent blood sampling not performed from a separate room (Table 2).



**Figure 1.** Serum melatonin levels and core body temperature values after pyridoxine and placebo administration in normal men.

## Discussion

In the present study we determined the effect of a single oral dose of 100 mg pyridoxine given in the afternoon on serum melatonin levels, core body temperature and sleep pattern in normal adult men. We used a calculated dose related to body weight (1.5 mg/kg body weight), which is much higher than the daily basal requirement of pyridoxine, taking in account the intestinal absorption and liver metabolism [21]. The three circadian rhythms examined revealed similar results after pyridoxine and placebo. Animal studies revealed that in pyridoxine deficient rats, melatonin synthesis was reduced and the addition of pyridoxine restored the levels of pineal melatonin to values observed in control animals' [22]. Also, the administration of melatonin increased brain pyridoxal phosphokinase activity [23]. A mild hypothermia was observed in female rats during pyridoxine treatment. The hypothermic effect appeared by day 3 of treatment. The reduction in core body temperature was greater early in the day [24]. It is not clear whether the hypothermic effect of pyridoxine was mediated through the release of melatonin, as melatonin levels were not determined in this study. Positive immunohistochemical staining for pyridoxine-5-phosphate oxidase was demonstrated in mammalian hypothalamic paraventricular nucleus [25]. Also, positive staining to brain pyridoxal kinase was demonstrated in human brain [26].

The effect of pyridoxine on dreaming was investigated in adult men treated with 100 mg pyridoxine or placebo prior to bedtime for 4 consecutive days. The data suggested that pyridoxine may act by increasing cortical arousal during periods of rapid eye movement (REM) sleep. The authors suggested that this action of pyridoxine might result from the conversion of tryptophan to serotonin [27].

Pharmacological doses of pyridoxine significantly decreased pituitary hormone levels [3]. Others failed to observe any effect of acute or chronic pyridoxine treatment on anterior pituitary hormones in amenorrheic women [28]. In young children and infants single IV dose of pyridoxine given at 21:00h, but not when given at 09:00h, significantly increased serum melatonin levels three hours later. In this study, only two blood samples were obtained from each subject for the determination of melatonin levels [29]. These findings are in contrast with our results and may be due to differences in the populations studied, the route and dose of pyridoxine administered and frequency of blood sampling.

Rat brain serotonin levels were increased after tryptophan load. Coadministration of pyridoxine, significantly increased serotonin levels in the hypothalamus when tryptophan intake was in excess, but not with a diet of basal tryptophan requirement [30]. Oral L-5-hydroxytryptophan administered to normal children in the late evening hours significantly increased serum melatonin levels [29].

In our study, oral administration of pyridoxine may be associated with retarded absorption and hepatic metabolism of pyridoxine, which may alter the available dose of the vitamin in the circulation [31]. Considering that our subjects were in good health and probably were not consuming tryptophan or pyridoxine in excess, which may account for the lack of effect of pyridoxine on melatonin levels in the current study.

Our data suggest that acute oral administration of 100 mg pyridoxine given in the afternoon to normal adult men has no immediate effect on serum melatonin levels, core body temperature or sleep quality.

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