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Influences of Twilight on Diurnal Variation of Core Temperature, Its Nadir, and Urinary 6-Hydroxymelatonin Sulfate during Nocturnal Sleep and Morning Drowsiness

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A B S T R A C T

This study aimed at elucidating the physiological significance of dusk and dawn in the circadian rhythm of core temperature (T_{core}) and urinary 6-hydroxymelatonin sulfate in humans during sleep and the waking sensation just after rising. Seven female and four male students served as participants. Participants retired at 2300 h and rose at 0700 h. They were requested to sit on a chair and spend time as quietly as possible during wakefulness, reading a book or listening to recorded light music. Two lighting conditions were provided for each participant: 1) Light-Dark (LD)-rectangular light change with abrupt decrease from 3,000 lx to100 lx at 1800 h, abrupt increase from 0 lx to 3,000 lx at 0700 h. 2) LD-twilight light change with gradual decrease from 3,000 lx to 100 lx starting at 1700 h (twilight period about 2 h), with gradual increase from 0 lx to 3,000 lx starting at 0500 h (twilight period about 2 h). The periods of 0 lx at night were from 2300 h to 0700 h on the first day and from 2300 to 0500 h on the second day. Nadir time advanced significantly under the influence of the LD-twilight condition. The amount of 6-hydroxymelatonin sulfate in urine collected at 0200 h was significantly higher under LD-twilight in comparison with LD-rectangular light. Morning drowsiness tended to be lower under LD-twilight. Our results suggest that in architectural design of indoor illumination it is important to provide LD-twilight in the evening and early morning for sleep promotion in healthy normal people and/or light treatment in elderly patients with advanced dementia.

***Key words**: circadian rhythm, core temperature, 6-hydroxymelatonin sulfate, dusk, dawn*

Introduction

In modern society people are awake until late evening, resulting in exposure to artificial bright light. Exposure to bright light in the evening may worsen digestion of carbohydrates in the evening meal[1] and reduce some immunological parameters (white blood cell count, interferon-γ, interleukin-4, CD69 and transforming growth factor-β1)[2]. Modern technology of artificial illuminance has deprived people of experiencing natural twilight. The influences of evening exposure to bright light without artificial twilight on health maintenance must be studied more systematically in terms of physiological anthropology.

It is well known that bright light exposure causes phase responses in circadian rhythm of core temperature in humans[3,4]. It is also well known that the extremities play an important role in heat-loss mechanisms[5] and in the rapid onset of sleep[6]. Higher skin temperatures of the extremities could accelerate the fall of core temperature in the late evening, and this is important for inducing deeper sleep. Recent studies indicate that manipulation

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of skin temperature not only promotes sleep[7–10] but also improves vigilance[11].

Light has been shown to influence melatonin secretion[12–14]. However, the studies were mostly done under a constant light condition. Natural light changes gradually. It remains to be elucidated more systematically how a living system reacts physiologically under such gradually changing illumination in comparison to constant illumination.

According to Boulos et al.[15], locomotor activity in hamsters may have a wider range of entrainment under the influence of simulated twilights (Light-Dark (LD) -twilight) in comparison to abrupt transitions between light and darkness (LD-rectangular), suggesting that twilight transitions may increase the strength of the LD Zeitegeber. It was reported that the circadian phase of melatonin secretion advanced under the influence of dawn simulation in humans[16–18].

Thus, twilight seems to influence circadian physiology. However, the influence of twilight on core and skin temperatures and their circadian phase, melatonin secretion and waking sensation just after rising remains to be studied in humans. Therefore, the purpose of the present experiment was to clarify whether twilight or rectangular light changes could influence these parameters mentioned above during and just after nocturnal sleep.

Participants and Methods

Participants

Seven female and four male students served as participants. They were all physically and psychologically healthy. Females served as participants during follicular phases. Their physical characteristics were as follows: age, 21.3 ± 0.5 yrs (mean ± SEM), (range 18–25 yrs); stature, 1.64 ± 0.03 m (range 1.50–1.85 m); body mass, 55.7 ± 2.4 kg (range 45–70 kg); body mass index calculated by weight/height^2, 20.71 ± 0.59 kg/m^2 (range 17.99–26.03 kg/m^2); and body surface area, 1.56 ± 0.05 m^2 (range 1.33–1.87 m^2) [calculated with weight in kg and height in cm, as weight$^{0.444}$ × height$^{0.663}$ × 88.83 cm/kg]. Short-sleeved shirts with knee-length trousers were loosely worn by males and short-sleeved shirts with sleeveless one-piece suits by females. Clothing material was 100% cotton. These clothes had nearly 0.3 clo. At night, the same clothing was worn with the addition of a bath towel. Clothing which did not exert pressure on the skin was worn during the experiments. Participants did not have any sleep disorders for at least one month prior to the start of the experiment. Experimental design was approved by the Ethics Committee at the Sekisui House Heart-ful Living R&D Institute. The purpose and risk of the experiment were fully explained to all participants. All of them gave their written consent as participants. They could leave the experiment at any time whenever they wanted. Some reward was paid to them by Sekisui House Heart-ful Living R&D Institute for their attendance.

Experimental Design

Figure 1 depicts the experimental design. Participants entered a bioclimatic chamber (size: 5.1 m in length by 3.8 m in width, controlled at 27 °C and a relative humidity of 60%) at 1000 h on the 1st day. Participants retired at 2300 h and rose at 0700 h on the next day. They were requested to sit on a chair and spend time as quietly as possible during wakefulness, reading a book or listening to recorded light music. Isocaloric meals were provided at 0800, 1200 and 1800 h, and a light snack was served at 1500 h. Total daily caloric intake of food was adjusted to 2300 kcal in males and 1800 kcal in females.

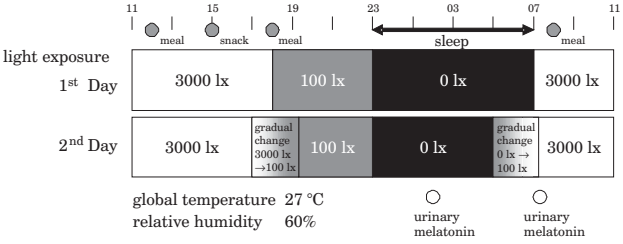

Fig. 1. Experimental protocol. First day: light-dark cycle with rectangular light to dark change and vice versa. Second day: light-dark cycle with twilight light to dark change and vice versa. Global temperature: 27 °C, relative humidity: 60%. Closed circles: meal times. Open circles: times for the collection of urine for the measurement of 6-hydroxymelatonin sulfate level.

Two lighting conditions were provided for each participant: 1) LD-rectangular light change with abrupt decrease from 3,000 lx to100 lx at 1800 h, and abrupt increase from 0 lx to 3,000 lx at 0700 h. 2) LD-twilight light change with gradual decrease from 3,000 lx to 100 lx starting at 1700 h (twilight period about 2 h), and gradual increase from 0 lx to 3,000 lx starting at 0500 h (about 2 h) (Figure 1). We set the time of dawn at 0700 h instead of 0600 h in the middle of twilight (0500–0700 h) because light less than 2,000 lx is ineffective in regulating neuroendocrine hypothalamic functions in participants with closed eyelids[19]. The periods of 0 lx at night were from 2300 h to 0700 h on the first day and from 2300 to 0500 h on the second day. Light intensity was controlled at eye level. To ensure compliance with the scheduled sleep episodes, participants wore an actigraph (Actiwatch-16, Mini-Mitter Company Inc., Bend, OR, USA) on their wrist. Participants emptied their bladder on retiring at 2300 h, at 0200 h and again on waking at 0700 h. We collected a urinary sample from each participant at 0200 h and 0700 h. The 6-hydroxymelatonin sulfate content of these samples was analyzed at SRL Laboratory, Inc. (Tokyo, Japan). The experiment was carried out at the Sekisui House Heart-ful Living R&D Institute, Kyoto, Japan from April 27th, 2005 to June 5th, 2005.

Physiological parameters

Rectal temperature (T_{core}) was measured every min by a logger (LT-8A, Gram, Saitama, Japan) using a ther-

mistor probe (LT-ST08-11, accuracy ± 0.01 °C, Gram) inserted 12 cm beyond the anal sphincter. Skin temperatures were measured by thermistor sensors (LT-ST08-12, accuracy ± 0.01 °C, Gram) fixed to the skin surface at seven sites with thin, air-permeable adhesive surgical tape. The seven sites were mid-forehead ($T_{forehead}$), frontal chest (T_{chest}), right mid-thigh (T_{thigh}), right leg (T_{leg}), instep of the right foot (T_{foot}), right forearm (T_{arm}), and back of the right hand (T_{hand}).

Urinary 6-hydroxymelatonin sulfate concentration of the urine samples at 0200 h and 0700 h was analyzed by an enzyme-linked immunosorbent assay (ELISA, IBL, Hamburg, Germany). For comparison of hormone levels between participants, the results were standardized as rates per milligram of creatinine[20].

Each morning after collecting urine at 0700 h, sleep was estimated with the Kwansei-Gakuin Sleepiness Scale (KSS)[21]. KSS is a subjective rating scale of drowsiness that has been translated into Japanese, and which is based on the Stanford Sleepiness Scale (SSS).

Data analysis

Raw temperature data were inspected and segments that had been lost (due to slippage of the temperature sensor) were estimated by interpolation. The raw data from each participant were averaged every 30 min. Maximum and minimum values of rectal temperature of each participant were evaluated from the raw data. The times when acrophase and bathyphase occurred were individually derived from the raw data by cosinor analysis.

Comparison of values obtained between the two lighting conditions was made using a two-way analysis of variance (ANOVA) with repeated measures. ANOVA was applied separately for five periods: 1100–1800 h, 1800–2200 h, 2200–0200 h, 0200–0600 h and 0600–1000 h. Observing the raw data, we selected these times from the viewpoint of the elevation and descent in body temperature, which seemed important in terms of circadian rhythms. Multiple comparisons of differences were performed with Dunnett's multiple comparison test. Values for 6-hydroxymelatonin sulfate concentration and sleepiness as well as the actigraphy results were compared by paired Student *t*-tests. Data were generally expressed as mean ± SEM. Statistical significance was assessed at 1% and 5% levels.

Results

The results of T_{core} rhythms (as assessed by cosinor analysis) under the influence of LD-rectangular light change and LD-twilight light change for all participants are summarized in Table 1. Mean nadir time with SEM was 0418 ± 0018 h with LD-twilight change and 0507 ± 0027 h with LD-rectangular light change. The values were significantly different ($p<0.05$), suggesting that the T_{core} phase occured earlier with LD-twilight change than with LD-rectangular light change. On the contrary, mesor and amplitude did not differ between the two conditions. The maximum and minimum values are also summarized, but they did not differ between the two conditions.

Figure 2 compares the temporal changes in mean T_{core} (a), $T_{forehead}$ (b) and T_{chest} (c) between LD-rectangular light change and LD-twilight light change. T_{core}, $T_{forehead}$ and T_{chest} were significantly higher with LD-twilight

TABLE 1
THE RESULTS OF THE PHASES OF TCORE RHYTHMS AND MAXIMUM AND MINIMUM VALUES

	Nadir time*		Amplitude		Mesor		Maximum		Minimum	
	Rectangular	Twilight	Rectangular	Twilight	Rectangular	Twilight	Rectangular	Twilight	Rectangular	Twilight
S-1	6:59	5:32	0.37	0.25	36.79	36.90	37.25	37.35	36.34	36.44
S-2	2:12	3:03	0.41	0.41	36.83	36.81	37.30	37.36	36.23	36323
S-3	2:53	2:37	0.27	0.33	36.16	36.18	36.79	36.85	35.49	35.71
S-4	5:23	3:33	0.35	0.34	37.03	36.97	37.38	37.31	36.52	36.51
S-5	6:00	4:48	0.28	0.32	36.74	36.82	37.22	37.25	36.42	36.32
S-6	6:24	4:45	0.23	0.17	36.86	36.94	37.22	37.17	36.43	36.47
S-7	5:57	5:27	0.30	0.30	36.57	36.69	36.96	37.03	36.22	36.21
S-8	4:58	4:49	0.53	0.45	37.03	36.98	37.58	37.50	36.26	36.32
S-9	6:10	3:41	0.35	0.09	37.04	36.82	37.42	37.32	36.52	36.48
S-10	5:22	4:59	0.37	0.47	36.85	36.83	37.23	37.31	36.14	36.05
S-11	3:55	4:00	0.17	0.17	36.94	36.94	37.26	37.23	36.57	36.45
Ave	5:07	4:18	0.33	0.33	36.80	36.81	37.24	37.24	36.29	36.29
SEM	0:27	0:18	0.03	0.03	0.08	0.07	0.06	0.05	0.09	0.07

*$p<0.05$.
Individual bathyphase (nadir) times, amplitude, mesor, maximum and minimum values of 24 h T_{core} rhythm under two kinds of light conditions: rectangular and twilight. Note that nadir time occurred significantly earlier under twilight conditions ($p<0.05$).

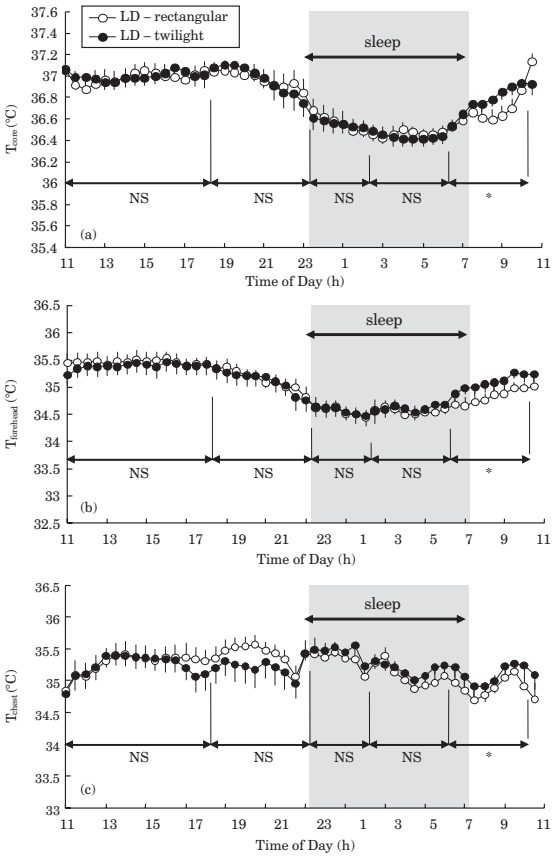

*Fig. 2. A comparison of temporal changes of mean T_{core} (a), $T_{forehead}$ (b), T_{chest} (c) under two kinds of light-dark cycles. Closed circles: twilight. Open circles: rectangular. Shaded area: sleep. NS: not significant. * p <0.05*

light change than with LD-rectangular light change from 0600 to 1000 h ($p<0.05$).

Figure 3 compares the temporal changes in mean T_{hand} (a), T_{thigh} (b), T_{leg} (c), and T_{foot} (d) between LD-rectangular light change and LD-twilight light change. T_{thigh}, T_{leg} and T_{foot} were significantly lower with LD-twilight light change than with LD-rectangular light change from 1100 to 1800 h ($p<0.05$).

Figure 4 compares the two conditions, LD-rectangular light change and LD-twilight light change, with regard to temporal changes in T_{core} between 1800 and 2200 h (a), 2200 and 0600 h (b) and 0600 and 1000 h (c) with Dunnett's multiple comparison test. There were no significant differences in changes in T_{core} between 1800 and 2200 h (a) and between 2200 and 0600 h (b). Between 0600 and 1000 h, T_{core} was significantly higher ($p<0.05$) at 0700 h than at 0600 h under LD-twilight light change, although there were no significant differences between 0600 and 0700 h under LD-rectangular light change. T_{core} kept a higher value under LD-twilight light change than LD-rectangular light change between 0600 and 1000 h (c).

Figure 5 compares urinary levels of 6-hydroxymelatonin sulfate at 0200 h between LD-rectangular light change and LD-twilight light change. Mean urinary 6-hydroxymelatonin sulfate levels were 34.12 ± 7.46 (ng/mgCRE) with LD-rectangular light change and 43.48 ± 8.26 (ng/mgCRE) with LD-twilight change at 0200 h, and 71.62 ± 13.68 (ng/mgCRE) in LD-rectangular light and 72.81 ± 13.03 (ng/mgCRE) with LD-twilight at 0700 h. The values (compensated by creatinine) obtained at 0200 h were significantly ($p<0.01$) higher under the LD-twilight change than LD-rectangular light change. However,

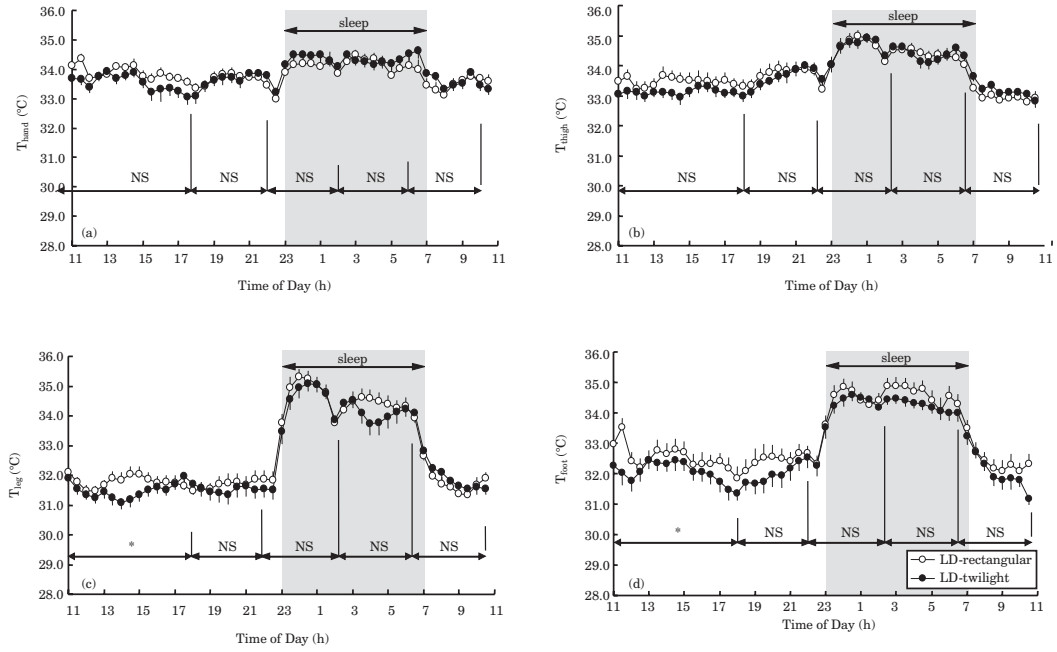

*Fig. 3. A comparison of temporal changes of mean T_{hand} (a), T_{thigh} (b), T_{leg} (c) and T_{foot} (d) under two kinds of light-dark cycles. Closed circles: twilight. Open circles: rectangular. Shaded area: sleep. NS: not significant. * p <0.05*

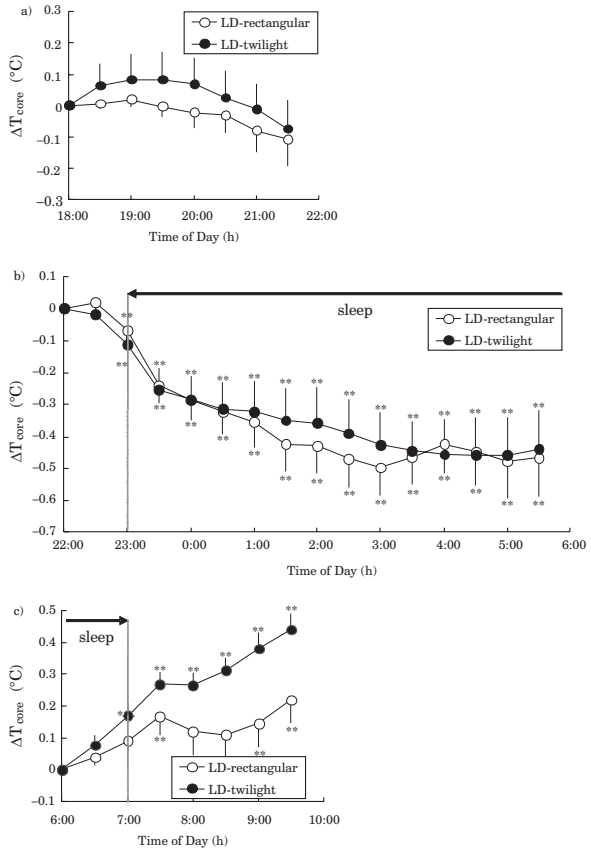

*Fig. 4. A comparison of temporal changes of ΔT_{core} from 1800 to 2200 h (a), 2200 to 0600 h (b) and 0600 to 1000 h (c). Closed circles: twilight. Open circles: rectangular. * p <0.05, ** p <0.01*

the values obtained at 0700 h did not differ between the two light conditions.

Figure 6 compares results from the KSS questionnaire, which was carried out immediately after collecting urine as soon as the participants rose in the morning. The mean score tended to be lower with LD-twilight light change than with LD-rectangular light change (p= 0.083), suggesting that the participants tended to wake with no drowsiness under the LD-twilight condition.

Mean sleep end time and sleep efficiency with SEM were 0655 ± 0001 h and 81.7 ± 2.66%, respectively, with

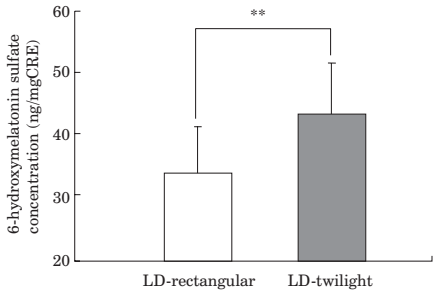

*Fig. 5. A comparison of 6-hydroxymelatonin sulfate levels at 0200 h during sleep under the influence of twilight and rectangular light conditions. ** p <0.01*

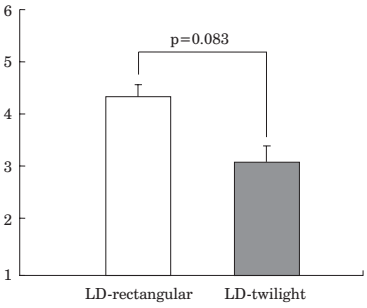

Fig. 6. A comparison of the scores from the KSS test. Open column: rectangular. Shaded column: twilight. p <0.083.

LD-twilight light change and 0654 ± 0001 h and 82.7 ± 2.21%, respectively, with LD-rectangular light change as scored by the actigraphy software. There were no significant differences between the values of the two conditions.

Discussion

Nadir time advanced significantly under the influence of the twilight condition (Table 1). This is similar to the report[16–18] that dawn simulation phase-advanced melatonin rhythm. The morning rise of core temperature was significantly quicker under the twilight condition (Figure 4c), while the evening fall in temperature did not vary between the two light conditions (Figure 4a). These suggest that twilight light change is effective in the determination of nadir time. An alternative interpretation for the advance of nadir time might be that the bright light of 3,000 lx during the previous daytime could phase--advance. In order to test this, we compared the circadian phase of rectal temperature of two female participants for two consecutive days under the influence of diurnal bright light exposure (3,000 lx). However, we could not find any differences of nadir time in rectal temperature. With these in mind, twilight light change might have played a role in determination of nadir time.

Gradual decrease and gradual increase of room temperature[22] and of both room temperature and light[23], in the evening and morning, respectively, also caused the earlier occurrence of nadir time. These suggest that both cycled T_a and twilight have the same significance in inducing phase advance.

6-hydroxymelatonin sulfate in urine collected at 0200 h were significantly higher under LD-twilight in comparison with LD-rectangular light. The values collected at 0700 h did not differ between the two light conditions. Why did LD-twilight induce higher melatonin metabolites? A recent study showed that urinary norepinephrine and vanillylmandelic acid (VMA), which are related to the parasympathetic nervous system, decreased significantly at 0200 h under the influence of LD-twilight in comparison to LD-rectangular light[24]. It has been reported that increased daytime light can induce a reduction in nocturnal sympathetic activity and/or increase in nocturnal melatonin level in humans[25]. Therefore, LD-

-twilight might also have reduced nocturnal sympathetic activity and increased nocturnal melatonin level.

The LD-twilight condition started 1 hour earlier than the LD transition from 3,000 lx to 100 lx at 1800 h in the LD-rectangular condition. Thus, there is a possibility that this time lag caused an increase in nocturnal melatonin secretion in the LD-twilight condition. However, it has been reported that nocturnal melatonin secretion can be suppressed by exposure to light of several hundred lx for only 30 min[26] and the light intensity of the twilight condition at 1800 h was 1,550 lx in the present study. Therefore, our results suggest that the twilight light change per se, not the 1 hour time lag, caused the increase in nocturnal melatonin secretion.

Why did the melatonin collected at 0700 h not differ? Core temperature rose significantly higher under LD--twilight than LD-rectangular light (Figure 4a-c), suggesting that activity of the sympathetic nervous system had already become higher, resulting in suppression of melatonin even under LD-twilight.

Morning drowsiness tended to be lower under LD--twilight. This is probably related to quicker increase of core temperature in the early morning, which might have led to the reduction in drowsiness.

LD-twilight is physiologically significant in increasing core temperature earlier in the morning, increasing melatonin secretion at 0200 h, and somewhat reducing morning drowsiness. With these in mind, when designing indoor illumination it is important that architects provide LD-twilight in the evening and early morning for sleep promotion in healthy normal people and/or light treatment in elderly patients with advanced dementia[27].

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UTJECAJ SUMRAKA NA DNEVNU PROMJENU BAZALNE TJELESNE TEMPERATURE, NJEGOVE SUPROTNE TOČKE I URINARNOG 6-HIDROKSI-SULFATA TIJEKOM NOĆNOG SPAVANJA I JUTARNJE MAMURNOSTI

SAŽETAK

Cilj ovog istraživanja bio je utvrditi psihološku značajnost sumraka i zore u cirkadijanom ritmu bazalne tjelesne temperature i urinarnog 6-hidroksi-sulfata tijekom spavanja te u osjećaju buđenja. Ispitanike su činili sedam studentica i četiri studenta. Ispitanici su lijegali u 23.00 sata i budili se u 7.00 sati. Zamoljeni su da tijekom budnosti, prije lijeganja, sjede na stolcu, što tiše, čitajući knjigu ili slušajući laganu glazbu. Svakom ispitaniku ponuđena su dva svjetlosna ugođaja: 1) lagano tamno (LD) pravokutno svijetlo s naglim zatamnjenjem sa 3000 lx na 100 lx u 18.00 sati i

naglim osvjetljenjem sa 0 lx do 3000 lx u 7.00 sati; 2) sumrak s postupnim zatamnjenjem sa 3000 lx na 100 lx počevši u 17.00 sati (trajanje sumraka oko 2 sata) i postupno osvjetljenje sa 0 lx na 3000 lx počevši u 5.00 sati (trajanje sumraka oko 2 sata). Period s 0 lx prvi dan istraživanja trajao je od 23.00 sata do 7.00 sati, dok je drugi dan istraživanja trajao od 23.00 sata do 5.00 sati. Vrijeme najniže točke značajno se povećavalo pod utjecajem uvjeta sumraka. Količina 6-hidroksi-sulfata u urinu, uzetog oko 2.00 sata, značajno je veća pod utjecajem uvjeta sumraka nego kod uvjeta lagano tamnog pravokutnog svijetla. Jutarnja mamurnost uglavnom je bila niža u slučajevima uvjeta sumraka. Naši rezultati pokazuju da se u arhitektonskom dizajnu unutarnje rasvjete treba koristiti ugođaj sumraka za večer te za rano jutro zbog poboljšanja spavanja kod zdravih normalnih ljudi i/ili zbog svjetlosne terapije starijih ljudi s uznapredovalom demencijom.