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Melatonin Metabolism and Living Conditions among Children on Weekdays and Holidays, and Living Factors Related to Melatonin Metabolism

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The purpose of this study was to make clear the aspects of salivary melatonin metabolism and living conditions among school children during weekdays and the day following holidays. Additionally, the purpose was to examine living factors related to melatonin metabolism. The analysis samples were 47 healthy 3rd and 4th grade children in an elementary school. This investigation was carried out during a week in July 2009. Saliva samples were collected three times (6:30pm: evening, 9:30pm: night, 6:30am: morning) at two different periods (weekdays and the day following holidays). Weekdays were from Wednesday to Thursday (weekday-weekday: W-W) and the day following holidays were from Sunday to Monday (holiday-weekday: H-W). Responses to a questionnaire about living conditions were recorded over the course of a week. The results were as follows: 1) Half of the subjects had their highest melatonin concentration at the morning measurement instead of the night measurement. 2) During the W-W period, the percentages of the subjects who had their highest salivary melatonin concentration at the night and morning measurements were not significantly different, and the mean concentrations at the night and morning measurements were also not significantly different. In contrast, during the H-W period, a majority had their highest melatonin concentration at the morning measurement, and the mean concentration was clearly higher at the morning measurement than at the night measurement. 3) Sleeping hours tended to be longer on holidays than on weekdays. Furthermore, bedtimes and wake times were significantly later on holidays than on weekdays. It was also found that electronic media and outdoors play hours were longer on holidays than on weekdays, while study hours were shorter on holidays than on weekdays. 4) Subjects whose salivary melatonin concentration had its highest value at the morning measurement had later bedtimes and wake-times, longer electronic media hours, and shorter outdoor play hours than those whose salivary melatonin concentration had its highest value at the night measurement. These findings suggest that the differences of the living conditions influence the melatonin metabolism among the children.

Keywords: elementary school children, salivary melatonin concentration, biological rhythm, sleeping-wakening

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1. Purpose

In Japan, children with physical disorders, who are neither ill nor healthy, started being reported in the early 1960s (Masaki, 2000). Since then, the disorders have become so prevalent that they are now obvious even to the layman. One of the underlying causes for such physical disorders is believed to be an irregular lifestyle. More specifically, a night or late-night orientated lifestyle has become prevalent, and quite a few studies have reported that irregular daily rhythms have shortened children's sleeping hours (Kohyama, 2000; Committee for School Health, Japanese Society of Child Health, 2001: Suzuki and Noi, 2007; Noi et al., 2008).

In addition to shortened sleeping hours associated with irregular sleeping habits, studies focusing on irregular sleep schedules have been conducted. For example, Ishihara et al. (1995) found that the standard deviation of bedtime increased with the school year. Tanaka et al. (2000) reported that children having irregular bedtimes tended to miss breakfast and complained of poor physical condition in the morning, and Suzuki and Noi (2007) reported that children who went to bed late or whose bedtime varied greatly tended to have sleep problems or general malaise. These findings provide good grounds for supporting “A proposal concerning children’s sleep” (Committee for School Health, Japanese Society of Child Health, 2001), which recommends maintenance of a regular sleep schedule.

Irregular sleeping habits in children are also considered to be associated with school and nursery teachers’ impressions that more children complain of poor physical condition in the morning following a holiday. Most of the previous studies about children’s lifestyle including sleeping habits analyzed trends on weekdays, and there have been only a few studies that compared children’s daily activities on weekdays with those on holidays. Monden (2001) compared children’s lifestyle and physical condition on a Friday with those on a non-school Saturday, and his results are noteworthy. His report showed that unhealthy living activities on a holiday increased the number of complaints of subjective symptoms, which suggested that the sleeping-waking rhythm in children is disturbed on holidays. However, this study was conducted when every other Saturday was a non-school day in Japan, and no such study has been conducted since every Saturday became a non-school day. Further, there has been no study that investigated the sleeping-waking rhythm in children on weekdays and on days immediately following holidays using a biochemical approach.

As is well known, the activity rhythm of mammals is regulated by the circadian clock, which is situated in the suprachiasmatic nuclei, and its phase is adjusted by melatonin (Fukada and Hirota, 2000). Thus, observing the melatonin rhythm in humans allows one to evaluate their sleeping-waking rhythm. In practice, many studies have evaluated the sleeping-waking rhythm using the melatonin rhythm phase (Uchiyama et al., 2000; Miyazaki et al., 2001; Buxton et al., 2003; Noi et al., 2009).

With this motivation, the purpose of this study was to clarify melatonin metabolism and daily activities among children on weekdays and holidays, as well as lifestyle factors that affect melatonin metabolism.

2. Methods

2.1. Subjects and study period

The subjects of this study were 72 healthy third and fourth graders at a public elementary school in Saitama Prefecture with a set schedule including regular start (around 8:20AM) and finish times (around 2:50PM or 3:40PM). Of these, 10 children whose saliva samples, which were needed for the analysis of melatonin concentrations, were not collected at all sampling times, and 15 children whose amounts of saliva in their samples were insufficient to be analyzed were excluded, and the remaining 47 children (22 boys and 25 girls) were the subjects of this analysis. The study was conducted for 7 days from July 7 to 13, 2009.

Consent for this study was obtained from the subjects’ school at a staff meeting via the principal and the yogo teacher. Then, thorough written and oral explanations of the purpose and method of the survey, noting that participation was optional and privacy was protected, were given to the students at a homeroom meeting and to their parents at a briefing session that was organized. Only children from whom written consent was obtained were subjects in this study. After the survey, a result report was prepared for each of the subjects with contact information for any questions that they may have about the results, and it was given to the subjects and their parents via their homeroom teachers.

2.2. Survey methods

In this study, salivary melatonin concentrations were measured, and a daily activity survey was conducted.

Salivary melatonin concentrations are known to be related to serum melatonin concentrations and urinary 6-hydoxymelatonin sulphate (6HMS) excretion rates, and they are recognized as reliable indices for evaluating biological rhythm (Nowak et al., 1987). Thus, because of the methodological limitations of a field study, salivary melatonin concentrations were measured in this study. Saliva was collected when the subjects were at rest using a saliva sample collection device (Salivette®, produced by Sarstedt

Ltd., Nümbrecht, Germany, and obtained from Assist Ltd., Tokyo, Japan). The melatonin concentrations in the samples were determined by a Direct Saliva Melatonin ELISA kit (Bühlmann Laboratories AG, Schönenbuch, Switzerland) at Toyo Kensa Center. Three melatonin concentrations per specimen were obtained, and the mean was used for the analysis. In addition, when the measurement value was higher than the standard range of the calibrators (0.5-50.0 pg/ml), 50 pg/ml was used for analysis. Saliva was collected three times referring to the method by Noi et al. (2009): 18:30 (evening measurement), 21:30 (night measurement), and 6:30 the following morning (morning measurement), and during two periods: Wednesday to Thursday (weekday-weekday: W-W) and Sunday to Monday (holiday-weekday: H-W) of a week that had no special school events. In this study, measurements were conducted on Wednesday and Thursday to represent "a weekday", and on Sunday and Monday to represent "a day following holiday". All salivary samples were collected at home and transported refrigerated to school. The subjects and their parents were asked to:

i) Avoid having food or drink except water one hour prior to sample collection,
ii) Rinse the mouth with water 15 minutes prior to sample collection and stay relaxed in a dim room with no light on until sample collection,
iii) Avoid having bananas, cherries, corn, coffee, juice, or soft drinks on the sample collection days.

For the daily life survey, a named, self-completed questionnaire was filled out. Referring to the surveys conducted by the Japanese Society of School Health (2010) and by Noi et al. (2008), the questionnaire consisted of the following 6 items:

i) Bedtime of the previous night,
ii) Wake-time of that morning,
iii) TV, video, or DVD viewing hours on the previous day (TV viewing hours),
iv) Computer or TV game play hours on the previous day (game play hours),
v) Outside play or exercise hours on the previous day, excluding physical education classes (outside play hours), and
vi) Study hours on the previous day.

Sleeping hours were obtained from the recorded bedtime and wake-time, and electronic media hours were obtained from the recorded TV viewing hours and game play hours. The subjects were asked to complete the survey every morning using the same questionnaire for seven consecutive days.

2.3. Analytical methods

In this study, the following three things were investigated.

First, the subjects' melatonin metabolism on W-W and H-W was investigated. The raw data were observed for the chronological change in salivary melatonin concentrations of each subject to determine the measurement time when the salivary melatonin concentration had its highest value for each subject during each W-W and H-W measurement period. Then, using two-way repeated measure ANOVA with period and measurement time as factors, the mean salivary melatonin concentrations at each measurement time in each period were compared. When the results of ANOVA showed main effects when significant interactions were present, then Bonferroni multiple comparisons were performed.

Secondly, the subjects' daily activities were investigated. One-way repeated measure ANOVA was performed first, and when the results showed significant differences, Bonferroni multiple comparisons were performed to compare the means of each questionnaire item on each day. However, because the saliva collection times were predetermined, the daily schedule related to sleep on the collection days may have been constrained. Thus, bedtime on Wednesday and Sunday and wake-time on Thursday and Monday were excluded from the analysis. Then, based on the results of the analysis, the separation between weekdays and holidays was set, and daily activities were compared between the periods using paired *t*-tests.

Thirdly, living activities that affect salivary melatonin concentrations were investigated. The subjects were divided into groups according to the measurement time when their melatonin concentration was highest, and their daily activities were compared using Student's *t*-tests, for which the melatonin on W-W and daily activities on weekdays were paired, and the melatonin on H-W and daily activities on holidays were paired, and then combined for analysis. The subjects (n=6) who had the same value on several measurements were excluded in this analysis.

The significance level was 5% ($p<0.05$), with a significant trend taken to be 10% ($0.05 \le p < 0.1$).

3. Results

3.1. Melatonin metabolism

Figure 1 shows the chronological changes in salivary melatonin concentrations for each subject. During the W-W period, 23 subjects (48.9%) had their highest salivary melatonin concentration at the night measurement, and 22 subjects (46.8%) had their highest concentration at the morning measurement, while during the H-W period, 15 subjects (31.9%) had their highest concentration at the night measurement, and 28 subjects (59.6%) had their highest concentration at the morning measurement. Two subjects (4.3%) during the W-W period and four subjects (8.5%) during the H-W period showed identical concentrations at the evening and night measurements or at the night and morning measurements.

Next, the mean salivary melatonin concentrations at each measurement time during the W-W and H-W periods were obtained to observe the transitions (**Figure 2**). The results showed that the transitions in the melatonin concentrations during the W-W period were clearly different from those during the H-W period. Thus, using two-way repeated measure ANOVA with period and measurement time as factors, the mean salivary melatonin concentrations at each measurement time in each period were compared. The results are shown in **Table 1**. The results showed significant differences in the period and measurement time as the main effects and in the interaction between the period and measurement time. The results of Bonferroni multiple comparisons performed subsequently showed significant differences in the morning measurement (W-W < H-W) with respect to the period, and the W-W measurement (evening < night and morning) and the H-W measurement (evening < night and morning, night < morning) with respect to the measurement time.

3.2. Daily activities

Table 2 shows the mean and standard deviation on each day of each item in the daily activities questionnaire. The items that showed a significant difference between days were bedtime, wake-time, TV viewing hours, game play hours, electronic media hours, and outdoor play hours. In contrast, no significant differences were observed in sleeping hours and study hours between days. The means and the test results showed marked differences in bedtime between Monday, Tuesday, and Thursday and Friday and Saturday, and in wake-time between Tuesday, Wednesday, and Friday and Saturday and Sunday. Similarly, there were marked differences in

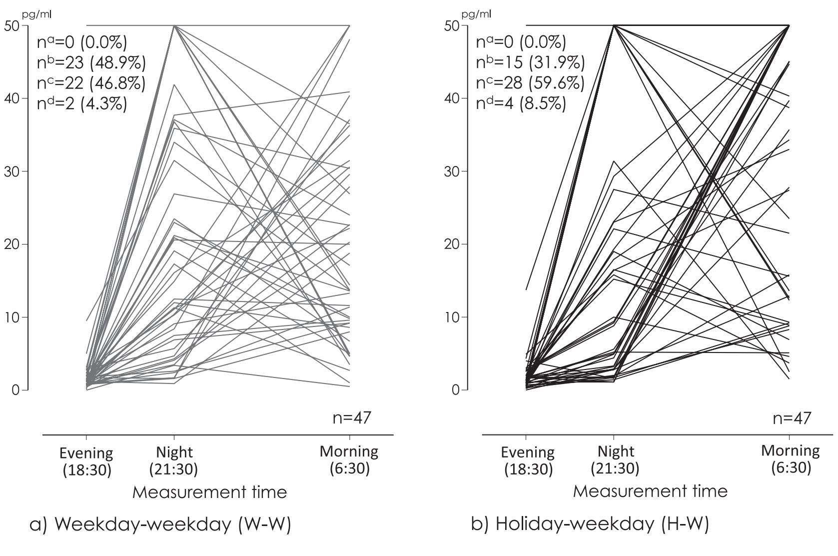

Figure 1 Changes of salivary melatonin concentration for each subject on Wednesday to Thursday (weekday-weekday: W-W) and Sunday to Monday (holiday-weekday: H-W). n^a =number of people who had their highest salivary melatonin concentration at evening measurement (18:30). n^b =night measurement (21:30). n^c =morning measurement (6:30). n^d =evening measurement=night measurement or night measurement=morning measurement.

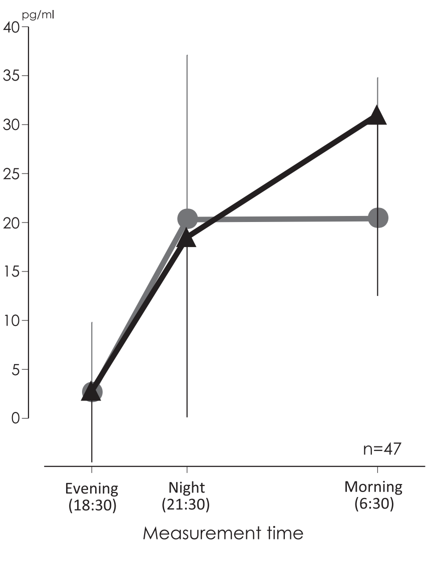

Figure 2 Changes of salivary melatonin concentration classified by weekday and the day following holiday. Values are mean±S.D.. ● shows the Wednesday to Thursday (weekday-weekday: W-W) change. ▲ shows the Sunday to Monday (holiday-weekday: H-W) change.

Table 1 Salivary melatonin concentration classified by period factor and measurement time factor.

		Measurement Time			Main effect ª		Interaction ª
		Evening (18:30)	Night (21:30)	Morning (6:30)	Period ᵇ	Time ᶜ	Period × Time
Period	Weekday-weekday: W-W	2.6±7.2	20.3±16.8	20.4±14.4	4.799*	45.215*	7.787*
	Holiday-weekday: H-W	2.8±7.3	18.5±18.4	31.0±18.5			

Data was analyzed by two-way repeated measures ANOVA and multiple comparison. Values are mean±S.D. (pg/ml). n=47. a: F value (*p<0.05). b; The period detected significant difference as a result of multiple comparison by Bonferroni's method was morning (W-W < H-W). c; The measurement time detected significant difference as a result of multiple comparison by Bonferroni's method were weekday (evening < night/morning) and holiday (evening < night/morning, night < morning).

Table 2 Mean and S.D. of bedtime, wake-time, sleeping hours, TV viewing hours, game play hours, electronic media hours, outdoor play hours and study hours for each day.

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	F value ª	Multiple comparison by Bonferroni's method ᵇ
Bedtime	21:38±42.9	21:42±53.7		21:38±48.7	22:00±41.3	21:58±44.1		5.127*	Mon<Fri/Sat Thu<Fri
Wake-time		6:41±28.3	6:36±26.7		6:34±30.8	7:14±68.9	7:02±58.7	11.225*	Tue<Sat Wed/Fri<Sat/Sun
Sleeping hours	9°03′±45.4	8°54′±57.1		8°56′±52.0	9°14′±74.1	9°04′±66.2		1.433	N.S.
TV viewing hours	1°27′±67.9	1°28′±79.7	1°20′±76.1	1°21′±76.4	1°46′±77.6	1°54′±100.0	1°58′±97.3	5.923*	Wed<Sun Thu<Fri/Sun
Game play hours	0°17′±29.2	0°16′±28.6	0°18′±28.3	0°15′±25.8	0°20′±34.6	0°50′±83.8	0°40′±53.0	5.862*	Tue/Thu<Sun
Electronic media hours	1°43′±72.5	1°44′±83.4	1°38′±78.7	1°37′±82.5	2°09′±92.7	2°45′±152.3	2°39′±122.8	11.308*	Mon/Tue/Wed/Thu <Sat/Sun
Outdoor play hours	0°55′±71.0	1°14′±86.9	1°00′±69.1	1°00′±71.1	1°20′±117.6	1°58′±117.2	1°56′±116.8	6.148*	Mon/Wed/Thu<Sat/Sun
Study hours	1°11′±54.0	1°08′±53.2	1°06′±55.4	1°23′±101.5	0°46′±35.4	0°47′±48.6	1°00′±65.9	2.652	N.S.

Bedtime on Wednesday and Sunday, wake-time on Thursday and Monday and sleeping hours on Wednesday to Thursday and Sunday to Monday were excluded from the analysis. Data was analyzed by one-way repeated measures ANOVA and multiple comparison by Bonferroni's method. Values are mean±S.D.. n=47 (outside play hours is n=46). a: *p<0.05. b: N.S. is not significant difference.

TV viewing, game play, electronic media, outdoor play, and study hours between Monday, Tuesday, Wednesday, and Thursday and Saturday and Sunday, while on Friday they were not significantly different from the other weekdays or holidays.

Based on these results, the following were selected: for bedtime, Monday, Tuesday, and Thursday as weekdays, and Friday and Saturday as holidays; for wake-time, Tuesday, Wednesday, and Friday as weekdays, and Saturday and Sunday as holidays; and for the other items, Monday, Tuesday, Wednesday, and Thursday as weekdays, and Saturday and Sunday as holidays.

Table 3 shows comparisons of daily activities on weekdays and holidays. The results showed a significant difference or trend in all items between weekdays and holidays. They showed trends that bedtime and wake-time were later on holidays than on weekdays, and sleeping hours were longer on holidays than on weekdays. It was also found that electronic media and outdoor play hours were longer on holidays than on weekdays, while study hours were shorter on holidays than on weekdays. The standard deviations of all the items except bedtime were found to be higher on holidays than on weekdays.

3.3. Measurement time when salivary melatonin concentration was highest and daily activities

Table 4 shows the results of the daily activities questionnaire of the two groups formed according to the measurement time when their salivary melatonin concentration was highest. The results showed no significant differences in sleeping hours, game play hours, and study hours between the group whose salivary melatonin concentration was highest at the night measurement (the night group) and the group whose salivary melatonin concentration was highest at the morning measurement (the morning group). However, bedtime was significantly later in the morning group (22:01±36.8 min) than in the night group (21:36±40.2 min), and there was a significant trend for wake-time to be later in the morning group (7:01±50.3 min) than in the night group (6:44±36.3 min). Similarly, significant differences or trends were observed between the two groups in TV viewing hours (night group, 1 h14±51.7 min; morning group, 2 h02± 95.1min), in electronic media hours (night

Table 3 Mean and S.D. of bedtime, wake-time, sleeping hours, TV viewing hours, game play hours, electronic media hours, outdoor play hours and study hours on weekdays and holidays.

	Weekday	Holiday	t value a
Bedtime	21:41±40.1	21:58±36.8	3.734*
Wake-time	6:38±24.6	7:09±56.3	4.440*
Sleeping hours	8°57′±40.2	9°10′±61.2	1.965†
TV viewing hours	1°22′±69.0	1°55′±92.0	3.477*
Game play hours	0°16′±22.6	0°44′±60.3	3.449*
Electronic media hours	1°38′±72.2	2°40′±129.5	4.757*
Outdoor play hours	1°01′±64.5	2°05′±110.4	4.508*
Study hours	1°11′±49.8	0°52′±52.4	2.192*

These data were selected: for bedtime, Monday, Tuesday, and Thursday as weekdays, and Friday and Saturday as holidays; for wake-time, Tuesday, Wednesday, and Friday as weekdays, and Saturday and Sunday as holidays; and for the other items, Monday, Tuesday, Wednesday, and Thursday as weekdays, and Saturday and Sunday as holidays. Data was analyzed by paired *t*-tests. Values are mean±S.D.. n=47 (outside play hours is n=46). a: *p<0.05, † 0.05≦p<0.10.

Table 4 Mean and S.D. of bedtime, wake-time, sleeping hours, TV viewing hours, game play hours, electronic media hours, outdoor play hours and study hours of the two groups formed according to the measurement time when their salivary melatonin concentration was highest.

	Group which their melatonin concentration was highest at ...		
	night measurement	morning measurement	t value a
Bedtime	21:36±40.2 (n=38)	22:01±36.8 (n=50)	3.091*
Wake-time	6:44±36.3 (n=38)	7:01±50.3 (n=50)	1.794†
Sleeping hours	9°08′±42.6 (n=38)	9°00′±58.8 (n=50)	0.724
TV viewing hours	1°14′±51.7 (n=38)	2°02′±95.1 (n=50)	3.049*
Game play hours	0°24′±33.9 (n=38)	0°37′±57.0 (n=50)	1.231
Electronic media hours	1°38′±67.1 (n=38)	2°39′±127.2 (n=50)	2.911*
Outdoor play hours	1°57′±107.4 (n=37)	1°20′±85.1 (n=49)	1.758†
Study hours	1°08′±51.5 (n=38)	0°55′±50.4 (n=50)	1.151

Bedtime on Wednesday and Sunday, wake-time on Thursday and Monday, sleeping hours on Wednesday to Thursday and Sunday to Monday and the other items on Friday were excluded from the analysis. Data was analyzed by Student's *t*-tests. Values are mean±S.D.. a: *p<0.05, †0.05≦p<0.10

group, 1 h38±67.1 min; morning group, 2 h39±127.2 min), and in outdoor play hours (night group, 1 h57±107.4 min; morning group, 1 h20± 85.1 min).

4. Discussion

It is a social responsibility of school health science to draw a picture of children's biological rhythms and daily activities that is as accurate as possible. In order to gain knowledge that will help fulfill this responsibility, this study was conducted with the purpose of clarifying melatonin metabolism in children and factors in daily life that affect it. Another aim was to identify the indirect cause for poor physical condition, which has been felt to be present in children on days following holidays, from the biological rhythm perspective.

No subjects in this study had their highest salivary melatonin concentration at the evening measurement (18:30), but some subjects showed it at the morning measurement (6:30) instead of the night measurement (21:30) (Figure 1).

Uchiyama et al. (2000) reported that staying up late delays the onset time of melatonin secretion. Saliva was collected only three times a day in this study, so the exact time when melatonin secretion peaked in each subject could not be identified. However, a higher salivary melatonin concentration at the morning than at the night measurement suggests a phase delay in melatonin secretion. Melatonin has sleep inducing actions, and those who had their highest melatonin value at the morning measurement were considered to be sleepier at 6:30 than at 21:30 and found it difficult to get up. Thus, about half (the W-W period, 46.8%; the H-W period, 59.6%) of the subjects may be experiencing “difficult mornings”.

Also, “difficult mornings” seem to be more prevalent on days following holidays than on weekdays. Figures 1 and 2 and Table 1 show that, during the W-W period, the percentages of the subjects who had their highest salivary concentration at the night and morning measurements were not significantly different, and the mean salivary melatonin concentrations at the night and morning measurements were also not significantly different. In contrast, during the H-W period, a majority had their highest melatonin concentration at the morning measurement, and the mean concentration was clearly higher at the morning measurement than at the night measurement. These results suggest that even more subjects suffer from “difficult mornings” following holidays.

The bedtime, wake-time, and sleeping hours on weekdays shown in Table 3 were also compared with those reported by the Japanese Society of School Health (2010); there were small differences, but none were statistically significant. Thus, it is suggested that the difference in melatonin rhythm between weekdays and day following holidays was not peculiar to the subjects of this study but may be a health issue for many children in Japan.

The results of this study also showed that the daily activities of the subjects varied depending on the day of the week, more specifically, weekday or holiday (Tables 2 and 3). Sleeping hours tended to be longer on holidays than on weekdays, and bedtime and wake-time were markedly later on holidays than on weekdays. Recently, it has been suggested that the difference in sleeping hours between weekdays and holidays can serve as an objective index of sleep deprivation (Mikami and Matsushita, 2010), so this result of our study raises a concern about sleep deprivation in the subjects. Furthermore, electronic media and outdoor play hours were longer on holidays than on weekdays, while study hours were shorter on holidays than on weekdays. These results suggest that children spend their free time on holidays on electronic media and outdoor play, and mostly agree with the results reported by Monden (2001), in which the daily activities of fifth and sixth graders on Friday (weekday) were compared with those on Saturday (holiday). However, the results of our study, in which daily activities were surveyed for one week, did not show a significant difference in daily activities, except in wake-time, between Friday and Saturday. One possible reason for the present results being different from the earlier results is that the earlier survey was conducted when every other Saturday was a non-school day in Japan, while the present study was conducted when every Saturday was a non-school day. Although further studies are needed to investigate this possibility, our results provide useful school health science data for improving the school system.

The results of this study also showed great between and within individual differences in salivary melatonin concentrations. Despite this, salivary melatonin concentrations are considered useful for evaluating biological rhythm (Nowak et al., 1987), suggesting that focusing on the rhythm is necessary for analyzing living activities. With this motivation, in this study, the daily activities of groups formed according to the measurement time when their salivary melatonin concentration was highest were analyzed. According to the results, a clear difference in daily activities was also confirmed between those who had their highest salivary melatonin concentration at the night measurement and those who had their highest concentration at the morning measurement (Table 4).

As is well known, exposure to light during the nighttime suppresses melatonin secretion in humans (Lewy et al., 1980; Brainard et al., 1997; Zeitzer et al., 2000), while exposure to light during the daytime increases nocturnal melatonin secretion. For example, Mishima et al. (2001) reported that elderly insomniacs who received daytime light illumination therapy had improved symptoms and increased nocturnal melatonin concentrations. Another interesting report described that high illumination photodynamic therapy reduced sleep disturbance and fatigue in children with school refusal and childhood chronic fatigue syndrome (Miike, 2005). Other recent studies have also suggested that physical exercise during the daytime (Miyazaki et al., 2001; Buxton et al., 2003) and regular mealtimes (Shibata, 2008) may affect biological rhythm. Social factors such as time announcements and environmental factors such as temperature, humidity, noise, and vibration are also considered to affect the human biological rhythm (Uchida, 2005).

Melatonin is synthesized from L-tryptophan through a four-step enzymatic pathway via 5-hydroxy-tryptophan, serotonin, and N-acetylserotonin (Fukada and Hirota, 2000). Serotonin neurons are known to be activated by walking, mastication, and rhythmic movement (Arita, 2000; Sano et al., 2001; 2002). In addition to these reports about melatonin and serotonin, it has been reported that television viewing at bedtime is associated with sleep disturbances (Owens et al., 1999) and physical activity promotes good sleep (Nixon et al., 2009), which are noteworthy.

Thus, outdoor play during the daytime promotes exposure to light and physical activity, whereas TV viewing and TV game play are considered to have negative effects on biological rhythm, not only because they prevent children from having exposure to light and physical activity, but also because they can prevent experiencing a dark environment at night. Noi et al. (2009) reported that a long-term camp, during which participants were exposed to a dark environment at night and light during the daytime, had moderate exercise, regular mealtimes, and time-conscious behavior, improved melatonin rhythm in participants during the camp compared with before and after the camp. Their findings also help explain our results given in Table 4, i.e. the differences in daily activities between those whose salivary melatonin concentration was highest at the night measurement and those whose concentration was highest at the morning measurement.

As described above, this study clarified the melatonin rhythm on W-W period and H-W period and daily activities on weekdays and holidays, and the differences in daily activities between those whose salivary melatonin concentration was highest at the night measurement and those whose concentration was highest at the morning measurement.

However, this study had the following limitations, which require further study. First, the melatonin rhythm of the subjects was examined based on three measurement values (evening, night, and morning measurements) under two conditions (W-W and H-W, i.e. from Wednesday evening to early Thursday morning and from Sunday evening to early Monday morning, respectively). Under conditions where the intensity of illumination is kept constant, melatonin secretion starts about 14 to 16 hours after waking up, and at the same time, core temperature starts going down; then, after two or three hours, this causes drowsiness and, after four hours, melatonin secretion reaches its peak (Uchiyama et al., 2000). Considering this, estimating the peak of the melatonin rhythm based on three measurements is a limitation of this study. When considering the fact that salivary melatonin concentrations vary by individual and also fluctuate within individuals, another possible limitation of this study that will require further study comes from the fact that we have taken the measured value of Wednesday and Thursday as representatives for the weekday, and Sunday and Monday as representatives for days following a holiday.

The second major limitation is that the number of subjects in this study was not large, although they shared the same sleeping context as subjects in previous studies. Currently, there is no evidence that the relationship between melatonin concentration and daily activities on weekdays is the same as that on holidays. Therefore, they originally needed to be investigated separately. In addition, the subjects were third and fourth graders, who were old enough to be able to fill in the questionnaires, and young enough that the majority of the girls could be assumed to be pre-menarcheal, but the effect of menstruation or gender was not considered. We suggest further studies should be conducted with a larger number of subjects and a wider range of ages.

A third major limitation is that the characteristics of the 25 subjects who were excluded from analysis because their saliva samples were not taken at all the sampling times or the amounts of saliva in their samples were insufficient to be analyzed were not clarified. The main reason for saliva samples not being taken at all sampling times was that the subjects forgot to collect them, but insufficient amounts of saliva in their samples may have some physiological implication, which would be a topic for further study.

5. Conclusion

This study was conducted to clarify melatonin metabolism and living activities among children. The following results were obtained:

1) Changes in salivary melatonin concentrations at the evening, night, and morning measurements showed that about half of the subjects had their highest melatonin concentration at the morning measurement instead of the night measurement.

2) During the W-W period, the percentages of the subjects who had their highest salivary melatonin concentration at the night and morning measurements were not significantly different, and the mean concentrations at the night and morning measurements were also not significantly different. In contrast, during the H-W period, a majority had their highest melatonin concentration at the morning measurement, and the mean concentration was clearly higher at the morning measurement than at the night measurement.

3) Sleeping hours tended to be longer on holidays than on weekdays, and bedtime and wake-time were markedly later on holidays than on weekdays. It was also found that electronic media and outdoor play hours were longer on holidays than on weekdays, while study hours were shorter on holidays than on weekdays.

4) In contrast, subjects whose salivary melatonin concentration had its highest value at the morning measurement had later bedtimes and wake-times, longer electronic media hours, and shorter outdoor play hours than those whose salivary melatonin concentration had its highest value at the night measurement.

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References

Arita, H. (2000) Serotonergic system and circadian rhythm. Clinical Neuroscience 18: 1150-1152 (in Japanese).

Brainard, G.C., Rollag, M.D. and Hanifin, J.P. (1997) Photic regulation of melatonin in humans: ocular and neural signal transduction. Journal of Biological Rhythms 12: 537-546.

Buxton, O.M., Lee, C.W., L'Hermite-Baleriaux, M., et al. (2003) Exercise elicits phase shifts and acute alterations of melatonin that vary with circadian phase. American Journal of Physiology, Regulatory, Integrative and Comparative Physiology 284: R714-R724.

Committee for School Health, The Japanese Society of Child Health (2001) Proposal concerning the child's sleep. The Journal of Child Health 60: 817-819 (in Japanese).

Fukada, Y. and Hirota, T. (2000) Pineal organ and circadian rhythm. Clinical Neuroscience 18: 1147-1149 (in Japanese).

Ishihara, K., Eguchi, Y. and Miyake, S. (1995) Changes in sleep-wake habits with grade in primary and junior high school pupils. Journal of Sleep and Environments 3: 90-97 (in Japanese with English abstract).

Japanese Society of School Health (2010) Enterprise report on the health status surveillance among the child. Tokyo: Japanese Society of School Health (in Japanese).

Kohyama, J. (2000) Problems associated with child sleep environments. Clinical Psychiatry 42: 1309-1316 (in Japanese).

Lewy, A.J., Wehr, T.A., Goodwin, F.K., et al. (1980) Light suppresses melatonin secretion in humans. Science 210: 1267-1269.

Masaki, T. (2000) Some developmental disorders and the physical disorders among Japanese children: our methodology of the study on some developmental disorders. Japanese Journal of Physical Education, Health and Sport Sciences 45: 267-273 (in Japanese with English abstract).

Miike, T. (2005) Childhood chronic fatigue syndrome. Progress in Medicine 25: 1299-1304 (in Japanese with English abstract).

Mikami, A. and Matsushita, M. (2010) Comorbidity of depression and sleep disorders. Japanese Journal of Clinical Psychiatry 39: 645-651 (in Japanese).

Mishima, K., Okawa, M., Shimizu, T. et al. (2001) Diminished melatonin secretion in the elderly caused by insufficient environmental illumination. The Journal of Clinical Endocrinology and Metabolism 86: 129-134.

Miyazaki, T., Hashimoto, S., Masubuchi, S., et al. (2001) Phase-advance shifts of human circadian pacemaker are accelerated by daytime physical exercise. The American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 281: R197-R205.

Monden, S. (2001) A study on lifestyle and health of pupils –Analysis based on the five-day week school system. Japanese Journal of School Health 43: 61-72 (in Japanese with English abstract).

Nixon, G.M., Thompson, J.M.D., Han, D.Y., et al. (2009) Falling asleep: the determinants of sleep latency. Archives of Disease in Childhood 94: 686-689.

Noi, S., Shimosato, S., Suzuki, R., et al. (2008) The fundamental study of "learning of life and body": from the results of question, knowledge, living and physical condition investigation. Japanese Journal of School Health 49: 439-451 (in Japanese with English abstract).

Noi, S., Shikano, A., Suzuki, R., et al. (2009) The influence to melatonin metabolism of children by a long-term camp. Japan Journal of Human Growth and Development Research 41: 36-43 (in Japanese with English abstract).

Nowak, R., McMillen, I.C., Redman, J. et al. (1987) The correlation between serum and salivary melatonin concentrations and urinary 6-hydroxymelatonin sulphate excretion rates: two non-invasive techniques for monitoring human circadian rhythmicity. Clinical Endocrinology 27: 445-452.

Owens, J., Masim, R., McGuinn, M., et al. (1999) Television-viewing habits and sleep disturbance in school children. Pediatrics 104: e27.

Sano, S., Kaba, M., Sakamoto, M., et al. (2001) Activation of serotonergic nervous system by human rhythmical exercise (II). Neuroscience Research Supplement 25: s69.

Sano, S., Kaba, M., Sakamoto, M., et al. (2002) Activation of serotonergic system by step exercise in humans. The Bulletin of Hokuriku University 25: 39-48 (in Japanese).

Shibata, S. (2008) Role of circadian clock in the clock and time perception. Japanese Journal of Animal Psychology 58: 21-31 (in Japanese with English abstract).

Suzuki, R. and Noi, S. (2007) The relationship between sleeping habits and sleeping problems and/or physical discomforts among junior high school students. Japan Journal of Human Growth and Development Research 36: 21-26 (in Japanese with English abstract).

Tanaka, H., Taira, K., Arakawa, M., et al. (2000) Lifestyle for proper sleep securing related to the physical and mental health conservation in adolescence. Journal of School Mental Health 3: 57-62 (in Japanese).

Uchida, S. (2005) Sport and biological rhythm. The Journal of Clinical Sports Medicine 22: 623-627 (in Japanese).

Uchiyama, M., Okawa, M., Shibui, K., et al. (2000) Poor compensatory function for sleep loss as a pathogenic factor in patients with delayed sleep phase syndrome. Sleep 23: 553-558.

Zeitzer, J.M., Dijk, DJ., Kronauer, R.E., et al. (2000) Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression. The Journal of Physiology 526: 696-702.

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