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Clinical Study

Salivary Melatonin Concentrations in a Sitting and a Standing Position

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This study aimed to examine differences in salivary melatonin concentrations between the sitting and standing positions. Ten subjects (age range: 20–22 years; 5 males and 5 females) participated in the study and provided written consent. In the sitting condition, the subjects sat on a chair from 23:30 h to 1:00 h, whereas in the standing condition, the subjects sat on a chair from 23:30 h to 0:30 h and stood on the floor from 0:30 h to 1:00 h. Saliva samples were obtained at 0:25 h and 1:00 h from all subjects. The experiment was performed under a dim light condition (<10 lx). No significant differences were observed in melatonin concentrations between the two positions at 0:25 h; however, melatonin concentrations in the standing condition were significantly greater than those in the sitting condition at 1:00 h. These results show that melatonin secretion is significantly greater in the standing position than that in the sitting position.

1. Background

Melatonin, a hormone secreted by the pineal gland, is measured during human circadian rhythm studies because the secretion is controlled by the light-dark signal from the suprachiasmatic nucleus (SCN). The SCN, which is responsible for circadian rhythms, maintains the melatonin secretion, body temperature, and other hormones such as cortisol. Melatonin exhibits a 24 h secretion pattern, and its concentration is high at night and low during the day. The melatonin secretion pattern, particularly dim light melatonin onset, is used as a circadian phase marker [1]. Furthermore, melatonin secretion is acutely suppressed by bright light [2]. Light-induced melatonin suppression shows a dose-response relationship depending on light intensity [3, 4] and duration of light exposure [5]. Melatonin secretion is less susceptible to behavioral and environmental masking factors compared with other circadian rhythm markers such as core temperature and cortisol levels.

However, previous studies have reported that postural change from a supine to a standing position significantly increases the melatonin secretion [6, 7]. A study [7] revealed no significant difference in plasma melatonin level with postural change from a sitting to a standing position. Many recent studies have evaluated salivary melatonin concentration because it exhibits a good correlation with blood melatonin concentration [8]; furthermore, collecting saliva samples is less invasive. In this study, we examined the differences in salivary melatonin concentrations between the subjects in standing and sitting positions.

2. Methods

2.1. Subjects. The study included ten subjects (age range: 20–22 years; 5 males and 5 females) after providing written consent. The subjects were not administered medications during the experimental period and had no history of psychiatric or sleep disorders. Five female subjects with regular menstrual cycles (25–34 days) participated in the experiment during their menstrual cycle (1–3 days). The individual length of each female's menstrual cycle was considered when planning the appointments for the experiments. All subjects were instructed to abstain from alcohol and caffeine for a day before the experiment. Furthermore, they were instructed to

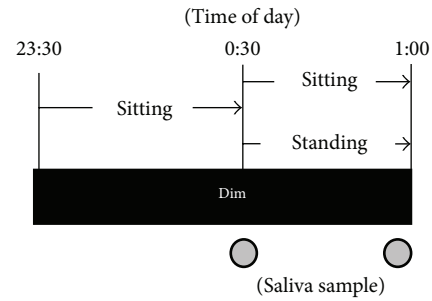

Figure 1: Time schedule. Subjects sat in a chair from 23:30 h to 1:00 h in the sitting condition. In the standing condition, they remained seated from 23:30 h to 0:30 h and then stood on the floor from 0:30 h to 1:00 h. The experiment was conducted under a dim light condition (<10 lx).

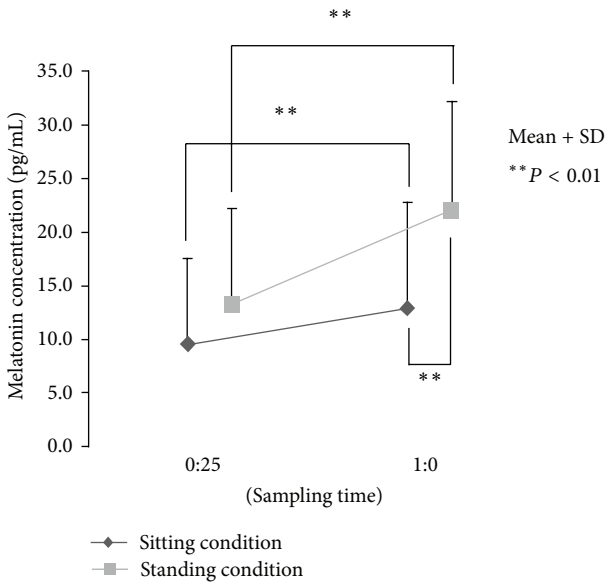

Figure 2: Mean (+standard deviation (SD)) melatonin concentrations in each position before (0:25 h) and after (1:00 h) changing the position.

maintain a regular sleep-wake schedule (sleep at 00:00 h and wake up between 08:00 h and 09:00 h) and avoid napping during the day from a week prior to the experiment.

2.2. Experimental Procedure. The experiments were conducted at the Research Center for Human Environmental Adaptation, Kyushu University, in October 2011. These experiments were approved by the ethical committee of the Faculty of Design, Kyushu University. Experiments in the sitting and standing positions were conducted on 2 different nights at a rate of a posture condition per night. On the day of the experiment (Figure 1), the subjects entered the experimental chamber at 23:20 h, and the experiment was initiated at 23:30 h under a dim lighting condition (<10 lx). In the sitting condition, the subjects sat on a chair from 23:30 h to 1:00 h, whereas in the standing condition, the subjects sat on a chair from 23:30 h to 0:30 h and stood on the floor from 0:30 h to 1:00 h. All subjects were instructed to remain awake during the experiment and were allowed to watch a movie. The illuminance level of the movie display was maintained at <2 lx because bright light from the display could possibly suppress melatonin secretion [9]. Saliva samples were obtained at 0:25 h and 1:00 h. The ambient temperature in the experimental chamber was maintained at 25°C with 50% relative humidity.

2.3. Measurement of Salivary Melatonin Concentration. Saliva samples obtained with a salivette (Salisoft, Sarstedt, Germany) were centrifuged at 1000 ×g for 2 min and frozen at −30°C until assay. The salivette swab was made from polypropylene and polyethylene because cotton swabs reportedly influence melatonin assay results [10]. Melatonin concentrations were analyzed in duplicate with a commercially available radioimmunoassay kit (Direct Saliva Melatonin RIA; Bühlmann Laboratories, Allschwil, Switzerland), and mean values of duplicate determinations were used for analysis. The sensitivity of the assay kit was 0.9 pg/mL. The intra- and interassay coefficients of variance were 2.6%–20.1% and 6.6%–16.7%, respectively.

2.4. Data Analysis. Statistical analyses were performed using SPSS version 11.5 (SPSS, Inc., Chicago, IL, USA). A two-way within-subject factor repeated-measures analysis of variance (ANOVA) was used to assess the differences in melatonin concentrations over time and between posture conditions. A repeated-measures t-test was used for the post hoc analysis. A P value of < 0.05 was considered statistically significant.

3. Results

Figure 2 illustrates the time course of the mean melatonin concentrations in the two positions. Two-way repeated-measures ANOVA revealed a significant effect of time interval ($F_{1,9} = 64.1$; $P < 0.01$) and an interaction between time interval and posture ($F_{1,9} = 13.8$; $P < 0.01$). Post-hoc comparison revealed a significant increase in melatonin concentrations at 1:00 h in both positions. Although no significant differences in melatonin concentrations were observed between the 2 positions at 0:25 h, the melatonin concentration at 1:00 h was significantly higher in the standing position than that in the sitting position. As illustrated in Figure 3, the repeated-measures t-test indicated that the melatonin concentration was significantly higher in the standing position than that in the sitting position.

4. Discussion

The melatonin concentrations at 1:00 h were significantly higher in the standing position than those in the sitting position, indicating that melatonin secretion is affected by posture, a finding similar to that of previous studies [6, 7]. The concentration of various plasma components changes with postural change [11]. Most melatonin may be bound to blood proteins [12, 13], and the bounding melatonin is not secreted into saliva. Indeed, salivary melatonin closely reflects

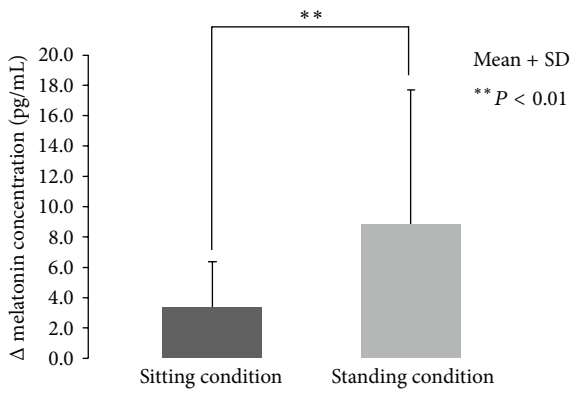

Figure 3: The increase in melatonin concentration from 0:25 h to 1:00 h in each position.

changes in free (unbounded) melatonin levels in plasma [14]. Therefore, a shift in posture from a sitting to a standing position may affect the salivary melatonin concentration.

However, a study has reported no significant difference in plasma melatonin level with a postural change from a sitting to a standing position [7]. This study included two female subjects, and their menstrual phases were not mentioned. While the majority of studies reported that the menstrual cycle does not affect melatonin secretion, some studies have suggested the effects of menstrual cycle on melatonin secretion [15]. Furthermore, in the present study, salivary melatonin levels were measured, while a previous study measured plasma melatonin levels [7]. Because only free melatonin in plasma is secreted into saliva, the present findings may have been determined by variations in different plasma components with the postural change [11]. Furthermore, the time of sample collection may have contributed to the inconsistent result, because these samples were collected at midnight (0:25 h and 1:00 h) in our study. In a previous study [7], the melatonin level in the sitting position significantly increased compared to that in the supine position after the late evening (23:00 h–1:00 h), although there was no difference in the melatonin levels between the sitting and the supine position until late evening (21:00 h–23:00 h). Postural changes may not affect the melatonin level during the early period of melatonin secretion.

In addition, our results raise questions about a possible masking effect of posture on salivary melatonin concentration. Previous findings showed a significant increase in melatonin level in the standing and sitting positions compared to that in the supine position [7]. However, many studies have evaluated human circadian rhythms on the basis of salivary melatonin concentrations in the sitting position [16, 17]. The masking effect of posture might be limited.

While previous studies reported a significant difference in melatonin secretion between standing and supine positions [6, 7], the present study revealed that the salivary melatonin secretion significantly increased in the standing position than that in the sitting position. Because salivary melatonin level generally reflects free melatonin level in blood [14], free melatonin level in blood might change in different postures. In general, only free hormones are physiologically active. According to the International Agency for Research on Cancer, working in shifts involving circadian disruption is probably carcinogenic (Group 2A) because women working in the long night shifts are at higher risk of breast cancer compared with women who do not work at night [18, 19]. Melatonin secretion and pattern are well known to be affected by light [20]. Moreover, some studies reveal that light-induced melatonin suppression is a probable risk factor for cancer [21, 22]. Furthermore, melatonin might have a role in regulating human circadian rhythms and sleep [23]. Considering the melatonin suppression can contribute to health risks including carcinogenesis and insomnia, the risk may depend on the posture. A previous study reported that melatonin level in the sitting position significantly increased compared to that in the supine position [7]. Sitting posture should have an accelerated effect on melatonin secretion as well as the standing posture; however, the present study showed a significant difference with increased melatonin levels between the standing and sitting positions. Most kinds of work might not contribute to a carcinogenic risk because workers sit and/or stand during work. However, melatonin concentrations may significantly change in other situations such as spaceflight because changes in melatonin concentrations with posture may be related to a shift in body fluids. Further studies should be conducted to evaluate light-induced melatonin suppression in different positions.

Conflict of Interests

The authors declare that they have no competing interests.

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