〈Original Article〉

Sleep quality and mental stress influence salivary melatonin concentrations

Yasumi Taki[1)], Tadayuki Iida[2)], Meiho Nakayama[3)], Yasushi Nakagami[1)], Kazuhiro Maruta[4)], Ryoji Teradaira[4)] and Yasuhiro Ito[1)]

Summary We compared different factors to explore an association between melatonin and sleep quality. In 26 healthy young women, we measured the salivary melatonin concentration, and used the Pittsburgh sleep quality index (PSQI), State-Trait Anxiety Inventory (STAI), Self-Rating Depression Scale (SDS), and NEO Five-Factor Inventory (NEO-FFI). We obtained additional findings: 1) subjects with a poor sleep quality had a higher nocturnal concentration of salivary melatonin associated with more intensive recent mental stress (as demonstrated by the STAI and SDS scores), and 2) the nocturnal melatonin concentration is recommended 100 pg/mL or below for studying sleep quality using salivary melatonin concentrations as a measure in healthy young women. The results from the present study suggested that nocturnal melatonin was associated with stress scores in the subjects with a poor sleep quality and that most recent mental stress, such as depression and anxiety, may have an impact on the nocturnal melatonin level. Moreover, the nocturnal melatonin concentration should be desirably 100 pg/mL or below, which is considered to be a daily level, for studying sleep quality using a salivary melatonin concentration as a measure in healthy young women.

Key words: Salivary melatonin, Sleep quality, Mental stresses, Personality inventory

1. Introduction

Melatonin and serotonin are the major hormones affecting the circadian rhythm. Melatonin is produced from serotonin in the pineal body and shifts human circadian rhythms to the night phase. This means that melatonin exhibits a circadian rhythm whereby blood melatonin is increased in the night and decreased in the day due to the transmission of light information involved in the changes of day length and brightness to inside the body by MT_2 receptors and, thereby, regulates the mechanism of circadian rhythms[1)]. This evidence was obtained from many reports, including the melatonin ingestion studies by Haimov et al.[2)] and Attenburrow et al.[3)] and the report on the association between the lifestyle and a nocturnal melatonin level by Mishima et al.[4)] However, these reports mostly addressed the circadian rhythms for the purpose of

[1)]Department of Physiology, School of Health Sciences, Fujita Health University.
[2)]Department of Public Health, School of Medicine, Fujita Health University.
[3)]Department of Otolaryngology Head &Neck Surgery, Nagoya City University Medical School.
[4)]Department of Biochemistry, School of Health Sciences, Fujita Health University.
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Correspondence: Yasuhiro Ito, Department of Physiology, School of Health Sciences, Fujita Health University, Toyoake, Aichi 470-1192, Japan

insomnia treatment. In the present study, we measured the salivary melatonin concentrations, sleep quality, and mental stress in young women who had no insomnia and lived a healthy daily life. The saliva can be sampled noninvasively and, therefore, this sampling does not constitute a mental stressor or affect sleep. Sleep was evaluated using the Pittsburgh sleep quality index[5] questionnaire. As a stress measure, the questionnaires STAI[6] and SDS[7] were used. Based on the scores obtained from these questionnaires, we discussed the factors influencing the salivary melatonin concentration and sleep quality.

2. Subjects and Methods

1. Subjects

The study included 26 young healthy female volunteers (mean age: 21.8 ± 0.6 years old) who had no subjective sleep disorders. Written informed consent was obtained from all subjects, and the study was in compliance with our university rules for human experimentation. Additionally, this study was approved by the ethics review committee of Fujita Health University No. 09-037.

2. Sampling

The saliva samples were collected in the proliferative phase (follicular phase of the ovarian cycle) considered to involve mental stability to eliminate any impacts of the estrous cycle, before lunch during 11:00 - 13:00 in the day and immediately before bedtime at night. For saliva sampling, a Salivette® from the Sarstedt Co., Ltd was used.

3. Measurement of salivary melatonin

Salivary melatonin was measured using a Buehlmann Direct Saliva Melatonin ELISA kit from Bühlmann Laboratories AG. This product employs a high-sensitive polyclonal Kennaway G280 anti-melatonin antibody to be used for the RIA technique[8-9].

4. Sleep evaluation

Sleep was evaluated with a questionnaire used commonly worldwide, the Pittsburgh sleep quality index (PSQI)[5]. The PSQI defines 5.5 points or above as the cut-off value, i.e., a "poor sleep quality"[5].

5. Evaluation of stress intensity

Stress intensity was evaluated using the questionnaires: State-Trait Anxiety Inventory (STAI) as a measure of anxiety[6], and Self-Rating Depression Scale (SDS) as a measure of depression[7]. The STAI includes separate measures of state and trait anxiety, and we used the popular 20-item version[10]. We defined 42 points or above for state anxiety and 45 points or above for trait anxiety as "intensive anxiety". In the SDS, we defined 40 points or above as a "high level of depression"[7].

6. Personality analysis (personality inventory)

The personality of the subjects was analyzed using the questionnaire NEO Five-Factor Inventory (NEO-FFI)[11]. In this analysis, the big-five personality traits[12] of the individual, i.e., neuroticism, extraversion, openness, agreeableness, and conscientiousness, can be scored.

7. Statistical analysis

Student's t-test was used to compare the salivary concentrations of melatonin between day and night. A Pearson product moment correlation coefficient of Microsoft Office Excel 2003 was used to calculate correlation coefficient between individual values.

3. Results

1. Salivary melatonin

The salivary concentration of melatonin was 30.1 ± 20.6 pg/mL for the day and 131.5 ± 92.3 pg/mL for the night, being significantly higher for the night ($p<0.000005$) (Table 1).

2. PSQI score[5]

The subjects had PSQI scores ranging from 1 to 10 points, with a mean score of 5.3 ± 2.4. Sixteen subjects had scores below the cut-off value, i.e., 5.5 points[5], and the remaining ten subjects had scores of 5.5 points or above (Table 1).

2-1. Association between PSQI score and salivary melatonin concentration

Relationship between the PSQI score and salivary melatonin concentration for all subjects (n = 26) are shown. The correlation coefficient was r = - 0.140 for the day and r = -0.013 for the night, indicating no association between the PSQI score and salivary melatonin concentration.

2-2. Group with good sleep quality (n = 16)

Relationship for the group with a PSQI score below 5.5 points, i.e., good sleep quality, are shown below. The correlation coefficient was r = 0.341 for the day and r = 0.173 for the night, indicating no association between the PSQI score and salivary melatonin concentration.

2-3. PSQI scores and the group with a nocturnal salivary concentration of melatonin of 100 pg/mL or below[13] (n = 13)

Relationship between the PSQI score and salivary melatonin concentration when assuming that a regular nocturnal level of melatonin is about 100 pg/mL or below based on the reports from Hashimoto et al.[13] and Mishima et al.[4] are shown below. The correlation coefficient was r = 0.004 for the day and r = -0.719 ($p < 0.005$) for the night . Thus, if the nocturnal salivary concentration of melatonin is 100 pg/mL or below, a larger amount of secretion is associated with a reduced sleep difficulty (Fig. 1).

3. STAI score[6]

The state anxiety scores for the subjects ranged from 25 to 60 points, with a mean score of 43.9 ± 10.6. Thirteen subjects had 41 points or below, corresponding to a "moderate level", and the remaining 13 subjects had 42 points or above, corresponding to a "high level". The trait anxiety scores for the subjects ranged from 27 to 64 points, with a mean score of 46.1 ± 9.4 points (Table 1). Twelve subjects had 44 points or below, corresponding to a "moderate level", and the remaining 14 subjects had 45 points or above, corresponding to a "high level".

3-1. Association between STAI and PSQI scores

The correlation coefficient between state anxiety

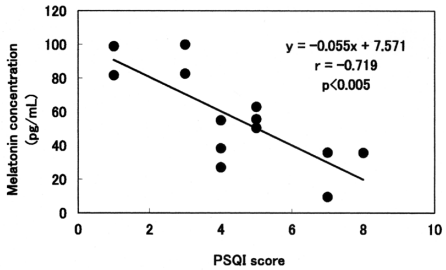

Fig. 1 Association between PSQI score and nocturnal melatonin concentration. For the subjects with a nocturnal melatonin concentration of 100 pg/mL or below (n = 13). There was a significant inverse correlation between PSQI score which is an index of the sleep quality and nocturnal melatonin (r = - 0.719, $p<0.005$).

Table 1 Subject characteristics and salivary melatonin. Mean values ± 1 standard deviation (SD) for each data

Subjects			salivary melatonin (pg/mL)		PSQI scores	SDS scores
n	age	gender	daytime	night		
26	21.8±0.6	F	30.1±20.6	131.5±92.3	5.3±2.4	41.3±7.6

STAI scores		NEO-FFI scores				
state anxiety	trait anxiety	neuroticism	extraversion	openness	agreeableness	conscientiousness
43.9±10.6	46.1±9.4	27.5±8.4	26.6±7.1	30.9±4.9	29.1±6.6	28.4±6.1

Note: PSQI = Pittsburgh sleep quality index as an index of sleep quality, SDS = Self-Rating Depression Scale as a measure of depression, STAI = State-Trait Anxiety Inventory as a measure of each of state anxiety and trait anxiety. NEO-FFI = NEO Five-Factor Inventory as a measure of big-five personality traits.

and PSQI scores and between trait anxiety score and PSQI scores was r = 0.311 and r = 0.000, respectively, indicating that there was no association.

3-2. Association between state anxiety score and salivary melatonin concentration

3-2a. Group with good sleep quality (n = 16)

In the group with a PSQI score below 5.5 points, i.e., a good sleep quality, the association between the state anxiety score and salivary melatonin concentration was as follows: the correlation coefficient was r = -0.139 for the day and r = 0.211 for the night, indicating that there was no association between the stress (anxiety) status in the daytime of the current day and salivary melatonin level in the subjects with a good sleep quality.

3-2b. Group with poor sleep quality (n = 10)

In the group with a PSQI score of 5.5 points or above, i.e., a poor sleep quality, the association between the state anxiety score and salivary melatonin concentration was as follows: the correlation coefficient was r = -0.296 for the daytime and r = 0.668 ($p < 0.05$) for the night , indicating that a higher level of stress (anxiety) in the daytime of the current day was associated with a higher salivary concentration of melatonin in the subjects with a poor sleep quality (Fig. 2).

3-3. Association between trait anxiety score and salivary melatonin concentration

3-3a. Group with good sleep quality (n = 16)

In the group with a PSQI score of 5.5 points or below, i.e., a good sleep quality, the association between the trait anxiety score and salivary melatonin concentration was as follows: the correlation coefficient was r = -0.141 for the day and r = 0.135 for the night, indicating that there was no association between an increased (or decreased) sensitivity to stress (anxiety) and the salivary melatonin level in the subjects with a good sleep quality.

3-3b. Group with poor sleep quality (n = 10)

In the group with a PSQI score of 5.5 points or above, i.e., a poor sleep quality, the association between the trait anxiety score and salivary melatonin concentration was as follows: the correlation coefficient was r = -0.335 for the day and r = 0.423 for the night, indicating that there was no association between the trait anxiety score and salivary melatonin concentration in the subjects with a poor sleep quality.

4. SDS score (Depression)[7]

The SDS scores for the subjects ranged from 30 to 51 points, with a mean score of 41.3 ± 7.6. Eleven subjects had 39 points or below corresponding to a "moderate level" and the remaining 15 subjects had 40 points or below corresponding to a "high level".

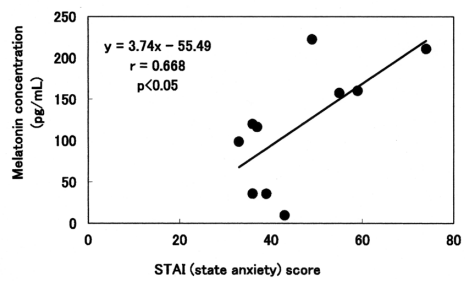

Fig. 2 Association between STAI (state anxiety) score and nocturnal melatonin concentration. For the subjects with a PSQI score of 5.5 points or above (n = 10). There was a significant correlation between state anxiety and nocturnal melatonin (r = 0.668, p<0.05).

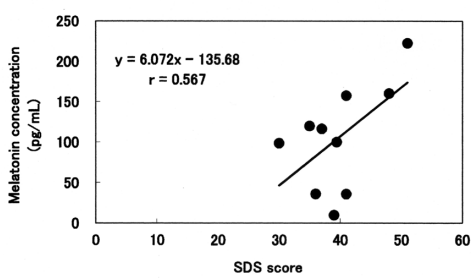

Fig. 3 Association between SDS (depression) score and nocturnal melatonin concentration. For the subjects with a PSQI score of 5.5 points or above (n = 10). There was a weak correlation between depression and nocturnal melatonin (r = 0.567, ns).

4-1. Association between SDS and PSQI scores

Relationship between the SDS and PSQI scores was only r = 0.209, indicating that there was no association.

4-2. Association between SDS score and salivary melatonin concentration

4-2a. Group with good sleep quality (n = 16)

In the group with a PSQI score of 5.5 points or below, i.e., a good sleep quality, the relationship between the SDS score and salivary melatonin concentration was as follows: r = 0.022 for the day and r = 0.265 for the night, indicating that there was no association.

4-2b. Group with poor sleep quality (n = 10)

In the group with a PSQI score of 5.5 points or above, i.e., a poor sleep quality, the association between salivary melatonin and SDS was as follows: the correlation coefficient was r = 0.018 for the day and r = 0.666 (p = 0.06) for the night, indicating a trend whereby, in the group with a high PSQI score, a higher level of stress (depression) was associated with a higher nocturnal melatonin concentration (Fig. 3).

5. NEO-FFI personality analysis

The mean scores for the subjects were as follows: 27.5 ± 8.4 (neuroticism: N), 26.6 ± 7.1 (extraversion: E), 30.9 ± 4.9 (openness: O), 29.1 ± 6.6 (agreeableness: A), and 28.4 ± 6.1 (conscientiousness: C).

5-1. Association between score for NEO-FFI personality analysis and PSQI (n = 26)

The correlation coefficient between the big-five personality traits and sleep quality was as follows: r = 0.566 (p<0.005) for the association between neuroticism and PSQI, r = 0.386 (p = 0.06) for extraversion and PSQI, r = 0.347 for openness and PSQI, r = 0.165 for agreeableness and PSQI, and r = 0.567 (p<0.005) for conscientiousness and PSQI. This shows correlations between the personality traits and sleep quality (Fig. 4, 5).

5-2. NEO-FFI score and salivary melatonin concentration

There was no particular association between the NEO-FFI personality score and salivary melatonin. However, the group with a nocturnal melatonin concentration of 100 pg/mL or below (n = 12) had the following correlation factors: r = -0.561 (p=0.06) for the association between neuroticism and melatonin: r = -0.507 for extraversion and melatonin, r = 0.022 for openness and melatonin, r = -0.441 for agreeableness and melatonin, and r = -0.756 (p<0.001) for conscientiousness and melatonin (Fig. 6). These results show correlations between the personality traits and melatonin secretion and mean that a higher point of conscientiousness is associated with a lower nocturnal melatonin level. A similar trend was observed for neuroticism.

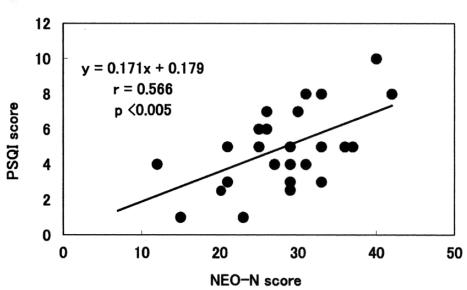

Fig. 4 Association between NEO-N and PSQI scores. For all the subjects (n = 26). There was a significant correlation between neuroticism scores and PSQI scores (r = 0.566, p < 0.005).

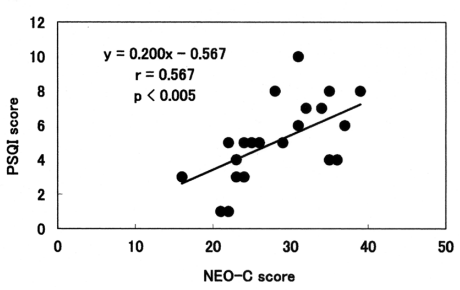

Fig. 5 Association between NEO-C and PSQI scores. For all the subjects (n = 26). There was a significant correlation between conscientiousness scores and PSQI scores (r = 0.567, p < 0.005).

4. Discussion

Voultsios et al.[8] reported that salivary melatonin is highly correlated with plasma melatonin and, therefore, a circadian rhythm can be evaluated based on salivary melatonin concentrations. In addition, melatonin does not accumulate in the blood because of its short serum half-life of 28.4 minutes[14], and the melatonin detected could have been most recently synthesized. These previous findings indicate that salivary melatonin can be a useful tool for studying the circadian rhythm of an individual noninvasively. The subjects in this report all showed a higher level of nocturnal melatonin. A MT_2 melatonin receptor plays an important role in adjusting circadian rhythms[4], and natural sleep occurs using melatonin or MT_2-receptor agonists[15]. Natural sleep produces normal circadian rhythms and is considered to lead to the treatment of sleep disorders. However, the association between the melatonin level and sleep quality remains to be fully elucidated. In the present study, we determined the salivary melatonin concentrations of the day and night as well as Pittsburgh sleep quality index (PSQI) scores in healthy adult women (mean age: 21.8 years old) who had no regular self-conscious sleep disorders. Additionally, we used the NEO-FFI for personality analysis as well as the SDS[7] and STAI[6] as a psychological measure. Unfortunately, a comparison among all the subjects revealed that there was no association between the salivary melatonin concentration and PSQI score and, however, that a larger amount of melatonin secretion was associated with a reduced sleep difficulty in the subjects with a nocturnal salivary melatonin concentration of 100 pg/mL or below[4, 13]. In other words, a larger amount of melatonin produced most recently (if the amount is 100 pg/mL or below) may be associated with a higher sleep quality. In contrast, the subjects with a poor sleep quality demonstrated a stronger trend whereby a higher depression level and, at the same time, a higher anxiety level, was associated with a larger amount of nocturnal melatonin. This suggests that stress deteriorates sleep quality and "may promote melatonin production". On the other hand, we failed to find an association between sleep quality and stress based on the PSQI and a psychological questionnaire, i.e., SDS or STAI, alone. Hori et al.[16] found in a dexamethasone/CRH stress test that an increased stress reaction in the hypothalamic-pituitary-adrenal axis was associated with a deteriorated sleep quality, and suggested that mental stress could affect sleep quality. Although our study employed a simple method of scoring the mental stress level, we found an association among stress reaction, sleep quality, and melatonin by incorporating salivary melatonin concentrations. Melatonin provides a biological defense through its antioxidative action[17] and potentially responds to oxidative stress caused by a change of diet or lifestyle due to mental stress[18]. In addition, the suggested association between the big-five personality traits and sleep quality requires further investigation. As a matter of course, a poor sleep quality is more likely to increase the melatonin level and, therefore, we should investigate the association between the big-five personality traits and melatonin.

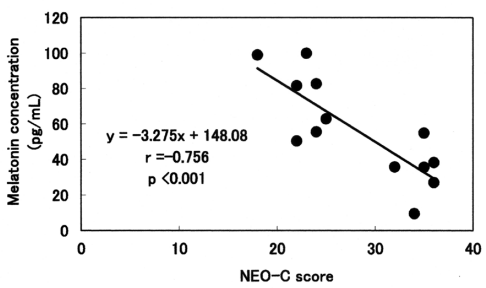

Fig. 6 Association between NEO-C score and nocturnal melatonin concentration. For the subjects with a nocturnal melatonin concentration of 100 pg/mL or below (n = 13). There was a significant inverse correlation between conscientiousness score and nocturnal melatonin ($r = -0.756$, $p < 0.001$).

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