Relationships between Salivary Melatonin Levels, Quality of Sleep, and Stress in Young Japanese Females

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Abstract: A decrease in the quality of sleep is believed to cause anxiety and worsen depression. Comparisons of salivary melatonin levels with different factors including quality of sleep, state and trait anxieties, and depression, were conducted to examine whether there is a relationship between melatonin, presumably associated with sleep, and psychological stress. The saliva of healthy young females was collected during the daytime and before they went to bed at night (when they were awake and resting in a sitting position), and salivary melatonin levels were measured. The quality of sleep was scored using the Pittsburgh Sleep Quality Index (PSQI)—a questionnaire method. State and trait anxieties, and depression were scored using other questionnaire methods: the State-Trait Anxiety Inventory (STAI) and Self-Rating Depression Scale (SDS), respectively. The following findings were obtained: (1) Salivary melatonin levels measured during the daytime and before going to bed were higher in females with a high depression score, compared to those with a low score, and there was a correlation between the depression scores and salivary melatonin levels measured at night; and (2) salivary melatonin levels measured before going to bed at night (in a sitting position) were higher in females with a high state anxiety score, suggesting a correlation between state anxiety scores and salivary melatonin levels during the night. Both depression and a sense of anxiety are forms of psychological stress. Therefore, it is assumed that, when a person is under psychological stress, the action of melatonin as a ligand on its receptor is reduced. Meaning psychological stress may induce oxidative stress in the body. On the other hand, no correlation was noted between the quality of sleep and salivary melatonin levels during the night, presumably because saliva was collected when the subjects were awake and sitting, rather than sleeping.

Keywords: salivary melatonin, sleep quality, anxiety, depression symptoms
Introduction

Melatonin and serotonin are important hormones responsible for regulating circadian rhythms. Melatonin, produced from serotonin in the pineal body, guides the body of a person to the night phase. This occurs because melatonin conveys optical information related to the day length and light-dark cycle into the body to control the mechanism of circadian rhythms. It has been reported that circadian rhythm disturbances, including insomnia, increase the level of psychological stress and causes depression. A high level of psychological stress is believed to cause oxidative stress, which leads to a number of disorders. There are a large number of young Japanese people, females in particular, with depression. In addition, the secretion of female hormones in young women is reduced in the presence of lifestyle changes or mental stress, affecting the menstrual cycle. Furthermore, approximately 50% of females have experienced physical/mental discomfort or premenstrual syndrome between ovulation and the start of menstruation. In those with depression, there was a correlation between the severity of mental symptoms and that of physical symptoms. Thus, the subjective assessment of depression-related mental discomfort, as well as the objective assessment of a stress-related reduction in female hormone secretion, must be considered.

As this is an important public health issue, we examined the relationship between circadian rhythm disturbance and psychological stress using endogenous biomarkers. We focused attention on melatonin, one of the factors responsible for regulating circadian rhythms, as it has antioxidant properties. A survey was conducted assuming that good-quality sleep represents a normal circadian rhythm. The present study aimed to examine the relationships between the quality of sleep in daily life, psychological stress, and salivary melatonin levels measured at night, and to determine whether or not salivary melatonin levels measured during the night serve as an index for the quality of sleep or an effective psychological stress marker. In this survey, although the reference to an individual personality was avoided, we chose the participants who are living an approximated lifestyle. Specifically, participants belong to the same university, the same major, and the same class. As saliva sampling is a noninvasive procedure and does not serve as a psychological stressor, it did not affect the participants. The Pittsburgh Sleep Quality Index (PSQI), a widely known questionnaire, was used to assess the status of the participant’s sleep. To assess the level of psychological stress, senses of anxiety and depression were scored using the State-Trait Anxiety Inventory (STAI) and Self-Rating Depression Scale (SDS) (both well-known questionnaires), respectively.

Materials and Methods

Participants

Participants were 48 healthy Japanese female students (mean age: 21.8 ± 0.7 years old) with no subjective symptoms of sleep disorders who attended the same faculty of the same university, and shared a common lifestyle. In addition, all participants were nonsmokers. All participants provided their informed consent.

Saliva collecting

Saliva collecting was conducted in the proliferation phase (or the follicular phase of the ovarian cycle), in which females are usually in the most stable mental condition, in order to prevent the estrous cycle from influencing results. The saliva collecting method is shown in Figure 1. The menstrual cycle and survey period were identified through an interview survey. The menstrual phase was identified based on self-reports. In the menstrual (after the start of menstruation), proliferative (13 to 15 days), and secretory (24 to 26 days) phases, a questionnaire survey was conducted.

![Figure 1](image-url)

Figure 1. Survey schedule developed while taking into account the estrous cycle. Menstrual phase: Within 3 days of the onset of menstruation. Proliferation phase (follicular phase): 13 to 15 days from the onset of menstruation. Secretory phase (luteal phase): 23 to 25 days from the onset of menstruation. Saliva was collected in the proliferation phase.
As the purpose was to examine salivary melatonin levels in daily life, the participants underwent daytime sampling in a sitting position prior to eating lunch (between 11:00 and 13:00). Melatonin is synthesized from serotonin in the pineal body and leads the human body to the night phase by transmitting optical information concerning the daytime length and changes in light and dark through the MT$_2$ receptor, regulating the mechanism of circadian rhythms. Melatonin, therefore, increases night. They all underwent night-time sampling prior to going to bed when they were awake in a sitting position in a bright room (between 22:00 and 24:00). The protocol required neither eating nor drinking (except water) for one hour prior to saliva collection, so that food debris would not stimulate salivation. Saliva sampling was conducted for three minutes, using Salivette® container (SARSTEDT, Germany) without citric acid. After saliva sampling, the Salivette® containers were centrifuged as soon as possible for 5 minutes at 1500 × g. For the melatonin measurement, all samples were kept frozen at −20 °C until assay.

**Measurement of salivary melatonin**

An enzyme linked immunoassay kit (Buehlmann DirectSaliva Melatonin ELISA (EK-DMS), Buehlmann Laboratories AG, Germany) was used for the salivary melatonin assay process, according to the procedure based in the manual. This product employs a high-sensitive polyclonal Kennaway G280 anti-melatonin antibody, and the system requires 200 μL of saliva along with a detector with a wavelength of 450 nm (Multiskan JX detector, Thermoelectron, USA).

**Assessment of sleep**

The quality of sleep was assessed using the PSQI, a questionnaire developed by Buysse and used in a number of countries. The questionnaire consists of nineteen self- and five objective-assessment-based questions. The following items were assessed on a scale between 0 and 21 points: the time of falling asleep, sleep length and efficiency, and sleeping and awakening difficulties. Zero points represent no problem, and 21 points indicate severe problems in relation to all assessment items. In general, a score of 5.5 (6) or higher represents a “poor quality sleep” (Table 1).

**Assessment of anxiety using a scale**

Anxiety was assessed on a scale using a questionnaire. The STAI is a test method developed by Spielberger in 1983. It consists of State Anxiety (designed to deter-

| Table 1. Assessment methods using the Pittsburgh Sleep Quality Index (PSQI), the State-Trait Anxiety Inventory (STAI), and the Self-Rating Depression Scale (SDS). |
|---|---|---|---|---|
| **PSQI** | The Pittsburgh Sleep Quality Index (PSQI) is combined to form seven component scores. Each of which has a range of 0–3 points. In all cases, a score of 0 indicates no difficulty, while a score of 3 indicates severe difficulty. The seven component scores are then added to yield one global score, with a range of 0–21 points, 0 indicating no difficulty and 21 indicating severe difficulties in all areas. The normal revel in global score is 5.5 or less points. |
| **STAI** | The State-Trait Anxiety Inventory (STAI) is a psychological inventory based on a 4-point Likert scale. Scores range from 20 to 80, with higher scores correlating with greater anxiety. |
| **SDS** | The Self-Rating Depression Scale (SDS) is a short self-administered survey to quantify the depressed status of a patient. There are ten positively worded and ten negatively worded questions. Each question is scored on a scale of 1–4. The score range from 25–100. |

| **State-Anxiety** | **Trait-anxiety** |
|---|---|
| 51 or more | 55 or more |
| 42–50 | 45–54 |
| 31–41 | 34–44 |
| 22–30 | 24–33 |
| 21 or less | 23 or less |

| **SDS** |
|---|
| 25–49 | Normal range |
| 50–59 | Mildly depressed |
| 60–69 | Moderately depressed |
| 70 and above | Severely depressed |
mine whether a person is in a state of anxiety) and Trait Anxiety (to determine whether a person often feels anxiety because of one’s psychological trait) scales. A commonly used version of STAI (Form-X), including 20 items, was employed in the present study. A score of 42 or higher on the State Anxiety scale represents “a state of anxiety,” and 45 or higher on the Trait Anxiety scale represents a trait associated with anxiety. The STAI is an assessment method widely used in psychology, psychiatry, internal medicine, psychosomatic medicine, surgery, and sociology (Table 1).

Assessment of depression using a scale
Depression was assessed on a scale using a questionnaire. The SDS is a self-assessment-based scale developed by Zung to assess depression. A score below 40 represents “no depression,” 40 to 50 “mildly depressed,” and 50 or above “moderately depressed.” The SDS, a widely used assessment method, is implemented when a patient is diagnosed with “a possible psychological problem” by a physician as a first-stage-procedure (Table 1).

Data analyses
Statistical data are presented in the following form: ‘mean ± standard deviation.’ All P-values and confidence limits were based on two-tailed calculations. A P-value level of less than 0.05 was deemed significant. For analyses, salivary melatonin levels were compared between participants with and without problems, as determined based on their scores on each of the above-mentioned scales. Comparisons of salivary melatonin levels were conducted between participants with (1) a PSQI score of 5 or lower and 6 or higher, (2) a State Anxiety score of 41 or lower and 42 or higher, (3) a Trait Anxiety score of 44 or lower and 45 or higher, and (4) an SDS score of 39 or lower and 40 or higher. The results were analyzed using the Mann-Whitney U test, a non-parametric test. The analyses were conducted in this manner because salivary melatonin concentrations were not normally distributed. The F-test was used to compare salivary melatonin levels during the daytime and at night. To calculate correlation coefficients between salivary melatonin levels and PSQI, STAI, and SDS, the Pearson Product Moment Correlation Coefficient of Microsoft Office Excel 2010 was used. For measurement of an effect size, Cohen’s d was calculated. This is a measure of the strength of a sample-based estimate of that quantity. Cohen’s d is defined as the difference between two means divided by a standard deviation for the data.

Ethical considerations
The study was conducted with the approval of the ethics committee of Fujita Health University (No. 10-075), and published in a manner so that individuals could not be identified.

Results
The psychological characteristics of the participants, including melatonin concentrations, are shown in Table 2. One participant for whom saliva was collected with poor melatonin measurement accuracy was excluded from analysis, therefore, the number of participants was set to 47.

Salivary melatonin concentration
Figure 2 shows salivary melatonin concentrations for the two conditions. According to a comparison involving all subjects (n = 47), the salivary melatonin level at night (29.600 ± 18.231 pg/mL) was significantly higher than that during the daytime (2.457 ± 0.617 pg/mL), F(1,46) = 872.687, P < 0.0000.

Relationship between PSQI scores (quality of sleep) and salivary melatonin levels
The PSQI scores received by the participants ranged between 1 and 13 (mean: 5.5 ± 3.1). Twenty-eight females received five or fewer points (excellent quality of sleep), while nineteen scored six or higher (poor quality of sleep). There was no significant correlation between PSQI scores involving all participants (n = 47) and salivary melatonin levels. Additionally, no correlation was noted between PSQI scores and melatonin levels during the night (correlation coefficient = −0.0079, P = 0.9580; Fig. 3A) or day (correlation coefficient = 0.2382, P = 0.1068; Fig. 4A). The participants were divided into two groups: those with a PSQI score of 5 or lower (n = 28), and those with a score of 6 or higher (n = 19). Salivary melatonin levels at night were compared for each group. The mean melatonin level at night was...
Table 2. The table shows data for all 47 participants, including melatonin concentrations. A State-Trait Anxiety Inventory score represents state and trait anxieties, and the Self-rating Depression Scale scores depression. A Pittsburgh Sleep Quality Index (PSQI) score represents the quality of sleep.

| Subjects no. | Sex | Age | Daytime Salivary Melatonin (pg/mL) | Night time Salivary Melatonin (pg/mL) | Pittsburgh Sleep Quality Index (PSQI score) | State-Trait Anxiety inventory | Self-rating depression scale |
|--------------|-----|-----|-----------------------------------|--------------------------------------|---------------------------------------------|-----------------------------|-----------------------------|
|              |     |     |                                   |                                      |                                             | State Anxiety score | Trait Anxiety score | SDS score |
| 1            | F   | 21  | 2.628                             | 27.383                               | 8                                           | 38              | 43              | 36         |
| 2            | F   | 23  | 1.665                             | 27.503                               | 1                                           | 39              | 50              | 42         |
| 3            | F   | 22  | 1.736                             | 19.039                               | 7                                           | 42              | 47              | 40         |
| 4            | F   | 21  | 1.616                             | 42.284                               | 4                                           | 49              | 58              | 45         |
| 5            | F   | 21  | 1.636                             | 37.341                               | 6                                           | 36              | 37              | 35         |
| 6            | F   | 22  | 2.507                             | 54.602                               | 5                                           | 51              | 58              | 44         |
| 7            | F   | 23  | 2.340                             | 35.529                               | 3                                           | 66              | 54              | 56         |
| 8            | F   | 21  | 1.595                             | 18.653                               | 4                                           | 43              | 48              | 35         |
| 9            | F   | 22  | 2.990                             | 10.477                               | 8                                           | 39              | 46              | 41         |
| 10           | F   | 22  | 2.256                             | 34.734                               | 3                                           | 42              | 48              | 36         |
| 11           | F   | 21  | 2.480                             | 36.530                               | 10                                          | 37              | 34              | 37         |
| 12           | F   | 22  | 2.237                             | 38.708                               | 2                                           | 36              | 42              | 31         |
| 13           | F   | 22  | 2.963                             | 32.920                               | 3                                           | 51              | 59              | 47         |
| 14           | F   | 21  | 3.247                             | 2.901                                | 13                                          | 46              | 54              | 51         |
| 15           | F   | 22  | 2.200                             | 7.146                                | 1                                           | 37              | 36              | 35         |
| 16           | F   | 21  | 1.762                             | 10.551                               | 7                                           | 36              | 39              | 36         |
| 17           | F   | 21  | 3.112                             | 55.993                               | 3                                           | 55              | 65              | 49         |
| 18           | F   | 21  | 2.647                             | 41.399                               | 4                                           | 40              | 48              | 44         |
| 19           | F   | 22  | 3.289                             | 18.918                               | 5                                           | 52              | 58              | 49         |
| 20           | F   | 22  | 2.439                             | 54.330                               | 8                                           | 74              | 65              | 65         |
| 21           | F   | 21  | 2.684                             | 39.900                               | 5                                           | 48              | 73              | 53         |
| 22           | F   | 22  | 2.795                             | 16.654                               | 10                                          | 44              | 44              | 45         |
| 23           | F   | 23  | 2.256                             | 38.112                               | 6                                           | 34              | 37              | 34         |
| 24           | F   | 23  | 2.766                             | 21.551                               | 5                                           | 35              | 51              | 36         |
| 25           | F   | 22  | 2.349                             | 2.814                                | 4                                           | 28              | 44              | 35         |
| 26           | F   | 22  | 2.386                             | 4.684                                | 5                                           | 48              | 50              | 47         |
| 27           | F   | 21  | 2.654                             | 27.810                               | 3                                           | 35              | 36              | 30         |
| 28           | F   | 22  | 1.523                             | 7.837                                | 2                                           | 35              | 36              | 29         |
| 29           | F   | 22  | 1.507                             | 11.752                               | 4                                           | 49              | 42              | 36         |
| 30           | F   | 22  | 4.255                             | 6.369                                | 7                                           | 50              | 51              | 43         |
| 31           | F   | 22  | 3.140                             | 32.152                               | 11                                          | 32              | 33              | 38         |
| 32           | F   | 22  | 2.600                             | 36.522                               | 12                                          | 56              | 65              | 49         |
| 33           | F   | 22  | 3.061                             | 6.363                                | 4                                           | 25              | 27              | 35         |
| 34           | F   | 23  | 2.270                             | 55.168                               | 1                                           | 37              | 47              | 40         |
| 35           | F   | 21  | 1.727                             | 15.860                               | 6                                           | 59              | 56              | 47         |
| 36           | F   | 22  | 2.043                             | 33.741                               | 2                                           | 34              | 41              | 39         |
| 37           | F   | 22  | 2.460                             | 45.264                               | 13                                          | 54              | 69              | 50         |
| 38           | F   | 21  | 1.532                             | 45.650                               | 10                                          | 59              | 58              | 48         |
| 39           | F   | 22  | 2.980                             | 68.537                               | 5                                           | 45              | 43              | 40         |
| 40           | F   | 22  | 2.080                             | 30.363                               | 4                                           | 41              | 45              | 41         |
| 41           | F   | 21  | 2.669                             | 47.849                               | 3                                           | 49              | 50              | 45         |
| 42           | F   | 21  | 3.069                             | 56.014                               | 6                                           | 49              | 44              | 51         |
| 43           | F   | 22  | 1.469                             | 1.274                                | 7                                           | 43              | 45              | 39         |
| 44           | F   | 22  | 2.929                             | 16.917                               | 5                                           | 41              | 44              | 41         |
| 45           | F   | 23  | 3.029                             | 63.612                               | 5                                           | 60              | 64              | 50         |
| 46           | F   | 21  | 3.490                             | 45.140                               | 8                                           | 55              | 42              | 41         |
| 47           | F   | 21  | 2.414                             | 6.338                                | 2                                           | 38              | 40              | 37         |
| Average      |     |     | 21.8                              | 29.5997                               | 5.5                                          | 44.5            | 48.2            | 42.0       |
| SD           |     |     | 0.617                             | 18.2307                               | 3.1                                          | 10.0            | 10.3            | 7.3        |
Salivary melatonin (pg/mL)

Figure 2. Comparison of salivary melatonin levels during the daytime and night. The salivary melatonin level at night (29.600 ± 18.231 pg/mL) was significantly higher than that during the daytime (2.457 ± 0.617 pg/mL).

Figure 3. Correlations between the indices and salivary melatonin levels during the night. (A–D) represent the relationships between salivary melatonin levels during the night and Pittsburgh Sleep Quality Index (PSQI), State Anxiety, Trait Anxiety, and Self-Rating Depression Scale (SDS) scores, respectively. Significant correlations were noted between salivary melatonin levels during the daytime and State Anxiety, Trait Anxiety, and SDS scores. *P < 0.05.

Relationship between State Anxiety scores and salivary melatonin levels

The State Anxiety scores received from the participants ranged between 25 and 74 (mean: 44.5 ± 10.0). Twenty-one females received 41 or fewer points (moderate level of anxiety), and twenty-six scored 30.486 ± 18.9753 pg/mL for the 5-or-lower group and 28.2928 ± 17.4997 pg/mL for the 6-or-higher group. There was no significant difference between the two groups (U(28,19) = −0.2818; P = 0.7781) in which the quality of sleep varied (Fig. 5A). The Cohen’s d between both groups was 0.119 signifying negligible effect. The melatonin level during the daytime was 2.4204 ± 0.5158 pg/mL for the 5-or-lower group and 2.5112 ± 0.7543 pg/mL for the 6-or-higher group; there was no significant difference attributed to the varying quality of sleep between the two groups (U(28,19) = −0.4986; P = 0.6181; Fig. 6A). The Cohen’s d between both groups was 0.146 signifying negligible effect.
42 or higher (high level of anxiety). There was a significant correlation between State Anxiety scores involving all participants (n = 47) and salivary melatonin levels at night; no correlation was noted between State Anxiety scores and melatonin levels during the daytime (correlation coefficient $= 0.4161$, $P = 0.0036$; Fig. 3B) or at night (correlation coefficient $= 0.1155$, $P = 0.4396$; Fig. 4B). The participants were divided into two groups: those with a State Anxiety score of 41 or lower (n = 21) and those with 42 or higher (n = 26). Salivary melatonin levels at night were compared for each group. The melatonin level at night was $24.5812 \pm 14.6538$ pg/mL for the 41-or-lower group and $33.6532 \pm 20.0376$ pg/mL for the 42-or-higher group; there was no significant difference between the two groups ($U(21,26) = -0.9415; P = 0.3464$; Fig. 6B). The Cohen’s $d$ between both groups was 0.260 signifying a small effect.

### Relationship between Trait Anxiety scores and salivary melatonin levels

The Trait Anxiety scores received from the subjects ranged between 27 and 73 (mean: $48.2 \pm 10.3$) points. Twenty females received 44 or fewer points (moderate level of anxiety), and 27 scored 45 or higher (high level of anxiety). Although there was a significant correlation between Trait Anxiety scores received by all participants (n = 47) and salivary melatonin levels at night (correlation coefficient $= 0.3808$, $P = 0.0083$) (Fig. 3C), no correlation was noted between Trait Anxiety scores and melatonin levels during the daytime (correlation coefficient $= 0.1150$, $P = 0.4416$;
The participants were divided into two groups: those with a Trait Anxiety score of 44 or lower (n = 20) and those with a score 45 or higher (n = 27). Salivary melatonin levels at night were compared for each group. The melatonin level at night was 26.39 ± 18.25 pg/mL for the 44-or-lower group and 31.97 ± 18.19 pg/mL for the 45-or-higher group; there was no significant difference between the two groups (U (20,27) = −1.0973; P = 0.2725; Fig. 5C). The Cohen’s d between both groups was 0.307 signifying a small effect. The melatonin level during the daytime was 2.46 ± 0.57 pg/mL for the 44-or-lower group and 2.43 ± 0.66 pg/mL for the 45-or-higher group; there was no significant difference between the two groups (U (20,27) = −0.0215; P = 0.9828; Fig. 6C). The Cohen’s d between both groups was 0.002 signifying negligible effect.

Relationship between SDS scores (depression levels) and salivary melatonin levels

The SDS score ranged between 29 and 65 (mean: 42.0 ± 7.3). Nineteen participants received a score of 39 or lower (a moderate degree of depression) and 28 received 40 or higher (a high degree of depression). Figure 3D shows that there was a significant correlation between the total SDS score of all participants (n = 47) and melatonin levels at night (correlation coefficient = 0.3775, P = 0.0089). Figure 4D, on the other hand, shows a moderate correlation between the total SDS score and melatonin levels during the daytime (correlation coefficient = 0.2712, P = 0.0651). The participants were divided into two groups: those with an SDS score of 39 or lower (n = 19) and those...
with a score of 40 or higher (n = 28). Salivary melatonin levels at night were compared for each group. Figure 5D shows the melatonin level at night was 22.7979 ± 15.3805 pg/mL for the 39-or-lower group and 34.6381 ± 18.7935 pg/mL for the 40-or-higher group; there was a significant difference between the two groups (U(19,28) = −2.6447; P = 0.0082). The Cohen’s d between both groups was 0.841 signifying a large effect. Figure 6D shows the melatonin level during the daytime was 2.2123 ± 0.5060 pg/mL for the 39-or-lower group and 2.6384 ± 0.6374 pg/mL for the 40-or-higher group; there was a significant difference between the two groups (U(19,28) = −2.4279; P = 0.0152). The Cohen’s d between both groups was 0.707 signifying a large effect.

Discussion

In a healthy person, a larger amount of melatonin is excreted by the pineal body at night because it is regulated by signals sent by the suprachiasmatic nucleus. As melatonin controls circadian rhythms through the MT₁ and MT₂ melatonin receptors, the amount of melatonin secretion is considered to affect the quality of sleep. Since MT₂ melatonin receptors play an important role in helping a person fall asleep naturally,
the administration of melatonin and MT$_2$ receptor antagonists is believed to be effective. On the other hand, people with depression are advised to be exposed to bright sunlight in the morning, which sends signals to the suprachiasmatic nucleus and lowers the melatonin level. In general, blood and salivary melatonin levels increase when a person is lying down in the dark at night, and, therefore, the level of secretion is low when a person is awake and sitting in a well-lit room. This is the environment adopted in the present study. Furthermore, the research participants were Japanese people, who usually live in bright rooms with a luminance level of 300 to 750 lux (Japanese Industrial Standards for Residential Lighting). For this reason, in the present study, salivary melatonin levels in most participants were low even during the night, and the distribution may have been biased.

The present study aimed to examine whether or not salivary melatonin levels serve as an index to determine the quality of sleep or an effective psychological stress endogenous biomarker. In the present experiment, the relationship was noted between the quality of sleep and salivary melatonin levels. It should be noted, however, that depression is a type of psychological stress common in young Japanese females. Depression-related scores were significantly correlated with salivary melatonin levels at night, and showed a moderate correlation with salivary melatonin levels during the daytime, which suggested that the salivary melatonin level can be useful as a biomarker of depression. If the biomarker can be applied to the prevention of psychiatric disorders, it will be an effective preventive medicinal approach. As salivary melatonin levels show a strong correlation with blood plasma levels, circadian rhythms can be assessed based on changes in salivary melatonin levels. This is because melatonin, whose serum half-life is short at approximately 28.4 minutes, does not accumulate in the blood, and, therefore, the melatonin detected can be determined as recently synthesized. Although the participants of the study had no subjective symptoms of sleep disorders, the results of the SDS and STAI suggest that they felt a certain level of psychological stress. Salivary melatonin levels at night were correlated not only with depression scores, but also anxiety scores. However, the State Anxiety scale aims to determine a psychological state at one point, whereas the Trait Anxiety scale scores the traits of persons vulnerable to anxiety. For this reason, salivary melatonin levels showed a weaker correlation with anxiety than depression scores.

The results suggest that the sense of depression, as well as living environments and lifestyle habits which induce it, increase salivary melatonin levels for two possible reasons: anxiety increases oxidative stress in the body and therefore a larger volume of melatonin is produced as its antioxidant action effectively protects the body; and the effects of melatonin, as a ligand, on its receptors decrease. With the aim of promoting the application of salivary melatonin to preventive medicine, its relationships with psychiatric conditions will be examined.

Author Contributions
Conceived and designed the experiments: YI, TI, RT. Analyzed the data: YI, TI, YY, MT, YN. Wrote the first draft of the manuscript: YI. Contributed to the writing of the manuscript: MT, KK, YN, RT. Agree with manuscript results and conclusions: YI, TI, YY, YN, KK, YN, RT. Jointly developed the structure and arguments for the paper: YI, TI, YN, RT. Made critical revisions and approved final version: YI, TI, RT. All authors reviewed and approved of the final manuscript.

Funding
This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports Culture and Technology of Japan [23500823] and Fujita Health University Grant.

Competing Interests
Author(s) disclose no potential conflicts of interest.

Disclosures and Ethics
As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.
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