Influence of eye colors of Caucasians and Asians on suppression of melatonin secretion by light

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Running head: human eye color and melatonin suppression by light
Abstract

This experiment tested effects of human eye pigmentation depending on the ethnicity on suppression of nocturnal melatonin secretion by light. Ten healthy Caucasian males with blue, green or light brown irises (light-eyed Caucasians) and eleven Asian males with dark brown irises (dark-eyed Asians) volunteered to participate in the study. The mean ages of the light-eyed Caucasians and dark-eyed Asians were 26.4 ± 3.2 and 25.3 ± 5.7 years, respectively. The subjects were exposed to light (1000 lx) for two hours at night. The starting time of exposure was set to two hours before the time of peak salivary melatonin concentration of each subject, which was determined in a preliminary experiment. Salivary melatonin concentration and pupil size were measured before exposure to light and during exposure to light. The percentage of suppression of melatonin secretion by light was calculated. The percentage of suppression of melatonin secretion two hours after the start of bright light exposure was significantly larger in light-eyed Caucasians (88.9 ± 4.2%) than in dark-eyed Asians (73.4 ± 20.0%) (p < 0.01). No significant difference was found between pupil sizes in light-eyed Caucasians and dark-eyed Asians. These results suggest that sensitivity of melatonin to light suppression is influenced by eye pigmentation and/or ethnicity.

Keywords: Pigmentation, retina, iris, circadian rhythm, ethnic
Introduction

Melatonin is secreted from the pineal gland during the subjective night, and its secretion is controlled by the suprachiasmatic nucleus (SCN), which is the central circadian pacemaker. In 1980, it was demonstrated that the nocturnal melatonin was suppressed during exposure to bright light at 2500 lx in humans (25). More recent studies have shown that human nocturnal melatonin secretion can be suppressed by exposure to a light of several hundred luxes (2, 27, 38). It has been reported that there are individual differences in melatonin suppression by light (15, 23, 24, 31). The magnitude of melatonin suppression by light is influenced by several factors, such as recent history of exposure to light (13, 31), season (14, 30), seasonal affective disorder (29, 33) and delayed sleep phase syndrome (1). However, attention has not given to the effect of eye color and ethnicity on melatonin suppression.

Some studies have shown that physiological responses to light depend on eye color. It has been shown that intraocular light scattering is greater in blue-eyed Caucasians than in brown-eyed people (17) and that blue eyes permitted increased light transmittance through the iris and the surrounding eye wall (34). As for patients with seasonal affective disorder, it has been reported that blue-eyed patients show a large summertime increase in cone sensitivity in comparison to darker-eyed patients (32) and that darker-eyed patients are significantly more depressed and fatigued than blue-eyed patients (12). Furthermore, it has been suggested that brown/black-eyed people show delayed chronotype and sleep phase in comparison to
blue/gray-eyed people (36). There have also been some studies showing ethnic differences in sleep and depression (19, 22). These previous studies suggest that the eye color may affect the circadian modulation to light and may affect suppression of nocturnal melatonin secretion in the pineal gland.

We therefore hypothesized that the magnitude of melatonin suppression is larger in Caucasians with blue and light brown eyes than in Asians with dark brown eyes. The purpose of the present study was to examine the influence of eye pigmentation on suppression of nocturnal melatonin secretion by exposure to light.

**Methods**

The study was conducted in Japan in the winter. The subjects were ten Caucasian males with blue/green irises or light brown irises (light-eyed Caucasians) and eleven Asian males with dark brown irises (dark-eyed Asians). The mean ages of the light-eyed Caucasians and dark-eyed Asians were 26.4 ± 3.2 and 25.3 ± 5.7 years, respectively. All of the subjects were researchers or teachers or university students, and they spent most of the day time indoors. All subjects had been living in Akita for at least six months. The subjects gave written informed consent for participation in the study, which was approved by the Ethics Committee of Akita University School of Medicine. Before the experiment, each subject completed a questionnaire on morningness-eveningness preferences (16) and sleep
habits (habitual bedtime, habitual rising time, etc.). None of the subjects had any sleep complaints, and none of the subjects were taking medications or working night shifts. Characteristics of each group are shown in Table 1.

Beginning one week prior to the start of the experiment, the subjects were instructed to keep their habitual sleep-wake rhythms and habitual daily activity pattern. Subjects were instructed not to consume caffeine-containing drinks or alcohol and not to perform intense exercise 24 hours prior to the experiments.

It is necessary to know the nocturnal phase of melatonin secretion of each subject to select the timing of exposure to bright light. The period of exposure to light was set to the rising phase of melatonin secretion of each subject. In a baseline experiment, we measured dim-light salivary melatonin concentration at night. Each subject spent at night (21:00 to 6:00) under dim light (DL) (<15 lx) in the experimental room, and saliva samples were collected every hour to determine the phase of salivary melatonin concentration. Participants were allowed to sleep during the experiment. A strong correlation between circadian variation in saliva and plasma melatonin concentration has been confirmed (28, 35). The saliva samples were kept frozen at –30 °C until analysis. After centrifugation (for 5 min at 3000 rpm), melatonin concentrations in the saliva samples were determined using an ELISA kit (Direct Saliva Melatonin ELISA, Bühlmann, Switzerland). One Asian and one Caucasian subject were excluded because their peak melatonin concentration could not be
detected. The mean times of peak salivary melatonin concentration of the light-eyed group and dark-eyed group were 3:20 ± 1:19 and 4:33 ± 1:13, respectively. This difference was not significant.

The experiment for determining the percentage of melatonin suppression was conducted within 2 weeks after the preliminary experiment. The protocol of the second experiment was based on those of previous studies (1). The subjects were instructed to keep their habitual sleep-wake rhythm and habitual daily activity pattern. The subjects came to the laboratory 5.5 hours before the time of peak melatonin concentration determined in the baseline experiment, and they were asked to sleep for three hours starting five hours before the time of peak melatonin concentration. Then they were awakened and exposed to bright light (1000 lx) for two hours. The starting time of exposure was set to two hours before the time of peak salivary melatonin concentration of each subject, which was determined in a preliminary experiment. White fluorescent lamps (FLR-40S W/M 4200K, Matsushita Electric Industrial Co. Ltd., Osaka, Japan) placed on the ceiling were used for light sources. The illuminance level in the direction of gaze was measured at the subject’s eye level using a light meter (CL-200, KONICA MINOLTA HOLDINGS, INC., Tokyo, Japan). During the exposure, each subject sat on a chair and watched an unexciting movie to fix his eyes on a 15 inch LCD display placed about 2 meters in front of the subject. The illuminance level from the LCD display was very small (< 5 lx) and the effect of LCD display on melatonin
suppression was thought to be negligible small. Saliva samples were collected before sleep and before exposure to the light and every hour during the period of exposure. Based on the value before exposure to light, the absolute change of melatonin suppression and percentage of melatonin suppression were calculated. The percentage of suppression of melatonin concentration induced by the light was defined as \[
\frac{(\text{melatonin concentration before exposure to light} - \text{melatonin concentration after exposure to light})}{\text{melatonin concentration before exposure to light}} \times 100.
\]

Pupil size of the right eye was measured before light exposure and every hour during the exposure to light using an electronic pupillometer (DK-101, Scalar Corporation, Tokyo, Japan).

Student’s t-test was used to assess the statistical significance of differences. Welch’s test was also used when variance of two groups was not equal.

**Results**

Changes in salivary melatonin concentration before exposure and during exposure to the light in each subject are shown in Fig. 1. Salivary melatonin concentrations significantly increased before exposure to light and then decreased after exposure to light in both groups. There were large individual differences in salivary melatonin concentration. The mean values and standard deviations of melatonin concentration are shown in Table 1. Although
mean melatonin concentration before exposure to light in dark-eyed Asians was large in
comparison to that in light-eyed Caucasians, no significant difference was found. Only
salivary melatonin concentration two hours after exposure to light was significantly larger in
dark-eyed Asians (17.4 ± 3.6 pg/ml) than in light-eyed Caucasians (6.05 ± 1.46 pg/ml)
(p<0.05, Welch’s test). Mean absolute change of melatonin suppression one hour and two
hours after the start of exposure to light were larger in dark-eyed Asians than in light-eyed
Caucasian, but no significant differences were found (Table 1).

Fig. 2 shows the changes in percentages of melatonin concentration in each subject.
The values are expressed as percentage of melatonin suppression based on the value before
exposure to light (0 h). There were large individual variations in the percentages of
melatonin suppression in dark-eyed Asians in comparison to light-eyed Caucasians. No
significant difference was found between the percentages of suppression of melatonin
concentration one hour after the start exposure to light in light-eyed Caucasians (69.8 ±
12.5%) and dark-eyed Asians (52.9 ± 26.5%). However, the percentage of suppression two
hours after the start of exposure to light was significantly larger in light-eyed Caucasians
(88.9 ± 4.2%) than in dark-eyed Asians (73.4 ± 20.0%) (p < 0.01, Welch’s test).

Fig. 4 shows the changes in pupil size. Pupil size significantly decreased after exposure
to light. No significant difference was found between pupil size in light-eyed Caucasians
and dark-eyed Asians.
Discussion

A difference between melatonin suppression in light-eyed Caucasians and that in dark-eyed Asians was found in percentage change but not in absolute change of melatonin suppression. Since percentage values have been used to evaluate melatonin suppression by light in most previous studies (1, 6, 11, 13, 15, 29, 30, 33, 38), it seems to have been difficult to evaluate melatonin suppression using absolute values, the main reason being large individual difference in melatonin secretion level. In the present study, absolute values of melatonin suppression became large when melatonin level was large before light exposure. A strong correlation was found between melatonin level before light exposure and absolute change in melatonin suppression during light exposure ($r=0.96$, $n=19$, $p<0.01$). This means that the large absolute changes in melatonin suppression during light exposure in dark-eyed Asians were caused by large melatonin levels before light exposure. Therefore, using percentage of melatonin suppression is reasonable for comparing melatonin suppression by light because the effects of individual differences in melatonin level before exposure to light can be excluded. However, it is not easy to conclude which value (absolute change or percentage change) is more significant in terms of the human circadian system or photo physiology. Integration with additional case would be useful to have reliable data.

In the present study, it was found that the percentage of melatonin suppression by
exposure to light was significantly larger in light-eyed Caucasians than in dark-eyed Asians.

What is the possible cause of the eye color-dependent difference found in the present study? A previous study has shown that intraocular light scattering, which is called straylight, is dependent on pigmentation of eye (17). Straylight in blue-eyed Caucasians was larger than that in darker-eyed non-Caucasians. It has been reported that light transmittance through the iris is higher in light-blue-eyed people than in dark-brown-eyed people (34). It has been suggested that eye color-dependent difference in straylight is caused by not only light transmittance through the eye wall but also reflection from the fundus oculi (34). There is a retinal pigment epithelium in the fundus oculi. One of the roles of the retinal pigment epithelium is to absorb light and to prevent light scattering. The color of fundus oculi is brownish-red in dark-brown-eyed Asians and orange in light-blue-eyed Caucasians. Therefore, the amount of dispersion of light in fundus oculi is thought to be larger in light-eyed Caucasians with less pigmentation than in dark-eyed people. Recent studies have been shown the existence of photosensitive retinal ganglion cells as circadian photoreceptors, in addition to rods and cones (4, 5). In the present study, these photoreceptors might have received more light by dispersion of light in light-eyed Caucasians. Thus, larger suppression of melatonin secretion is thought to be induced in light-eyed Caucasians.

Individual difference in melatonin suppression in light-eyed Caucasians was small, but a large individual difference was found in dark-eyed Asians. The small individual difference
in light-eyed Caucasians could be caused by the ceiling effect. It is known that there is a
dose-response relationship between light intensity and melatonin suppression (2, 6, 27, 38)
and that saturation of the melatonin suppression response was occurred by exposure to bright
light (38). Therefore, it is thought that small individual variation at higher percentage of
melatonin suppression in light-eyed Caucasians is caused by saturation of melatonin
suppression response.

In the present study, the percentage of melatonin suppression in some dark-eyed Asians
were as large as those in light-eyed Caucasians, indicating that some ethnic factors other than
eye color may have contributed to the difference between melatonin suppression in dark-eyed
Asians and that in light-eyed Caucasians. Further study is needed to examine the effect of
eye color and ethnic separately. Furthermore, light history of the subjects (13, 31) may have
contributed to the difference between melatonin suppression in dark-eyed Asians and that in
light-eyed Caucasians. It is needed to control this confounding factor in the future to test the
effects of eye color on melatonin suppression. It is known that pupil size also contributes to
suppression of melatonin secretion. It has been reported that magnitude of melatonin
suppression is larger when the pupil is dilated (7, 11). In the present study, there was no
significant difference between pupil size in light-eyed Caucasians and in dark-eyed Asians.
Therefore, it is thought that the eye color-dependent difference in melatonin suppression was
not caused by pupil size.
In the present study, we could not match the habitual bed time and sleep time of the two groups. Since it has been reported that there was no correlation between habitual bed time and magnitude of suppression of melatonin by light (15), the differences in sleep habits between two groups may not affect melatonin suppression by light in the present study. On the other hand, it has been reported that eye color may affect sleep habits (36). Although the reliability of that study is uncertain since data were collected using the Internet, it was found that wake-up time in light-eyed groups was earlier than that in the dark-eyed group. In the present study, the bed time in blue/gray-eyed subjects was earlier than that in brown/black-eyed subjects. It is known that various cultural and social factors affect human sleep habits, and it is impossible to conclude causal relationships between eye colors and habitual bed time from the small sample in the present study.

Variations in physiological traits of humans have been induced by human adaptation to given environments. It is known that white skin color in Caucasian is the result of adaptation to a short duration of sunshine (3). White skin facilitates absorption of ultraviolet rays, promotes vitamin D synthesis and prevents rickets in high-latitude places with a short duration of sunshine in winter. Although it is not clear whether Caucasians acquired blue eyes as a result of adaptation to a short duration of sunshine, blue eyes might have been advantageous for prevention of seasonal affective disorders in ancient times without artificial lighting.
It has been reported that illuminance and spectrum of artificial light affects various human physiological response, such as body temperature, heart rate, alertness and sleep and circadian rhythm (8-10, 18, 20, 21, 26, 37). The attention to the eye color or ethnic difference has been not paid in these previous studies. It is interesting to study the effects of eye color or ethnic difference on such physiological responses to light in the future.

In summary, there was significant difference between suppression of melatonin secretion in light-eyed Caucasians and that in dark-eyed Asians. The percentage of melatonin suppression in light-eyed Caucasians was significantly larger than that in dark-eyed Asians. These results suggest that the variation of melatonin suppression by light is related to eye pigmentation and/or ethnicity.

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Legends to figures

Figure 1. Changes in salivary melatonin concentration before and after exposure to light in each subject (thin lines). 0 hours means two hours before the time of peak melatonin concentration in each subject. The mean value of melatonin concentration

Figure 2. Individual variations and changes in percentages of melatonin suppression after exposure to light. Each value is the percentage of suppression based on the value before exposure to light (0 hours).

Figure 3. Changes in mean percentages of melatonin suppression after exposure to light. Open circles show data for light-eyed Caucasians and closed circles show data for dark-eyed Asians. The percentage of suppression two hours after the start of exposure to light was significantly larger in light-eyed Caucasians (88.9 ± 4.2%) than in dark-eyed Asians (73.4 ± 20.0%) (p < 0.01). All data are expressed as means + S.D.

Figure 4. Pupil size before and after exposure to light. No significant difference was found between pupil size in light-eyed Caucasians and dark-eyed Asians. All data are expressed as means + S.D.
Table 1. Characteristics of the subjects

<table>
<thead>
<tr>
<th></th>
<th>Light-eyed Caucasians n = 9</th>
<th>Dark-eyed Asians n = 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>26.4 (3.2)</td>
<td>25.3 (5.7) Ns</td>
</tr>
<tr>
<td>ME Score*</td>
<td>54.0 (8.5)</td>
<td>51.3 (8.5) Ns</td>
</tr>
<tr>
<td>Bedtime (clock time)</td>
<td>23:42 (0:37)</td>
<td>00:57 (0:38) p&lt;0.01</td>
</tr>
<tr>
<td>Rising time (clock time)</td>
<td>07:44 (0:50)</td>
<td>07:41 (0:39) Ns</td>
</tr>
<tr>
<td>Sleep hour (hour)</td>
<td>8.04 (0.99)</td>
<td>6.73 (0.46) p&lt;0.01</td>
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</table>

* morningness-eveningness preference score

All data are expressed as means (+/- standard deviation)
Table 2. Changes in melatonin concentration before exposure (0 hours) and during exposure to light (1 hour and 2 hours)

<table>
<thead>
<tr>
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<th>0 hour (pre exposure)</th>
<th>1 hour</th>
<th>2 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light-eyed Caucasians</td>
<td>Dark-eyed Asians</td>
<td>Light-eyed Caucasians</td>
</tr>
<tr>
<td>Melatonin concentration (pg/ml)</td>
<td>54.3 (10.85)</td>
<td>78.8 (11.9)</td>
<td>18.9 (5.34)</td>
</tr>
<tr>
<td>Absolute change of melatonin suppression (pg/ml)</td>
<td>-</td>
<td>-</td>
<td>35.5 (18.9)</td>
</tr>
<tr>
<td>Percentage of melatonin suppression (%)</td>
<td>-</td>
<td>-</td>
<td>69.8 (12.5)</td>
</tr>
</tbody>
</table>

All data are expressed as means (+/- standard deviation)
Fig. 1

![Graph showing melatonin concentration (pg/ml) vs Relative clock (hour) for Light-eyed Caucasians and Dark-eyed Asians.]
Fig. 2

Percentage of melatonin suppression (%)

Relative clock (hour)

Light-eyed Caucasians

Dark-eyed Asians

Light

Light
Fig. 3

- Dark-eyed Asians
- Light-eyed Caucasians

Percentage of melatonin suppression (%)

Relative clock (hour)

*
Fig. 4

![Graph showing pupil size (mm²) before and after exposure for Light-eyed Caucasians and Dark-eyed Asians.]

- Light-eyed Caucasians
- Dark-eyed Asians

Before exposure

After exposure