

# The Consideration Of Melatonin Concentration and Subjective Evaluation in the Various Bathing Methods

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## Summary

**Aims:** In Japan, although evening bathing as a means of both cleaning and relaxation has been a well-established custom from ancient times, the prevalence of morning baths has increased in Japan in recent years. The effects of morning bathing on human sleep quality have not yet been revealed. Thus, we aimed to clarify the effects different bathing method on human sleep quality.

**Methods:** The first night, the subject entered the laboratory at 19:00 and went to bed at 24:00. On the second day, the subject got up at 07:00 and either showered, took a mist sauna, or did not bathe, between 07:10 and 07:20. Each hour after that, the subject completed an alpha attenuation test (AAT), Kwansei-Gakuin sleeping scale (KSS) test and subjective evaluations from 09:00 to 17:00. We measured the melatonin concentrations at 22:30, 23:00, 23:30, and 24:00 of the second day. On the third day, the subject got up at 07:00 and finished at 07:30. Key finding: We found that the melatonin concentrations after morning showering were significantly lower than those after no bathing at 23:00, 23:30, and 24:00. In addition, the selections of “coziness” on the subjective evaluation after the mist sauna were significantly higher than those after no bathing at 7:20. The selection of “fatigue” after the mist sauna bathing was significantly lower than that after showering during the task period at 14:00.

**Significance:** Our findings indicate that the morning mist sauna bathing method produced more good feelings and prevented fatigue compared to other bathing methods.

**Keywords:** Melatonin; Showering; Mist sauna; Quality of sleeping

## Introduction

In Japan, evening bathing as a means of both cleaning and relaxation has been a well-established custom from ancient times. The effects of bathing, its environment, the method of bathing, the time of day and similar details have been studied extensively in Japan. For example, Lee et al. [1] reported that both a full immersion bath and a mist sauna are effective in facilitating recovery from muscle fatigue. Sauna therapy was shown to improve the cardiac index, mean pulmonary wedge pressure, systemic and pulmonary vascular resistance, and cardiac function [2]. Furthermore, Hashiguchi et al. [3] reported that after bathing, low relative humidity and high air velocity have negative effects on humans, i.e., a decrease in body temperature and dryness of the skin and eyes.

On the other hand, taking a bath in the morning has increased in prevalence in Japan in recent years. According to a 2011 internal study by the Urban Research Institute (Tokyo Gas Co., Tokyo), many people in Japan are switching from bathing at the end of the day to a morning shower (more than 4 times/week: 15.8%, 1-3 times/week: 23.2% in summer, n=3,267). In a previous study, we evaluated the effects of morning bathing on human physiological function and work efficiency during the day. We found that compared to other bathing methods, a morning mist sauna was safe and maintains the skin temperature. Moreover, it was found that morning mist sauna bathing improves work efficiency compared to other bathing methods during the day following the morning bathing [4]. However, the effects of morning bathing methods on people's sleep quality have not yet been revealed.

According to Akerstedt et al. [5] melatonin controls circadian rhythms through the MT1 and MT2 melatonin receptors, and the amount of melatonin secretion is thought to affect the quality of sleep. MT2 melatonin receptors play an important role in helping a person fall asleep naturally, and the administration of melatonin and

MT2 receptor agonists is thought to aid sleep [6,7]. In light of these findings, in the present study we focused on melatonin and one of the factors responsible for sleep quality, thermoregulation. As adequate sleep is an important public health issue, we investigated sleep quality and the relationships between morning bathing methods and salivary melatonin levels measured after morning bathing, including showering, mist sauna bathing, and no bathing as a control.

## Materials and Methods

### Subjects

Ten male healthy students ( $24 \pm 3$  yrs,  $174 \pm 6.1$  cm,  $70 \pm 7.6$  kg) participated in this study. The subjects were asked to maintain their regular sleep-wake cycle during the 7 days of the experiment. Informed consent for participation in the study, which was approved by the bioethics committee of the Graduate School of Engineering, Chiba University, was obtained from all subjects.

### The conditions of the experiment

**We investigated three bathing methods:** Showering (S), mist sauna (M) and no bathing (C) as the control. For each condition, the subjects

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Received: May 23, 2016; Accepted: June 07, 2016; Published: June 14, 2016

Citation: Lee S, Kadek HS, Fujimura H, Shimomura Y, Katsuura T (2016) The Effects of Different Bathing Methods on Melatonin Response and a Subjective Evaluation. *Physiother Rehabil* 1: 112. doi: [10.4172/2573-0312.1000112](https://doi.org/10.4172/2573-0312.1000112)

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spent three days and two nights in the laboratory. The temperature of the three conditions was controlled as follows: In the mist sauna condition the subject was splashed with the mist of fine drops of warm water (40°C) in a sitting position. During showering (40°C), the subject was drenched in a sitting position outside the bathtub. In the no-bathing condition (air temp. 26°C), the subject sat on a chair outside the bathtub in the same manner as for the showering.

Three experiments were conducted at the same time of day on separate days. The protocol consisted of three experiments on three different days which were 1 week apart from session to session. The order of the three conditions was counterbalanced among the 10 subjects. We provided each subject with the same diet. However, the menus of dinner, breakfast, and lunch were different.

### Experimental environment

The air temperature and relative humidity of the laboratory were controlled at 26°C and 50%, respectively. The laboratory consisted of a pre-room and a bathroom. The subjects were in the pre-room at all times except for urination, bowel movements, and bathing. The lighting of the pre-room was controlled as follows: 7:00 to 9:00 at 300 lx, 9:00 to 17:00 at 800 lx, and 17:00 to 24:00 at 100 lx. The subjects bathed at 7:00 on the second day.

### Procedure

The first night, the subject entered the laboratory at 19:00 and went to bed at 24:00. On the second day, he got up at 07:00, and took a bath (showering, mist sauna bathing, or no bathing) between 07:10 and 07:20. To avoid sleeping later in the day, the subjects performed an alpha attenuation test (AAT), a Stroop color-word test, and a mental arithmetic task every hour from 09:00 to 17:00. We obtained saliva from each subject to measure the salivary melatonin concentration at 22:30, 23:00, 23:30 and 24:00 of the second day. In addition, the subjects took the Kwansai-Gakuin sleeping scale (KSS) test before and after the mental task as for drowsiness level and also a subjective evaluation mental test after it. The KSS is a scale developed for the Japanese based on the Stanford Sleeping Scale (SSS) devised by Hoddes in 1972, and it consists of 22 items.

The protocol required neither eating nor drinking (except water) for 1 hr prior to the saliva collection, so that food debris would not stimulate salivation. On the third day, the subject got up at 07:00 and finished bathing/not bathing at 07:20 (Figure 1).

### Saliva collection

All of the subjects underwent night-time saliva sampling prior to going to bed when they were awake in a sitting position at 22:30, 23:00, 23:30 and 24:00 of the second day. The saliva was collected in a commercially available saliva-collecting tube (Salivette, Sarstedt, Nümbrecht, Germany). The saliva tube with plain cotton swabs placed into the subject's mouth and moved around until it became heavy (5 min), then placed back into the tube. The samples were then centrifuged (3000 rpm × 5 min) and the cotton plug discarded and frozen at -20°C for the later analysis of melatonin levels.

Melatonin samples (200 µL) were sent to a commercial laboratory (SRL, Tokyo) for analysis by radioimmunoassay kits (Bühlmann Laboratories, Allschwil, Switzerland) following the manufacturer's instructions. These kits use the Kennaway G280 antibody [8,9]. The intra-assay coefficient of variation for the melatonin concentration was <10%.

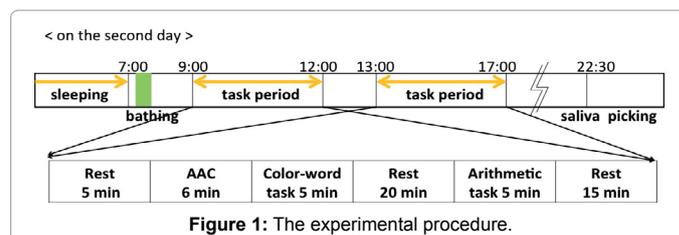


Figure 1: The experimental procedure.

### The subjective evaluation

For the subjective evaluation of the bathing methods, the subjects were asked to record their feelings with a visual analog scale (VAS) method. The contents of the subjective evaluation consisted of the items such as “comfort”, “coziness”, “feel refresh” and “fatigue”.

### The measurement items and the statistical analysis

We measured the subjects' salivary melatonin and subjective evaluation throughout the experiment. The second day salivary melatonin values and subjective evaluations were used as the parameter. For the salivary melatonin value was conducted a one-way repeated-measures analysis of variance (ANOVA) (bathing method factor) and the subjective evaluations values were conducted a two-way repeated-measures analysis of variance (ANOVA) (bathing method factor × time factor). When a significant F-value was found, we performed Bonferroni's test and Holm's post-hoc test. All statistical analyses were performed using SPSS 18.0J (SPSS, Tokyo). Probability values <0.05 were considered significant. Data are shown as mean ± S.E. unless otherwise stated.

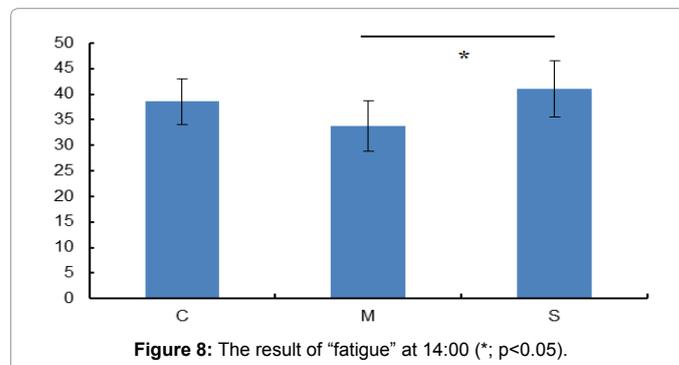
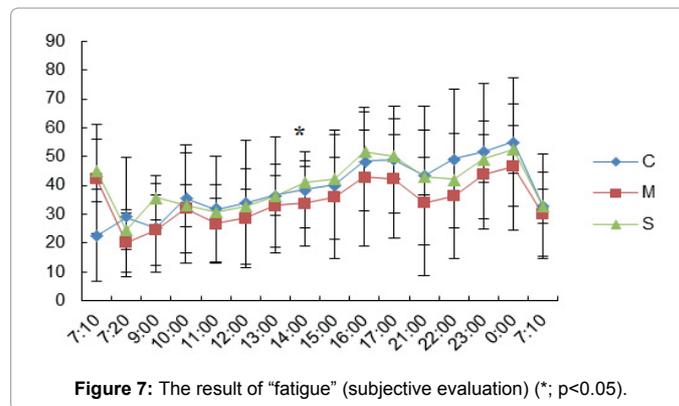
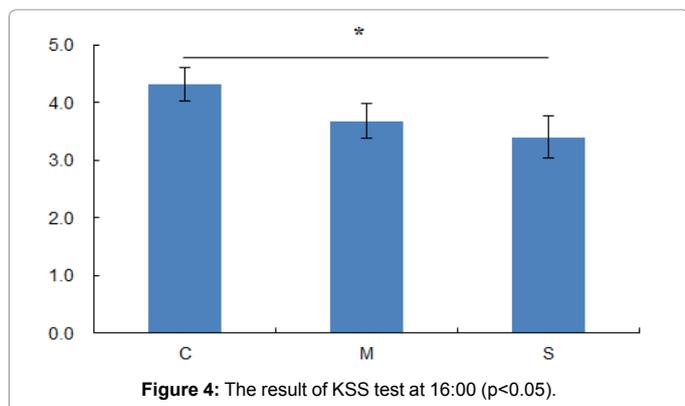
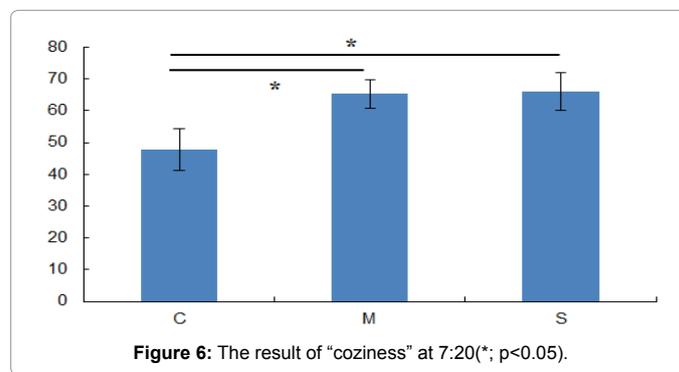
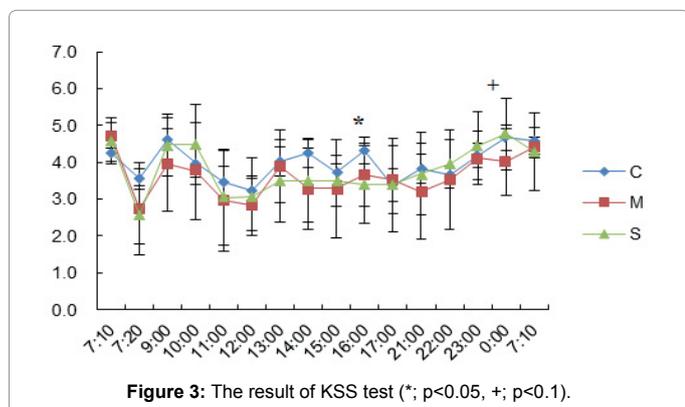
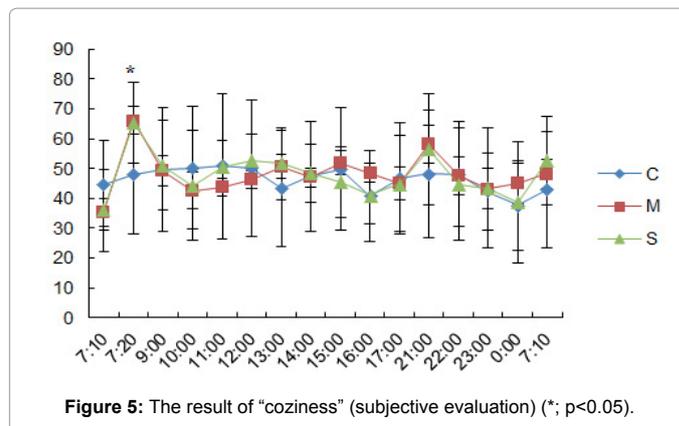
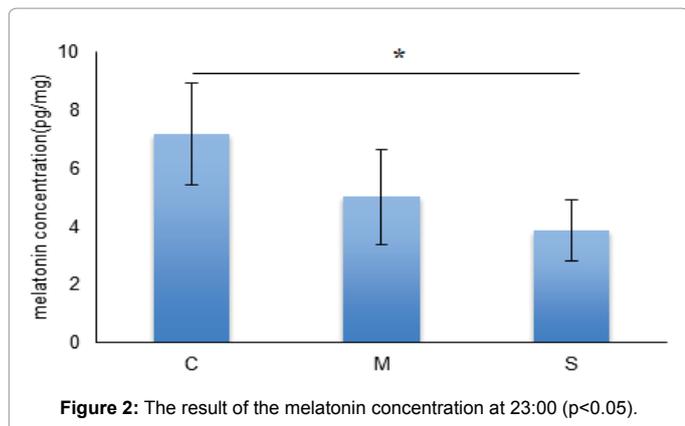
### Results

The melatonin concentrations in the shower condition were significantly lower than those in the no bathing condition at 23:00 ( $p=0.01$ ), 23:30 ( $p=0.025$ ), and 24:00 ( $p=0.038$ ) (Figure 2). The KSS test results were significantly lower in the shower condition compared to the no bathing condition at 16:00 and tended to be lower at 24:00 (Figures 3 and 4); this result is data of nine persons because one person missed his KSS test). The showering and mist sauna conditions also resulted in significantly higher “coziness” item in the subjective evaluation compared to the no-bathing condition at 7:20 ( $p<0.05$ ) (Figures 5 and 6). In addition, the fatigue levels in the mist sauna condition were significantly lower than those in the showering condition at 14:00 ( $p<0.05$ ) (Figures 7 and 8).

### Discussion

In the analysis of melatonin concentrations, a significant main effect was revealed for bathing condition ( $p<0.05$ ). The morning shower melatonin concentrations were significantly lower compared to those for no bathing at 23:00, 23:30, and 24:00. There was no significant difference between the morning mist sauna bathing and the shower and no-bathing conditions. Based on these results, we concluded that the morning mist sauna had no effect on sleeping. In addition, we suspected that the morning showering decreased the subjects' sleep quality compared to the other conditions.

The reasons for these results are difficult to determine because melatonin is affected by various factors such as the sleep/wake rhythm, the light condition, the bathing water temperature, thermoregulation, the subjects' mental stress, life habit, life environment and more. We thus further investigated factors that affect melatonin, in light of the following studies' findings.



Melatonin was reported to play an important role in the sleep/wake rhythm [10,11]. In addition, individuals 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 [12,13]. On the other hand, Deacon et al. [14] verified that salivary melatonin increases when a person is lying down in the dark at night and the secretion is low when a person is awake and sitting up in a well-lit room.

Furthermore, we investigated the relationship between melatonin and thermoregulation. The pineal gland with its melatonin hormone is implicated in thermoregulation. In certain species of rodents [15-18] and birds [19], melatonin administration increases non-shivering thermogenesis and improves cold resistance. In rats exposed to several types of environmental stimuli, a 3 min immersion in cold water was found to be the most potent stimulus increasing serum melatonin

concentrations [20]. In human case, since a number of winter swimmers report having taken up the practice of ice-water immersions to cure, among other things, insomnia [21], serum melatonin concentrations

have been measured to determine whether brief exposures to cold might induce changes in the excretion of this hormone known to promote sleep [22].

However, the above-described findings might not concern the melatonin concentration examined in the present study, because we controlled the environmental conditions (light, temperature), wake-sleep cycle, and temperature of the bathing water.

Meanwhile, Ito et al. [23] verified that the state of depression-as well as the living environments and lifestyle habits that induce it - increases salivary melatonin levels. In an earlier study, we found that a morning mist sauna was safe and that it maintains skin temperature compared to other bathing methods. We also found that the morning mist sauna bathing improves work efficiency compared to other bathing methods during the work day following the morning bathing [4]. In the present study, we verified that the morning showering and mist sauna were not effective improving from sleeping quality in this study. We concluded that changes in the melatonin were not observed because we did not provide controls for the subjects' mental stress and daily lifestyle.

The shower condition produced KSS test results were significantly lower than those obtained in the no-bathing condition at 16:00 and tended to be lower at 24:00. In the "coziness" item of the subjective evaluation, the showering and mist sauna conditions produced significantly higher scores compared to the no-bathing condition at 7:20. In addition, the fatigue level in the mist sauna condition was significantly lower than that in the showering condition at 14:00. From the subjects' subjective evaluations, we confirmed that the morning showering did not produce drowsiness during the day compared to the no-bathing condition. Moreover, a morning mist sauna might reduce the level of fatigue during the day compared to morning showering.

## Conclusions

In conclusion, we found that both morning mist sauna and showering were not effective at improving the subjects' sleeping quality from the endocrinological viewpoint. However, we confirmed that morning showering did not result in drowsiness compared to the no bathing condition during the day. In addition, our results showed that a morning mist sauna might reduce the level of fatigue during the day compared to morning showering.

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