Urinary 8-hydroxydeoxyguanosine Levels and Psychological Reactions after Sleep Deprivation

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Abstract: The aim of this study was to investigate whether changes in urinary 8-hydroxydeoxyguanosine (8-OH-dG), an oxidative stress indicator, occur or not, and how psychological reactions change, after one night of sleep deprivation (SD) and after 7 hour recovery sleep opportunities during three recovery days. Twenty healthy subjects participated in this study. We measured their urinary 8-OH-dG levels and psychological reactions using the Profile of Mood States (POMS) for 5 days: on the baseline day, the post-vigil day, and on 3 recovery days after SD. The urinary 8-OH-dG levels and subscale scores of POMS were analyzed using repeated analysis of variance (ANOVA). In the psychological reactions, the values of Vigor on the post-vigil day and 2nd recovery day were significantly lower than on the baseline day. Fatigue and confusion on the post-vigil day were significantly higher than on the baseline day, and on the 1st and 2nd recovery days were relatively higher compared to the baseline day but returned to baseline level on the 3rd recovery day. The urinary 8-OH-dG levels did not change significantly after SD, on the post-vigil day or on the 3rd recovery days. These results suggest that the effect of one night of SD on psychological reactions continued for 2 or 3 days, and SD might not influence urinary 8-OH-dG levels despite marked changes in psychological reactions.

Key words: urinary 8-hydroxydeoxyguanosine, oxidative stress, psychological reactions, POMS, sleep deprivation.

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Introduction

The amount of 8-hydroxydeoxyguanosine (8-OH-dG) excreted in urine can be used as an indicator of DNA repair capacity, and also as a potential marker of oxidative DNA damage [1, 2]. In addition, urinary excretion of 8-OH-dG is considered to reflect generalized repair activity against the formation of 8-OH-dG in cellular DNA [3, 4], and it is rather easily analyzed by high-performance liquid chromatography coupled to an electrochemical detector (HPLC-ECD). Urinary 8-OH-dG has been proposed as a good and non-invasive indicator of oxidative stress in vivo. Therefore, many studies, but not all, have shown that various demographic, occupational, lifestyle and psychological reactions, such as age, gender, smoking, alcohol intake, body mass index, perceived workload and psychological stressors, are associated with the urinary 8-OH-dG level[1–5].

Some studies have reported a relationship between sleep and oxidative stress, using rats [5–7]. For example, the levels of glutathione, an antioxidant, were significantly reduced in the hypothalamus of sleep-deprived rats compared with controls [6]. There was increased activity of superoxide dismutase (SOD), an antioxidant enzyme, in the cerebral cortex and brain stem of paradoxical sleep deprived rats, but reduced activity in their hippocampus, thalamus, and hypothalamus [7]. These results suggest that sleep deprivation (SD) may influence oxidative stress. On the other hand, Gopalakrishnan et al. reported that prolonged wakefulness may not cause oxidative damage at the lipid or protein level, and that it can not represent an oxidative stress for the brain or for peripheral tissue, such as liver and skeletal muscles, in rats [8]. Matsumoto et al. reported that the length of habitual sleep had no effect on urinary 8-OH-dG levels in humans [9]. On the other hand, Kasai et al. reported higher urinary 8-OH-dG levels in shift workers [1]. Thus, many studies have reported a relationship between sleep and oxidative stress, but definite findings have not been obtained yet, particularly in human studies.

In our previous study, we suggested that SD induces temporary human psychological reactions, such as fatigue and confusion [10]. The relationship between psychological reactions and oxidative stress has also been investigated. Irie et al. provided evidence that human psychological reactions can be associated with increased 8-OH-dG levels in the leukocytes of healthy female subjects [5]. In another study of theirs, some psychological reactions indicated positive and significant correlations with the 8-OH-dG levels in leukocytes [11]. These results suggest that psychological reactions may be linked to 8-OH-dG levels, and it could be important to investigate the changes of urinary 8-OH-dG and psychological distress simultaneously.

The present study aims to clarify whether SD modifies urinary 8-OH-dG and changes in psychological reactions after SD. In addition, we expected to clarify the relationship between urinary 8-OH-dG levels and sleep loss in experimental human subject research, be-
cause some reports on the relationship were conducted in field research [9]. We examined the change of urinary 8-OH-dG levels and psychological reactions using the Profile of Mood State (POMS) [12, 13] after one night (approximately 40 h) of SD in human subjects.

Methods

Subjects
The subjects were 20 healthy, non-smoking men (age: mean = 22.1 ± 2.2 yr, range = 18-26) who responded to an advertisement posted at our college. We chose the subjects so that their demographic characteristics, such as normal habitual sleeping hours, no obesity, good health condition, and no medication, were generally equal, in order to exclude any factors that might influence urinary 8-OH-dG levels [1, 2, 5]. Informed consent was obtained after an explanation of all procedures. The subjects were screened for physical or mental health problems, including sleep disorder, by inquiry into their medical history and a vital check, and the Japanese version of the 60 item General Health Questionnaire (GHQ-60) developed by Goldberg was conducted to measure the subjects' current mental health condition[14, 15].

The subjects had no history of chronic medical illness, psychiatric illness or sleep disorder, and no prescribed medication regimen, such as taking antidepressants, hypnotics or sedative medications, in the previous 1 year. The GHQ-60 score of the subjects ranged from 0 to 12, with a mean of 3.4 ± 3.1. We made sure of their good mental health condition because each of their GHQ-60 scores were under 16 [16]. They had no problems in any of the health-checks (Height: mean = 172.2 ± 5.6 cm, weight: mean = 61.2 ± 7.9 kg, Body Mass Index: mean = 20.8 ± 2.0). We confirmed that their bedtimes were between 23:00 and 1:00 and their daily sleep ranged from 6 to 8h (mean sleep time = 6.6 ± 0.80 h).

Study design and procedure
Our study procedures were reviewed and approved by the Human Ethics Committee for Epidemiological Research at the University of Occupational and Environmental Health, Japan. All subjects were randomly allocated to experimental days, requiring a total of 6 days. Our study took place from December 2006 to January 2008.

The subjects' physical and mental condition was examined in our laboratory on Day 0, the adaptation day. They were fitted with a Lifecorder \(^\text{®}\) for daily activity monitoring. The purpose of monitoring the subjects by Lifecorder \(^\text{®}\) was to confirm how much activity they carried out during the investigation periods, and particularly, that they did not have excessive exercise, because of an increase in urinary 8-OH-dG levels after excessive exercise [17]. They were monitored during all the investigation periods.

Their bedtime on Day 0 was 23:30, and they had a 7 h sleep at their homes. Following waking at around 6:30 on Day 1, the baseline day, the subjects visited our laboratory, their mid-stream urine was collected, and they completed the Profile of Mood Status (POMS) at 7:
Afterwards, they underwent the same procedure at 15:00 and 23:00. The subjects stayed up from 23:00 on Day 1, the baseline day, to 7:30 on Day 2, the post-vigil day, in the laboratory. SD continued for a total of approximately 40 h until 23:30 on Day 2. For the 40 h SD, the subjects’ activities were monitored by Lifecorder® as mentioned above, and we also used the 40 h SD to check their wakefulness and physical condition. The subjects followed the schedule of Day 1 in the daytime of Day 2. Their bedtime on Day 2 was 23:30, and they had a 7 h sleep. The subjects kept the same schedule for the daytime of Days 3-5, the 1st-3rd recovery days. They went to bed at around 23:30 and got up at 6:30 and had approximately 7 h sleep during Days 3-5. The experiment was completed at 23:30 on Day 5. In addition, we provided all the subjects with the same meals, and prohibited them from consuming alcohol and caffeine during the periods.

Materials and analysis of human urinary 8-OH-dG

Urinary 8-OH-dG was analyzed according to the method of Kasai [18]. Human urine samples were mixed with the same volume of a dilution solution containing the ribonucleoside marker 8-hydroxyguanosine. 20 μl diluted urine samples were injected into an HPLC-1 (MCI Gel CA08F, 7 μM, chloride form, 1.5×120 mm, 2% acetonitrile in 0.3 mM sulfuric acid, 50 μM/min, 65) via the guard column (1.5×40 mm), so that they were fractionated by using HPLC-1.

The 8-OH-dG fraction was automatically collected based on the marker 8-hydroxyguanosine peak position as detected at 254 nm, and was injected into the second reserve phase column (40°C, 5% methanol in 10 mM phosphate buffer, pH 6.7). The 8-OH-dG fraction was collected based on the marker 8-hydroxyguanosine peak position as detected at 254 nm.

That was automatically injected into the HPLC-2 column (Shiseido Capcell Pak C18, 5 μM, 4.6×250 mm 40°C, 5% methanol in 10 mM sodium phosphate buffer, pH 6.7), so that it was fractionated by HPLC-2. 8-OH-dG was detected by using a Choulochem II EC detector (ESA) with a guard cell (5020) and an analytical cell (5011) (applied potentials were as follows: guard cell = 350 mV, E1 = 170 mV, and E2 = 300 mV). A guard column before the anion exchange column was backwashed with 0.5 M ammonium sulfate: acetonitrile (7:3 v/v).

Creatinine (Cre), which was used for normalizing the urinary 8-OH-dG, was simultaneously analyzed with 8-OH-dG in the HPLC-1 by the method of Kasai [13].

Profile of Mood States (POMS)

The psychological reactions of the subjects were evaluated by the POMS, developed by McNair et al., a 65-item 5-point, Likert-type, multiple-dimensional questionnaire on moods [12, 13]. Scores for 6 scales are calculated: Tension-Anxiety (T-A), Depression-Dejection (D), Anger-Hostility (A-H), Vigor (V), Fatigue (F), and Confusion (C).
Statistical analysis

The urinary 8-OH-dG levels and subscales of POMS as dependent variables were analyzed using repeated analysis of variance (ANOVA) for subjects, investigation day, and Days 1-5. The investigation times of day (TOD) (7:00, 15:00, & 23:00) were analyzed as independent variables. Mixed models were employed to account for systematic inter-individual variation. Day as an independent variable was included to investigate changes after SD, and TOD was used to consider the control of the diurnal rhythm. The statistical analyses were performed with the SPSS Statistics 17.0 statistical package.

Results

The subjects’ sleeping hours (duration of stay in bed) on each experimental day except the post-vigil day after SD were checked by a Lifecorder® (Mean: Day1 = 422.1 ± 23.4 min; Day2, SD: Day3 = 422.2 ± 17.9 min; Day4 = 419.0 ± 25.3 min; Day5 = 418.7 ± 28.3 min). Repeated ANOVA showed no significant differences in the main effects of Day [F (3, 76) = 0.15, n.s.]. The subjects’ sleeping hours on the baseline day and the 3 recovery days were approximately the same as their ordinary sleeping hours. Physical activity based on the consumption of calories monitored by the Lifecorder® was highest on the baseline day, but no significant differences in the main effects of Day were observed [F (4, 95) = 1.75, n.s.].

Table 1 shows the results of all the analyses in the present study. The scores of Vigor, Fa-

### Table 1. Results of the POMS scores and the urinary 8-OH-dG levels during experimental days

<table>
<thead>
<tr>
<th>Variables</th>
<th>Day1 Mean (SD)</th>
<th>Day2 Mean (SD)</th>
<th>Day3 Mean (SD)</th>
<th>Day4 Mean (SD)</th>
<th>Day5 Mean (SD)</th>
<th>Repeated ANOVA F value</th>
<th>F value</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>POMS</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Tension-Anxiety</td>
<td>7.6 (4.9)</td>
<td>5.1 (3.5)</td>
<td>6.0 (4.6)</td>
<td>6.0 (5.9)</td>
<td>4.7 (4.8)</td>
<td>4.7 (4.8)</td>
<td>3.9 (4.0)</td>
<td>3.7 (4.4)</td>
</tr>
<tr>
<td>Depression-Depression</td>
<td>13.6 (7.9)</td>
<td>14.8 (8.3)</td>
<td>7.9 (8.8)</td>
<td>7.6 (7.1)</td>
<td>9.5 (5.6)</td>
<td>9.5 (5.6)</td>
<td>10.3 (5.6)</td>
<td>10.1 (5.7)</td>
</tr>
<tr>
<td>Anger-Hostility</td>
<td>5.0 (3.5)</td>
<td>4.1 (3.2)</td>
<td>4.7 (3.8)</td>
<td>4.0 (4.1)</td>
<td>3.9 (3.8)</td>
<td>3.9 (3.8)</td>
<td>3.6 (4.7)</td>
<td>3.7 (4.1)</td>
</tr>
<tr>
<td>Vigor</td>
<td>6.3 (3.3)</td>
<td>6.4 (3.5)</td>
<td>7.9 (6.7)</td>
<td>7.6 (6.6)</td>
<td>9.5 (5.9)</td>
<td>9.5 (5.9)</td>
<td>10.3 (5.9)</td>
<td>10.1 (5.7)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>6.7 (6.6)</td>
<td>6.1 (6.9)</td>
<td>7.6 (6.6)</td>
<td>9.5 (6.4)</td>
<td>10.3 (7.7)</td>
<td>10.3 (7.7)</td>
<td>10.5 (7.7)</td>
<td>11.9 (8.0)</td>
</tr>
<tr>
<td>Confusion</td>
<td>6.1 (3.3)</td>
<td>6.1 (3.4)</td>
<td>10.1 (3.2)</td>
<td>9.2 (3.5)</td>
<td>7.3 (3.6)</td>
<td>7.3 (3.6)</td>
<td>7.7 (3.6)</td>
<td>6.6 (3.7)</td>
</tr>
<tr>
<td>Urinary 8-OH-dG</td>
<td>3.3 (0.8)</td>
<td>3.4 (0.8)</td>
<td>3.2 (0.6)</td>
<td>3.2 (0.6)</td>
<td>3.2 (0.6)</td>
<td>3.2 (0.6)</td>
<td>3.2 (0.6)</td>
<td>3.3 (0.6)</td>
</tr>
</tbody>
</table>

TOD: Time of day (7:00, 15:00 & 23:00); Day 1: baseline day, Day 2: Post-vigil day, Day 3: 1st recovery day, Day 4: 2nd recovery day, Day 5: 3rd recovery day, Day*TOD: interaction analysis of Day & TOD; POMS: Profile of Mood States, Urinary 8-OH-dG: urinary 8-Hydroxydeoxyguanosine, †: P<0.10, **: P<0.01, ***: P<0.001, (n = 20)
Fatigue and Confusion were significantly different in the main effects of Day (Vigor: \( F (4, 295) = 4.67, P < 0.01 \); Fatigue: \( F (4, 295) = 8.58, P < 0.001 \); Confusion: \( F (4, 295) = 5: 15, P < 0.001 \)). In multiple comparisons, the value of Vigor on the post-vigil day and on the 2nd recovery day was significantly lower than that on the baseline day. In addition, the value of Vigor on the 1st recovery day was higher than that on the baseline day, but not significantly. Fatigue and Confusion on the post-vigil day were significantly higher than on the baseline day, and on the 1st and 2nd recovery days were higher than baseline, but not significantly (Fig. 1). No significant Day or TOD interactions were observed in any of the
scales of the POMS. The urinary 8-OH-dG levels were not significant in the main effects of Day or TOD (Day: \( F(4,295) = 0.36, \text{n.s.} \), TOD: \( F(2,297) = 1.17, \text{n.s.} \)).

**Discussion**

We measured psychological reactions using the POMS and urinary 8-OH-dG as an oxidative stress indicator on the baseline, the post-vigil and 3 recovery days after one night of SD to investigate physiological and psychological changes and recovery. Among the psychological reactions, the present study indicates that there was a significant deterioration in fatigue and confusion, and vigor also declined significantly on the post-vigil day. Fatigue and confusion recovered slightly after one ordinary 7 h sleep opportunity, and these reactions returned to the baseline levels on the 3rd recovery day, whereas vigor significantly decreased after two ordinary 7 h sleep opportunities. The former results are in agreement with our previous study. The latter result first came to light in the present study. It may be necessary to take more than two ordinary sleep opportunities after one night of SD for the recovery of general moods. In addition, it is obvious that the psychological reactions deteriorate remarkably with SD.

We equalized generally demographic characteristics such as age, smoking history, habitual sleeping hours, BMI, health condition, and medication to exclude any factors which might influence urinary 8-OH-dG levels [1, 2, 5]. The subjects ate the same meals and their exercise was restricted to avoid any change of urinary 8-OH-dG levels by the intake of antioxidant nutrients and physical activities, which are known as potent inducers of oxidative stress in experiment periods. In our study, one night of SD had no effect on urinary 8-OH-dG levels during Day 1 to Day 5, in contrast to the significant deterioration of psychological reactions. Irie et al. suggest a positive correlation between psychological reactions and 8-OH-dG levels in leukocytes [11], but these are epidemiological studies, not experimental studies. In addition, it is possible that the urinary 8-OH-dG levels were not influenced by the temporal deterioration of psychological reactions or acute sleep loss.

The present experimental study supports the findings of animal studies performed by Gopalakrishnan et al., who assessed the oxidant production, (SOD) activity, lipid peroxidation, and protein oxidation of long-term sleep-deprived rats, and observed no oxidative damage at the lipid or protein level, and no constant change in SOD activity or oxidant production in the cerebral cortex or peripheral muscle. However, before making a conclusion in a human study, it may be necessary to deal more carefully with the effects of the repair mechanism of oxidative stress, such as SOD activity, or the existence of unknown confounding factors [9]. There is room for argument on these points. Further investigation might be needed on the relationship between sleep loss and oxidative stress with plural oxidation stress markers or antioxidants, to distinguish between the increase of oxidative stress and the reinforcement of the repair mechanism of oxidative stress. On the other hand, it is important for
industry to know the results of the deterioration of psychological reactions after one night of SD in our study, and the decline of cognitive performances after SD in our previous study, because of considerations of assessment or measures against low performance and efficiency and failure in work [10].

In conclusion, we experimentally investigated the changes of urinary 8-OH-dG levels and psychological reactions after one night of SD. We found that an increase of urinary 8-OH-dG excretion was not induced by one night of SD. While the subjects’ psychological condition deteriorated remarkably after one night of SD, it may be that such a temporal deterioration of psychological reactions did not influence the urinary 8-OH-dG levels.

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References


断眠による尿中 8-hydroxydeoxyguanosine と心理的反応

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要旨: 一晩の夜間断眠とその後 3 日間各 7 時間の回復睡眠期間中に、全身の酸化ストレス指標である尿中 8-hydroxydeoxyguanosine (8-OH-dG) 値に変化が起こるか、同時に心理的反応の変化を調査する目的で、当院保健室に所属する健康な非喫煙男性 20 名を対象に基準日、断眠後日、回復 3 日間の計 5 日間、被験者の尿を採取し、尿中 8-OH-dG 値を測定すると同時に、心理的反応を Profile of Mood States (POMS) を用いて測定し、尿中 8-OH-dG 値と POMS の下位尺度について日別の変化を分析した結果、心理的反応について、断眠後日と回復 2 日目の活気は、基準日と比べ有意に低値であった。断眠後日の大便と無理は基準日より有意に高値を示し、その後漸減、回復 3 日目には基準日と同程度まで回復した。尿中 8-OH-dG 値は、有意な変化を認めなかった。以上の結果一晩の夜間断眠後、心因性のストレス反応が数日間持続することを示唆する一方、尿中 8-OH-dG 値は、心理的反応の著明な変化に関わらず断眠による影響を受けない可能性も示唆された。

キーワード: 尿中 8-hydroxydeoxyguanosine、酸化ストレス、心理的反応、POMS、断眠。

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