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Original article

Effect of mats with "A Distinctive 4-Layer 3-Dimensional Structure" on sleep quality and nocturnal blood glucose: A crossover trial.

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Abstract

***Purpose*:** Relationships between the decline in sleep quality and glucose metabolism disorders have been reported recently. The present study examined effects on sleep quality, melatonin secretions, and nocturnal blood glucose from a test product via a mat with "a distinctive 4-layer 3-dimensional structure".

***Methods*:** Enrolled subjects were 12 adults who had complaints about sleep (4 men and 8 women, at the age of 51.9 ± 7.2). A controlled, crossover clinical trial was performed to examine the test product of a mat (AiR SX: Nishikawa) for 2 weeks, as well as the control product of bedding for 2 weeks; a 2-week washout was set between tests. Participants were equipped with a continuous blood glucose monitoring meter (Free Style Libre).The presence or absence of subjective symptoms, anthropometric measurements, biochemical examinations of blood, and examinations of urinary melatonin metabolites in collected urine were evaluated before the test and for 2 weeks after the commencement of the test.

***Results*:** Subjective sleep qualities in the test group were shown in the following. "Daytime dysfunction" in Pittsburgh Sleep Quality Index (PSQI) suggested an improvement tendency ($p < 0.1$), and OSA sleep inventory showed improvements in "Unpleasant feelings" ($p < 0.05$), "Sense of release" ($p < 0.1$) and "Shallow sleep" ($p < 0.1$). In urinary melatonin metabolite examination, there was no significant change in 6-sulfatoxymelatonin, N-acetylserotonin and serotonin. However, in the test group, 6-hydroxymelatonin ($p < 0.1$) and melatonin ($p < 0.05$) tended to remain high. In the analysis of nocturnal blood glucose for 2 weeks, the ratio of blood glucose less than 80 mg/dL, was confirmed to decrease in the test group on Days 9 and 11 ($p < 0.1$).

***Conclusion*:** This study suggested a possibility that melatonin secretion increased and the frequency of nocturnal blood glucose decreased as a result of the improvements of sleep quality.

***KEY WORDS*:** sleep quality, melatonin, nocturnal hypoglycemia, continuous glucose monitoring

Introduction

A decline in sleep quality is one of the changes which occur simultaneously with aging. Furthermore, people in Japan, from children to adults, tend to have chronic sleep deprivation [1)]. Unidentified complaints are frequently recognized in not only middle and old age but also childhood and puberty, which are related to diverse lifestyle diseases and mentalhealth [2-6)]. From a view point of preventive medicine, it is vital to maintain a high quality of sleep.

There exists a bidirectional relation between sleep and glucose metabolism. Patients with diabetes mellitus, which is a typical disease characterized by strong glycative stress, accompany various forms of sleep disorders at a rate of 40% [7)]. Sleep apnea syndrome (SAS), which is a typical disease characterized by the decline of sleep quality, is frequently accompanied by obesity and diabetes mellitus [5-14)].

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To reduce glycative stress, the prevention and treatment must be performed simultaneously for glucose metabolism and sleep disorders.

Our previous research confirmed that subjects with insufficient sleep had a large accumulation of advanced glycation end products (AGEs) in the skin [15]. The data results of 24-hour continuous blood glucose monitoring showed that between the groups with a sleep time of 7 hours or more and the group with fewer than 6 hours, the frequencies of postprandial hyperglycemia differed, and during sleep deprivation, a blood glucose spike (a steep rise in blood glucose, which peaked at 140 mg/dL or greater) occurred more frequently [16]. The blood glucose spike [17, 18] simultaneously induces co-occurring formations of a variety of aldehyde (we call it an aldehyde spark) [16, 19, 20] and triggers tissue injuries and cell damage initially from vascular endothelial cells. Therefore, the blood glucose spike has been gaining attention as a fundamental causative risk for cardiovascular and cerebrovascular events.

To maintain a high quality of sleep, it is desirable to utilize bedding suitable to individual conditions. We have examined mental and physical effects of mats with a distinctive 4-layer 3-dimensional structure. The first trial regarding the use of the test product for 4 weeks, which was uncontrolled and open-labeled, confirmed improvements in subjective symptoms regarding sleep, a significant elevation of insulin-like growth factor-I (IGF-I) (+10.2%), and a significant elevation of high-density lipoprotein-cholesterol (HDL-C) (+7.5%). This trial suggested that growth hormone secretion increased, which was due to the improvements in sleep quality, and lipid metabolism improved [21]. The second trial, a non-controlled open-label trial, confirmed a significant decrease of HbA1c (−2.3%) in addition to improvements of subjective conditions. However, there was no significant change in urinary melatonin metabolite examination [22]. The reason why no change in melatonin secretion was detected was that the degree of darkness in bedrooms was not under a fixed condition and thus, the levels of the melatonin secretions were widely dispersed. The present clinical trial was performed as a controlled, crossover trial using a control product under the fixed condition of darkness.

Methods

Subjects

Potential research participants were recruited, totaling 43 healthy men and women at 40-64 years of age who had subjective symptoms of slight sleep disorders such as sleep latency and shallow sleep. Potential subjects received a screening (SCR) as follows: anthropometric measurements, a hematologic test, serum biochemical examinations, urine examination, Pittsburgh Sleep Quality Index (PSQI) [23], OSA sleep inventory [24], and an inquiry by a doctor. From those meeting inclusion criteria and not meeting exclusion criteria, 12 subjects were selected based on the following ranking: among the potential subjects who had a score of 6 or higher in PSQI-J, subjects were selected by ranking them in the top 12 with total scores of the three factors below. First, ordering was determined on each item. Using the total ranking based on three rankings, 12 subjects were decided according to ascending order. Additionally, an outlier and background factors of subjects were considered.

1. Descending order of the scores of PSQI
2. Ascending order of the scores of the second factor (initiation and maintenance of sleep) in OSA sleep inventory
3. Descending order of HbA1c level

The inclusion criteria are as follows:

[1] Men and women of age 40-64 at the time of informed consent for participation
[2] Healthy individuals who do not currently undergo any treatment for a disease
[3] Individuals who have subjective symptoms of slight sleep disorders, such as sleep latency and shallow sleep
[4] Individuals who have habitual sleep patterns of bedtime (lights out) and rising, with bedtime before midnight and a 4-hour or longer sleeping time
[5] Individuals who have habitual eating patterns of finishing dinner 3 hours before bedtime and not eating a snack until rising
[6] Individuals who have working schedules of working 5 days a week with a day off on Saturdays and Sundays
[7] Individuals who are fully informed with the purpose and content of the trial, have an ability to understand and consent to the information, and agree with voluntary participation in the written consent form
[8] Individuals who are able to visit and receive examinations on an assigned date
[9] Individuals who are accepted for participation in the trial by a principal investigator

The exclusion criteria are as follows:

[1] Individuals who have a disease and undergo medical treatments
[2] Individuals who have a past and present medical history of mental disease, sleep disorder, high blood pressure, diabetes, dyslipidemia and other serious diseases
[3] Individuals who have a possibility, under treatments or a medical history of SAS
[4] Individuals who have or have a possibility of night urination, prostatic hyperplasia and overactive bladder
[5] Individuals who have habitually ingested medication for medical treatments over the last month (except for a portion history for headache, menstrual pain and cold)
[6] Individuals who have a past and present history of serious disorders of the liver, kidneys, heart, lungs, and blood
[7] Individuals who have a serious comorbidity or anamnesis in their digestive organs
[8] Individuals whose body mass index (BMI) is 25 kg/m^2 or higher
[9] Individuals who made 200 mL of blood donation during the past month or 400 mL or more of blood donation within the last 3 months
[10] Individuals who have an experience of feeling ill or deterioration in condition due to blood sampling
[11] Individuals who have a possibility of development of allergic symptoms against the test item or grave allergic symptoms against other food or medicaments

[12] Individuals who are liable to have a seasonal allergy such as pollenosis and use medication (except for instillation and collunarium)
[13] Individuals who habitually ingest supplements carrying effects related to blood glucose level or containing vitamin C, or who plan to ingest them (except individuals who quit the ingestion at the time of informed consent)
[14] Individuals who habitually drink an average of 60 g/day or more of alcohol content
[15] Individuals who may possibly change life habits, such as going on a long trip
[16] Individuals who are pregnant or lactating or who may possibly be pregnant
[17] Individuals who will or may undergo an MRI scan during this clinical trial
[18] Individuals who are participating in another human clinical trial at present or who have participated in another human clinical trial within the last 3 months
[19] Individuals who or whose family is employed for enterprises that develop, produce, or sell products of health and/or functional food or cosmetics
[20] Individuals who the principal investigator has judged as inappropriate for this clinical trial

The changes in the number of analysis sets for the trial are shown as below (***Fig. 1***). The age of the 12 subjects (4 men and 8 women) was 51.9 ± 7.2 years (men: 53.5 ± 7.9 years, women: 51.1 ± 7.2 years).

Trial design

This study was a controlled, crossover trial, using a control product. The test product was a mat with a distinctive 4-layer 3-dimensional structure (AiR SX: Nishikawa, Tokyo, Japan). The control product was a mat, which was commonly sold, with a single-layer flat-surface structure made of urethane. Test and control products were a single size (9 × 97 × 200 cm). Both the mats and specified sheets were provided by Nishikawa (founded 1566).

To randomly assign the 12 subjects into two groups, Stratified Block Randomization was employed, where stratification factors were age, sex, and the second factor (initiation and maintenance of sleep) of OSA sleep inventory in SCR. The assignment manager made a final confirmation that there was no significant difference between the two groups.

Arm structures of the two groups were as follows:
1. The test product (a mat with a distinctive 4-layer 3-dimensional structure) → the control product (a mat with a single-layer flat-surface structure)
2. The control product (a mat with single-layer flat-surface structure) → the test product (a mat with distinctive 4-layer 3-dimensional structure)

Subjects switched from a *futon* mattress, which they usually used, to a test or control product and used the test or control product for 2 weeks (Period I). After the 2-week washout period, they used the other product, which was different from the product that they used during Period I,

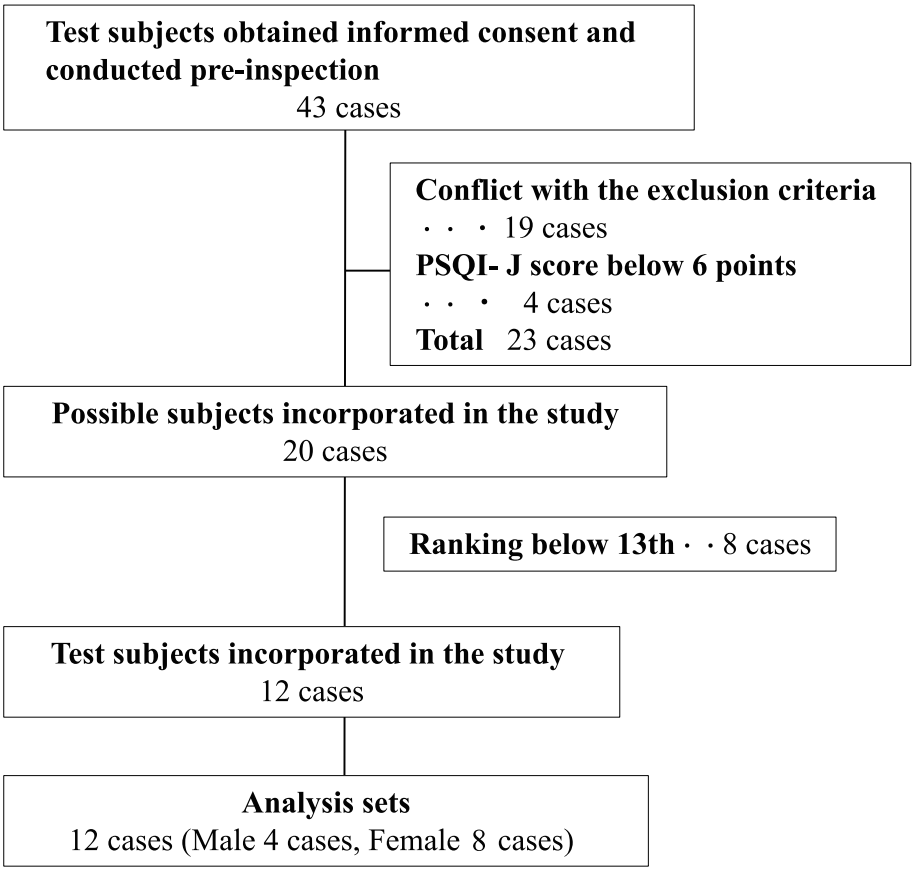

Fig. 1. **The number of analysis sets.**
PSQI- J, Pittsburgh sleep quality index, Japanese version.

for 2 weeks (Period II). The test group consisted of the test product users with the total of Period I and II. The control group consisted of the control product users with the total of Period I and II.

Before starting the test (Period I) and 2 weeks after the commencement of the test (Period I), examinations were performed: confirmations of subjective symptoms, anthropometric measurements, blood examinations, early morning collecting urine of collected urine during night, and an inquiry by a doctor. During the test (Period I), the research participants measured blood glucose using a continuous blood glucose monitoring meter (Free Style Libre). After the completion of the 2-week washout period, examinations were performed before the commencement of the test (Period II) and 2 weeks after the commencement of the test (Period II), examinations were performed, which was in the same manner of Period I: confirmations of subjective symptoms, anthropometric measurements, blood examinations, measurements of urinary melatonin metabolite, and an inquiry by a doctor. During the test (Period II), the research participants measured blood glucose using a glucose monitoring meter. The research participants recorded the presence or absence, or degrees of adverse events, life habits and simple diet surveys in their daily notes during both test periods. The period of the present trial was from February 2018 to April 2018.

Assessment items

Subjective symptoms

The quality of sleep was evaluated using the PSQI [23]. According to the scoring of PSQI, the PSQI global score (PSQIG) was calculated by totaling seven component scores; component scores consisted of subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction.

Further, the present trial employed the OSA sleep inventory MA version [24], which is a psychological scale to evaluate self-reflection regarding sleep on rising. Answers of the survey were obtained with numeric values in a four-level assessment questionnaire. Collected data were summed up in each item factor; the first factor: sleepiness on rising, the second factor: initiation and maintenance of sleep, the third factor: frequent dreaming, the fourth factor: refreshing, and the fifth factor: sleep length.

Anthropometric measurement

For anthropometric measurement, body height, body weight, body fat, BMI, systolic and diastolic pressure, and pulse rate were measured. Body composition was measured using a body composition monitor (DC-320, Tanita, Tokyo, Japan).

Blood examination

Using blood samples, hormones in blood such as IGF-I, dehydroepiandrosterone-sulfate (DHEA-s), and cortisol were measured in LSI Medience (Tokyo, Japan).

Urinary melatonin metabolite measurement

The specimen was the first morning sample of urine after getting up. The research participants collected the first urine by themselves after rising and delivered the sample to the medical institution of clinical trial implementation. Using the urine test body, 6-sulfatoxymelatonin (SaMT), 6-hydroxymelatonin (HaM), melatonin (MEL), N-acetylserotonin (NAS), serotonin (5-hydroxytryptamine: 5HT), and N-acetyl-5-methoxykynuramine (AMK) were measured in Professor Hattori Research Laboratory, Liberal Arts and Sciences, Tokyo Medical and Dental University (Ichikawa, Chiba, Japan).

Continuous blood glucose monitoring

The subjects were equipped with a continuous blood glucose monitoring meter (Free Style Libre; Abbot Japan, Tokyo, Japan) on the back part of their upper arm. Blood glucose levels (GLU) were continuously measured throughout the day and night during both periods: from the observation Period I/before the commencement (Visit-1) to the observation Period I/2 weeks after the commencement (Visit-2), and from the observation Period II/before the commencement (Visit-3) to the observation Period II/2 weeks after the commencement (Visit-4).

Statistical analysis

Statistical analysis was employed by a statistical analysis software SAS (SAS 9.4: SAS Institute Japan, Tokyo, Japan) or SPSS (Statistics19: IBM Japan, Tokyo, Japan).To verify the validity of the crossover design, the order effect was confirmed by repeated measure analysis of variance. After the confirmation, a paired-t test was performed. Scores which were obtained by PSQI and OSA sleep inventory were processed as nonparametric, and the comparison of differences between groups employed Wilcoxon signed rank sum test. Hazard rates lower than 5% indicated a significant difference and hazard rates lower than 10% indicated a marginal significance. As for outliers and missing values, outliers were not removed from the data. However, when data were not obtained due to troubles in the examination or some data had a grave problem regarding data reliability, they were regarded as missing values, and substitute values were not employed.

Ethical consideration

This study was implemented in compliance with the Helsinki Declaration (amended by the 64th WMA General Assembly, Fortaleza, Brazil, 2013) and the Ethical Guidelines for Medical and Health Research Involving Human Subjects established by the Ministry of Health, Labour and Welfare, and Ministry of Education in Japan. Further, general foundational juridical person Society for Glycative Stress Research (Tokyo, Japan) held Ethics Review Committee on Research Involving Human Subjects to deliberate ethics and validity for this study, and this study was approved (GSE 2018-001). Clinical trial registration to UMIN was obtained in advanced (UMIN #000031144).

Results

Subjective symptoms

Two-week use of the test product induced improvements in subjective symptoms.

In PSQI-J, sleep quality, sleep latency, sleep disturbances, daytime dysfunction and PSQI-J, scores significantly improved in both the test group and the control group 2 weeks after the commencement of use in comparison to before use. Sleep duration significantly improved 2 weeks after the commencement of use in comparison to before use, only in the test group. However, there was no significant difference between groups. Degree of the improvement in daytime dysfunction was greater in the test group than the control group and the quantity of change was marginally significant in the test group in comparison to the control group ($p = 0.058$, ***Table 1***).

In OSA sleep inventory, which is a psychological scale to evaluate self-reflection regarding sleep on rising, the first factor of sleepiness on rising, the second factor of initiation and maintenance of sleep, the fourth factor of refreshing, and the fifth factor of sleep length significantly improved 2 weeks after the commencement of use in comparison to before use, in both the test group and the control group. The degree of improvement of the first factor of sleepiness on rising is higher in the test group than the control group, the amount of change 2 weeks after the commencement of use, was marginally significant ($p = 0.060$, ***Table2***). Among items, degree of improvement 2 weeks after the commencement of use in "Unpleasant feelings" significantly improved in the test group comparing the control group ($p = 0.024$). In items of "Sense of release" and "Shallow sleep", the amount ofofof changes in improvement of the test group at 2 weeks after the commencement of use was marginally significant compared to the control group ($p = 0.075$, $p = 0.091$).

Table 1. Sleep quality evaluation

			Before	2 weeks	p value	
PSQI-J	Sleep quality	Test	2.1 ± 0.1	1.0 ± 0.0**	**0.001**	
		Control	2.0 ± 0.1	1.1 ± 0.1**	**0.002**	
	Sleep latency	Test	2.1 ± 0.2	1.1 ± 0.3**	**0.010**	
		Control	1.9 ± 0.3	1.1 ± 0.3*	**0.031**	
	Sleep duration	Test	1.6 ± 0.1	1.1 ± 0.2*	**0.014**	
		Control	1.5 ± 0.2	1.2 ± 0.2	0.103	
	Sleep efficiency	Test	0.2 ± 0.1	0.1 ± 0.1	0.317	
		Control	0.3 ± 0.2	0.3 ± 0.1	0.655	
	Sleep disturbance	Test	0.8 ± 0.1	0.5 ± 0.2*	**0.046**	
		Control	0.9 ± 0.1	0.3 ± 0.1**	**0.005**	
	Use of sleep inducers	Test	0.0 ± 0.0	0.0 ± 0.0	1.000	
		Control	0.0 ± 0.0	0.0 ± 0.0	1.000	
	Daytime dysfunction	Test	1.3 ± 0.2	0.3 ± 0.1**	**0.006**	†
		Control	0.9 ± 0.2	0.4 ± 0.2*	**0.014**	
	PSQIG	Test	8.0 ± 0.5	4.0 ± 0.7**	**0.002**	
		Control	7.5 ± 0.7	4.3 ± 0.7**	**0.006**	

Results are expressed as mean ± SEM, *p < 0.05, **p < 0.01 vs Before, † p < 0.05 vs Control, Wilcoxon signed-rank test, n = 12. PSQI-J, Pittsburgh Sleep Quality Index (Japan version) questionnaire; PSQIG, PSQI global score; SEM, standard error mean.

Table 2. OSA sleep Questionnaire.

			Before	2 weeks	p value	
OSA sleep Questionnaire	First factor (Sleepiness on rising)	Test	40.17 ± 4.52	70.61 ± 5.23**	**0.003**	†
		Control	44.53 ± 6.24	66.95 ± 5.09*	**0.012**	
	Second factor (Initiation and maintenance of sleep)	Test	34.97 ± 4.81	66.02 ± 4.25**	**0.003**	
		Control	33.73 ± 3.36	59.28 ± 4.28**	**0.005**	
	Third factor (Worries)	Test	74.04 ± 7.03	79.17 ± 3.84	0.463	
		Control	71.42 ± 7.72	77.29 ± 3.86	0.310	
	Fourth factor (Refreshing)	Test	41.69 ± 4.35	71.58 ± 4.57**	**0.003**	
		Control	44.81 ± 5.02	65.09 ± 4.02*	**0.011**	
	Fifth factor (Sleep length)	Test	52.88 ± 5.96	75.54 ± 4.17**	**0.010**	
		Control	52.75 ± 6.15	75.71 ± 5.43*	**0.016**	

Results are expressed as mean ± SEM, *p < 0.05, **p < 0.01 vs before, † p < 0.05 vs Control, Wilcoxon signed-rank test, n = 12. OSA, obstructive sleep apnea syndrome; SEM, standard error mean.

Anthropometry

Weight, somatic fat rate, and BMI showed significant decrease in the test group between before use and 2 weeks after the commencement of use (***Table 3***).

Endocrine markers

For the endocrine examination, age-related hormones, IGF-I and DHEA-s, sleep-related hormones, urinary melatonin metabolites (SaMT, HaMT, MEL, NAS, 5HT and AMK), and stress-related hormones, cortisol were measured (***Table 4***, ***Fig. 2***).

There were no significant changes in IGF-I, DHEA-s and cortisol during the observation periods.

As for urinary melatonin metabolite examinations, SaMT, NAS and 5HT showed no significant change. In comparison between groups, the value of HaMT at the time of after 2 weeks was significantly higher in the test group than in the control group ($p = 0.036$), although HaMT showed no significant change between before and after the test. There was no significant change in MEL in the test group between before and after use, while the control group showed significant decrease compared to before use ($p = 0.019$). In

Table 3. Anthropometry.

			Before	2 weeks	p value
Height	cm	Test	163.63 ± 1.83	- ± -	-
		Control	163.63 ± 1.83	- ± -	-
Weight	kg	Test	60.19 ± 1.68	59.63 ± 1.61**	0.004
		Control	59.87 ± 1.7	59.76 ± 1.67	0.491
Body fat	%	Test	28.78 ± 1.97	28.31 ± 1.96*	**0.028**
		Control	28.58 ± 1.89	28.35 ± 1.98	0.494
BMI	–	Test	22.45 ± 0.39	22.23 ± 0.37**	**0.003**
		Control	22.33 ± 0.38	22.27 ± 0.38	0.296
Blood pressure (systolic)	mmHg	Test	116.8 ± 3.7 †	113.3 ± 3.1	0.176
		Control	111.0 ± 3.2 †	111.3 ± 3.4	0.817
Blood pressure (diastolic)	mmHg	Test	67.8 ± 3.4	67.2 ± 3.0	0.698
		Control	65.2 ± 2.8	67.1 ± 3.1	0.280
Pulse	/min	Test	69.3 ± 2.7	68.6 ± 2.8	0.701
		Control	66.3 ± 2.6	67.8 ± 2.4	0.437

Data are expressed as mean ± SEM, *p < 0.05, **p < 0.01 vs before, † p < 0.05 vs Control, paired t test, n = 12. BMI, body mass index; SEM, standard error mean.

Table 4. Blood, urine, salivary examination.

Hormonal examination				Before	2 weeks	p value
Serum	IGF-I	ng/mL	Test	124.3 ± 10.1	119.1 ± 14.4	0.473
			Control	114.7 ± 8.2	124.7 ± 14.0	0.256
	DHEA-s	μg/dL	Test	128.8 ± 17.1	121.3 ± 16.7	0.325
			Control	138.4 ± 22.0	124.4 ± 16.0	0.098
	Cortisol	μg/dL	Test	7.90 ± 0.75	7.83 ± 0.67	0.913
			Control	8.98 ± 0.96	7.63 ± 0.61	0.157
Urine	SaMT	ng/mg Cre	Test	9.878 ± 2.932	9.117 ± 2.725	0.370
			Control	11.132 ± 3.715	12.342 ± 3.904	0.761
	HaMT	pg/mg Cre	Test	20.431 ± 5.791	20.951 ± 4.136	0.940 #
			Control	15.371 ± 2.761	12.394 ± 2.327	0.201 #
	MEL	pg/mg Cre	Test	19.547 ± 6.878	15.614 ± 3.795	0.446 †
			Control	22.297 ± 5.412	10.679 ± 3.000*	**0.019** †
	NAS	pg/mg Cre	Test	78.618 ± 21.251	60.171 ± 13.923	0.249
			Control	70.475 ± 16.237	45.550 ± 7.744	0.064
	5HT	ng/mg Cre	Test	20.468 ± 1.757	21.012 ± 3.106	0.886
			Control	22.479 ± 2.037	20.700 ± 2.854	0.413

Data are expressed as mean ± SEM, *p < 0.05 vs before, † p < 0.05, # p < 0.1 vs Control, paired t test, n = 12. IGF-I, insulin-like growth factor-I; DHEA-s, dehydroepiandrosterone-sufate; SaMT, 6-sulfatoxymelatonin; HaMT, 6-hydroxymelatonin; MEL, melatonin; NAS, N-acetylserotonin; 5HT, serotonin (5-hydroxytryptamine); SEM, standard error mean.

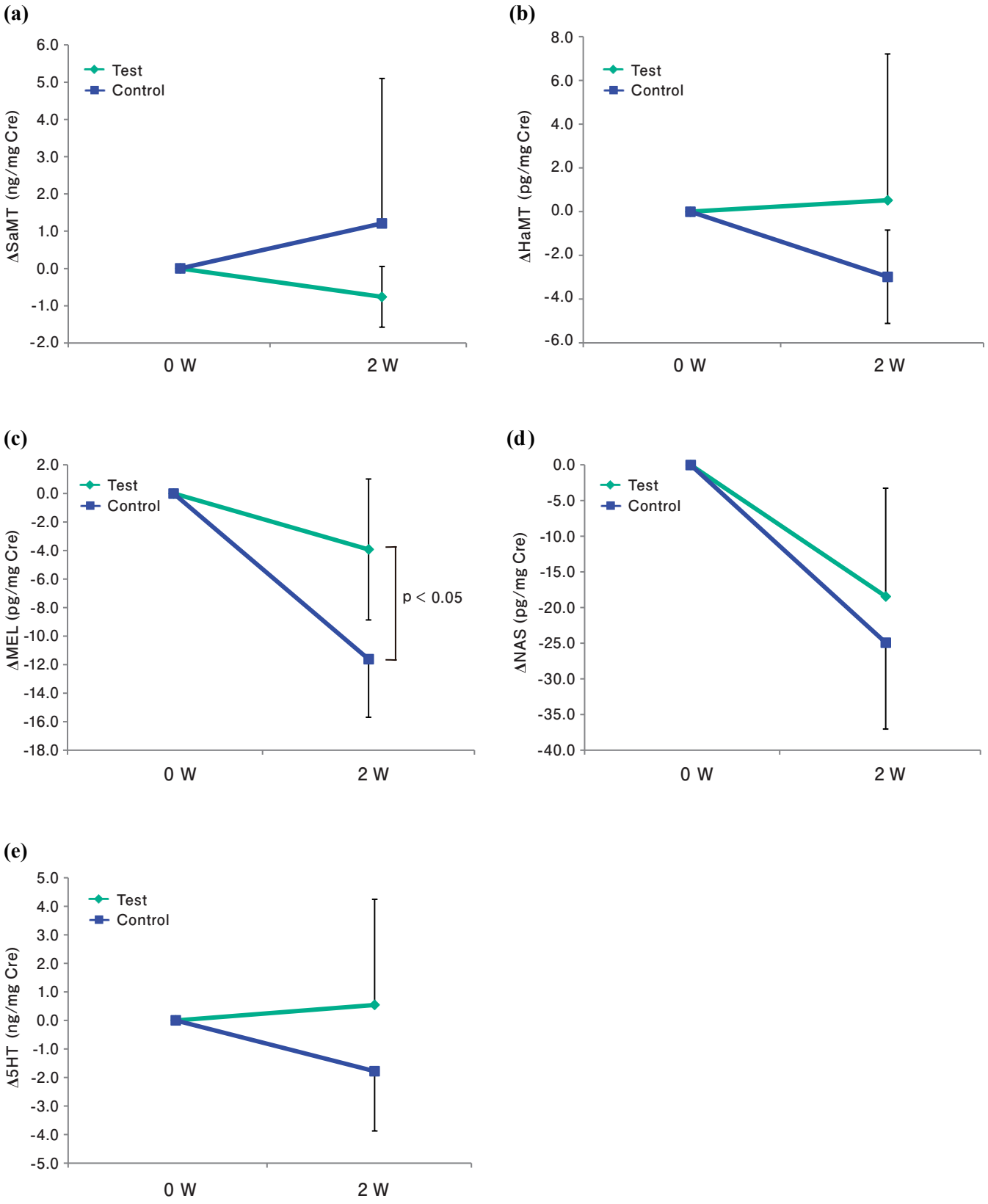

Fig. 2. **Changes of melatonin metabolites in urine.**

a: SaMT, **b**. HaMT, **c**. MEL, **d**: NAS, **e**: 5HT. Results are expressed as mean ± SEM, n = 12, paired t test vs control.

SaMT, 6-Sulfatoxymelatonin; HaMT, 6-Hydroxymelatonin; MEL, melatonin; NAS, N-acetylserotonin; 5HT, serotonin (5-hydroxytryptamine); Cre, creatinine; SEM, standard error mean.

the comparison between the groups, a significant difference was recognized in quantity of change ($p < 0.05$, ***Fig. 2-c***).

AMK of subjects in this trial was below sensitivity of detection.

Carbohydrate metabolism markers

In 2-week continuous blood glucose monitoring, there was no difference in mean glucose level during sleeping hours (00:00 – 06:00) and 24 hours between the test group and the control group (***Table 5***). There was no difference between the test group and the control group in appearance ratio of high glucose level (GLU 140 mg/dL or higher) and appearance ratio of low glucose level (lower than GLU 80 mg/dL) for 2 weeks (Day 1 – Day 14). However, in daily notes, appearance ratios of low blood glucose on Days 9 and 11 were marginally significant between groups ($p = 0.081$, $p = 0.075$); appearance ratios were lower in the test group than the control group.

Safety

No adverse event was recognized due to the test product during the observation period.

Discussion

Outline of results

This trial, which was a controlled, crossover study, examined influences on the sleep quality and the nocturnal blood glucose change by using the test mat for 2 weeks with subjects who were healthy men and women at 40-64 years of age at the time of obtaining their informed consent. Enrolled subjects were 12 adults (4 men and 8 women), with a mean age of 51.9 ± 7.2 years (men: 53.5 ± 7.9 years, women: 51.1 ± 7.2 years).

Assessment of sleep-related subjective symptoms in PSQI-J suggested that daytime dysfunction showed an improvement tendency (marginally significant) in the test group ($p < 0.1$). In OSA sleep inventory, "Unpleasant feelings" significantly improved ($p < 0.05$) in the test group. "Sense of release" ($p < 0.1$) and "Shallow sleep" ($p < 0.1$) showed an improvement tendency in the test group. These findings suggested improvements in subjective symptoms by using the test product.

The urinary melatonin metabolite examinations showed that there was no significant change of SaMT, NAS and 5HT in urine. However, HaMT tended to remain high in the test group ($p < 0.1$) and melatonin significantly remained high

Table 5. Continuous glucose monitoring.

Average GLU						p value
During sleep (24-6 o'clock)	mg/dL	Test	86.65	±	2.37	0.849
		Control	86.03	±	3.42	
Morning (6-12 o'clock)	mg/dL	Test	94.62	±	1.89	0.832
		Control	95.27	±	3.30	
Afternoon (12-18 o'clock)	mg/dL	Test	107.22	±	2.68	0.892
		Control	107.71	±	3.73	
Night (18-24 o'clock)	mg/dL	Test	106.78	±	2.51	0.913
		Control	106.37	±	3.72	
24 hours	mg/dL	Test	98.83	±	2.01	0.994
		Control	98.85	±	3.27	
Portion						
GLU 140 mg/dL or larger						
(Average of 1st day-7th day)	%	Test	5.85	±	1.30	0.145
		Control	8.14	±	2.06	
(Average of 8th day-14th day)	%	Test	5.23	±	1.66	0.787
		Control	4.37	±	1.13	
(Average of 1st day-14th day)	%	Test	5.49	±	1.43	0.301
		Control	7.03	±	2.00	
GLU below 80 mg/dL						
(Average of 1st day-7th day)	%	Test	13.93	±	5.59	0.593
		Control	18.53	±	5.48	
(Average of 8th day-14th day)	%	Test	11.28	±	2.99	0.138
		Control	23.95	±	6.02	
(Average of 1st day-14th)	%	Test	12.72	±	4.07	0.280
		Control	20.49	±	5.40	

Data are expressed as mean ± SEM, n = 12. GLU, glucose values measered by Free style Libre.

in the test group ($p < 0.05$). Judging from these findings, there is a possibility that the quantity of melatonin secretion during sleeping at night was larger due to the use of the test product.

In the examination of the 2-week continuous blood glucose monitoring, there was no difference in average GLU level between groups. There was no difference between groups in appearance ratio of high GLU and low GLU during measurement periods. However, in daily notes, appearance ratio of low blood glucose on Days 9 and 11 showed a tendency for differences between groups; appearance ratios of low blood glucose were lower in the test group than the control group ($p < 0.1$). It was assumed that in the use of the test product, homeostasis of blood glucose remained better during night sleeping.

Body weight, body fat and BMI in the safety assessment items showed a significant decrease in the test group from before the use to 2 weeks after the commencement of the use. In the adverse event investigation, a temporary cold was recognized. It disappeared in a short period of time and the symptoms were transient. The trial continued by the doctor's judgement. No adverse event was recognized due to the test product.

Assessment of subjective symptoms

This product has been examined in 2 clinical trials before [21, 22]. The present study is the third clinical trial.

Assessment of PSQI-J confirmed in the first trial that the use of the test product improved subjective symptoms. Scores of the following items were significantly improved [21]: sleep quality ($2.1 \pm 0.1 \rightarrow 1.5 \pm 0.2$, $p = 0.008$), sleep latency ($2.3 \pm 0.3 \rightarrow 1.7 \pm 0.3$, $p = 0.034$), sleep disturbance ($1.4 \pm 0.2 \rightarrow 1.0 \pm 0.0$, $p = 0.046$), and daytime dysfunction ($1.7 \pm 0.1 \rightarrow 0.7 \pm 0.2$, $p = 0.002$). PSQIG significantly improved from a high-degree disorder (9.5 ± 0.4) to a low-degree disorder (7.1 ± 0.7, $p = 0.005$).

The second trial confirmed that the following items were improved due to the use of the test product for 2 weeks [22]: scores of sleep quality ($2.0 \pm 0.4 \rightarrow 0.8 \pm 0.6$, $p = 0.006$), sleep latency ($2.0 \pm 0.9 \rightarrow 0.8 \pm 1.0$, $p = 0.016$), sleep duration ($1.7 \pm 0.5 \rightarrow 1.0 \pm 0.8$, $p = 0.011$), sleep disturbance ($1.3 \pm 0.5 \rightarrow 0.7 \pm 0.5$, $p = 0.034$), and daytime dysfunction ($1.5 \pm 0.8 \rightarrow 0.5 \pm 0.7$, $p = 0.026$) significantly improved. PSQIG significantly improved from a high-degree disorder (9.0 ± 1.7) to a low-degree disorder (3.9 ± 2.1, $p = 0.005$).

The third clinical trial showed improvement in subjective symptoms of PSQI-J due to the use of this product for 2 weeks. The scores of the following items in PSQI-J significantly improved: sleep quality ($2.1 \pm 0.1 \rightarrow 1.0 \pm 0.0$, $p < 0.01$), sleep latency ($2.1 \pm 0.2 \rightarrow 1.1 \pm 0.3$, $p < 0.01$), sleep duration ($1.6 \pm 0.1 \rightarrow 1.1 \pm 0.2$, $p < 0.05$), sleep disturbance ($0.8 \pm 0.1 \rightarrow 0.5 \pm 0.2$, $p < 0.05$), and daytime dysfunction ($1.3 \pm 0.2 \rightarrow 0.3 \pm 0.1$, $p < 0.01$). PSQIG significantly improved from a high-degree disorder (8.0 ± 0.5) (before the use of the test product) to a low-degree disorder (4.0 ± 0.7, $p < 0.01$).

Those three clinical trials obtained mostly the same results. The present study has high repeatability in improvement of effects on subjective symptoms.

As for OSA sleep inventory, the first trial recognized that the following scores significantly improved: the first factor: "Sleepiness on rising" ($15.29 \pm 3.75 \rightarrow 20.23 \pm 5.37$, $p = 0.041$), the second factor: "Initiation and maintenance of sleep" ($11.87 \pm 3.83 \rightarrow 17.47 \pm 3.92$, $p = 0.012$), and the fourth factor: "Refreshing" ($14.23 \pm 3.84 \rightarrow 19.30 \pm 5.79$, $p = 0.038$).

The third trial recognized that the following scores significantly improved: the first factor: "Sleepiness on rising" ($13.40 \pm 5.22 \rightarrow 23.53 \pm 6.05$, $p < 0.01$), the second factor: "Initiation and maintenance of sleep" ($11.66 \pm 5.55 \rightarrow 22.01 \pm 4.91$, $p < 0.01$), the fourth factor: "Refreshing" ($13.89 \pm 5.02 \rightarrow 23.85 \pm 5.28$, $p < 0.01$), and the fifth factor: "Sleep length" ($17.62 \pm 6.88 \rightarrow 25.18 \pm 4.82$, $p < 0.01$).

The present trial was the first controlled trial for the test product. In comparison to the control product between groups, PSQI-J had no item that had a significant difference between groups. However, the score of daytime dysfunctions in the test group showed an improvement tendency (difference between groups, $p = 0.058$). OSA sleep inventory recognized that improvement in change rate of item of "Unpleasant feelings" was significantly high in the test group ($p < 0.05$). The ratios of changes in improvements of the following factors were higher (marginally significant) in the test group between groups; the first factor: "Sleepiness on rising" ($p = 0.060$), item of "Sense of release" ($p = 0.075$) and item of "Shallow sleep" ($p = 0.091$).

Using the control product in the present study significantly improved various subjective symptoms. It seemed that the control product was more suitable than the bedding that the participants had used in their daily life. Therefore, it was assumed that the test products were less likely to extract differences between groups.

Sleep quality and carbohydrate metabolism

Patients with diabetes mellitus, which is a typical disease characterized by strong glycative stress, accompanies various forms of sleep disorders at a rate of 40%. SAS, which is a typical disease characterized by the decline of sleep quality, frequently accompanies obesity and diabetes [7]. Briefly, there was a bidirectional correlation between sleep and carbohydrate metabolism. To reduce glycative stress, the prevention and treatment for both glucose metabolism and sleep disorders must be performed simultaneously.

Prior research revealed that the intensity of AGE-derived skin autofluorescence (SAF), which is a glycative stress marker, was affected by the length of sleep duration. In individuals with a shorter sleep duration, the SAF value line showing changes by age was located with a shift in hyperdeviation [15]. Briefly, individuals with a shorter sleep duration (shorter than 4 sleeping cycles) had a larger quantity of AGE accumulation in the skin, while individuals with a longer sleep duration (5 sleeping cycles or more) showed low glycative stress. Individuals with high sleep quality had less glycative stress.

We have analyzed relationships between sleep quality and blood glucose alternation recently, employing a continuous blood glucose monitoring meter. The data results showed that with enough sleep duration, postprandial blood glucose level increased mildly after getting up and did not exceed 140 mg/dL, while having a 3-to-4-hour sleep duration, postprandial blood glucose level after breakfast sometimes increased drastically [16]. Insufficient sleep can induce postprandial hyperglycemia.

Blood glucose spike refers to a phenomenon of hyperglycemia elevated sharply to 140 mg/dL or higher, after having a meal, regardless of normal fasting blood glucose level [17, 18]. Blood glucose spikes easily cause vascular endothelial dysfunctions. Repeated blood glucose spikes quickly advance arteriosclerosis and cause diverse physical disorders. Particularly, blood glucose spikes have been recognized to trigger "aldehyde spark" [16, 19, 20]. Due to the decyclization in part of glucose, exposed aldehyde group (-CHO) of glucose in linear-chain form induces, with a chain-reaction behavior, the production of diversified types of aldehyde.

By avoiding sleep deprivation, less frequent blood glucose spikes would lead to an inhabitation of aldehyde spark, which could contribute to health preservation.

Assessment of melatonin metabolites

The present study measured melatonin and melatonin metabolites (HaMT, SaMT and AMK) of collected urine sample to evaluate the effects on melatonin secretions by the use of the test product. The synthesis and metabolic pathway of melatonin are shown in ***Fig. 3*** [25].

The secretion of melatonin is controlled by detecting brightness and darkness with the retina. The secretion of melatonin in human is activated in the dark and is reduced in the light [26]. This is referred to as melatonin oscillation. During bright daytime, the blood concentration is low (10-50 pmol/L) and during dark night, the blood concentration is high (200-500 pmol/L). The oscillation of melatonin decreases along with aging. Therefore, for the assessment of melatonin secretion, measuring concentration of melatonin in blood, salivary and spot urine samples is incorrect. Determining the quantity of melatonin metabolites in collected urine is the most suitable. Thus, the present study employed measurement of typical melatonin metabolites such as HaMT [27-29], SaMT [30-34] and AMK [35-39] in the collected urine samples.

It is recognized that urinary melatonin, which has little variation between day and night, is not associated with blood

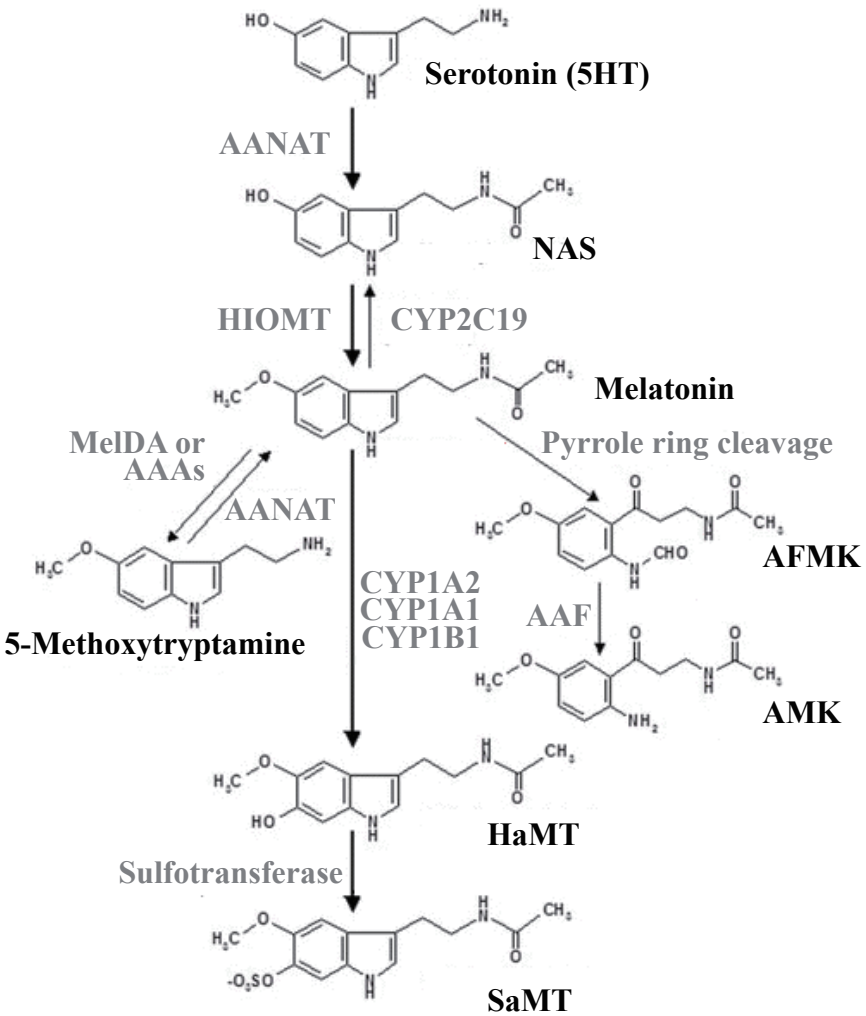

Fig. 3. **Melatonin metabolism.**

5HT, serotonin (5-hydroxytryptamine); AFMK, N(1)-acetyl-N(2)-formyl-5-methoxykynuramine; AMK, N-acetyl-5-methoxykynuramine; HaMT, 6-Hydroxymelatonin; SaMT, 6-Sulfatoxymelatonin; AANAT, arylalkylamine N-acetyltransferase; HIOMT, hydroxyindole-O-methyltransferase ; MelDA, melatonin deacetylase; AAAs, aryl acylamidases; AAF, arylamine formamidase; CYP, cytochrome P450 monooxygenase or dealkylase. Quoted and modified from Reference 7).

melatonin. HaMT, which is a melatonin metabolite, exists 100-1,000 times as much as melatonin in urine and has correlation with blood melatonin, as is widely known [28].

SaMT decreases in patients with diabetes [31] and growth hormone deficiencies [32]. Using these urinary melatonin metabolites as markers, researchers have reported on changes in nurses with 3 working shifts [33], changes in night-shift workers [34], and influences of room temperature while sleeping [30].

AMK is an oxidative melatonin metabolite [35]. AMK has an anti-oxidative effect by itself [36]. AMK is recognized to inhibit the proliferation of pigment cells and tyrosinase activities [38, 39] in the skin.

Melatonin is metabolized mainly to AFMK and AMK in the brain. AMK has an ability to induce long-term memory [40]. Administration of AMK promotes learning and memory in mice tests. Melatonin inhibits the aggregation of amyloid β in Alzheimer's disease [41]. Melatonin is a potential countermeasure for the prevention and improvement of dementia (cognitive impairment), which is expected.

The decline of melatonin secretion is one of the factors for several physical changes caused by aging. The aged are more likely to have sleep disorders than the young, which is induced by this factor. Among changes with aging, melatonin is involved in the decline of immune strength, the increase in the frequency of carcinogenicity, and the abnormality in cholesterol metabolism. It has been reported that melatonin plays an important role in the maintenance of ovarian functions, such as fertilization and implantation, and growth of bones [26].

Melatonin and carbohydrate metabolism

Effects on carbohydrate metabolism by melatonin have been gaining the most attentions these days. Melatonin has no inhibitory effect on the formation of AGEs but has effects to promote the degradation of AGEs. Melatonin administration at bed time subdues high postprandial glucose after having breakfast the next morning. These combined actions can reduce glycative stress.

Mechanisms of how melatonin improves carbohydrate metabolism and reduces glycative stress are shown in ***Fig. 4***.

Firstly, melatonin has an anti-oxidative effect [42-52]. This is based on two activities: a direct action to remove reactive oxygen species (ROS) and an action to promote the activity of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase [26, 53]. In addition, metabolites of AFMK and AMK also have anti-oxidative effects.

Secondly, melatonin has the effect of promoting the degradation of AGEs [54]. In glucose metabolism such as diabetes, endoplasmic reticulum (ER) stress in β-cells of the islet exacerbates insulin secretion decreases [55]. AGEs increase ER stress in β-cells of the islet and decreases the synthesis and secretion of insulin [56]. The degradation of AGEs reduces ER stress, which could induce the recovery of decreased insulin secretion.

The third mechanism is a hormone-mediated activity. Melatonin lowers the secretion of glucocorticoid from the adrenal cortex; for example, cortisol. Glucocorticoid not only promotes acceleration of protein catabolism and gluconeogenesis but also accelerates insulin tolerance, which

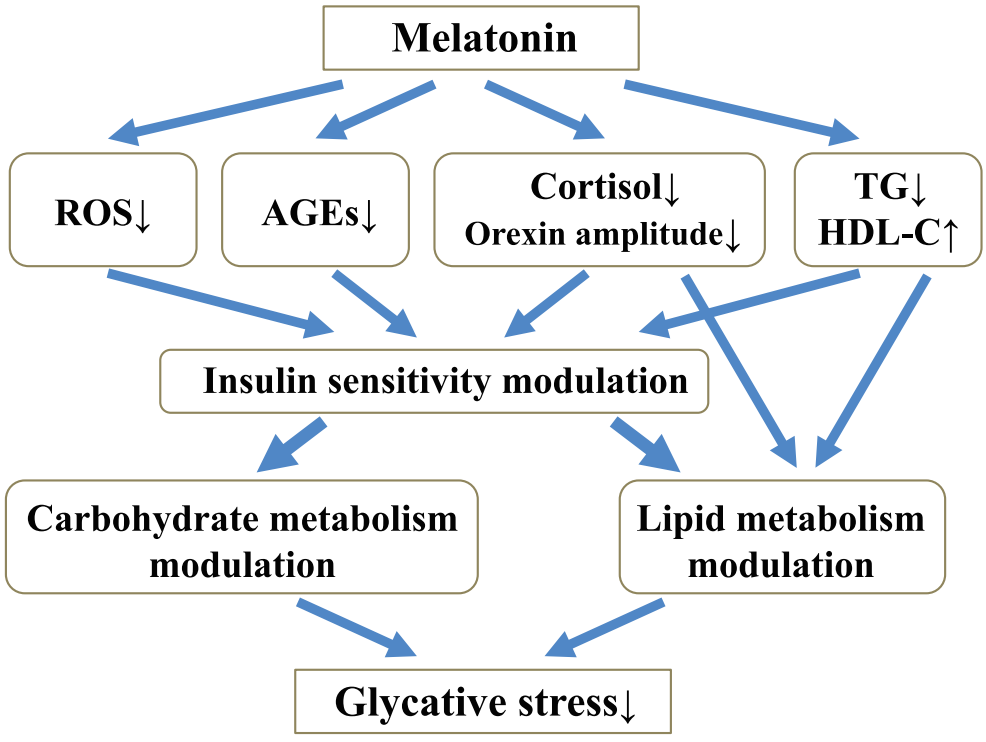

Fig. 4. **Melatonin and glycative stress.**

ROS, reactive oxygen species; AGEs, advanced glycation end products; TG, triglyceride; HDL-C, high-density lipoprotein-cholesterol.

causes hyperglycemia. Cortisol, which accounts for 95% of glucocorticoid activities, has direct effects on lipid metabolism. Cortisol, which is secreted due to acute stress, has lipolysis and promotes mitochondrial use of carbohydrates, lipids and amino acid. Contrarily, cortisol in chronic excessive state inhibits peroxisome proliferator-activated receptor γ (PPARγ) of white fat, causing adiposity [57].

Orexin, which is a type of brain hormone, has the ability to regulate arousal. Orexin shows low levels when sleeping at night and secretes around daybreak as well as diurnal time (orexin oscillation). Orexin secretion is affected by blood glucose: in low blood glucose, its secretion is promoted and in high blood glucose, its secretion is inhibited [58-60]. Oscillation of orexin levels decreases with aging. Orexin and blood glucose changes bidirectionally affect each other, and it is essential to maintain proper orexin oscillation for the control of blood glucose levels. Melatonin recovers the variations of orexin by lowering the accelerated secretion of orexin during night.

The fourth actuating path is the effects of melatonin on the improvement in lipid metabolism. In the experiment with the rat model, which presented hyperlipidemia, melatonin administration decreased triglyceride (TG) and LDL-C. HDL-C increased even in healthy rats [61-63]. As melatonin administration improves insulin tolerance, some areas are improved via insulin effects. Pinealectomized rats were lacking in the secretion of melatonin and showed the disorder of glycolipid metabolism [64]. It was revealed that melatonin played an influential role in maintaining the homeostasis for glycolipid metabolism. Ingestion of melatonin at bed time subdues the rise of postprandial blood glucose level next morning [65].

As the four actuating paths of melatonin were mentioned above, melatonin reduces glycative stress, having direct effects on glycolipid metabolism in most cases and functioning partly via insulin effects. Though the mechanism of effect on β cells of the pancreas is not simple, melatonin reduces fasting insulin in the condition of hyperinsulinemia, such as borderline or early stage type-2 diabetes mellitus [66]. In skeletal muscles, effects of insulin are enhanced, and glucose uptake is activated [67, 68], which results in the decrease of HOMA-IR (homeostasis model assessment of insulin resistance). It is assumed that the insulin resistance is reduced. Furthermore, the synthesis and secretion of insulin is decreased in the condition of intensive oxidative stress and glycative stress where the formation of AGEs increases and ER stress in pancreatic beta cells increases. Under these conditions, the secretion of insulin from β cells of the pancreas is partly supported by melatonin, which has an anti-oxidative effect and an anti-glycative effect (to promote the degradation of AGEs). The reduction of the melatonin secretion is a risk factor for the onset of type-2 diabetes mellitus [69]. In addition, melatonin is involved in the hypertrophy and activation of brown adipose tissues [70]. It is considered that melatonin is related to controlling not only the glycolipid but also whole energy metabolism.

The present trial recognized the tendency that the use of the test product maintained the quantity of HaMT in the collected urine and the quantity of melatonin at high level. It is considered that the elevation ofof the sleep quality resulted in the high leveled quantity of melatonin secretion. When preserving sleep quality at this level, the varied melatonin effects mentioned above can be obtained.

Assessment of continuous blood glucose monitoring

The previous trial confirmed that HbAlc significantly decreased from 5.31 ± 0.23% to 5.19 ± 0.17% using the test product (−2.6%, p = 0.003). The present trial recognized that the appearance frequency of low blood glucose was lower in the test group than the control group for several days, which was marginally significant. We examined the mechanisms of how the improvement in nocturnal blood glucose, which was induced by the sleep quality improvements, are associated with the improvement of carbohydrate metabolism (HbA1c improvement).

Low blood glucose adversely affects cardiovascular events and dementia, [71, 72] as is known. Recently, reports regarding nocturnal hypoglycemia have been increasing. Nocturnal hypoglycemia lowers the quality of life in healthy individuals as well as patients with diabetes [73-76]. Nocturnal hypoglycemia frequently induces postprandial hyperglycemia (blood glucose spike) after eating breakfast [77]. Blood glucose spikes and the followed aldehyde spark cause glycation of hemoglobin. As symptoms of low blood glucose at night, changes in having dreams (a vivid dream or a scary, disturbing dream), night sweat and headache in the morning are listed. However, in several cases, no subjective symptoms were recognized. Employing an electro-cardiogram for hypoglycemia while sleeping, prolonged the QT interval and activated sympathetic nerves [78] are detected. Decline of sleep quality, not having a deep and sound sleep, can lead to physical weariness, feelings of tiredness, and mood disorder during daytime, influencing daytime activities. Additionally, academic performance and work situation can be influenced, which can result in school absence, work absence, low academic performance, and low productivity in work. Long-term effects can lead to deterioration of memory and decline of cognitive function.

Previous research findings regarding nocturnal hypoglycemia are summarized below.

The healthiest condition in nocturnal blood glucose is a state in which homeostasis is maintained, in a fairy limited range, having neither hyperglycemia nor hypoglycemia. Contrarily, hypoglycemia could lead to burdens such as sympathetic nerve stimulation and pituitary-adrenal cortex axis stimulation (cortisol) secretion, which promotes the decline of sleep quality. As an initial change of glycolipid dysbolism in healthy persons (non-diabetic persons), there is a possibility that low blood glucose appears.

Nocturnal hypoglycemia is associated with postprandial hyperglycemia (blood glucose spike) during daytime. However, the details are unidentified in its mechanism; as in which is the cause and which is the result. Judging from the findings of the present study, the improvement in nocturnal hypoglycemia due to the use of the test product or an appropriate bedding reduced postprandial hyperglycemia (blood glucose spike) and mitigated followed aldehyde spark and glycative stress markers, such as HbA1c, were favorably affected, as is interpreted.

Research limitations

It is very difficult for a clinical trial for bedding to select a control product. If we choose a mat of poor quality for a control product, it would be easier to obtain differences comparing the test product. However, there

would be an apprehension that research participants would feel uncomfortable. If the control product is determined to be luxury bedding, it will be exceedingly difficult to extract differences in data. In the present study, the control product was decided as a product of higher quality than a common marketed product (a product of higher quality than the bedding that the participants used), as was judged. In the use of the controlled product, some assessment items in subjective symptoms and other physical markers were recognized to have significantly improved between the before and after. It is considered that this could be a factor that significant differences between groups were not unambiguously detected.

Data results of the continuous blood glucose monitoring test were reflected by individual personal life, such as time of going to bed, getting up, having a meal, and contents of the meal. Individual variation and massive data required considerable labor for the analysis. The present study chose, as analysis markers, average blood glucose during sleep, maximum blood glucose value, minimum blood glucose value, time zone of hyperglycemia, and time zone of hypoglycemia. It is challenging to determine at this stage whether these are correct or not. Further examination is required to settle this issue.

Safety

There were no adverse events reported in this trial. It was judged that the test product has no issue regarding safety.

Conclusion

An improvement tendency of the test group was recognized in subjective symptoms regarding sleep. Melatonin and HaMT, which is a melatonin metabolite, indicated a tendency of remaining at a high level, and the frequency of nocturnal hypoglycemia tended to decrease. These findings suggested a possibility that by using the test product, the secretion of melatonin increased and the homeostasis in nocturnal hypoglycemia was stable. The present study had a small number of subjects and further examination is needed. A possibility of contribution to health promotion was suggested with sleep quality improvement due to appropriate bedding.

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Conflict of Interest Statement

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