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Original article

Effects of mats with "A Distinctive 4-Layer 3-Dimensional Structure" on sleep quality and menpause: An open-label study

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Abstract

***Objectives*:** "Quality of sleep" plays an important role for the maintenance of homeostasis of the body. The deterioration of sleep quality induces diverse lifestyle-related disorders. The present study with an open-label trial verified the effects of a test product, a bed mattress with a "distinctive 4-layer 3-dimensional structure", regarding sleep quality and menopausal syndrome.

***Methods*:** Potential research participants, 38 women who had complaints about sleep quality underwent a questionnaire survey using the Japanese version of the Pittsburgh Sleep Quality Index (PSQI-J) and the Simplified menopausal index (SMI). Among them, 12 women whose scores of PSQI-J and SMI were high (age: 48.2 ± 0.7, PSQIG: 7.8 ± 0.6, SMI: 53.5 ± 3.3) were selected. The test product mattresses (Nishikawa Co., Tokyo, Japan) were used for 8 weeks, and alternations in physical information were examined in an open trial. The present study was conducted with the approval of an ethics committee.

***Results*:** Data results suggested significant improvements in PSQI-J scores 8 weeks after the commencement of the trial. The global score of PSQI significantly improved from 7.8 ± 0.6 to 5.4 ± 0.6 ($p = 0.004$). SMI scores improved significantly from 53.5 ± 3.3 to 42.5 ± 4.5 ($p = 0.003$). Assessments of hormones indicated no significant differences in estradiol, progesterone, luteinizing hormone, and follicle stimulating hormone. Values of dehydroepiandrosterone-sulfate (DHEA-s) changed from (149.9 ± 76.5 μg/dL) → 4 weeks (124.9 ± 63.9 μg/dL) → 8 weeks (146.9 ± 59.2 μg/dL). No adverse event was reported.

***Conclusions*:** Through the usage of the test products, it was suggested that "improvements in sleep quality" resulted in the mitigation of menopausal symptoms, though secretions of menopause-related hormones did not improve. It is considered that appropriate bed mat usage is an effective and safe measure of supplementary guidance for women with severe symptoms of menopause.

***KEY WORDS*:** mats with a "Distinctive 4-Layer 3-Dimensional Structure," sleep quality, menopausal syndrome, dehydroepiandrosterone (DHEA)

Introduction

The deterioration of sleep quality affects diverse functions on the body. Prolonged and habitual sleeping disruptions and/or chronic sleeping disorders raise risks of onsets of diseases such as a cardiovascular disease, diabetes, obesity, and depression, and worsen life prognosis. Appropriate measures for problematic sleep are considered to contribute to the improvement in clinical prognosis of lifestyle-related diseases [1]. Diabetes, which is strongly related to glycative stress, and sleeping issues bidirectionally affect each other, in particular. Therefore, it is required to assess and control "sleep quality" for the treatment and management of diabetes and a patient's quality of life (QOL) [2-4]. It is also known that the decline of sleep quality affects endocrine functions. Steiger *et al.* advocated "a hypothesis of the control by hormones on sleep". In fact, secretions of diverse hormones, such as growth hormone/insulin-like growth

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factor-I (IGF-I), melatonin, orexin, cortisol, insulin, and somatotropin/somatostatin, affect the homeostasis of sleep[5].

We have conducted multiple clinical trials with people who had a decline in "sleep quality" due to problems with bedding quality, to examine how improvements of "sleep quality" affect human bodies[6-10]. Findings show that there was a remarkable difference in physical changes between individuals or subject groups. That is, symptoms due to "sleep quality" deterioration were individually different through lifestyles, environments, and genetic factors. As an example of hormones, not all hormones indicate the same level of alternation.

Menopausal syndrome in middle-aged and older women is a disease induced by alternations in female hormones. Menopausal symptoms are unidentified complaints such as burning sensation, dizziness, hot flushes, transpiration, and irritability, as well as sleep problems, which is designated as menopausal insomnia[11].

We conducted a trial in the present study, an uncontrolled open-label study with premenopausal women with a slight sleep disorder and menopausal symptoms. Effects of the test product of a bed mat with "a distinctive 4-layer 3-dimensional structure" was verified, regarding effects of sleep quality improvements, which was induced by the test product usage, on menopause-related hormones.

Methods

Target

Potential research participants were recruited: 38 women aged between 45 and 54 years of age at the time of the obtainment of consent, who underwent screening and pre-examination test (SCR) with body measurements, blood and urine tests, the Japanese version of the Pittsburgh Sleep Quality Index (PSQI), the Oguri-Shirakawa-Azumi (OSA) sleep inventory MA version, the Simplified Menopausal Index (SMI), the Menstrual Distress Questionnaire (MDQ), and a medical interview by a doctor. Among them, 12 research participants were selected, who satisfied the inclusion criteria, did not match the exclusion criteria, and had PSQI-J score 6 or higher and SMI score 26 or higher.

Key inclusion criteria were as follows:

1) Premenopausal female aged between 45 and 54 at the time of informed consent
2) Individuals who are healthy and have no chronic physical disease including skin disease
3) Individuals who are aware of mild sleep disorders such as waking up in the middle of the night (midway awakening), waking up early in the morning (early morning awakening), and not achieving deep sleep (deep sleep disorder)
4) Individuals who are aware of menopausal symptoms (irritability, anxiety, mood discomfort, insomnia, hot flushes, *etc*.)
5) Individuals who work daytime from 3 to 5 days a week and have a day off for Saturday, Sunday, and public holidays
6) Individuals who sleep for over 4 hours, regularly going to bed (lights-out) and waking up with lights-out time before 0 a. m.
7) Individuals who are sleeping alone
8) Individuals who do not have a habit of drinking alcohol
9) Individuals who are fully informed regarding the purpose and contents of the test, has an ability of consent, voluntarily applied for participation with a full understanding, and agreed to participate in the test with written informed consent
10) Individuals who can come to the designated venue on the date to undergo the examination
11) Individuals judged appropriate for this study by the responsible doctor

Key exclusion criteria were as follows:
Individuals (who)

1) contracted a disease and are receiving medical treatments
2) under treatment or with history of mental disorders, sleep disorders, hypertension, diabetes, lipid metabolism abnormality, or other serious disorders
3) have a history of and/or contract serious diseases (uterine disease, hepatic, renal, cardiovascular, respiratory, hematologic, *etc*.)
4) have a serious history and/or contract digestive disease and comorbidities
5) receiving hormone replacement therapy
6) are suspected, receive treatment of, or have a history of sleep apnea syndrome
7) have or are suspected with the night urination or overactive bladder
8) receiving/received medical drug treatment for the past one month except for temporary relief medication for headache, menstrual pain, common cold, *etc*.
9) BMI are 30.0 kg/m^2 or more
10) have a habit to use functional foods and/or are planning to use those foods during test periods
11) donated 200 mL of blood in the past month or more than 400 mL within 3 months
12) with possible changes of life style during test periods and with possibility of being unable to use the test product mat due to long-term trip, *etc*.
13) are participating and/or had participated in other clinical studies within the last 3 months
14) are or are possibly pregnant, or are breastfeeding
15) are judged as not appropriate to this study by a responsible doctor

Trial design

The present study was an uncontrolled open-label study.

The test product was a bed mattress with a distinctive 4-layer 3-dimensional structure, "AiR SX" (Nishikawa Co. Ltd., Chuo-ku, Tokyo, Japan). The size of the test product was a single size (9 × 97 × 200 cm). The mats with the specified sheets were provided by Nishikawa. At the commencement of this trial, the mattress which research participants used was replaced with the test product.

Before the commencement of the test, 4 weeks, and 8 weeks after the commence of the test, research participants underwent body measurements, the Japanese version of Pittsburgh Sleep Quality Index (PSQI-J), the OSA sleep inventory MA version, SMI, MDQ, the Anti-Aging QOL

Common Questionnaire (AAQOL), SF-36v2 acute version, blood specific-designed examination: estradiol (E2), progesterone, dehydroepiandrosterone-sulfate (DHEA-s), luteinizing hormone (LH), and follicle-stimulating hormone (FSH), and a medical interview by a doctor. Other than these examinations, before and 8 weeks after the commencement of the trial, hematological examination, blood biochemical examination, and urine tests were performed. Research participants recorded a living diary on the presence or absence of adverse events, lifestyle habits, and dietary and excise habits. The trial period was between May and July of 2022.

Assessment Items

Subjective symptoms (sleeping)

Qualities of sleep were assessed by PSQI-J, and the OSA sleep inventory MA version [12]. In accordance with scoring system in the totals table of questionnaire sheets in PSQI, the items of sleep quality, time to fall asleep, sleep duration, sleep efficiency, difficulty sleeping, use of sleep inducers, daytime difficulty waking were scored, and PSQI global score (PSQIG) was calculated. In assessment standard, 5 or lower points were no sleep disorder, 6 or higher points were a sleep disorder. A mild disorder was from 6 to 8 points, and a severe disorder was 9 points or higher [13].

Research participants answered the OSA sleep inventory MA version, which was a psychological scale to evaluate self-reflection at wake-up time, with numbering in 4-point scale regarding go-to-bed time, get-out-of-bed time, and sleep duration. Data results were calculated with each factor, Factor 1/sleepiness on rising, Factor 2/initiation and maintenance of sleep, Factor 3/frequent dreaming, Factor 4/refreshing, and Factor 5/sleep length.

Subjective symptoms (menopausal disorder and QOL)

Subjective symptoms of menopausal disorder and QOL were evaluated, employing SMI [14], MDQ [15], AAQOL [16], and SF-36v2 acute version [17].

SMI provided a 4-level evaluation, Severe, Moderate, Mild, and Absent, for the following 10 items: 1. Hot flushes, 2. Sweats, 3. Cold constitution of waist, hands, and feet, 4. Shortness of breath or palpitation, 5. Difficulty in falling asleep or shallow sleep, 6. Easy excitability or irritability, 7, Nervousness and self-depression, 8. Headache, dizziness or nausea, 9. Easy fatigability, and 10. Shoulder stiffness, lumbago or joint pain. Evaluation standard indicated Normal: 0–25, Need of attention in daily life: 26–50, Need of visit to an obstetrician, or a specialized doctor in menopausal disorder: 51–65, and Need for a long-term (longer than 6 months) medical treatment plan: 66–80, and Need of complete examinations of clinical departments: 81–100.

Assessments in MDQ provided a 4-grade evaluation from 1. Absence to 4. Strong, with 54 question items regarding dysmenorrhea and/or dysmenorrhea-related conditions, respectively after menses, during menses, and after menses. Recall method (Atype) was employed for answering questions. Each item was classified into three categories, "physical symptoms", "mental symptoms", and "daily-life troubles". Data results were calculated by scoring from 0 to 4 points.

AAQOL had questions regarding two types of complaints about "physical and mental symptoms", and assessed with a 5-level evaluation:1. Never, 2. Rarely, 3. A few, 4. Middle level, 5. High level.

Answer scoring of SF-36v2 acute version was performed with 36 items for the following factors, employing a Likert scale (5 levels):

Physical functioning (PF), Role physical (RP), Bodily pain (BP), General health (GH), Vitality (VT), Social functioning (SF), Role emotional (RE), Mental health (MH), physical component summary (PCS), mental component summary (MCS), and role/social component summary (RCS).

Body measurement

Body height, weight, somatic fat rate, body mass index (BMI), contraction and diastolic blood pressure, and pulse rate were measured in the body measurement. A multi frequency segmental body composition analyzer was employed for the examination of body composition (MC-180: Tanita corporation, Itabashi-ku, Tokyo, Japan).

Blood specifically-designed examination

Estradiol (E2), progesterone, DHEA-s, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were measured.

Hematological examination

Measurements were performed in white blood-cell count (WBC), red blood-cell count (RBC), hemoglobin content (Hb), hematocrit value (Ht), mean red cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count (Plt).

Blood biochemical examination

The present study performed measurements of total protein (TA), albumin (quantitative) (Alb), blood urea nitrogen (BUN), creatinine (Cre), uric acid (UA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CPK), total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), non-HDL cholesterol, total bilirubin (T-Bil), sodium (Na), potassium (K), chloride (Cl), Calcium (Ca), magnesium (Mg), iron (Fe), fasting blood glucose (FPG), and C-reactive protein (quantitative) (CRP).

The blood examinations were performed in Ueno-Asagao Clinic (Director: Takahiro Ono, Tokyo, Japan) and measurements were performed in Hoken Kagaku, Inc. (Yokohama, Kanagawa, Japan).

Statistical analysis

Values of examination results were calculated in the

ground total sheet, using Microsoft Office Excel 2016 (Microsoft Corp.). As fundamental statistics, the mean values, standard deviation, and standard error were calculated. Values that were used for statistical analysis were measured values and alternation quantity from the value before the usage. Statistical analysis was performed using appropriate statistical software such as SAS (SAS 9.4) or SPSS (Statistics 26). For all tests, significance level was 5 % in two-tailed tests.

Statistical data of examination points were analyzed in a paired t-test with comparison among the points of before the usage, 4 weeks, and 8 weeks after the commencement of the usage. Scores that were obtained in the questionnaire surveys, and data obtained in urine examinations were treated as non-parametric, and Wilcoxon signed-rank sum test was conducted for comparison among groups.

Ethical review

The present study was conducted with an ethical approval obtained from the committee of "medical research involving human subjects" of a general incorporated association: Society for Glycative Stress Research (April 15, 2022, GSE #2022-002). The present study was conducted in the compliance with the Declaration of Helsinki (Amendment: October 2013) and "Ethical Guidelines for Medical and Biological Research Involving Human Subjects (Ministry of Education, Culture, Sports, Science and Technology/Ministry of Health Labour and Welfare, December 22, 2014). An informed consent process was provided to potential research participants prior to initiating the research, and obtained written consents were the voluntary agreement of research participants. Pre-registration for a clinical trial to University Hospital Medical Information Network, UMIN Clinical Trials Registry (UMIN-CTR) was conducted (registered number: UMIN #000047493). Furthermore, considering the social situation of COVID-19 infections and impacts, we conducted the present study with the exercise of extreme caution in the accordance with the guidelines for infection prevention of COVID-19 by the medical institution for the trial, only in the case where study directors of the entrusted, the principal investigator, and the ethical committee of medical research judged that the trial was able to be conducted safely.

Results

An open-label trial was conducted to examine effects of a test product mattress with 8 weeks usage regarding the quality of sleep, QOL, and menopausal syndrome. Research participants were premenopausal women aged between 45 and 54 years of age at the time of obtainments of consents.

Twelve female research participants started to undergo this trial and all the participants completed the trial. The 12 participants were the target for analysis. The age of 12 female participants in the group of the test product is 48.2 ± 0.7. The score of PSQIG at inclusion was 7.8 ± 0.6. SMI was 53.5 ± 3.3.

Assessment of subjective symptoms (sleeping) ***Table 1***

Subjective symptoms obtained by PSQI-J suggested that "sleep quality" ($p = 0.014$) and "daytime difficulty waking" ($p = 0.046$) significantly improved 4 weeks after the usage, compared before the usage. After 8 weeks, "sleep quality" ($p = 0.002$) and "sleep duration" ($p = 0.025$) showed significant improvements. As a result, PSQIG significantly improved from 7.8 ± 0.6 (before usage) to 6.3 ± 0.7 (4 weeks after usage) ($p = 0.007$). Moreover, PSQIG significantly improved to 5.4 ± 0.6 eight weeks after the usage ($p = 0.004$).

The examinations of OSA sleep inventory, which was a psychological scale to evaluate self-reflection at wake-up time, indicated that Factor 2: initiation and maintenance of sleep significantly improved ($p = 0.034$) 4 weeks after the usage in comparison with before the usage. Factor 1: sleepiness on rising ($p = 0.041$) and Factor 4: refreshing ($p = 0.010$) significantly improved 8 weeks after the usage.

Subjective symptoms (menopausal symptoms and QOL)

Examinations of **SMI** indicated that "difficulty in falling asleep or shallow sleep" significantly improved after 4 weeks compared with before the usage ($p = 0.041$). "difficulty in falling asleep or shallow sleep" ($p = 0.011$), "easy fatigability" ($p = 0.023$), and "shoulder stiffness", "lumbago or joint pain" ($p = 0.035$) significantly improved after 8 weeks compared with before the usage. As a result, the score of SMI significantly improved from 53.5 ± 3.3 (before usage) to 42.5 ± 4.5 (8 weeks after the usage) ($p = 0.003$, ***Table 2***).

MDQ (before menses): compared with before the usage, after 4 weeks, "experienced drowsiness and took a nap" ($p = 0.039$), "headache" ($p = 0.046$), and "edema (abdomen, breast, and legs)" ($p = 0.020$) significantly improved. Compared with before the usage, after 8 weeks, "inactive for learning and working" ($p = 0.014$), "headache" ($p = 0.020$), "break into a cold sweat" ($p = 0.046$), and "to lose confidence and blame oneself" ($p = 0.034$) significantly improved. As a result, the score of daily-life troubles in MDQ (before menses) significantly improved after 5 weeks, compared with before the usage. (***Table 3-a***).

MDQ (during menses): compared with before the usage, after 4 weeks, "experienced drowsiness and took a nap" ($p = 0.011$) significantly improved. After 8 weeks, "experienced drowsiness and took a nap" ($p = 0.016$), and "be upset" ($p = 0.025$) significantly improved (***Table 3-b***).

MDQ (after menses): compared with before the usage, after 4 weeks, and 8 weeks, no item significantly improved. (***Table 3-c***).

In Anti-Aging QOL Common Questionnaire (AAOQL), among 33 items of physical symptoms, 3 items indicated significant improvements: "to gain weight easily", "dizziness", and "lumbago", and among 21 items of mental symptoms, 5 items indicated significant improvements after 8 weeks: "a shallow sleep", "to take time in falling asleep", "poor concentration", "sleep deprivation due to anxiety", and "unreasonable anxiety" (***Table 4***).

Table 1. Sleep-related subjective symptoms.

		Before	4 weeks		8 weeks	
		Mean SEM	Mean SEM	P value	Mean SEM	P value
PSQI-J	Sleep quality	2.0 ± 0.0	1.5 ± 0.2	**0.014**	1.1 ± 0.1	**0.002**
	Time to fall asleep	1.6 ± 0.3	1.5 ± 0.3	0.706	1.1 ± 0.3	0.058
	Sleep duration	1.8 ± 0.2	1.5 ± 0.2	0.083	1.3 ± 0.2	**0.025**
	Sleep efficiency	0.3 ± 0.2	0.2 ± 0.1	0.317	0.1 ± 0.1	0.157
	Difficulty sleeping	1.0 ± 0.1	0.8 ± 0.1	0.157	0.9 ± 0.1	0.564
	Use of sleep inducers	0.0 ± 0.0	0.0 ± 0.0	1.000	0.0 ± 0.0	1.000
	Daytime difficulty waking	1.2 ± 0.2	0.8 ± 0.2	**0.046**	0.9 ± 0.3	0.366
	PSQIG	7.8 ± 0.6	6.3 ± 0.7	**0.007**	5.4 ± 0.6	**0.004**
OSA	Factor 1: sleepiness on rising	42.3 ± 2.1	46.1 ± 2.2	0.071	46.6 ± 2.3	**0.041**
	Factor 2: initiation and maintenance of sleep	39.6 ± 1.5	44.5 ± 2.2	**0.034**	44.9 ± 2.3	0.060
	Factor 3; freaquent dreaming	51.1 ± 2.4	51.5 ± 2.2	0.683	52.3 ± 2.4	0.221
	Factor 4: refreshing	42.1 ± 1.5	45.6 ± 1.9	0.100	48.1 ± 2.1	**0.010**
	Factor 5: sleep length	41.3 ± 2.4	42.5 ± 2.4	0.575	45.5 ± 3.1	0.083

Analyzed by Wilcoxon signed-rank sum test, n = 12. PSQI- J, Japanese version of the Pittsburgh Sleep Quality Index; PSQIG, PSQI global score; OSA, OSA sleep inventory MA version; SEM, standard error mean.

Table 2. Menopause-related subjective symptoms: SMI.

	Before	4 weeks		8 weeks	
	Mean SEM	Mean SEM	P value	Mean SEM	P value
1. Hot flushes	2.5 ± 0.7	2.5 ± 0.6	1.000	2.5 ± 0.7	1.000
2. Sweats	6.2 ± 0.6	5.7 ± 0.8	0.589	5.3 ± 0.6	0.334
3. Cold constitution of waist, hands, and feet	7.8 ± 1.2	6.5 ± 1.3	0.196	5.8 ± 1.0	0.066
4. Shortness of breath or palpitations	3.3 ± 1.2	3.0 ± 0.9	0.739	2.7 ± 0.8	0.414
5. Difficulty in falling asleep or shallow sleep	10.8 ± 0.9	8.1 ± 1.1	**0.041**	7.1 ± 1.2	**0.011**
6. Easy excitability or irritability	6.7 ± 1.1	5.3 ± 1.2	0.103	6.7 ± 1.4	1.000
7. Nervousness and self-depression	3.7 ± 0.7	3.1 ± 0.6	0.334	2.8 ± 0.7	0.063
8. Headache, dizziness or nausea	2.1 ± 0.6	2.3 ± 0.5	0.590	1.7 ± 0.5	0.257
9. Easy fatigability	5.1 ± 0.5	4.3 ± 0.6	0.238	3.6 ± 0.6	**0.023**
10. Shoulder stiffness, lumbago or joint pain	5.5 ± 0.4	4.6 ± 0.6	0.083	4.3 ± 0.5	**0.035**
Score	53.5 ± 3.3	45.4 ± 4.3	0.059	42.5 ± 4.5	**0.003**

Analyzed by Wilcoxon signed-rank sum test, n = 12. SMI, Simplified menopausal index; SEM, standard error mean.

Table 3-a. Menopause-related subjective symptoms: MDQ.

MDQ (before menses)	Before	4 weeks		8 weeks	
	Mean SEM	Mean SEM	P value	Mean SEM	P value
Weight gain	1.8 ± 0.3	2.2 ± 0.2	0.103	1.8 ± 0.3	0.564
Insomnia	1.2 ± 0.2	1.4 ± 0.3	0.257	0.8 ± 0.2	0.103
Feel like crying	0.9 ± 0.3	1.0 ± 0.3	0.706	0.6 ± 0.3	0.103
Inactive for learning and working	1.7 ± 0.2	1.3 ± 0.3	0.096	1.2 ± 0.2	**0.014**
Stiffness in the shoulders and neck	2.1 ± 0.3	1.9 ± 0.3	0.480	1.5 ± 0.3	0.107
Forgetfulness	1.1 ± 0.3	1.3 ± 0.3	0.480	1.0 ± 0.3	0.792
Difficulty in thinking clearly	1.1 ± 0.3	1.1 ± 0.3	1.000	0.8 ± 0.3	0.429
Experienced drowsiness and took a nap	1.8 ± 0.3	1.2 ± 0.3	**0.039**	1.3 ± 0.3	0.083
Headache	1.3 ± 0.4	0.9 ± 0.3	**0.046**	0.7 ± 0.3	**0.020**
Skin irritation	1.8 ± 0.4	1.5 ± 0.3	0.257	1.6 ± 0.4	0.414
Feeling sad	1.2 ± 0.3	1.0 ± 0.3	0.414	1.0 ± 0.3	0.414
Have trouble breathing	0.5 ± 0.2	0.4 ± 0.2	0.564	0.3 ± 0.1	0.180
Feeling kind	0.7 ± 0.2	0.5 ± 0.2	0.317	0.7 ± 0.2	1.000
Feeling honest	0.5 ± 0.2	0.8 ± 0.1	0.180	0.8 ± 0.2	0.257
Reluctance to leave the house	1.3 ± 0.3	1.2 ± 0.3	0.317	0.9 ± 0.3	0.132
Lower abdominal pain	1.7 ± 0.3	1.2 ± 0.3	0.105	1.3 ± 0.3	0.131
Dizziness	0.9 ± 0.3	0.8 ± 0.2	0.706	0.6 ± 0.2	0.103
Easily agitated	1.1 ± 0.3	0.8 ± 0.2	0.558	0.8 ± 0.2	0.518
Tightness in the chest	0.3 ± 0.1	0.2 ± 0.2	0.706	0.3 ± 0.1	1.000
Difficulty in socializing with others	1.3 ± 0.3	1.1 ± 0.2	0.257	1.0 ± 0.3	0.280
Feeling anxious	1.4 ± 0.3	1.1 ± 0.3	0.180	1.1 ± 0.3	0.180
Lumbago	1.7 ± 0.3	1.4 ± 0.3	0.257	1.7 ± 0.3	1.000
Break into a cold sweat	0.3 ± 0.1	0.3 ± 0.1	1.000	0.6 ± 0.2	**0.046**
Nausea	0.1 ± 0.1	0.0 ± 0.0	0.317	0.0 ± 0.0	0.317
Restlessness	1.1 ± 0.3	1.0 ± 0.3	0.564	1.0 ± 0.3	0.564
Hot flush	0.7 ± 0.3	0.8 ± 0.3	0.564	0.8 ± 0.2	0.739
Difficulty concentrating	1.3 ± 0.3	1.4 ± 0.3	0.480	1.0 ± 0.3	0.180
Breast pain	1.4 ± 0.3	1.5 ± 0.3	0.706	1.5 ± 0.3	0.706
Impaired judgment	0.9 ± 0.3	1.0 ± 0.3	0.317	0.8 ± 0.3	0.655
Easy fatigability	1.8 ± 0.3	1.8 ± 0.3	0.564	1.5 ± 0.2	0.157
Abdominal fullness feeling	1.5 ± 0.3	1.4 ± 0.3	0.706	1.0 ± 0.2	0.058
Change in appetite	1.7 ± 0.4	1.4 ± 0.3	0.180	1.5 ± 0.3	0.480
Suicidal feeling	0.4 ± 0.3	0.4 ± 0.3	1.000	0.4 ± 0.3	1.000
Feeling happy	0.6 ± 0.2	0.4 ± 0.1	0.480	0.5 ± 0.2	0.564
Tinnitus	0.2 ± 0.1	0.4 ± 0.2	0.180	0.2 ± 0.1	1.000
Distracted	1.4 ± 0.3	1.3 ± 0.3	0.706	0.8 ± 0.3	0.066
Edema (abdomen, breast, and legs)	1.8 ± 0.3	1.3 ± 0.3	**0.020**	1.5 ± 0.3	0.234
Often cut fingers, break plates, and make mistakes.	0.3 ± 0.2	0.3 ± 0.3	1.000	0.2 ± 0.2	0.317
Irritability	2.0 ± 0.3	1.9 ± 0.3	0.655	1.8 ± 0.3	0.317
Body hurts	1.2 ± 0.3	1.1 ± 0.3	0.655	0.8 ± 0.3	0.103
Be upset	1.2 ± 0.3	1.2 ± 0.3	1.000	0.9 ± 0.3	0.257
Palpitaion	0.3 ± 0.1	0.4 ± 0.2	0.317	0.6 ± 0.2	0.083
Depressed	1.5 ± 0.3	1.3 ± 0.3	0.414	1.3 ± 0.3	0.527

Reduced efficiency in work and study	1.2 ± 0.3	1.3 ± 0.3	0.157	1.0 ± 0.3	0.706
Awkward movement	0.5 ± 0.3	0.3 ± 0.2	0.655	0.3 ± 0.1	0.655
Numbness in the limbs	0.0 ± 0.0	0.1 ± 0.1	0.317	0.1 ± 0.1	0.317
Change in food preferences	0.8 ± 0.3	0.5 ± 0.3	0.180	0.6 ± 0.3	0.480
Nervousness	0.8 ± 0.4	0.8 ± 0.4	1.000	0.8 ± 0.3	1.000
Blurry or missing parts	0.9 ± 0.3	0.8 ± 0.3	0.516	0.9 ± 0.3	1.000
Impulsive	1.0 ± 0.3	0.7 ± 0.3	0.103	0.8 ± 0.3	0.257
Irritability, quarrels with close people. scold a child	1.7 ± 0.3	1.6 ± 0.3	0.655	1.3 ± 0.3	0.257
To lose confidence and blame oneself	1.4 ± 0.3	1.1 ± 0.3	0.157	0.9 ± 0.3	**0.034**
Take a day off from work (school)	0.2 ± 0.2	0.1 ± 0.1	0.317	0.0 ± 0.0	0.317
Easily moved to tears	0.7 ± 0.3	0.8 ± 0.30	0.564	0.6 ± 0.3	0.706
Physical symptoms	22.1 ± 2.8	20.2 ± 2.8	0.123	18.9 ± 2.8	0.059
Mental symptoms	15.7 ± 3.3	13.9 ± 3.0	0.451	13.3 ± 3.1	0.112
Daily-life troubles	19.9 ± 3.2	18.4 ± 3.1	0.303	15.3 ± 3.3	**0.050**

a) Before menses, **b**) During menses, **c**) After menses. Analyzed by Wilcoxon signed-rank sum test, n = 12. MDQ, Menstrual Distress Questionnaire; SEM, standard error mean.

Table 3-b.

MDQ (during menses)	Before	4 weeks		8 weeks	
	Mean SEM	Mean SEM	P value	Mean SEM	P value
Weight gain	1.4 ± 0.3	1.6 ± 0.3	0.414	1.3 ± 0.3	0.317
Insomnia	1.2 ± 0.2	1.0 ± 0.3	0.480	0.7 ± 0.2	0.084
Feel like crying	0.4 ± 0.1	0.4 ± 0.1	1.000	0.3 ± 0.1	0.317
Inactive for learning and working	1.3 ± 0.3	1.1 ± 0.2	0.257	1.0 ± 0.2	0.157
Stiffness in the shoulders and neck	1.7 ± 0.3	1.9 ± 0.3	0.477	1.3 ± 0.3	0.272
Forgetfulness	0.9 ± 0.2	1.0 ± 0.2	0.783	0.8 ± 0.2	0.739
Difficulty in thinking clearly	0.9 ± 0.3	0.9 ± 0.3	1.000	0.6 ± 0.2	0.305
Experienced drowsiness and took a nap	1.9 ± 0.2	1.2 ± 0.2	**0.011**	1.0 ± 0.3	**0.016**
Headache	0.9 ± 0.3	0.8 ± 0.3	0.589	0.4 ± 0.2	0.084
Skin irritation	1.0 ± 0.2	1.0 ± 0.2	1.000	0.7 ± 0.2	0.103
Feeling sad	0.7 ± 0.2	0.5 ± 0.2	0.414	0.5 ± 0.2	0.317
Have trouble breathing	0.4 ± 0.2	0.4 ± 0.1	1.000	0.3 ± 0.1	0.317
Feeling kind	0.7 ± 0.2	0.6 ± 0.2	0.564	0.9 ± 0.2	0.180
Feeling honest	0.8 ± 0.3	0.9 ± 0.2	0.414	1.0 ± 0.2	0.257
Reluctance to leave the house	1.9 ± 0.3	1.4 ± 0.3	0.131	1.4 ± 0.3	0.109
Lower abdominal pain	1.5 ± 0.2	1.4 ± 0.2	0.564	1.3 ± 0.2	0.157
Dizziness	0.7 ± 0.2	0.7 ± 0.2	1.000	0.4 ± 0.2	0.180
Easily agitated	0.7 ± 0.2	0.7 ± 0.2	1.000	0.6 ± 0.1	0.706
Tightness in the chest	0.2 ± 0.1	0.0 ± 0.0	0.157	0.3 ± 0.1	0.564
Difficulty in socializing with others	1.7 ± 0.3	1.5 ± 0.2	0.480	1.1 ± 0.3	0.066
Feeling anxious	0.8 ± 0.2	0.8 ± 0.2	0.655	0.6 ± 0.2	0.083
Lumbago	1.6 ± 0.3	1.1 ± 0.3	0.096	1.2 ± 0.3	0.206
Break into a cold sweat	0.3 ± 0.2	0.3 ± 0.1	0.564	0.6 ± 0.2	0.083

Nausea	0.1 ± 0.1	0.0 ± 0.0	0.317	0.0 ± 0.0	0.317
Restlessness	0.7 ± 0.2	0.6 ± 0.2	0.564	0.4 ± 0.2	0.257
Hot flush	0.6 ± 0.2	0.7 ± 0.2	0.564	0.7 ± 0.2	0.739
Difficulty concentrating	1.2 ± 0.3	1.0 ± 0.2	0.739	0.8 ± 0.2	0.257
Breast pain	0.5 ± 0.2	0.8 ± 0.2	0.083	0.7 ± 0.2	0.157
Impaired judgment	0.7 ± 0.3	0.7 ± 0.2	0.706	0.6 ± 0.2	1.000
Easy fatigability	1.8 ± 0.2	1.6 ± 0.1	0.414	1.3 ± 0.2	0.160
Abdominal fullness feeling	1.1 ± 0.3	1.1 ± 0.2	1.000	0.8 ± 0.2	0.083
Change in appetite	1.3 ± 0.3	1.2 ± 0.2	0.655	1.3 ± 0.3	1.000
Suicidal feeling	0.1 ± 0.1	0.2 ± 0.2	0.317	0.2 ± 0.2	0.317
Feeling happy	0.8 ± 0.2	0.7 ± 0.2	0.480	1.0 ± 0.3	0.480
Tinnitus	0.2 ± 0.1	0.3 ± 0.1	0.157	0.2 ± 0.1	1.000
Distracted	1.1 ± 0.2	1.1 ± 0.3	1.000	0.8 ± 0.2	0.317
Edema (abdomen, breast, and legs)	1.2 ± 0.2	1.2 ± 0.2	1.000	1.3 ± 0.3	0.706
Often cut fingers, break plates, and make mistakes.	0.2 ± 0.1	0.2 ± 0.1	1.000	0.1 ± 0.1	0.317
Irritability	1.3 ± 0.2	1.3 ± 0.3	1.000	1.0 ± 0.3	0.103
Body hurts	1.2 ± 0.3	1.0 ± 0.3	0.480	0.9 ± 0.3	0.257
Be upset	0.8 ± 0.2	0.7 ± 0.1	0.317	0.4 ± 0.1	**0.025**
Palpitaion	0.3 ± 0.1	0.3 ± 0.1	1.000	0.3 ± 0.1	1.000
Depressed	1.1 ± 0.2	0.9 ± 0.2	0.317	0.9 ± 0.3	0.527
Reduced efficiency in work and study	1.2 ± 0.3	0.9 ± 0.2	0.180	1.0 ± 0.3	0.706
Awkward movement	0.7 ± 0.3	0.4 ± 0.2	0.414	0.8 ± 0.3	0.706
Numbness in the limbs	0.0 ± 0.0	0.1 ± 0.1	0.317	0.1 ± 0.1	0.317
Change in food preferences	0.6 ± 0.3	0.2 ± 0.1	0.103	0.3 ± 0.2	0.083
Nervousness	0.7 ± 0.3	0.4 ± 0.3	0.480	0.5 ± 0.2	0.655
Blurry or missing parts	0.8 ± 0.3	0.8 ± 0.3	0.725	0.9 ± 0.3	0.739
Impulsive	0.5 ± 0.2	0.4 ± 0.1	0.706	0.6 ± 0.2	0.739
Irritability, quarrels with close people. scold a child	1.3 ± 0.3	1.1 ± 0.2	0.480	0.7 ± 0.2	0.103
To lose confidence and blame oneself	0.9 ± 0.3	0.5 ± 0.2	0.157	0.6 ± 0.2	0.180
Take a day off from work (school)	0.2 ± 0.1	0.0 ± 0.0	0.157	0.0 ± 0.0	0.157
Easily moved to tears	0.4 ± 0.1	0.5 ± 0.2	0.564	0.3 ± 0.1	0.157
Physical symptoms	17.3 ± 1.5	16.8 ± 1.5	0.919	14.8 ± 2.0	0.253
Mental symptoms	10.9 ± 2.1	9.5 ± 1.9	0.530	9.4 ± 2.2	0.505
Daily-life troubles	18.5 ± 2.5	15.3 ± 2.0	0.422	13.2 ± 2.4	0.075

a) Before menses, **b**) During menses, **c**) After menses. Analyzed by Wilcoxon signed-rank sum test, n = 12. MDQ, Menstrual Distress Questionnaire; SEM, standard error mean.

Table 3-c.

MDQ (after menses)	Before	4 weeks		8 weeks	
	Mean SEM	Mean SEM	P value	Mean SEM	P value
Weight gain	0.7 ± 0.2	0.9 ± 0.2	0.317	0.5 ± 0.2	0.458
Insomnia	1.0 ± 0.2	0.9 ± 0.2	0.739	0.7 ± 0.2	0.157
Feel like crying	0.3 ± 0.2	0.3 ± 0.2	1.000	0.2 ± 0.1	0.157
Inactive for learning and working	0.7 ± 0.1	0.5 ± 0.2	0.317	0.5 ± 0.2	0.317
Stiffness in the shoulders and neck	1.6 ± 0.3	1.3 ± 0.3	0.083	1.1 ± 0.3	0.058
Forgetfulness	0.6 ± 0.2	0.8 ± 0.2	0.414	0.8 ± 0.2	0.414
Difficulty in thinking clearly	0.3 ± 0.2	0.4 ± 0.2	0.564	0.5 ± 0.2	0.317
Experienced drowsiness and took a nap	0.7 ± 0.2	0.4 ± 0.1	0.083	0.5 ± 0.2	0.317
Headache	0.3 ± 0.1	0.3 ± 0.2	0.317	0.3 ± 0.2	0.317
Skin irritation	0.4 ± 0.2	0.4 ± 0.1	1.000	0.3 ± 0.1	0.317
Feeling sad	0.3 ± 0.2	0.3 ± 0.1	1.000	0.4 ± 0.1	0.564
Have trouble breathing	0.3 ± 0.2	0.3 ± 0.1	0.564	0.3 ± 0.1	0.655
Feeling kind	0.8 ± 0.3	1.0 ± 0.2	0.334	1.3 ± 0.3	0.131
Feeling honest	0.8 ± 0.3	1.1 ± 0.3	0.257	1.4 ± 0.3	0.066
Reluctance to leave the house	0.4 ± 0.2	0.6 ± 0.2	0.157	0.6 ± 0.1	0.317
Lower abdominal pain	0.1 ± 0.1	0.1 ± 0.1	1.000	0.2 ± 0.1	0.564
Dizziness	0.3 ± 0.2	0.5 ± 0.2	0.157	0.4 ± 0.2	0.317
Easily agitated	0.4 ± 0.2	0.4 ± 0.2	1.000	0.3 ± 0.2	0.739
Tightness in the chest	0.1 ± 0.1	0.1 ± 0.1	1.000	0.3 ± 0.1	0.317
Difficulty in socializing with others	0.5 ± 0.2	0.6 ± 0.1	0.317	0.4 ± 0.1	0.564
Feeling anxious	0.5 ± 0.2	0.3 ± 0.1	0.317	0.5 ± 0.2	1.000
Lumbago	0.9 ± 0.3	0.8 ± 0.3	0.480	0.7 ± 0.3	0.317
Break into a cold sweat	0.3 ± 0.1	0.2 ± 0.1	0.317	0.3 ± 0.1	0.317
Nausea	0.1 ± 0.1	0.0 ± 0.0	0.317	0.0 ± 0.0	0.317
Restlessness	0.3 ± 0.2	0.3 ± 0.2	0.317	0.3 ± 0.1	1.000
Hot flush	0.6 ± 0.2	0.4 ± 0.1	0.414	0.4 ± 0.2	0.480
Difficulty concentrating	0.5 ± 0.2	0.5 ± 0.2	1.000	0.4 ± 0.2	0.317
Breast pain	0.1 ± 0.1	0.3 ± 0.1	0.157	0.1 ± 0.1	1.000
Impaired judgment	0.2 ± 0.1	0.3 ± 0.2	0.157	0.3 ± 0.1	0.157
Easy fatigability	1.1 ± 0.2	0.8 ± 0.2	0.157	0.6 ± 0.2	0.058
Abdominal fullness feeling	0.3 ± 0.2	0.3 ± 0.1	1.000	0.3 ± 0.1	1.000
Change in appetite	0.3 ± 0.1	0.4 ± 0.1	0.157	0.3 ± 0.1	1.000
Suicidal feeling	0.2 ± 0.2	0.1 ± 0.1	0.317	0.1 ± 0.1	0.317
Feeling happy	1.0 ± 0.2	1.1 ± 0.2	0.739	1.1 ± 0.3	0.739
Tinnitus	0.2 ± 0.1	0.3 ± 0.1	0.157	0.2 ± 0.1	1.000
Distracted	0.4 ± 0.2	0.5 ± 0.2	0.317	0.3 ± 0.1	0.564
Edema (abdomen, breast, and legs)	0.4 ± 0.1	0.5 ± 0.2	0.564	0.8 ± 0.2	**0.046**
Often cut fingers, break plates, and make mistakes.	0.2 ± 0.1	0.1 ± 0.1	0.317	0.1 ± 0.1	0.317
Irritability	0.8 ± 0.3	0.7 ± 0.2	0.564	0.8 ± 0.3	1.000
Body hurts	0.8 ± 0.2	0.6 ± 0.2	0.180	0.5 ± 0.2	0.103
Be upset	0.5 ± 0.2	0.3 ± 0.1	0.157	0.3 ± 0.1	0.157
Palpitaion	0.3 ± 0.1	0.3 ± 0.1	1.000	0.3 ± 0.1	1.000

Depressed	0.4 ± 0.2	0.5 ± 0.2	0.564	0.6 ± 0.2	0.157
Reduced efficiency in work and study	0.4 ± 0.1	0.4 ± 0.1	1.000	0.4 ± 0.1	1.000
Awkward movement	0.1 ± 0.1	0.2 ± 0.2	0.317	0.3 ± 0.1	0.083
Numbness in the limbs	0.0 ± 0.0	0.0 ± 0.0	1.000	0.1 ± 0.1	0.317
Change in food preferences	0.2 ± 0.1	0.3 ± 0.1	0.317	0.2 ± 0.1	1.000
Nervousness	0.4 ± 0.2	0.4 ± 0.3	1.000	0.5 ± 0.2	0.317
Blurry or missing parts	0.8 ± 0.3	0.8 ± 0.3	0.725	0.7 ± 0.3	0.414
Impulsive	0.5 ± 0.2	0.4 ± 0.1	0.655	0.4 ± 0.2	0.564
Irritability, quarrels with close people. scold a child	0.6 ± 0.2	0.7 ± 0.1	0.317	0.5 ± 0.2	0.317
To lose confidence and blame oneself	0.5 ± 0.2	0.3 ± 0.2	0.157	0.4 ± 0.2	0.317
Take a day off from work (school)	0.0 ± 0.0	0.0 ± 0.0	1.000	0.0 ± 0.0	1.000
Easily moved to tears	0.3 ± 0.1	0.3 ± 0.1	1.000	0.3 ± 0.1	0.564
Physical symptoms	9.6 ± 1.7	9.0 ± 1.7	0.395	8.1 ± 2.1	0.208
Mental symptoms	7.7 ± 2.5	7.7 ± 1.9	0.759	8.5 ± 2.0	0.405
Daily-life troubles	7.3 ± 1.9	7.8 ± 2.3	0.673	7.0 ± 2.2	0.587

a) Before menses, **b**) During menses, **c**) After menses. Analyzed by Wilcoxon signed-rank sum test, n = 12. MDQ, Menstrual Distress Questionnaire; SEM, standard error mean.

Table 4. QOL-related subjective symptoms: AAQOL.

AAQOL	Before		4 weeks			8 weeks		
	Mean	SEM	Mean	SEM	P value	Mean	SEM	P value
Physical symptoms								
Tired eyes	3.9	± 0.3	3.9	± 0.2	1.000	4.0	± 0.2	0.655
Blurry eyes	3.6	± 0.2	3.3	± 0.4	0.388	3.2	± 0.3	0.248
Eye pain	1.9	± 0.4	2.1	± 0.4	0.603	1.9	± 0.3	1.000
Stiff shoulders	4.3	± 0.3	3.9	± 0.3	0.206	3.8	± 0.3	0.129
Mascular pain / stiffness	3.2	± 0.4	3.3	± 0.4	0.914	3.1	± 0.4	0.792
Palpitations	1.8	± 0.3	1.7	± 0.3	0.655	1.8	± 0.2	0.706
Shortness of breath	1.7	± 0.2	1.7	± 0.2	1.000	1.8	± 0.3	0.589
Tendency to gain weight	3.5	± 0.3	2.6	± 0.3	**0.206**	2.9	± 0.3	**0.038**
Weight los/; thin	1.1	± 0.1	1.3	± 0.2	0.180	1.6	± 0.3	0.059
Lethargy	3.5	± 0.2	3.3	± 0.3	0.564	3.2	± 0.3	0.206
No feeling of good health	2.5	± 0.3	2.5	± 0.3	1.000	2.4	± 0.3	0.763
Thirst	2.2	± 0.4	2.3	± 0.3	0.405	2.3	± 0.4	0.317
Skin problems	2.8	± 0.3	2.3	± 0.1	0.129	2.3	± 0.3	0.161
Anorexia	1.8	± 0.3	2.3	± 0.2	0.068	2.0	± 0.2	0.317
Early satiety	1.8	± 0.3	1.6	± 0.2	0.706	1.5	± 0.2	0.257
Epigastralgia	1.4	± 0.2	1.0	± 0.0	0.059	1.2	± 0.1	0.180
Liable to catch cold	1.4	± 0.2	1.2	± 0.1	0.083	1.3	± 0.2	0.157
Coughing and sputum	1.3	± 0.2	1.3	± 0.1	1.000	1.5	± 0.2	0.317
Diarrhea	1.8	± 0.3	1.4	± 0.2	0.198	1.7	± 0.2	0.480
Constipation	2.5	± 0.3	2.1	± 0.4	0.288	2.3	± 0.3	0.429
Gray hair	1.8	± 0.3	1.7	± 0.3	0.480	2.0	± 0.3	0.527
Hair loss	3.6	± 0.2	3.2	± 0.3	0.236	3.4	± 0.3	0.414

Headache	2.5 ± 0.2	2.2 ± 0.3	0.157	2.2 ± 0.3	0.206
Dizziness	2.5 ± 0.3	2.1 ± 0.3	0.129	1.8 ± 0.2	**0.011**
Tinnitus	1.4 ± 0.2	1.6 ± 0.2	0.157	1.6 ± 0.2	0.317
Difficulty hearing	1.8 ± 0.3	1.6 ± 0.2	0.180	1.7 ± 0.3	0.317
Lumbago	3.3 ± 0.4	2.8 ± 0.3	0.083	2.6 ± 0.3	**0.014**
Arthralgia	2.2 ± 0.4	1.8 ± 0.3	**0.046**	2.0 ± 0.3	0.157
Edematous	2.8 ± 0.2	2.5 ± 0.3	0.336	2.3 ± 0.4	0.161
Easily breaking into a sweat	3.2 ± 0.2	3.1 ± 0.2	0.763	3.1 ± 0.3	0.748
Frequent urination	2.8 ± 0.3	2.4 ± 0.3	0.190	2.4 ± 0.3	0.129
Hot flush	2.1 ± 0.3	2.2 ± 0.3	0.655	2.1 ± 0.3	1.000
Cold skin					
Mental symptoms	2.8 ± 0.3	2.3 ± 0.3	0.103	2.4 ± 0.3	0.257
Irritability	3.3 ± 0.4	2.8 ± 0.3	0.096	2.8 ± 0.4	0.096
Easily angered	3.0 ± 0.4	2.6 ± 0.4	0.132	2.7 ± 0.4	0.206
Loss of motivation	2.8 ± 0.4	2.7 ± 0.4	0.763	2.3 ± 0.4	0.160
No feeling of happiness	2.5 ± 0.3	2.5 ± 0.3	1.000	2.1 ± 0.3	0.132
Nothing to look forward to in life	2.8 ± 0.4	2.5 ± 0.3	0.157	2.3 ± 0.3	**0.038**
Daily life is not enjoyable	2.5 ± 0.3	2.3 ± 0.3	0.180	2.2 ± 0.3	0.157
Loss of confidence	2.5 ± 0.3	2.7 ± 0.4	0.480	2.6 ± 0.4	0.739
Reductance to talk with others	2.4 ± 0.3	2.5 ± 0.3	0.739	2.6 ± 0.4	0.527
Depressed	2.3 ± 0.3	2.3 ± 0.3	1.000	2.0 ± 0.3	0.454
Feeling of usefulness	2.1 ± 0.3	2.3 ± 0.3	0.739	2.2 ± 0.3	0.739
Sallow sleep	4.0 ± 0.2	2.8 ± 0.3	**0.013**	2.3 ± 0.4	**0.005**
Difficulty in falling asleep	3.8 ± 0.3	3.2 ± 0.3	**0.035**	2.6 ± 0.3	**0.012**
Pessimism	2.8 ± 0.4	2.7 ± 0.4	0.317	2.5 ± 0.3	0.206
Lapse of memory	3.0 ± 0.3	2.7 ± 0.4	0.527	2.5 ± 0.4	0.058
Inablity to concentrate	2.8 ± 0.3	2.3 ± 0.3	**0.034**	2.3 ± 0.4	**0.038**
Inability to solve problems	2.2 ± 0.3	2.3 ± 0.3	0.655	2.0 ± 0.3	0.480
Inability to make judgments readily	2.3 ± 0.3	2.1 ± 0.3	0.180	2.1 ± 0.4	0.317
Inability to sleep because of worries	2.3 ± 0.3	2.0 ± 0.3	0.157	1.8 ± 0.3	**0.014**
A sense of tension	2.7 ± 0.4	2.5 ± 0.4	0.317	2.3 ± 0.3	0.103
Feeling of anxiety for no special reason	2.5 ± 0.4	1.8 ± 0.2	**0.024**	1.8 ± 0.3	**0.046**
Vague feeling of fear	1.8 ± 0.3	1.5 ± 0.2	0.103	1.4 ± 0.2	0.103

Analyzed by Wilcoxon signed-rank sum test, n = 12. QOL, quality of life; AAQOL, Anti-Aging QOL Common Questionnaire; SEM, standard error mean.

Examinations of **SF-36v2 acute version** indicated that compared with before the usage, after 8 weeks, "Vitality (VT)", "Mental health (MH)", "Vitality NBS (VT_N)", "Mental health NBS (MH_N)", and "mental component summary (MCS)" significantly improved (***Table 5***).

Body composition and blood pressure (Table 6)

Measurement items of body weight, percent body fat, fat percent, lean body mass, muscle mass, BMI, and basal metabolism demonstrated no significant difference, after 4 and 8 weeks compared before the usage.

Specifically-designed examination of blood (Table 6)

In examinations of E2, progesterone, LH, and FSH, there were no items with a significant change, after 4 and 8 weeks compared to before the usage (***Figs. 1-a, b. c, and d***). A significant decrease was confirmed in DHEA-s from 149.9 ± 22.1 μg/d: before usage to 124.9 ± 18.4 μg/dL: after 4 weeks ($p = 0.027$). However, at the point of 8 weeks, the level increased from 4 weeks to the level of 146.9 ± 59.2 μg/dL (almost the same as the level before usage) (***Fig. 1-e***).

Hematological examination (Table 6)

MCV significantly increased from before the usage: 95.8 ± 1.0 fL to after 8 weeks: 96.8 ± 0.8 fL ($p = 0.039$). MCHC significantly decreased from before the usage: 32.2 ± 0.2 % to after 8 weeks: 31.8 ± 0.2 % ($p = 0.049$).

Blood biochemical examination (Table 6)

In comparison with before the usage, after 8 weeks, significant decreases were confirmed in TP (7.41 ± 0.14 g/dL → 7.09 ± 0.08 g/dL, $p = 0.020$), Alb (4.66 ± 0.09 g/dL → 4.38 ± 0.07 g/dL, $p = 0.004$), Ca (9.65 ± 0.12 mg/dL → 9.27 ± 0.12 mg/dL, $p = 0.004$), and Mg (2.37 ± 0.05 mg/dL → 2.22 ± 0.04 mg/dL, $p = 0.029$).

Urine examination

Urine examination showed no items with a significant change after 8 weeks of the usage.

Table 5. QOL-related subjective symptoms: SF-36v2.

SF36v2	Before		4 weeks			8 weeks		
	Mean	SEM	Mean	SEM	P value	Mean	SEM	P value
Physical functioning (PF)	92.10	1.70	88.80	2.80	0.084	89.60	2.90	0.389
Role physical (RP)	94.81	2.41	91.68	3.47	0.416	95.84	1.93	0.593
Bodily pain (BP)	68.30	5.50	74.80	6.30	0.172	76.00	5.30	0.085
General health (GH)	64.80	4.60	64.30	4.20	1.000	68.90	5.80	0.330
Vitality (VT)	43.77	4.74	45.35	5.92	0.166	54.70	6.06	**0.032**
Social functioning (SF)	88.54	5.64	84.38	6.18	0.340	90.63	5.36	0.891
Role emotional (RE)	88.21	3.15	89.58	3.42	0.799	88.88	3.30	0.932
Mental health (MH)	63.30	3.60	63.80	4.30	0.952	72.50	4.70	**0.024**
Physical functioning NBS (PF_N)	52.81	0.92	50.98	1.54	0.071	51.43	1.56	0.321
Physical role functioning NBS (RP_N)	54.31	1.11	52.86	1.60	0.416	54.78	0.89	0.593
Bodily pain NBS (BP_N)	46.67	2.50	49.61	2.87	0.192	50.19	2.42	0.075
General health NBS (GH_N)	53.35	2.36	53.08	2.17	0.929	55.43	3.01	0.414
Vitality NBS (VTVT_N)	43.76	2.31	44.50	2.88	0.380	49.08	2.95	**0.032**
Social role NBS (SF_N)	52.55	2.54	50.67	2.78	0.344	53.48	2.41	0.787
Emotional role functioning NBS (RE_N)	51.53	1.41	52.14	1.53	0.799	51.85	1.47	0.932
Mental health NBS (MH_N)	48.64	1.80	48.85	2.18	0.858	53.28	2.40	**0.022**
Physical component summary：PCS	52.83	2.17	52.63	2.30	0.844	51.78	2.70	0.308
Mental component summary：MCS	45.33	2.23	46.66	2.45	0.328	51.24	2.73	**0.004**
Role/social component summary：RCS	53.57	2.46	52.07	2.85	0.754	52.73	2.58	0.433

Analyzed by Wilcoxon signed-rank sum test, n = 12. QOL, quality of life; SEM, standard error mean.

Table 6. Physical findings

		Before		4 weeks			8 weeks		
		Mean	SEM	Mean	SEM	P value	Mean	SEM	P value
Physical examination									
Body weight	kg	52.3	± 2.1	52.7	± 2.2	0.134	52.5	± 2.3	0.540
Body fat percentage	kg	26.7	± 1.9	26.8	± 2.0	0.743	26.4	± 1.9	0.547
Fat mass	kg	14.3	± 1.5	14.5	± 1.6	0.429	14.3	± 1.6	0.929
Lean body mass	kg	38.0	± 1.0	38.2	± 1.0	0.412	38.3	± 0.9	0.488
Muscle mass	kg	35.9	± 0.9	36.0	± 0.9	0.486	36.1	± 0.9	0.462
BMI	–	21.2	± 0.7	21.4	± 0.7	0.095	21.3	± 0.8	0.494
Basal metabolic rate	kcal	1,097.3	± 29.9	1,103.8	± 30.5	0.309	1,103.8	± 30.8	0.458
BP systolic	mmHg	106.0	± 3.3	104.8	± 3.3	0.686	104.4	± 3.0	0.669
BP diastolic	mmHg	67.5	± 2.6	65.0	± 1.9	0.281	66.6	± 1.9	0.534
Pulse rate	拍/分	71.4	± 2.3	74.5	± 3.5	0.174	73.8	± 2.6	0.150
Menopause-related hormones									
E2	pg/mL	142.7	± 56.5	116.4	± 33.4	0.712	97.4	± 19.9	0.479
Progesteron	ng/mL	3.5	± 1.7	1.7	± 0.8	0.071	5.8	± 2.8	0.262
DHEA-s	μg/dL	149.9	± 22.1	124.9	± 18.4	**0.027**	146.9	± 17.1	0.789
LH	mIU/mL	11.9	± 3.6	13.0	± 4.6	0.498	12.1	± 4.4	0.944
FSH	mIU/mL	25.6	± 10.1	27.3	± 11.3	0.796	26.9	± 12.1	0.866
Biocemistry									
TP	g/dL	7.4	± 0.1				7.1	± 0.1	**0.020**
Alb	g/dL	4.7	± 0.1				4.4	± 0.1	**0.004**
BUN	mg/dL	10.9	± 0.7				11.5	± 0.5	0.497
Cre	mg/dL	0.67	± 0.03				0.67	± 0.03	0.672
UA	mg/dL	4.0	± 0.3				4.0	± 0.3	0.957
AST	U/L	15.8	± 0.7				16.3	± 0.7	0.377
ALT	U/L	14.0	± 1.1				13.1	± 1.3	0.421
γ-GTP	U/L	23.6	± 3.6				27.9	± 7.7	0.421
ALP	U/L	56.3	± 3.7				57.8	± 4.4	0.347
LDH	U/L	162.5	± 5.9				159.1	± 5.5	0.455
CPK	U/L	71.0	± 11.0				68.7	± 7.5	0.791
TC	mg/dL	220.6	± 6.6				215.7	± 6.3	0.199
TG	mg/dL	77.9	± 12.8				77.8	± 11.0	0.987
LDL-C	mg/dL	121.0	± 5.5				119.2	± 5.7	0.443
HDL-C	mg/dL	79.7	± 3.6				76.1	± 3.9	0.104
T-Bil	mg/dL	0.70	± 0.08				0.68	± 0.05	0.860
Na	mEq/L	139.8	± 0.6				139.0	± 0.4	0.121
K	mEq/L	4.4	± 0.1				4.3	± 0.1	0.236
Cl	mEq/L	104.8	± 0.6				105.8	± 0.4	0.111
Ca	mg/dL	9.7	± 0.1				9.3	± 0.1	**0.004**
Mg	mg/dL	2.4	± 0.1				2.2	± 0.0	**0.029**
Fe	μg/dL	109.3	± 10.3				101.6	± 8.7	0.374
FPG	mg/dL	84.7	± 1.8				83.4	± 1.7	0.364
CRP	mg/dL	0.08	± 0.04				0.05	± 0.01	0.433

Hematology					
WBC	/μL	5,983 ± 388		5,742 ± 344	0.578
RBC	×10^4/μL	440.8 ± 9.9		438.4 ± 8.2	0.665
Hb	g/dL	13.6 ± 0.2		13.5 ± 0.2	0.449
Ht	%	42.2 ± 0.7		42.4 ± 0.6	0.661
MCV	fL	95.8 ± 1.0		96.8 ± 0.8	**0.039**
MCH	pg	30.9 ± 0.4		30.7 ± 0.3	0.579
MCHC	%	32.2 ± 0.2		31.8 ± 0.2	**0.049**
Plt	×10^4/μL	28.2 ± 1.6		27.3 ± 1.5	0.055

Analyzed by Wilcoxon signed-rank sum test, n = 12. QOL, quality of life; SEM, standard error mean.

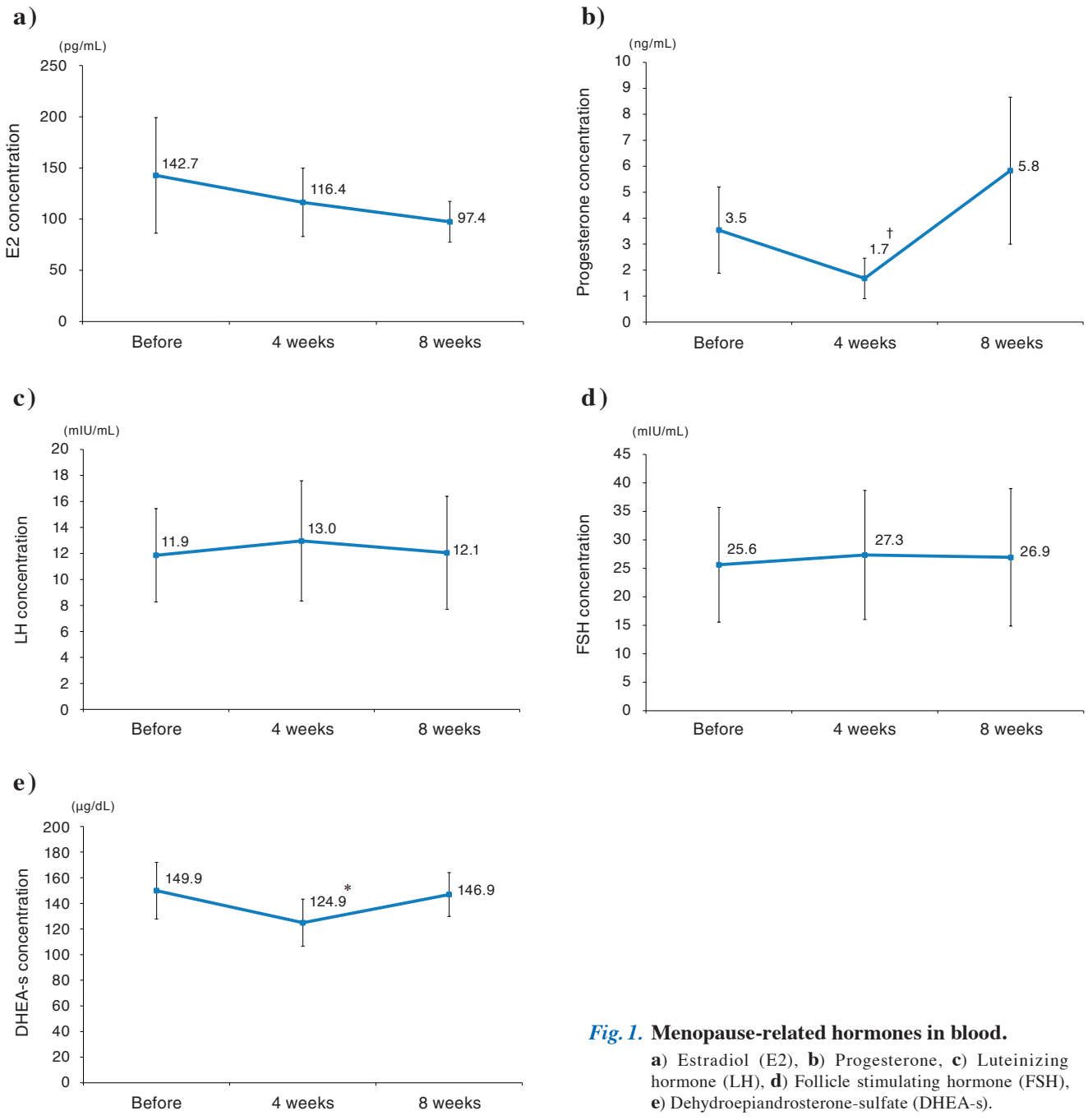

Fig. 1. **Menopause-related hormones in blood.**

a) Estradiol (E2), **b**) Progesterone, **c**) Luteinizing hormone (LH), **d**) Follicle stimulating hormone (FSH), **e**) Dehydroepiandrosterone-sulfate (DHEA-s).

Discussion

Improvement effects of "sleep quality": Comparison with previous studies

We have conducted clinical trials for this test product five times in total, and the present trial is the sixth study [6-10]. For PSQI-J in the 5 previous studies, sleep quality and sleep latency significantly improved in all the 5 studies, and similarly, daytime dysfunction in 4 out of 5 studies. Sleep disturbance and sleep duration significantly improved in 3 out of 5 studies. PSQIG, which is the global score for the total assessment, significantly improved in all 5 out of 5 studies. Consequently, we obtained almost the same results in the 5 trials and improvement effects regarding subjective symptoms are highly reproducible. It is suggested that the usage of the test product improved "the quality of sleep".

The first trial confirmed that the following improvements of subjective symptoms in PSQI-J significantly improved through the usage for 4 weeks. (All descriptions are the mean value ± standard error) [6]: sleep quality (2.1 ± 0.1 → 1.5 ± 0.2, p = 0.008), sleep latency (2.3 ± 0.3 → 1.7 ± 0.3, p = 0.034), sleep disturbance (1.4 ± 0.2 → 1.0 ± 0.0, p = 0.046), and daytime dysfunction (1.7 ± 0.1 → 0.7 ± 0.2, p = 0.002). PSQIG significantly improved from severe disorder (9.5 ± 0.4) to mild disorder (7.1 ± 0.7) (p = 0.005).

The second trial confirmed the following improvements of subjective symptoms in PSQI-J significantly improved through the usage for 4 weeks. (All descriptions are the mean value ± standard error) [7]: sleep quality (2.0 ± 0.4 → 0.8 ± 0.6, p = 0.006), sleep latency (2.0 ± 0.9 → 0.8 ± 1.0, p = 0.016), sleep duration (1.7 ± 0.5 → 1.0 ± 0.8, p = 0.011), sleep disturbance (1.3 ± 0.5 → 0.7 ± 0.5, p = 0.034), and daytime dysfunction (1.5 ± 0.8 → 0.5 ± 0.7, p = 0.026). PSQIG significantly improved from severe disorder (9.0 ± 1.7) to no disorder (3.9 ± 2.1) (p = 0.005).

The third trial confirmed the following improvements of subjective symptoms in PSQI-J significantly improved through the usage for 2 weeks. (All descriptions are the mean value ± standard error) [8]: sleep quality (2.1 ± 0.1 → 1.0 ± 0.0, p < 0.01), sleep latency (2.1 ± 0.2 → 1.1 ± 0.3, p < 0.01), sleep duration (1.6 ± 0.1 → 1.1 ± 0.2, p < 0.05), sleep disturbance (0.8 ± 0.1 → 0.5 ± 0.2, p < 0.05), and daytime dysfunction (1.3 ± 0.2 → 0.3 ± 0.1, p < 0.01). PSQIG significantly improved from mild disorder (8.0 ± 0.5) to no disorder (4.0 ± 0.7) (p < 0.01).

The fourth trial confirmed the following improvements of subjective symptoms in PSQI-J significantly improved through the usage for 4 weeks. (All descriptions are the mean value ± standard error) [9]: sleep quality (2.0 ± 0.0 → 1.0 ± 0.1, p = 0.001), sleep latency (1.8 ± 0.2 → 0.7 ± 0.2, p = 0.004), and daytime dysfunction (1.5 ± 0.2 → 0.4 ± 0.2, p = 0.012). PSQIG significantly improved from mild disorder (8.2 ± 0.4) to no disorder (4.2 ± 0.6) (p = 0.006).

The fifth trial confirmed the following improvements of subjective symptoms in PSQI-J significantly improved through the usage for 8 weeks. (All descriptions are the mean value ± standard error) [10]: sleep quality (2.0 ± 0.4 → 0.8 ± 0.4, p = 0.002), sleep latency (1.8 ± 0.8 → 0.8 ± 0.9, p = 0.008), sleep duration (1.8 ± 0.4 → 1.1 ± 0.7, p = 0.007) and habitual sleep efficiency (0.8 ± 0.9 → 0.0 ± 0.0, p = 0.023). PSQIG significantly improved from mild disorder (8.8 ± 1.9) to no disorder (3.8 ± 1.3) (p < 0.01).

As the assessment standard, 5 or lower points were no sleep disorder, 6 or higher points were a sleep disorder. A mild disorder is from 6 to 8 points, and a severe disorder is from 9 points or higher [9].

In the present study (the sixth trial), only two items, sleep quality (2.0 ± 0.0 → 1.5 ± 0.5 → 1.1 ± 0.5, p = 0.002) and sleep duration (1.8 ± 0.8 → 1.5 ± 0.8 → 1.3 ± 0.7, p = 0.025) demonstrated significant effects after 8 weeks. Possibilities were suggested in data results of the present study. Through the replacement of a bed mattress to the test product, qualities of sleep improved, physical and mental stress decreased, and menopausal syndrome was partially mitigated (sleep deprivation, fatigue, *etc.*). However, secretion quantity of hormones did not improve, and menopausal symptoms (hot flushes, cold constitution, *etc.*) did not show changes. These symptoms could inhibit sleep partially, where physical and mental stresses could remain.

In PSQIG assessments, mild disorder significantly improved from before the usage (7.8 ± 2.0) to 4 weeks (6.3 ± 2.5) and to 8 weeks (5.4 ± 2.1) (p = 0.004). This did not reach the range of no disorder, whose score is less than 5 points. However, the score of PSQIG was characteristic. In comparison with the previous 5 trial, where the mean value was 8.0 or higher (the first and second trial: severe disorder and the third, fourth, and fifth trial: mild disorder), PSQIG of the present study (before the usage) was the lowest. The score of the last observation point was the second highest among the previous 5 trials.

The research participants in the present trial were premenopausal women with severe menopausal symptoms. Thus, characteristics of this trial were 1) The causative factor of deteriorated quality of sleep was strong physical and mental stress, which was related to menopausal syndrome. 2) Only replacement of a mattress lead to a partial relief of physical and mental stress but effects were limited, not improving the declined female hormones. For treatments and managements of menopausal symptoms, attentions must be paid to the deterioration of sleep quality, which is induced by physical and mental stress, as a latent possibility. Other than hormone replacement therapy, it is recommendable to use appropriate bedding.

Sleep and physical and mental stress: steroid hormones

Metabolism pathway of steroid hormones are shown in ***Fig. 2*** [18, 19].

Observing previous studies on the behaviors of steroid hormone metabolites, it was suggested that when "sleep quality" improved, DHEA metabolites (androsterone: AN and etiocholanolone: Et) significantly increased, and cortisol metabolites did not change (***Table 7***) [6]. These findings suggested the possibility of the increase of DHEA formation due to increased qualities of sleep. It is considered that load levels of physical and mental stress of research participants in this previous study were not particularly strong.

However, it is assumed that research participants in the present study had severe stress due to menopausal syndrome. In people with excessive physical and mental stress, stress is relieved, when their sleep quality improves. Consequently, cortisol secretion, which is at somehow high level, decreases, and stress level returns to the normal or almost normal level.

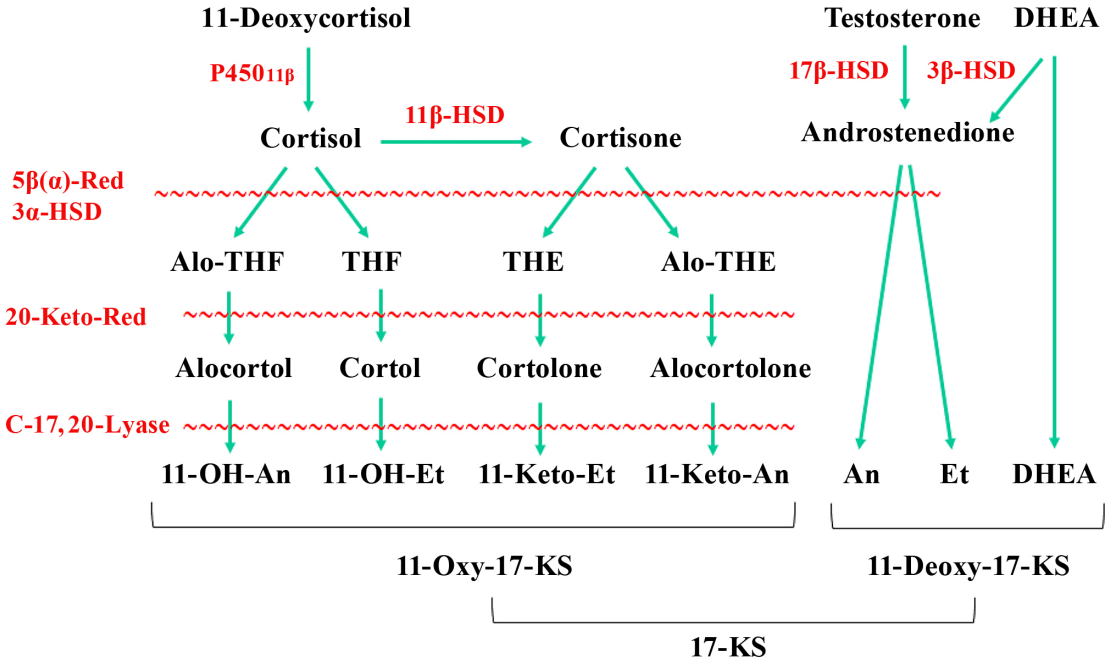

Fig. 2. **Metabolic pathways of steroid hormones.**

The figure is prepaed based on the contents of References 18) and 19). DHEA, dehydroepiandrosterone; THF, tetrahydrocortisol; THE, tetrahydrocortisone; An, androsterone; Et, ethiocolanolone; KS, ketosteroid; P450, cytochrome P450; HSD, hydroxysteroid dehydrogenase; Red, reductase.

Table 7. Impact of improved "sleep quality" in normal subjects with psychosomatic stress: Increased DHEA metabolites.

		Before	4 weeks	P value
DHEA-derived metabolites				
Free DHEA	mg/day	0.19 ± 0.09	0.15 ± 0.04	0.630
An	mg/day	0.73 ± 0.18	1.02 ± 0.24	**0.037**
Et	mg/day	0.80 ± 0.18	1.03 ± 0.21	**0.023**
Cortisol-derived metabolites				
Free cortisol	μg/day	30.35 ± 5.73	26.59 ± 5.80	0.232
11-keto-An	mg/day	0.02 ± 0.00	0.02 ± 0.00	1.000
11-keto-Et	mg/day	0.19 ± 0.06	0.20 ± 0.06	0.516
11-hydroxy-An	mg/day	0.58 ± 0.18	0.48 ± 0.13	0.098
11-hydroxy-Et	mg/day	0.17 ± 0.07	0.18 ± 0.07	0.607

Results are expresses as mean ± SEM, n = 11. Reference 6) Takabe W, et al. Glycative Stress Res. 2016; 3: 110-123. DHEA, dehydroepiandrosterone; An, androsterone; Et, ethiocolanolone; SEM. Standard error mean. Source:

Observing steroid hormone metabolites, it is assumed that cortisol metabolites (11-OH-An and 11-OH-Et), which increase due to excessive stress, decrease. However, the present study was not able to elucidate this point.

"Sleep quality" related hormones

Three hormones, melatonin, orexin, and cortisol play important roles for the control of "sleep quality". It is required to have an understanding of characteristics in these three hormones to maintain "sleep quality" at the optimal state.

Melatonin controls "sleepquality" during the night [20-22]. The level of melatonin blood concentration is kept low during daytime, and levels become higher around the evening via instruction from the circadian clock. The level before sleeping is the highest. This prepares an initiation of sleep. Melatonin is affected by light and dark apart from the circadian clock. When retina cells of eyeballs sense light, the secretion of melatonin ceases.

Orexin regulates an arousal state during daytim [23-25]. The blood concentration of orexin starts to rise around dawn via the instruction from the circadian block, and the body is awakened from the state of sleep. Orexin blood concentration is kept high during daytime. Thus, concentration is heightened, basal metabolism is raised, and work efficiency is elevated. The hormone named as orexin was discovered in 1996 by Masashi Yanagisawa *et al.* of the University of Tsukuba (The report of the discovery of orexin was published in 1998) [23]. Diverse physical and mental stress in a daily life stimulate the secretion of orexin. Stresses during daytime induce the increase of orexin leading to the elevation of vitality. However, increased orexin induced by stresses during night heightens the arousal activities and lower "sleep quality". Nocturnal awakening (arousal during sleep) and premature arousal (early arousal) are induced.

Cortisol, a stress hormone, plays a role of the stimulation of "sleep quality" [26-29]. The concentration of cortisol during daytime is kept at a high level. Cortisol starts to decrease in accordance with the circadian clock around the early evening, and decreases more when sleep becomes deep. Around dawn, cortisol starts to increase. When the concentration level reaches the threshold value, it provides arousal stimulations to the sleep state, which induces arousal. Similar to orexin, the rise of cortisol is an arousal stimulus. The secretion of cortisol increases due to physical and mental stresses apart from the circadian clock. Therefore, when cortisol does not decrease during night due to prolonged chronic stresses, latency sleep is worsened (the time it takes a person to fall asleep after turning the lights out) and the quality of sleep is also worsened. Diverse factors act in an additive manner regarding physical and mental stresses. For an example, a bedding-dysfunction-related factor and a menopause-related factor in stresses, in factors-addition manner, raise the concentration level of cortisol.

Moving on to the menopause-related stresses, we discuss conditions of research participants before the trial regarding impacts on the secretions of theses hormones.

When physical and mental stress, which is related to menopausal syndrome, becomes severe, "sleep quality" becomes poor [30-32]. Normally, the level of cortisol before bedtime is at its minimum value. However, when stresses raise the secretion of cortisol, the cortisol level does not decrease to the normal level of the sufficiently decreased state. As a result, sleep latency is worsened (the time it takes to fall asleep is increased). When the concentration of cortisol during sleep is higher than normal, "sleep quality" is deteriorated, which induces nocturnal awakening (middle wakening). When the level of cortisol, which is high during sleep, starts to rise at dawn, it reaches, earlier than normal, the threshold level, which leads to early morning awakening.

The deterioration of "sleep quality" induces the decline of melatonin secretion. It is considered that the relationship between these two factors is bidirectional. When "sleep quality" is lowered artificially, for an example, photic stimulation induces the decrease of melatonin secretion and "sleep quality" decreases [33].

In the present study, it is assumed that melatonin secretions of the research participants decreased in comparison with the optimal level as their "sleep quality" was declined via the exacerbation of the cortisol secretion due to physical and mental stresses of menopausal symptoms.

The deteriorated "sleep quality" affects daytime activities and orexin secretion [34, 35]. Even when people feel that "They feel somehow extremely drowsy", people tend to be lazy with less vitality and their activity quantity is reduced. A causative factor for daytime dysfunction (difficulties in staying awake during daytime) is the deterioration of orexin secretion, as is considered.

In the present study, it is supposed that before the trial, research participants had "daytime dysfunction" via the decrease in orexin secretion during daytime, as they had menopausal symptoms and physical and mental stresses that were caused by these symptoms, which induced the state of a decline in "sleep quality".

Transition of DHEA

Next, we discuss transitions of DHEA-s in the present study.

One possibility is that in reaction to physical and mental stresses, the formation of DHEA increases as compensation. In this case, when stresses decrease, the level of DHEA returns to normal (the compensation is removed and DHEA decreases).

There are some unknown issues in control mechanisms of DHEA. It is known that corticotrophin, insulin, and prolactin are promotive factors for DHEA formations. It is known that when physical and mental stresses are created, cortisol increases and cortisol/DHEA-s ratio increase. When physical and mental stresses are mitigated, cortisol decreases and cortisol/DHEA-s ratio decreases.

A previous study (astaxanthin research paper) shown in ***Table 8*** [36], reported that both DHEA-s and cortisol decreased, and DHEA-s/cortisol ratio showed an increasing tendency: 10.9 → 13.0 (4 weeks) → 12.0 (8 weeks), (cortisol/DHEA-s ratio showed a decreasing tendency). These alternations can be interpreted as by the administration of astaxanthin. "Loads of physical and mental stresses decreased, and DHEA-s returned to the state with no load of stresses; DHEA-s had increased to retaliate against stresses in a compensation manner." This finding means that in the

Table 8. Cortisol and DHEA-s after astaxanthin intake.

		Before	4 weeks	8 weeks
Cortisol	μg/dL	9.22 ± 3.28	6.89 ± 2.23**	7.12 ± 2.48**
DHEA-s	μg/dL	84.05 ± 43.24	82.43 ± 45.37	71.38 ± 34.56**

Results are expresses as mean ± SEM, n = 20, **p < 0.01 vs Before. Source: Reference 36) Iwabayashi M, et al. Anti-Aging Medicine 6(4): 15-21, 2009. Astaxanthin, AstarealR (AstaReal Inc., Tokyo, Japan) at a daily dose of 12 mg per day; SEM. Standard error mean.

case of the increased DHEA-s via compensation, there is a possibility that "the level of anti-stress hormone, DHEA-s decreases along with the reduction of physical and mental stress".

From this perspective of these mechanisms, we try to explain the alternation of DHEA-s in the present study (***Fig. 1-e***).

The research participants were premenopausal women with severe menopausal symptoms. Thus, it was expected that stresses, which they had due to menopausal symptoms, were intense to a great extent.

Alternation for the first 4 weeks:

149.9 ± 76.5 μg/dL → 124.9 ± 63.9 μg/dL (p = 0.027)

This finding is interpreted that for 4 weeks the improvement of "sleep quality" and the mitigation of physical and mental stresses led to the return of DHEA secretion to the prior level (that is, significantly decreased), as DHEA secretion had increased in compensation. The improvements of "sleep quality" after 4 weeks were confirmed in the significant improvement in PSQIG of PSQI-J score, and the significant improvement in the score of "Factor 2: initiation and maintenance of sleep" of the OSA sleep inventory.

Findings confirmed that some of menopausal concomitant symptoms improved, which indicated the mitigation effects on physical and mental stresses. Improved items after 4 weeks were MDQ before menses: "experienced drowsiness and took a nap", "headache", and "edema (abdomen, breast, and legs)" as well as MDQ during menses: "experienced drowsiness and took a nap". A reason for the improvements of MDQ was "the decrease of physical and mental stress".

Alternation from 4 weeks to 8 weeks:

124.9 ± 63.9 μg/dL → 146.9 ± 59.2 μg/dL (p = 0.001)

We considered that research participants, whose stresses had improved to almost normal level, increased the formation and blood concentration of DHEA due to the continuous state of "sleep quality" improvement from 4 weeks to 8 weeks.

Moreover, glucocorticoid is classified into active glucocorticoid and inactive glucocorticoid. Both of them are regulated by 11β-hydroxysteroid dehydrogenase (11β-HSD) [37]. In adipocytes, 11β-HSD1 converts cortisone to cortisol, and 11β-HSD2 converts cortisol to cortisone.

Under the load of stress, the gene expression of 11β-HSD1 increases and cortisol increases. In the state of the elevation of glucocorticoid and inflammatory cytokine, the gene expression of 11β-HSD1 increases. However, cortisol decreases and cortisone increases, as the formation of DHEA increases through the increase of "sleep quality", which leads to the increase of peroxisome proliferator-activated receptor γ (PPARγ) and the inhibition of 11β-HSD1 activities. PPARγ has an increasing activity of adiponectin as a transcription factor, and acts leading to the improvement of insulin resistance and glucose tolerance.

In the present trial, cortisol in the bloodstream was not measured. Therefore, this interpretation is an assumption. However, this is not conflicted to the assumed mechanism described above. We intend to develop a clinical plan for the verification of this hypothesis.

DHEA, which is corticosteroid, has a steroid nucleus. There are free-form of DHEA and sulfated-form of DHEA (DHEA-s), which is stable in the body. DHEA-s is a steroid hormone and exists abundantly in the body. More than 50 types of hormones are produced from DHEA-s such as sex hormone (E2, progesterone), glucocorticoid (cortisol, *etc.*), mineralocorticoid, and anabolic steroid [38]. This phenomenon is designated as intracrine action [38].

The compounding processes of the synthesis of steroid hormones as materials of cholesterol are shown in ***Fig. 6*** [39]. The intracrine actions correspond to the processes in the formations of every kind hormone starting at DHEA-s.

DHEA secretions decrease due to aging [40]. The decline in secretion of DHEA-s is called adrenopause, which is involved in the decline of immunity and resistance against stress, as well as the increase of onset risks in lifestyle-related diseases such as metabolic syndrome, fatty liver, diabetes, hyperlipidemia, hypertension, and osteoporosis [41]. In examinations of the present study, estradiol was measured at the highest activity value among stability-form of DHEA-s, progesterone, and estrogen.

Menopause-related hormones

FSH and LH, which are secreted from hypophysis, stimulate the ovary to promote secretions of female hormones (estrogen and progesterone). When functions of ovary deteriorated and secretions of female hormones decrease around late 30s and 40s of females, FSH and LH gradually increase to urge female hormone secretions. The decline in female hormones is a major reason for the onset of menopausal syndrome as a mechanism [42,43]. Therefore, replacement method of female hormones is used as a

treatment for menopausal symptoms.

In the present clinical trial, a hypothesis was proposed. Via the decrease of "sleep quality, 1) Menopausal symptoms would improve. 2) Secretions of menopause-related hormones would improve. Examinations of this hypothesis was performed. As a specific and substantial expectation, it was expected that estrogen and progesterone would increase, and FSH and LH would decrease. It was assumed that the DHEA increase and intracrine actions would be a mechanism for the increase of female hormones (estrogen and progesterone).

Menopausal symptoms and DHEA

Secretions of sex hormones decline due to aging [40, 42, 43]. Menopausal symptoms, which a large number of people experience around 50 years old, are individually different in the manner of appearance and the level of severity. Causative factors for these differences are related to DHEA (intracrine, in particular) [44].

First, we review the outline of menopausal syndrome.

Estrogen and progesterone regulate diverse functions of the female body. These hormones are significantly related to the formation of female-specific body figure, the maintenance of regular menstruation, the stimulation to ovulation, and the capability for pregnancy and delivery.

As described previously, female hormones start to be active from the mid to late teens, reach peak at 30s, and then, the secretions of female hormones start to decrease leading to the cessation of ovarian functions around 50 years old, and then menopause occurs. Ten years before and after menopause is called climacterium, and diverse physical disorders, which are not diseases, occur [42, 43]. The generic term for symptoms caused by the fluctuation of estrogen, which is produced in the uterus, is designated as menopausal syndrome.

Menopausal symptoms are multifarious, such as hot flushes, dizziness, palpitation, headache, stiff shoulders, emotional instability, and easy fatigue. However, there exist some people who have limited subjective symptoms. Why do they live a life without feeling menopausal symptoms? One theory indicates that DHEA, which is the source of steroid hormones, sufficiently continues production.

When the period of menopause is past and the formation of estrogen in the ovary ceases, estradiol in the blood is depleted, the value of estradiol is less than 10 pg/mL or too small to be measured, in almost all cases of females in their 70s.

However, there are a small minority of people, where E2 of 20–30 pg/mL can be measured. In this case, estrogen should not be formed in the ovary, but still remains replenished in the blood. The reason is that DHEA actively forms in the adrenal gland, and then, progesterone and/or E2 form via intracrine actions [44]. There is a possibility for females with the sufficient secretion of DHEA to gain the benefits and live a life without suffering from menopausal symptoms in their 40s and 50s.

Alternations in menopause-related hormones

In conclusion, though the usage of a test product to improve "the quality of sleep", no improvement in the hormone secretion state were recorded. Increases in progesterone and E2 were not observed and no significant decrease of LH and FSH was observed. DHEA-s, where an expectation for an intracrine action was not obtained, did not show a significant increase.

DHEA-s significantly decreased after 4 weeks and returned after 8 weeks to the prior level.

The reason for this phenomenon was described previously. The increased level of DHEA-s was shown before the usage; the increase had been caused by the menopause-related physical and mental stresses in a compensation manner. It seemed that after 4 weeks, the DHEA formation decreased, returning to the normal level (the state with less stress), because the stress load decreased and the compensation was removed. From 4 weeks to 8 weeks, due to the continuous improvement of "sleep quality", the DHEA formation increased and them DHEA-s value increased (after 8 weeks).

Progesterone showed a similar transition (similarity in the change curve). The similarity of the change curves is not inconsistent with the "possibility that the intracline action of DHEA occurred as speculated."

Safety

During and after the present trial, no adverse reaction effects induced by the usage of the test product mattress were reported. The safety of the test product was confirmed.

Conclusions

The root cause of menopausal symptoms is the decline of female hormone secretion. One fundamental treatment for the relief of menopausal symptoms is a hormone replacement therapy. In the present study, through the usage of a test product mattress "sleep quality" was improved and physical and mental stresses with menopausal symptoms partially decreased. However, this did not lead to the improvements in the secretions of menopause-symptom-related hormones.

It was shown that regarding the improvements of menopausal insomnia, the treatment of hypnotic sedative is more effective than hormone replacement therapy [30]. The present trial suggested that the usage of an appropriate bedding, which is suitable for the individual user, improves "sleep quality". Through the improved "quality of sleep", physical and mental stresses, which induced by menopausal syndrome, were relieved and menopausal symptoms were relieved. It is considered that the usage of an appropriate bed mattress is an effective and safe measure as a supplementary guidance for women with severe symptoms of menopause.

Conflict of Interest

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