Glycative Stress Research

Online edition : ISSN 2188-3610
Print edition : ISSN 2188-3602
Received : June 18, 2017
Accepted : July 20, 2017
Published online : September 30, 2017

Original article

Effect of mats with "A Distinctive 4-Layer 3-Dimensional Structure" on sleep quality, anti-oxidative and immunological function.

Mari Ogura [1,2], Wakako Takabe [1], Masayuki Yagi [1], Masashi Furukawa [3], Yoji Shimura [3], Midori Ando [3], Yoshikazu Yonei [1]

1) Anti-Aging Medical Research Center and Glycative Stress Research Center, Faculty of Life and Medical Sciences, Doshisha University, Kyoto, Japan
2) Kyoto Bunkyo Junior College, Kyoto, Japan
3) Japan Research Laboratory of Sleep Science, Nishikawa Sangyo Co. Ltd., Tokyo, Japan

Abstract

***Purpose*:** Recently, various causal relationships between the deterioration of sleep quality and various diseases are reported. In this research, the possibility of improving the quality of sleep by using a mattress with "Distinctive 4-Layer 3-Dimensional Structure", a test product, and its effects on glycolipid metabolism and immune function were verified.

***Method*:** The subjects were eleven males and females having dissatisfaction with their sleep (4 males and 7 females (49.8 ± 6.5 years). A non-controlled open-label test was conducted targeting the subjects for the change on their physical information while they were using the mattress test product. The test products were provided by Nishikawa Sangyou Co., Ltd. (Tokyo, Japan). The confirmation of their subjective symptoms, physical measurements, biochemical examination of blood and urine were conducted before the start of experiment and 4 weeks after the start of experiment. This research was conducted after an ethical approval was obtained from an ethical committee.

***Results*:** Significant improvements in the sleep quality; time to fall asleep, difficulty sleeping and daytime difficulty waking were observed using the evaluation by Pittsburgh sleep quality marker, Japanese version (PSQI-J) 4 weeks after the start of the test. PSQI global score (PSQIG) was improved from 9.0 ± 1.7 to 3.9 ± 2.1. According to a glycolipid metabolism test, HbA1c significantly decreased from 5.31 ± 0.23% to 5.19 ± 0.17%, but there was no difference in fasting plasma glucose, LDL-cholesterol, HDL-cholesterol and triglyceride. According to an endocrine examination, dehydroepiandrosterone-sulfate (DHEA-s; –13.8%, p = 0.016), cortisol (–15.7%, p = 0.025) decreased, but there was no difference in cortisol/DHEA-s ratio. According to an immunological examination, the number of CD56 – CD16 + cells among the subsets of NK cells remarkably increased (+61.1%, p = 0.020).

***Conclusion*:** The improvement of subjective symptoms related to sleep, decrease of HbA1c, decrease of cortisol and decrease of DHEA-s were observed. Further study is anticipated for the significance of the increase of CD56–CD16+ NK cells accompanied by the improvement of the sleep quality.

***KEY WORDS*:** sleep quality, HbA1c, cortisol, natural killer (NK) cell, CD56-16+ cells

Introduction

Type-2 diabetes is a typical disease with strong glycative stress. It is well known that sleep disorders such sleep apnea syndromes reduce sleep quality, and it is deeply involved in the onset and progression of type-2 diabetes [1-7], diabetic retinitis [8,9] and diabetic nephropathy [10,11]. Our laboratory has reported that skin advanced glycation end product (AGE) fluorescence intensity, a glycative stress marker, of those who lack of sleep is strong [12]. Furthermore, as a result of an experiment on melatonin, a sleep-related hormone, for the purpose of examining the relationship between the sleep quality and glycative stress, melatonin did not affect the inhibition on the generation of AGEs [13] and AGEs/RAGE (receptor for AGEs) signal [14] ; however, it promoted the decomposition of AGEs with α-diketone structure [15].

Maintaining high sleep quality is important also from the viewpoint of preventive medicine. In order to maintain high sleep quality, it is desirable to use the bedding materials the most suitable to each condition.

The mattress with a "Distinctive 4-Layer 3-Dimentional Structure" had obtained a good reputation through a

Contact Address: Professor Yoshikazu Yonei, MD, PhD
Anti-Aging Medical Research Center,
Graduate School of Life and Medical Sciences, Doshisha University
1-3, Tatara Miyakodani, Kyotanabe, Kyoto, 610-0394 Japan
Phone/Fax: +81-774-65-6394 Email: yyonei@mail.doshisha.ac.jp
Co-authors; Ogura M, m-ogura@po.kbu.ac.jp ; Takabe W, wtakabe@mail.doshisha.ac.jp ; Yagi M, yagi@yonei-labo.com ; Furukawa M, furukawa-m@nishikawasangyo.co.jp; Shimura Y, shimura@nishikawasangyo.co.jp; Ando M, andoh-mi@nishikawasangyo.co.jp

questionnaire since its release. The author et al. conducted a non-controlled open-label test for the purpose of verifying the effect of the mattress with a "Distinctive 4-Layer 3-Dimensional Structure" on physical information including the sleep quality 4 weeks after using it as a test product in place of the bed that had been used so far [16]. The result showed the improvements of subjective symptoms concerning sleep quality, the promotion of growth hormone (GH)/insulin-like growth factor-I (IGF-I) secretion and the alleviation of oxidative stress.

In this research, for the purpose of reconfirming the effect of this mattress on the sleep quality and oxidative stress after 4 weeks of use, and also verifying its effect on immune function anew, a non-controlled open-label test was conducted targeting 11 male and female subjects who reported having dissatisfaction with their sleep, where the items for immune markers were focused on in addition to the markers relating to sleep.

Methods

Subjects

Thirty-two healthy males and females, aged from 40 to 65 years, 1) not obese or skinny 2) with dissatisfaction in sleep including difficulty sleeping, nocturnal awaking, early-morning awaking and sound sleep difficulty were recruited for this research. They were interviewed, and 12 of them were selected based upon the subject selection standards and subject exclusion standards with the target of a 1:1 male-to-female ratio. Because one of them dropped out due to personal reason, the subjects consisted of 4 males (48.3 ± 8.1 years) and 7 females (50.7 ± 5.9 years), 11 in total (49.8 ± 6.5 years).

The subject selection standards are as follows:

1) Healthy males and females who are 40 years or older and younger than 65 years when the letter of consent was written.
2) Healthy persons who are not receiving any treatment for disease at present
3) Persons with body mass index (BMI) of 18.5 kg/m^2 or more and less than 25.0 kg/m^2
4) Persons with dissatisfaction in sleep including difficulty sleeping, nocturnal awaking, early-morning awakening or sound sleep disorder
5) Persons whose time from going to bed (lights-out time) to waking up is more than 4 hours
6) Persons whose times for going to bed and waking up are regular and whose time for going to bed (lights-out time) is before 24:00
7) Persons who sleep in futon usually
8) Persons who can sleep in the test mattress during the test period
9) Persons who can sleep alone during the test period for sleeping time assessment

Exclusion standards are as follows:

1) Persons who suffer some chronic disease and are receiving drug treatment
2) Persons suspected of having sleep apnea syndrome (SAS), or under treatment or having a history of treatment.
3) Persons having nocturia, prostatic hyperplasia or overactive bladder or those who are suspected of any of them
4) Persons having a history of any of serious liver disease, kidney disease, cardiac disease, lung disease, digestive system disease (including gastric resection), organ damage, diabetes, thyroid gland disorder and other serious disease or those are suffering these diseases
5) Persons having skin disorders including atopic dermatitis and dermal hypersensitivity

Research design

An open-label test without control group was conducted in this research.

As same as previously reported, the mattress with "Distinctive 4-Layer 3-Dimensional Structure" (&Free Mattress SA ™; Regular HF2521R; Nishikawa Sangyo Co., Ltd., Chuo-ku, Tokyo, Japan) [16] was used as test product. The test products with a single size (9 × 97 × 200 cm) were provided together with sheets for exclusive use with the mattress by Nishikawa Sangyo Co., Ltd. At the start of this test, the futon that the subjects had been using was switched to those test products. There has been no serious adverse event concerning the safety of the products since the product started selling in February 2015 until March 2017.

Before the test started and 4 weeks after the test started, the confirmation of subjective symptoms, physical measurements, biochemical examination of blood, the tests of oxidative stress, glycative stress, mental and physical stress and immune-related tests were conducted. The subjects recorded the presence or absence or degree of adverse event, daily habits and meal and exercise habits on their living diary during the test period. The test period was from November 2016 to March 3017.

Evaluation items

Subjective symptoms

The Pittsburg Sleep Quality Marker (SQO-J) [17] was used for the evaluation of sleep quality. In accordance with their PSQI questionnaire, scoring method, and spreadsheet, the quality, time to fall asleep, sleeping time, sleep efficiency, difficulty sleeping, use of sleeping pills and daytime difficulty waking were scored and the calculation of PSQI global score (PSQIG) were implemented.

An Anti-aging quality of life (QOL) Common Questionnaire (AAQOL) [18] was used for the evaluation of subjective symptoms. Subjective symptoms were divided into "physical symptoms" and "mental symptoms," and further divided into 5 stages from point 1 to 5 point and the scores were evaluated.

Physical measurements

As physical measurements, height, weight, body fat percentage, BMI, systolic and diastolic blood pressures and pulse were measured. A body composition test was also conducted using a body composition analyzer (DC-320; Tanita Co., Itabashi-ku, Tokyo, Japan).

Blood examination

A peripheral blood examination and biochemical examination were conducted using blood samples, and the results of the biochemical examination of blood, oxidative stress marker, glycative stress marker, endocrine marker and immunological marker were shown. A general biochemical examination and IFG-I, dehydroepiandrosterrone-sulfate (DHEA-s) and cortisol were measured using serum or plasma

at the Health Science Research Institute (Hodogaya-ku, Yokohama, Japan). The measurements of oxidative stress (OS) and antioxidant power (AP) using plasma were conducted in our laboratory [19]. The immunological examination using whole blood (not only plasma) was conducted in the Institute of Health and Life Science (Chiyoda-ku, Tokyo, Japan) [20-23].

Urine test

The first morning urine samples were used. The subjects collected their own first morning urine sample and recorded "urine volume," "morning urination time," and "last night urination time," and calculated generation speed from the urinary concentration of each marker. As oxidative stress-related examinations, 8-hydroxy-2'-deoxyguanosine (8-OHdG) [24] and isoprostane (15-isoprostane F2t [25] were measured in the Japan Institute for the Control of Aging (JaICA), Nikken Seil Co., Ltd. (Fukuroi, Shizuoka, Japan). A Melatonin metabolic product (6-hydroxymelatonine sulfate (SaMT) [26] was measured in LSI Medience Corporation (Chiyoda-ku, Tokyo, Japan).

Statistical analysis

A statistical analysis software, SAS (SAS 9.4; SAS Institute, Japan, Minato-ku, Tokyo, Japan) or SPSS (Statistics 19; Nihon IBM, Chuo-ku, Tokyo, Japan) was used for statistical analysis and a paired-t test was conducted. A risk ratio of less than 5% was regarded as a significant difference and less than 10% was regarded as a significant trend. Regarding outliers and missing value, the missing value was not particularly set. However, when data could not be obtained because of the trouble due to the test, or there was big problem in the reliability of data, it was treated as a missing value and an alternative value was not used.

Ethical review

This research was conducted in compliance with the Helsinki Declaration (revised in the WMA General Assembly in 2013, Fortaleza and the ethical guidance for Medical and Health Research Involving Human Subjects (announced by the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare. The validity of this research was deliberated and approved (GSE #2016-006) by the ethical review committee concerning the "research involving human subjects" of the Society for Glycative Stress Research (Nakano-ku, Tokyo, Japan). This research was previously registered for clinical test (UMIN #000025755).

Results

ASubjective symptoms

The following improvements in subjective symptoms were observed by the use of the test product for 4 weeks:

According to PSQI-J, significant improvements were observed in the scores of sleep quality ($p = 0.006$), time to fall asleep ($p = 0.016$), sleeping time ($p = 0.011$), difficulty sleeping ($p = 0.034$) and daytime difficulty waking ($p = 0.026$, ***Table 1***). PSQIG was significantly improved from high difficulty level (9.0 ± 1.7) to low difficulty level (3.9 ± 2.1, $p = 0.005$).

According to AAQOL, 2 items of stiff shoulders ($p = 0.014$) and lethargy ($p = 0.020$) out of 33 items of physical symptom and 1 item of shallow sleep ($p = 0.014$) out of 21 items of mental symptom were significantly improved; however, the item of pessimism ($p = 0.046$) significantly increased (***Table 2***). There was no significant difference in other items.

Anthropometry

No significant change was observed in height, BMI, body fat or pulse (***Table 3***).

Blood biochemical examination

Although the result of the biochemical examination of blood showed that the level of alkaline phosphatase (ALP; −6.5%, $p = 0.017$) significantly lowered and Na (−0.7%, $p = 0.019$) significantly lowered, however, both changes were within standard values (***Table 4***). No significant difference was observed in liver function, renal function or serum protein.

Table 1. Sleep quality evaluation.

		Before	4 weeks	p value
PSQI-J	Sleep quality	2.0 ± 0.4	0.8 ± 0.6 **	**0.006**
	Time to fall asleep	2.0 ± 0.9	0.8 ± 1.0 *	**0.016**
	Sleeping time	1.7 ± 0.5	1.0 ± 0.8 *	**0.011**
	Sleep efficiency	0.5 ± 0.7	0.1 ± 0.3 †	0.059
	Difficulty sleeping	1.3 ± 0.5	0.7 ± 0.5 *	**0.034**
	Use of sleep inducers	0.0 ± 0.0	0.0 ± 0.0	1.000
	Daytime difficulty waking	1.5 ± 0.8	0.5 ± 0.7 *	**0.026**
	PSQIG	9.0 ± 1.7	3.9 ± 2.1 **	**0.005**

Results are expressed as mean ± SEM, paired t test, n = 11. PSQI-J, Pittsburgh Sleep Quality Index (Japan version) questionnaire; PSQIG, PSQI global score; SEM, standard error mean.

Table 2. AntiAging QOL Common questionnaire.

	Before	4 weeks	p value
Physical symptoms			
Tired eyes	3.3 ± 0.9	2.6 ± 1.3 †	0.052
Eye pain	1.8 ± 0.6	1.4 ± 0.5 †	0.059
Stiff shoulders	3.5 ± 1.2	2.6 ± 1.2 *	**0.014**
Muscular pain/stiffness	2.9 ± 1.3	2.3 ± 1.1 †	0.085
Lethargy	3.0 ± 1.0	2.1 ± 0.3 *	**0.020**
No feeling of good health	2.8 ± 1.0	2.3 ± 1.0 †	0.084
Easily breaking into a sweat	1.8 ± 0.9	2.3 ± 0.9 †	0.096
Mental symptoms			
Shallow sleep	4.0 ± 0.6	2.9 ± 0.8 *	**0.014**
Difficulty in falling asleep	3.5 ± 1.1	2.8 ± 0.9 †	0.071
Pessimism	2.3 ± 0.5	2.6 ± 0.7 *	**0.046**
Inability to solve problems	2.2 ± 0.8	2.1 ± 0.7	0.655
Inability to sleep because of worries	2.9 ± 0.9	2.4 ± 0.7 †	0.063
A sense of tension	2.5 ± 1.0	2.5 ± 0.5	0.763

Data are expressed as mean ± SEM, paired t test, n = 11. SEM, standard error mean.

Table 3. Anthropometry.

		Before	4 weeks	p value
Height	cm	164.23 9.65	– –	–
Weight	kg	57.71 ± 7.10	57.69 ± 6.97	0.898
Body fat	%	26.87 ± 7.03	27.11 ± 7.16	0.396
BMI	–	21.35 ± 1.22	21.35 ± 1.21	0.882
Blood pressure (systolic)	mmHg	110.4 ± 11.6	105.3 ± 13.2	0.170
(diastolic)	mmHg	70.4 ± 10.6	69.3 ± 14.6	0.623
Pulse	/min	63.5 ± 9.2	68.3 ± 11.3 †	0.060

Data are expressed as mean ± SEM, paired t test, n = 11. BMI, body mass index; SEM, standard error mean.

Table 4. Blood, urine, salivary examination.

Blood chemistry		Before	4 weeks	p value
AST	U/L	21.1 ± 6.5	19.5 ± 3.6	0.295
ALT	U/L	16.8 ± 8.2	18.1 ± 6.1	0.411
γ-GTP	U/L	17.3 ± 11.8	17.9 ± 10.0	0.490
ALP	U/L	207.1 ± 31.3	193.6 ± 27.7 *	**0.017**
LDH	U/L	171.5 ± 41.5	161.3 ± 30.8 †	0.074
CPK	U/L	135.2 ± 112.0	84.9 ± 20.9	0.142
Uric acid	mg/dL	4.68 ± 1.49	4.65 ± 1.20	0.871
BUN	mg/dL	13.5 ± 5.0	14.1 ± 4.6	0.630
Creatinin	mg/dL	0.70 ± 0.11	0.71 ± 0.08	0.620
Na	mEq/L	141.5 ± 1.9	140.5 ± 1.4 *	**0.019**
K	mEq/L	3.97 ± 0.29	4.17 ± 0.30	0.153
Cl	mEq/L	104.1 ± 3.0	105.1 ± 2.0	0.128
Ca	mg/dL	9.47 ± 0.44	9.33 ± 0.26	0.152
Fe	μg/dL	111.5 ± 30.9	116.3 ± 48.0	0.706
Total protein	g/dL	7.41 ± 0.52	7.31 ± 0.24	0.386
Albumin	g/dL	4.45 ± 0.34	4.37 ± 0.24	0.377
Oxydative stress markers				
OS	mg/mL	34.8 ± 7.0	32.3 ± 5.7	0.107
AP	umol/L	2681 ± 327	2616 ± 255	0.481
AP/OS ratio	–	79.1 ± 14.6	83.1 ± 15.0	0.137
8-OHdG [creatinine-adjusted, urine]	ng/mg crea	8.30 ± 2.66	7.79 ± 1.68	0.405
Isoprostane [creatinine-adjusted, urine]	ng/mg crea	2.40 ± 0.72	2.46 ± 0.75	0.848
Glycative stress relate d markers				
FPG	mg/dL	88.5 ± 8.4	87.8 ± 5.4	0.817
HbA1c [NGSP]	%	5.31 ± 0.23	5.19 ± 0.17 **	**0.003**
Total cholesterol	mg/dL	225.4 ± 37.1	231.4 ± 36.4	0.496
LDL-C	mg/dL	133.6 ± 31.3	139.2 ± 28.7	0.391
HDL-C	mg/dL	72.5 ± 12.8	74.5 ± 14.7	0.465
TG	mg/dL	75.7 ± 31.8	76.6 ± 30.4	0.880
Hormonal examination				
(Serum)				
IGF-I	ng/mL	136.5 ± 48.5	145.8 ± 54.2 †	0.083
DHEA-s	μg/dL	166.5 ± 85.9	143.6 ± 79.1 *	**0.017**
Cortisol	μg/dL	9.75 ± 2.04	8.22 ± 1.85 *	**0.025**
Cortisol/DHEA-s ratio	—	0.076 ± 0.055	0.073 ± 0.046	0.563
(Urine)				
SaMT	ng/mL	29.0 ± 19.4	27.1 ± 15.7	0.484
	ng/h/kg	19.4 ± 7.6	18.2 ± 9.2	0.513

Data are expressed as mean ± SEM, paired t test, n = 11. OS, oxidative stress; AP, antioxidant power; 8-OHdG, 8-hydroxy-2’ -deoxyguanosine; FPG, fasting plasma glucose; IGF-I, insulin-like growth factor-I; DHEA-s, dehydroepiandrosterone-sufate; SaMT, 6-hydroxy-melatonin sulfate; SEM, standard error mean.

Oxidative stress markers

No significant difference was observed in oxidative stress markers; OS, AP or 8-OHdG (***Table 4***).

Glycative stress markers

HbA1c of glycative stress significantly lowered from 5.31 ± 0.23% to 5.19 ± 0.17% (−2.3%, p = 0.003, ***Table 4***). No significant difference was observed in fasting plasma glucose (FPG), total cholesterol (TC), low-density-lipoprotein-cholesterol (LDL-C), high-density-lipoprotein-cholesterol (HDL-C) or triglyceride (TG).

Endocrine markers

As the endocrine examination, IGF-I and DHEA-s relating to hormone age, urinary SaMT as a sleep-related hormone and cortisol involved in physical and mental stress were measured (***Table 4***). Serum IGF-I tended to increase from 136.5 ± 48.5 ng/mL, the level before the start of use, to 145.8 ± 54.2 ng/mL after 4 weeks (+6.8%, p = 0.083). Both serum DHEA-s (−13.8%. p = 0.017) and cortisol (−15.7%, p = 0.025) significantly lowered; however, there was no significant change in the cortisol/DHEA-s ratio. There was no significant change in SaMT in urine.

Immunological Markers

In a peripheral blood test, although white blood cell (WBC) count significantly lowered (−11.9%, p = 0.033), there was no significant change in white blood cell fractions (***Table 5***). There was no significant change in serum C-reactive protein (CRP), an inflammatory reaction marker.

In immunity evaluation tests, there was no significant difference in immunity function scores, and there was also no significant difference in its subitems (T cells score, CD8+ CD28+ T cell score, CD4/CD8 cell ratio score, Naïve T cell score, Naïve Memory T cell score, B cell score or natural killer [NK] cell score). The B cell score significantly lowered from 341.8 ± 144.0 /μL to 310.5 ± 123.9 /μL (−9.2%, p = 0.044). Although there was no significant change in CD56+CD16− cells and CD56+CD16+ cells out of the NK cell subsets, the immunity of the CD56−CD16+ cells remarkably increased from 0.18 ± 0.09% to 0.29 ± 0.15% (+61.1%, p = 0.020).

Safety

There has been no adverse event during the observation period.

Discussion

In this experiment, the improvement in sleep-related subjective symptoms, decrease of WBC by biochemical examination of blood (−11.9%, p < 0.05) or changes in FPG, LDL-C, HDL-C or TG by glycolipid metabolization tests were not observed, but the decrease of HbA1c (−2.3%, p < 0.01) was observed. The upward trend in IGF-I (+6.8%, p < 0.1) and decreases of cortisol (−15.7%, p < 0.05) and DHEA-s (−13.8%, p < 0.05) were observed through an endocrine examination, but no change in cortisol/DHEA-s ratio was observed. No change was observed in oxidative stress markers; 8-OHdG in urine (previous value 8.3 ± 2.7 ng/mg crea) or isorostane. As for immunity evaluation, the decrease in B cell count and a remarkable increase of CD56−CD16+ cells, a subset of NK cells (+64.4%, p < 0.05) was recognized.

In a previous test using the same mattress[16], no improvement in sleep-related subjective symptoms or no change in FPG, HbA1c, LDL-C or TG by a glycolipid metabolization test using the test mattress for 4 weeks was observed, but a rise of HDL-C level (+7.5%, p < 0.01) was observed. In an endocrine examination, the rise of IGF-I level (+10.2%, p < 0.01) was recognized, but no change in cortisol, DHEA-s or cortisol/DHEA-s ratio was observed. The decrease of 8-OHdG in urine, an oxidative stress maker, was recognized (previous value: 9.9 ± 0.8 ng/mg crea, −28/3%. p < 0.01), but no change in isoprostane was observed.

As the result of comparison between the results of this test and those of previous tests[16], the improvement of sleep-related subjective symptoms, an upward trend in IGF-I and the improvement of one item of glycolipid metabolism markers (rise of HDL-C level in the previous test, and the fall of HbA1c level in this test) were observed in common. Regarding the difference in oxidative stress markers, it is presumed that because the level of 8-OHdG at this time was lower than previous one, there was no room to improve it.

• Anti-aging medicine

Anti-aging medicine is a preventive medicine focusing on aging. It does not resist natural aging but aims at the prevention and treatment of morbid aging and deterioration.

In the field of anti-aging medicine, as a result of the investigation of the characteristics of centenarians (people who are 100 years old or older, have no dementia, cancer or any other major diseases and live independently, not bedridden), it is known that the decisive factor for longevity is to keep the balance of "exercise," "nourishment" and "rest (sleep)," in addition to genetic and organic factors.

• Relationship between anti-aging medicine and sleep

As a result of the investigation on the relationship between sleeping time and lifespan, persons whose sleeping time is 7 hours live longest and those who's sleeping time is 8 hours or longer or less than 6 hours have a higher risk of death[27]. Regarding the relationship between sleeping time and the risk of the onset of type-2 diabetes, a questionnaire for sleeping state and a monitoring inspection on sleep breathing overnight (polysomnography) were conducted, and the presence or absence of the onset of type-2 diabetes during a year after that was assessed. As a result, the risk of the onset of type-2 diabetes was lowest among the subjects whose sleeping time was 7-8 hours[28]. It is also reported that as the relationship between sleeping time and obesity, the children whose sleeping time is short tend to be more overweight[29].

"Melatonin," "growth hormones" and "cortisol", which are sleep-related factors, are closely related to each of anti-aging medicines. It is recognized that "melatonin" has an antioxidant effect and an immunity-activating effect and that growth hormones have the effects to keep bones, muscles, skin and others in healthy condition, and these hormones are considered to have anti-aging effects. Cortisol is said to have the effect of raising blood pressure and inhibiting the secretion of growth hormone with circadian secretion rhythm. The secretion of cortisol is inhibited at night and

Table 5. Immunological assessment.

Peripheral blood		Before	4 weeks	p value
WBC	/μL	4972.7 ± 1496.7	4381.8 ± 1340.0 *	**0.033**
Neutrophil	%	58.40 ± 8.64	59.52 ± 5.41	0.520
Lymphocyte	%	33.28 ± 7.81	32.23 ± 5.42	0.425
Monocyte	%	5.78 ± 2.37	5.42 ± 1.22	0.443
Eosinophil	%	2.01 ± 1.06	2.31 ± 1.27	0.280
Basophil	%	0.53 ± 0.30	0.53 ± 0.31	1.000
Inflammtory reaction				
CRP	mg/dL	0.0457 ± 0.0660	0.0318 ± 0.0425	0.228
Immunity evaluation				
Immunity Function Score	–	16.1 ± 0.8	15.6 ± 1.1	0.257
T cell score	–	2.2 ± 0.4	2.0 ± 0.4	0.157
CD8+CD28+ T cell score	–	2.1 ± 0.3	2.1 ± 0.3	1.000
CD4/D8 ratio score	–	1.6 ± 0.7	1.5 ± 0.7	0.317
Naïve T cell score	–	2.8 ± 0.4	2.7 ± 0.5	0.317
Memory T cell score	–	2.3 ± 0.6	2.3 ± 0.5	1.000
B cell score	–	3.0 ± 0.0	3.0 ± 0.0	1.000
NK cell score	–	2.1 ± 0.3	2.1 ± 0.3	1.000
T cell CD3+	%	69.65 ± 4.97	70.73 ± 6.31	0.395
B cell CD20+	%	19.44 ± 5.97	18.36 ± 4.04	0.201
CD4T CD4+	%	50.62 ± 7.34	51.91 ± 6.65	0.378
CD8T CD8+	%	13.49 ± 3.71	12.66 ± 3.31	0.162
CD4 subsets Naïve T	%	44.12 ± 11.36	44.28 ± 9.02	0.884
CD4 subsets Memory T	%	55.88 ± 11.36	55.72 ± 9.02	0.884
CD8 T cell subset CD28+	%	71.78 ± 15.41	70.39 ± 12.12	0.541
NK subsets CD56+CD16−	%	2.99 ± 2.25	2.75 ± 1.30	0.526
NK subsets CD56+CD16+	%	10.82 ± 6.24	10.55 ± 3.87	0.870
NK subsets CD56−CD16+	%	0.18 ± 0.09	0.29 ± 0.15 *	**0.020**
Neutrophil	/μL	2780.7 ± 1288.2	2554.9 ± 931.1	0.354
Lymphocyte	/μL	1732.3 ± 370.6	1646.3 ± 402.0	0.137
T cell	/μL	1208.1 ± 285.1	1158.3 ± 275.6	0.122
B cell	/μL	341.8 ± 144.0	310.5 ± 123.9 *	**0.044**
CD4+ T cell	/μL	885.5 ± 275.1	856.8 ± 239.0	0.274
CD8+ T cell	/μL	236.4 ± 92.5	210.6 ± 88.9 †	0.093
CD4/CD8 ratio	–	4.212 ± 1.894	4.451 ± 1.585	0.297
Naïve T cell	/μL	373.0 ± 83.1	373.5 ± 102.0	0.972
Memory T cell	/μL	512.5 ± 258.6	483.4 ± 184.2	0.296
Naïve / Memory ratio	–	0.865 ± 0.438	0.842 ± 0.342	0.533
NK cell	/μL	181.0 ± 96.0	171.5 ± 68.9	0.775
CD8+CD28+ cell	/μL	164.0 ± 65.9	146.6 ± 66.3 †	0.074

Data are expressed as mean ± SEM, paired t test, n = 11. WBC, white blood cell; CRP, C-reactive protein; NK, natural killer; SEM, standard error mean.

increases in daytime, which leads to an anti-aging effect.

It is considered that a good quality of sleep leads to the increase of secretions of melatonin and growth hormones and if there is an adjustment of the rhythm of cortisol secretion, it leads to anti-aging effects. A good quality of sleep is considered to be one of the secrets to extend healthy life expectancy.

The accumulation of the evidences regarding the relationship between sleeping time and SAS and diabetes is remarkable and the relationship between these sleeping problems and diabetes has been suggested, but its mechanism has not been clarified in many respects. As the achievement of this test, it was recognized that the significant decrease of HbA1C, a glycative metabolic marker (previous value 5.31%, –23%, $p < 0.001$), was observed. HbA1c reflects average glucose value for 3 to 4 weeks. It is common that people use a mattress for a long time more than 5 years. If the improved glucose control is carried out for a long time like this case, it can be expected that the accumulation of AGEs is decreased.

Here, the relation between sleep and glycative metabolism is discussed from the relationship among melatonin, GH/IGF-I and cortisol of stress hormone. They are paid attention to as the hormones deeply related with sleep and mental and physical stress from the perspective of anti-aging medicine.

• Melatonin

There are several reports concerning melatonin's effect on glycolipid metabolism. Membrane-bound melatonin receptors MT1/MT2 reduce cAMP in cells through Gi-protein, and which itself acts toward the inhibition of insulin secretion. Meanwhile melatonin receptor signal promotes the phosphorylation of insulin receptor by controlling cAMP sensitivity and phosphotyrosine phosphatase activity,[30] and furthermore, it mobilizes calcium irons into cytoplasm by activating phospholipase C/IP3 pathways, and ultimately insulin secretion increases[31]. Diabetic rats were experimentally dosed with melatonin and then the levels of TG, free fatty acid and TC in the blood were improved, and furthermore, the level of tumor necrosis factor-α (TNF-α) lowered 50%[32]. On the contrary, in the case where the pineal gland was resected, insulin resistance increased, showed hyperinsulinemia and advanced to type-2 diabetes[33]. It was reported that in a human experiment where females in menopause and after menopause were dosed with melatonin, the level of HDL-C rose[34]. Melatonin plays a role of alleviating glycative stress by promoting the decomposition of AGEs[15].

In this experiment, no significant change in SaMT in urine was observed. Melatonin's contribution to the improvement of HbA1c is considered to be not great. The average age of the subjects in this experiment was 49.8 ± 6.5 years. It is unclear to what extent the function of the pineal gland of the persons in these ages deteriorates and whether there is a room for improvement in recovery of melatonin secretion ability.

• GH/IGF-I

GH/IGF-I hormones also effect glycative metabolism. GH has a blood glucose raising action and it is mobilized at the time of low blood glucose, in particular, in order to maintain blood glucose level. GH decreases insulin sensitivity, but IGF-I increases insulin sensitivity[35]. It is considered that even if patients lacking GH are dosed with GH for a long time, their blood glucose level, HbA1c level or insulin resistance will not change[35,36].

In the previous test of the mattress, IGF-I levels significantly increased in association with the improvement in the quality of sleep[16], and in the test of this time also, its increasing trend was observed. The hyper-secretion of IGF-I hormones possibly increased insulin sensitivity, but it is not clear to what extent it contributed to the improvement of glycative metabolism in this case.

• DHEA-s

The quality of sleep also effects steroid hormones. In general, the levels of serum DHEA-s decrease in association with the decrease of the quality of sleep[37-40]. According to an investigation targeting 6,465 subjects 50 years old or older, the levels of DHEA-s of those having low quality of sleep were low, but it had no relationship with the sleeping time[41]. In the case of female subjects, there is a correlation between nocturnal awakening and DHEA-s[40] and DHEA-s increases by the sleep deprivation stimulus of one night[42]. It is reported that the quality of sleep has positive correlations with HbA1c, TG and TC, and it has negative correlation with DHEA-s[39].

Some reports say that the replenishment of DHEA improves the quality of sleep[43] and some other reports say that the results are uneven[44]. DHEA is partially converted into estradiol and testosterone by intracrine action in the living body, but as there are individual differences in rates of conversion, it causes the differences in the quality of sleep[44]. When considering these phenomena that occur in the living body, although DHEA-s decrease by the quality of sleep in association with aging, it seems like they play a role as anti-stress hormones by increasing responding to mental and physical stress.

By the achievement of this test, a significant decrease in DHEA-s (–13.8%, $p < 0.05$) was observed through the use of the test product. It is probably that the quality of sleep was improved, the stimuli as mental and physical stress decreases and, as a result, DHEA-s decreased. Although DHEA-s have the ability to improve insulin resistance, because DHEA-s decreased in this test, it did not contribute to lowering of HbA1c.

• Cortisol

Cortisol is secreted from adrenal cortex by being stimulated by adrenocorticotropic hormone (ACTH) secreted from the pituitary gland. ACTH/cortisol hormones have circadian rhythm by biological clock and they show high levels from early morning to before noon and low levels from evening to night time. Cushing's syndrome is a disorder associated with cortisol hyper-secretion and a dexamethasone suppression test (DST) is conducted for its diagnosis. In this examination, when healthy persons took dexamethasone (Decadron) orally, ACTH concentration in blood almost completely lowered in normal condition and the concentration of cortisol also lowered. On the contrary, in the next morning, in the case of persons with Cushing's syndrome, even though ACTH was decreased by dexamethasone, cortisol concentration in the blood shows a high level the next morning because cortisol is produced from adrenal tumor. As a result of DST conducted on 20 patients with obese-type and type-2 diabetes and having SAS, 5 of them (25%) showed positive result; however, they were treated with continuous positive airway pressure (CPAP) and they showed negative result in DST[45].

As the result of analysis between nurses' shift work and

their cortisol in blood, cortisol on the second day of their midnight shift showed a significantly high level immediately before the start of work, more than the concentration change by circadian rhythm [46]. The secretion of cortisol is adjusted by endogenous biological clock and sleep suppressively affects the secretion of cortisol. Therefore, the rise of cortisol levels at midnight shift is considered to be caused by disrupted sleep.

As the result of comparison of serum cortisol levels before sleep deprivation and 24 hours after sleep deprivation targeting 223 soldiers (20.8 ± 2.1 years), significant increases of cortisol levels from 486.6 ± 102.1 nmol/L to 508.8 ± 89.5 nmol/L were observed [47]. In other words, sleep deprivation acts as an endogenous stressor. The decline of the quality of sleep is considered to act as an endogenous stressor through complex paths.

At the discussion on the patients with serious atopic dermatitis, accompanied by sleeping disturbances, their cortisol levels before treatment (before admission to hospital) were low and they increased after treatment (after discharge from hospital) [48]. When the intensity of mental and physical stress is high, it is not that the variation of cortisol secretion occurred due to the disturbance of the circadian rhythm caused by sleep disturbance, but that the secretion of cortisol is inhibited by the inhibition of the endocrine system itself, and it is considered that cortisol secretion recovered through treatment.

There is a method for the evaluation of mental and physical stress using the blood concentration levels of cortisol and DHEA-s. Cortisol reflects the degree of mental and physical stress and DHEA-s reflect the resistance against stress; therefore the stress balance is evaluated by cortisol/DHEA-s ratio (or its inverse ratio) [49]. In this test, a significant decrease in cortisol (–15.7%, $p < 0.05$) was observed. Presumably, it is because the mental and physical stress was alleviated because the sleep quality was improved, and as a result, the secretion of cortisol was inhibited.

The significant decrease of DHEA-s (–13.8% m $p < 0.05$) reflects the fact that the resistance against stress becomes weak, and it can be interpreted that DHEA-s values that had been increased by stress load in compensatory so far returned to the original values because the stress load was alleviated. Because both cortisol and DHEA-s decreased to a similar degree, no significant difference in cortisol/DEHA-s ratio, stress hormone balance, was observed. Similar changes were observed in an open test where astaxanthin was administered and the decreases of DHEA-s (–15.1%, $p < 0.001$) and cortisol (–22.8%, $p = 0.002$) and the decreasing trend of cortisol/DHEA-s ($p < 0.1$) were observed [50].

Cortisol has hyperglycemic action and insulin resistance acceleration action. In this test, cortisol decreased 15.7%, and as a result, insulin resistance was improved. These improvements possibly contributed to the alleviation of postprandial hyperglycemia and the decrease of HbA1c.

Immunological makers

In this test, the method by Hirokawa K [22, 23] was used for the evaluation of immunities.

There was no significant change in each score of T cell count, CD8+CD28+ T cell count, CD4/CD8 cell ratio, naïve T cell count, naïve/memory T cell ratio, B cell count and NK cell count, before and after the test. The immunity score of comprehensive judgment of immunities was 16.1 ± 0.8 before the test and 15.6 ± 1.1 after 4 weeks, and there was no significant difference before and after the test. All immunities were at the third stage (grade III), the range requiring improving measures, out of 5 stages of immunity grades.

From the comparison before and after the test, significant differences were observed in CD56–CD16+ cells, a subset of NK cells (0.177 ± 0.093% → 0.291 ± 0.154%) and a number of B cells (341.8 ± 144.0 /μL → 310.5 ± 123.9 /μL). Moreover, significant trends were observed in CD8+ T cells (236.4 ± 92.5 /μL → 210.6 ± 88.9 /μL) and CD8+CD28+ T cells (164.0 ± 65.9 /μL → 146.6 ± 66.3 /μL). The B cell count significantly decreased and CD8+ T cells also showed a decreasing trend.

When NK cells encounter virus-infected cells and tumor cells, they kill or wound them. NK cells have 3 subgroups, which are CD56+CD16+, CD56+CD16– and CD56–CD16+ [51, 52]. CD56+CD16+ cells are ordinarily NK cells, CD56+CD16– cells have less amount of cytokine to kill the cells such as perforin and their killing ability is low, and CD56–CD16+ cells are small in number and do not increase or decrease greatly. As the result of this test, CD56–CD16+ cells significantly increased from 0.177 ± 0.093% before the test to 0.291 ± 0.154% 4 weeks after ($p < 0.05$). This is an extremely rare phenomenon and has been never been reported. It is possible that as the result of improvement of the quality of sleep, CD56–CD16+ cells increased.

There are several reports concerning CD56–CD16+ NK cells' role [53, 55]. In the case of a patient with acquired immunodeficiency syndrome caused by the infection of human immunodeficiency virus (HIV), because CD56–CD16+ NK cells increase and affect the state of disease, it had been considered that these cells might be involved in the decline of immune function [53]. However, it is also considered that NK cell activation failure in acquired immunodeficiency syndrome (AIDS) might be caused by the lack of activated CD56–CD16+ NK cells [54]. In other words, in the case of patients with AIDS, non-activated CD56–CD16+ NK cells increase, but activated CD56–CD16+ NK cells are lacking and this is the cause of NK cell dysfunction.

Among the case reports of ocular muscle type myasthenia gravis, there is a report that CD56–CD16+ NK cells increased [55]. In this case, it is reported that as a result of the treatment by immunosuppressant drugs, the symptom was alleviated and at the same time, CD56–CD16+ NK cells decreased. In the case myasthenia gravis, an autoimmune disorder, immune function is in an accelerating condition and an excessive number of CD56–CD16+ NK cells are involved there. It is presumed that the number of CD56–CD16+ NK cells was decreased via the dosing of immunosuppressant drugs.

As the result of the observation of the changes of IFN-γ over time by NKp46 and NKp30 receptor stimulation, CD56–CD16+ NK cells produce IFN-γ 2-4 hours after stimulation, but CD56+CD16+ NK cells produce IFN-γ 16 hours after stimulation or later [56]. CD56–CD16+ NK cells are said to be important for the early reaction of natural immunity.

In this test, if you consider that cortisol with immunosuppressive functions decreased, and as a result, inhibition was removed and the number of CD56–CD16+ NK cell increased, which may be easily understood. It is possible that this acted on the natural immunity, which had been inhibited, to be restored. However, the significance of the increase of CD56–CD16+ NK cells is a task for the

future.

Following this, CD4 and CD8 are discussed. CD4+ T cells play an important role for human immune system, and called CD4 cells, Th cells, T4 cells and helper T cells. Their role is to send signals to other immune systems such as CD8+ T cells (Killer T cells or cytotoxic T cells), and CD8+ T cells activated by it destroy infected cells. In the cases of untreated HIV-1 patients and the users of immunosuppressant drugs, CD4+ T cells are depleted or mostly depleted. As a result, their immunity decreases and they easily get infected with opportunistic infection.

CD4+ T cells assist B cells to produce immune globulin, and CD8+ T cells inhibit the production of immune globulin.

As standard values, the CD4+ T cell count is 500-1,600 /μL, the CD8+ T cell count is 300-900 /μL and the CD4/CD8 ratio is 0.6-2.9 /μL [57].

The CD4+ T cell count is used as an index for the treatment of AIDS. If the CD4+ T cell count is less than 350 /μL, treatment is required. If it is less than 200 /μL, the HIV positive patient is diagnosed with AIDS. The examination of the CD4+ T cell count is used also as an efficacy evaluation of treatment.

The CD4/CD8 ratio is an index showing the balance between CD4+ T cells and CD8+ T cells. When CD4+ T cells and CD8+ T cells are the most appropriately balanced, the immune functions most effectively. The CD4/CD8 ratio decreases with the increase of mental and physical stress and increases with age.

The values measured before the test were as follows: CD4+ T cell count was 885.5 ± 275.1 /μL, within the normal range, CD8+ T cell count was 36.4 ± 9.5 /μL, rather low, and CD4/CD8 ratio was 4.212 ± 1.894, rather high.

Although it is not significant, the CD4/CD8 ratio tended to increase from 4.212 ± 1.894 to 4.451 ± 1.585. It is safe to assume that it increased because the subjects were released from a mentally and physically stressed state.

The reason why the B cell count decreased from 341.8 ± 144.0 /μL before the test to 310.5 ± 123.9 /μL 4 weeks after the start of test is unknown. In this test, as whole, the number of WBC significantly decreased and those of neutrophil and lymphocytes decreased, although not significantly. For this specific test, it is presumed that natural immunity rose in light of the changes in NK cell fractions, and as a result, B cells and neutrophil did not have a chance to work.

Conclusion

As a result of a pilot test on the use of mattresses of test product for 4 weeks, targeting 11 male and female subjects realizing a mild degree of sleep disorders, the improvements in sleep-related subjective symptoms, the decrease of WBC by biochemical examination of blood, decrease of HbA1c, and as a hormone system, the rising trend of IGF-I, decrease of cortisol and decrease of DHEA-s were observed. The decrease in cortisol level reflects the alleviation of mental and physical stress associated with the improvement of the quality of sleep. Because cortisol enhances insulin resistance, the decrease of cortisol possibly favorably affects the change of glucose level and contributes to the decrease of HbA1c.

In the immune function score, the decreases in the B cell count and a remarkable increase of CD56+ cell count of a subset of NK cells were observed. Although no improvement in oxidative marker was observed in this test, the improvement in secretion of GH/IGF-I in association with the improvement of the quality of sleep, and the improvement of the balance of stress hormones were observed. In immunity evaluation, a remarkable increase of CD56−CD16+ NK cells was observed, that is a very interesting result from the perspective of natural immunity. Further analysis of the significance of the increase in the number of CD56−CD16+ cells associated with the improvement of the quality of sleep is anticipated in the future.

Acknowledgements

This work was supported by JSPS KAKENHI Grant Number 26350917.

Conflict of Interest Statement

The present study was partly supported by Nishikawa Sangyo Co.,Ltd.

Reference

1) Kashine S, Kishida K, Funahashi T, et al. Selective contribution of waist circumference reduction on the improvement of sleep-disordered breathing in patients hospitalized with type 2 diabetes mellitus. Intern Med. 2011; 50: 1895-1903.
2) Tamura A, Kawano Y, Watanabe T, et al. Obstructive sleep apnea increases hemoglobin A1c levels regardless of glucose tolerance status. Sleep Med. 2012; 13: 1050-1055.
3) Yamada K, Nakayama H, Kato T, et al. Prevalence and clinical characteristics of unremembered nocturnal eating in diabetic subjects: Kurume sleep trouble in obesity and metabolic disorders (KUSTOMED) study. Endocr J. 2013; 60: 1059-1063.
4) Tamada D, Otsuki M, Kashine S, et al. Obstructive sleep apnea syndrome causes a pseudo-Cushing's state in Japanese obese patients with type 2 diabetes mellitus. Endocr J. 2013; 60: 1289-1294.
5) Shimizu K, Yamamoto T, Shirai K. Arterial stiffness, as monitored by cardio-ankle vascular index, is affected by obstructive sleep apnea, blood glucose control, and body weight: A case with 8 years follow up. Int Med Case Rep J. 2016; 9: 231-235.
6) Nagayoshi M, Punjabi NM, Selvin E, et al. Obstructive sleep apnea and incident type 2 diabetes. Sleep Med. 2016; 25: 156-161.

7) Kawada T. Untreated sleep apnea syndrome and glycemic control in patients with type 2 diabetes. J Diabetes. 2017; 9: 717.
8) Shiba T, Takahashi M, Hori Y, et al. Evaluation of the relationship between background factors and sleep-disordered breathing in patients with proliferative diabetic retinopathy. Jpn J Ophthalmol. 2011; 55: 638-642.
9) Nishimura A, Kasai T, Tamura H, et al. Relationship between sleep disordered breathing and diabetic retinopathy: Analysis of 136 patients with diabetes. Diabetes Res Clin Pract. 2015; 109: 306-311.
10) Fujihara K, Kodama S, Horikawa C, Y et al. The Relationship between diabetic neuropathy and sleep apnea syndrome: A meta-analysis. Sleep Disord. 2013; 2013: 150371.
11) Furukawa S, Saito I, Yamamoto S, et al. Nocturnal intermittent hypoxia as an associated risk factor for microalbuminuria in Japanese patients with type 2 diabetes mellitus. Eur J Endocrinol. 2013; 169: 239-246.
12) Nomoto K, Yagi M, Arita S, et al. Skin accumulation of advanced glycation end products and lifestyle behaviors in Japanese. Anti-Aging Med. 2012; 9: 165-173.
13) Moniruzzaman M, Takabe W, Yonei Y. Melatonin is not a carbonyl scavenger. Glycative Stress Res. 2016; 3: 1-4.
14) Mamun-Or-Rashid ANM, Takabe W, Yonei Y. Melatonin has no direct effect on inflammatory gene expression in CML-HSA stimulated RAW264.7 cells. Glycative Stress Res. 2016; 3: 141-151.
15) Takabe W, Mitsuhashi R, Parengkuan L, et al. Cleaving effect of melatonin on crosslinks in advanced glycation end products. Glycative Stress Res. 2016; 3: 38-43.
16) Takabe W, Ogura M, Yagi M, et al. Effect on sleep quality of bedding with a high user rating in a post-marketing survey: A non-controlled open-label study. Glycative Stress Res. 2016; 3: 110-123.
17) Doi Y, Minowa M, Uchiyama M, et al. Psychometric assessment of subjective sleep quality using the Japanese version of the Pittsburgh Sleep Quality Index (PSQI-J) in psychiatric disordered and control subjects. Psychiatry Res. 2000; 97: 165-172.
18) Oguma Y, Iida K, Yonei Y, et al. Significance evaluation of Anti-Aging QOL Common Questionnaire. Glycative Stress Rese. 2016; 3: 177-185.
19) Sato K, Yagi M, Yonei Y. A new method for measuring oxidative stress using blood samples. Glycative Stress Res. 2015; 2: 15-21.
20) Hirokawa K, Utsuyama M, Makinodan T. Immunity and aging. In; Principles and Practice of Geriatric Medicine, eds. Pathy MSJ, Sinclair AJ, Morley JE , pp.19-36. John Wiley & Son, Van Hoboken, NJ, 2006.
21) Hirokawa K, Utsuyama M, Kikuchi Y, et al. Scoring of immunological vigor: Trial assessment of immunological status as a whole for elderly people and cancer patients. In; Immunosenescence, ed. Pawelec G, Landes Bioscience, Abingdon, UK, 2007.
22) Hirokawa K. An objective assessment of immunological status and function recovering. International Life Sciences Institute (ILSI). 2012; 129: 5-17. (in Japanese)
23) Hirokawa K. Immunity and aging: The mecganism, assessment and recovery. Ochanomizu Medical Journal. 2013; 61: 17-36. (in Japanese)
24) Toyokuni S, Hirao A, Wada T, et al. Age- and sun exposure-dependent differences in 8-hydroxy-2'-deoxyguanosine and N-(carboxymethyl)lysine in human epidermis. J Clin Biochem Nutr. 2011; 49: 121-124.
25) Hozawa A, Ebihara S, Ohmori K, et al. Increased plasma 8-isoprostane levels in hypertensive subjects: The Tsurugaya Project. Hypertens Res. 2004; 27: 557-561.
26) Kawamoto T, Takabe W, Maruyama Y, et al. Relationships between urinary melatonin metabolites and glycative stress and body functional age. Glycative Stress Res. 2016; 3: 15-22.
27) Kripke DF, Garfinkel L, Wingard DL, et al. Mortality associated with sleep duration and insomnia. Arch Gen Psychiatry. 2002; 59: 131-136.
28) Gottlieb DJ, Punjabi NM, Newman AB, et al. Association of sleep time with diabetes mellitus and impaired glucose tolerance. Arch Intern Med. 2005; 165: 863-867.
29) Sekine M, Yamagami T, Hamanishi S, et al. Parental obesity, lifestyle factors and obesity in preschool children: Results of the Toyama Birth Cohort study. J Epidemiol. 2002; 12: 33-39.
30) Nishida S. Metabolic effects of melatonin on oxidative stress and diabetes mellitus. Endocrine. 2005; 27: 131-136.
31) Sharma S, Singh H, Ahmad N, et al. The role of melatonin in diabetes: Therapeutic implications. Arch Endocrinol Metab. 2015; 59: 391-399.
32) Nishida S, Segawa T, Murai I, et al. Long-term melatonin administration reduces hyperinsulinemia and improves the altered fatty-acid compositions in type 2 diabetic rats via the restoration of Delta-5 desaturase activity. J Pineal Res. 2002; 32: 26-33.
33) Nishida S, Sato R, Murai I, et al. Effect of pinealectomy on plasma levels of insulin and leptin and on hepatic lipids in type 2 diabetic rats. J Pineal Res. 2003; 35: 251-256.
34) Tamura H, Nakamura Y, Narimatsu A, et al. Melatonin treatment in peri- and postmenopausal women elevates serum high-density lipoprotein cholesterol levels without influencing total cholesterol levels. J Pineal Res. 2008; 45: 101-105.
35) Oliveira CR, Salvatori R, Barreto-Filho JA, et al. Insulin sensitivity and β-cell function in adults with lifetime, untreated isolated growth hormone deficiency. J Clin Endocrinol Metab. 2012; 97: 1013-1019.
36) Ikeda H, Kudo M. Long-term follow-up results of growth hormone therapy for patients with adult growth hormone deficiency. Hormones (Athens). 2016; 15: 45-53.
37) Makino S, Fujiwara M, Handa H, et al. Plasma dehydroepiandrosterone sulphate and insulin-like growth factor I levels in obstructive sleep apnoea syndrome. Clin Endocrinol (Oxf). 2012; 76: 593-601.
38) Maggio M, Colizzi E, Fisichella A, et al. Stress hormones, sleep deprivation and cognition in older adults. Maturitas. 2013; 76: 22-44.
39) Ko SB. Night shift work, sleep quality, and obesity. J Lifestyle Med. 2013; 3: 110-106.
40) Kische H, Ewert R, Fietze I, et al. Sex hormones and sleep in men and women from the general population: A cross-sectional observational study. J Clin Endocrinol Metab. 2016; 101: 3968-3977.
41) Jackowska M, Kumari M, Steptoe A. Sleep and biomarkers in the English Longitudinal Study of Ageing: associations with C-reactive protein, fibrinogen, dehydroepiandrosterone sulfate and hemoglobin. Psychoneuroendocrinology. 2013; 38: 1484-1493.
42) Selvi Y, Kiliç S, Aydin A, et al. The effects of sleep deprivation on dissociation and profiles of mood, and its association with biochemical changes. Noro Psikiyatr Ars. 2015; 52: 83-88.

43) Sorwell KG, Urbanski HF. Causes and consequences of age-related steroid hormone changes: Insights gained from nonhuman primates. J Neuroendocrinol. 2013; 25: 1062-1069.
44) Caufriez A, Leproult R, L'Hermite-Balériaux M, et al. Effects of a 3-week dehydroepiandrosterone administration on sleep, sex steroids and multiple 24-h hormonal profiles in postmenopausal women: A pilot study. Clin Endocrinol (Oxf). 2013; 79: 716-724.
45) Tamada D, Otsuki M, Kashine S, et al. Obstructive sleep apnea syndrome causes a pseudo-Cushing's state in Japanese obese patients with type 2 diabetes mellitus. Endocr J. 2013; 60: 1289-1294.
46) Miyauchi F, Kimura K, Hirano M, et al. Effects of working at night on plasma cortisol concentrations and BMI in female nurses. Japanese Journal of Occupational Medicine and Traumatology (JJOMT). 2012; 60: 348-352. (in Japanese)
47) Sun X-Y, Song H-T, Yang T-S, et al. Effects of sleep deprivation on neuroendocrine hormones in servicemen. Sleep and Biological Rhythms. 11: 274-277.
48) Fukuda H, Suzuki T, Saotome A, et al. Efficacy of inpatient treatment for atopic dermatitis evaluated by changes in serum cortisol levels. J Dermatol. 2013; 40: 43-47.
49) Yonei Y, Takabe W. Aging assessment by Anti-Aging Medical Checkup. Health Evaluation and Promotion. 2015; 42: 459-464.
50) Iwabayashi M, Fujioka N, Nomoto K, et al. Efficacy and safety of eight-week treatment with astaxanthin in individuals screened for increased oxidative stress burden. Anti-Aging Med. 2009; 6: 15-21.
51) Deniz G, Akdis M, Aktas E, et al. Human NK1 and NK2 subsets determined by purification of IFN-gamma-secreting and IFN-gamma-nonsecreting NK cells. Eur J Immunol. 2002; 32: 879-884.
52) Hagberg N, Berggren O, Leonard D, et al. IFN-α production by plasmacytoid dendritic cells stimulated with RNA-containing immune complexes is promoted by NK cells via MIP-1β and LFA-1. J Immunol. 2011; 186: 5085-5094.
53) Mavilio D, Lombardo G, Benjamin J, et al. Characterization of CD56–/CD16+ natural killer (NK) cells: A highly dysfunctional NK subset expanded in HIV-infected viremic individuals. Proc Natl Acad Sci U S A. 2005; 102: 2886-2891.
54) Naluyima P, Eller MA, Laeyendecker O, et al. Impaired natural killer cell responses are associated with loss of the highly activated NKG2A(+)CD57(+)CD56(dim) subset in HIV-1 subtype D infection in Uganda. AIDS. 2014; 28: 1273-1278.
55) Nguyen S, Morel V, Le Garff-Tavernier M, et al. Persistence of CD16+/CD56–/2B4+ natural killer cells: A highly dysfunctional NK subset expanded in ocular myasthenia gravis. J Neuroimmunol. 2006; 179: 117-125.
56) De Maria A, Bozzano F, Cantoni C, et al. Revisiting human natural killer cell subset function revealed cytolytic CD56 (dim) CD16+ NK cells as rapid producers of abundant IFN-gamma on activation. Proc Natl Acad Sci USA. 2011; 108: 728-732.
57) Bofill M, Janossy G, Lee CA, et al. Laboratory control values for CD4 and CD8 T lymphocytes. Implications for HIV-1 diagnosis. Clin Exp Immunol. 1992; 88: 243-252.