Glycative Stress Research

Online edition : ISSN 2188-3610
Print edition : ISSN 2188-3602
Received: November 20, 2015
Accepted : December 25, 2015
Published online : March 31, 2016

Original Article

Relationships between urinary melatonin metabolites and glycative stress and body functional age

Takuya Kawamoto [1)], Wakako Takabe [1)], Yusuke Maruyama [2)], Atsuhiko Hattori [2)], Yoshikazu Yonei [1)]

1) Anti-Aging Medical Research Center and Glycative Stress Research Center, Graduate School of Life and Medical Sciences, Doshisha University, Kyoto, Japan
2) College of Liberal Arts and Sciences, Tokyo Medical and Dental University, Chiba, Japan

Abstract

***Objective*:** This study was conducted to evaluate the associations between 6-hydroxy-melatonin sulfate (SaMT), a urinary metabolite of melatonin, and body functional age as determined through anti-aging health checkups and lifestyle.
***Methods*:** The study population comprised 25 elderly subjects with no exercise contraindication (14 males and 11 females, 73.2 ± 6.5 years), who had participated in walking exercises in earlier studies conducted in the Yurin area in Shimogyo-ku, Kyoto, Japan. Body functional age data from anti-aging health checkups were evaluated using the Age Management Check® (Ginga Kobo). Nocturnal spot urine SaMT excretion volume was measured by ELISA, and the correlations with SaMT excretion volume were analyzed by sex and for both sexes together.
***Results*:** Simple correlation analysis revealed a significant correlation with SaMT excretion volume in terms of hormonal age ($r = 0.479$, $p = 0.028$), hormonal age gap ($r = 0.533$, $p = 0.013$), and alcohol consumption ($r = 0.600$, $p = 0.004$) for the entire study population (both sexes together); hormonal age ($r = 0.652$, $p = 0.030$) and alcohol consumption ($r = 0.789$, $p = 0.004$) for males; and vascular age gap ($r = 0.769$, $p = 0.009$) for females. The gap was defined as the difference between functional age and true age. Logistic analysis revealed the absence of body parameters that were significantly correlated with urinary SaMT excretion.
***Conclusion*:** The present preliminary study found no definite association between melatonin secretion, as indicated by urinary SaMT excretion, and physical data including body functional ages. Further investigation will be necessary with an increased sample size.

***KEY WORDS*:** elderly person, anti-aging health checkup, body functional age, 6-hydroxymelatonin sulfate (SaMT), melatonin

Introduction

Advanced glycation end products (AGEs) of proteins are involved in the onset and progression of lifestyle-related diseases such as diabetic complications and arteriosclerosis, and aging-related diseases [1,2)]. The process of AGE production is mediated by various reactions, such as oxidation and crosslinking, and it is thought to be influenced by lifestyle factors such as diet, exercise, sleep, and stress [1,2)].

Our laboratory previously measured skin fluorescent AGEs in healthy subjects in a noninvasive manner, and found increased AGE content and wide individual differences with aging [3)]. The broad individual differences have been suggested to be due to the involvement of lifestyle factors such as smoking, drinking, and a lack of sleep [4)]. Although melatonin is known to be a sleep-related hormone [5)], only a few studies describe the associations between melatonin and body functional ages or senescence risk factors. The present study, which included a population who had undergone anti-aging health checkups for body functional age over many years, was undertaken to evaluate the association between melatonin secretion and physical data.

Methods

Subjects

The study population comprised 25 elderly persons (14 males and 11 females, 73.2 ± 6.5 years) with no exercise contraindication, living in the Yurin area in Shimogyo-ku,

Contact Address: Professor Yoshikazu Yonei, MD, PhD
Anti-Aging Medical Research Center and Glycative Stress Research Center,
Graduate School of Life and Medical Sciences, Doshisha University
1-3, Tataramiyakodani, Kyotanabe-shi, Kyoto, 610-0394 Japan
Phone/Fax: +81-774-65-6394 E-mail: yyonei@mail.doshisha.ac.jp
Co-authors: Kawamoto T, dmo2005@mail4.doshisha.ac.jp;
Maruyama Y, sobs0930@yahoo.co.jp; Takabe W, wtakabe@mail.doshisha.ac.jp;
Hattori A,ahattori.las@tmd.ac.jp

(c) Society for Glycation Stress Research

Kyoto, Japan, who had participated in walking exercise as an intervention, motivated by the use of a pedometer and by the health information in printed materials from in earlier studies [6-8].

Before starting this study, study subjects were provided a full explanation regarding the study period, study site, study contents, and study methodology, and also about the benefits and disadvantages expected with participation in the study. Written informed consent was obtained from each subject.

Anti-aging health checkup

In April 2014, an anti-aging health checkup was implemented to evaluate the five functional age parameters (myological, skeletal, hormonal, neurological, and vascular), to quantify indicators of glycative stress, and to evaluate subjective symptoms with the Anti-Aging QOL Common Questionnaire [9,10]. The five functional ages were calculated from these databases using the Age Management Check® System (Ginga Kobo, Nagoya, Japan).

Indicators that were needed to calculate the functional ages were measured using bioelectrical impedance analysis (Physion MD; Nippon Shooter, Kyoto, Japan) [11] for muscle mass, an ultrasound technique (A-1000; Omron Colin, Tokyo, Japan) for bone strength, Wisconsin Card SortingTest (WCST) [12] for higher brain function, and a fingertip acceleration plethysmograph (SDP-100, Fukuda Denshi Co., Ltd., Tokyo, Japan) for the degree of arteriosclerosis. Blood biochemical testing was performed for endocrine assessments. A glycative stress test was performed using AGE reader™ (DiagnOptics, Groningen, Netherlands) [3,4].

Measurement of urinary melatonin metabolites

At each anti-aging health checkup described above, a urine sampling kit and a dedicated recording form were handed to each subject. On urine sampling days in May 2014, he or she collected urine upon awakening in the morning, and recorded urine volume, morning urination time, and the previous night's urination time on the form.

The urine samples were examined for the concentrations of the melatonin metabolite 6-hydroxy-melatonin sulfate (SaMT) [13] and creatinine. Urinary melatonin is known to exhibit little fluctuation between day and night, with no association with blood melatonin, whereas 6-hydroxy-melatonin, a melatonin metabolite, is known to occur in the urine at levels 100-1000 times higher than melatonin, and it is correlated with blood melatonin [14]. Numerous studies employing these urinary melatonin metabolites as indicators have been undertaken to examine data changes among nurses in three shifts [15], profile changes in night workers [16], and the influence of room temperature during sleep [17].

In addition, the absolute urinary creatinine level is nearly constant per unit time, irrespective of water and dietary consumption; data can be corrected for the influence of urine volume by dividing the spot urine albumin measurement by the creatinine measurement from the same urine sample, and quantitative assay may be possible with the use of spot urine samples. Concentrations were measured using a SaMT measurement kit (Melatonin Sulfate ELISA; IB International GmbH, Hamburg, Germany) and a creatinine measurement kit (LabAssay Creatinine; Wako Chemicals USA, Richmond, VA, USA),.

Statistical analyses

Measured values were compared using IBM SPSS Statistics 21 (SPSS Inc., Chicago, IL, USA). Logistic analysis was performed for both sexes together and by sex, associations between corrected SaMT concentration (ng/mg creatinine) or SaMT excretion volume (μg/h) and five functional ages (myological, skeletal, neurological, vascular, and hormonal), age gap (functional age – real age) for the five functional ages, smoking amount, alcohol consumption, exercise volume, water intake, and sleep time, with 2-variate correlation were analyzed using Pearson product-moment correlation analysis. The level of significance was set at 5%.

Ethical standards

Before the start of the present study, each patient was given a full explanation not only about study period, study site, study contents, and study methodology, but also about the benefits and disadvantages expected with their participation in the study. Written informed consent was obtained from each subject. This study was conducted after approval by the Ethics Committee of Doshisha University (Application No. 0832, 14089).

Results

Functional ages

The true and functional ages of the subjects and lifestyle assessments with the Anti-Aging QOL Common Questionnaire are shown in ***Table 1***.

Overall means were higher than the true age only for hormonal age, whereas the other functional ages (myological age, skeletal age, neurological age, vascular age, glycation age) were younger than the true age.

Urinalysis results

Urine sampling records and measurements of urinary SaMT and urinary creatinine are shown in ***Table 2*** and ***Fig. 1***.

Correlations with measurements of the various parameters are shown in ***Table 3***. A significant correlation with SaMT excretion volume was found with hormonal age ($r = 0.479$, $p = 0.028$), hormonal age gap ($r = 0.533$, $p = 0.013$), and alcohol consumption ($r = 0.600$, $p = 0.004$), for the entire study population (both sexes together); with hormonal age ($r = 0.652$, $p = 0.030$) and alcohol consumption ($r = 0.789$, $p = 0.004$) for males; and with vascular age gap ($r = 0.769$, $p = 0.009$) for females. The corrected SaMT concentration was not correlated with any parameter in any case.

Subjects were divided according to SaMT excretion volume, which was found to be correlated with some parameters, into two groups: those with a high SaMT excretion volume and those with a low SaMT excretion volume. Univariate logistic regression analysis of data from each group revealed no significant association with any of the various functional ages or blood test parameters. Significant trends were found for hormonal age ($p = 0.058$) and IGF-I ($p = 0.058$). Even after adjustments for hormonal age, vascular age, dehydroepiandrosterone -sulfate (DHEA-s),

Table 1. Results of body functional age and lifestyle behavior.

	Total			Male			Female		
	mean		SD	mean		SD	mean		SD
Age (years)	73.2	±	6.5	74.6	±	4.8	71.5	±	8.1
Myological age (years)	56.1	±	4.2	57.1	±	2.5	51.1	±	21.0
Skeletal age (years)	69.1	±	14.8	69.6	±	16.2	54.9	±	5.6
Hormonal age (years)	78.8	±	6.9	77.4	±	7.8	68.6	±	13.6
Neurological age (years)	69.4	±	16.4	73.0	±	15.4	80.7	±	5.1
Vascular age (years)	65.4	±	9.4	63.6	±	10.3	65.0	±	17.3
Glycation age (years)	59.5	±	18.4	66.0	±	13.6	67.5	±	8.2
Number of cigarettes (/day)	0.0	±	0.0	0.0	±	0.0	0.0	±	0.0
Alcohol consumption (Gou*/day)	0.9	±	1.5	1.4	±	1.7	0.3	±	0.6
Drinking frequency (day/week)	3.5	±	3.2	5.0	±	2.8	1.6	±	2.7
Exercise (day/week)	3.2	±	2.5	4.3	±	2.7	1.9	±	1.6
Sleep hours (hours)	7.0	±	0.3	7.1	±	1.6	6.9	±	0.7

SD, standard deviation; Glycation age = (AF−1.112) / 0.0175 ; AF, auto fluorescence measured by AGE Reader ; *Gou = 180 mL of Sake equivalent (Sake is Japanese rice wine containing 15-18% ethanol).

Table 2. Urine melatonin metabolite.

	Total			Male			Female		
	mean		SD	mean		SD	mean		SD
Urine volume (ml)	306.5	±	105.3	279.2	±	82.1	342.0	±	125.1
Urine deposit time (min)	317.6	±	128.7	263.8	±	120.9	382.1	±	111.0
Urine SaMT concentration (ng/ml)	24.6	±	21.1	26.0	±	21.6	22.7	±	21.5
Urine creatinine concentration (mg/dL)	100.1	±	62.5	115.3	±	65.1	80.3	±	56.0
Corrected SaMT (ng/mg Creatinine)	23.1	±	14.5	24.1	±	20.2	26.7	±	13.9
Urine discharged SaMT (μg/h)	1.0	±	0.6	1.1	±	0.7	1.0	±	0.6

SaMT, 6-hydroxy-melatonin sulfate; SD, standard deviation.

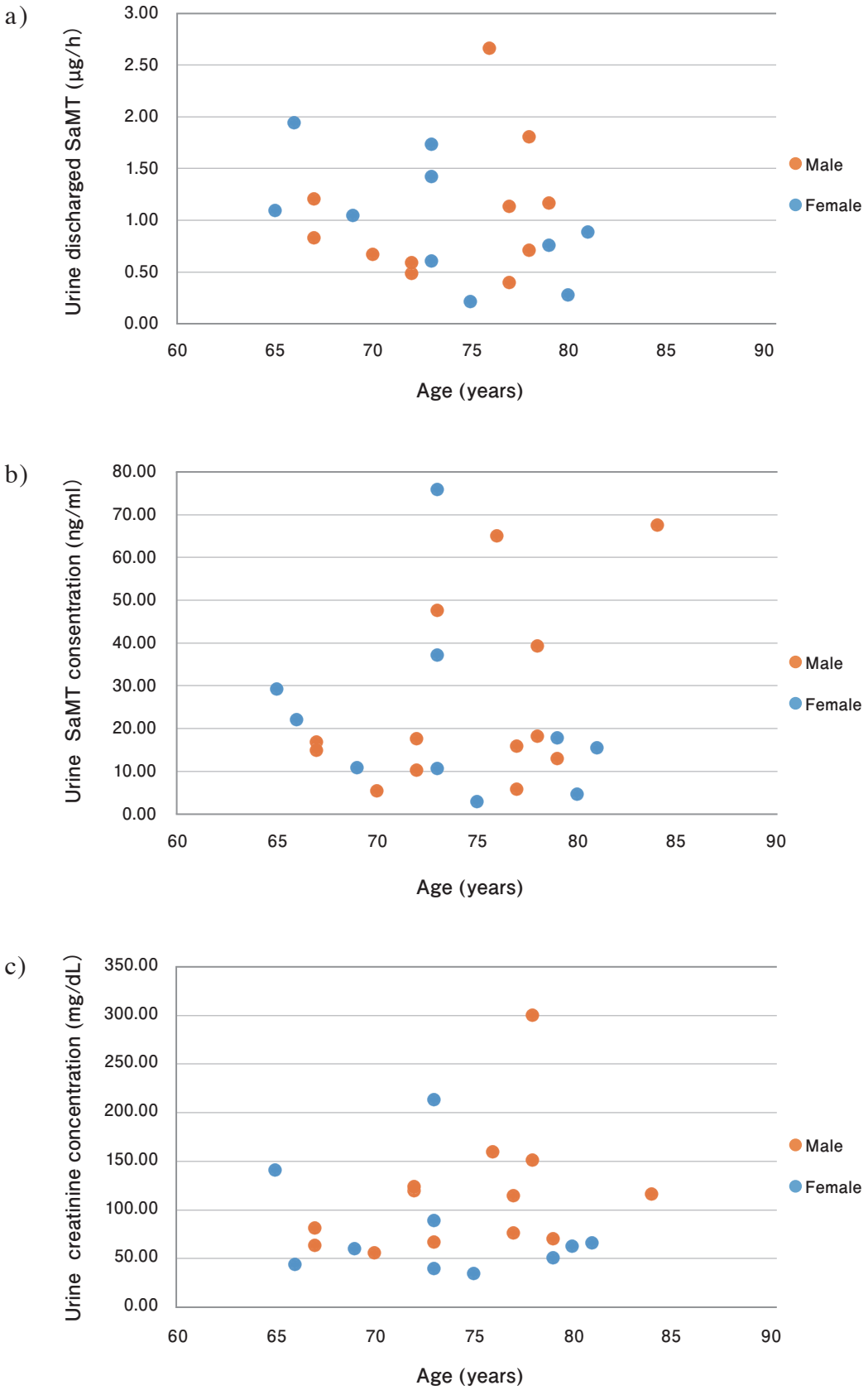

Fig. 1. **Scatter plots of urine SaMT and creatinine data.**

a) Urine discharged SaMT, b) Urine SaMT concentration and c) Urine creatinine concentration. SaMT, 6-hydroxy-melatonin sulfate, n = 25.

Table 3. Results of correlation analysis.

	Total		Male		Female	
	Coefficient (R)	p value	Coefficient (R)	p value	Coefficient (R)	p value
Myological age (years)	-0.149	0.730	0.273	0.416	-0.531	0.114
Skeletal age (years)	-0.162	0.520	-0.194	0.568	-0.106	0.771
Hormonal age (years)	0.479	0.028	0.652	0.030	0.235	0.514
Neurological age (years)	0.000	0.999	-0.196	0.563	0.229	0.524
Vascular age (years)	-0.028	0.905	-0.265	0.431	0.478	0.162
Glycation age (years)	0.080	0.730	0.256	0.447	-0.097	0.789
Myological age gap (years)	0.131	0.572	0.187	0.472	0.615	0.058
Skeletal age gap (years)	-0.122	0.598	-0.243	0.398	0.159	0.661
Hormonal age gap (years)	0.533	0.013	-0.284	0.079	0.624	0.054
Neurological age gap (years)	0.046	0.844	0.550	0.428	0.422	0.225
Vascular age gap (years)	0.041	0.859	-0.267	0.328	0.769	0.009
Glycation age gap (years)	0.124	0.592	-0.326	0.581	0.063	0.862
IGF-1 (ng/mL)	-0.420	0.065	-0.584	0.059	-0.200	0.606
DHEA-s (μg/dL)	-0.097	0.069	-0.338	0.309	0.426	0.253
Number of cigarettes (/day)	—	—	—	—	—	—
Alcohol consumption (Gou*/day)	0.600	0.004	0.789	0.004	0.237	0.509
Drinking frequency (day/week)	0.001	0.995	-0.374	0.258	0.482	0.158
Exercise (day/week)	0.259	0.257	0.246	0.466	0.333	0.348
Sleep hours (hours)	-0.160	0.489	-0.249	0.460	0.095	0.794

by correlation analysis

IGF-1, insulin-like growth factor-1; DHEA-s, dehydroepiandrosterone-sulfate; gap=functional age minus chronological age; Glycation age = (AF－1.112) / 0.0175 ; AF, auto fluorescence measured by AGE Reader; *Gou = 180 mL of Sake equivalent (Sake is Japanese rice wine containing 15-18% ethanol).

insulin-like growth factor-1 (IGF-1), and alcohol consumption, which had shown simple correlations, multivariate analysis showed no significant associations.

Discussion

Our body has a biological clock, by which energy metabolism is controlled. Disruption of the biological clock can trigger a broad range of diseases, including lifestyle-related diseases. While the basic rhythm of the biological clock is known to occur in approximate 25-hr cycles, it is adjusted to the earth's rotation rhythm, *i.e.*, 24 hr, in the presence of brightness/darkness information and day/night information generated through rhythm melatonin secretion, as well as sleep-wake information generated through growth hormone secretion. In modern society, where people can act around the clock, and tend to have rhythm derangements, the roles of melatonin as a transmitter of day/night information and brightness/darkness information, are expected to become increasingly important in the future [5, 18].

Melatonin has major effects on sleep. Quantitative reduction and qualitative deterioration of sleep cause various health issues, including brain and mental functional disorders and lifestyle-related diseases, as well as increased mortality rates [19, 20]. In reproductive medicine settings, lack of sleep, qualitative deterioration of sleep, and lack of melatonin secretion have been shown to reduce ovarian function, resulting in decreased success rates for infertility treatment [21]. Sleep time of less than 6 hr increases the risks of obesity and metabolic syndrome, and diabetes mellitus and hypertension, eventually leading to increases in total mortalities and cardiovascular diseases [19]. A lack of sleep (shortened sleep time) raises 24 hr blood pressure, causing nocturnal hypertension and abnormalities in nocturnal blood pressure non-dipper type infra-day blood pressure fluctuation in depressive states and elderly sleep disorders [22]. In sleep apnea syndrome (SAS), the risk of night onset of cardiovascular disease is increased 2 to 3 fold; in SAS patients with nocturnal hypertension, the risk increases synergistically [19]. In hypertensive patients, bedtime administration of melatonin or a melatonin agonist seems to lower 24 hr blood pressure, especially nocturnal blood pressure, and the restoration of the normal circadian rhythm is expected to lessen these risks.

Simple correlation analysis revealed a positive correlation between SaMT excretion volume and hormonal age. Hormonal age is determined on the basis of growth hormone and DHEA-s. People with high levels of melatonin secretion are expected to have sleep of high quality, much growth hormone secretion, and high IGF-1 levels; however, the reverse results were obtained in the present study. Generally, serum IGF-1 decreased with aging; in people with increased insulin resistance, compensatory increases in IGF-1 are observed. This may account for the negative associations found with melatonin secretion and IGF-1. Since serum DHEA-s decreases with aging [23], this parameter has been thought to be correlated with melatonin secretion, which also decreases with aging. Hormonal age is an indicator established in view of both IGF-1 and DHEA-s, and in the present study, it may have been influenced mainly by IGF-1.

Logistic analyses revealed tendencies for statistically insignificant but somewhat strong negative correlations with IGF-I in the entire study population and in the male group. DHEA-s levels were highly variable in the entire study population, the male group, and the female group, and there seemed to be little association. Further investigation will be necessary with an increased sample size.

A positive correlation was found between alcohol consumption and SaMT excretion. The subjects did not drink in excess. These results are attributable to reasonable amount of alcohol intake, not immediately before bedtime, which seems to have helped stress relief and sleep induction.

No correlation was found between SaMT and sleep time. This is attributable to the fact that the data used in the study involved a time lag of about 1 month from subjective evaluation of living activities to urine sampling. In addition to sleep time, melatonin secretion is largely involved by bed time and daytime light exposure; therefore, it is recommended that such background factors should also be investigated in the future.

While melatonin is profoundly associated with sleep and other lifestyle factors, other factors, including exercise, are considered to make large contributions to functional ages. Although associations between melatonin and lifestyle-related diseases have been demonstrated in animal studies, they have not always yielded results consistent with those in human studies.

Melatonin suppresses increases in blood pressure and heart beats, and reduces urinary adrenalin in spontaneously hypertensive rats [24]. Obayashi *et al.* [22] studied 114 hypertensive elderly subjects (≥60 years) for the association between urinary melatonin excretion volume (an indicator of endogenous melatonin) and nocturnal blood pressure change pattern. They revealed that the mean nocturnal percentage reduction of systolic blood pressure (adjusted for age, diabetes mellitus, and daytime activity volume) was significantly higher in the high urinary melatonin excretion group than in the low urinary melatonin excretion group. Hence, melatonin secretion acts to reduce nighttime blood pressure in hypertensive elderly subjects. However, a comparison of urinary melatonin metabolite and noradrenalin levels in night shift nurses and day shift nurses showed that the relationship between the two parameters was variable on duty and at rest, with no significant correlation found [15].

In an animal study, the body weight loss, adrenal/thymus atrophy, and increased total cholesterol produced with antiglucocorticoid administration in mature rats were mitigated by administration of melatonin; antiglucocorticoid effects were observed [25]. In healthy humans, corticosteroid secretion capacity did not change significantly after administration of melatonin [26].

In rats fed a high cholesterol diet, increased serum lipid and lipoprotein levels and fatty infiltration of the liver are partially mitigated by the administration of melatonin. In this case, lipase activity is not influenced [27]. Melatonin acts on rat pancreatic β cells to promote insulin synthesis and secretion [28]. In a rat model of non-insulin-dependent diabetic mellitus (Otsuka Long-Evans Tokushima Fatty rats / OLETF rats), prolonged treatment with melatonin decreased plasma triglyceride and total cholesterol by 39% and 27%, respectively, and improved both the unsaturated polyfatty acid content and the 20:3n-6/20:4n-6 ratio to the extent of nearly a normal fatty acid composition [29]. Melatonin deficiency due to pinealectomy is increased by insulin

resistance in OLETF rats[30]. The above results led to the expectation of amelioration of hyperlipidemia and the recovery from normal insulin resistance with prolonged treatment with melatonin; however, no such findings were obtained with the sample size in the present study.

Conclusion

People with high levels of melatonin secretion are expected to have sleep of high quality, elevated growth hormone secretion, and high IGF-1 levels; however, the reverse results were obtained in the present study. In addition, reasonable amount of alcohol intake seemed to help stress relief and sleep induction, allowing SaMT excretion to increase. In the present study, logistic analysis did not reveal a definite association between melatonin secretion as indicated by urinary SaMT excretion volume and physical data, including body functional age, further investigation will be necessary with an increased sample size.

Acknowledgements

This work was supported by the Japanese Council for Science, Technology and Innovation, SIP (Project ID 14533567), "Technologies for creating next-generation agriculture, forestry and fisheries" (funding agency: Bio-oriented Technology Research Advancement Institution, NARO) and JSPS KAKENHI Grant Number 26350917.

Conflict of Interest Statement

The authors state that the performance of this study entailed no issues representing a conflict of interest.

References

1) Ichihashi M, Yagi M, Nomoto K, et al. Glycation stress and photo-aging in skin. Anti-Aging Medicine. 2011; 8: 23-29.
2) Nagai R, Jinno M, Ichihashi M, et al. Advanced glycation end products and their receptors as risk factors for aging. Anti-Aging Medicine. 2012; 9: 108-113.
3) Nomoto K, Yagi M, Arita S, et al. Skin accumulation of advanced glycation end products and lifestyle behaviors in Japanese. Anti-Aging Medicine. 2012; 9: 165-173.
4) Nomoto K, Yagi M, Arita S, et al. A survey of fluorescence derived from advanced glycation end products in the skin of Japanese: Differences with age and measurement location. Anti-Aging Medicine. 2012; 9: 119-124.
5) Yonei Y, Hattori A, Tsutsui K, et al. Effects of melatonin: Basics studies and clinical applications. Anti-Aging Medicine. 2010; 7: 85-91.
6) Miyazaki R, Ishi K, Ichikawa H, et al. Community Medicine and Anti-Aging: Effects of combining a long-term pedometer-based physical activity program with anti-aging medical checkups on health and anti-aging medical indicators in community-dwelling older adults (Yurin study 1). Anti-Aging Medicine 2010; 7: 143-152.
7) Miyazaki R, Ayabe M, Ishii K, et al. Longitudinal association between the daily step count and the functional age in older adults participating in a 2.5-year pedometer-based walking program: The YURIN Study. Anti-Aging Medicine. 2013; 10: 60-69.
8) Miyazaki R, Kotani K, Tsuzaki K, et al. Effects of a year-long pedometer-based walking program on cardiovascular disease risk factors in active older people. Asia Pac J Public Health. 2015; 27: 155-163.
9) Yonei Y, Mizuno Y. The human dock of tomorrow: Annual health checkup for anti-aging. Ningen Dock. 2005; 19: 5-8.
10) Yonei Y, Takabe W. Aging assessment by anti-aging medical checkup. Health Evaluation and Promotion. 2015; 42: 459-464.
11) Yonei Y, Miwa Y, Hibino S, et al. Japanese anthropometric reference data: special emphasis on bioelectrical impedance analysis of muscle mass. Anti-Aging Medicine. 2008; 5: 63-72.
12) Ito H. Supporting system for anti-aging medical checkups, "Age Management Check R". Modern Physician 26: 605-608, 2006. (in Japanese)
13) Mahlberg R, Tilmann A, Salewski L, et al. Normative data on the daily profile of urinary 6-sulfatoxymelatonin in healthy subjects between the ages of 20 and 84. Psychoneuroendocrinology. 2006; 31: 634-641.
14) Attachment. Melatonin-Sulfate Urine ELISA (6-Hydroxymelatonin Sulphate, 6-Sulfatoxymelatonin), IBL International GmbH, Hamburg, Germany. http://www.ibl-international.com/en/6-hydroxymelatonin-sulphate-melatoninsulfat-elisa, accessed at December 4th, 2015.
15) Yamauchi H, Iwamoto M, Harada N. Effects of night work on urinary excretion rates of 6-sulfatoxymelatonin and norepinephrine in hospital nurses. International Medical Journal. 2003; 10: 185-190,
16) Roach GD, Lamond N, Dorrian J, et al. Sleep Health at Work: Changes in the concentration of urinary 6-sulphatoxymelatonin during a week of simulated night work. Ind Health. 2005; 43: 193-196.
17) Kondo M, Tokura H, Wakamura T, et al. Physiological significance of cyclic changes in room temperature around dusk and dawn for circadian rhythms of co re and skin temperature, urinary 6-hydroxymelatonin sulfate, and waking sensation just after rising. J Physiol Anthropol. 2007; 26: 429-436.
18) Hattori A. Melatonin and melatonin receptor agonists. The Medical Frontline (Saishin Igaku). 2014; 69: 1032-1039. (in Japanese)
19) Kario K. Sleep and lifestyle-related diseases. The Journal of Adult Diseases. 2012; 42: 576-583. (in Japanese)

20) Miyazaki S, Kitamura T. Antiaging from the viewpoint of brain and sleep. Japanese Journal of Geriatric Psychiatry. 2015; 26: 615-623. (in Japanese)
21) Jinno M, Tamura H, Yonei Y. Anti-aging medicine and reproductive health. Anti-Aging Medicine. 2012; 9: 6-13.
22) Obayashi K, Saeki K, Iwamoto J, et al. Nocturnal urinary melatonin excretion is associated with non-dipper pattern in elderly hypertensives. Hypertens Res. 2013; 36: 736-740.
23) Nomoto K, Arita S, Yonei Y. Development of a model of functional endocrine age in Japanese people: Serum dehydroepiandrosterone-sulfate (DHEA-s) concentration as an index of aging. Anti-Aging Medicine. 2011; 8: 69-74.
24) Fujii M, Akamine Y, Kuroda K, et al. Effect of melatonin on the pathogenesis of hypertension in the Spontaneous Hypertensive Rats (SHR). Japanese Journal of Applied Physiology. 1993; 23: 525-532. (in Japanese)
25) Aoyama H, Mori N, Mori W. Anti-glucocorticoid effects of melatonin on adult rats. Acta Pathol Jpn. 1987; 37: 1143-1148.
26) Ito Y, Oiwake H, Takeda R, et al. Adrenocortical hormone secretory regulation action of melatonin and its action on arteriosclerosis. Proceedings of Daiwa Securities Health Foundation. 1999; 22: 120-127. (in Japanese)
27) Mori N, Aoyama H, Murase T, et al. Anti-hypercholesterolemic effect of melatonin in rats. Acta Pathol Jpn. 1989; 39: 613-618.
28) Iizuka Y. Effect of melatonin on serum glucose and insulin levels in rats. Medicine and Biology. 1996; 133: 65-67. (in Japanese)
29) Nishida S, Segawa T, Murai I, et al. Influence of long term melatonin administration on the lipid metabolism in the non-insulin dependent diabetes mellitus model rats. Proceedings of the Japanese Conference on the Biochemistry of Lipids. 2000; 42: 220-223. (in Japanese).
30) Segawa T, Murai I, Matsumura M, et al. Effects of pinealectomy on insulin resistance in Otsuka Long-Evans Tokushima Fatty rats: Physiological role of melatonin in the pathogenesis of type 2 diabetes. The Nihon University Journal of Medicine. 2003; 45: 125-136.