博士論文

Epidemiological study on the relationship between PTSD symptoms and sleep habits, meal habits, and mental health of people who suffered natural disasters

（大規模自然災害被災者の心的外傷後ストレス障害、睡眠健康、食習慣、精神衛生についての疫学的研究）

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General Introduction

General Introduction

This PhD thesis consists of four steps as follows.

The 1st step: 1-1: Questionnaire study to understand the current situation of the circadian typology of Japanese children in comparison with Czech children
1-2: Intervention study to test the Tryptophan-Serotonin-Melatonin Scheme.

The 2nd step: Epidemiological study on the relationship between the circadian typology and PTSD symptoms on the young participants who suffered the Great Hanshin-Awaji Earthquake in their childhood.

The 3rd step: Intervention study on such young participants who suffered the Great Hanshin-Awaji Earthquake in their childhood using a leaflet on "Go to bed early, Get up early and Do not forget to have a breakfast".

The 4th step: Epidemiological study on the relationship between PTSD symptoms and circadian typology in the people who experienced the landslide disaster which occurred 6 years ago (on 29th November 2006).

Diversification of lifestyles and also evening-typed life in advance has created problems in the basic living habits of children. This may contribute to a variety of adverse effects, such as a decrease in motivation for learning, a decrease in physical and mental energy, and possibly even an increase in several health disorders. Shifting to evening-typed life for young children might be being accelerated, especially due to the ongoing 24-hour commercialization of society [1]. Especially in Japan, environmental factors such as mobile phones [2], convenience stores open 24 hours [3][4], midnight broadcasting programs [5], watching TV program before sleep [6], and playing computer game [7], seem to function as zeitgebers of circadian oscillators and increase the tendency for Japanese students to be evening-typed. In addition, habitual playing of video games reduces sleep health regardless of the countries where they are living [6, 8-13].

Two oscillators hypothesis driving human diurnal rhythms is general in chronobiological field: one is the main clock having period of a little bit longer than 24 hours and another is the second or "slave" clock which is driving sleep-wake cycles having of 33 to 40 hours period [14]. Persistent lower amplitudes of several zeitgebers may promote an inner-desynchronized protocol between the main clock, which drives the autonomous nervous system, and the slave clock, which controls the sleep-wake cycle (Double-oscillations theory [15]). As a result, 24-hour commercialization society may reduce the amplitudes of environmental daily cycles of light, social activities, and food intake. Ishihara (2001) [16] reported that the sleep duration of Japanese children shortened one hour over the thirty years of 1970-2000. Shifting to an evening-type life for young children are progressing [17] and there is no sign to stop this current.

Shinomiya et al. (2004) [18] reported that Italian children were significantly morning-typed than Japanese children. Another previous research has shown that diurnal rhythms and sleep health in early childhood may be highly sensitive to the living environment including light and regularity and timing of meals for Japanese children [19]. However, amount of data might be not sufficient on current circadian typology of Japanese children. As the former half of the 1st step of this thesis, questionnaire study was performed to understand the current situation of the circadian typology of Japanese children in comparison with Czech children.

Tryptophan is a nutritionally essential amino acid that can be absorbed exclusively from meals in humans. It is metabolized via 5-hydroxytryptamine (serotonin) to melatonin by a series of 4 enzymes in the pineal body [20, 21]. Vitamin B6 functions as coenzyme of the enzyme to modify 5-hydrooxytryptophan to serotonin [20, 21]. So intake of the Vitamin B6 at breakfast is indirectly related to serotonin synthesis.

Serotonin is known as a precursor to melatonin. A lack of serotonin causes depression, panic disorder, obsessive compulsive disorder, sleep disorders and eating disorders [22] and induces aggression, anxiety/aggression-driven depression, impulsive behavior and suicidal attempts [23, 24]. Serotonin thus has a strong relationship with mental health. In the past two decades, serotonin reuptake inhibitors (SSRIs) have come to be widely used for the treatment of affective disorders including depression, although there are controversies on whether SSRIs are effective or not for the treatment of depression in children and adolescents because of the shortage of coincident scientific evidence of SSRIs for young humans [25].

Exposure to sunlight in the daytime appears to trigger synthesis of serotonin in the pineal body [26]. This action is hypothesized to occur mainly in the morning hours, because the amount of tryptophan consumed with supper has neither significant effects on Morningness-Eveningness (M-E) scores nor an effect on sleep habits, as shown by another study on young Japanese children performed in 2005 [27].

Melatonin is synthesized in the pineal body of the hypothalamic area and secreted at night. Melatonin level in the serum can be well and positively correlated with that in the saliva [28-31]. Secretion of melatonin exhibits circadian rhythms and is suppressed by bright light at night [32, 33]. Even room lights such as fluorescent lamps can attenuate melatonin excretion duration at night [34]. Evening lighting conditions are also said to affect circadian rhythms [35, 36] and mental health in mice [37]. Tryptophan intake at breakfast is effective for the onset and offset of sleep in young children [38]. Moreover, questionnaire surveys showed that young children exposed to sunlight for more than 30 minutes after having sources of protein at breakfast are more morning-typed than those exposed for less than 30 minutes [39], and that the more amount of vitamin B6 young children take at breakfast, the more they are morning-typed [40].

These findings imply that morning tryptophan and vitamin B6 intake and following exposure to sunlight would promote synthesis of serotonin in the daytime and further that of melatonin at night. However, it is difficult to test the hypothesis only with questionnaire studies. To test this hypothesis, an intervention field study would be effective. Therefore, an intervention field experiment was performed on university students to test the hypotheses as the latter half of the 1st step of this thesis.

Sleep disturbance is one of 4 factors indicating PTSD symptoms as assessed by the Impact Event Scale-Revised (IES-R) and the main symptom exhibited in PTSD [41-43]. In the former domain, symptoms related to sleep were characterized by intrusion of traumatic memories or other threatening themes into dreams. Insomnia and nightmares, an extreme manifestation of this problem, have even been referred to as the "hallmark" of PTSD [44]. On the other hand, the study to assess the quality of sleep and its architecture in injured victims of traffic accidents one year after the accident reported that the main problem in PTSD is sleep misperception rather than actual sleep alteration [45].

Posttraumatic stress disorder (PTSD) is the most frequently reported psychiatric morbidity among victims of natural disasters [46]. Mental health problems of natural disaster survivors have been reviewed by many studies [47-53]. A study interviewed to the parents whose infants suffered the 1995 Great Hanshin – Awaji Earthquake in Japan five months after the earthquake [54] reported the five points as follows. 1: The severer they've been devastated, the easier the PTSD symptoms appeared and lasted long; 2: Lifestyle stability alleviates psychological damage caused by the disaster; 3: The infants whose parents with anxiety or PTSD symptoms developed their PTSD symptoms significantly; 4: There is no significant correlation between age, sex, and the appearance of the symptoms; 5: A lot of infants as victims complained sleep disorders and fear to earthquake origin and showed regression. It tended to become prolonged.

Hayano (2005) [55] found that daily stress disturbed rest during sleep, induced psychiatric disorders, and accumulated cardiac and/or blood vessel fatigue. Loss of family members or close friends, basis for human life, and security feeling were the most stressful things. A natural disaster caused victims to have PTSD or adjustment disorders and increased a risk for cerebral apoplexy, cardiac infarct, and so on.

However, there have been few studies on the relationship between PTSD symptoms and circadian typology. Previous study aims to determine the relationship between Post Traumatic Stress Disorder (PTSD) and current circadian typology and sleep habits of adults who experienced the Great Hanshin-Awaji Earthquake (on 17th January 1995) after becoming adults [56]. The study reported that people who suffered severe damage from a disaster and who currently show severe PTSD symptoms are more evening-typed and have a lower quality of sleep. However, whether PTSD symptoms of young people who suffered the Hanshin-Awaji earthquake in childhood could be reduced by the morning-typed life has been remained to be studied. So an epidemiological study was performed to examine this relationship as the 2nd step.

Kuroda et al. [56], which was the first study to indicate a positive correlation between evening-typed lifestyle and PTSD symptoms (morning-type lifestyle and reduced PTSD symptoms), mentioned that the limitation of this study is that the questionnaire study cannot say the causal relationship between morning-typology and reduced PTSD. And they discussed that intervention to improve their quality of sleep and promote a morning-typed lifestyle may be an effective way to reduce PTSD symptoms. An intervention study on young participants who suffered the Great Hanshin-Awaji earthquake in childhood using a leaflet on “Go to bed early, Get up early and Do not forget to have a breakfast” and a sleep diary was done as the 3rd step.

A landslide caused by the torrential rain attacked Albay, Philippines on November 29, 2006. The mortality was about 1000 included missing people [57]. A lot of people lost their house and lands.

An epidemiological study was performed on a total of 271 adolescents who had been evacuated from their homes 3 months after Typhoon Morakot in Taiwan [58]. The prevalence of PTSD related to the typhoon was 25.8% and adolescents with PTSD had more severe depression and internalizing, externalizing, social, thought and attention problems than those without PTSD [58]. Even 4 years after the Parnitha earthquake in Greece, 22% of survivors reported subjective distress and intense fear during the earthquake, and the participation in rescue operations positively correlated with greater post-earthquake psychological stress [59]. The psychological consequence of earthquakes may be serious and long-lasting even when the magnitude of the earthquake is moderate [59]. At 0.5 and 3 years after a severe earthquake (7.3 on the Richter scale) in Taiwan, the estimated rate of victims with posttraumatic stress symptoms (PTSS) was 23.8% and 4.4%, respectively, and PTSS scores were tightly correlated with QOL scores (showing less severe symptoms with higher QOL) [60]. Qu et al. (2012)[61] reported that 8 months after the Sichuan earthquake, the prevalence of PTSD symptoms was 19.9%, and the prevalence of depression was 29.0% and that earthquake experience had significant correlation with PTSD and depression [61]. As just described, a great disparity exists in the reported rates of PTSD symptoms across different studies and the number of such studies has been limited.

The 4th step of this study aims, first, to estimate the rate of PTSD symptoms among survivors after 6 years from the landslide and, second, to explore what factors had strong linkage with severe PTSD, from an epidemiological view point.

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The 1st Step-1

Please show this original publication as the 1st step-1.

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SHORT PAPER

Comparative study on circadian typology and sleep habits of Japanese and Czech infants aged 0–8 years

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Abstract

Average bedtime and sleep duration of Japanese infants aged 0–8 years were later by 1.3 h and shorter by 1 h, respectively, than those of Czech infants. Japanese infants were predominantly more evening-type than Czech infants, while Japanese parents (mostly mothers) were significantly more morning-type than Czech mothers. Correlation value (r-value) between the morningness-eveningness (M-E) scores of infants and mothers was 0.405 in Japanese participants, whereas it was relatively low (0.297) in Czech ones. Czech infants were reported as being depressed more frequently than Japanese ones who were reported as being more frequently angry than the Czech infants.

Key words: bedtime, Czech Republic, infants and mothers, Japan, morningness-eveningness scores.

INTRODUCTION

A survey on young children's health in 2000 reported that the percentage of Japanese children going to bed at 22.00 hours or later is approximately 50% of 3 year olds.[1] The ratio of evening-type infants showing a score of less than 14 points in the morningness-eveningness questionnaire (MEQ) constructed by Torsvall and Åkerstedt[2] increased between 2003–2007 in Kochi (T. Harada *et al.*, unpublished data, 2007). Such evening-type infants can be produced due to several factors related to the 24 h-society; for example, direct effects such as watching TV and playing games,[3] and indirect effects such as mothers[4] going to stores open in the evening and night and using mobile phones, can be on the circadian typology of infants. To evaluate, relatively, the circadian typology and sleep habits of infants living in Japan, which is an extremely evening-type society (kindergarten starts at 08.30–09.30 hours), this study compared the two characteristics shown by Japanese infants aged 0–6 years with infants living in the Czech republic (0–8 years) which is an extremely morning-type society (kindergarten starts at 07.30–08.30 hours in contrast).

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METHODS

Japanese and Czech versions of the MEQ originally constructed by Torsvall and Åkerstedt, and an original questionnaire on sleep habits[5] and mental health, were completed in June or November 2007 to avoid the severe summer and winter seasons. The original questionnaire on sleep habits included questions such as, "How frequently does your child get angry in usual life because of only a little trigger?" and "How frequently does your child have depressed mood in usual life?", with the possible responses being (1) often, (2) sometimes, (3) rarely, and (4) not at all.

Respondents included 697 (360 girls, 337 boys) infants attending one of nine kindergartens governed by Kochi City in Kochi Prefecture in Japan (33°N) and 627 (305 girls, 322 boys) infants attending kindergartens drawn from all over the Czech Republic (49–51°N) (mainly South Bohemia). The questionnaire was distributed to all or a number of infants attending the randomly selected kindergartens, and responses were received for 70% and 86% of Japanese and Czech infants, respectively. Parents (95% or more mothers) answered the questionnaire for themselves and their infants. Data were statistically analyzed with the use of SPSS software (version 12.0 J for Windows; SPSS, Chicago, IL, USA). Because M-E distributions were not normal (Shapiro–Wilk test, Japanese infants: statistic value = 0.975, $P < 0.001$; Japanese parents: statistic value = 0.980, $P < 0.001$), non-parametric analysis was adopted for the comparison of M-E scores.

The first part was a Japanese version of the MEQ Torsvall and Åkerstedt[2] originally constructed for students.[5] The MEQ is used to measure an individual's diurnal preference. Three of the seven questions included in the MEQ pertain to sleep onset timing in the evening, three to sleep offset timing in the morning, and one to peak timing of activity during the daytime. Each question allows for choice (scored from 1 to 4). The M-E score is the sum of the seven answers. The minimum possible score is 7 (extreme evening-type), and the maximum possible score is 28 (extreme evening-type). The questionnaire currently used most widely was constructed by Horne and Östberg[6] which was lengthened to include 19 items. Correlations have been examined in M-E scores in the two versions: one by Torsvall and Åkerstedt,[2] the other by Horne and Östberg.[6] High correlation values were seen in junior high students aged 11–15 years (K. Ishihara, unpublished communication, 2000; $r = 0.673$–0.762; Pearson correlation test: $P < 0.001$), and for 18–25-year-old students in occupational and physical therapy training school (T. Harada, unpublished data, 2000; $r = 0.736$; Pearson correlation test: $P < 0.001$), whereas there was no such validation data for the Czech version of the questionnaire. However, there was significant negative correlation between ME scores and time gaps in wake-up time and bedtime between weekdays and holidays in both the Japan and Czech versions. Therefore, the Czech and Japanese versions of the Torsvall and Åkerstedt questionnaire are validated.

The study followed the guidelines established by the Journal, Chronobiology International, for the conduct of research on human subjects.[7] We carefully explained the concepts and purpose of the study orally and in writing to kindergarten teachers or nurses and to the parents of the infants, emphasizing that questionnaires were completely unregistered and that answers were only to be used for academic purposes. After the explanation, all parents agreed to participate in the research study survey, which was administered at home.

RESULTS

Czech infants aged 0–8 years who were all kindergarten attendants were more morning-type than Japanese infants, while Czech parents were more evening-type than Japanese mothers (Fig. 1).

Morningness-eveningness (M-E) scores became lower ($P < 0.001$) (more evening-type) according to age both in Japanese and Czech infants, while Japanese infants were more evening-type than Czech ones between the ages of 2 to 6 years (Table 1). Both age and being Japanese/Czech significantly and independently correlated with M-E scores (Two-way ANOVA, effect of age: $df = 1$, $F = 108.3$, $P < 0.001$; effect of being Japanese/Czech: $df = 1$, $F = 161.3$, $P < 0.001$). Means and SD for weekday bedtimes of Japanese and Czech infants were 21.5 ± 0.9 ($n = 690$) and 20.1 ± 0.6 ($n = 627$), respectively (Mann–Whitney U-test, $P < 0.001$). Means and SD for weekday wake-up times were 7.0 ± 0.6 ($n = 627$) for Czech infants and 6.7 ± 0.3 ($n = 630$) for Japanese ones (Mann–Whitney U-test, $P < 0.001$).

The mean (± SD) sleep hours of Japanese infants was 9.5 h (± 0.8) ($n = 689$) on weekdays, significantly shorter than for Czech infants (10.6 ± 0.7 [$n = 624$]) (Mann–Whitney U-test, $P < 0.001$).

Morningness-eveningness (M-E) scores of Czech infants were much higher than those of their parents (Mann–Whitney U-test, $z = -12.33$, $P < 0.001$), whereas those of Japanese infants were relatively similar to those of their parents (Mann–Whitney U-test, $z = -2.09$, $P = 0.03$). The correlation value (r-value) between the M-E scores of infants and mothers was 0.405 in Japanese participants, whereas it was relatively low (0.297) in Czech participants. The average bedtime and sleep duration of Japanese infants was later by 1.3 h and shorter by 1 h, respectively, than those of Czech infants.

The average score (and SD) of "anger" was 2.91 (± 0.88) for Japanese infants, which was significantly lower (more frequently angry) than 3.28 (± 0.74) for Czech infants (% "often angry": Japan 9.3, Czech republic 1.3;

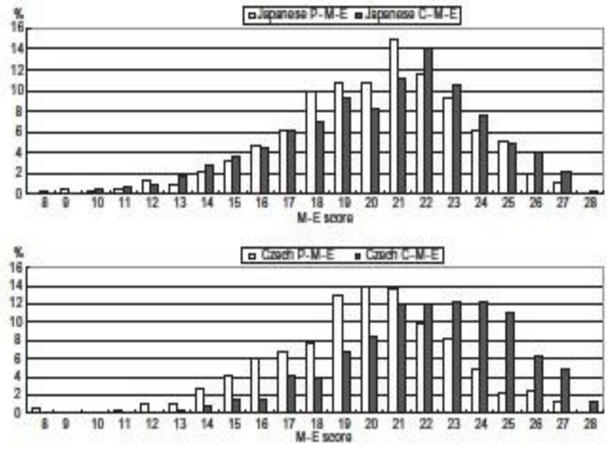

Figure 1 Distributions of morningness-eveningness (M-E) scores shown by Japanese (upper panel) and Czech (lower panel) infants (black bars) and their parents (white bars) (Mean ± SD [n]: Japanese infants, 20.4 ± 3.6 [626]; Japanese parents, 20.0 ± 3.2 [682]; Czech infants, 22.1 ± 3.1 [626]; Czech parents, 19.6 ± 3.3 [604]).

χ^2-test, χ^2-value = 80.09, df = 3, $P < 0.001$). The average score (and SD) of "depressed mood" was 3.58 (± 0.66) for Japanese infants, which was significantly higher (less frequently depressed) than 2.31 (± 0.79) for Czech infants (% "often depressed": Japan 0.7, Czech 12.6; χ^2-test, χ^2-value = 593.92, df = 3, $P < 0.001$). Infants who felt "often angry" were significantly more evening-typed than those who felt angry less frequently in both countries (Kruskal–Wallis test, Czech republic: χ^2-value = 13.02, df = 3, $P = 0.005$; Japan: χ^2-value = 12.87, df = 3, $P = 0.005$). There was no such relationship between the frequency of feeling depressed and M-E scores.

DISCUSSION

There are two possibilities as to why Czech infants are more morning-type than Japanese ones. First, very severe bedtime discipline performed in general in the Czech Republic seems to play an important role in the extreme early bedtime and morning-type circadian typology of Czech infants. Such strong discipline, which links to the independency of infants' diurnal rhythm to that of the parents (Fig. 1), may be related to the earlier starting time of Czech kindergartens by >1 h than Japanese ones. Second, the children's lifestyle is possibly affected by their parents' lifestyle, especially that of the mother. Another hypothesis for the reason for the morning typology of Czech infants could be the morning-type social system for Czech parents compared to that experienced by Japanese parents. For example, most official bus and train services start at 04.00–05.00 hours in the Czech Republic and at 05.00–06.00 hours in Japan. Czech laborers in factories start work at 06.00 hours, whereas Japanese laborers start at 08.00–09.00 hours. Many Czech and Japanese official services open at 07.30–08.00 hours and 09.00 hours, respectively. The short sleep time in Japanese infants, which may be influenced by the 24-h commercial society, could have some impact on their mental health.

Although it would be possible for the data of the M-E questionnaire for babies to be compared to that from elder children and students because of the "common" questionnaire, one limitation arising from the M-E questionnaire exists in this study: extreme morning-type babies (score >28) may be undetected. A further limitation is that the ME questionnaire may be limited in detecting the M-E preference of babies of 0–2 years, and in addition there may be geographical bias for the respondents. The mental and physical health of the parents seems to influence the mental health of the infants' in regards to angry and depressed mood. This question remains to be solved in future study.

ACKNOWLEDGMENTS

We thank all the members of the kindergartens who cooperated with this survey. This study was performed as an official international research project based on the Academic Exchanging Agreement between the University of South Bohemia and Kochi University.

Table 1 Relationship of circadian typology of Japanese and Czech infants to age (Mean ± SD [n])

	0 year	1 year	2 years	3 years	4 years	5 years	6 years	7 years	8 years
Japan	22.4 ± 3.1 (18)	23.0 ± 2.7 (61)	21.5 ± 3.0 (92)	20.3 ± 3.3 (141)	19.4 ± 3.6 (131)	19.6 ± 3.7 (146)	19.0 ± 4.3 (27)	–	–
Czech	27.0 (1)	23.7 ± 3.8 (20)	23.7 ± 2.5 (15)	23.8 ± 2.6 (72)	22.0 ± 3.0 (100)	22.3 ± 2.9 (173)	21.0 ± 3.2 (103)	20.7 ± 3.0 (70)	21.9 ± 2.2 (27)

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The 1st Step-2

(This part is English version translated from the manuscript which has been already published in *Japanese Journal of Physiological Anthropology* (in Japanese) and the published manuscript was attached as Appendix of this thesis.)

A tryptophan-rich breakfast and exposure to light improve sleep and mental health in Japanese students

(Subtitle: Effect of intervention as introduction to taking protein and vitamin rich breakfast and following sun-light exposure on sleep and mental health of Japanese university soccer team members inhabiting Kochi Prefecture)

ABSTRACT

Objective of this study is to evaluate the effects of intervention, as asking Japanese university students to get foods including protein and Vitamin B6 (especially recommending fermented soy-beans and banana) at breakfast and to be exposed to sun-lights after that for one month (October-November, 2008), on sleep-wake cycle during the intervention. Participants of 83 attending university soccer club were divided to three groups (G1: no intervention; G2: asking them to have protein resources and Vitamin B6 resource at breakfast; G3: asking them to do as G2 plus sun light exposure after breakfast). Sleep diary was noted by themselves through one month of intervention period to evaluate the effects of it. Phase advance tended to be shown at all 4 phase points (bed time, sleep-in-time, med-point of sleep, wake-up time) in the last week period of intervention month only in G3 (P=0.100,0.148,0.070,0.148). The integrated intervention to breakfast and the following light exposure might be effective for evening-typed Japanese students to be shifted to be more morning-typed only within 2-4 weeks.

Introduction

Diversification of lifestyles and advanced eveningness has created problems in the basic living habits of children. This may contribute to a variety of adverse effects, such as a decrease in motivation for learning, a decrease in physical and mental energy, and possibly even an increase in several health disorders. Shifting to an evening-type life for young children might be being accelerated, especially due to the ongoing 24-hour commercialization of society [1]. Two oscillators hypothesis driving human diurnal rhythms is general in chronobiological field: one is the main clock having period of a little bit longer than 24 hours and another is the second or “slave” clock which is driving sleep-wake cycles having of 33 to 40 hours period [2]. Extreme evening-type habits have danger to induce the inner de-synchronization between the two clocks which has afraid to induce the mental disease like as depression syndrome. Therefore, such shifting to evening-typed life might be dangerous for children to keep healthful growth especially from the view point of mental health.

Tryptophan is a nutritionally essential amino acid that can be absorbed exclusively from meals in humans. It is metabolized via 5-hydroxytryptamine (serotonin) to melatonin by a series of 4 enzymes in the pineal body [3, 4]. Vitamin B6 functions as coenzyme of the enzyme to modify 5-hydrooxytryptophan to serotonin. So intake of the Vitamin B6 at breakfast is indirectly related to serotonin synthesis. Serotonin is known as a precursor to melatonin. A lack of serotonin causes depression, panic disorder, obsessive compulsive disorder, sleep disorders and eating disorders [5] and induces aggression, anxiety/aggression-driven depression, impulsive behavior and suicidal attempts [6, 7]. Serotonin thus has a strong relationship with mental health. In the past two decades, serotonin reuptake inhibitors (SSRIs) have come to be widely used for the treatment of affective disorders including depression, although [8] there are controversies on whether SSRIs are effective or not for the treatment of depression in children and adolescents because of the shortage of coincident scientific evidence of SSRIs for young humans.

Exposure to sunlight in the daytime appears to trigger synthesis of serotonin in the pineal body [9]. This action is hypothesized to occur mainly in the morning hours, because the amount of tryptophan consumed with supper has neither significant effects on Morningness-Eveningness (M-E) scores nor an effect on sleep habits, as shown by another study on young Japanese children performed in 2005 [10].

Melatonin is synthesized in the pineal body of the hypothalamic area and secreted at night. Melatonin level in the serum can be well and positively correlated with that in the saliva [11-14]. Secretion of melatonin exhibits circadian rhythms and is suppressed by bright light at night [15, 16]. Even room lights such as fluorescent lamps can attenuate melatonin excretion duration at night [17]. Evening lighting conditions are also said to affect circadian rhythms [18, 19] and mental health in mice [20]. Tryptophan intake at breakfast is effective for the onset and offset of sleep in young children [21]. Moreover, questionnaire surveys showed that young children exposed to sunlight for more than 30 minutes after having sources of protein at breakfast are more morning-typed than those exposed for less than 30 minutes [22], and that the more young children take in Vitamin B6 at breakfast, the more they exhibit morning typology [23].

Although these findings imply that morning tryptophan and Vitamin B6 intake and following exposure to sunlight would promote synthesis of serotonin in the daytime and further that of melatonin at night, it is difficult to test the hypothesis only with questionnaire studies. This hypothesis cannot be tested by questionnaire work and would require an intervention field experiment. An intervention field experiment was thus performed on university students to test the hypotheses.

Participants and Methods

The intervention program was administered to 83 subjects (male, 19-22 years old, average age: 20.32) belonging to a university soccer club. Forty seven subjects answered to the integrated questionnaire before the intervention period. They were divided into three groups (G1, n = 19: no intervention; G2, n = 13: asked to have protein-rich foods such as fermented soybeans and Vitamin B6-rich foods such as bananas at breakfast; G3, n = 17: the same breakfast contents as G2 and sunlight exposure after the meal). This university football club includes only men. To separate the participants equally, integrated questionnaires were administered to all participants before the intervention period. The questionnaire consisted of the diurnal-type scale constructed by Torsvall and Åkerstedt [24], questions on sleep habits and meal habits [25], an irritation index, and FFQ (Food Frequency Questionnaire).

We made nine groups initially based on the scores of the diurnal-type scale (three groups: morning-type, middle-type, evening-type) and FFQ (three groups: good, mid, bad). After that we divided participants into three groups for each of the nine groups with random number list arbitrarily. There were no significant differences among the body height, body mass and age of the three groups. All participants were asked to keep a sleep diary throughout the 30 days of the intervention period, which was November in 2008. The sleep diary involved the question about sleep habits (bed time, falling asleep time, awakening time), and about subjective mood (mood of the day, mood on awakening). Participants of G2 and G3 were asked to report their breakfast contents, and G3 were also asked to answer the duration of their time spent under sun light after breakfast each day. The implementation score was calculated from the sum of days when they had “high protein content breakfast” and “exposure to >30 min-exposure to sunlight”.

For the statistical analysis, the “implementation score” was defined as how many days participants had a protein-rich food (1 point) at breakfast and, further, exposed to sunlight for more than 30 min after breakfast (1 point). The 30-day-long intervention period was divided into 3 parts (FWP: First week period, MP: Medium period of 16 days, LWP: Last week period). The “high implementation group” was defined as 50%

participants who marked higher implementation score in both breakfast contents (G2 and G3) and exposure to sunlight after breakfast (G3). The other 50% participants group was defined as "the low implementation group". The software used for statistical analysis was SPSS 12.0 J for Windows (SPSS Inc., Chicago, IL, USA). *Kai-square*-test was used for categorized variables, and Mann-Whitney U-test was used for ranked variables. Pearson's correlation analysis was performed to test the relationship between two numerical variables.

Before the beginning of the study, participants received a full explanation with the code of the guideline for a study targeting humans [26], including that the results of the study would be used only for academic purposes, and all participants completely agreed to participate in the study.

Results

In G1, it was shown that the phase of middle point of sleep from FWP to MP tended to be delayed (Wilcoxon's signed rank sum test: z=-1.851, p=0.064), whereas in LWP the delayed phase went back to the original one in FWP (z=-2.201, p=0.028) **(Table 1-A).**

In G2, although the phase of wake up time tended to be delayed from FWP to MP (z=-1.962, p=0.050), bed time, falling asleep time, and middle point of sleep were not delayed **(Table 1-B)**.

In G3, all of the 4 phase points were not delayed from FWP to MP at all, by contraries, their bed time and wake up time tended to be in advance from MP to LWP (bed time: z=-1.647, p=0.100; wake up time: z=-1.810, p=0.070)(**Table 1-C**)(**Figure 1-A, B, C, D**) .

In G2, there were "negative" (which means higher implementation correlates with earlier times) correlations between implementation score and bed time (Pearson's correlation test r=-0.520, p=0.069), wake up time (r=-0.731, p=0.004), and those at middle point of sleep (r=-0.500, p=0.082) in FWP. In addition, there were also significant the "negative" correlations between implementation score and bed time (r=-0.734, p=0.004), falling asleep time (r=-0.628, p=0.007), wake up time (r=-0.544, p=0.055), and middle point of sleep (r=-0.727, p=0.005) in MP (**Figure 2-A, B, C, D**). However, in LWP, there was no significant correlation between implementation score and subjective moods.

In G3, there were the "negative" correlations between implementation score and wake up time (r=-0.586, p=0.017), mood of the day (r=0.428, p=0.099), and mood on waking up (r=0.561, p=0.024) in FWP. In MP there were also "positive" correlation (which means higher implementation correlates with better mood) between implementation rate and mood of the day (r=0.515, p=0.041) and mood on waking up (r=0.713, p=0.001) (**Figure 3-A, B**).

Discussion

These results indicated that intervention on not only breakfast contents but also sunlight exposure after breakfast was important to induce phase advance, which might lead to their wake-up-mood and daytime mood improved.

That phenomenon may imply a hypothesis as follows: "Such intervention promotes the synthetic pathway of tryptophan-serotonin-melatonin, and the increased peaks of serotonin and melatonin which function as stronger inner-zeitgeber for circadian clock. Both the phase advance and higher serotonin level in daytime could promote the mental health."

Their implementation score declined toward the end of intervention. This decline needs something to encourage their motivation to the intervention.

In a previous research using questionnaires, a link between tryptophan intake and the diurnal-type score was shown only for children aged 0 to 8years [21]. High levels of melatonin in the blood plasma at night are thought to have a large effect on sleep onset and the quality of sleep itself. Night-time blood plasma melatonin levels are many times higher in early childhood than during adolescence [27].Sensitivity to light of young children is much higher than adolescents and young adults such as university students, because young children have wider pupil responding to light and more transparent lens, and their serotonin and melatonin level are also far higher. So such intervention program may be effective to make young children's phase of their circadian clock in advance.

Circumstances surrounding children and adolescents such as light condition, meal habits, and social environment are changing rapidly with wide-spread 24-hour commercialization society in Japan. This change makes people's amplitude of circadian oscillations declined and also the phase of their rhythms irregular. Harada [1] reported the women students attending junior high schools used mobile-phone intensively at night and that the intensive usage leaded to their evening-typed life style. Abuse of mobile-phone can cause to disappear the circadian rhythms.

We don't know whether we will develop, in the future, a new genetic potential to keep a well-synchronized two oscillators even under environmental weak zeitgebers with irregular phase and lower amplitudes. We also do not know whether we already have such genetic potential. Anyway, it is probably difficult to change, within only one or two recent decades, physiological characteristics we've evolved over the time course of tens of thousands years. So we should consider how we adjust our circumstances to maintain clear 24-hour cycle and to function as good synchronizer (or zeitgeber) for human beings as "diurnal animal". The intervention in this study: "tryptophan and vitamin B6 intake at breakfast, followed by the exposure to sunlight" can be a strategy for us to live regularly keeping diurnal rhythms without inner de-synchronization.

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Table 1 Average and Standard deviation of four phase points (Bed time, Falling asleep time, Awakening time, and Middle point of sleep) in First week period (FWP), Middle period (MP), and Last week period (LWP) in three groups.

A G1	**Bed time in FWP**	**Bed time in MP**	**Bed time in LWP**	**Falling asleep in FWP**	**Falling asleep in MP**	**Falling asleep in LWP**
Ave.	24.539	24.739	24.449	24.874	25.045	24.803
N	19	19	18	19	19	18
SD	0.905	0.758	0.912	0.902	0.741	0.912
	Awakening time in FWP	**Awakening time in MP**	**Awakening time in LWP**	**Mid point in FWP**	**Mid point in MP**	**Mid point in LWP**
Ave.	8.212	8.383	8.179	5.416	5.779	5..294
N	19	18	18	19	18	18
SD	0.733	0.813	0.647	1.631	1.451	1.601
B G2	**Bed time in FWP**	**Bed time in MP**	**Bed time in LWP**	**Falling asleep in FWP**	**Falling asleep in MP**	**Falling asleep in LWP**
Ave.	24.554	24.401	24.570	24.845	25.847	25.009
N	12	12	12	12	12	12
SD	0.579	0.721	0.814	0.666	0.773	0.792
	Awakening time in FWP	**Awakening time in MP**	**Awakening time in LWP**	**Mid point in FWP**	**Mid point in MP**	**Mid point in LWP**
Ave.	8.234	8.445	8.542	5.385	5.493	5.784
N	12	12	12	12	12	12
SD	0.493	0.359	0.559	1.071	1.243	1.299
C G3	**Bed time in FWP**	**Bed time in MP**	**Bed time in LWP**	**Falling asleep in FWP**	**Falling asleep in MP**	**Falling asleep in LWP**
Ave.	24.519	24.605	24.394	24.856	24.904	24.743
N	16	16	16	16	16	16
SD	0.640	0.625	0.663	0.603	0.555	0.717
	Awakening time in FWP	**Awakening time in MP**	**Awakening time in LWP**	**Mid point in FWP**	**Mid point in MP**	**Mid point in LWP**

Ave.	8.438	8.472	8.080	5.527	5.592	5.155
N	15	16	16	15	16	16
SD	1.174	0.903	0.745	1.093	0.933	1.318

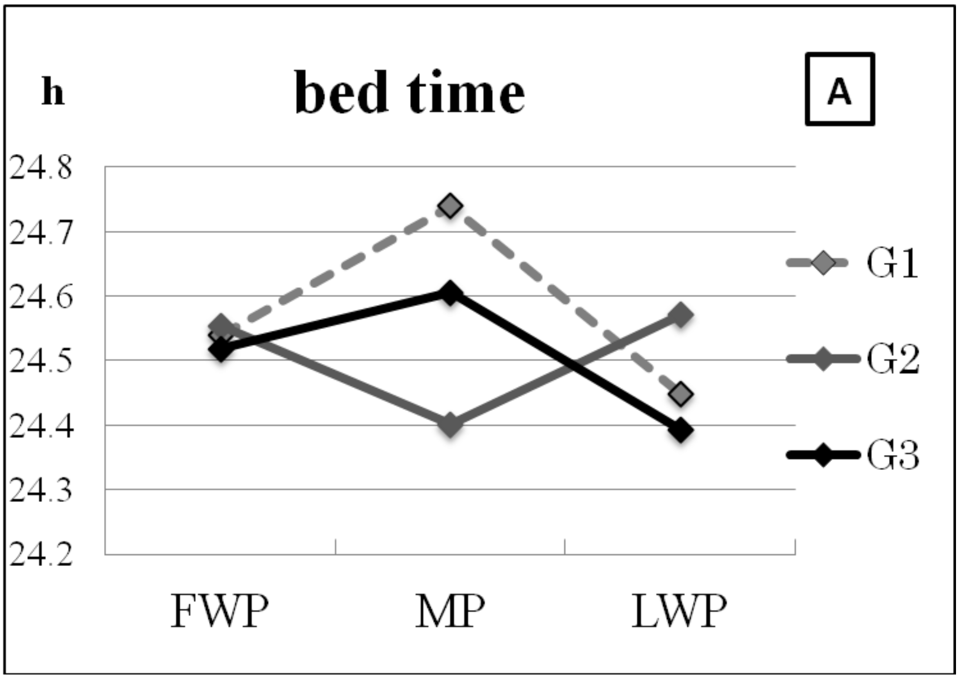

Figure 1-A Comparison of bed time during intervention among three groups.

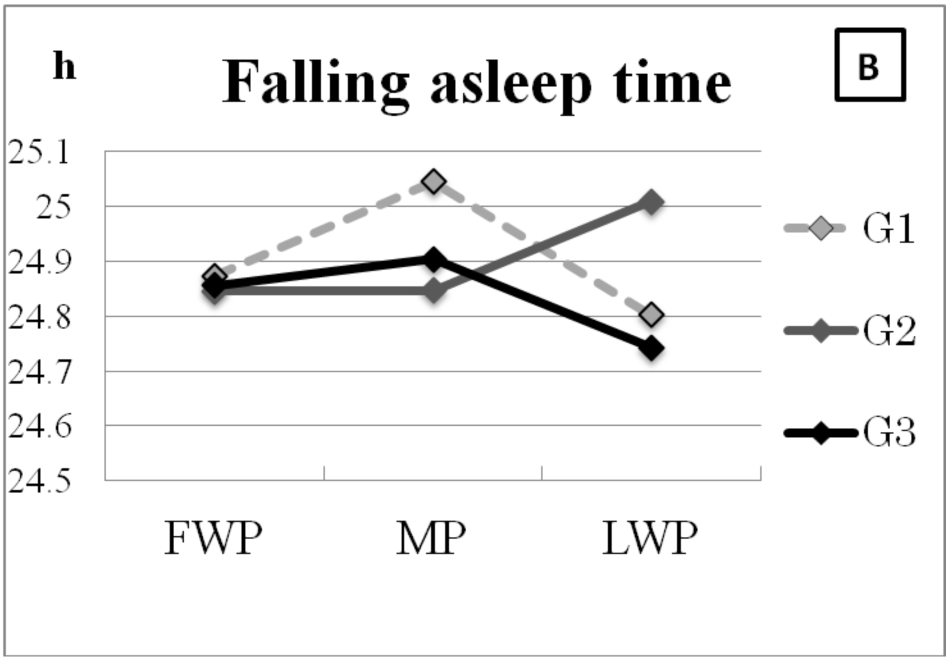

Figure 1-B Comparison of falling asleep time during intervention among three groups.

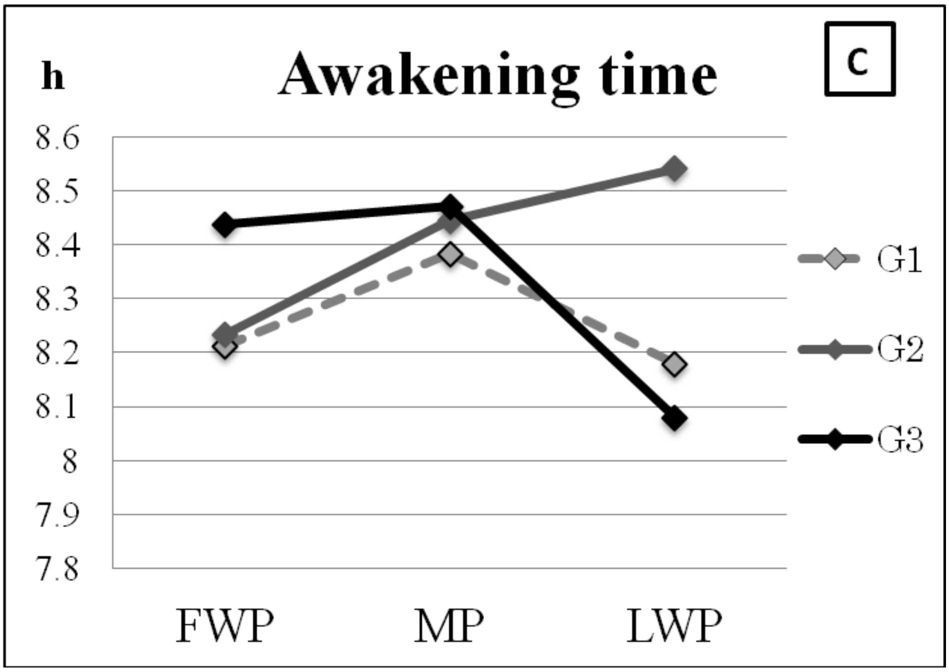

Figure 1-C Comparison of awakening time during intervention among three groups.

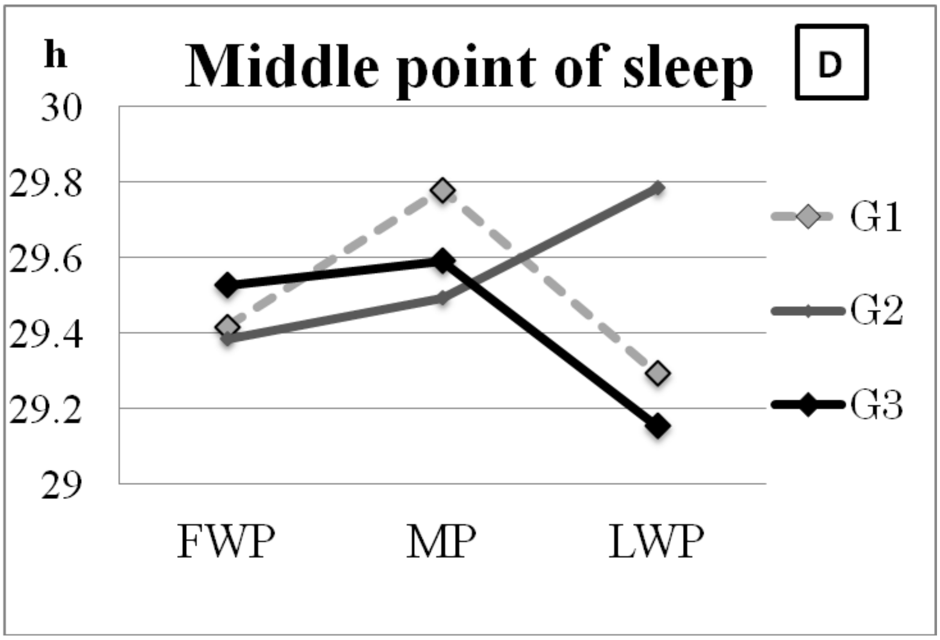

Figure 1-D Comparison of Middle point of sleep during intervention among three groups.

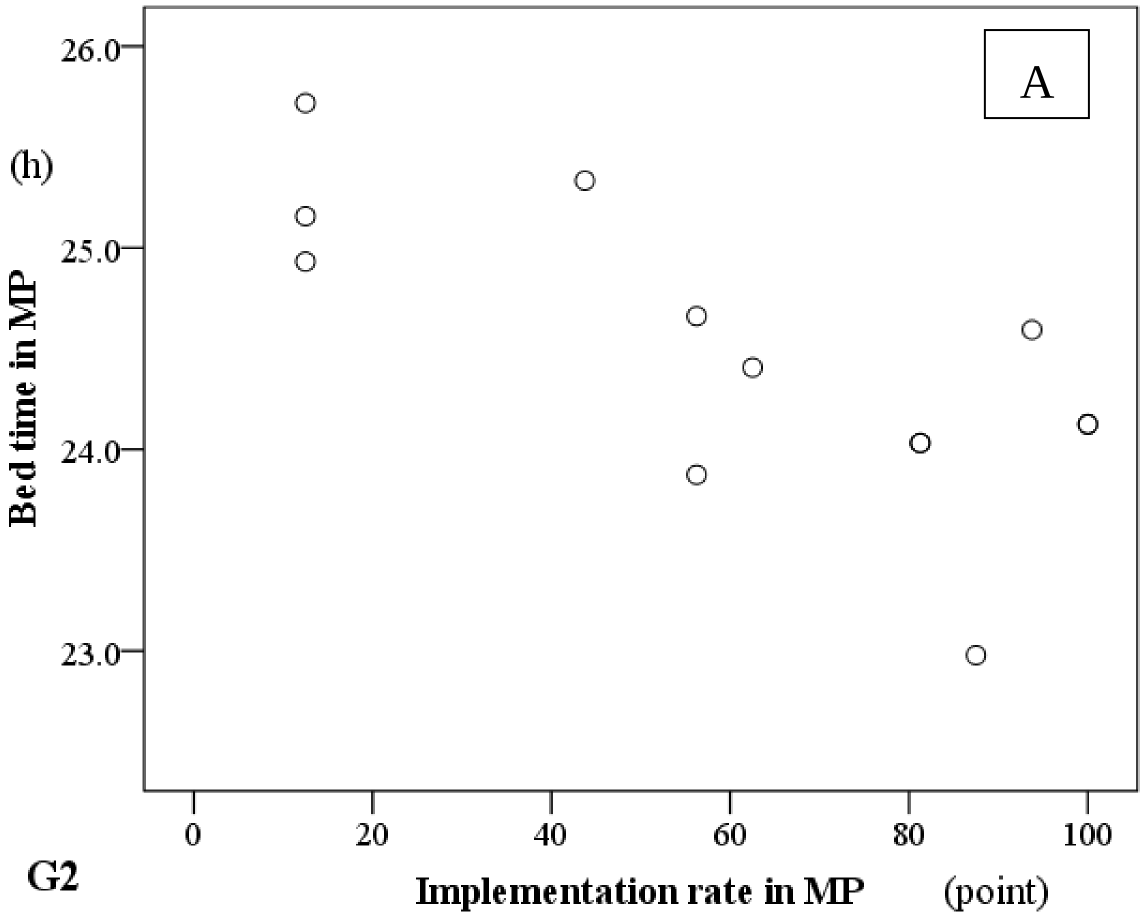

Figure 2-A Negative (which means higher implementation correlates with earlier times) correlation between implementation score and bed time (Pearson's correlation test r=-0.734, p=0.004) in MP in G2.

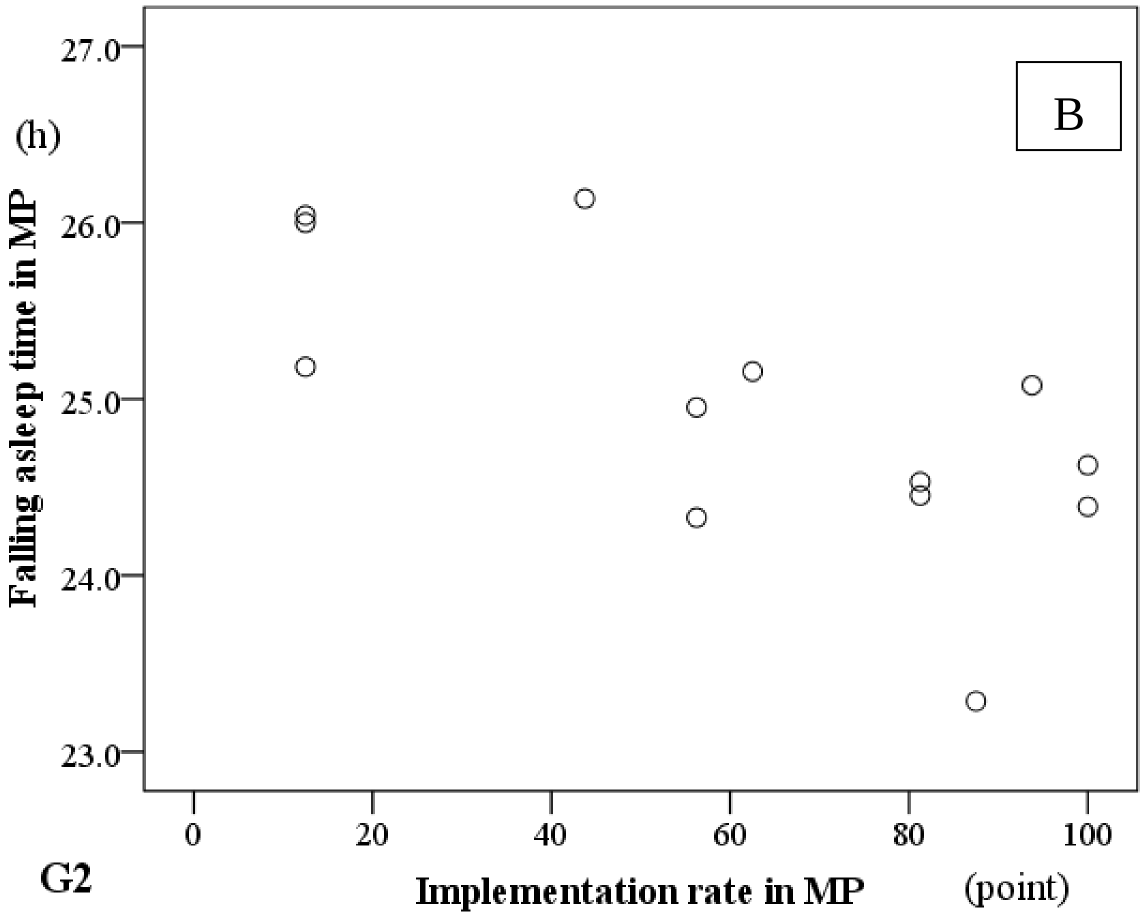

Figure 2-B Negative (which means higher implementation correlates with earlier times) correlation between implementation score and falling asleep time (Pearson's correlation test r=-0.628, p=0.007) in MP in G2.

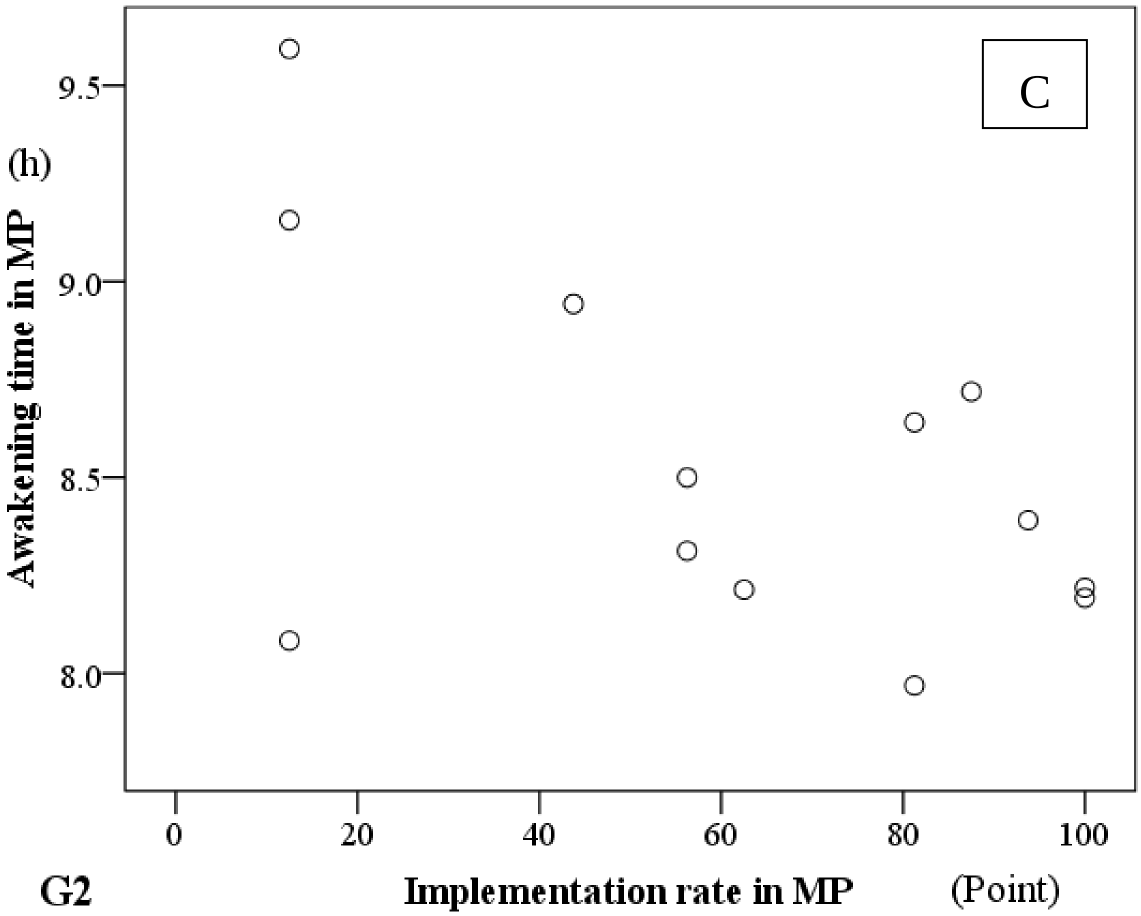

Figure 2-C Negative (which means higher implementation correlates with earlier times) correlation between implementation score and awakening time (Pearson's correlation test r=-0.544, p=0.055) in MP in G2.

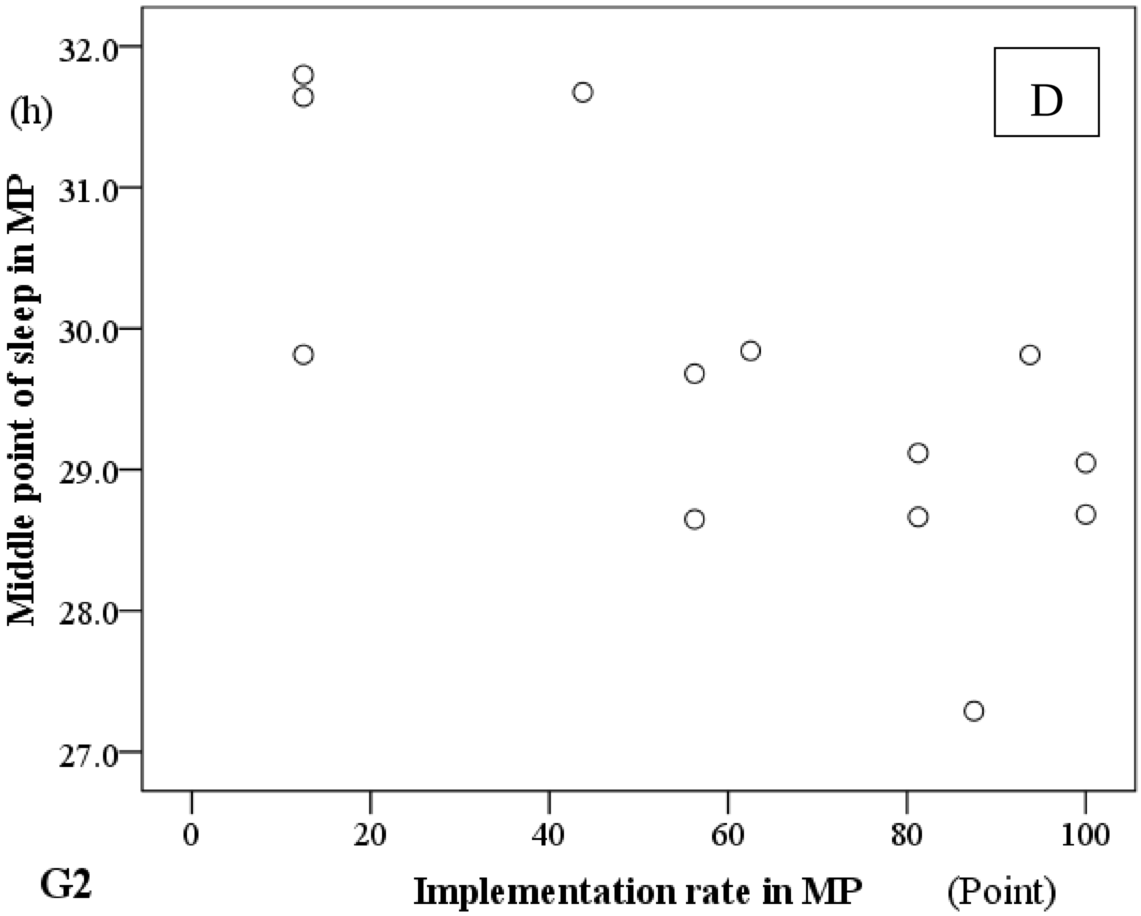

Figure 2-D Negative (which means higher implementation correlates with earlier times) correlation between implementation score and middle point of sleep (Pearson's correlation test r=-0.727, p=0.005) in MP in G2.

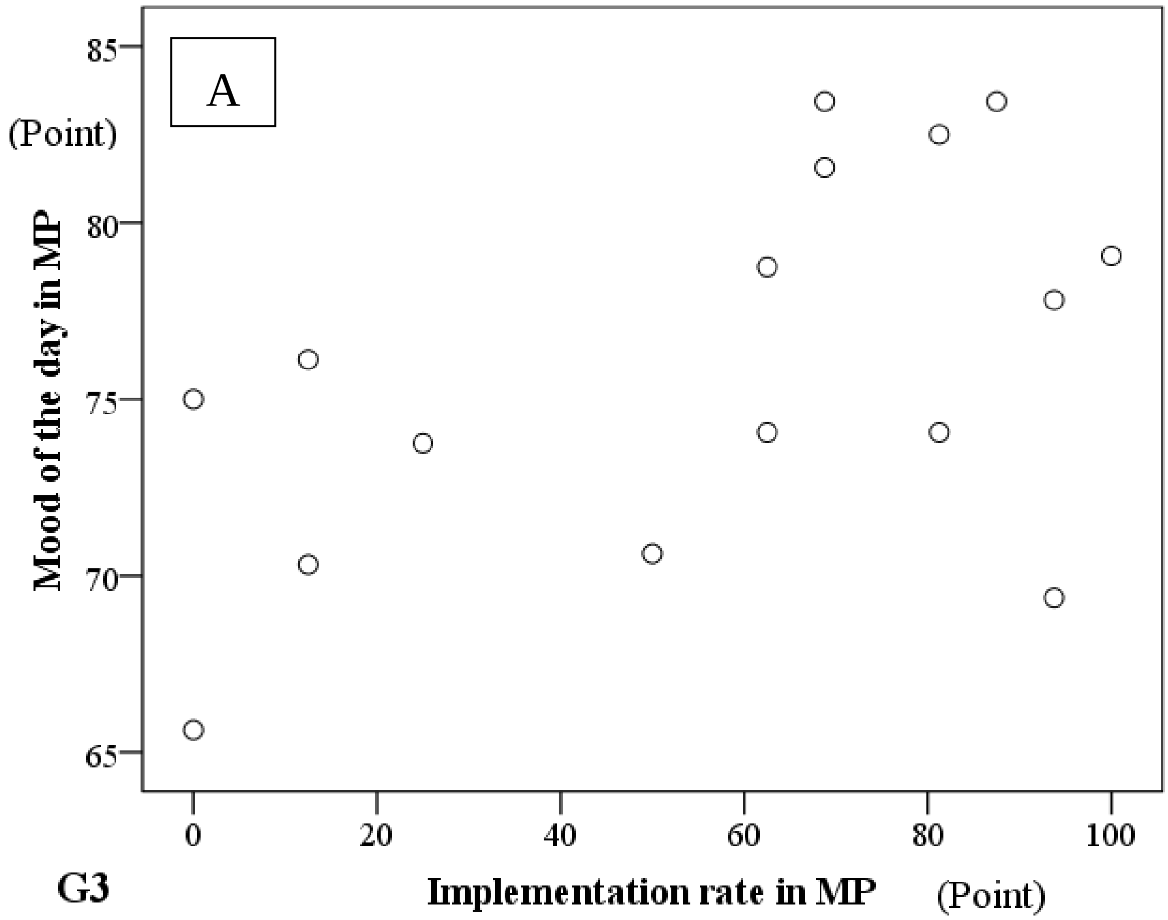

Figure 3-A In MP there were positive (which means higher implementation correlates with better mood) correlation between implementation rate and mood of the day ($r=0.515$, $p=0.041$) in G3.

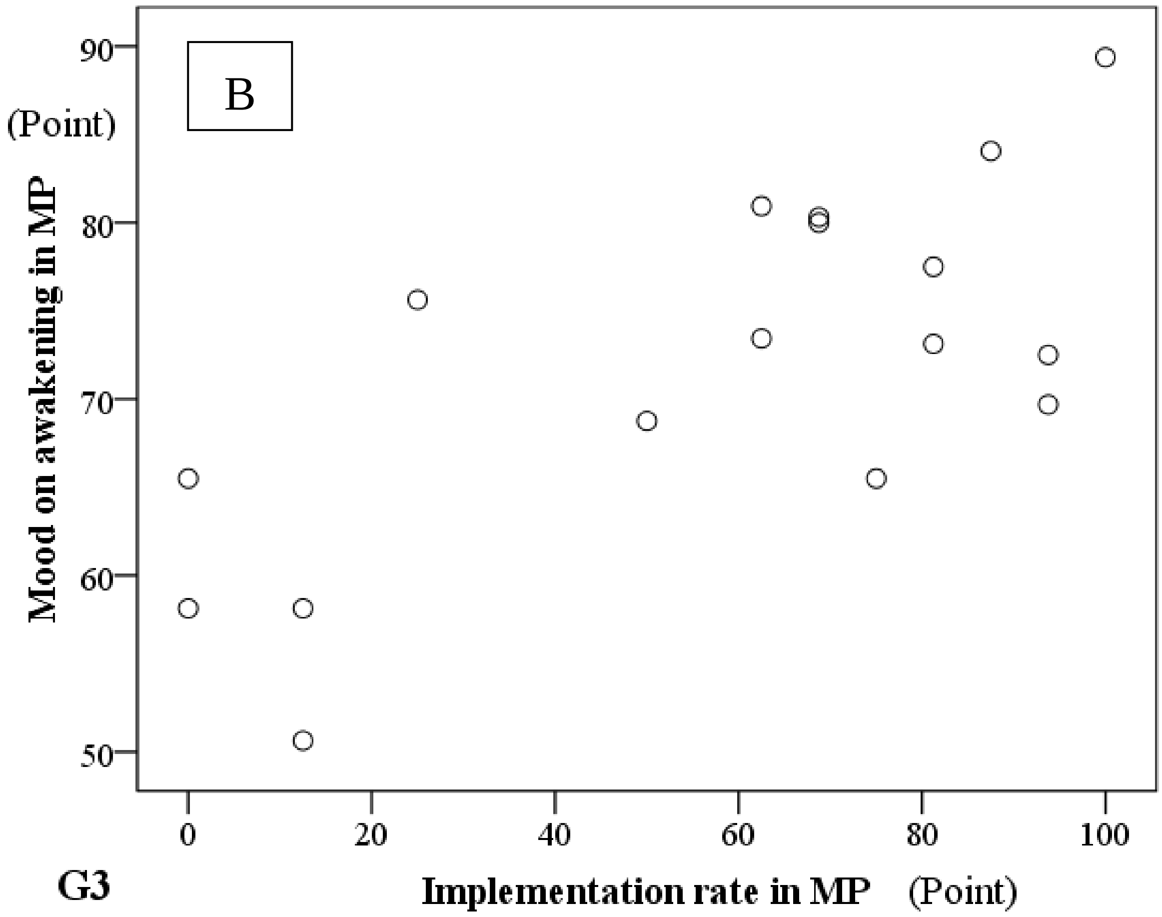

Figure 3-B In MP there were positive (which means higher implementation correlates with better mood) correlation between implementation rate and mood on waking up (r=0.713, p=0.001) in G3.

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RESEARCH Open Access

A tryptophan-rich breakfast and exposure to light with low color temperature at night improve sleep and salivary melatonin level in Japanese students

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Abstract

Background: Epidemiological studies in Japan have documented an association between morning type and a tryptophan-rich breakfast followed by exposure to sunlight in children. The association may be mediated by enhanced melatonin synthesis, which facilitates sleep at night. However, melatonin is inhibited by artificial light levels with high color-temperature common in Japanese homes at night. In this study, we investigated whether a combination of tryptophan-rich breakfast and light with low color-temperature at night could enhance melatonin secretion and encourage earlier sleep times.

Methods: The intervention included having breakfast with protein- and vitamin B6 - rich foods and exposure to sunlight after breakfast plus exposure to incandescent light (low temperature light) at night (October-November, 2010). The participants were 94 members of a university soccer club, who were divided into 3 groups for the intervention (G1: no intervention; G2: asked to have protein-rich foods such as fermented soybeans and vitamin B6-rich foods such as bananas at breakfast and sunlight exposure after breakfast; G3: the same contents as G2 and incandescent light exposure at night). Salivary melatonin was measured around 11:00 p.m. on the day before the beginning, a mid-point and on the day before the last day a mid-point and on the last day of the 1 month intervention.

Results: In G3, there was a significantly positive correlation between total hours the participants spent under incandescent light at night and the frequency of feeling sleepy during the last week ($p = 0.034$). The salivary melatonin concentration of G3 was significantly higher than that of G1 and G2 in combined salivary samplings at the mid-point and on the day before the last day of the 1 month intervention ($p = 0.018$), whereas no such significant differences were shown on the day just before the start of the intervention ($p = 0.63$).

Conclusion: The combined intervention on breakfast, morning sunlight and evening-lighting seems to be effective for students including athletes to keep higher melatonin secretion at night which seems to induce easy onset of the night sleep and higher quality of sleep.

Keywords: Salivary melatonin, Tryptophan, Protein rich breakfast, Sunlight exposure, Lighting with low color temperature

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Background

Tryptophan is an essential amino acid that can be absorbed exclusively from meals in humans. It is metabolized via 5-hydroxytryptamine (serotonin) to melatonin by a series of 4 enzymes in the pineal body [1,2]. Serotonin is known as a precursor to melatonin. A lack of serotonin causes depression, panic disorder, obsessive-compulsive disorder, sleep disorders and eating disorders [3] and induces aggression, anxiety/aggression-driven depression, impulsive behavior and suicidal attempts [4,5]. Serotonin thus has a strong relationship with mental health. In the past two decades, serotonin reuptake inhibitors (SSRIs) have come to be widely used for the treatment of affective disorders including depression, although [6] there are controversies whether SSRIs are effective or not for the treatment of depression in children and adolescents because of the shortage of coincident scientific evidence of SSRIs for young humans.

Exposure to sunlight in the daytime appears to trigger synthesis of serotonin in the pineal body [7]. This action is hypothesized to occur mainly in the morning hours, because the amount of tryptophan consumed with supper has neither significant effects on Morningness-Eveningness (M-E) scores nor an effect on sleep habits, as shown by another study on young Japanese children performed in 2005 [8].

Melatonin is synthesized in the pineal body of the hypothalamic area and secreted at night. Melatonin level in the serum can be well and positively correlated with that in the saliva [9-12]. Secretion of melatonin exhibits circadian rhythms and is suppressed by bright light at night [13,14]. Even room lights such as fluorescent lamps can attenuate melatonin excretion duration at night [15]. Evening lighting conditions are also said to affect circadian rhythms [16,17] and mental health in mice [18]. Tryptophan intake at breakfast is effective for the onset and offset of sleep in young children [19]. Moreover, questionnaire surveys showed that young children exposed to sunlight for more than 30 minutes after having sources of protein at breakfast are more morning-typed than those exposed for less than 30 minutes [20], and that the more young children take in vitamin B6 at breakfast, the more they exhibit morning typology [21].

Although these findings imply that morning tryptophan and vitamin B6 intake and following exposure to sunlight would promote synthesis of serotonin in the daytime and further to melatonin at night, it is difficult to test the hypothesis only with questionnaire studies. Moreover, this melatonin synthesis might be inhibited by exposure to short-wave (blue) light including light emitted from fluorescent lamps. This hypothesis cannot be tested by questionnaire work and would require an intervention field experiment. An intervention field experiment for was thus performed on university students to test the hypotheses.

Methods

The intervention program was administered to 94 subjects (male, 19-22 years old, average age: 20.33) belonging to a university soccer club. 63 subjects answered to the integrated questionnaire before the intervention period. They were divided into three groups (G1, n = 20: no intervention; G2, n = 22: asked to have protein-rich foods such as fermented soybeans and vitamin B6-rich foods such as bananas at breakfast and sunlight exposure after breakfast; G3, n = 21: the same contents as G2 and incandescent light exposure at night). This university football club includes only men. All the members used fluorescent lamps (white light) for the lighting at night. To estimate the effects of the one month interventions, integrated questionnaires were administered to all participants three times: before the start of the intervention period, immediately after the end of the intervention period, and one month after the end of the intervention period (See note on Figure 1). The questionnaires which were administered before the intervention and 1 month after the intervention consisted of the diurnal-type scale constructed by Torsvall and Åkerstedt [22], questions on sleep habits and meal habits [23], an irritation index, the General Health Questionnaire (GHQ), the Sense of Coherence (SOC) questionnaire, and FFQ (Food Frequency Questionnaire). The questionnaire just after the intervention period of 1 month consisted of self-assessment questions asking how many days during the month-long intervention period they followed the recommendations for breakfast content (the first point), sunlight exposure after breakfast (the second point) and the use of light bulbs that emit lower color temperature light at night (the third point). We made nine groups initially based on the scores of the diurnal-type scale (three groups: morning-type, middle-type, evening-type) and FFQ three groups: good, mid, bad). After that we divided participants into three groups for each of the nine groups with random number list arbitrarily. There were no significant differences among the body height, body mass and age of the three groups.

All participants were asked to keep a sleep diary throughout the 30 days of the intervention period, which was October-November in 2010. The sleep diary involved the question, "How was the depth of your last night's sleep?" to which participants answered every morning. The choices for answer were "deep", "relatively deep", "relatively shallow" and "shallow".

Incandescent light bulbs were distributed one by one to the participants in the G3 group, and these participants were asked to install the light bulb in the room in which they slept at night. The G1 and G2 members were

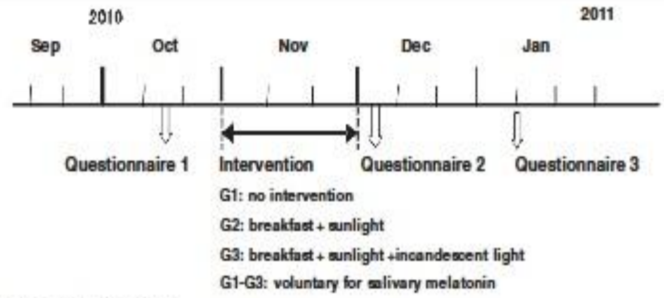

Figure 1 The schedules of the interventions.

asked to switch fluorescent lamps on and the G3 ones were asked to switch incandescent light bulbs on, instead, when they got back to their residences after sunset. After the incandescent light was set, illumination intensity was measured (Table 1). Participants of G2 and G3 were asked to report their breakfast contents, and G3 were also asked to answer the duration of their time spent under incandescent light each day. 63 of 94 (67%) participants answered the first questionnaire and 51 of 63 (81%) kept sleep diaries for 1 month.

The implementation score was calculated from the sum of days that had "high protein content breakfast" and "exposure to >30 min-exposure to sunlight". For night exposure to low temperature light, the implemental score was defined as the mean hours (per night for 30 days) when participants were exposed to the low temperature light

Table 1 Illumination value (Lux) of all subjects in the third group (G3)

	Standing under the light*						Sitting as usual#					
	Fluorescent			Incandescent			Fluorescent			Incandescent		
	Max	Min.	Ave	Max	Min	Ave	Max	Min	Ave	Max	Min	Ave
A	579	571	574	76	74	74	173	158	166	27	25	26
B	845	601	774	42	38	40	143	122	130	30	18	24
C	186	160	170	18	18	18	34	32	33	9	9	9
D	358	344	352	21	15	18	108	100	105	23	9	18
E	1120	1050	1019	32	28	30	89	86	87	18	15	17
F	1042	1013	1025	42	38	40	272	246	257	5	5	5
G	644	572	604	52	50	51	168	103	144	21	19	19
H	3997	2479	2942	70	61	63	284	277	281	39	38	38
I	1200	1002	1106	24	23	23	205	122	163	7	8	7
J	1155	1106	1132	41	41	41	196	184	189	8	6	6
K	657	651	654	72	70	70	65	64	64	20	18	20
L	173	137	148	26	8	19	134	128	130	18	9	18
M	1092	908	1025	377	360	370	597	263	341	50	37	50
N				64	59	61	1			131	114	131
O	505	433	465	160	133	150	231	145	86	39	9	39
P	1307	1193	1264	438	431	434	42	41	41	45	45	45
Q	591	542	572	140	129	136	149	146	147	24	20	24
R	1376	1078	1213	544	483	511	66	49	52	9	8	9
S	1	0	1	346	254	316	1	1	1	24	19	24
T	31	30	30	207	193	202	65	58	61	29	23	29

The surface of illumination meter sensor was put vertically just in front of eyes of participants who are standing* or sitting# and illumination value was measured.

Table 2 Estimates of the extent to which subjects in groups G2 and G3 carried out the intervention

Question: On a scale of 0 to 100, how would you estimate your confidence in your response? The question is "To what extent did you carry out this intervention program during this one month intervention period?	
1. Estimate for the whole protocol. (G2, G3)	score /100
2. Estimate for "taking protein-rich and Vitamin B6-rich foods at breakfast". (G2, G3)	score /100
3. Estimate for "exposure to sunlight after the breakfast". (G2, G3)	score /100
4. Estimate for "exposure to low color temperature light emitted from incandescent bulbs at night". (G3)	score /100

emitted from the incandescent bulb. After the intervention period for 30 days, the participants were asked to mark the scores on "To what extent do you satisfy on your own carrying out each of the two (G2) or three (G3) contents of intervention as a whole for 30 days, marking as 0-100 points as 'satisfaction score' ?" (Table 2).

The salivary melatonin was measured of 10 subjects which were randomly selected from each group because of financial limitation for the chemical analysis (30 participants in total) three times: the day before the start of intervention, at the mid-point (two weeks past in the intervention) and the day before the last day of the intervention. Participants were asked to extract their own saliva at around 23:00 and keep it in a freezer. They turned off the lights when they went to bed (ranging from 23:00 to 2:00).

The saliva samples were collected around 23:00 with cylindrical cotton (1 cm diameter, 3 cm long) which was put under the tongue for 3 min. The saliva samples were kept frozen at −25°C until analysis for 1 or 2 weeks. After centrifugation (1000 × g for 5 min), melatonin concentrations in the saliva samples were determined using an ELISA kit (Direct Saliva Melatonin ELISA, Bulmann, Switzerland).

For the statistical analysis, the "implementation rate" was defined as how many days participants had a protein-rich food (1 point) and Vitamin B6-rich food (1 point) at breakfast and, further, exposed to sunlight for more than 30 min after breakfast (1 point). Participants reported how many minutes they were exposed to low-color temperature lights during the 30 intervention days. The 30-day-long intervention period was divided into 3 parts (FWP: First week period, MP: Medium period of 16 days, LWP: Last week period). The "high implementation group" was defined as 50% participants who marked higher implementation rate in both breakfast contents and exposure to sunlight after breakfast (G2 and G3) and also were exposed to longer hours when they were exposed to the low temperature lights each

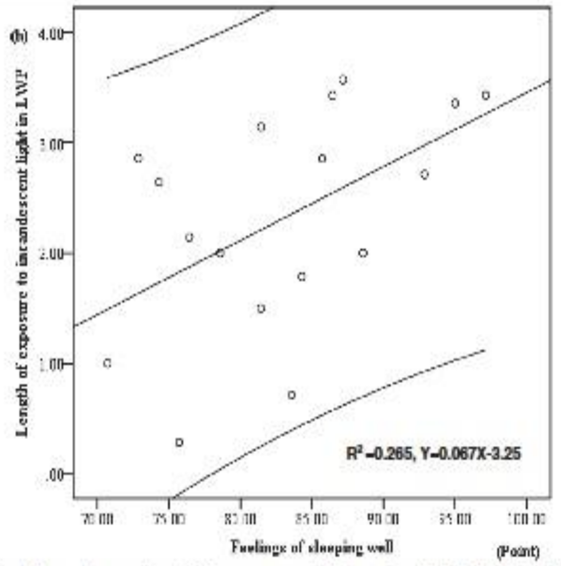

Figure 2 Positive correlation between hours when subjects were exposed to incandescent light at night and the index of feeling of sleeping well in Last Week Period. Upper and lower lines of linear regression line show 95% confidence estimate.

night (G3). The other 50% participants group was defined as "the low implementation group".

The software used for statistical analysis was SPSS 12.0 J for Windows (SPSS Inc., Chicago, IL, USA). χ^2-test was used for categorized variables and Mann-Whitney U-tests was used for ranked variables. Pearson's correlation analysis was performed to test the relationship between two numerical variables.

Before the beginning of the study, participants received a full explanation with the code of the guideline for a study targeting humans [24], including that the results of the study would be used only for academic

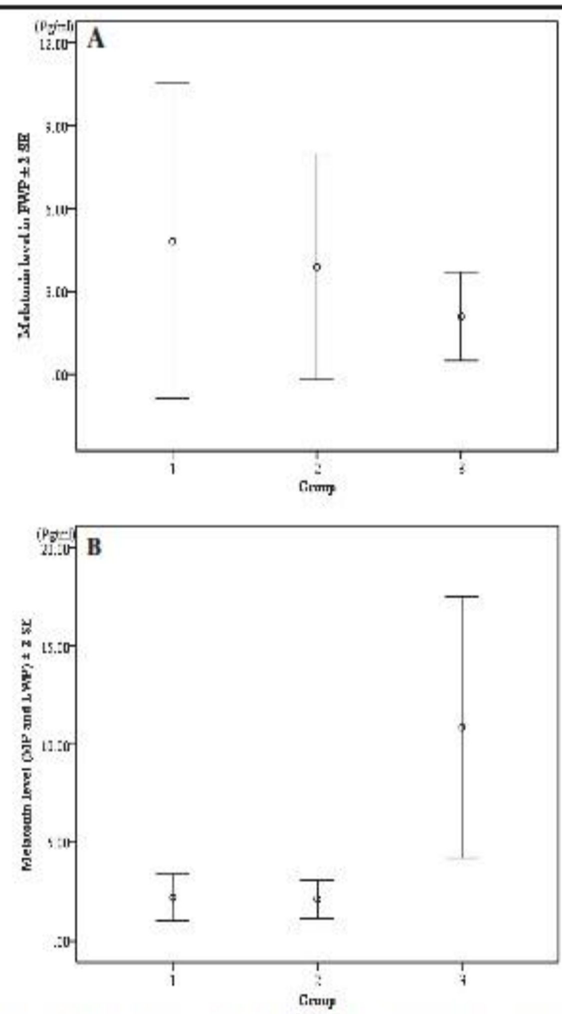

Figure 3 Comparison of salivary melatonin concentration among the three groups. Group 1: no intervention; Group 2: Recommendation of high protein breakfast and exposure to sunlight; Group 3: Same as Group 2 plus the recommendation of exposure to low color temperature light emitted from an incandescent light bulb. **A**: Melatonin level in the saliva collected on the day just before the intervention (Kruskal Wallis test χ^2-value = 0.92, df = 2, p = 0.63); **B**: Melatonin in the saliva collected at the mid-point and on the day before the last day of the intervention (Bonferroni multiple comparison test: G1 versus G3, p = 0.018; G2 versus G3, p = 0.011).

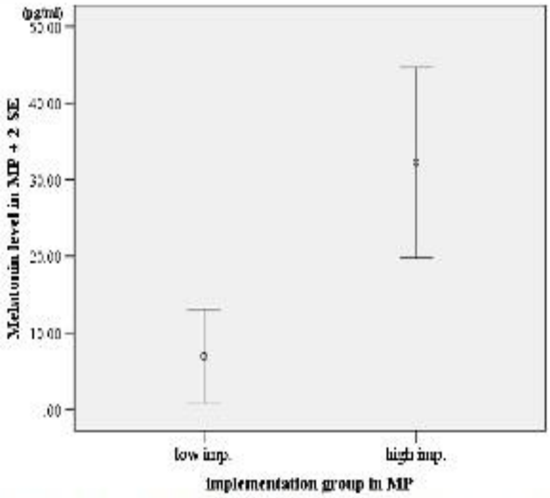

Figure 4 Comparison of melatonin level between "high implementation group" and "low implementation group" of G3 participants. See the text for details.

purposes, and all participants completely agreed to participate in the study.

Results

Sleep diary data and salivary melatonin concentration during the 30 days of the intervention

There was significant positive correlation between hours spent under incandescent light at night and the feeling of sleeping well in Last Week Period (LWP) (Pearson's correlation test: $r^2 = 0.265$, $p = 0.034$) (Figure 2).

The concentration of salivary melatonin shown by the participants of G3 was significantly higher than that of G1 and G2 in the mid-point and the day before the last day of the intervention (Bonferroni multiple comparison test: G1 versus G3, $p = 0.018$; G2 versus G3, $p = 0.011$), whereas there were no significant differences among the three groups on the day just before the start of the intervention (Kruskal Wallis test: χ^2-value = 0.92, df = 2, $p = 0.63$) (Figure 3).

The "high implementation group" tended to show a higher concentration of salivary melatonin in MP than the "low implementation group" did in G3 (Mann-Whitney U-test: $z = -2.000$, $p = 0.071$) (Figure 4). Participants of G3 tended to follow the morning intervention recommendations (high protein breakfast and sunlight exposure) on more days than G2 participants did (Mann-Whitney U-test: FWP; $z = -1.952$, $p = 0.053$, MP; $z = -1.628$, $p = 0.105$, LWP; $z = 1.253$, $p = 0.221$) (Figure 5). The implementation rate in FWP tended to be higher than in MP (Wilcoxon's signed rank sum test; G2:, $z = -1.851$, $p = 0.064$; G3:, $z = -1.914$, $p = 0.056$) and LWP (G2:, $z = -2.298$, $p = 0.022$; G3:, $z = -2.898$, $p = 0.004$). The implementation rate in MP tended to be also higher than in LWP in G2 and G3 (G2:, $z = -1.681$, $p = 0.093$; G3:, $z = -2.533$, $p = 0.011$).

Several parameters before and after the intervention period

There was a significantly positive correlation between the implementation satisfaction index (Maximum score: 100, Table 2) and the regularity of time to take breakfast and supper (Kendall tau-b test: breakfast, $r = 0.058$, $p = 0.038$; supper, $r^2 = 0.057$ $p = 0.036$).

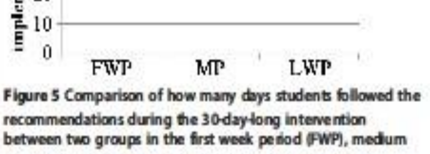

Figure 5 Comparison of how many days students followed the recommendations during the 30-day-long intervention between two groups in the first week period (FWP), medium period of 2 weeks (MP) and the last week period (LWP).

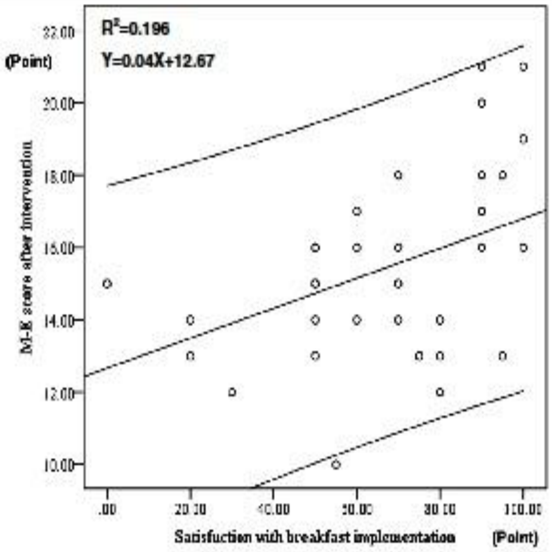

Figure 6 Positive correlation between M-E scores and the index of satisfaction on the implementation of the intervention on breakfast with high protein foods. Upper and lower lines of linear regression line show 95% confidence estimate.

There was a significant positive correlation between the index of how many days among the 30 days subjects were satisfied in their own implementation of the intervention (of having a breakfast that includes high protein foods) and M-E scores one month after the intervention (higher scores showing morning-type) (Pearson's correlation test: $r^2 = 0.195$, $p = 0.006$) (Figure 6).

There was a significant positive correlation between the number of nights when participants were exposed to incandescent light during the month-long intervention and the regularity index of meal time, not only for breakfast, but also for lunch and supper, just after the intervention (Kendall tau b-test: $r = -0.574$, $p = 0.007$, $r^2 = 0.146$, $p = 0.084$, $r^2 = 0.215$, $p = 0.029$). Participants

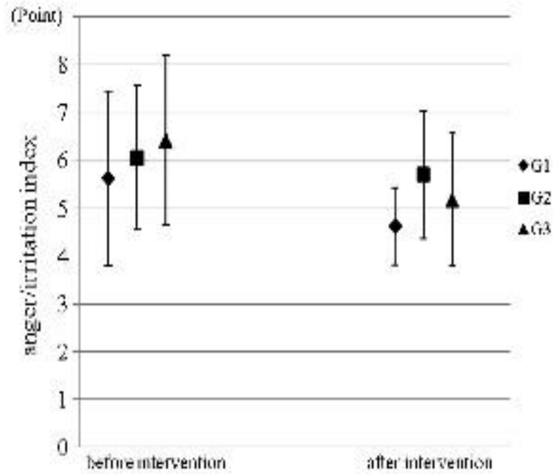

Figure 7 Comparison of the anger/irritation index before and after the intervention.

who ate breakfast more frequently for one month after the intervention showed a lower frequency of having late night snacks during that month (Kendall tau-b test: $r^2 = -0.142$, $p = 0.003$) than those who ate breakfast less frequently.

The participants in G3 after the intervention showed a lower anger/irritation index than before the intervention (Wilcoxon's signed rank sum test: $z = -3.072$, $p = 0.002$), whereas there was only the tendency towards reduced irritation in G1 ($z = -1.786$, $p = 0.074$) and no differences were seen in G2 ($z = -0.954$, $p = 0.340$) (Figure 7). Two components of the anger/irritation index, the frequency to be irritated and the frequency to become angry due to small trigger, were also reduced after the intervention in comparison with before the intervention in G3 (Wilcoxon's signed rank sum test: irritation, $z = -2.496$, $p = 0.013$; anger;, $z = 2.714$, $p = 0.007$).

Discussion

This study showed that a triple intervention concerning breakfast content, sunlight exposure after breakfast and exposure to low temperature light emitted from incandescent bulbs is a powerful method for inducing secretion of high amounts of melatonin by the pineal gland in human adults. Underlying mechanisms can be hypothesized to consist of two components. The first is that serotonin synthesis from tryptophan taken at breakfast may be enhanced by the exposure to sunlight just after taking breakfast. The second is that the high potential of melatonin synthesis based on the high serotonin synthesis in the pineal during daytime might be available due to the night exposure to the "low temperature light" emitted from incandescent bulbs. Although many reports have shown that melatonin secretion is suppressed by light emitted from fluorescent lamps including short wave length (with around 460 nm of wave length) components [25-27], and especially short wave length light [27-30], this study newly implies that the combined behaviors of modifying breakfast content, receiving sunlight exposure and receiving exposure to low color temperature lighting at night can facilitate achievement of high plasma melatonin at night in humans.

Melatonin, a hormone secreted from the pineal gland, causes the core body temperature to decrease and induces sleep [31-33]. High plasma melatonin levels at night may play an important role in sleep onset and sleep quality [34]. In this study, the longer time participants spent under incandescent lights at night, the significantly higher scores they marked to feel deep sleep. This better sleep quality might be due to high plasma melatonin levels.

This intervention study supports the hypothesis that the triple intervention of having sources of tryptophan and vitamin B6 at breakfast, following up breakfast with exposure to sunlight and the exposure to low temperature lights as night lighting can stimulate the synthesis of serotonin and succeeding melatonin synthesis at night and that these hormones work as natural anti-depression drugs and/or natural sleeping pills and make students more-morning typed and improve their mental health.

A limitation of this study as a "field intervention experiment" is that we did not include a control group with low-tryptophan breakfast, sunlight exposure, and exposure to low temperature light to find out the importance of the intake of tryptophan at breakfast for the mechanism of tryptophan-serotonin-melatonin pathway more clearly. This study was not a "physiological experiment" to set up several experimental groups and control all the environmental conditions, and such experiment remains to be conducted in the future. Another limitation of this study is that it was performed only with men, whereas the inclusion of participants from a female sports club could add important data on gender differences in response to breakfast modulation and the change in lighting at night.

Consent

Written informed consent was obtained from the participants for publication of this report and any accompanying images.

Competing interest

The authors declare that they have no competing interests.

Authors' contributions

KW: planned the study, conducted the intervention experiments, analyzed data, participated in the discussion of the results, and drafted the manuscript. SY: conducted the intervention experiments, analyzed data, and participated in the discussion of the results. OA: participated in the discussion of the results. MK: participated in the discussion of the results. TN: planned the study and participated in the discussion of the results. MN: planned the study, analyzed data on nutrition and participated in the discussion of the results. HT: planned the study, analyzed data and participated in the discussion of the results. TH: supervised the project, participated in the discussion of the results, and edited the manuscript. All authors read and approved the final manuscript.

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The 2nd Step

The current circadian typology and sleep habits of people who experienced the Great Hanshin-Awaji Earthquake in their childhood

Introduction

Disasters and PTSD

On 17th January 1995, the Great Hanshin-Awaji earthquake with a magnitude of 7.2 as the Richter scale attacked Hanshin-Awaji area in Japan. The earthquake killed 6,434 people [1]. Many studies investigating the psychological states of natural disaster victims, especially earthquakes such as in Italy [2], Armenia [3], and China [4], have been demonstrated.

Kato et al. [5] assessed the frequency of short-term, post-traumatic symptoms among survivors of the Hanshin-Awaji earthquake using the Post-Traumatic Symptom Scale. This paper reported that subjects experienced sleep disturbances, depression, hypersensitivity, and irritability during the third week after the earthquake.

Half and three years after a severe earthquake (7.3 on the Richter scale) in Taiwan, the estimated rate of survivors with posttraumatic stress symptoms (PTSS) was 23.8% and 4.4%, respectively, and PTSS scores were highly correlated with QOL scores (less severe symptoms with higher QOL) [6].

Even 4 years after the Parnitha earthquake in Greece, 22% of survivors reported subjective distress and intense fear during the earthquake, and participation in rescue operations positively correlated with greater post-earthquake psychological stress [7]. The psychological consequence of earthquakes may be serious and long-lasting even when the magnitude of the earthquake is moderate [7].

Circadian Typology and Sleep Habits

The ongoing 24-hour commercialization society accelerated shifting to evening- typed life for many people [8]. As a result, 24-hour commercialization society may reduce the amplitudes of environmental daily cycles of light, social activities, and food intake. Persistent lower amplitudes of several zeitgebers may induce an inner-desynchronized protocol between the main clock, which drives the autonomous nervous system, and the slave clock, which controls the sleep-wake cycle (Double-oscillations theory [9]). So, evening-typed diurnal rhythms, which are linked to the shortage of sleep due to late bedtimes and also poor quality of sleep [10-15], may lead to lower moods and higher levels of irritation than morning-typed ones [16].

PTSD, Sleep Disturbance and Circadian Typology

A previous study to assess the quality of sleep and its architecture in injured victims of traffic accidents one year after the accident found that the problem in PTSD is mainly sleep misperception rather than actual sleep alteration [17]. On the other hand, Wang et al. [18] reported that sleep disturbance is one of 4 factors indicating PTSD symptoms as assessed by the Impact Event Scale-Revised (IES-R) and the main symptom exhibited in PTSD. Some studies were consistent [19, 20].

Previous study aims to determine the relationship between Post Traumatic Stress Disorder (PTSD) and current circadian typology and sleep habits of adults who experienced the Great Hanshin-Awaji Earthquake after becoming adults [21]. Kuroda et al. [21] found that people who damaged strongly from a disaster and who currently show severe PTSD symptoms are more evening-typed and have a lower quality of sleep. However, whether PTSD symptoms of young people who suffered the Hanshin-Awaji earthquake in childhood can be reduced by the morning-typed life was remained to be studied. This study aims to determine the relationship between Post Traumatic Stress Disorder (PTSD) and current circadian typology and sleep habits of people who experienced the Great Hanshin-Awaji Earthquake in their childhood in January 1995.

Participants and Methods

Questionnaire Study

An integrated questionnaire was administered to 275 people aged 19-37 (mean age: 21.9 years) in Hyogo Prefecture (35°N), Japan in March 2012, with responses received from 275 people (females: 173, males: 93, unknown: 9) which were all available for analysis. The questionnaire consisted of basic questions about attributes such as age and sex, questions on sleep habits and sleep quality, the Torsvall-Åkerstedt Diurnal Type Scale [22] and a Japanese version [23] of the Impact of Event Scale- Revised (IES-R) which has been usually used as PTSD scores [24] composed of 22 questions, 8 questions related to “intrusion”, 6 on “hyper-arousal” and 8 on “avoidance-numbing” (**Table 1**). The original questions on sleep habits which Harada et al. [25] originally constructed have been used in several papers [26-31].

Statistical Analysis

The data was statistically analyzed using Mann-Whitney U-tests and Pearson’s correlation analysis with SPSS 20.0 statistical software. Diurnal Type scale scores were expressed as means plus or minus the standard deviation (Mean ± SD).

Criterion for High and Low Damage Groups

Participants were divided into a High Damage Group (HDG) and a Low Damage Group (LDG) based on the IES-R scores. Participants who scored 25 or more in the IES-R were placed in HDG and those who scored less than 25 were placed in LDG.

The study followed the guidelines established by the *Chronobiology International* Journal for the conduct of research on human subjects [32]. Before administrating the questionnaires, each participant was given a written explanation that detailed the concepts and purposes of the study and stated that their answers would be used only for academic purposes. After the above explanation, all participants agreed completely with the proposal. The study was also permitted by the ethic committee in the Laboratory of Environmental Physiology, Graduate School of Integrated Arts and Sciences, Kochi University which carried out an ethical inspection of the contents of the questionnaire

Results

55 participants (20.8%) scored 25 or more can be decided to be the persons who have PTSD symptoms **(Figure 1).** There is no significant difference of bedtime, wake-up time, and sleep duration in weekday between HDG (High Damage Group) and LDG (Low Damage Group).

HDG participants exhibited significantly worse sleep quality than LDG participants (Mann-Whitney U-test: Z=-4.637, p<0.001) **(Figure 2)**. There was no significant difference in sleep latency (p>0.05) (χ^2 test: χ^2-value=0.481, df=2, p=0.786). HDG participants woke more frequently during sleep **(Figure 3)** and had more difficulty in falling asleep than LDG participants (woken during sleep: χ^2-value=48.517, df=1, p<0.001, difficulty in falling asleep: χ^2-value=13.378, df=2, p=0.001).

HDG participants exhibited significantly worse subjective sleep quality than LDG participants (Mann-Whitney U-test: z=-3.348, p=0.001) **(Figure 4)**. LDG participants fell asleep easily and slept deeply with higher frequency than HDG participants (p=0.005, p=0.011), although there was no difference in mood at awakening in the morning between the two groups (p>0.05).

LDG participants tended to be more morning-typed than HDG participants (HDG: 14.17, LDG: 15.12, z=1.659, p=0.097) **(Figure 5)**. Despite of degree of PTSD, significant positive correlation was shown between morning-typology and high sleep quality (HDG: ME versus Monroe r=-0.420, p=0.002, ME versus Subjective QOS r=-0.580, p<0.001; LDG: ME versus Monroe r=-0.304, p<0.001, ME versus Subjective QOS r=-0.359, p<0.001).

Significant relationship of ME scores didn't appear to the IES-R score (ANOVA Variable factor: ME score, p=0.710), while that of Monroe's QOS did so (Higher quality of sleep with lower IES-R score; ANOVA Variable factor: Monroe, p<0.001).

Discussion

In this study, 20.8% participants scored 25 or more can be decided to be the persons who have PTSD symptoms. Kun et al. reported that the estimated prevalence rate of PTSD symptoms of younger adults was 28.6% (n=364; <15-34 years) [33]. Their survey was conducted three months after the 2008 Sichuan earthquake. Another previous study which was undertaken 15 months after the same earthquake reported that the prevalence rate of PTSD symptoms of younger adults was 8.0% (n=138; <60 years) [34]. In this study, estimated PTSD symptoms remains even 17 years after the Great Hanshin-Awaji Earthquake. A previous study found that PTSD became chronic in 46% of all patients who developed the disorder [35], and Kessler et al. reported that one-third of individuals who developed PTSD after traumatic event did not have remission of the disorder after ten years [36]. Whether the rate of PTSD in children which suffered such disaster was kept for long years has been remained unclear. Anyway, results of this study suggested that long-lasting mental health care services were required by the people who experienced severe disasters.

This study showed that there was no correlation between diurnal type and PTSD symptoms directly, but morning-typed participants had significantly good sleep quality. And it also resulted in that people who suffered the Great Hanshin-Awaji earthquake in childhood and, moreover, currently have PTSD had difficulty in achieving high sleep quality. A previous study was conducted to investigate the relationship between PTSD and current circadian typology and sleep habits of adults who exposed to the Great Hanshin-Awaji Earthquake after becoming adults. They reported that old people who showed severe PTSD symptoms had a lower quality of sleep and were more evening-typed [21]. Because the PTSD symptoms are relatively moderate in young adults, the relationship between circadian typology and the IES-R score might be unclear in this study.

There are three possible hypothetical and physiological pathways to reduce PTSD symptoms which might be reduced by the morning-typed life. The first possible mechanism is that people who had better quality of sleep could consolidate the memory system during REM sleep and delete the scarce memory of the earthquake, because dreaming during REM sleep consolidates the memorizing system in the brain [37, 38].

The second is via a high amount of serotonin synthesis in the morning due to a rich-protein breakfast [31, 39, 40] followed by light exposure after that [41]. Such high serotonin level in the brain could improve mental health in the daytime.

The third one is better inner synchronization of the main and slave clocks [42]. This strong coupling of the two clocks could improve their mental health and reduce the severity of PTSD symptoms. The clocks of morning-type people might be also well entrained to zeitgebers such as light, temperature and social cues.

Intervention to improve their quality of sleep and promote a morning-typed lifestyle may be an effective way to reduce PTSD symptoms. The causal relationship between the morning-typed life and reduced PTSD symptoms can be a research question to be tested in the future.

Conclusion and Limitation

Intervention to improve their quality of sleep and promote a morning-typed lifestyle might be an effective method to reduce PTSD symptoms. The limitation of this study is that the rate of PTSD symptoms the IES-R score reported in our study is only measured by IES-R which is self-reported tool. It is said that interpretation and generalization of the results should be treated with caution because IES-R was not designated to estimate diagnostic prevalence of PTSD [43]. Moreover questionnaire study cannot say the causal association between morning-typology and reduced PTSD. Some experimental or intervention study for the causal relationship is remained in the future.

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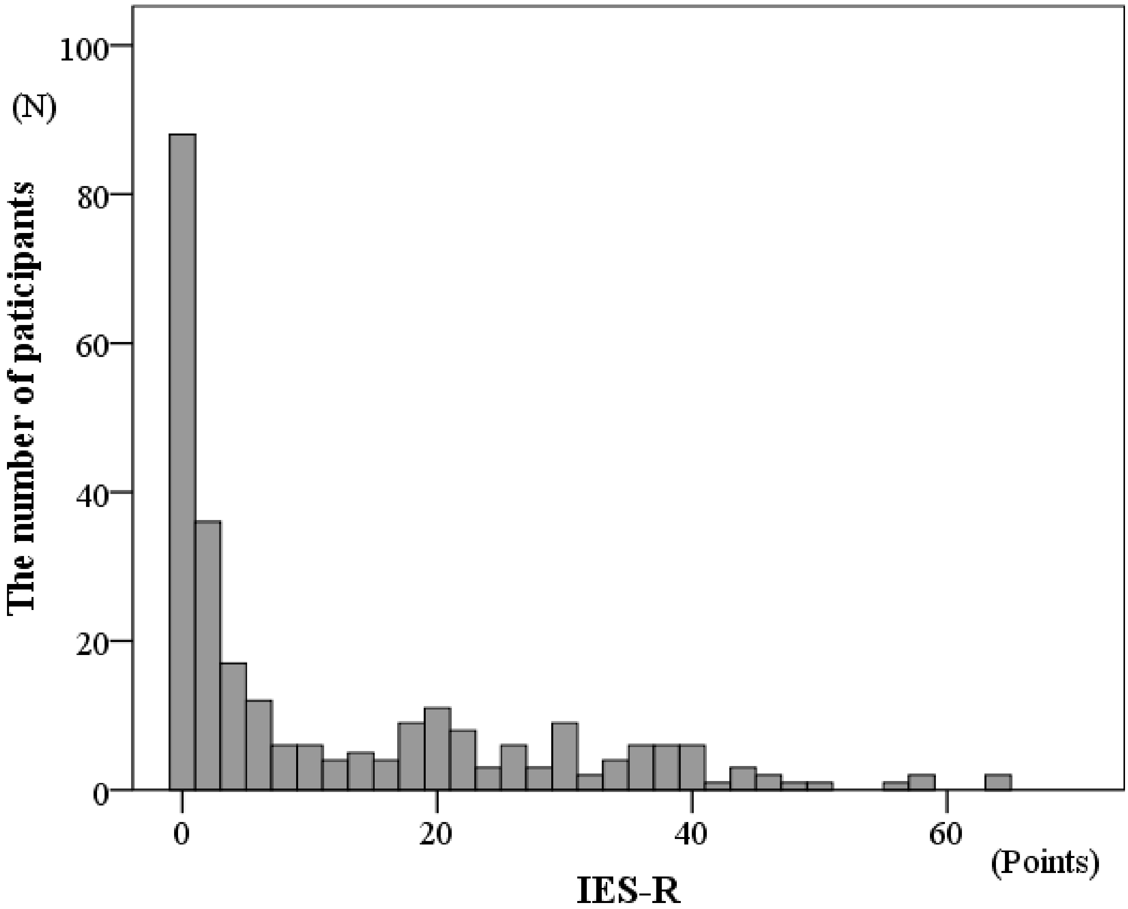

Figure 1 55 participants (20.8%) scored 25 or more on IES-R. It can be decided to be the persons who have PTSD symptoms.

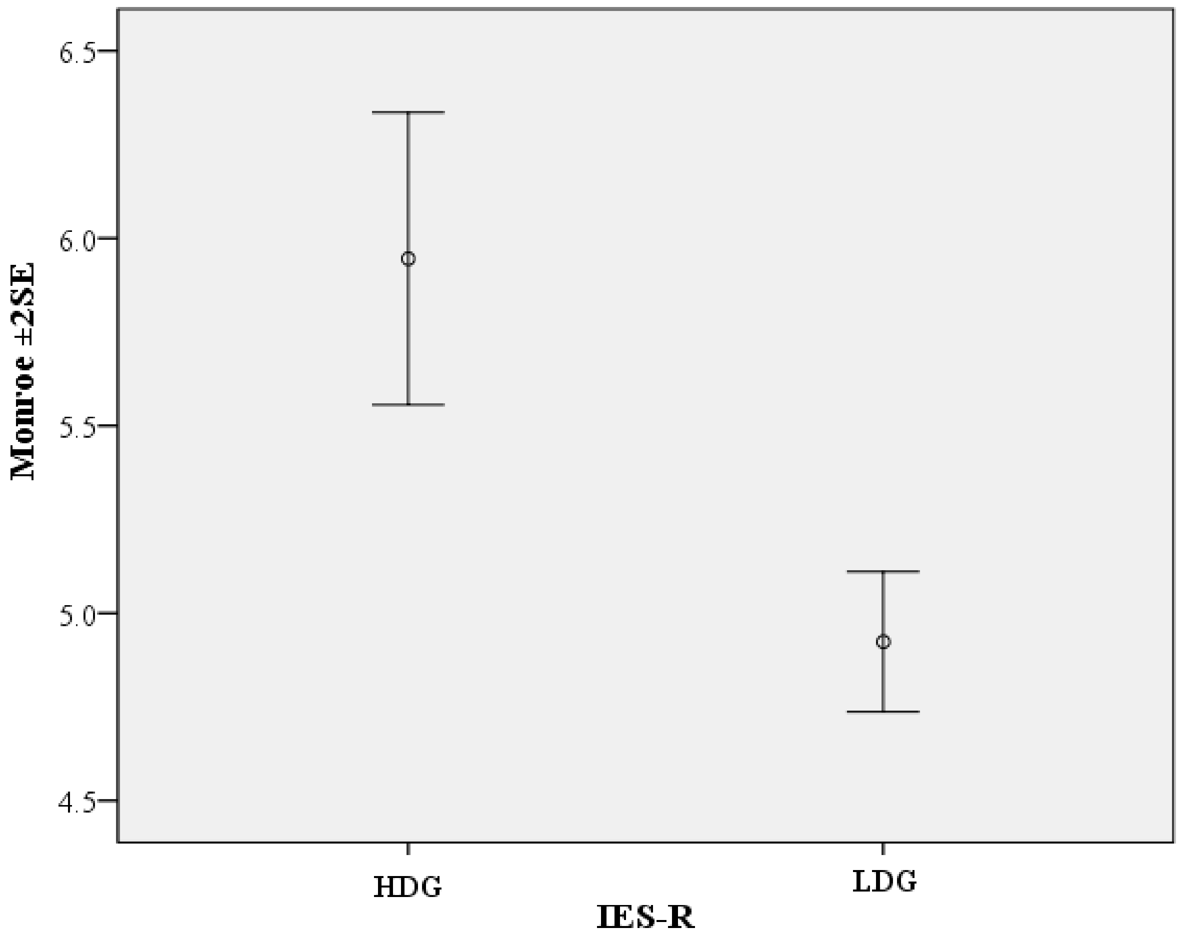

Figure 2 Comparison of current (March 2012) PTSD scores of participants of a High Damage Group (HDG) and a Low Damage Group (LDG) of the Hanshin-Awaji Great Earthquake (Jan 1995). HDG participants exhibited significantly worse sleep quality than LDG participants (Mann-Whitney U-test: Z=-4.637, p<0.001).

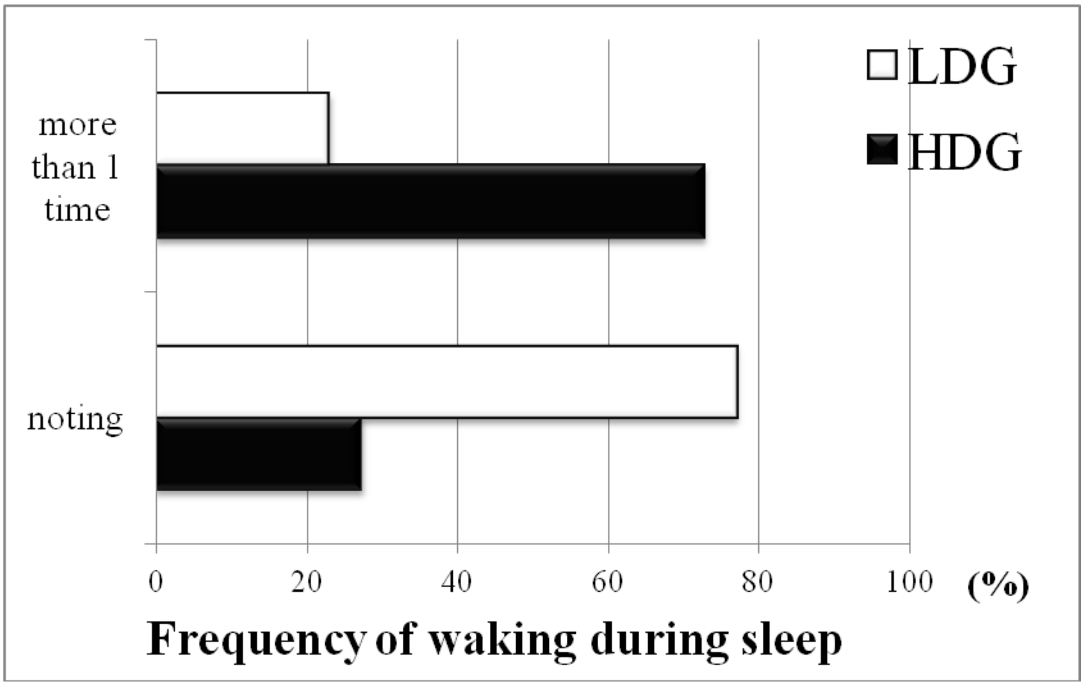

Figure 3 Comparison of frequency of waking during sleep of between High Damage Group (HDG) and Low Damage Group (LDG). HDG participants woke more frequently during sleep than LDG participants (χ^2 test: χ^2-value=48.517, df=1, p<0.001).

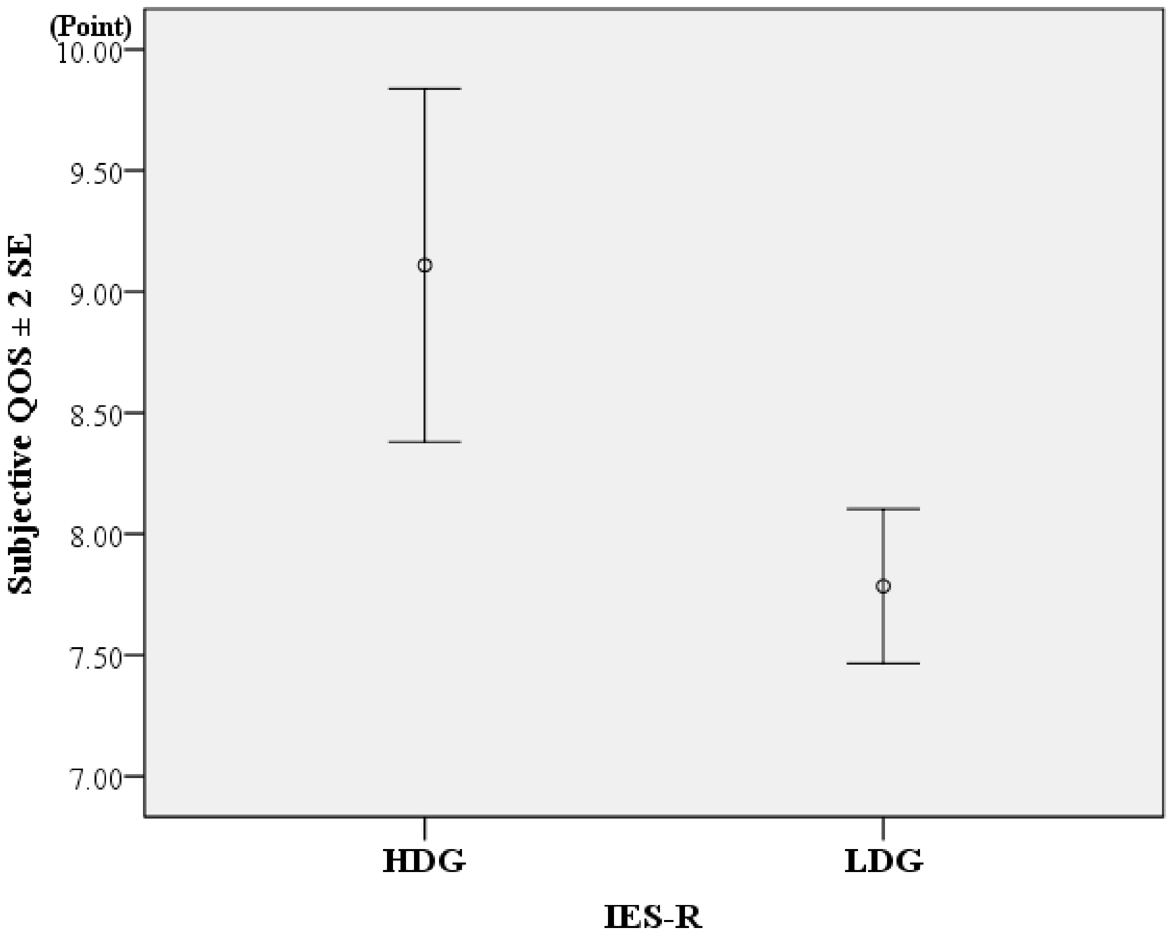

Figure 4 Comparison of subjective quality of sleep index between High Damage Group (HDG) and Low Damage Group (LDG). HDG participants exhibited significantly worse sleep quality than LDG participants (Mann-Whitney U-test: z=-3.348, p=0.001)

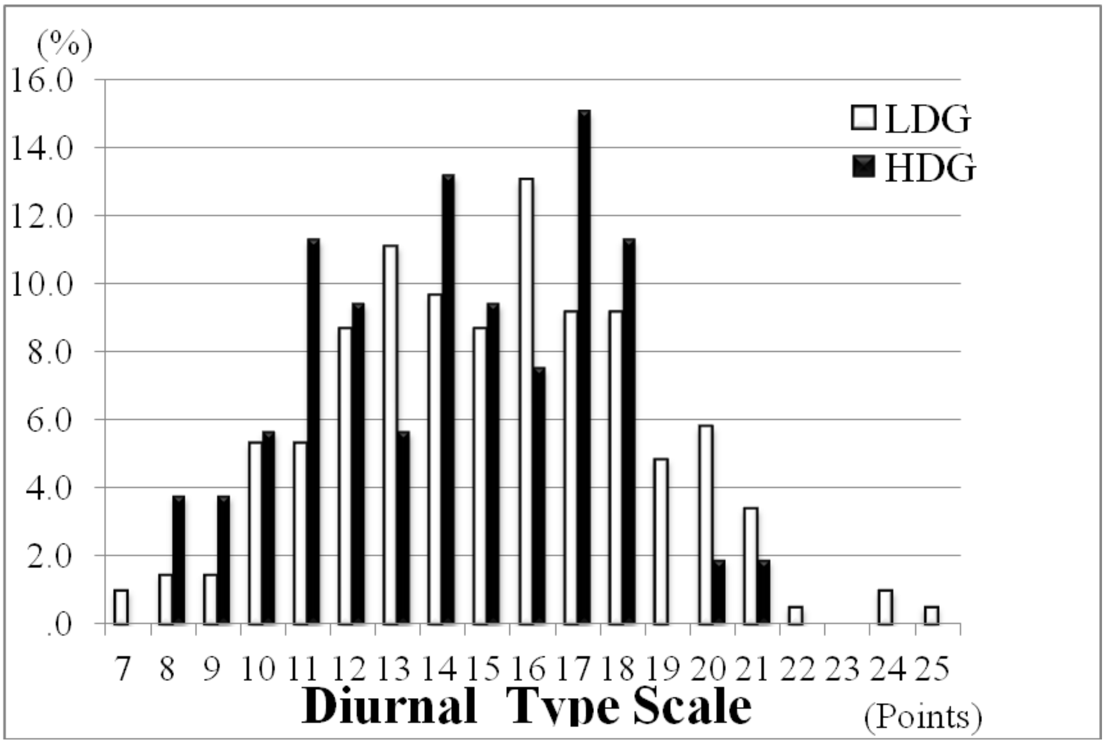

Figure 5 Comparison of diurnal type scale between High Damage Group (HDG) and Low Damage Group (LDG). LDG participants tended to be more morning- typed than HDG participants (HDG: 14.17, LDG: 15.12, Mann-Whitney U-test: z=1.659, p=0.097).

The 3rd Step

Intervention program for young people who suffered a natural disaster, Great Hanshin-Awaji Earthquake in their childhood

Introduction

Natural disaster and PTSD

Several problems in mental health of natural disaster survivors have been reviewed by many studies [1-4]. An epidemiological study was performed on a total of 271 adolescents who had been evacuated from their homes into a safe place 3 months after Typhoon Morakot in Taiwan. The prevalence of PTSD related to the typhoon was 25.8% and adolescents with PTSD had more severe depression and internalizing, externalizing, social, thought and attention problems than those without PTSD [5]. Even 4 years after the Parnitha earthquake in Greece, 22% of survivors reported subjective distress and intense fear during the earthquake and participation in rescue operations positively correlated with greater post-earthquake psychological stress [6]. The psychological consequence of earthquakes may be serious and long-lasting even when the magnitude of the earthquake is moderate [6]. At 0.5 and 3 years after a severe earthquake (7.3 on the Richter scale) in Taiwan, the estimated rate of victims with posttraumatic stress symptoms (PTSS) was 23.8% and 4.4%, respectively, and PTSS scores were tightly correlated with QOL scores (with less severe symptoms linking to higher QOL) [7]. Qu et al reported that 8 months after the Sichuan earthquake, the prevalence of PTSD symptoms was 19.9%, and the prevalence of depression was 29.0% and that earthquake experience had significant correlation with PTSD and depression [8].

PTSD, Sleep Disturbance and Circadian Typology

In general, evening-typed diurnal rhythms are linked to the shortage of sleep due to late bedtimes and also poor quality of sleep [9-14]. Morningness-Eveningness preference shows the phase relationship of circadian clocks to the environmental zeitgebers and can be an appropriate indicator of circadian phase in human circadian clocks in ordinary life [15, 16].

Sleep disturbance is one of 4 factors indicating PTSD symptoms as assessed by the Impact Event Scale-Revised (IES-R) and the main symptom exhibited in PTSD [17-19]. In the former domain, symptoms related to sleep are characterized by intrusion of traumatic memories or other threatening themes into dreams. Insomnia and nightmares, an extreme manifestation of this problem, have even been referred to as the “hallmark” of PTSD [20]. On the other hand, the study to assess the quality of sleep and its architecture in injured victims of traffic accidents one year after the accident reported that the main problem in PTSD is sleep misperception rather than actual sleep alteration [21].

Previous study aimed to determine the relationship between PTSD and current circadian typology and sleep habits of adults who experienced the Great Hanshin-Awaji Earthquake (on 17th January 1995) after becoming adults [22]. The study reported that people who had suffered severe damage from a disaster and who currently showed severe PTSD symptoms were more evening-typed and had a lower quality of sleep. Wada et al. (unpublished) indicated that people who had suffered a disaster in childhood and currently had PTSD also showed a difficulty in achieving high sleep quality. As a hypothesis, these people with PTSD are possible to be treated by an intervention to improve their quality of sleep and promote a morning-typed lifestyle, and this intervention might become an effective method to reduce PTSD symptoms.

This study aims to evaluate the effects of an intervention on sleep and mental health including PTSD symptoms of such young participants using a leaflet on “Go to bed early, Get up early and Do not forget to have a breakfast” and a sleep diary.

Participants and Methods

The intervention program was administered to 96 subjects belonging to school of nursing. To estimate the effects of the one month intervention, integrated questionnaires were administered to all participants two times: before the start of the intervention period and one or two months after the end of the intervention period (See legend of **Figure 1**). Ninety-four of 96 (98%) participants answered the first questionnaire and 72 of 94 (77%) kept sleep diaries for 21 days and answered the second questionnaire. Participants aged 38 or more were eliminated. Finally 66 participants (male: 7, female: 57, unclear: 2, 19-37 years old, average age: 22.73) were used for analysis.

All participants were asked to keep a sleep diary throughout the 21 days of the intervention period, which was in November or December of 2012. To counterbalance the effect of the day length, two intervention periods were set across the winter solstice. Twenty-three subjects participated in the intervention in November and 43 participants did in December. The sleep diary consisted of questions on sleep habits (including falling asleep time and awakening time), physical condition, mood on awakening, awakening frequency during night sleep, and the implementation status of 5 contents of intervention, which were as follows: Exposed to early morning sunlight (1st), Protein rich food taken at breakfast (2nd), Exposed to sunlight after breakfast (3rd), Cut back on the time to watch TV at night (4th), Cut back on the time to be exposed to fluorescent light at night (5th).

Participants marked their implementation status every day. For the 1st and 3rd contents, they marked circle or × for "Yes" or "No" and answered how many minutes they had been exposed to sunlight. For the 2nd content, they chose one from four choices for breakfast contents (a double circles: they had nutritionally well balanced breakfast; a circle: they had breakfast including staple diet; a triangle: they had something; a ×-marking: they had nothing). For the 4th content, they marked a circle when they had not watched the TV program. If they had watched it on TV, they did a ×-marking and filled how many minutes they had watched. For the 5th content, they filled how many hours they had stayed under fluorescent light.

For the statistical analysis, the "implementation score" was calculated. A circle of the 1st, 3rd, and 4th contents meant 1 point and a x-mark was 0 point. A double circle of the 2nd content was 1 point, a circle was 0.67 points, a triangle was 0.33 points, and a ×-mark did 0 points. On the 5th content, "0 hour" converted to 1 point and "the maximum hours" among all participants each day converted to 0 point. The other hours were rationally converted to the mark distributed from 0 and 1 points, in each day.

The 21-day-long intervention period was divided into 3 parts (FWP: First week period, MWP: Middle week period, LWP: Last week period). The "high implementation group (HIG)" was defined as 50% participants who marked higher implementation scores in each or all of 5 contents. The other 50% participants group was defined as "the low implementation group (LIG)".

The questionnaire administered before the intervention consisted of basic questions about attributes such as age and sex, the diurnal-type scale constructed by Torsvall and Åkerstedt [23], questions on sleep habits, quality of sleep, and a Japanese version [24] of the Impact of Event Scale- Revised (IES-R) which had been usually used as PTSD scores [25] composed of 22 questions, 8 questions related to "intrusion", 6 on "hyper-arousal" and 8 on "avoidance-numbing" (**Table 1**). One or two months after the intervention period, the second questionnaire was administered once which consisted of questions on continuous implementation of the 5 intervention-contents and questions on the change in the consciousness to "light" and "meal" in addition to all contents of the first questionnaire. The original questions on sleep habits which Harada et al. [26] originally constructed have been used in several papers [27-32].

The software used for statistical analysis was SPSS 20.0 J for Windows (SPSS Inc., Chicago, IL, USA). χ^2-test was used for categorized variables and Mann-Whitney U-tests was used for ranked variables. Pearson's correlation analysis was performed to test the relationship between two numerical variables.

The study followed the guidelines established by the Journal, *Chronobiology International* for the conduct of research on human subjects [33]. Before administrating the questionnaires, each participant was given a written explanation that detailed the concepts and purposes of the study and stated that their answers would be used only for academic purposes. After the above explanation, all participants agreed completely with the proposal. The study was also permitted by the ethic committee in the Laboratory of Environmental Physiology, Graduate School of Integrated Arts and Sciences, Kochi University which carried out an ethical inspection of the contents of the questionnaire

Results

Only 1 person was over the cutoff point (between 24 and 25) before intervention (**Figure 2**). This high-traumatic person's comprehensive sleep health tended to be improved through the intervention (Wilcoxon test: z=1.941, p=0.052) (**Table 1**).

The others' (non high-traumatic persons) diurnal type scale before intervention was significantly lower (evening-type) than that after intervention (Wilcoxon test: z=2.004, p=0.045) (**Figure 3**). Sleep latency of the others was significantly shortened through the intervention (z=-2.004, p=0.045) (**Figure 4**).

The wake-up time of the high implementation group on the 2nd content (Protein rich food taken at breakfast) tended to become earlier than that of the low implementation group (Mann-Whitney U-test: z=1.807, p=0.071). Monroe's sleep quality index in the high implementation group on the 3rd content (Exposed to sunlight after breakfast) was significantly improved (z=2.540, p=0.011) and their sleep latency tended to be shorten than those of the low implementation group (z=1.902, p=0.057). Participants in the high implementation group on the 4th content (Cut back on the time to watch TV at night) significantly shifted morning typed than those in the low implementation group (z=-2.518, p=0.012). Subjective sleep quality index of the high implementation group of the 5th content (Cut back on the time to be exposed to fluorescent light at night) was significantly more improved than that of the low implementation group (z=2.159, p=0.031) (**Table 2**).

Discussion

It was difficult to verify the effect of intervention to ease-up the PTSD symptoms because of absence of the participants who had severe PTSD. In the previous intervention study focused on the elderly who experienced the Great Hanshin-Awaji Earthquake (on 17th January 1995) after becoming adults, the prevalence of PTSD was 31.7% (Kuroda, Wada et al., unpublished). Jia et al. found that there were significant distinctions in the prevalence of PTSD (22.5% vs. 8.0%) and general psychiatric morbidity (42.0% vs. 25.4%) between elder and younger adult survivors after an earthquake [34]. They concluded that compared with the younger adults, the elderly survivors were more likely to develop PTSD and general psychiatric morbidity [34]. The underlying mechanisms that the elderly were more likely to develop psychological problems after disasters are still unclear. But Pekovic et al. argued that due to the fact that an elder often already feels frail because of chronic health conditions, impaired cognitive abilities and decreased sensory awareness, the impact of an unexpected disaster may be overwhelming [35]. Additionally Kessler et al. found reported that one-third of individuals who develop PTSD after trauma did not have remission of the disorder after ten years [36]. The disaster-related impact seems more severe and lasting than we thought when the elderly were involved in the impaired psychological recovery [37]. It had past seventeen years since the Great Hanshin-awaji Earthquake. The reason of absence of the young participants who had severe PTSD might be related to that the post-disaster vulnerability to PTSD could be age-dependent.

On the other hand, the remains of participants significantly shifted their circadian phase in advance in the sleep-wake rhythms and got better quality of sleep. Intervention study of the same sort for university students belonging to football club concluded that the combined intervention on breakfast, morning sunlight and evening-lighting seems to be effective for students including athletes to keep higher melatonin secretion at night, because this intervention successfully induced easy onset of the night sleep and higher quality of sleep in these athletes [38]. Another previous intervention study (using a leaflet introducing the benefits of morningness and technical issues to make morning-typed life) for the elderly who experienced the Great Hanshin-Awaji Earthquake when they were adult reported that intervention to improve participants' quality of sleep and promote a morning-typed lifestyle may be an effective way to reduce PTSD symptoms (Kuroda, Wada et al., unpublished). These results suggested that the leaflet intervention would be an effective way to ease up PTSD symptoms for people who suffered natural disaster in childhood and have currently severe PTSD.

Conclusion and Limitation

Intervention to improve their quality of sleep and promote a morning-typed lifestyle may be an effective method to reduce PTSD symptoms. The limitation of this study is absence of the young participants who had severe PTSD in this study. Because of this it is difficult to verify the effect of intervention to PTSD symptoms. It is remained for future studies to test the effect of the leaflet intervention on the ease-up of PTSD in the young people who suffered the East-Japan Great Earthquake on 11th March, 2011.

Acknowledgements

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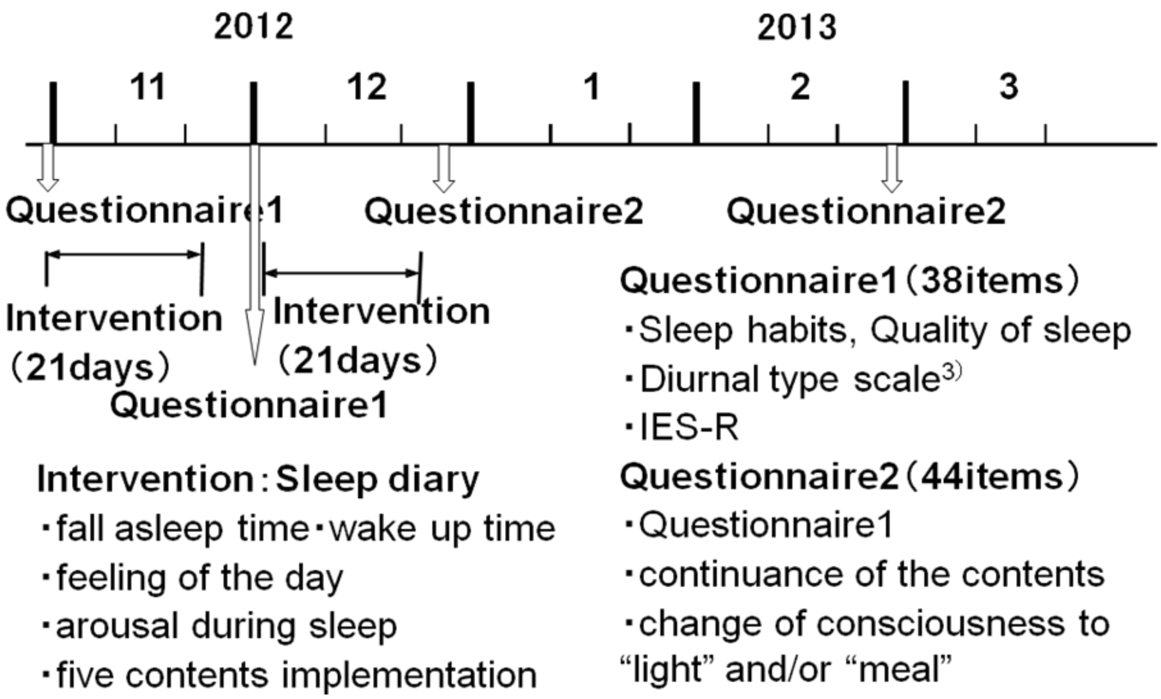

Figure 1: Schematic presentation of the schedule of the intervention program.

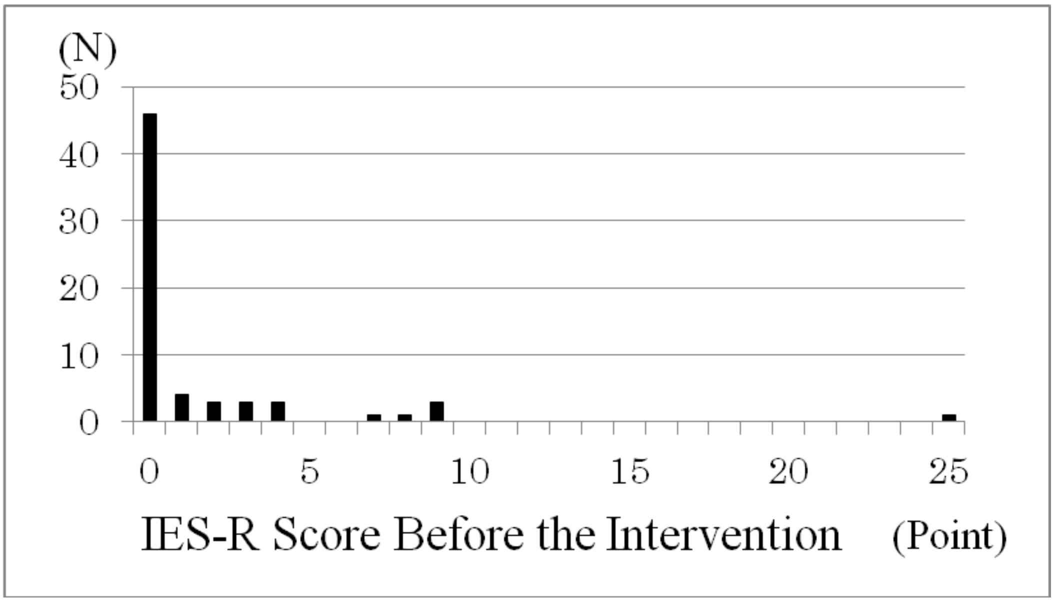

Figure 2: IES-R score before the intervention. Only 1 person was over the cut-off point (between 24 and 25).

Table 1: Change in comprehensive variables on sleep health of the high-traumatic person with the IES-R score of 25. This sleep health of this person tended to be improved through the intervention (Wilcoxon test: z=1.941, p=0.052).

Variables	Before	After	Change
Bed time (h)	25.00	24.50	Improved
Fall asleep time (h)	25.50	25.25	Improved
Awakening time (h)	6.00	6.00	Stable
Sleep duration (hs)	4.50	4.75	Improved
Middle point (h)	3.75	3.63	Improved
ME-Awakening (p)	12	6	Worsen
ME-Asleep (p)	5	7	Improved
Activity peak (p)	2	3	Improved
Intrusion (p)	8	7	Improved
Avoidance (p)	8	10	Worsen
Hyperarousal (p)	9	5	Improved
Arousal during Sleep (t)	2	1	Improved
Sleep latency (m)	30.0	45.0	Worsen
Wake up latency (m)	0.0	0.0	Stable
Difficulty falling asleep (p)	3	2	Improved
Difficulty wake up (p)	1	1	Stable
Sleep deeply (p)	3	3	Stable
Mood on awakening (p)	3	3	Stable

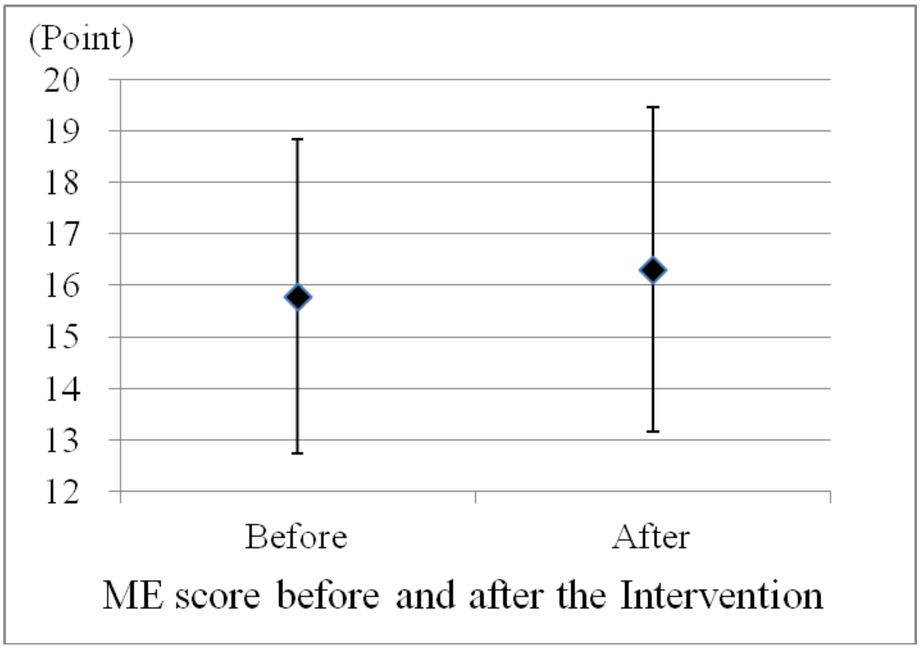

Figure 3: Comparison of diurnal type scale between before and after the intervention of non high-traumatic persons who showed less than 25 of IES-R score. The diurnal type scale after intervention was significantly higher (more morning-typed) than that before the intervention (Wilcoxon test: z=2.004, p=0.045)

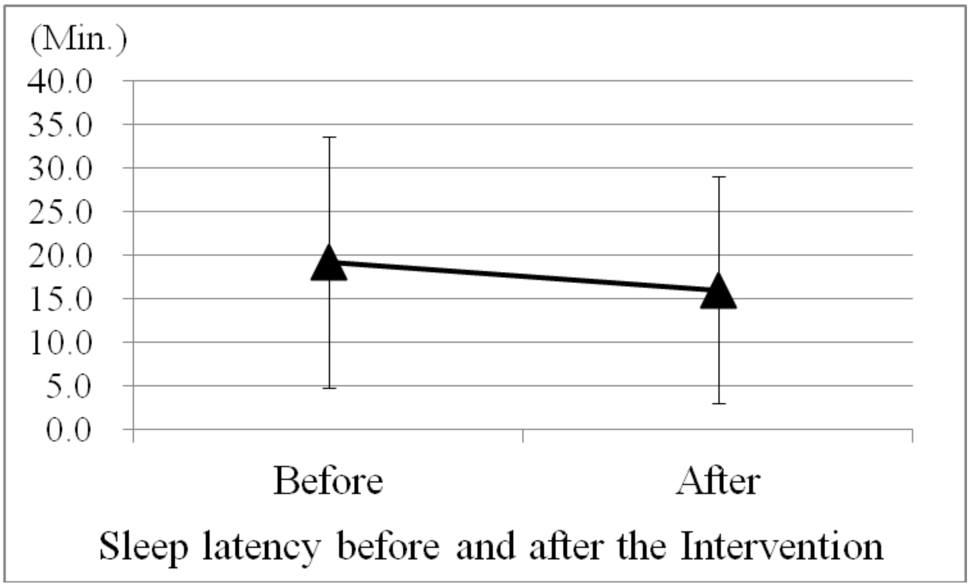

Figure 4: Comparison of sleep latency between before and after the intervention of the non high-traumatic persons. It was significantly shortened through the intervention (Wilcoxon test: z=-2.004, p=0.045).

Table 2: Contents-segregated and cross-sectional analysis on the effects of the intervention to the 65 subjects on the variables of sleep health and circadian typology. The results of Mann-Whitney tests were shown on whether the high-implementatsion group was more impromed than the low-impementation one in each item.

◎: sig improve ($p<0.05$)　　○: tend improve ($0.05 \le p<0.1$)

	Awakening time	Sleep latency	ME score	Monroe	Quality of sleep
1st content (Early morning sunlight)					
2nd content (Protein rich breakfast)	○				
3rd content (Sunlight after breakfast)		○		◎	
4th content (Limitation watch TV			◎		
5th content (Limitation use fluorescent)					◎

The 4th Step

The current circadian typology and sleep habits of people who experienced the Landslide in Philippines

Introduction

Natural disaster and PTSD

Mental health problems of natural disaster survivors have been reviewed by many studies [1-7]. Posttraumatic stress disorder (PTSD) is the most frequently reported psychiatric morbidity among victims of natural disasters [8]. An epidemiological study was performed on a total of 271 adolescents who had been evacuated from their homes to a safe place 3 months after Typhoon Morakot in Taiwan. The prevalence of PTSD related to the typhoon occupied 25.8% of all victims and adolescents with PTSD had more severe depression and internalizing, externalizing, social, thought and attention problems than those without PTSD [9]. Even 4 years after the Parnitha earthquake in Greece, 22% of survivors reported subjective distress and intense fear during the earthquake and participation in rescue operations positively correlated with greater post-earthquake psychological stress [10]. The psychological consequence of earthquakes may be serious and long-lasting even when the magnitude of the earthquake is moderate [10]. At 0.5 and 3 years after a severe earthquake (7.3 on the Richter scale) in Taiwan, the estimated rate of victims with posttraumatic stress symptoms (PTSS) was 23.8% and 4.4%, respectively, and PTSS scores were tightly correlated with QOL scores (less severe symptoms linked to higher QOL) [11]. Qu et al reported that 8 months after the Sichuan earthquake, the prevalence of PTSD symptoms occupied 19.9% and the prevalence of depression did 29.0% of all victims and that earthquake experience had significant correlation with PTSD and depression [12]. As just described, a great disparity exists in the reported rates of PTSD symptoms across different studies. Such inconsistency may be attributed to the differences in the magnitude of the earthquakes, proximity to the epicenters, the time of assessment, the methods of sampling, and the measures used [3, 13].

PTSD, Sleep Disturbance and Circadian Typology

In general, evening-typed diurnal rhythms are linked to the shortage of sleep due to late bedtimes and also poor quality of sleep [14-19]. Morningness-Eveningness preference shows the phase relationship of circadian clocks to the environmental zeitgebers and can be an appropriate indicator of circadian phase in human circadian clocks in ordinary life [20, 21].

Sleep disturbance is one of 4 factors indicating PTSD symptoms as assessed by the Impact Event Scale-Revised (IES-R) and the main symptom exhibited in PTSD [22-24]. In the former domain, symptoms related to sleep are characterized by intrusion of traumatic memories or other threatening themes into dreams. Insomnia and nightmares, an extreme manifestation of this problem, have even been referred to as the “hallmark” of PTSD [25]. On the other hand, the study to assess the quality of sleep and its architecture in injured victims of traffic accidents one year after the accident reported that the main problem in PTSD is sleep misperception rather than actual sleep alteration [26].

Typhoon “Reming” induced a landslide disaster in Philippines

A landslide caused by the torrential rain attacked Albay, Philippines on November 29, 2006. The mortality was about 1000 which included missing people [27]. A lot of people lost their house and lands. Previous study determined the relationship between PTSD and current circadian typology and sleep habits of adults who experienced the Great Hanshin-Awaji Earthquake (on 17th January 1995) after becoming adults [28]. This study reported that people who had suffered severe damage from a disaster and currently showed severe PTSD symptoms were more evening-typed and had a lower quality of sleep.

This study aims, first, to estimate the rate of PTSD symptoms among survivors after 6 years from the landslide, and, second, to explore what factors had strong linkage with severe PTSD.

Participants and Methods

Questionnaire Study

An interview on the contents of an integrated questionnaire was administered in December 2012 to 109 people from two villages in Albay, Philippines. One village was attacked by the landslide disaster which occurred in 2006 on site and another was set by government as a settlement area. All of respondents were 101(collection rate was 93%). Respondents under 20 years old and 70 or more were eliminated. So, remains were 88 (male: 16, female: 72, Ave.: 41.39±12.34 [SD]). University students having experience of the interviewing for social research interviewed participants in local language.

The questionnaire consisted of basic questions about attributes such as age, sex and annual income, questions on sleep habits and sleep quality, the Torsvall-Åkerstedt Diurnal Type Scale [29] and Impact of Event Scale- Revised (IES-R) which has been usually used as PTSD scores [30] composed of 22 questions, 8 questions related to intrusion, 6 on hyper-arousal and 8 on avoidance-numbing. The original questions on sleep habits which Harada et al. [31] originally constructed have been used in several papers [32-37]. After interview, it became apparent that annual income they answered possessed insufficient reliability. Therefore, it was eliminated.

Statistical Analysis

The data were statistically analyzed using Mann-Whitney U-tests and Pearson's correlation analysis with SPSS 21.0 statistical software. Diurnal Type scale scores were expressed as means plus or minus the standard deviation (Mean ± SD).

Criterion for High and Low Damage Groups

Participants were divided into four groups based on their IES-R scores (24 or less: non PTSD, 25-48: low PTSD, 49-66: Middle PTSD, 67 or more: High PTSD).

Ethical consideration

The study followed the guidelines established by the Chronobiology International Journal for the conduct of research on human participants [38]. Before administrating the questionnaires, each participant was given a written explanation that detailed the concepts and purposes of the study and stated that their answers would be used only for academic purposes. After the above explanation, all participants agreed completely with the proposal. The study was also permitted by the ethic committee in the Laboratory of Environmental Physiology, Graduate School of Integrated Arts and Sciences, Kochi University which carried out an ethical inspection of the contents of the questionnaire

Results

80 participants (90.9%) scored 25 or more can be decided to be the persons who have PTSD symptoms**.**

Age (Pearson's correlation test: r=0.278, p=0.009: higher age with higher PTSD score) (**Figure 1**), Monroe's quality of sleep index (r=0.212, p=0.048: lower quality of sleep with higher PTSD one), and Mental health (r=-0.449, p<0.001: worse mental health with higher PTSD one) (**Figure 2**) had significant correlation between IES-R scores. QOL(r=-0.199, p=0.066) did not have significant correlation between IES-R scores. There was no significant association between IES-R scores and the diurnal type scale (r=-0.075, p=0.486).

Participants were divided into four groups based on their IES-R scores (24 or less: non PTSD, 25-48: low PTSD, 49-66: Middle PTSD, 67 or more: High PTSD). There were significant difference in Age (ANOVA: F=4.524, df=3, p=0.005, BMI (F=4.617, df=3, p=0.005), and Mental health (F=7.477, df=3, p<0.001) among the four groups. Participants in Middle PTSD group were significantly younger than Low PTSD group and High PTSD group (Bonferroni correction: vs. Low PTSD, p=0.019; vs. High PTSD, p=0.018) (**Figure 3**). There was no significant difference in age between Non PTSD and Low PTSD group or High PTSD group (Bonferroni correction: vs. Low PTSD, p=0.641; vs. High PTSD, p=0.674) (**Figure 3**). Participants in Non PTSD group had significantly higher BMI than Middle PTSD group and High PTSD group (Bonferroni correction: vs. Middle PTSD, p=0.004; vs. High PTSD, p=0.015) (**Figure 4**). Mental health of participants in High PTSD group was significantly better than the others (Bonferroni correction: vs. Non PTSD, p=0.001; vs. Low PTSD, p=0.003; Middle PTSD, p=0.032) (**Figure 5**).

Participants who had lower PTSD symptoms had dinner at regular time with higher frequency (χ^2 test: χ^2 value=14.746, df=9, p=0.098), and lost higher number of family-members and/or close-friends (χ^2 value=7.399, df=3, p=0.060) (**Figure 6, 7**).

Participants who had protein-rich food at breakfast (46.27 points) exhibited significantly lower IES-R scores than participants (57.87 points) who had no protein-rich food (Mann-Whitney U-test: z=-2.871, p=0.004) (**Figure 8**).

A significant relationship (ANOVA: F=7.874, df=1, p=0.006) between contents of breakfast and IES-R scores didn't disappear even with the covariance of Age (ANCOVA: p=0.011), BMI (p=0.006), Monroe's sleep quality index (p=0.008), regularity of dinner (p=0.005) and whether they lost or not family member (p=0.010). However, p-value was risen from 0.006 to 0.125 when ANCOVA was performed with covariance of mental health index.

Discussion

Numerous studies have examined the risk factors of PTSD among earthquake survivors. The frequently reported risk factors include female gender, older age, low level of education, degree of exposure to earthquake, death in the family, loss of friends, loss of property, greater fear during the earthquake, number of traumatic experiences, inadequate social support, and previous psychiatric illness [13, 39-41].

In this study, Age and Mental health index showed strong correlation with PTSD score. Jia et al. found that the elderly survivors were more likely to develop PTSD and general psychiatric morbidity than the younger adults [42]. They discussed that the post-disaster vulnerability to PTSD and general psychiatric morbidity is still age-dependent [42]. The underlying mechanisms that the elderly were more likely to develop psychological problems after disasters are still unclear. But Pekovic et al. argued that due to the fact that an elder often already feels frail because of chronic health conditions, impaired cognitive abilities and decreased sensory awareness, the impact of an unexpected disaster may be overwhelming [43]. The disaster-related impact seems more severe and lasting than we thought when the elderly were involved in the impaired psychological recovery [44].This current study's results did not deny that theory.

The result of χ^2 test indicated that, in addition to age and mental health index, loss of family member and dinner regularity also had significant relation with PTSD score. Death in the family has been often suggested as significant risk factor for PTSD symptoms, though the contents or extent of the risk would be inconstant. Some studies indicated that death in the family was painful event for survivors [42, 45-49], whereas other previous studies reported that loss of a family member was not a significant predictor [40, 41, 50], or was not strong but significant [13]. Chan et al. discussed that it might be led from cultural difference and whom they lost [49]. Their study suggested that loss of a child was a strong predictable factor of the severity of PTSD symptoms for parents [49]. It was not able to know whom the survivors lost in this study. It is remained to reveal the relationship between "who" were dead for survivors and PTSD-severity for future study.

In this study, the estimated rates of probable PTSD were 90.9% (25 or more on IES-R). Although such estimated rates have great differences among studies, the high rate over 90% is much higher rate than those in previous studies [9-13]. The reason why the estimated rates were extremely high is still unclear. It has been described in the first paragraph of the Discussion session that there are the eleven risk factors which have been reported frequently. Because the questionnaire used in this study had not included these contents at all, it was difficult to imply or discuss the reason why the rates of probable PTSD symptoms were extremely high. It is remained for future studies.

The most interesting result in this study was that participants who had protein-rich food at breakfast (46.27 points) exhibited significantly lower IES-R scores than those (57.87 points) who had no protein-rich food. Moreover, this significant relationship between protein rich breakfast and low IES-R scores did not disappear when ANCOVA was performed with the covariance as one of "age", "BMI", "Monroe's sleep quality index", "regularity of dinner", and "whether they lost or not family member". These would indicate that protein-rich food intake at breakfast may ease up the traumatic symptoms through the mental health improvement due to the syntheses promotion of serotonin [37] and dopamine [51]. It would be, especially, a possible hypothesis that higher serotonin synthesis in the morning at pineal from tryptophan taken at breakfast might soften the PTSD symptoms through the mental health improved. Intervention to improve their quality of sleep and promote a morning-typed lifestyle may be an effective method to reduce PTSD symptoms.

Limitation

There are several limitations to this study. First, the small sample size may have possibility to produce spurious statistical findings. Second, several additional and important factors (such as education level, number of traumatic experiences, loss of property and so on) that might affect PTSD symptoms were not included in this study. Third, the rate of PTSD symptoms reported in our study is only measured by self-rated tool. Chan et al reported that IES-R was not designated to estimate diagnostic prevalence of PTSD, so interpretation and generalization of the results should be treated with caution [49]. Forth, questionnaire study cannot make clear causal association between PTSD and some possible factors. An experimental or intervention study for the causal relationship is remained in the future.

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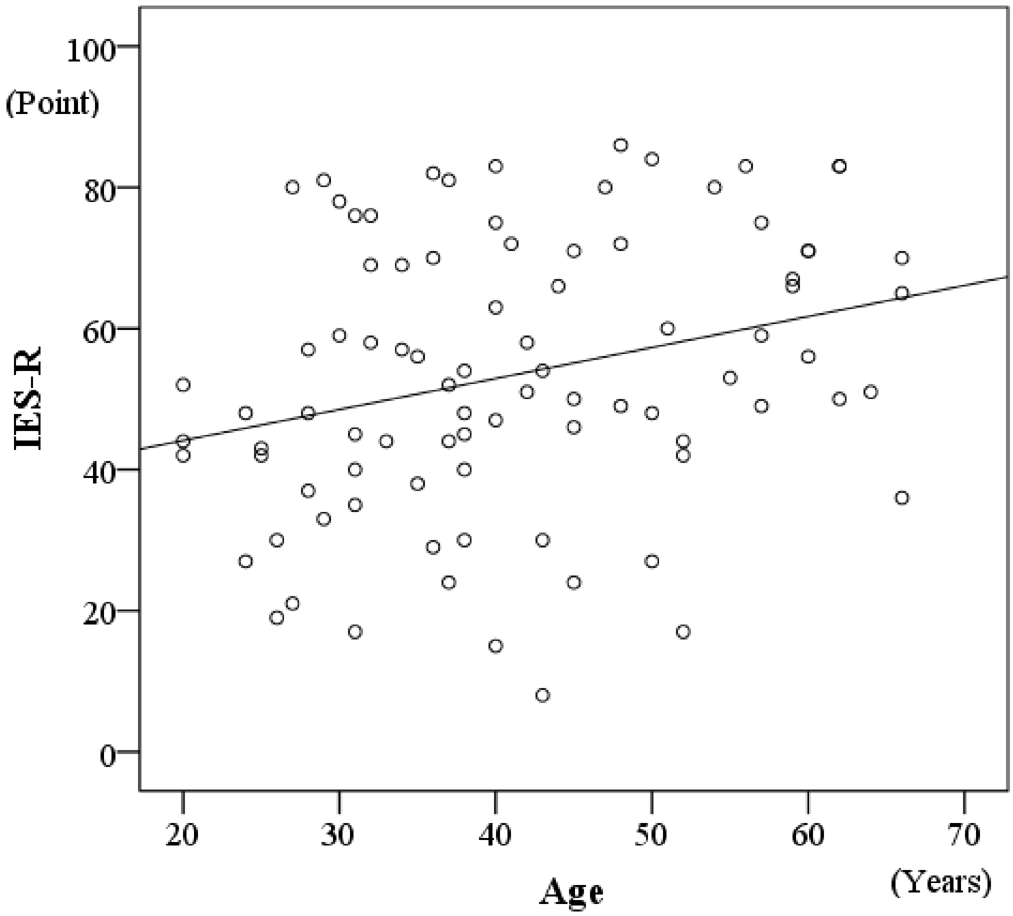

Figure 1: Significant and positive correlation between Age and IES-R score (higher score means severer PTSD) (Pearson's correlation test: r=0.278, p=0.009).

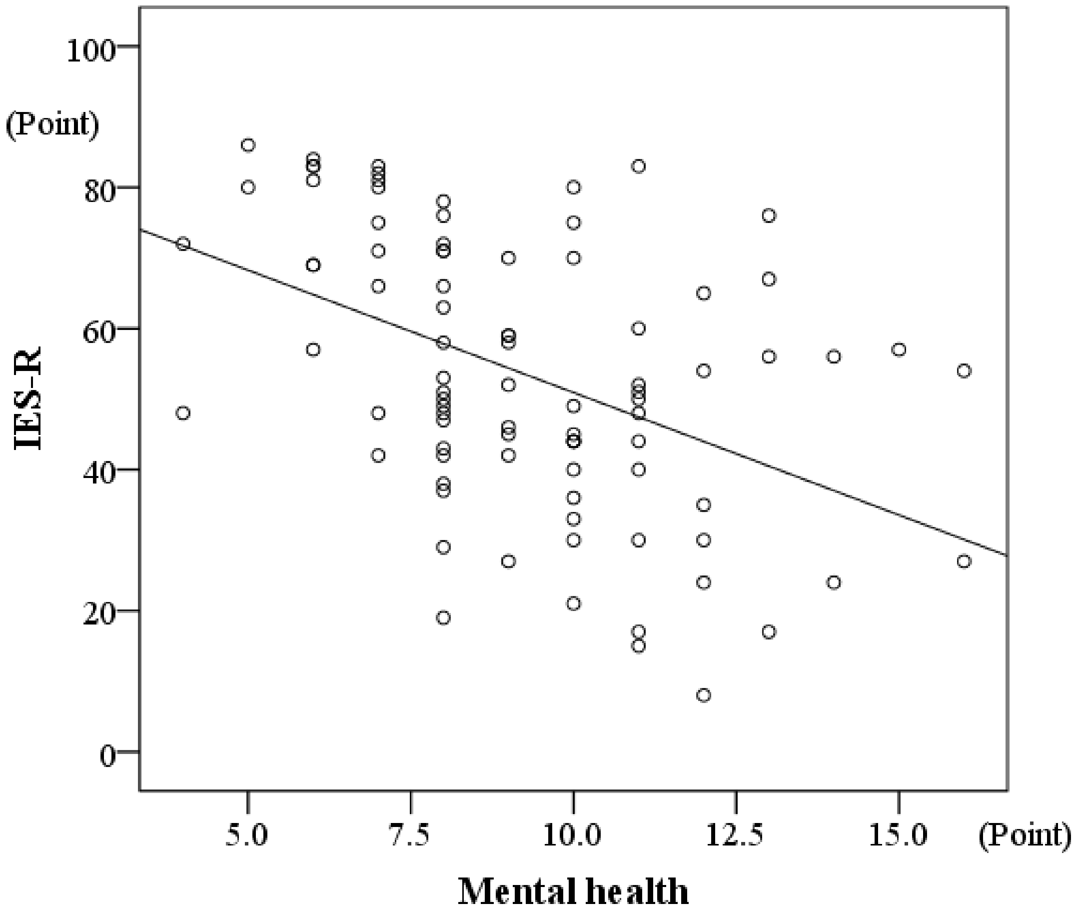

Figure 2: Significant and negative correlation between mental health and IES-R scores (higher score means severer PTSD) (Pearson's correlation test: r=-0.449, p<0.001).

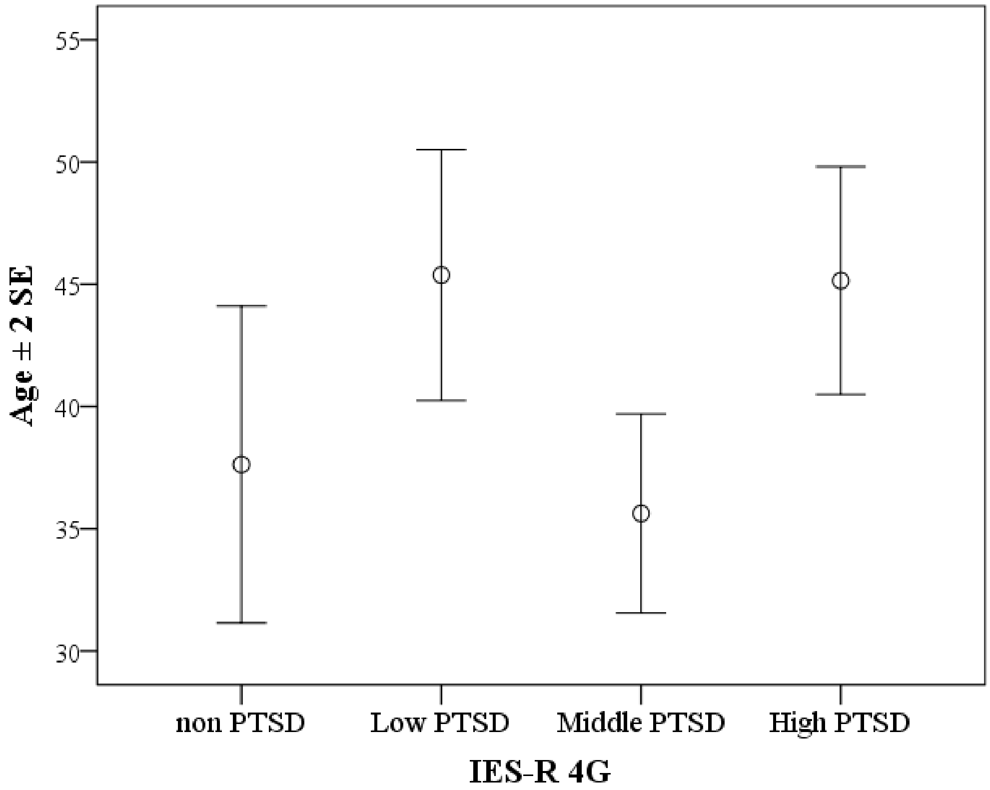

Figure 3: Relationship between age and rank of PTSD scores. Participants were divided into four groups based on their IES-R scores (24 or less: non PTSD, 25-48: low PTSD, 49-66: Middle PTSD, 67 or more: High PTSD). There were significant difference between intergroup on Age (ANOVA: F=4.524, df=3, p=0.005). Participants in Middle PTSD group were significantly younger than Low PTSD group and High PTSD group (Bonferroni correction: vs. Low PTSD, p=0.019; vs. High PTSD, p=0.018).

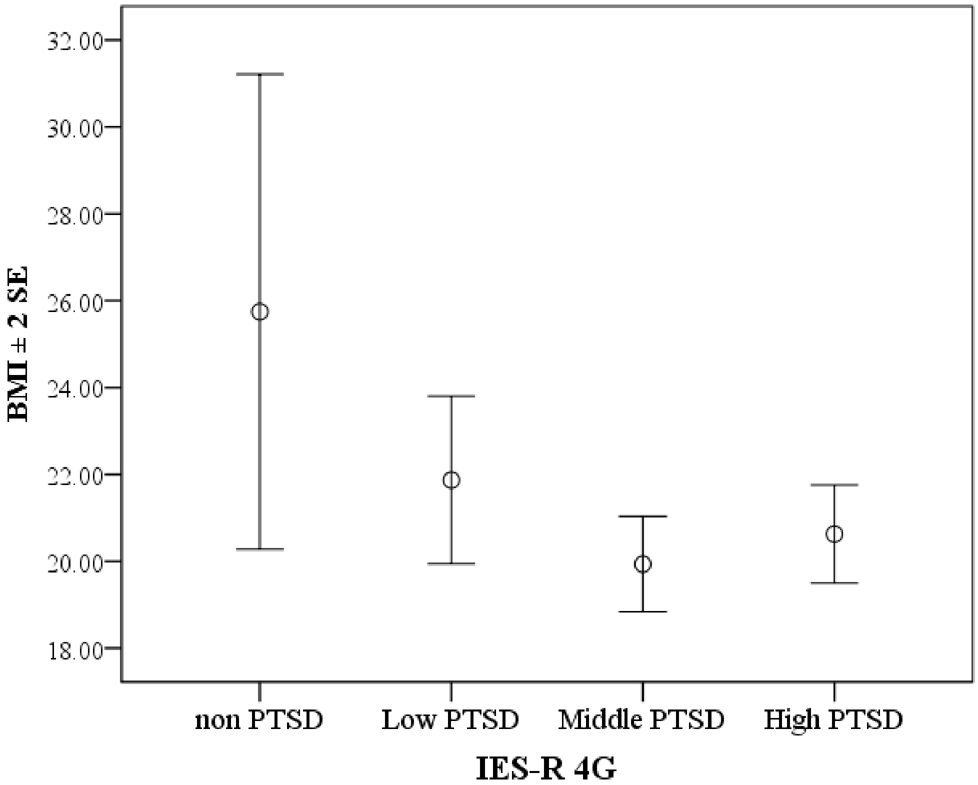

Figure 4: Relationship between BMI scores and rank of PTSD scores (ANOVA: AF=4.617, df=3, p=0.005). Participants in Non PTSD group had significantly higher BMI than Middle PTSD group and High PTSD group (Bonferroni correction: vs. Middle PTSD, p=0.004; vs. High PTSD, p=0.015).

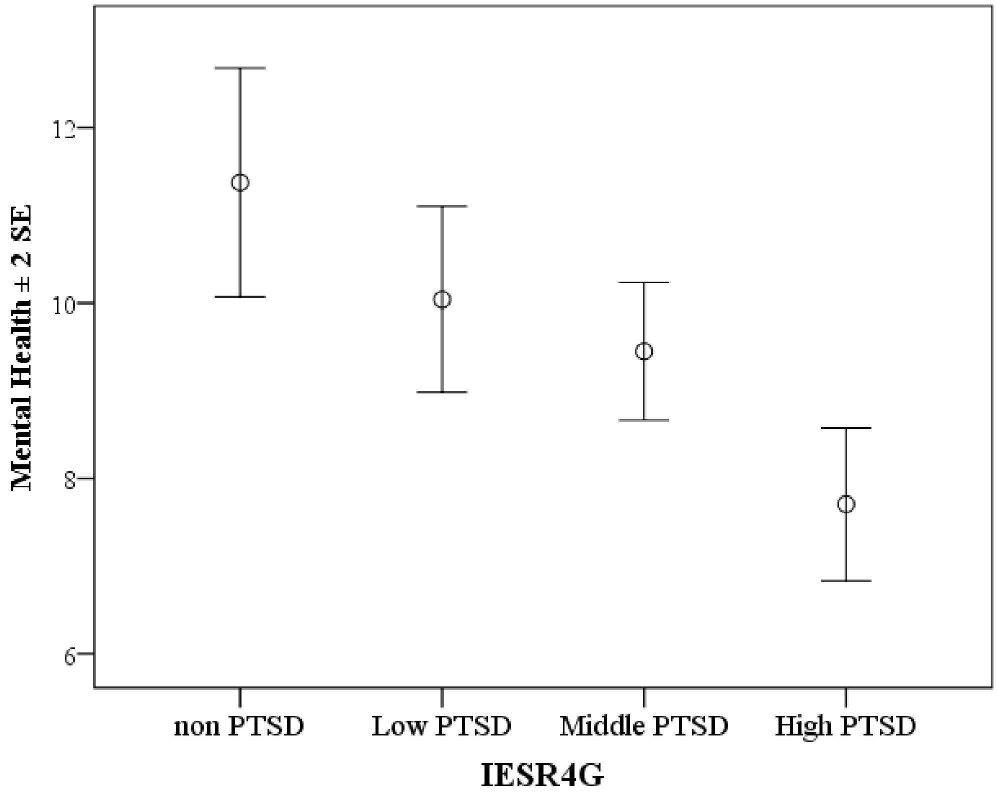

Figure 5: Relationship between mental health and rank of PTSD scores (ANOVA: F=7.477, df=3, p<0.001). Mental health of participants in High PTSD group was significantly better than the others (Bonferroni correction: vs. Non PTSD, p=0.001; vs. Low PTSD, p=0.003; Middle PTSD, p=0.032).

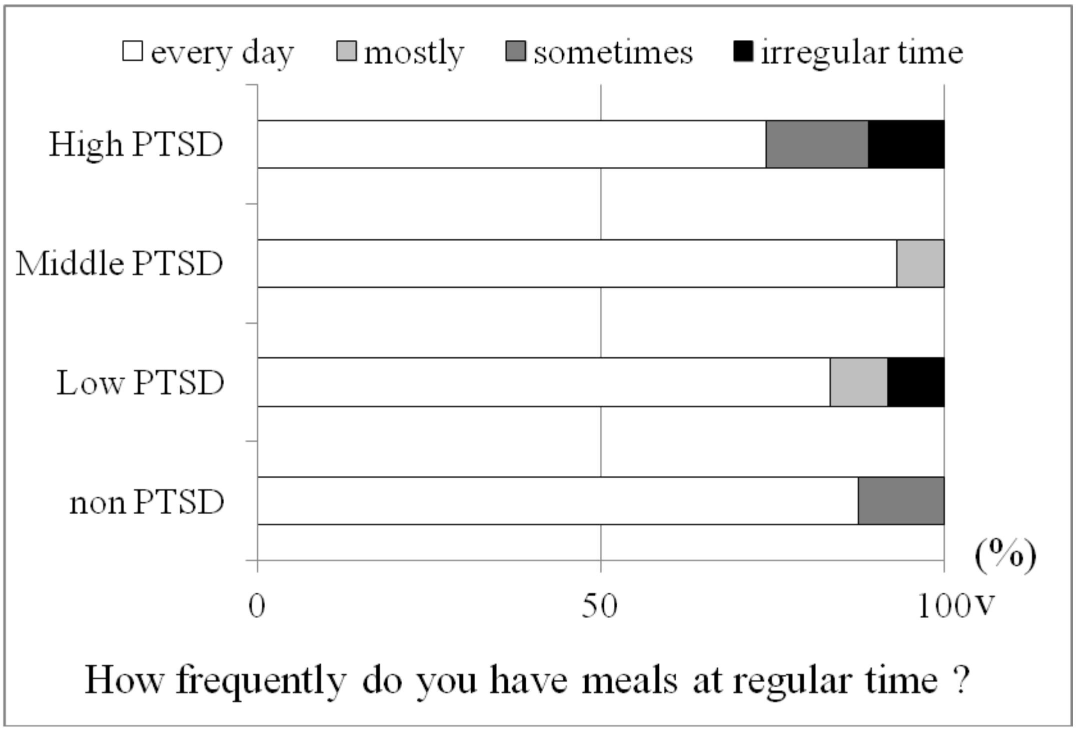

Figure 6: Relationship between regularity of dinner time and PTSD scores. High PTSD group tended to show significantly higher ratio of participants having meals at irregular time with high frequency than the other PTSD groups (χ^2 test: χ^2 value=14.746, df=9, p=0.098).

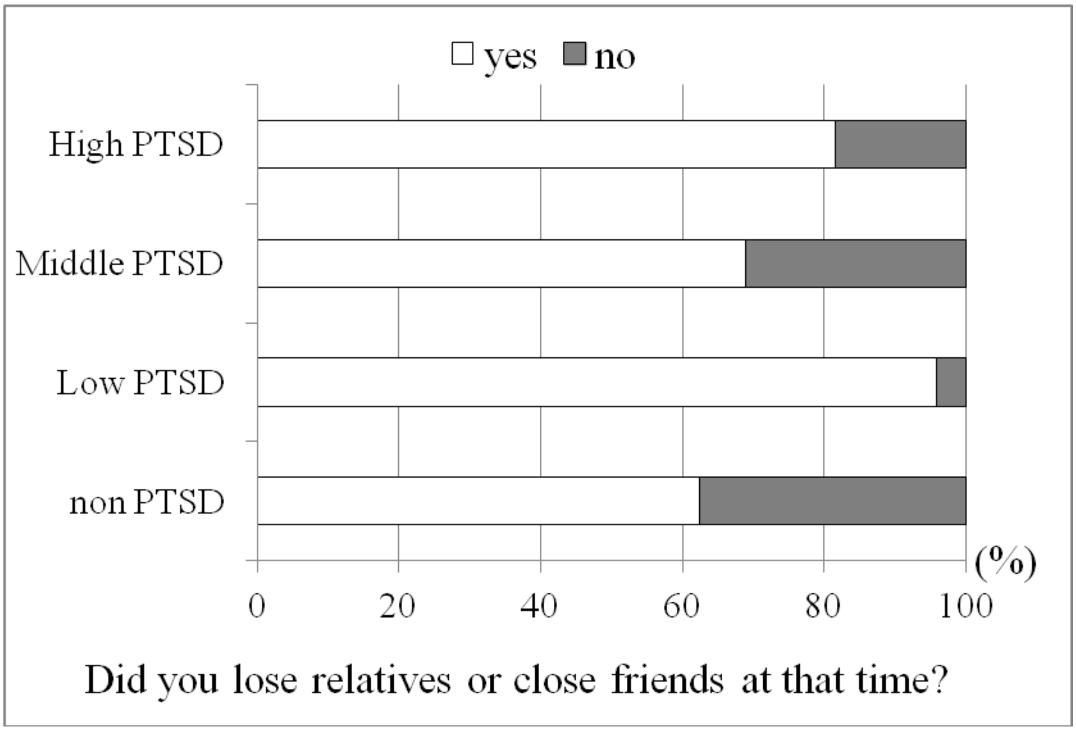

Figure 7: Relationship between whether participants lost their relative or close-friend and PTSD scores. Non PTSD group showed lower ratio of participants with a relative or close-friend lost at this disaster than the other groups (χ^2 value=7.399, df=3, p=0.060).

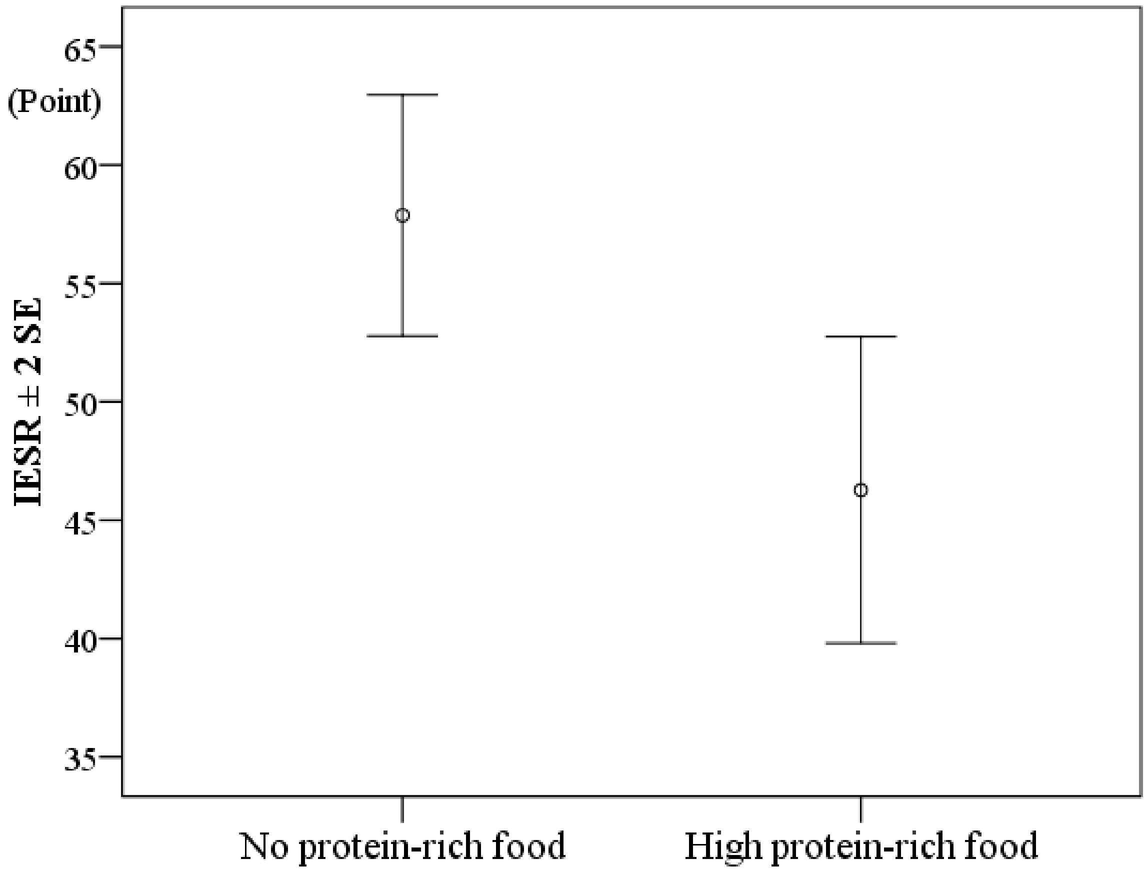

Figure 8: Participants who had protein-rich food at breakfast (46.27 points) exhibited significantly lower IES-R scores than those (57.87 points) who had no protein-rich food (Mann-Whitney U-test: z=-2.871, p=0.004)

General Discussion

General Discussion

The following hypothesis was supported by the intervention study for university student belongs to sports club and would be applied to practical life of victims who suffered current PTSD symptoms.

"A triple intervention of taking sources of tryptophan and vitamin B6 at breakfast (1), following up breakfast with exposure to sunlight (2) and the exposure to low temperature lights as night lighting (3) can stimulate the synthesis of serotonin and succeeding melatonin synthesis at night. These hormones work as natural anti-depression drugs and/or natural sleeping pills and make students more-morning typed and improve their mental health."

This intervention study for university athletes showed that the triple intervention was a powerful method for inducing secretion of high amounts of melatonin by the pineal gland in human adults in this study.

The month-long intervention experiment resulted in overall improvements of frequency of breakfast consumption and regularity of meal timings of the university soccer team members. Many students who received breakfast intervention shifted their circadian phase in advance. Moreover, they reported that students who implemented one or three contents of the triple intervention groups showed a significantly lower frequency to become angry or irritated after the intervention. Participants in the control group with no intervention did not improve such problems in mental health compared to before the intervention [1].

A joint study (Fukushige et al., submitted) was administered to examine, more directly, the tryptophan- serotonin- melatonin hypothesis. Thirty-seven healthy men (aged 21.6 ± 0.49 years) lived their own rooms in a residence for the experiment for 5 days and 4 nights. Participants were randomly divided into the two groups which have taken rich tryptophan (476mg, RICH GROUP) and poor tryptophan at breakfast (55mg, POOR GROUP). Furthermore, each group divided into two groups which were exposed to blight light (> 5000lx, BL GROUP) and dim light (< 50lx, DIM GROUP) during daytime (07:30-18:00). Under the BL condition, salivary melatonin level in the RICH group tended to be higher than that in the POOR group at 4^{th} day after start of the experiment (p=0.076). However, under the DIM condition, such tendency was not shown. It was concluded that the tryptophan intake from breakfast and the daytime bright light exposure were effective for melatonin secretion and better night sleep.

The tryptophan-serotonin-melatonin hypothesis was being valid with supports by some experimental studies. Underlying mechanisms can be hypothesized to consist of two components. The first is that serotonin synthesis from tryptophan taken at breakfast may be enhanced by the exposure to sunlight just after taking breakfast. Reports of Moore et al. (2000) [2] and Zheng et al. (2004) [3] support the hypothesis that tryptophan contained in protein and consumed at breakfast is converted to serotonin in the morning (assisted by sunlight exposure) leading to improvement in mental health. The second is that the high potential of melatonin synthesis based on the high serotonin synthesis in the pineal during daytime might be available due to the night exposure to the "low temperature light" emitted from incandescent bulbs.

Many reports have shown that melatonin secretion is suppressed by light emitted from fluorescent lamps including short wave length (with around 460 nm of wave length as absorbance peak of melanopsin) components [4-8]. This study newly implies that "an integrated effectiveness" of modifying breakfast content, receiving sunlight exposure and receiving exposure to low color temperature lighting at night can facilitate achievement of high plasma melatonin at night in humans.

Posttraumatic stress disorder (PTSD) is the most frequently reported psychiatric morbidity among victims of natural disasters [9-11]. Numerous studies have examined the risk factors of PTSD among earthquake survivors. The frequently reported risk factors include female gender, older age, low level of education, degree of exposure to earthquake, death in the family, loss of friends, loss of property, greater fear during the earthquake, number of traumatic experiences, inadequate social support, and previous psychiatric illness [12-15]. A previous study was performed to estimate the prevalence of posttraumatic stress disorder (PTSD) and assess determinants related to PTSD symptoms among adult earthquake survivors after the 2008 Wenchuan earthquake in China. This previous study also found that older age, female gender, unmarried/divorced/widowed, ethnic minority, death of family member, no household income, and damaged household were independent risk factors for PTSD symptoms in heavily damaged areas [16].

This thesis newly demonstrated that participants who had protein-rich food at breakfast (46.27 points) exhibited significantly lower IES-R scores than participants (57.87 points) who had no protein-rich food, and a significant relationship between contents of breakfast and IES-R scores didn't disappear when a ANOVA was performed this relationship with the covariance of Age, BMI, Monroe's sleep quality index, regularity of dinner, and lost or not family in the 4th step (related to Philippines's landslide). It is impossible to probe a causal relationship by the questionnaire study. For instance, on the relation between lower quality of sleep and PTSD symptoms severity, both form a positive feedback loop including a mechanism of memory consolidation by REM sleep function. In addition to that, intake protein-rich food (tryptophan) which is possible to reduce PTSD symptom both through high serotonin level and also promotion of sleep quality due to high level of melatonin can be put as a explanatory variable to PTSD symptoms. Some intervention or experimental studies are expected in the future.

Because meal habits and/or light conditions are familiar with our daily common life, the triple interventions as having sources of tryptophan and vitamin B6 at breakfast (1), following up breakfast with exposure to sunlight (2) and the exposure to low temperature lights as night lighting (3) can be applicable for children and people who suffered natural disasters and with PTSD symptoms. The implication of the triple interventions can stimulate the synthesis of serotonin and succeeding melatonin synthesis at night and that these hormones work as natural anti-depression drugs and/or natural sleeping pills. These two hormones could make students more-morning typed and improve their mental health. Therefore, this intervention recommended in this thesis would be applicable for most of all people living in this 24-hour commercialization society in Japan.

Reference in General Discussion

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Appendix

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研究報告

高知県内の運動部所属大学生への朝食・光曝露介入が介入中の睡眠・精神衛生に及ぼす影響

EFFECT OF INTERVENTION AS INTRODUCTION TO TAKING PROTEIN AND VITAMIN RICH BREAKFAST AND FOLLOWING SUN-LIGHT EXPOSURE ON SLEEP AND MENTAL HEALTH OF JAPANESE UNIVERSITY SOCCER TEAM MEMBERS INHABITING KOCHI PREFECTURE

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ABSTRACT

Objective of this study is to evaluate the effects of intervention, as asking Japanese university students to get foods including protein and Vitamin B6 (especially recommending fermented soy-beans and banana) at breakfast and to be exposed to sun-lights after that for one month (October-November, 2008), on sleep-wake cycle during the intervention. Participants of 83 attending university soccer club were divided to three groups (G1: no intervention; G2: asking them to have protein resources and Vitamin B6 resource at breakfast; G3: asking them to do as G2 plus sun light exposure after breakfast). Sleep diary was noted by themselves through one month of intervention period to evaluate the effects of it. Phase advance tended to be shown at all 4 phase points (bed time, sleep-in-time, med-point of sleep, wake-up time) in the last week period of intervention month only in G3 (P=0.100,0.148,0.070,0.148). The integrated intervention to breakfast and the following light exposure might be effective for evening-typed Japanese students to be shifted to be more morning-typed only within 2-4 weeks.

Key words: intervention experiment, rich tryptophan and Vitamin B6 breakfast, Sleep-wake cycle

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1. 緒言

日本の子ども達や学生が置かれる環境は、24時間型社会の進展に伴い、20−30年前とは完全に一変した。ヒトの体内時計は2つの駆動体から成るという説（2振動体説：約25時間の周期を持つ主時計、約2日周期の第2時計）が一般的であるが、主時計の重要な同調因子は光であり、第2時計の場合社会的同調因子や食事時刻などが関与していると考えられている[1]。

一方、第2時計が駆動していると考えられているヒトの睡眠−覚醒リズムには、天然の入眠剤（生理活性物質）としてメラトニンの関わりが深い。メラトニンは間脳の視床下部にある松果体で、セロトニンを基質として主に夜間に生合成・分泌される。血中メラトニン濃度はサーカディアンリズムを示し、夜間の短波長光（昼光色蛍光灯に多く含まれる）により分泌抑制される[2,3]。

メラトニンの合成基質となるセロトニンも同じく松果体で合成される脳内生理活性物質として働き、枯渇するとうつ病、パニック障害、強迫性障害、片頭痛、睡眠障害、摂食障害を引き起こすことから、精神衛生に影響を与える物質である[4]。セロトニンは、生体内では必須アミノ酸のTryptophan からトリプトファン 5-カルボキシラーゼと芳香属-L-アミノ酸デカルボキシラーゼの 2 つの酵素により5-ヒドロキシトリプトファンを経て合成され、メラトニンの原料となるため[5]、睡眠とも深いつながりを持つ。また、5-ヒドロキシトリプトファンからセロトニンへの過程でビタミン B6(PLP；ピリドキサール 5 リン酸　5' -phos-phate)が補酵素として働く[6]ため、ビタミン B6 摂取も間接的にセロトニン合成に関わりをもつ。

日常生活に於いて、朝食で魚や納豆などからトリプトファンを摂取するほど朝型で、寝付きがよく、起床困難を訴える頻度が高いという質問紙調査結果が 2−8 歳児から得られている[7]。また、朝食でのタンパク源摂取後、保育園（幼稚園）への登園までに 30 分以上太陽光暴露している幼児は暴露時間が 30 分に満たない幼児より朝型であり[8]、朝食時のビタミン B6 摂取量が高いほど幼児が朝型(Nakade *et al.*, unpublished)であることも質問紙調査結果で分かっている。これらの質問紙研究結果は朝食で摂取されたトリプトファン、ビタミン B6 摂取が太陽光暴露によって効果的にセロトニン、メラトニン合成が促進されていることを暗示している。また、質問紙調査におけるこれらの明確な相関関係は 8 歳児まででのみ見られる現象である。これは、メラトニンの夜間血中濃度は、生後 3−8 年の時期にきわだって濃度が高く[9]、この年齢児のセロトニン・メラトニン合成量の高さを反映していると理解できる。しかしながら、朝食でのタンパク源やビタミン B6 高含有量食材摂取とその後の太陽光曝露が高いセロトニンやメラトニンの合成分泌量が、内的同調因子や入眠剤として子どもたちを朝型化と精神衛生の向上をもたらしているという仮説の検証は質問紙調査結果では不可能である。

そこで、本研究では、これら一連の仮説の一端として、倫理上の観点から、運動部所属の学生達を対象に睡眠健康や精神衛生を改善する方向の介入フィールド実験を行った。

2. 方法

高知県内の大学でサッカー部に所属する男子学生83名のうち、研究協力受諾者47名を、3グループ(G1：無介入、G2：介入1カ月間朝食に納豆とバナナを例示しタンパク源と野菜類を摂取するよう事前口頭介入、G3：G2の介入に加え朝食後の太陽光曝露の事前口頭介入)に分けた。3群に分ける際、あらかじめ簡易型朝型夜型質問紙[10]に回答してもらい、朝型夜型度の平均値を中心とした50%を中間型グループ、両側それぞれ25%を夜型グループ、朝型グループとし、更に食事摂取状況調査[11]を行い、良好、中間、不良の3グループにわけ、合計9グループをつくった。それぞれのグループから乱数表を用いて任意に3グループ(G1:　17名；G2: 13名；G3: 17名)に振り分けた。G1-G3ともほぼ全員が体脂肪率10%以下、身長165-185cmのアスリートであり、年齢は18-22歳であった。3グループ間で身長、体重、年齢に有意差は無かった(Kruskal-Wallis test: 身長, χ^2-value=1.000, df=2, P=0.607; 体重, χ^2-value=0.282, df=2, P=0.868; 年齢, χ^2-value=2.390, df=2, P=0.303)

介入は 2008 年の 11 月の一か月間に行った。その直前にサッカー部を講義室に集め、口頭による介入を行った。まず、3 グループ全体に「本研究はサッカー部の皆さんの健康を増進する形の介入研究であり、学術目的以外にデータを使用せず、厳重に保管する」など口頭で研究協力の了解を得た。尚、倫理的配慮については、Touitou *et al.*[12]が

示した倫理規範に沿った。口頭で全員の研究協力許可を得たところで、口頭介入に入った。まず全体に朝食の内容記載を含む睡眠日誌の介入期間中毎日自記載をお願いした。その時点で、まずG1の研究協力者に退出してもらった。その後G2、G3に対して介入期間中、タンパク質や野菜・果物を朝食でなるだけ摂取するようにお願いした。その際、安価で比較的取り組みやすい摂取推奨食材として比較的経済的負担の小さい納豆・バナナを推奨した。この時点でG2の研究協力者に退出してもらった。最後にG3に対しさらに、もうひとつの要請事項として、朝食後に30分以上の太陽光曝露をお願いした。

1ヶ月の介入期間を第1週期(FWP: First Week Period)、16日間の中間期(MP: Medium Period)、第4週期(LWP: Last Week Period)に分け、就床時刻、入眠時刻、起床時刻、睡眠中点の比較を行った。また、G2,G3では、毎日の朝食でのタンパク質高含有食品摂取の有無、G3では朝食摂取後の30分以上の太陽光曝露の毎日の有無を調べ得点化し指数とした。

タンパク質摂取指数、朝食後太陽光曝露指数とともに0-100点の「実施度」が設定された。

以下にそれぞれの指数、実施度の計算過程を説明する。

A. タンパク質摂取指数:

G2及びG3の睡眠日誌に含まれる、朝食内容の項目より、主な栄養素がタンパク質である食材を1品目以上摂取した日数（以下摂取日数）を30で割った値を介入期間全体の実践度、第1週期の摂取日数を7で割った値をFWP期の実践度、16日間の中間期の摂取日数を16で割った値をMPの実践度、第4週期の摂取日数を7で割った値をLWPの実践度とした。

B. 朝食後太陽光曝露指数:

G3の睡眠日誌に含まれる、太陽光曝露時間の項目より、朝食後の30分以上の曝露の記載があった日数（以下曝露日数）を30で割った値を介入期間全体の実践度、第1週期の曝露日数を7で割った値をFWPの実践度、16日間の中間期の曝露日数を16で割った値をMPの実践度、第4週期の曝露日数を7で割った値をLWPの実践度とした。

C. 実施度:

G3において、上記A.B.の両方を満たす日数、すなわち、朝食でタンパク質含有食品を1品目以上摂取し、かつその後30分以上太陽光に曝露している日数（以下実施日数）を30で割った値を介入期間全体の実践度、第1週期の実施日数を7で割った値をFWPの実践度、16日間の中間期の実施日数を16で割った値をMPの実践度、第4週期の実施日数を7で割った値をLWPの実践度とした。

実施度と事後の就床、入眠、起床、睡眠中点のそれぞれの時刻との間で相関分析を行った。

3. 結果

G1では、FWPからMPにかけて睡眠中点(統計値=-1.851, P=0.064)で位相後退の傾向が見られたが、LWPにはMPの時点で認められた位相後退が元に戻った(MPとLWPの間でWilcoxon test, 睡眠中点(統計値=-2.201, P=0.028)(表1-A)。

G2ではFWPからMPにかけて、起床時点での位相後退は見られた(Wilcoxon test: 統計値=-1.962, P=0.050)が、就床、就寝、中点において、位相後退は生じなかった(表1-B)。更にG3では4時点ともにFWPからMPにかけての位相後退は全く見られず、逆にMPからLWPにかけて就床と起床の位相点で逆に位相前進の傾向があった(Wilcoxon test, 就床:統計値=-1.647, P=0.100(図1-A); 起床:統計値=-1.810, P=0.070(図1-C)。G2とG3において、実践度との相関関係を見ると、G2では実践度が高いほど時刻が有意に早まる効果やその傾向がFWPの起床(Pearson's correlation test, r=-0.-0.731, P=0.004)・就床時刻(r=-0.520, P=0.069)、睡眠中点(r=-0.500, P=0.082)、さらにMPでも就床(Pearson's correlation test, r=-0.734, P=0.004)(図2-A)・入眠(r=-0.628, P=0.007)(図2-B)・起床時刻(r=-0.544, P=0.055)(図2-C)、睡眠中点(r=-0.727, P=0.005)(図2-D)で見られたが、主観的評価の部分やLWPでは有意な相関関係は見られなかった。G3ではFWPの起床時刻(Pearson's correlation test, r=-0.586, P=0.017)に加え、FWPやMPの1日の気分や寝起きの気分(Pearson's correlation test, FWP: 気分, r=0.428, P=0.099, 寝起きの気分, r=0.561, P=0.024, MP: 気分, r=0.515, P=0.041(図3-A), 寝起きの気分, r=0.713, P=0.001)(図

3-Ⅲ）が取り組むほど改善される有意な効果やその傾向が見られた。

表1：3グループ間における、就床時刻、入眠時刻、起床時刻、睡眠中点時刻の平均と標準偏差をFWP(介入最初の1週間)、MP(中間2週間)、LWP(最後の1週間)ごとに示した。

A

G1	就床 FWP	就床 MP	就床 LWP	入眠 FWP	入眠 MP	入眠 LWP	起床 FWP	起床 MP	起床 LWP	中点 FWP	中点 MP	中点 LWP
平均値	24.539	24.739	24.449	24.874	25.045	24.803	8.212	8.303	8.179	29.416	29.779	29.294
度数	19	19	18	19	19	18	19	18	18	19	18	18
標準偏差	0.905	0.758	0.912	0.902	0.741	0.912	0.733	0.813	0.647	1.631	1.451	1.601

B

G2	就床 FWP	就床 MP	就床 LWP	入眠 FWP	入眠 MP	入眠 LWP	起床 FWP	起床 MP	起床 LWP	中点 FWP	中点 MP	中点 LWP
平均値	24.554	24.401	24.570	24.845	24.847	25.009	8.234	8.445	8.542	29.385	29.493	29.784
度数	12	12	12	12	12	12	12	12	12	12	12	12
標準偏差	0.579	0.721	0.814	0.686	0.773	0.792	0.493	0.359	0.559	1.071	1.243	1.299

C

G3	就床 FWP	就床 MP	就床 LWP	入眠 FWP	入眠 MP	入眠 LWP	起床 FWP	起床 MP	起床 LWP	中点 FWP	中点 MP	中点 LWP
平均値	24.519	24.606	24.394	24.856	24.904	24.743	8.438	8.472	8.090	29.527	29.592	29.155
度数	16	16	16	16	16	16	15	16	16	15	16	16
標準偏差	0.640	0.625	0.663	0.603	0.555	0.717	1.174	0.903	0.745	1.093	0.933	1.318

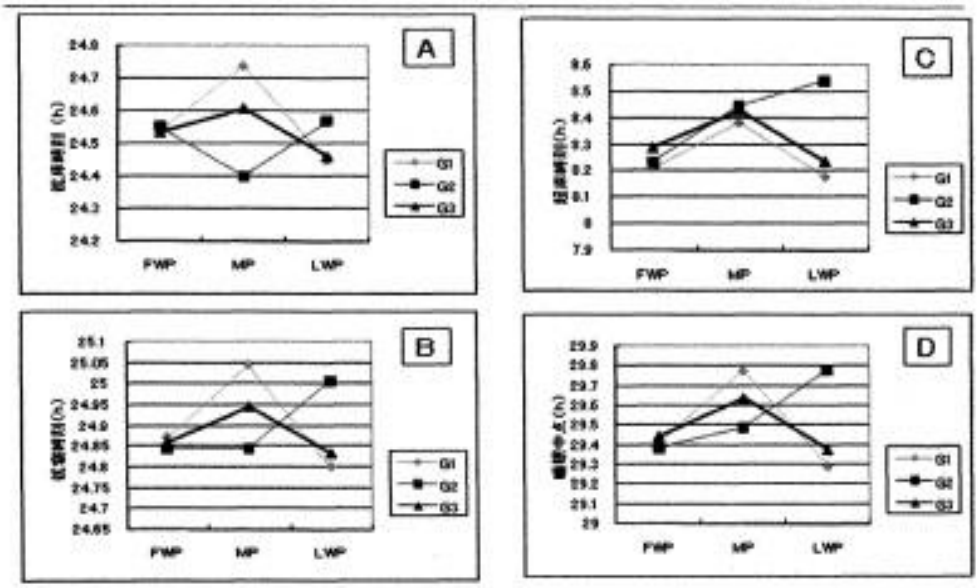
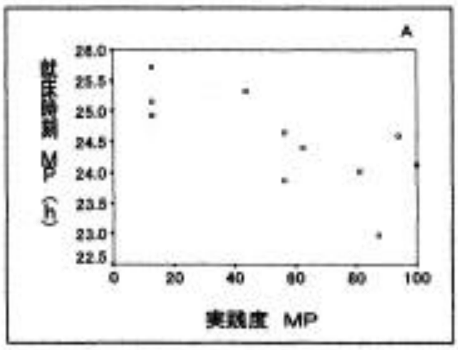
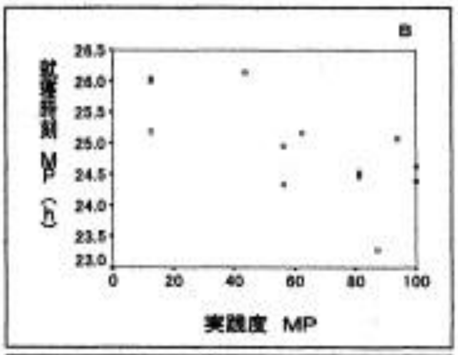
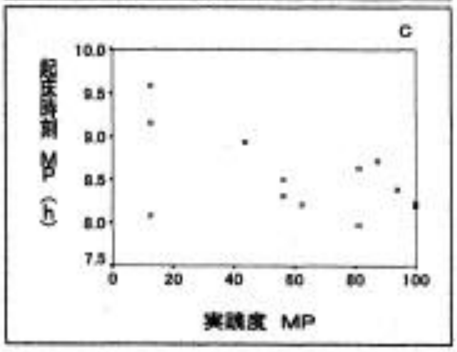


図1：サッカー部介入研究の結果。介入中の1ケ月の就床時刻、入眠時刻、起床時刻、睡眠中点の時刻位相の変化。グループ3は、朝食に納豆やバナナなどの摂取と摂取後の太陽光暴露を行うよう奨められたグループ。グループ2は納豆、バナナの 摂取のみを奨められたグループ。グループ1はそれらの推奨なしのグループ。4つの位相ポイントで、いずれも食事と光暴露の介入グループのみが介入期間の最終週(LWP: Last week period)に大きく位相前進している。(FWP:Firse week period:第1週； MP:Mid period: 中間の2週間)







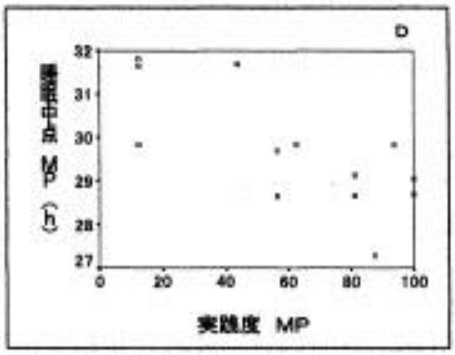


図2: グループ2における「朝食で納豆とバナナを」の Mid Period (実践期間のうち中間の2週間)における実践度と就床時刻(A)、入眠時刻(B)、起床時刻(C)、睡眠中点(D)との相関図。いずれも比較的明瞭な負の相関関係一実践度が高いほど4つの位相時点が早かった。

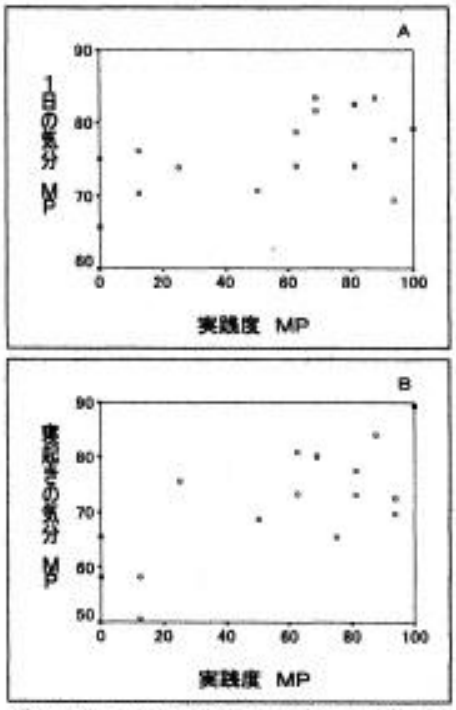


図3：グループ3における「朝食で納豆とバナナを摂取した後太陽光暴露を」の Mid Period (実践期間のうち中間の2週間)における実践度と1日の気分(daily mood)(A)、寝起きの気分(wake-up mood)(B)との相関図。いずれも比較的明瞭

な正の相関関係—実践度が高いほど1日の気分や寝起きの気分がよかった。

4. 考察

朝食への介入だけでなく、その後の太陽光暴露を促すことで、起床時刻の位相前進が約3週間の介入後から起こるだけでなく、それに伴って1日の気分や寝起きの気分など、精神衛生の改善が起こる可能性を本研究は暗示している。この現象の生理学的メカニズムの推察可能な仮説として、「トリプトファン—セロトニン—メラトニン合成系の増強を経由してこれらのホルモンピークが内的同調因子として、睡眠覚醒リズムの位相前進や日中の高い脳内セロトニン濃度による精神衛生の改善をもたらした」が挙げられるであろう。また、1ヶ月間の介入調査では、終盤の取り組みがやや低下することが示唆され、高いモチベーションを維持させるための中盤での働きかけが必要かもしれない。

若年の子どもたちは光に対する感受性が大学生も青年よりも高く[10]、緒言でも述べたようにセロトニン—メラトニン濃度が若年の子どもで際立って高い。これらのことから、朝食後の太陽光曝露介入は、若年の子どもに試してみる意義があるかもしれない。

24時間型社会が急速に進展する日本にあって、子どもたちを取り巻く光環境、食環境、社会的環境のどれを取って見ても、日周期サイクルの振幅が減少し、極端には規則的サイクルの成立まで危ぶまれることも想定できる。コンビニの登場で深夜であっても食物が手に入り、テレビの24時間放映やインターネットなども24時間使用可能であるので、これらのディスプレイからの光も光日周期サイクルを不規則化するであろう。携帯電話の夜間使用は特に女子中学生の間でその夜型化[10]に拍車をかけているが、携帯メイルのやりとりは一種の社会的同調因子の昼夜変動のメリハリを無くす要素になり得る。これほど環境の日周期サイクルのメリハリの弱い状況になったのは、人類歴史上かつてなかったことと言える。

このような同調因子の弱い状況でも体内時計の同調が乱れないような新しい環境適応能を人間が獲得するか、またはそのような機能的潜在性を人間は持っているのか今のところ知見は皆無であるが、数万年の人類の歴史で獲得した生理的形質が、ある程度の生理学的多型性があるとはいえ、そう簡単に変化するとも考えられない。むしろ、昼光性動物として持っている本来の体内時計の同調機構などを有効に発揮させるべく、私たちの体を取り巻く環境の24時間サイクルを明確に保持できるよう調整するべき時代に来ているのかもしれない。本研究の介入事項である「朝食に納豆・バナナとその後の太陽光曝露」はその調整の具体的1例となりえる可能性を秘めている。

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