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Original Article

Association between Subjective Sleep and Dietary Intake in University Students

Shuji Akagi[a] **and Sayaka Kihara**[b]

[a]*Department of Human Nutrition, Faculty of Contemporary Life Science, Chugokugakuen University, Niwase 83, Kitaku, Okayama, 701-0197, Japan,*
[b]*Department of Nutrition, Matsuo Internal Medicine Hospital, 3-7-1 Shiromachi, Mihara, Hiroshima, 723-0014, Japan*

Background
Inadequate sleep is known to be related to obesity-related diseases and also considered to be associated with dietary nutrition intake. Tracked dietary patterns have been reported which show that lifestyle during adolescence may be a determinant of overweight status and sleep behavior in adulthood. Thus, it is important to confirm the relationship between sleep behavior and dietary nutrition intake in younger individuals.
Objective
The aim of this study was to clarify the influence of ingested nutrients on poor sleep behavior.
Participants
We examined 177 university students from a sample of 187 (43 males, 144 females), with ages ranging from 18-24 years.
Measurable outcome
The Pittsburgh Sleep Quality Index (PSQI) was used to assess sleep behavior. Dietary intake was evaluated using a self-administered diet history questionnaire. Based on the PSQI results, the participants were divided into the favorable and unfavorable groups, and the amounts of nutrient intake were compared between them.
Results
Although none of the specific nutrients surveyed showed a relationship with poor sleep quality or long sleep latency, students with short sleep duration consumed a significantly lower amount of soluble fiber, as shown by multivariate analysis.
Conclusion
Our findings suggest that ingestion of soluble dietary fiber may be desirable for obtaining an adequate sleep time and prevention of obesity-related diseases. Nevertheless, additional studies including a longitudinal investigation are required to confirm our findings.

Keywords: Pittsburgh Sleep Quality Index, Self-administered diet history questionnaire, Short sleep duration, Soluble fiber

Introduction

Sleep inadequacy is an emerging public health problem and has been shown to be associated with weight gain, as well as obesity-related diseases such as diabetes, hypertension, dyslipidemia, and cardiovascular disease [1-3]. Since energy intake is a

Corresponding author: Shuji Akagi
Department of Human Nutrition, Faculty of Contemporary Life Science, Chugokugakuen University, Niwase 83, Kitaku, Okayama, 701-0197, Japan
TEL: +81 86 293 0247 Fax: +81 86 293 2798
E-mail: sjakagi@cjc.ac.jp

major contributor to higher body mass index (BMI), several studies have examined the association between sleep duration and total energy intake, and generally found an association between short sleep duration and higher total calorie intake [4]. As for nutrients, a Japanese study of middle-aged women indicated that poor sleep quality was significantly associated with high carbohydrate intake [5]. More recently, a meta-analysis that included up to 14,906 participants from Europe and the United States [6] showed a significant association between shorter sleep duration and higher saturated fatty acid intake in younger men and women between 20 and 64 years of age, as well as with higher levels of carbohydrates, lower total fat, and lower polyunsaturated fatty acid levels in older women aged 65–80. However, results from studies that assessed the relationship between nutrient intake and sleep duration are inconsistent.

Previous findings have revealed that both body weight and body composition during adolescence are important determinants of overweight status in adulthood [7, 8]. Moreover, a large population longitudinal study performed in Great Britain indicated that an energy-dense, high fat, and low fiber dietary pattern during adolescence was prospectively associated with increased and excess adiposity in adulthood [9]. Accordingly, for dealing with public health problems, it is crucial to confirm the influence of dietary nutrients on sleep behavior in younger individuals such as university students, and for preparing countermeasure strategies for preventing overweight conditions and obesity-related diseases such as diabetes, hypertension, and cardiovascular disease, as well as other medical conditions.

In the present study, to clarify which nutrients contribute to the deterioration of sleep behavior, we examined the association between dietary nutrient intake and sleep behavior, including sleep quality, onset latency, and duration, using a subjective evaluation method.

Materials and methods

Participants

A total of 187 students (43 males, 144 females; age range 18 to 24 years) enrolled in a department for registered dietitians at a university in Hiroshima prefecture, Japan, participated in this cross-sectional study. Those who met the following criteria were excluded from analysis; use of medication with known effects on sleep or daytime alertness (n = 8), self-reported extremely low calorie intake (< 600 kcal) (n = 1), and incomplete answers to the questionnaires (n = 1). The final cohort included in the subsequent analysis comprised 177 students. This study was conducted in July 2015.

Evaluation of dietary nutrients

A self-administered diet history questionnaire (DHQ) was used to assess quantities of dietary nutrients ingested by the participants during the previous month. Details of the DHQ and its validity have been previously reported [10, 11]. Briefly, the DHQ is a 16-page structured questionnaire with items relating to the consumption frequency of a total of 150 foods and beverages commonly consumed by the general Japanese population. Dietary intake in terms of energy and selected nutrients was estimated by applying an ad hoc computer algorithm to the 150 foods and beverages of the DHQ and the Standard Tables of Food Composition in Japan [12]. It has been well established that habitual alcohol consumption and smoking influence sleep behavior, but those were not evaluated because none of the participants had a history of either.

Assessment of subjective sleep

Subjective sleep was assessed using the Japanese version of the Pittsburgh Sleep Quality Index (PSQI) [13], which is a self-administrated questionnaire that examines sleep difficulty retrospectively for a one-month period, with a global score (PSQIS) ranging from 0 to 21, with a higher PSQIS value indicating a lower quality of sleep [13, 14]. By use of the questionnaire, the participants reported bedtime, sleep onset latency (SOL), and rise times, from which we also calculated total sleeping time (TST) by subtracting sleep onset time from rise time. According to the PSQI findings, the 177 participants were divided into the good PSQIS group, including those with a score ≥6, or the poor PSQIS group, which included participants with a score ≤5. Participants were also classified into the short SOL group (≤30 minutes) or prolonged SOL group (≥31 minutes), and into the good TST group (> 7 hours) or the short TST group (< 7 hours), using the above-mentioned calculation methods.

Anthropometric assessment

Height and body weight were measured to the nearest 0.1 cm and 0.1 kg respectively, with the participants wearing light clothes and no shoes. Data regarding age and gender were simultaneously obtained. Their BMI was then calculated by dividing weight by squared height (kg/m^2).

Statistical analysis

For energy adjustment, we used the percentage of total energy intake (% energy) from macronutrients and intake per 1,000 kcal (/1,000 kcal) for other nutrients based on obtained DHQ data. In this manner, we evaluated the intake of energy (kcal/day), protein (% energy), total fat (% energy), carbohydrate (% energy), total dietary fiber (g/1,000 kcal), insoluble dietary fiber (g/1,000 kcal), soluble dietary fiber (g/1,000 kcal), total fatty acid (g/1,000 kcal), saturated fatty acid (g/1,000 kcal), monounsaturated fatty acid (g/1,000 kcal), polyunsaturated fatty acid (g/1,000 kcal), n3-polyunsaturated fatty acid (g/1,000 kcal), n-6 polyunsaturated fatty acid (g/1,000 kcal), cholesterol (mg/1,000 kcal), vitamin A (μg/1,000 kcal), vitamin B1 (mg/1,000 kcal), vitamin B2 (mg/1,000 kcal), vitamin C (mg/1,000 kcal), niacin (mg/1,000 kcal), vitamin D (μg/1,000 kcal), vitamin E (mg/1,000 kcal), vitamin K (μg/1,000 kcal), pantothenic acid (mg/1,000 kcal), vitamin B6 (mg/1,000 kcal), vitamin B12 (μg/1,000 kcal), folic acid (μg/1,000 kcal), calcium (mg/1,000 kcal), iron (mg/1,000 kcal), sodium (mg/1,000 kcal), potassium (μg/1,000 kcal), phosphorus (mg/1,000 kcal), magnesium (mg/1,000 kcal), zinc (mg/1,000 kcal), and copper (mg/1,000 kcal).

Continuous variables are shown as the mean ± standard error (SE) and categorical variables are expressed as frequency. Mann Whitney's U test was used to compare continuous variables and a chi-square test for categorical variables to detect differences between two groups in regard to PSQIS, SOL, and TST, as noted above. Binomial logistic regression analysis was conducted to confirm the association between subjective sleep and the amount of dietary nutrient intake. The dependent variable was classification in the poor group, while independent variables were items that showed a p value less than 0.15 in Mann Whitney's U test for each category of PSQIS, SOL, and TST.

Statistical Product and Service Solution (SPSS) version 23.0 (IBM, Armonk, New York, USA) was utilized for statistical analysis. A p value of <0.05 was considered to indicate statistical significance.

Ethical approval

This study was approved by the ethics committee of Fukuyama University, and written informed consent was obtained from all participants.

Results

Assessment of measured parameters related to subjective sleep

Results of the PSQI are presented in Figure 1. The mean PSQI value was 5.9 ± 2.8, with 82 (46.3 %) of the participants experiencing poor sleep quality, 43 (24.3 %) with prolonged SOL, and 98 (55.4 %) with short TST.

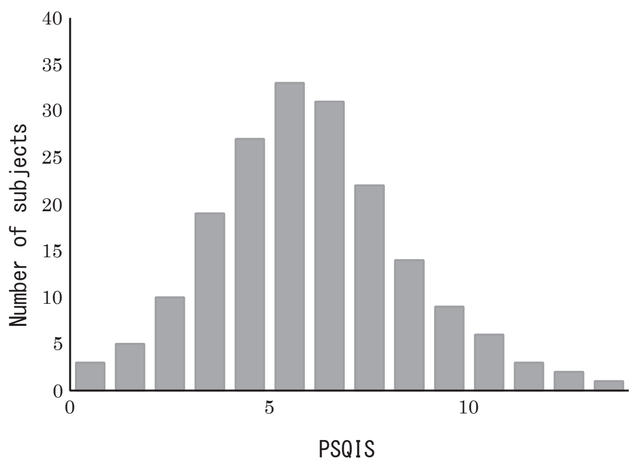

Figure 1 Histogram of participants' global scores on the Pittsburgh Sleep Quality Index (PSQIS)

Relationship between PSQIS and dietary nutrient intake

The differences of intake of dietary nutrients between the poor and good groups' PSQIS are shown in Table 1. Among participants with a score greater than 5 who experienced poor subjective sleep quality, the amount of ingested protein was significantly higher as compared to those with good sleep quality.

Table 1 Comparison of basic characteristics and nutrients intake between good and poor groups' PSQIS

	Good PSQIS (n=95)		Poor PSQIS (n=82)		
	Mean	SD	Mean	SD	p-value
Male: Female	22	73	20	62	0.981*
Age, years	19.9	0.1	20.0	0.2	0.977
Body Mass Index, kg/m^2	20.9	0.4	20.9	0.3	0.914
Nutrients					
Total energy, kcal/day	1,719.0	69.7	1,687.0	65.3	0.715
Carbohydrate, % energy	56.3	0.84	56.2	0.9	0.473
Total fat, % energy	29.3	0.7	28.7	0.7	0.557
Protein, % energy	12.5	0.2	13.1	0.3	0.048
Total dietary fiber, g/1,000 kcal	5.6	0.2	5.8	0.3	0.508
Insoluble dietary fiber, g/1,000 kcal	6.7	0.3	6.8	0.4	0.595
Soluble dietary fiber, g/1,000 kcal	1.41	0.05	1.43	0.07	0.959
Total fatty acids, g/1,000 kcal	2.86	0.07	2.80	0.07	0.290
Saturated fatty acids, g/1,000 kcal	9.4	0.3	9.4	0.3	0.685
Monounsaturated fatty acids, g/1,000 kcal	11.8	0.4	11.5	0.3	0.260
Polyunsaturated fatty acids, g/1,000 kcal	7.1	0.2	6.9	0.2	0.250
Cholesterol, mg/1,000 kcal	166.7	7.0	173.3	7.9	0.488
Vitamin A, μg/1,000 kcal	285.9	22.6	262.6	24.2	0.451
Vitamin B1, μg/1,000 kcal	0.38	0.01	0.40	0.01	0.117
Vitamin B2, μg/1,000 kcal	0.63	0.02	0.68	0.02	0.156
Vitamin C, mg/1,000 kcal	40.1	1.8	45.2	2.9	0.383
Niacine, μg/1,000 kcal	6.6	0.2	7.0	0.3	0.159
Vitamin D, mg/1,000 kcal	2.6	0.2	2.8	0.2	0.806
Pantothenic acid, μg/1,000 kcal	2.83	0.06	2.98	0.08	0.134
Vitamin K, μg/1,000 kcal	104.9	10.0	108.6	6.8	0.698
Vitamin E, mg/1,000 kcal	3.9	0.1	3.8	0.1	0.443
Vitamin B6, mg/1,000 kcal	0.47	0.02	0.49	0.02	0.257
Vitamin B12, μg/1,000 kcal	2.4	0.1	2.6	0.2	0.313
Folate, μg/1,000 kcal	131.1	4.7	137.8	6.8	0.149
Calcium, mg/1,000 kcal	227.5	11.1	242.0	11.2	0.318
Iron, mg/1,000 kcal	3.3	0.1	3.5	0.1	0.519
Sodium, mg/1,000 kcal	2,068.4	73.3	2,034.5	78.6	0.691
Potassium, mg/1,000 kcal	966.1	27.5	1,024.2	46.0	0.422
Phosphorus, mg/1,000 kcal	455.9	9.7	484.7	12.4	0.066
Magnesium, mg/1,000 kcal	104.3	2.3	110.4	3.9	0.622
Zinc, mg/1,000 kcal	3.95	0.06	4.08	0.08	0.092
Copper, mg/1,000 kcal	0.53	0.01	0.56	0.02	0.938

SD: standard deviation, *: chi-square test

Relationship between SOL and dietary nutrients' intake

There were significantly greater amounts of dietary energy and vitamin B2 intake, and a significantly lower total amount of dietary fiber noted in the students with prolonged SOL (Table 2).

Table 2 Comparison of basic characteristics and nutrients intake between short and prolonged SOL groups

	Short SOL (n=134)		Prolonged SOL (n=43)		
	Mean	SD	Mean	SD	p-value
Male: Female	34	100	8	35	0.294
Age, years	20.0	0.1	20.0	0.2	0.800
Body Mass Index, kg/m^2	20.6	0.2	21.3	0.5	0.293
Nutrients					
Total energy, kcal/day	1,700.0	54.0	1,734.0	102.9	0.050
Carbohydrate, % energy	56.5	0.7	55.6	1.3	0.203
Total fat, % energy	28.9	0.6	29.5	1.1	0.259
Protein, % energy	12.6	0.2	13.4	0.4	0.323
Total dietary fiber, g/1,000 kcal	6.2	0.6	5.6	0.2	0.034
Insoluble dietary fiber, g/1,000 kcal	4.0	0.1	4.4	0.3	0.452
Soluble dietary fiber, g/1,000 kcal	1.38	0.05	1.53	0.10	0.223
Total fatty acids, g/1,000 kcal	2.83	0.06	2.85	0.11	0.433
Saturated fatty acids, g/1,000 kcal	9.3	0.2	9.4	0.4	0.683
Monounsaturated fatty acids, g/1,000 kcal	11.7	0.3	11.7	0.5	0.487
Polyunsaturated fatty acids, g/1,000 kcal	7.0	0.2	7.1	0.3	0.387
Cholesterol, mg/1,000 kcal	167.8	6.0	173.6	10.3	0.535
Vitamin A, μg/1,000 kcal	271.1	18.7	293.1	18.7	0.571
Vitamin B1, μg/1,000 kcal	0.38	0.05	0.42	0.02	0.064
Vitamin B2, μg/1,000 kcal	0.64	0.02	0.72	0.03	0.014
Vitamin C, mg/1,000 kcal	41.5	2.0	46.3	3.4	0.107
Niacine, μg/1,000 kcal	6.7	0.2	7.1	0.4	0.390
Vitamin D, mg/1,000 kcal	2.6	0.1	2.9	0.3	0.750
Pantothenic acid, μg/1,000 kcal	2.9	0.1	3.1	0.2	0.104
Vitamin K, μg/1,000 kcal	104.6	5.1	118.2	10.1	0.114
Vitamin E, mg/1,000 kcal	3.8	0.1	4.0	0.2	0.349
Vitamin B6, mg/1,000 kcal	0.48	0.02	0.52	0.03	0.174
Vitamin B12, μg/1,000 kcal	2.34	0.11	2.86	0.02	0.909
Folate, μg/1,000 kcal	131.1	4.4	147.5	9.6	0.433
Calcium, mg/1,000 kcal	228.1	8.8	258.8	17.0	0.683
Iron, mg/1,000 kcal	3.4	0.1	3.7	0.2	0.487
Sodium, mg/1,000 kcal	2,041.8	58.3	2,020.4	118.5	0.387
Potassium, mg/1,000 kcal	973.4	23.5	1,065.2	80.5	0.554
Phosphorus, mg/1,000 kcal	462.4	8.3	499.6	19.4	0.393
Magnesium, mg/1,000 kcal	105.4	2.3	114.5	5.6	0.535
Zinc, mg/1,000 kcal	4.02	0.06	4.12	0.11	0.736
Copper, mg/1,000 kcal	0.54	0.01	0.55	0.02	0.701

SD: standard deviation, *: chi-square test

Relationship between TST and dietary nutrients' intake

Participants with short sleep duration had significantly greater amounts of total dietary fiber, soluble dietary fiber, and insoluble dietary fiber. No differences for sex or age, or in regard to either PSQIS or SOL were observed (Table 3).

Table 3 Comparison of basic characteristics and nutrients intake between good and short TST groups

	Good TST (n=79)		Short TST (n=98)		
	Mean	SD	Mean	SD	p-value
Male: Female	23	56	19	79	0.136
Age, years	19.9	0.2	20.0	0.2	0.857
Body Mass Index, kg/m^2	21.0	0.0	20.6	0.3	0.349
Nutrients					
Total energy, kcal/day	1,706.9	68.7	1,664.2	62.4	0.505
Carbohydrate, % energy	12.6	0.2	13.0	0.2	0.269
Total fat, % energy	28.6	0.7	29.4	0.7	0.226
Protein, % energy	56.7	0.8	55.9	0.9	0.165
Total dietary fiber, g/1,000 kcal	1.5	0.1	1.3	0.1	0.006
Insoluble dietary fiber, g/1,000 kcal	4.2	0.1	3.9	0.2	0.015
Soluble dietary fiber, g/1,000 kcal	5.9	0.2	5.5	0.3	0.010
Total fatty acids, g/1,000 kcal	2.81	0.07	2.85	0.07	0.341
Saturated fatty acids, g/1,000 kcal	9.2	0.3	9.5	0.3	0.806
Monounsaturated fatty acids, g/1,000 kcal	11.6	0.4	11.8	0.3	0.340
Polyunsaturated fatty acids, g/1,000 kcal	7.0	0.2	7.1	0.2	0.366
Cholesterol, mg/1,000 kcal	174.9	8.2	164.4	6.8	0.523
Vitamin A, μg/1,000 kcal	269.2	19.2	279.3	25.4	0.730
Vitamin B1, μg/1,000 kcal	0.39	0.01	0.39	0.01	0.627
Vitamin B2, μg/1,000 kcal	0.63	0.02	0.67	0.01	0.151
Vitamin C, mg/1,000 kcal	41.1	2.1	49.1	2.5	0.599
Niacine, μg/1,000 kcal	6.6	0.2	6.8	0.2	0.874
Vitamin D, mg/1,000 kcal	2.7	0.2	2.7	0.2	0.848
Pantothenic acid, μg/1,000 kcal	2.82	0.06	2.96	0.08	0.340
Vitamin K, μg/1,000 kcal	108.7	7.1	106.1	5.8	0.340
Vitamin E, mg/1,000 kcal	4.0	0.1	3.8	0.1	0.161
Vitamin B6, mg/1,000 kcal	0.48	0.02	0.49	0.02	0.174
Vitamin B12, μg/1,000 kcal	2.6	0.2	2.4	0.1	0.597
Folate, μg/1,000 kcal	130.6	5.0	137.3	6.1	0.895
Calcium, mg/1,000 kcal	230.0	13.0	237.3	9.8	0.252
Iron, mg/1,000 kcal	3.3	0.6	352.0	0.1	0.305
Sodium, mg/1,000 kcal	2,049.6	82.1	2,032.2	68.5	0.909
Potassium, mg/1,000 kcal	966.3	28.8	1,014.1	41.0	0.705
Phosphorus, mg/1,000 kcal	462.4	10.7	471.0	11.4	0.433
Magnesium, mg/1,000 kcal	105.2	2.6	109.1	3.4	0.946
Zinc, mg/1,000 kcal	4.02	0.07	4.04	0.08	0.413
Copper, mg/1,000 kcal	0.53	0.01	0.56	0.02	0.544

SD: standard deviation, *: chi-square test

Multivariate analysis

Regarding PSQIS and SOL, no specific nutrients showed an influence on poor sleep quality or prolonged SOL in results of binomial logistic regression analysis. However, multivariate analysis revealed that short sleep duration was significantly associated with a lower amount of soluble dietary fiber intake (OR: 95%, CI 0.111–0.765, p = 0.038) (Table 4).

Table 4 Influenced nutrients for short sleep duration

Nutrients	Odds Ratio (95%C.I.)	p value
Total dietary fiber	1.90 (0.77–4.68)	0.165
Soluble dietary fiber	0.21 (0.05–0.91)	0.037
Insoluble dietary fiber	0.55 (0.17–1.85)	0.377

Discussion

We conducted the present cross-sectional study of 177 university students to examine the relationships between dietary nutrients and sleep behavior, namely PSQIS, SOL, and TST. It is well-established that poor sleep quality, long sleep latency [15], and short sleep duration are associated with adverse health outcomes, including overall mortality [16], weight gain, and obesity-related diseases, such as type 2 diabetes [1], hypertension [2], and cardiovascular disease [3]. However, in the present study we found no specific nutrient that had a relationship with poor sleep quality or long sleep latency. On the other hand, participants with a short sleep duration (<7 hours) consumed significantly lower amounts of soluble fiber, which was shown in the findings of multivariate analysis. Epidemiological studies concerning habitual sleep duration and dietary intake have revealed an association between short sleep and higher total calories [4]. Moreover, cross-sectional studies have found a significant association between short sleep duration and a higher prevalence of weight gain or obesity in both children and adults [1]. It is generally assumed that the impact of short sleep on dietary intake is likely multi-factorial, with numerous mechanisms proposed and examined. Short sleep time results in extended hours of wakefulness, which is considered to provide additional opportunities to increase food intake, while it has also been observed that a changed dinner time to later than usual is a significant predictor of higher BMI, independent of age and sleep timing [17]. Furthermore, laboratory results revealed that the level of leptin, a satiety signal, is reduced after sleep restriction, while the level of ghrelin, an appetite stimulant, is increased [18]. Accordingly, short sleep duration is associated with body weight gain and obesity-related diseases.

There have been several reports regarding the association between nutrient intake and short sleep duration, and collectively they have suggested that short sleepers likely consume diets with higher fat and lower protein levels [6, 17]. However, results regarding carbohydrate composition remain controversial. As for food intake, an investigation of Japanese factory workers revealed that short sleepers (<6 hours) consumed fewer vegetables as compared to normal sleepers (6–9 hours) [5]. Recently, a large, nationally representative study conducted in the United States reported that short sleepers (5–6 hours) had a lower intake of dietary fiber than normal sleepers (7–8 hours) [4]. St-Onge *et al.* (2016) [19] also revealed that a lower consumption of dietary fiber was associated with lighter and restorable sleep with more arousals. In the present study, a significantly lower soluble dietary fiber intake was observed in participants with a short sleep duration. It has been reported that oligofructose, a prebiotic, contributes to weight loss and improved metabolic parameters such as insulin resistance in overweight or obese individuals [20]. Moreover, satiety, reduced energy and food intake, and increased levels of satiety peptides were also shown to result from consumption of prebiotic supplements that contained dexstrin in healthy human participants [21]. Consequently, it is suggested that an adequate ingested soluble dietary fiber intake may prevent overweight and obesity-related diseases, such as type 2 diabetes, hypertension, and cardiovascular disease, and may contribute to adequate sleep duration, thus leading to decreased rates of mortality and morbidity indirectly via improvement of metabolic disorders.

Several studies have been conducted regarding nutrients and foods that have an adverse influence on subjective sleep quality and/or sleep onset latency [5, 22, 23]. Katagiri *et al.* (2014) [5] showed that poor subjective sleep quality was associated with a low intake of vegetables and fish, and high intake of confectionary and noodles. It was also reported that a six-month diet intervention including energy restriction shortened sleep onset latency [23]. However, it remains unclear whether specific nutrients are harmful to both subjective sleep quality and sleep onset latency in healthy participants. In the present study as well, none of the nutrients assessed had an effect to significantly worsen sleep quality or sleep latency. Additional investigations are needed to further examine these issues.

It is well understood that inadequate sleep is associated with being overweight and obesity-related diseases. Being overweight in adolescence is known to be related to increased morbidity and mortality rates in adulthood, because both body weight and body composition in adolescent individuals are important determinants of an overweight condition in adulthood [7, 8]. On the other hand, a large British longitudinal study indicated that an energy-dense, high fat, and low fiber dietary pattern in adolescence was prospectively associated with increased and excess adiposity in adulthood [9]. Here, we examined the relationship between subjective sleep and dietary nutrient intake in university students between 18 and 24 years of age, and the results revealed that an adequate amount of soluble dietary fiber intake is beneficial for maintaining adequate sleep duration. Accordingly, better diet quality including increased soluble dietary fiber intake may improve not only sleep condition but also body composition, leading to reductions in the future risks of morbidity and mortality.

The present study has several limitations. We utilized a cross-sectional design, thus the results do not necessarily imply causal relationships. In the context of this study, a short sleeping time in university students may be caused by a low level of soluble dietary fiber intake, or alternatively, students with a short sleep duration may consume less soluble dietary fiber. A longitudinal design would be desirable to address such issues. Furthermore, our study's participants were not randomly selected. Rather, convenience sampling was used, thus generalization of the results should be made with caution. Also, we measured sleep duration using a subjective rather than objective method. Finally, sleep condition is affected by a number of factors in daily life, such as physical activity, commuting time, and current relationships with family members or friends. The impact of the association with sleep duration might have been modified by unknown confounders that were not assessed in this study. These methodological limitations should be addressed in a future study.

In conclusion, the present results suggest that ingestion of soluble dietary fiber may be useful for securing adequate sleep time as well as prevention of obesity-related diseases. However, an association shown in a cross-sectional study does not indicate causality, thus an additional longitudinal investigation is required to confirm our findings.

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