Table 6 The mixed model analyses outcome for detecting the baseline predictors of the SPS and SIAS scores at the 1-year follow ups

	N	SPS			SIAS		
		Mean	SE	*P*	Mean	SE	*P*
Gender							
Female	56	28.4	1.8	0.296	47.1	1.7	0.38
Male	57	31.0	1.8		49.2	1.7	
Age							
13–18	7	30.3	5.1	0.087	45.0	4.7	0.019‡
19–45	91	30.7	1.4		49.6	1.3	
≥46	15	22.7	3.3		40.3	3.1	
Educational status							
University	34	27.5	2.2	0.68	50.1	2.1	0.643
College	16	29.6	3.3		48.6	3.1	
High School	58	30.5	1.8		46.7	1.7	
Junior high school	5	33.2	6.1		47.4	5.8	
Marital status							
Married	39	28.4	2.2	0.740	45.5	2.0	0.223
Separated/divorced	3	32.2	7.7		45.4	7.2	
Single/never married	71	30.3	1.6		49.7	1.5	
Employment status							
Full-time employment	23	27.5	2.7	0.39	49.0	2.6	0.769
Full-time student	20	32.9	3.0		48.1	2.9	
Part-time/homemaker/retired	46	28.2	1.9		46.8	1.8	
Unemployed	24	32.0	2.7		50.0	2.6	
Onset of SAD							
≤12	17	40.0	3.1	0.001*	60.3	2.8	<0.0005*
13–18	65	26.9	1.7		46.1	1.5	
19–45	28	29.2	2.1		44.5	1.9	
Duration of SAD							
≤1	6	23.0	5.5	0.216	39.2	5.1	0.089
1<	104	30.0	1.3		48.2	1.2	
Number of sessions							
<12	18	33.3	3.8	0.338	51.5	3.6	0.341
12≤	95	29.5	1.3		47.9	1.3	
Benzodiazepine use							
No	76	30.3	1.5	0.521	49.7	1.4	0.059
Yes	37	28.6	2.2		45.0	2.0	
Antidepressant use							
No	60	29.2	1.8	0.705	48.6	1.7	0.716
Yes	58	30.1	1.7		47.7	1.6	
Current mood disorder							
No	86	30.0	1.4	0.775	47.1	1.3	0.106
Yes	27	29.1	2.6		51.5	2.4	
Current anxiety disorder							
No	102	29.1	1.3	0.121	47.4	1.2	0.067
Yes	11	35.6	3.9		54.6	23.7	
Severity							
SPS ≤ 33	48	21.4	1.6	<0.0005*	46.1	1.8	0.151
SPS ≥ 34	65	35.6	1.4		49.5	1.5	

Note: *$P < 0.05$.
Abbreviations: SAD, social anxiety disorder; SE, standard error; SPS, Social Phobia Scale.

did not diagnose avoidant personality disorder rigidly in the aforementioned way.

Fourth, there were some statistical issues in our study. Multiple *t*-tests may have increased the risk for type I errors. However, the magnitude of the treatment effectiveness was quantified by effect size as well as the percentage reduction. Besides, data for pre- and posttreatment SPS were not normally distributed, although those for the other measures were normally distributed. This might have had some effect on the statistical validity of our study. However, we conducted

post hoc Mann–Whitney analysis between completers and dropouts, and the result was not different.

Some may point out that we did not use the Liebowitz Social Anxiety Scale as the primary outcome, which is a widely used measure. Because this study was conducted as routine Japanese clinical work, follow-up assessments done by post- and self-reporting versions of this[35] have not been validated in Japan to date.

Moreover, a recent study[36] showed the effectiveness of attention training, which costs less than typical CBT. Although our program included attention training, we might be able to improve our program by emphasizing this component, according to the new findings.

Despite these limitations, this study provided evidence of long-term efficacy of group CBT for Japanese patients with generalized SAD. Although there is still room for improvement, our results favor the use of CBT for generalized SAD in Japan.

Conclusions

Group CBT resulted in improvements in Japanese patients with generalized SAD, and these improvements were maintained for up to 1 year after group CBT. We showed that older age at baseline, late onset, and lower severity of SAD were predictors of good outcome at 1-year follow-up for group CBT.

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Disclosure

The authors declare that they have no competing interests with regard to the present study.

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Original Contribution

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Assessment of Physical and Mental Health in Male University Students with Varying Sleep Habits

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Summary: Healthy sleep habits entail not only sleeping for a sufficient period (quantity) but also regularity of the sleep cycle and getting sound sleep (quality). University students often have erratic schedules that cause irregular sleep patterns even though sleep durations remain relatively constant. This study compared the physical and mental health of 90 male university students with different sleep habits. We created sleep habit scales using the Tokyo Metropolitan Institute for Neuroscience life habits inventory (TMIN-LHI; Miyashita, 1994) by performing a factor analysis and classifying sleeping habits based on regularity, quality, and quantity. Four types of sleep habits were identified by cluster analysis; good sleep was characterized by regular and high quality sleep but of relatively short sleep duration; long sleep was regular and relatively long but of low quality; short sleep was of high quality but short and irregular, while poor sleep was irregular, of low quality, and relatively long. The good sleep group had a significantly lower average waist circumference, and lower systolic and diastolic blood pressure. The long and poor sleep groups, which both had low quality sleep, scored lower than the national standard on the mental component summary (MCS) calculated from the Social Functioning-36 (SF-36) short-form health survey. Furthermore, the average MCS score of the poor sleep group was significantly lower than that of any other sleep habit group. Subjects with poor sleep also scored lowest on the Self-rating Depression Scale (SDS). In addition, the short and poor sleep groups were prone to glucose or lipid metabolism disorders. Maintaining good physical and mental health without sound sleep and a regular sleep cycle is difficult, even if sleeping hours are kept constant. Therefore, we included the assessment of regularity and quality in addition to hours of sleep in order to develop appropriate sleep guidelines for improved physical and mental health.

Key words university students, sleep habits, sleep hygiene, regularity of sleep, quality of sleep, sleep duration

INTRODUCTION

Metabolic syndrome and depression are now attracting considerable research attention. These disorders are closely related to various aspects of lifestyle, such as diet [1], exercise [2], and mental stress [3]. The Japanese population has a tendency to neglect sleep because of a national work ethic based on diligence [4]. In addition, working overtime is considered a virtue in Japan, and 24-h work operations are commonplace. Twenty-four hour stores are a regular feature and many young people work in late shifts. For them, work time and free time are more important than sleep duration [5].

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Abbreviations: ANOVA, analysis of variance; ASDA, American Sleep Disorders Association; BMI, body mass index; FBS, fasting blood sugar; HDL-C, high-density cholesterol; HMW-adiponectin, high molecular weight adiponectin; HOMA-IR, homeostasis model of assessment-insulin resistance; HSD, honestly significant difference; LDL-C, low-density cholesterol; ME, Morningness-Eveningness; MCS, mental component summary; n.s., not significant; PCS, physical component summary; QOL, quality of life; SD, standard deviation; SDS, Self-rating Depression Scale; SF-36, Social Functioning-36; TMIN-LHI, Tokyo Metropolitan Institute for Neuroscience life habits inventory.

However, irregular and insufficient sleep can cause daytime drowsiness and a lack of concentration, reduced quality of life, and a decline in performance. For example, Belenky [6] has indicated that once chronic sleep insufficiency affects performance, complete recovery is difficult even if adequate sleep was secured for three consecutive nights. When chronic sleep insufficiency gradually accumulates in an individual, performance is drastically reduced even if sleepiness is not felt. Lack of sleep and insomnia also cause mental disorders. Kaneita's study [7] on 24,686 Japanese adults ascertained that sleep duration of less than six h and more than eight h was associated with symptoms of depression. Sleep deficiency can affect physical health as well. Gangwish [8] found that subjects who slept less than four hours were 73% more obese than those who slept more than seven h. Tochikubo [9] reported a high blood pressure throughout the day in subjects who had only 3-4 h of sleep the previous night. Knutsona [10] demonstrated that one week of sleep insufficiency caused impaired glucose tolerance because of a decline in insulin sensitivity levels. Therefore, adequate sleep is important not only for performing normal daytime activities efficiently, but also for good physical and mental health.

University students often have erratic schedules that cause irregular sleep patterns. Kang [11] suggested that students with an irregular bedtime schedule might experience poor sleep quality. In addition, Buboltz [12] investigated that poor sleep habits might become a self-perpetuating cycle that students are unaware of and might be unable to alter. Chang [13] conducted a follow-up study on 1053 male university graduates for 34 years. Of these, 103 developed depression, and the risk of depression in those who had insomnia during their university days was twice as high as that in subjects who did not. Therefore, it has been suggested that poor sleep habits at a young age could have an effect on sleep habits in middle age in the same individual, and that sleep habits are not short-term but rather long-term factors.

When evaluating sleep, we tend to emphasize the number of hours (i.e., the quantity of sleep). However, maintaining a regular and sound sleep cycle are also important factors. Hayashi [14] stated that sleep habits could be measured in three dimensions (i.e., regularity, quality and quantity). In an earlier study, Takeuchi [15] identified these three factors of sleep from the Tokyo Metropolitan Institute for Neuroscience life habits inventory (TMIN-LHI) and classified sleep habits on the basis of these factors. Takeuchi's analysis takes into account not only the quantity of sleep but also examines other perspectives, by which the issue can be more comprehensively understood. Minimizing hidden perspectives in this way provided a better understanding of the sleep habits of university students. However, no physical or mental data were investigated in that study, so the relationship between health condition and sleep habits was ambiguous. University students often have physical and mental problems besides sleep disorders [16]. In this study, we used Takeuchi's method to compare the physical and mental condition of university students in order to clarify the effects of certain factors that cause differences in sleep habits. In addition, because sleep habits differ between sexes [17], we restricted our study to males in order to obtain a more exact analysis. Therefore, we investigated sleep habits (sleep hygiene) in male university students for the purpose of demonstrating the association between sleep habits and physical and mental health.

MATERIALS AND METHODS

Study subjects and duration

The subjects of this study were male students of Kurume University (18-29 years; average age: mean ± standard deviation (SD), 19.4±1.8 years). None of the subjects had any physical or mental disease and none were on regular medication. In addition, obesity can cause secondary sleep disturbances because of sleep apnea syndrome. Therefore, a body mass index (BMI) of 18.5 to 24.9 was set as an inclusion factor for this study. In total, 90 students were included in this study, which was carried out in June 2010. There were no aggravating circumstances such as examinations or long breaks before or after the testing day.

Questionnaires

We used the TMIN-LHI, which is a detailed questionnaire comprising two sections. The first section includes 60 questions based on sleep habits and other lifestyle issues. The second section is a Morningness-Eveningness (ME) questionnaire created by Horne and Ostberg [18] and translated into Japanese by Ishihara [19]. It comprises 19 items. We calculated the ME score for our subjects on the basis of their grade slips.

Preparation of the sleep habits scale and classification of subjects

Selected Items

From TMIN-LHI, 26 of 60 items from section 1 were excluded. These were nominal items or the items targeting certain people who offered a specific answer, and had large ceiling or floor effects. We eventually

conducted a factor analysis with 35 items (i.e., the remaining 34 items from section 1 and the ME score from section 2).

Factor Analysis

We extracted factors by principal factor analysis and ran a promax rotation. Three factors were chosen on the basis of scree plot. The characteristic values were 6.120, 3.030 and 2.591 respectively, and the cumulative percentage was 33.5%.

Following analysis of the 35 items from the TMIN-LHI, which was done five times, 18 items were excluded. The remaining 17 items had factor loadings of $\geqq 0.35$. The explanatory power was 53.4% and the accumulated contribution rate was 46.0%. The confidence coefficients (α) of the three factors were 0.878, 0.683, and 0.670 for the first, second and third factor, respectively.

The three factors focused on in this study are described in Table 1 along with their factor loadings. The first factor comprised 8 items: regular bedtime, regular wake-up time, irregular bedtime, irregular wake-up time, ME score, irregular sleep duration, breakfast habits, and exercise habits. This regularity factor along with its items was named the related Sleep Regularity scale. The second factor comprised 5 items: difficulty of sleep latency, time to fall asleep, mood on waking up in the morning, depth of sleep and experience of insomnia. This quality factor along with its items was named the related Sleep Quality scale. The third factor comprised 5 items: regular sleep duration, ideal sleep duration, regular wake-up time, value attached to sleep and time spent in commuting. This quantity factor along with its items was named the related Sleep Quantity scale.

Principal Component Analysis

Eight items from the first factor, five from the second factor and five from the third factor were subjected to principal component analysis, and each factor was given a standard score. We created a sleep habits scale by assuming the first score to be a scale score. The average score was initially fixed at 0. In the related Sleep Regularity scale, a positive score indicated mostly regular sleep habits and a negative score indicated mostly irregular sleep habits. In the related Sleep Quality scale, a positive score indicated a more sound sleep and a negative score indicated a very disturbed sleep. In the related Sleep Quantity scale, a positive score indicated longer sleep duration and more active procurement of sleep, and a negative score indicated shorter sleeping hours and more passive procurement of sleep.

Classifying Subjects

The Ward method of cluster analysis was selected

TABLE 1.
Extracted factors and factor loadings

	Items	Range	Factor Loadings
The First Factor α=0.878	Q1: regular bedtime	early – late	0.868
	Q4: regular wake-up time	early – late	0.745
	Q2: irregular bedtime	small – large	0.713
	Q5: irregular wake-up time	small – large	0.689
	ME score	Eveningness – Morningness	–0.657
	Q8: irregular sleep duration	small – large	0.652
	Q37a: breakfast habits	always – never	0.616
	Q49: exercise habits	never – always	–0.596
The Second Factor α=0.683	Q14: difficulty of sleep latency	easy – difficult	0.982
	Q13: time to fall asleep	short – long	0.529
	Q19: mood on waking up in the morning	pleasant – unpleasant	0.427
	Q20: depth of sleep	deep – light	0.417
	Q43: experience of insomnia	no – yes	0.408
The Third Factor α=0.670	Q7: regular sleep duration	short – long	1.002
	Q11: ideal sleep duration	short – long	0.574
	Q4: regular wake-up time	early – late	0.397
	Q12: value attached to sleep	important – unimportant	–0.381
	Q45: time spent in commuting	short – long	–0.377

for classifying the subjects on the basis of principal component score for each factor. Four clusters were determined because they were the most balanced and the clearest following the dendrogram.

Physical factors

The subjects were instructed to measure their own height and weight. Waist circumference was measured at the umbilical region by members of the same study staff. The subjects also measured their own resting blood pressure and pulse with an automatic manometer (BP-203RV Type C, Nippon Colin, Tokyo, Japan) in a sitting position. If systolic blood pressure was ≧130 mmHg or diastolic blood pressure was ≧85 mmHg, subjects measured their blood pressure again. In the event that the second blood pressure measurement was unacceptable, the study staff measured it with a mercurial column manometer.

Mental health

We evaluated mental health using the Japanese version of the Social Functioning-36 (SF-36) [20-22] short-form health survey and Japanese version of the Self-rating Depression Scale (SDS) [23].

The Japanese version of the SF-36, which was developed by Fukuhara [20-22], is a general scale used to measure quality of life over a one-month period. All items are scaled, and the higher the scores, the better the quality of life. Physical component summary (PCS) and mental component summary (MCS) were derived from the SF-36: physical functioning, role physical, bodily pain, general health perceptions, vitality, social functioning, role emotional, and mental health. PCS score and MCS score can be compared to national standard values directly (50=national standard score). Furthermore, we can evaluate physical quality of life (QOL) from PCS and mental QOL from MCS in a comprehensive manner.

Zung [24,25] developed SDS as simple test to assess depression, and Fukuda translated it into Japanese [23]. It comprises 20 items, all graded according to four ranks of 1-4 points each. We used an integrated scoring system as follows: ≦39 points indicated normal mental health, 40-49 points indicated slight depression, and ≧50 points indicated moderate depression.

Blood tests

Blood samples were taken and fasting blood sugar (FBS), immunoreactive insulin levels, high-density cholesterol (HDL-C), low-density cholesterol (LDL-C), leptin, des-acyl ghrelin, and high molecular weight adiponectin (HMW-adiponectin) were measured. In addition, the homeostasis model of assessment-insulin resistance (HOMA-IR) was calculated from FBS and immunoreactive insulin values; HOMA-IR= (immunoreactive insulin×FBS)/405 [26].

Statistical analysis and software

The mean and SD values of each item were expressed as mean ± SD. Shapiro-Wilk test was used as the normality test. If the item showed a normal distribution, one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) tests were used as parametric tests. In the event that the item did not have a normal distribution, the Kruskal-Wallis H test and Scheffe test were used as nonparametric tests. We used SPSS15.0J Base System SC Kit-software and set the significance level at $p<0.05$.

Ethical concerns

Prior to enrollment, subjects were informed about the purpose and method of this study, following which they asked to sign written informed consent forms. If the subjects were minors, we obtained their parents' approval. We ensured that no one of the subjects would be pressured or harmed because of nonparticipation. Personal data were strictly monitored to maintain confidentiality and to protect the privacy of subjects. This study was approved by the Kurume University Mii Campus Ethical Review Board.

RESULTS

Characteristics of each type of sleep habits

Figure 1 illustrates the four categories of sleep habits with the mean and SD of each scale score. The first type was the most regular and sound sleep, but there was a slight tendency towards shorter sleep duration. It was termed the "good sleep" type. The second type was somewhat regular and characterized by insomnia and longer sleeping period. It was called "long sleep" type. The third type was somewhat irregular but sound sleep and was characterized by the shortest sleep duration among all subjects. It was called "short sleep" type. The fourth type was the most irregular and was characterized by extreme insomnia and long sleep duration. It was referred to as the "poor sleep" type.

The upper section of Table 2 lists the averages for regular sleep duration, ideal sleep duration, regular bedtime, regular wake-up time, irregular sleep duration, irregular bedtime, irregular wake-up time and time to fall asleep for the four sleep habits groups. Regular sleep duration averaged 6.7±1.2 h. There were sig-

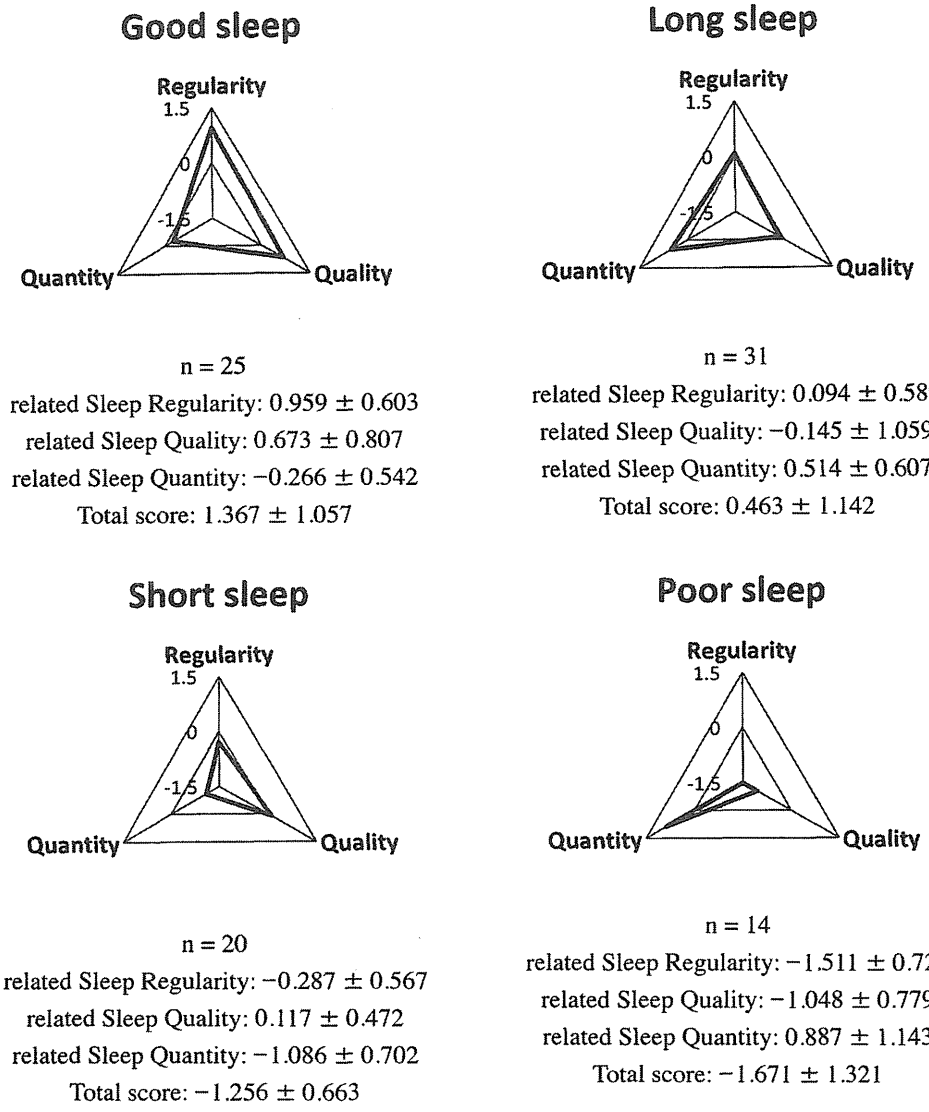

Fig. 1. Four types of sleep habits and corresponding scale scores (means ± SD).
Good sleep was the most regular and sound sleep, but there was a slight tendency towards shorter sleep duration. Long sleep was somewhat regular and characterized by insomnia and longer sleeping period. Short sleep was somewhat irregular but sound sleep and was characterized by the shortest sleep duration. Poor sleep was the most irregular and was characterized by extreme insomnia and long sleep duration.

nificant differences in regular sleep duration (multiple comparison; short sleep vs. good sleep, long sleep and poor sleep: $p<0.001$), ideal sleep duration (short sleep vs. long sleep: $p<0.05$ and poor sleep: $p<0.01$), regular bedtime (good sleep vs. long sleep: $p<0.01$, short sleep and poor sleep: $p<0.001$, long sleep vs. poor sleep: $p<0.001$, short sleep vs. poor sleep: $p<0.05$), regular wake-up time (good sleep vs. long sleep and poor sleep: $p<0.001$, long sleep vs. short sleep: $p<0.05$ and poor sleep: $p<0.001$, short sleep vs. poor sleep: $p<0001$), irregular sleep duration (good sleep vs. short sleep: $p<0.05$ and poor sleep: $p<0.001$, long sleep vs. poor sleep: $p<0.001$, short sleep vs. poor sleep: $p<0.001$), irregular bedtime (good sleep vs. short sleep: $p<0.01$ and poor sleep: $p<0.001$, long sleep vs. poor sleep: $p<0.001$, short sleep vs. poor sleep: $p<0.05$), irregular wake-up time (good sleep vs. short sleep: $p<0.05$ and poor sleep: $p<0.001$, long sleep vs. poor sleep: $p<0.001$, short sleep vs. poor sleep: $p<0.001$), and time to fall asleep (good sleep vs. poor sleep: $p<.001$, short sleep vs. poor sleep: $p<0.01$).

Physical characteristics of subjects

The middle section of Table 2 describes the physi-

TABLE 2.
Characteristics of each type of sleep habits (mean ± SD)

		Total	Good sleep	Long sleep	Short sleep	Poor sleep	p value
	n	90	25	31	20	14	
Sleep data	regular sleep duration (h)	6.7±1.2	6.7±0.8	7.3±0.7	5.3±0.9	7.1±1.5	<0.001*
	ideal sleep duration (h)	7.4±1.2	7.3±0.9	7.7±1.1	6.8±0.7	8.1±1.7	0.003*
	regular bedtime (o' clock)	24.7±1.3	23.6±1.1	24.6±0.9	25.2±1.0	26.1±1.1	<0.001*
	regular wake-up time (o' clock)	7.8±1.4	6.7±0.9	8.2±0.9	7.4±0.9	9.6±1.3	<0.001*
	irregular sleep duration (min)	71.0±62.9	32.4±35.6	60.0±49.6	79.5±53.6	152.1±67.1	<0.001*
	irregular bedtime (min)	94.0±73.6	50.0±25.8	78.7±42.3	117.0±79.6	173.6±102.7	<0.001*
	irregular wake-up time (min)	81.6±74.1	41.2±31.0	62.3±37.9	90.0±66.0	184.3±103.2	<0.001*
	time to fall asleep (min)	25.3±16.4	17.8±9.3	28.1±17.9	21.0±11.1	38.6±20.3	<0.001*
Physical data	Age (years)	19.4±1.8	19.0±0.9	19.6±2.1	19.2±2.4	19.9±1.3	n.s.
	BMI (kg/m^2)	21.0±1.7	20.2±1.6	21.2±1.8	21.3±1.5	21.3±1.8	n.s.
	waist circumference (cm)	72.1±5.1	69.9±4.7	72.1±5.6	73.9±3.9	73.4±5.2	0.040*
	systolic blood pressure (mmHg)	116.2±10.1	110.1±9.6	117.2±10.6	118.0±9.1	120.9±7.7	0.011*
	diastolic blood pressure (mmHg)	66.3±9.6	62.4±6.6	68.1±10.3	65.0±9.4	70.9±10.3	0.026*
	pulse (/min)	69.7±10.3	64.8±10.5	70.8±10.3	71.9±10.2	72.7±7.6	0.038*
Blood test data	FBS (mg/dl)	86.8±7.2	85.9±5.0	86.1±5.2	85.7±4.5	91.7±13.6	0.049*
	immunoreactive insulin (μIU/ml)	6.9±7.0	5.7±2.2	6.3±3.4	6.5±3.0	11.1±16.2	n.s.
	HOMA-IR	1.6±2.3	1.2±0.5	1.4±0.7	1.4±0.7	3.0±5.5	n.s.
	HDL-C (mg/dl)	61.2±10.0	63.5±9.5	57.7±8.4	65.0±11.8	59.3±9.3	0.035*
	LDL-C (mg/dl)	90.2±19.3	93.7±20.0	87.1±18.4	85.9±15.3	97.2±23.4	n.s.
	leptin (ng/ml)	2.4±1.1	2.0±0.8	2.5±1.3	2.7±1.0	2.4±1.0	0.024†
	desacyl-ghrelin (fmol/ml)	203.7±99.6	240.6±129.5	182.5±75.3	181.4±88.6	216.4±88.5	n.s.
	HMW-adiponectin (μg/ml)	5.2±2.7	5.9±2.7	5.1±2.8	4.9±2.7	4.6±2.5	n.s.

* significant difference (by ANOVA test), † significant difference (by Kruskal-Wallis H test), n.s = not significant.

cal characteristics of subjects associated with the four types of sleep habits. There were significant differences in waist circumference (multiple comparison; good sleep vs. short sleep: $p<0.05$), systolic blood pressure (good sleep vs. poor sleep: $p<0.05$), diastolic blood pressure (good sleep vs. poor sleep: $p<0.05$), and pulse (no difference in multiple comparison). However, no significant differences were observed in age or BMI among the subjects.

Mental health of subjects

Figure 2 shows data for the four types of sleep habits based on responses to PCS and MCS of SF-36. While PCS scores of all groups were higher than the national standard value (≧50), MCS scores of two groups (i.e., long sleep and poor sleep) were lower than the national average. There was a significant difference in MCS but no significant difference in PCS among the subjects. Scores for poor sleep were significantly lower than for the other types of sleep with regard to MCS (vs. good sleep: $p<0.001$, vs. long sleep: $p<0.05$, vs. short sleep: $p<0.01$). Figure 3 shows data for the four types of sleep habits based on responses to SDS. Scores for poor sleep were significantly higher than those for the other types of sleep (vs. good sleep: $p<0.001$, vs. long sleep: $p<0.05$, vs. short sleep: $p<0.05$).

Blood test results

The bottom section of Table 2 lists the results of blood tests for each group. There were significant differences in FBS (no difference in multiple comparison), HDL-C (long sleep vs. short sleep: $p<0.05$), and leptin (good sleep vs. short sleep: $p<0.05$), but no significant differences in immunoreactive insulin levels, HOMA-IR, LDL-C, des-acyl ghrelin, and HMW-

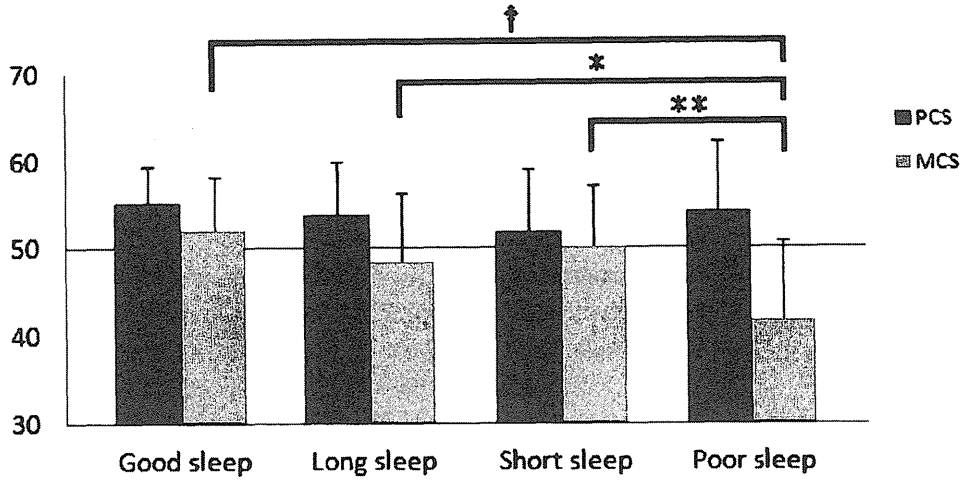

Fig. 2. PCS and MCS as measured by SF-36 (national standard value = 50, *p<0.05, ** p<0.01, †p<0.001 by Tukey's HSD test).
While PCS scores of all groups were higher than national standard value (≧50), MCS scores of long sleep and poor sleep were lower than it. Scores for poor sleep were significantly lower than for the other types of sleep with regard to MCS.

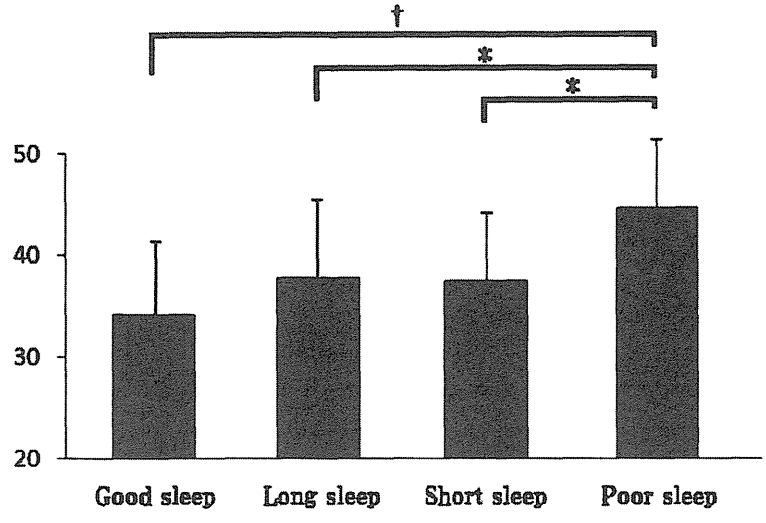

Fig. 2. SDS of four sleep habits (*p<0.05, †p<0.001 by Tukey's HSD test).
Poor sleep had significantly higher score when compared to the scores for the other types.

adiponectin among the subjects.

DISCUSSION

Methodology

The purpose of this study was to measure the impact of different sleep habits (sleep hygiene) on physical and mental health in a cohort of 90 male university students by classifying sleep habits. We developed comprehensive scales that described sleep regularity, quality, and quantity by factor analysis and principal component analysis. Cluster analysis was then employed to classify the sleep habits these students into four groups. Measures of sleep habits must consider not only the number of hours slept (quantity), but must also account for regularity and quality of sleep to

clearly identify those facets of sleep patterns that most influence mental and physical health. Takeuchi [15] classified sleep habits on the basis of all three factors, and this method proved to be more precise than methods that only evaluated single factors such as sleep duration. We investigated the physical and mental health of the present subjects after classifying sleep habits by means of Takeuchi's comprehensive scoring method. Our analysis underscores the importance of measuring all three dimensions to establish a consistent relation between sleep habits and physical and mental health.

Interpretation of each type of sleep habits

Good sleep is defined by consistent and normal sleep duration. Those subjects in the good sleep group kept very early hours and scored higher in Morningness. Daily changes of sleep duration, bedtime, and wake-up time were quite small. They also fell asleep easily. Good sleep is the exemplary sleeping pattern. In long sleep subjects, regular bedtimes were normal and regular, but regular wake-up time was later. In addition, both usual and ideal sleep durations were longer than the average. In other words, this group preferred to rise later. They fell asleep with difficulty and had lower quality sleep; that may cause them to oversleep. In the short sleep group, regular bedtimes were later and regular wake-up times were earlier. While this group fell asleep easily, they had irregular sleep habits. They preferred to stay up late and were less concerned about sleep because their ideal sleep duration was shorter than that of any of the other groups. These factors could be expected to result in short sleeping times. Subjects in the poor sleep group seemingly got plenty of sleep according to regular sleep duration, but sleep regularity was poor, and these subjects found it difficult to fall asleep. In addition, their ideal sleep duration was longer than that of any of the other groups. This group may lack sufficient sleep due to chronic insomnia.

In Takeuchi's study [15], several extracted sleep habits were similar to sleep disorders defined by the American Sleep Disorders Association (ASDA), including sleep deficit syndrome, circadian rhythm disorder, and delayed sleep phase syndrome. In the current study, both short and poor sleep subjects had lower than average total scale scores, indicating problematic sleep habits. Short sleep was similar to sleep deficit syndrome as defined by the ASDA in that there was a large gap between actual and ideal sleep durations. In contrast, poor sleep resembled exogenous circadian rhythm disorders such as jet lag. A change in time zone of over three hours causes jet lag with disturbance of sleep induction [27]. The sleep habits of subjects with poor sleep appeared similar to those of daily travelers suffering from jet lag. If the subjects in the poor sleep group graduate from university with the same sleep habits, they might not be able to conform to regular office hours. Thus, they should receive therapy to correct irregular lifestyle habits and to manage insomnia.

Physical characteristics of subjects

Lack of sleep may be linked to obesity due to increased food consumption [8]. There was a significant difference in average waist circumference between the short and the good sleep groups. However, the good sleep group had the second shortest average sleep duration; thus, shorter sleep duration alone does not lead to weight gain. If individuals continue to sleep for irregular hours and have an inconsistent circadian rhythm, the clock gene begins to express abnormalities, which may lead to easy accumulation of visceral fat [28,29]. Therefore, it is suggested that people with irregular as well as short sleep habits, like the short sleep group in this study, may be prone to visceral fat obesity even if their age and BMI are similar to individuals with good sleep habits.

There were significant differences in blood pressure and pulse between the poor and good sleep groups. Ishii [30] demonstrated that irregular sleep habits could disrupt autonomic nervous system function leading to hypertension and rapid pulse. In addition, Javaheri [31] reported that sleep disturbance could cause hypertension even in young healthy men without arteriosclerosis such as the poor sleep subjects in this study. Extremely irregular sleep habits with insomnia, as exhibited by the poor sleep group, may increase blood pressure and pulse due to an autonomic disorder.

Mental health of subjects

Apropos of the relationship between sleep and mental disease, insomnia and depression are often associated with poor sleep habits. Predictably, poor sleep, the type for which the related Sleep Quality scores were extremely low, indicated the worst mental condition. With respect to MCS measured on the basis of SF-36 and SDS scores, subjects in the poor sleep group had very bad scores that were significantly poorer than the scores of subjects of any other sleep type. SDS scores in this group were $\geqq 40$, which is considered to reflect a state of neurosis. People who have sleep habits like those in the poor sleep group are therefore prone to depression. The mental condition of subjects in the long sleep group, whose related Sleep Quality scores were second-lowest after those in the poor sleep

group, was also lower than national standard value in MCS in spite of surpassing that of subjects in the short sleep group whose total sleep habits scale score was far worse.

The timing of onset of depression and insomnia has not been sufficiently investigated. However, according to Ohayan [32], insomnia often precedes depression. Furthermore, for people who suffer from insomnia for more than one year, the risk of depression can be 40 times greater than that in those without insomnia [33]. It is important, therefore, that the symptoms observed in the poor sleep group of this study should be immediately resolved, and people whose sleep habits fall into the long sleep category should also be concerned about the relation between their sleep habits and mental condition.

Blood test results

Of the saccharometabolism items (FBS, immunoreactive insulin, and HOMA-IR), only FBS showed a significant difference among subjects with the four types of sleep habits. However, for all three items, the good sleep group had the lowest values and the poor sleep group showed the highest values. As mentioned above, an inconsistent circadian rhythm because of irregular sleep habits leads to abnormalities in the clock gene, which in turn may lead to visceral fat accumulation [28,29]. Eventually, insulin resistance and impaired glucose tolerance can also result.

Of the lipometabolism items (HDL-C and LDL-C), only HDL-C showed a significant difference between long sleep and short sleep. While subjects in the good sleep and short sleep groups had high levels of HDL-C, those in the long sleep and poor sleep groups had low levels of HDL-C. It has been suggested that deterioration in sleep quality and hypersomnia play a role in decreasing HDL-C. Contrary to our result, however, Bjorvatn [34] reported that sleep of short duration decreased HDL-C. This point, therefore, needs further study and consideration.

In the blood tests for leptin, des-acyl ghrelin, and HMW-adiponectin, only leptin showed a significant difference among subjects with the four types of sleep habits. Leptin and des-acyl ghrelin are appestat factors that are influenced by sleep. Sleep of short duration decreases leptin and increases des-acyl ghrelin, which in turn can bring about obesity due to enhanced appetite [35]. We predicted that leptin would decrease and des-acyl ghrelin would increase in the short sleep and poor sleep groups. However, there was no difference in des-acyl ghrelin, and leptin increased in subjects in the short sleep group. There was a significant difference in circulating leptin between the short and good sleep groups. Leptin levels often increase with obesity because increased visceral fat leads to leptin resistance [36]. Visceral fat accumulation resulting from a chronic lack of sleep and irregular sleep habits might cause leptin resistance. Most previous studies were short-term (i.e., they analyzed acute sleep insufficiency). The effects of long-term sleep habits have not been adequately considered. Other studies analyzed only sleep duration and not regularity and quality. Therefore, it has been suggested that irregular as well as short sleep habits over the long-term, like short sleep in this study, cause leptin resistance and a compensatory increase in leptin.

In this study, there was no significant difference in HMW-adiponectin between groups. However, HMW-adiponectin levels were highest in the good sleep group and lowest in the poor sleep group. Few studies have reported the relationship between adiponectin and sleep. There is a possibility that the small number of subjects in our study prevented us from finding a significant difference for this parameter. Therefore, the relationship between adiponectin and sleep requires further analysis with a larger study sample.

The limitations of this study include the inclusion of males only and the relatively small sample population. Triglyceride, which is one of the diagnostic criteria of metabolic syndrome, was not measured, so we were unable to estimate the relation between sleep habits and metabolic syndrome. We also did not ask about club activities and part-time jobs, factors which could impact regularity of sleep. In addition, TMIN-LHI is a not scale for scoring but a simply questionnaire, and is not subject to validation. Finally, the analyses in this study have a variability related to the subjects. University students have unique lifestyles and sleep habits compared to the general population, and their sleep characteristics may differ from those in heterogeneous subjects like general members of society or the aged.

CONCLUSION

We found significant differences in certain physical characteristics, mental health, and blood test results among four different sleep habit groups in male university students. Although all subjects in this study were young men with normal BMI, irregular sleep habits caused increases in waist circumference, blood pressure, and pulse, and impaired saccharometabolism and lipometabolism even if sleep duration was kept constant. In addition, the mental condition of subjects with poor quality sleep was not as good as that of sub-

jects with good quality sleep. It is important to gain a comprehensive understanding of sleep habits in order to maintain good physical and mental health among male university students. Further studies to validate these scales may be required in the fields of sleep medicine and preventive medicine.

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Effects of Oral L-Carnitine Administration in Narcolepsy Patients: A Randomized, Double-Blind, Cross-Over and Placebo-Controlled Trial

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Abstract

Narcolepsy is a sleep disorder characterized by excessive daytime sleepiness, cataplexy, and rapid eye movement (REM) sleep abnormalities. A genome-wide association study (GWAS) identified a novel narcolepsy-related single nucleotide polymorphism (SNP), which is located adjacent to the carnitine palmitoyltransferase 1B (*CPT1B*) gene encoding an enzyme involved in β-oxidation of long-chain fatty acids. The mRNA expression levels of *CPT1B* were associated with this SNP. In addition, we recently reported that acylcarnitine levels were abnormally low in narcolepsy patients. To assess the efficacy of oral L-carnitine for the treatment of narcolepsy, we performed a clinical trial administering L-carnitine (510 mg/day) to patients with the disease. The study design was a randomized, double-blind, cross-over and placebo-controlled trial. Thirty narcolepsy patients were enrolled in our study. Two patients were withdrawn and 28 patients were included in the statistical analysis (15 males and 13 females, all with *HLA-DQB1*06:02*). L-carnitine treatment significantly improved the total time for dozing off during the daytime, calculated from the sleep logs, compared with that of placebo-treated periods. L-carnitine efficiently increased serum acylcarnitine levels, and reduced serum triglycerides concentration. Differences in the Japanese version of the Epworth Sleepiness Scale (ESS) and the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) vitality and mental health subscales did not reach statistical significance between L-carnitine and placebo. This study suggests that oral L-carnitine can be effective in reducing excessive daytime sleepiness in narcolepsy patients.

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Introduction

Narcolepsy is a sleep disorder characterized by excessive daytime sleepiness, cataplexy (sudden loss of muscle tone in response to strong emotions), and rapid eye movement (REM) sleep abnormalities. Narcolepsy patients are at a higher risk of obesity and non–insulin-dependent diabetes mellitus [1–5], and show high levels of total cholesterol and triglycerides [6]. The prevalence of narcolepsy is 0.16% to 0.18% in Japan and 0.02% to 0.06% in the United States and Europe [7]. Following studies in dogs [8] and mice [9], a 95% loss of orexin (hypocretin)-producing cells in postmortem hypothalami from narcolepsy patients was reported [10,11]. It has been reported that both genetic and environmental factors contribute to its development [7]. Narcolepsy is closely associated with human leukocyte antigen (*HLA*)-*DQB1*06:02* [12,13]. Results from a genome-wide association study (GWAS) have revealed significant associations between narcolepsy and single nucleotide polymorphisms (SNP) located in the T-cell receptor α-locus and *P2RY11* [14,15]. Recently, we also conducted a GWAS and identified a novel narcolepsy-related SNP (rs5770917), located adjacent to the gene encoding carnitine palmitoyltransferase 1B (CPT1B) [16]. The mRNA expression levels of *CPT1B* were associated with this SNP and the expression levels were decreased according to the number of risk alleles (C) [16,17].

CPT1B is a rate-limiting enzyme in β-oxidation of long-chain fatty acids, which is mainly localized in the muscle mitochondrial outer membrane [18]. Conjugation of carnitine to long-chain fatty acyl coenzyme A (CoA) by CPT1B allows the transport of long-chain fatty acids into the mitochondrial matrix for subsequent β-oxidation. Several reports have indicated a role for fatty acid β-oxidation and the carnitine system in sleep regulation. Fasted

juvenile visceral steatosis ($jvs^{-/-}$) mice with systemic carnitine deficiency exhibit a higher frequency of fragmented wakefulness and REM sleep, and reduced locomotor activity [19]. These phenotypes in the fasted $jvs^{-/-}$ mice are similar to those in mouse models of narcolepsy [9,20]. In these mice, a significant reduction in the number of c-Fos-positive orexin neurons, hypothalamic prepro-orexin mRNA expression, and orexin-A concentration in the cerebrospinal fluid (CSF) was observed [19,21]. These findings indicate that the acylcarnitine availability is essential for normal sleep regulation and orexin cell functions. On the other hand, mice deficient in short-chain acyl-CoA dehydrogenase (encoded by *Acads*), an enzyme catalyzing the first step of β-oxidation, have shown significantly slower theta frequency during REM sleep [22]. Administration of acetyl-L-carnitine, which is known to restore β-oxidation in the mitochondria [23,24], significantly recovers slow theta frequency in the mutant mice.

In a previous study, we analyzed the expression level of *CPT1B* and measured the carnitine fractions in blood samples obtained from narcolepsy patients and healthy control subjects [17]. *CPT1B* expression was significantly higher in the narcolepsy patients than in the controls, and acylcarnitine levels were abnormally low in 21% of the narcolepsy patients while those of all the controls were within the normal range, suggesting that fatty acid β-oxidation is altered in narcolepsy [17]. Therefore, we hypothesized that promoting fatty acid oxidation by L-carnitine supplementation could alleviate narcolepsy symptoms.

Materials and Methods

Patients

Suitable study patients were identified from consecutive patients attending the Yoyogi sleep clinic affiliated to Neuropsychiatric Research Institute. Inclusion criteria were: age≥15 years and patients satisfying the diagnostic criteria of the 2nd edition of the International Classification of Sleep Disorders (ICSD-2) for narcolepsy with cataplexy. Written informed consent was obtained from all study participants. We did not obtain informed written consent from a legally acceptable representative because the participants of this study were all at least 20 years old and competent to consent. Thirty narcolepsy patients were enrolled in our study. Two patients were dropped out due to inability to follow regular visits defined in the study protocol and 28 patients were included in the statistical analysis (15 males and 13 females). The mean age ± standard deviation (SD) was 41.2±15.9 years. All the patients carried the *HLA-DQB1*06:02* and exhibited unambiguous cataplexy. Exclusion criteria were: pregnancy, potentially pregnant or lactating women; known hypersensitivity to L-carnitine; epilepsia; use of acenocoumarol or other experimental treatment during this study. The patients were unrelated Japanese individuals living in Tokyo or in neighboring areas. The protocol for this trial and supporting CONSORT checklist are available as supporting information; see Checklist S1 and Protocol S1. This study was approved by the Ethics Committee of the Tokyo Metropolitan Institute of Medical Science and the Ethics Committee of the Neuropsychiatric Research Institute. The gene analysis in this study was also approved by Human Genome, Gene Analysis Research Ethics Committee of the Faculty of Medicine and Graduate School of Medicine of the University of Tokyo.

Design

The trial was a randomized, double-blind, cross-over and placebo-controlled design of 16 weeks' duration. There were two, 8-week treatment periods, treatment period one and treatment period two. There were five specified visits at 0, 4, 8, 12 and 16 weeks. Patients were randomly assigned using a random number to L-carnitine during treatment period one, followed by placebo in treatment period two (group A), or placebo in treatment period one followed by L-carnitine in treatment period two (group B). An 8-week supply of the relevant treatment (L-carnitine or placebo capsules in a bottle) was labeled with that particular number. The randomization and labeling of the treatments were performed by a person who had not seen the patients. Oral L-carnitine (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) and placebo were supplied in identical capsules. Dosage was three L-carnitine capsules (170 mg×3 = 510 mg) or three placebo capsules per day, two capsules in the morning and one capsule in the evening. L-carnitine crosses the BBB through carnitine transporter OCTN2 [25]. Concomitant medication for narcolepsy such as psychostimulants (modafinil, methylphenidate, pemoline for excessive daytime sleepiness and clomipramine for cataplexy and hypnagogic hallucination) was available on the condition that the dosage and administration of the medication would not be altered during this study. This study was registered at the University hospital Medical Information Network (UMIN) (ID: UMIN000003760).

Efficacy variables and statistics

The primary outcome measure was the patient's subjective assessment of their sleepiness using total time for dozing off during the daytime in their sleep logs. The secondary outcome measures were as follows: the number of occurrences of dozing off during daytime, cataplexy and sleep paralysis in sleep logs; the Japanese version of the Epworth Sleepiness Scale (JESS) [26]; the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) vitality (VT) and mental health (MH) subscales [27,28]; Body Mass Index (BMI). Scores for JESS range from 0 to 24, with lower scores indicating less daytime sleepiness. Scores for SF-36 were transformed to norm-based scores with a Japanese population mean of 50 and a standard deviation (SD) of 10, with higher scores indicating a better health state. Actigraph data (Actiwatch, Phillips-Respironics, Tokyo) was collected for 11 days in both L-carnitine and placebo periods as auxiliary data to check the accuracy of sleep logs. We also evaluated the following biochemical measurements: serum levels of total carnitine, free carnitine, acylcarnitine, total cholesterol and triglycerides, measured by SRL Inc. (Tokyo, Japan). We did not adopt sleep log data for 2 weeks after the beginning of each treatment period in order to avoid a carry-over effect (treatment-period interaction). The total time and the number of times dozing off during daytime did not include scheduled daytime naps. Continuous variables were summarized as the mean and SD. Treatment effects, period effects and treatment-period interaction were analyzed using a 2-sample t-test. For the treatment effects, we used a one-tailed test because L-carnitine treatment was expected to improve narcolepsy symptoms relative to placebo controls. Baseline comparisons were performed with a 2-sample t-test. Categorical variables were summarized as percentages and the differences between groups were assessed using a chi-squared test. When data did not follow a normal distribution, non-parametric tests such as the Mann-Whitney U-test or the Wilcoxon signed-rank test were applied. Genotyping for SNP rs5770917 was performed using Taqman SNP genotyping assays (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's protocol. IBM SPSS Statistics 19 and Microsoft Office Excel 2007 were used for statistical analyses. Baseline SF-36 scores from three subjects were missing due to incomplete fill-in. We conducted a power analysis using G*Power [29,30] to determine the minimum sample size needed to detect a difference of 12 minutes in total time for dozing off during daytime. A total of 27 participants were required to achieve a power of 0.8 at

a = 0.05 (one-tailed test). Clinical research coordinators sent from Site Support Institute Co., Ltd. (SSI) supported doctors to improve the quality of this study implementation.

Results

A total of 30 narcolepsy patients were enrolled in this study between May, 2010 and July, 2010. Of the 30 enrolled patients, 28 completed all five visits defined in the study protocol (Fig. 1). There were no significant differences in the baseline characteristics of the study participants between the two groups (Table 1). The average BMI (± SD) of the 28 patients was 26.1±5.7, and 14 out of the 28 patients were obese. The average compliance (± SD) based on the number of capsules at clinic visits and patient logs was 98.2±2.7% on L-carnitine and 97.7±3.9% on placebo.

Efficacy results are shown in Table 2. Regarding the primary endpoint, patients treated with L-carnitine showed a significant reduction in the total time for dozing off during daytime as measured by sleep logs, compared with the placebo period (L-carnitine: 49±34 min/day slept; placebo: 58±37 min/day; $P = 0.048$). The number of naps in patients given L-carnitine was also decreased, but not significantly ($P = 0.14$). There were no significant improvements in JESS and SF-36 subscale (vitality and mental health) scores between L-carnitine and placebo periods. However, the JESS scores of both periods and SF-36 subscale scores for the L-carnitine period tended to be improved from the baseline. The average numbers of episodes of cataplexy and sleep paralysis were low with less than 0.05 per day in both periods and showed no significant differences between treatments. Regarding BMI, no significant differences between L-carnitine and placebo periods were found. No period effects and no treatment-period interactions were observed for any of the parameters described in Table 2. L-carnitine was well-tolerated with no side effects observed. Out of 28 patients, 13 patients carried a risk allele of SNP rs5770917 (2 of them were homozygous for the risk allele) and 15 patients did not carry it. We tested whether SNP rs5770917 affects the primary endpoint (total time for dozing off during daytime). SNP rs5770917 was not significantly associated with the change of total dozing off time between L-carnitine and placebo periods (mean change [±SD] of risk allele carrier and

Table 1. Baseline characteristics.

Demographics	L-carnitine First (group A)	Placebo First (group B)	*P* Value
Male (%)/Female (%)	8(53%)/7 (47%)	7 (54%)/6 (46%)	0.97
Age at start of trial (SD)	41 (17)	42 (16)	0.88
BMI (SD)	24.9 (3.9)	27.5 (7.2)	0.24
Patients with BMI≥25 (%)	7 (47%)	7 (54%)	0.70
JESS (SD)	13.7 (2.9)	13.8 (4.0)	0.93
SF-36 VT (SD)	45.9 (11.3)	44.9 (12.1)	0.82
SF-36 MH (SD)	51.4 (8.3)	49.4 (7.9)	0.53

In Japan, obesity is diagnosed as a BMI≥25 according to the classification of obesity developed by the Japan Society for the Study of Obesity.
VT, Vitality Mental health; MH, Mental health.
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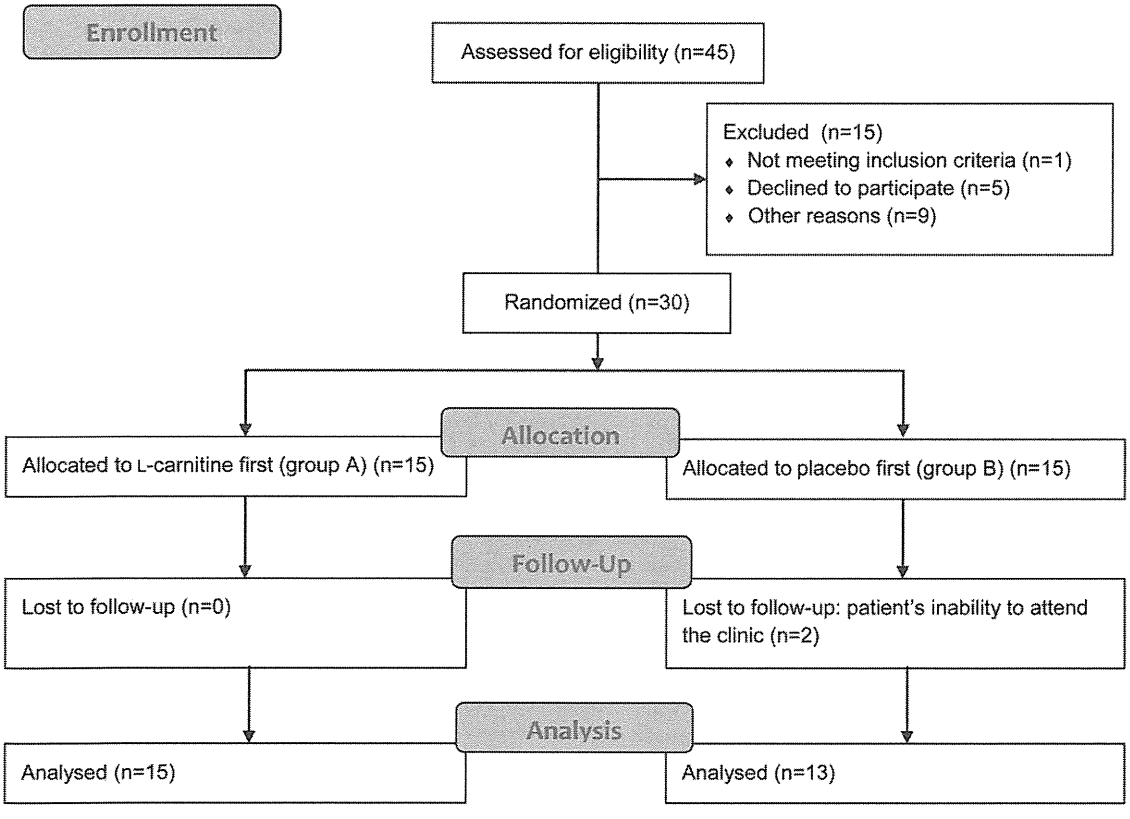

Figure 1. Study flow diagram.
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Table 2. Treatment effects of L-carnitine administration in narcolepsy patients.

Variable	L-carnitine	Placebo	*P* Value
Total nap time/day, min (SD)	49 (34)	58 (37)	0.048
No. of naps/day (SD)	1.3 (1.2)	1.4 (1.0)	0.14
No. of cataplexy/day (SD)	0.04 (0.07)	0.02 (0.04)	0.52*
No. of sleep paralysis/day (SD)	0.02 (0.06)	0.01 (0.03)	0.66*
JESS (SD)	12.7 (3.8)	12.7 (3.7)	0.48
difference to baseline (SD)	−1.0 (2.2)	−1.0 (3.8)	
SF-36 VT (SD)	47.5 (10.3)	45.5 (10.7)	0.13
difference to baseline (SD)	3.3 (7.1)	0.3 (10.0)	
SF-36 MH (SD)	50.4 (7.4)	49.9 (7.7)	0.39
difference to baseline (SD)	1.4 (8.2)	−0.2 (9.6)	
BMI (SD)	26.0 (5.8)	26.1 (5.9)	0.19
difference to baseline (SD)	−0.1 (0.6)	0.1 (0.7)	

*Parametric test assumptions were not available; thus the comparisons were performed with the Mann-Whitney U-test. For comparisons which were not analyzed using the Mann-Whitney U-test, the *P* values were calculated with a 2 sample t-test.
VT, Vitality Mental health; MH, Mental health.
Underlined variable, total nap time, indicates the primary endpoint of this study.
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non-carrier, 11.5±28.9 vs 6.4±26.2 minutes shortening; $P=0.62$ [2-sample t-test]).

Table 3 shows the effects of L-carnitine on metabolic characteristics. Narcolepsy patients with placebo showed relatively low levels of acylcarnitine (9.4±3.3 μmol/L; normal range: 6–23 μmol/L; 4 patients abnormally low), as we found abnormally low acylcarnitine levels in narcolepsy patients in the previous study [17]. The average level of acylcarnitine was significantly increased to 12.8±4.6 μmol/L ($P=3.1\times10^{-5}$), and 3 out of the 4 patients reached the normal levels by the administration of L-carnitine. Total and free carnitine levels were elevated by the treatment as we expected. Triglyceride levels during the L-carnitine treatment period were significantly decreased compared with those during the placebo period ($P=0.028$), and the average level dropped to the normal level (L-carnitine: 132.9±79.5 mg/dL; placebo: 168.7±111.4 mg/dL; normal range: 50–149 mg/dL). Four of the 28 patients were treated for hyperlipidemia. Triglyceride levels in the remaining 24 patients were compared between L-carnitine and placebo periods in order to eliminate the influence of the treatment for hyperlipidemia. A significant reduction for triglyceride levels was also observed in L-carnitine period (mean [±SD], L-carnitine: 122.8±81.1 mg/dL; placebo: 168.1±118.9 mg/dL; $P=0.014$). There was no significant difference for total cholesterol. No period effects and no treatment-period interactions were found for any of the parameters described in Table 3.

Table 3. Metabolic characteristics in L-carnitine and placebo periods.

Variable	L-carnitine	Placebo	*P* Value
Acylcarnitine (SD), μmol/L	12.8 (4.6)	9.4 (3.3)	3.1×10^{-5}
Total carnitine (SD), μmol/L	66.6 (10.7)	54.1 (9.4)	3.5×10^{-9}
Free carnitine (SD), μmol/L	53.8 (8.5)	44.6 (7.5)	1.2×10^{-8}
Triglycerides (SD), mg/dL	132.9 (79.5)	168.7 (111.4)	0.028
Total cholesterol (SD), mg/dL	213.8 (44.7)	206.3 (42.9)	0.96

The normal ranges for the laboratory tests are as follows: acylcarnitine 6–23 μmol/L; total carnitine 45–91 μmol/L, free carnitine 36–74 μmol/L, triglycerides 50–149 mg/dL and total cholesterol 150–219 mg/dL.
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Discussion

The present study revealed that total time for dozing off during daytime in narcolepsy patients, the primary endpoint, was significantly decreased by L-carnitine administration compared with placebo. Secondary outcome measures showed no statistically significant improvement between L-carnitine and placebo periods. However, the number of episodes of dozing off during daytime, JESS and SF-36 scores during the L-carnitine treatment period showed a tendency for improvement compared with those during the placebo period or the baseline. Limitations in the present study include statistical power. The power analysis was naturally performed for the primary endpoint and not the secondary endpoints. It is therefore possible that some of the positive trends in the secondary outcome measures may have been statistically significant with a larger sample size. The frequencies of cataplexy and sleep paralysis were found to be very low. The patients would have been successfully treated for these symptoms. In order to properly assess the effect on cataplexy and sleep paralysis, it would be effective to restrict concomitant medications for these symptoms. However, in conducting intervention studies that may make symptoms worse, the safety and ethics must be fully taken into consideration.

SNP rs5770917 adjacent to the gene encoding CPT1B has been found to be associated with narcolepsy [16]. CPT1B is a rate-limiting enzyme in β-oxidation of long-chain fatty acids. The expression levels were also associated with SNP rs5770917 genotype [16,17]. In this clinical trial, no significant association was observed between SNP rs5770917 and the primary endpoint. In addition, serum levels of total carnitine, free carnitine, acylcarnitine and triglycerides which were improved by L-carnitine were not significantly associated with SNP rs5770917. These results suggest that it may not be necessary to limit narcolepsy patients given L-carnitine using SNP rs5770917 genotype. However, the possibility of false negative cannot be denied because this study was not designed to assess the effect of SNP rs5770917, therefore a further study is required.

We observed that half of the study patients were obese. Average triglyceride levels during the placebo period were above the normal range, and 4 patients taking placebo showed abnormally low acylcarnitine levels (Table 2). These results reconfirmed previous studies [6,17], suggesting that fatty acid metabolism would be associated with the pathophysiology of narcolepsy. Oral

administration of L-carnitine was associated with triglyceride reduction and acylcarnitine increase, indicating that carnitine absorption and utilization are generally similar in narcolepsy patients and in normal controls. Triglyceride could be effectively utilized for fatty acid oxidation, resulting in the increase of acylcarnitine and facilitation of fatty acid oxidation. Treatment with L-carnitine could have the potential to reverse metabolic abnormalities in narcolepsy patients. However, there were no differences in BMI and total cholesterol between L-carnitine and placebo periods (Table 2). Generally, clinical trials for body weight and serum lipid profile in obese patients have been conducted for 6 months to 4 years [31]. The duration of the L-carnitine period in this study was only 8 weeks. Therefore, long term study is necessary to properly evaluate the effects of L-carnitine on obesity and lipid profile.

We set the endpoints for this study based on subjective measurements such as sleep logs, and questionnaires about daytime sleepiness (JESS) and QOL (SF-36 subscales), because our research focused on the changes in levels of sleepiness and psychological conditions in daily life of participants. Multiple sleep latency test (MSLT) is the standard test to objectively evaluate patients with excessive daytime sleepiness [32]. It would provide further information to use objective measurements such as MSLT for sleepiness in a future study. However, MSLT measures the tendency toward falling asleep (sleep propensity) in a lying position with few arousal signals from sensory input in a sleep laboratory. The outcome of MSLT measures reflect short-term "state" sleepiness and could be different from the "trait" sleepiness, the average conditions of many different situational sleep propensities that reflect the activities of daily life [33].

We tried to measure the changes of the global level of sleepiness using total dozing off time on sleep logs and JESS rating. They showed significant correlation with each other (correlation coefficient = 0.34, $P = 0.0051$), suggesting that total dozing off time on sleep logs could measure similar "trait" sleepiness as JESS. We selected total dozing off time on sleep logs as a primary outcome measure, because sleep logs are more accurate than other retrospective self-administered questionnaires including JESS. The participants can record their daily sleep status and symptoms in sleep logs before they forget. Furthermore, the total dozing off time calculated from the consecutive data can reduce the day-to-day variation. Clinical research coordinators instructed the participants how to record events in the sleep logs and confirmed that the sleep logs were correctly described.

A sleep actigraph as an objective measure is useful for determining nocturnal sleep timing and duration and can be worn for several weeks at a time [34]. We collected actigraph data in both L-carnitine and placebo periods and compared the actograms with the sleep logs, and observed that naps and dozing off time recorded in sleep logs approximately corresponded to the period of less motor activity, confirming that the participants understood and followed the instruction of clinical research coordinators well, and sleep log data could be utilized reliably for the comparison of sleepiness within the same subject. Incidentally, total motor activity, which is a principal measure of the actigraph, could be recorded in these study participants. We simply compared the total activity between L-carnitine and placebo periods, but the result showed no significant difference.

Low serum acylcarnitine levels have been observed in patients with chronic fatigue syndrome (CFS) [35–37], which is a clinically defined condition characterized by severe disabling fatigue and a combination of symptoms, such as musculoskeletal pain, difficulty in concentration and sleep disturbances [38]. It has been reported that L-carnitine supplementation resulted in significant improvements in fatigue severity after two months of supplementation [39]. Other results suggest that narcolepsy patients also feel consistently fatigued [40–42]. The SF-36 vitality subscale score of our participants was lower than that of national standards, and the score tends to be improved by L-carnitine administration, confirming that fatigue is common among narcolepsy patients (Table 2). These results also indicate that there might be a common pathological process underlying narcolepsy and CFS since both are accompanied by low serum acylcarnitine levels, a symptom that is improved by L-carnitine treatment.

In conclusion, L-carnitine is an effective and well-tolerated treatment for daytime somnolence in narcolepsy patients, but further studies with larger numbers of patients and long-term observation periods are required to confirm its efficacy and safety, and to clarify the mechanisms underlying its benefit.

Supporting Information

Checklist S1 CONSORT Checklist.
(DOC)

Protocol S1 Trial protocol.
(DOC)

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Author Contributions

Conceived and designed the experiments: TM KT YI MH. Performed the experiments: TM HK MO AI MH. Analyzed the data: TM HK MO AI MH. Contributed reagents/materials/analysis tools: TM AO YI MH. Wrote the paper: TM MH.

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