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Short Sleep Duration is a Risk of Incident Nonalcoholic Fatty Liver Disease: A Population-based Longitudinal Study

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ABSTRACT

Background & Aims: Previous cross-sectional studies revealed that short sleep duration has a close relationship with the presence of non-alcoholic fatty liver disease (NAFLD). We aimed to investigate the association between sleep duration and incident NAFLD.

Methods: In this historical cohort study of 12,306 participants (5,848 men and 6,458 women), we investigated the effect of sleep duration on incident NAFLD. NAFLD was defined as having fatty liver diagnosed by abdominal ultrasonography in the participants who consumed ethanol less than 30 g/day for men and 20 g/day for women. We divided the participants into four groups according to sleep duration: >7, >6-7, >5-6, and ≤5h. Cox proportional hazards models were performed to investigate the effect of sleep duration on incident NAFLD, adjusting for age, body mass index categories, alanine aminotransferase, triglycerides, high density lipoprotein-cholesterol, fasting plasma glucose, smoking status, alcohol consumption, systolic blood pressure, exercise.

Results: During the median 6.8-year follow-up for men and the 7.0-year follow-up duration for women, 2,280 participants (1,581 men and 699 women) developed NAFLD. In Cox proportional hazards models, sleep duration of ≤5 h in both men and women were revealed to be a significant risk for incident NAFLD, compared to men and women with a sleep duration of >7 h (men: hazard ratio 1.39, 95% confidence interval 1.13-1.72, p=0.002; women; 1.46, 1.05-2.04, p=0.023).

Conclusion: This is the first study showing that short sleep duration was a risk factor for incident NAFLD.

Key words: cohort study – non-alcoholic fatty liver disease – sleep duration – epidemiology.

Abbreviations: ALT: alanine aminotransferase; BMI: body mass index; CI: confidence interval; HDL: high density lipoprotein; HR: hazard ratio; NAFLD: non-alcoholic fatty liver disease; NAGALA: NAfld in the Gifu Area Longitudinal Analysis; OSAS: obstructive sleep apnea syndrome; TG: triglycerides.

INTRODUCTION

The proportion of adults whose sleep duration is less than 6 hours has increased from 34.7 % in 2005 to 39.5 % in 2015 in Japan [1]. Several longitudinal studies have demonstrated that short sleep duration and insomnia increased the risk of incident lifestyle diseases such as obesity [2], hypertension [3], diabetes, metabolic syndrome [4], cardiovascular disease [5] and mental illness such as depression [6]. Reported mechanisms of short sleep duration on incident various lifestyle diseases were: modulation of sleep caused irregular habits in diet and exercise [7, 8], decreased leptin and increased ghrelin, both of which influence appetite and energy balance [9, 10] and influence the hormones of hypothalamic-pituitary-adrenal system [11]. Therefore, early detection and resolution of sleep impairment could prevent the lifestyle disease.

The number of patients with non-alcoholic fatty liver disease (NAFLD), caused by ectopic fat accumulation in the liver has increased to 20-30 % in Japan [12]. Non-alcoholic fatty liver disease is not only one of the common causes of chronic liver disease [13], but also the cause of metabolic syndrome [14], type 2 diabetes [15] chronic kidney disease [16] and cardiovascular disease [17]. Therefore, the prevention and treatment of NAFLD are important for the prevention of further diseases such as type 2 diabetes, chronic kidney disease and cardiovascular disease.

Previous meta-analyses investigated the association between sleep duration or quality and the prevalence of NAFLD, and showed that short sleep duration was associated with NAFLD prevalence [18-20]. However, a few studies investigated the association between sleep duration and incident NAFLD [21] and evidenced that long sleep duration was associated with incident NAFLD. Thus, the relationship between sleep duration and NAFLD remains controversial.

Therefore, we focused especially on sleep duration and investigated the association between sleep duration and incident NAFLD in this retrospective cohort study.

METHODS

Study participants and study design

In this population-based longitudinal analysis of a medical examination program at the Asahi University Hospital (Gifu, Japan), we performed an investigation of the impact of sleep duration on the risk of incident NAFLD, using the NAGALA (NAfld in the Gifu Area, Longitudinal Analysis) database. The aim of this medical examination program is to detect chronic diseases and their risk factors, and to promote public health. This type of medical service called „a human dock" is very popular in Japan. In this center, more than 8,000 medical examinations are performed annually, 60% of them being monitoring visits, once or twice a year and 40% of them being done on new subjects. The details of NAGALA study and medical examination programs were expressed elsewhere [22]. In this study, we selected the data of the individuals who participated in this medical examination program from 2004 till 2015. The exclusion criteria were: the use of any kind of medication, known liver disease and fatty liver at baseline examination, ethanol consumption over 30 g/day for men and 20 g/day for women [15] and the participants in whom the data, including sleep duration, exercise habit, HDL-cholesterol, alcohol consumption, smoking status, were missed. Known liver disease was defined as viral hepatitis (assessment of hepatitis B antigen and hepatitis C antibody). The study followed the Declaration of Helsinki being approved by the Ethics Committee of Asahi University Hospital. In addition, written informed consent was obtained from each participant.

Data collection and measurements

The medical history and the data on smoking, alcohol habits, physical activity and sleep duration were obtained by a standardized self-administered questionnaire [22]. We evaluated alcohol consumption by asking about the type and amount of alcohol consumption per week during the prior month, then estimating the mean ethanol intake per week. We also categorized the participants into three groups by smoking status: never-, ex-, or current smoker. We investigated the participants' recreational and sports activities. We defined regular exercisers as participants who played any type of sports over one time per week regularly [23].

Definition of NAFLD

Abdominal ultrasonography, performed by trained technicians, was used for diagnosing fatty liver [24]. Gastroenterologists checked the images and diagnosed fatty liver being blind to the medical record of the participants. Liver brightness and liver contrast among the four known criteria (hepatorenal echo-contrast, liver brightness, deep attenuation and vascular blurring) were used for diagnosing fatty liver [24]. We excluded the participants who consumed ethanol over 30 g/day for men and 20 g/day for women [15].

Statistical analysis

We analyzed the data using the JMP ver. 13.0 software (SAS, Cary, NC, USA), and p-values <0.05 were considered significant. Values were expressed as mean (SD) for continuous variables and number (%) for categorical variables. First, we divided the participants into men and women because the number of participants with development of NAFLD differed in relation to gender. Moreover, we categorized the participants into four groups according to sleep duration: ≤5 h (the shortest), >5-6 h, >6-7 h and >7 h (the longest), which were often used in previous studies [2, 18, 25]. We also categorized the participants into four groups according to body mass index (BMI): lean, BMI <18.5 kg/m^2; normal body weight, ≥18.5-<23 kg/m^2; overweight, ≥23-<25 kg/m^2 obesity, ≥25 kg/m^2 [26, 27]. Categorical variables were compared among the groups by Pearson's qui-squared test, and continuous variables were compared by one-way analysis of variance and the Tukey honestly significant difference test, respectively.

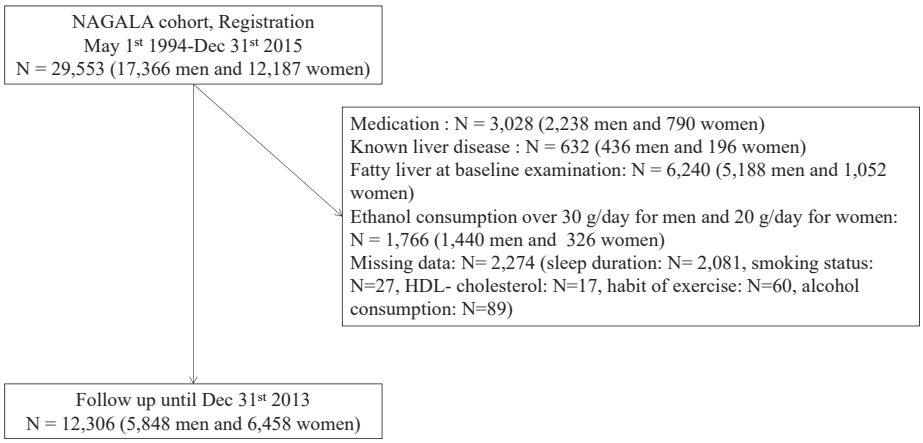


Fig. 1. Study flow diagram for the registration of participants. NAGALA: NAfld in Gifu Area, Longitudinal Analysis; NAFLD: non-alcoholic fatty liver disease.

We used Kaplan–Meier analysis for a graphical presentation of time to incident NAFLD, and log-rank test to evaluate difference among the groups according to sleep duration. We performed a Bonferroni correction and considered that a p value <0.0083 was statistically significant in the log-rank test.

Cox proportional hazards models were used to calculate adjusted hazard ratio (HR) and 95% confidence interval (CI) for incident NAFLD according to four categories of sleep duration. We adjusted for age, BMI categories, alanine aminotransferase (ALT), triglycerides (TG), high-density lipoprotein (HDL) cholesterol, smoking status, exercise habit, alcohol consumption, systolic blood pressure, fasting plasma glucose and sleep duration categories. We performed Cox hazards regression adjusted for BMI and sleep duration as continuous variables.

RESULTS

From May 1st 1994 to December 31st 2015, a total of 29,553 (17,366 men and 12,187 women) participants were registered in the NAGALA cohort. After we excluded 14,981 participants, 12,306 (5,848 men and 6,458 women) participants were introduced in this study (Fig. 1).

The baseline characteristics of the participants are summarized in Tables I and II. Women with sleep duration of >7 h (the longest) were significantly younger and had lower BMI than the other groups. On the other hand, men with the longest sleep duration and >6-7 h had significantly lower BMI than men with sleep duration of ≤5 h (the shortest), and men with the shortest sleep duration were significantly younger than the other groups.

Table I. Characteristics of study participants of the cohort study at the baseline examination according to sex

	Men	Women	p value
Number	5,848	6,458	
Age (years)	42.4 (8.9)	41.6 (8.4)	<0.001
Body mass index (kg/m^2)	22.1 (2.4)	20.7 (2.6)	<0.001
Body mass index categories			<0.001
Lean	372 (6.4)	1,236 (19.1)	
Normal body weight	3,421 (58.5)	4,175 (64.6)	
Overweight	1,389 (23.8)	675 (10.5)	
Obesity	666 (11.4)	372 (5.8)	
Systolic blood pressure (mmHg)	116.6 (13.6)	108.8 (13.6)	<0.001
Diastolic blood pressure (mmHg)	73.1 (9.5)	67.3 (9.3)	<0.001
Fasting plasma glucose (mmol/L)	5.3 (0.6)	5.0 (0.5)	<0.001
Hemoglobin A1c (%)	5.2 (0.5)	5.2 (0.4)	0.002
Triglycerides (mmol/L)	1.0 (0.7)	0.7 (0.4)	<0.001
High-density lipoprotein cholesterol (mmol/L)	1.3 (0.3)	1.6 (0.4)	<0.001
Triglycerides/High-density lipoprotein cholesterol ratio	0.9 (0.3)	0.5 (0.4)	<0.001
Creatinine (μmol/L)	83.6 (18.0)	61.0 (9.9)	<0.001
Estimated Glomerular filtration rate (ml/min/1.73m^2)	72.5 (12.7)	76.3 (14.6)	<0.001
Aspartate aminotransferase (IU/L)	18.2 (6.7)	16.2 (8.7)	<0.001
Alanine aminotransferase (IU/L)	20.2 (10.3)	14.1 (12.2)	<0.001
Gamma-glutamyltransferase (IU/L)	23.1 (20.4)	12.5 (8.4)	<0.001
Smoking status			<0.001
Never smoker	2,024 (34.6)	5,626 (87.1)	
Ex-smoker	1,531 (26.2)	416 (6.4)	
Current smoker	2,293 (39.2)	416 (6.4)	
Habit of exercise	1,116 (19.1)	989 (15.3)	<0.001
Alcohol consumption, g/wk	53.6 (57.9)	13.2 (28.1)	<0.001
Sleep duration			0.03
≤5h	889 (15.2)	1,039 (16.1)	
>5-6h	2,377 (40.6)	2,733 (42.3)	
>6-7h	2,068 (35.4)	2,128 (33.0)	
>7h	514 (8.8)	558 (8.6)	

Data are expressed as mean (SD) or number (%) of subjects. Lean was defined <18.5 kg/m^2, normal body weight was defined ≥18.5-<23 kg/m^2, overweight was defined ≥23-<25 kg/m^2 and obesity was defined ≥25 kg/m^2. p values by one-way analysis of variance for continuous variables and chi-squared test for categorical variables.

Table II. Characteristics of study participants at the baseline examination according to sex and sleep duration

Men					
Sleep duration (h)	≤5h	>5-6	>6-7	>7	p value
Number	889	2,377	2,068	514	
Age (years)	40.5 (8.1)	41.8 (8.5)†	43.4 (9.1)†,‡	44.2 (9.9)†,‡	<0.001
Body mass index (kg/m²)	22.4 (2.4)	22.2 (2.4)	22.1 (2.5)†	21.9 (2.4)†	0.002
Body mass index categories					0.112
Lean	53 (6.0)	135 (5.7)	149 (7.2)	35 (6.8)	
Normal body weight	498 (56.0)	1,388 (58.4)	1,219 (58.9)	316 (61.5)	
Overweight	222 (25.0)	585 (24.6)	465 (22.5)	117 (22.8)	
Obesity	116 (13.0)	269 (11.3)	235 (11.4)	46 (8.9)	
Systolic blood pressure (mmHg)	115.4 (12.5)	116.3 (13.2)	117.2 (14.3)†	117.5 (14.2)†	0.003
Diastolic blood pressure (mmHg)	71.9 (9.0)	72.8 (9.3)	73.7 (9.8)†,‡	73.8 (9.3)†	<0.001
Fasting plasma glucose (mmol/L)	5.4 (0.8)	5.3 (0.6)	5.3 (0.6)	5.3 (0.9)	0.109
HbA1c (%)	5.2 (0.5)	5.2 (0.5)	5.2 (0.5)‡	5.2 (0.6)‡	0.001
Triglycerides (mmol/L)	1.0 (0.6)	1.0 (0.6)	1.1 (0.7)†,‡	1.1 (0.7)†,‡	<0.001
HDL cholesterol (mmol/L)	1.3 (0.3)	1.3 (0.3)	1.3 (0.3)†,‡	1.3 (0.3)‡	<0.001
TG/HDL ratio	0.82 (0.68)	0.84 (0.78)	0.97 (0.84) †,‡	1.01 (0.82) †,‡	<0.001
Creatinine (μmol/L)	87.8 (12.1)	83.1 (23.9)	84.6 (12.6)†,‡	85.0 (12.1)†	<0.001
eGFR (ml/min/1.73m²)	74.7 (12.3)	73.6 (12.7)	71.0 (12.6)†,‡	70.2 (12.2)†,‡	<0.001
AST (IU/L)	18.1 (6.4)	18.1 (6.7)	18.4 (6.7)	18.4 (7.0)	0.566
ALT (IU/L)	20.3 (9.9)	20.5 (9.8)	20.1 (10.5)	19.6 (12.6)	0.313
GGT (IU/L)	21.8 (18.8)	22.5 (19.8)	23.9 (21.5)†	24.3 (20.4)	0.012
Smoking status*					<0.001
Never smoker	347 (39.0)	872 (36.7)	660 (31.9)	145 (28.2)	
Ex-smoker	215 (24.2)	620 (26.1)	541 (26.2)	155 (30.2)	
Current smoker	327 (36.8)	885 (37.2)	867 (41.9)	214 (41.6)	
Habit of exercise	131 (14.7)	462 (19.4)	416 (20.1)	107 (20.8)	0.003
Alcohol consumption, g/wk	44.4 (54.3)	51.0 (56.6)†	57.9 (59.0)†,‡	64.7 (61.5)†,‡	<0.001
Women					
Sleep duration (h)	≤5h	>5-6	>6-7	>7	p value
Number	1,039	2,733	2,128	558	
Age (years)	43.1 (8.5)	41.7 (8.3)†	41.1 (8.3)†,‡	39.8 (8.8)†,‡,§	<0.001
Body mass index (kg/m²)	20.9 (2.8)	20.8 (2.6)	20.5 (2.4) †,‡	20.4 (2.5)†,‡,§	<0.001
Body mass index categories					<0.001
Lean	183 (17.6)	481 (17.6)	443 (20.8)	129 (23.1)	
Normal body weight	649 (62.5)	1,783 (65.2)	1,390 (65.3)	353 (63.3)	
Overweight	129 (12.4)	301 (11.0)	194 (9.1)	51 (9.1)	
Obesity	78 (7.5)	168 (6.1)	101 (4.7)	25 (4.5)	
Systolic blood pressure (mmHg)	108.6 (13.8)	109.0 (13.7)	108.5 (13.6)	108.4 (13.2)	0.521
Diastolic blood pressure (mmHg)	67.0 (9.4)	67.5 (9.4)	67.2 (9.1)	67.0 (9.0)	0.412
Fasting plasma glucose (mmol/L)	5.0 (0.4)	5.0 (0.5)	5.0 (0.4)†	4.9 (0.4)†	0.003
HbA1c (%)	5.2 (0.3)	5.2 (0.4)	5.2 (0.4)†,‡	5.1 (0.4)	<0.001
TG (mmol/L)	0.6 (0.4)	0.7 (0.4)†	0.7 (0.4)†	0.7 (0.4)	0.006
HDL cholesterol (mmol/L)	1.7 (0.4)	1.6 (0.4)†	1.6 (0.4)†	1.6 (0.4)†,‡	<0.001
TG/HDL cholesterol ratio	0.41 (0.43)	0.46 (0.38) †	0.46 (0.33) †	0.47 (0.32) †	<0.001
Creatinine (μmol/L)	59.7 (6.5)	61.3 (10.0)†	61.4 (10.0)†	60.6 (9.7)	<0.001
eGFR (ml/min/1.73m²)	77.1 (14.4)	75.8 (14.5)	76.1 (14.8)	77.9 (14.8)‡,§	0.004
AST (IU/L)	16.5 (5.4)	16.2 (14.5)	16.1 (13.2)	16.0 (5.2)	0.723
ALT (IU/L)	14.6 (6.9)	14.0 (5.9)	14.0 (19.0)	14.0 (8.0)	0.599
GGT (IU/L)	12.9 (6.8)	12.6 (8.6)	12.3 (9.1)	11.8 (6.6)†	0.039

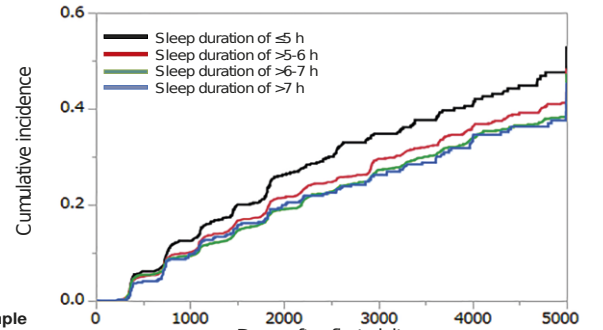
Table II (continued)

Smoking status					0.015
Never smoker	872 (83.9)	2,378 (87.0)	1,887 (88.7)	489 (87.6)	
Ex-smoker	79 (7.6)	176 (6.4)	129 (6.1)	32 (5.7)	
Current smoker	88 (8.5)	179 (6.6)	112 (5.3)	37 (6.6)	
Habit of exercise	131 (12.6)	445 (16.3)	340 (16.0)	73 (13.1)	0.011
Alcohol consumption, g/wk	13.3 (28.7)	13.4 (28.5)	13.5 (27.8)	11.2 (26.0)	0.373

Data are expressed as mean (SD) or number (%) of subjects. ALT: alanine aminotransferase; AST: aspartate aminotransferase; eGFR: estimated glomerular filtration rate; GGT, gamma-glutamyltransferase; HbA1c: hemoglobin A1c; HDL: high-density lipoprotein; TG: triglycerides. Lean was defined <18.5 kg/m^2, normal body weight was defined ≥18.5-<23 kg/m^2, overweight was defined ≥23-<25 kg/m^2 and obesity was defined ≥25 kg/m^2. p values by one-way analysis of variance for continuous variables and chi-squared test for categorical variables. The analyses of continuous among four groups were „performed by Tukey HSD test: †, p<0.05 versus sleep duration ≤5h, ‡, p<0.05 versus sleep duration >5-6h, § p<0.05 versus sleep duration >6-7h.

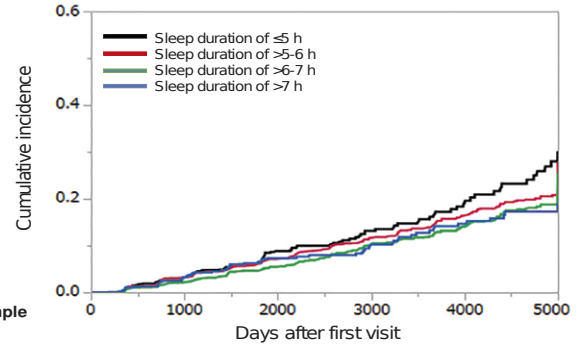
During the median 6.8-year follow-up for men and 7.0-year follow-up for women, 2,280 participants (1,581 men and 699 women) developed NAFLD. The 5 years cumulative incident rates of NAFLD were 23.5 % for men and 6.8 % for women with a sleep duration of ≤5 h, 19.4 % for men and 6.3 % for women with sleep duration of >5-6 h , 17.3 % for men and 4.9 % for women with sleep duration of >6-7 h and 17.2 % for men and 4.8 % for women with sleep duration of >7 h. Compared with the longest sleep duration group, the shortest sleep duration group was associated with a higher risk of incident NAFLD in both men (p = 0.007) and women (p = 0.005) (Figs. 2 and 3).

According to the Cox proportional hazards models, the shortest sleep duration was revealed to be a significant risk for incident NAFLD, compared to the longest sleep duration (men; hazard ratio [HR] 1.39, 95% confidence interval [95%CI] 1.13-1.72, p = 0.002, women; HR 1.46, 95%CI 1.05-2.04, p = 0.023) (Table III). Apart from sleep duration, BMI was a significant risk for incident NAFLD. Compared to lean subjects, the other participants, even those with normal body weight, had a significant risk for incident NAFLD (men: normal; hazard ratio [HR] 1.62, 95% confidence interval [95%CI] 1.20-2.20, p=0.002, overweight; HR 2.57, 95%CI 1.88-3.52, p<0.001, obesity; HR 3.56, 95%CI 2.57-4.93, p<0.001, women: normal; HR 4.30, 95%CI 2.73-6.75, p < 0.001, overweight; HR 9.38, 95%CI 5.84-15.1, p<0.001, obesity; HR 17.0, 95%CI 10.5-27.5, p<0.001). In addition, even if we used BMI and sleep duration as continuous variables in the Cox proportional hazards models, short sleep duration was a significant risk for incident NAFLD both in men and women (men; hazard ratio [HR] 0.91, 95% confidence interval [95%CI] 0.84-0.99, p = 0.045, women; HR 0.90, 95%CI 0.85-0.96, p < 0.001).



Sample size						
≤5h	889	790	712	679	664	645
>5-6h	2,377	2,158	1,975	1,880	1,826	1,767
>6-7h	2,068	1,888	1,732	1,631	1,567	1,483
>7h	514	468	428	408	391	372

Fig. 2. Kaplan–Meier analysis of incident NAFLD in men. The vertical axis is cumulative incidence of NAFLD and the horizontal axis is time as days. Log rank test to sleep duration of ≤5 h, sleep duration of >5-6 h; p =0.014, sleep duration of >6-7 h; p <0.001, sleep duration of >7 h; p =0.007, log rank test compared to sleep duration of 5-6 h, sleep duration of >6-7 h; p =0.183, sleep duration of >7 h; p =0.283, log rank test compared to sleep duration of 6-7 h, sleep duration of >7 h; p =0.773. Log rank tests were performed to investigate the association among the groups of sleep duration. Bonferroni correction was performed to correct familiar error and a p value <0.0083 was considered statistically significant.



Sample size						
≤5h	1,039	1,010	977	962	950	937
>5-6h	2,733	2,654	2,580	2,521	2,510	2,425
>6-7h	2,218	2,175	2,123	2,068	2,038	1,896
>7h	558	542	525	517	507	501

Fig. 3. Kaplan–Meier analysis of incident NAFLD in women. The vertical axis is cumulative incidence of NAFLD and the horizontal axis is time as days. Log rank test compared to sleep duration of ≤5 h, sleep duration of >5-6 h; p =0.249, sleep duration of >6-7 h; p =0.007, sleep duration of >7 h; p =0.005, log rank test compared to sleep duration of 5-6 h, sleep duration of >6-7 h; p =0.065, sleep duration of >7 h; p =0.123, log rank test compared to sleep duration of 6-7 h, sleep duration of >7 h; p =0.630. Log rank tests were performed to investigate the association among the groups of sleep duration. Bonferroni correction was performed to correct familiar error and a p value <0.0083 was considered statistically significant.

DISCUSSION

We investigated whether short sleep duration was a risk of incident NAFLD using a cohort study of over 12,000 Japanese

Table III. Cox proportional hazards for incident NAFLD

	Men		Women	
	HR (95%CI)	p value	HR (95%CI)	p value
Age, years	1.01 (0.99-1.01)	0.117	1.02 (1.01-1.03)	<0.001
Lean	1.00 (Reference)	-	1.00 (Reference)	-
Normal	1.62 (1.20-2.20)	0.002	4.30 (2.73-6.75)	<0.001
Overweight	2.57 (1.88-3.52)	<0.001	9.38 (5.84-15.1)	<0.001
Obesity	3.56 (2.57-4.93)	<0.001	17.0 (10.5-27.5)	<0.001
ALT, IU/L	1.01 (1.01-1.02)	<0.001	1.00 (0.99-1.00)	0.138
Triglycerides, mmol/L	1.21 (1.14-1.28)	<0.001	1.48 (1.26-1.75)	<0.001
HDL, mmol/L	0.55 (0.46-0.67)	<0.001	0.45 (0.35-0.58)	<0.001
Fasting plasma glucose, mmol/l	1.01 (1.00-1.02)	<0.001	1.01 (1.01-1.02)	<0.001
Systolic blood pressure, mmHg	1.01 (1.01-1.02)	0.001	1.01 (1.00-1.02)	<0.001
Alcohol consumption, g/wk	0.99 (0.99-1.00)	<0.001	0.99 (0.99-1.00)	0.064
Never smoker	1.00 (Reference)	-	1.00 (Reference)	-
Ex–smoker	1.01 (0.89-1.16)	0.846	0.66 (0.42-0.96)	0.03
Current smoker	0.98 (0.87-1.10)	0.738	1.21 (0.90-1.60)	0.195
Regular exerciser	0.80 (0.69-0.92)	0.001	0.86 (0.69-1.06)	0.151
Sleep duration (≤5h)	1.39 (1.13-1.72)	0.002	1.46 (1.05-2.04)	0.023
Sleep duration (>5-6h)	1.14 (0.95-1.37)	0.169	1.20 (0.91-1.61)	0.208
Sleep duration (>6-7h)	1.05 (0.88-1.27)	0.569	1.07 (0.81-1.45)	0.64
Sleep duration (>7h)	1.00 (Reference)	-	1.00 (Reference)	-

ALT: alanine aminotransferase; HDL: high-density lipoprotein; NAFLD: non-alcoholic fatty liver disease. Lean was defined body mass index (BMI) <18.5 kg/m^2, normal body weight was defined BMI ≥18.5-<23 kg/m^2, overweight was defined BMI ≥23-<25 kg/m^2 and obesity was defined BMI ≥25 kg/m^2.

participants. Our analyses revealed that the risk of incident NAFLD was significantly higher in participants with short sleep duration of ≤5 h than in those with sleep duration of >7 h, in both men and women. Previous meta-analysis revealed that short sleep duration was associated with higher prevalence of NAFLD. Moreover, several previous studies reported that short sleep duration was associated with the risk of incident obesity [2], hypertension [3], diabetes, metabolic syndrome [4] and cardiovascular disease [5]. To the best of our knowledge, our study is the first report to reveal that short sleep duration is a risk for incident NAFLD.

To support our findings, some potential explanations are proposed. Obesity and weight gain, both of which play key roles in the pathogenesis of NAFLD [28-30], were associated with short sleep duration by previous studies. In this study, both men and women with short sleep duration had higher BMI than the others. Moreover, short sleep duration was associated with decreased leptin [31, 32] and increased ghrelin [32], which influenced appetite, energy balance and weight gain [9, 10]. Decreased leptin levels increase appetite and causes obesity [33]. Increased ghrelin causes obesity by increasing dietary intake and decreasing fat utilization [34]. Furthermore, higher levels of inflammatory cytokines such as tumor necrosis factor-alpha and interleukin-6, involved in the pathogenesis of NAFLD, were detected in subjects with a short sleep duration [35-37]. Furthermore, short sleep duration was also reported to enhance the circadian misalignment cortisol [38] and hypothalamic-pituitary-adrenal axis stress [39]. Chronic overactivity in the hypothalamic-pituitary-adrenal axis leads to subclinical hypercortisolism that might be implicated in the development of NAFLD [40]. Previous studies also revealed that lifestyle is closely associated with sleep duration. In fact, individuals with a short sleep duration had dinner at late-night [8], which was a risk of hyperglycemia [41] or obesity [42]. To summarize all these findings, short sleep duration could be a risk of incident NAFLD.

The strengths of our study include using the same standardized diagnosis of fatty liver [22] and the standardized questionnaire for lifestyle factors, and the relatively large population-based longitudinal research. Our study also has some limitations. First, fatty liver was diagnosed by ultrasonography in this population. This may be inaccurate compared to liver biopsy, but ultrasonography detecting fatty liver is the common acceptance by authorities in the epidemiological studies [43]. Second, short sleep duration was reported to be associated with increased energy intake [44], which is well known to be the risk of incident NAFLD [45]. Unfortunately, however, we did not have the data of dietary intake and could not evaluate the association between sleep duration and dietary intake. Third, we did not investigate sleep quality. Poor sleep quality is associated with the prevalence of NAFLD [46]. Similarly, the symptoms such as sleep apnea and loud snoring suggestive of obstructive sleep apnea syndrome (OSAS) were found to be associated with NAFLD [47]. The physical activity links the OSAS and obesity. The association between physical activity and BMI or obesity has been reported

[48, 49]. Moreover, OSAS is associated with physical activity [50]. In addition, OSAS is caused by intermittent hypoxia and chronic inflammation via visceral fat obesity [51], similar to the development of NAFLD [52]. We did not have detail data on physical activity and OSAS in this study. We checked only the sleep duration from the questionnaire and did not evaluate the wake-up time, bedtime and shift pattern such as shift workers or day workers. Altered sleep timing was reported to affect increased energy intake [53], and a previous study revealed the association between shift work and metabolic syndrome [54]. In addition, insomia and mental illness such as depression or anxiety has been reported to be related to each other [55]. We did not evaluate the medical history of mental illness; however, we excluded the participants who used any kind of medication, including medication for mental illness. Fourth, several previous studies revealed U-shaped association of sleep duration with mortality [5], cardiovascular disease [4], stroke [56] and metabolic syndrome [4]. We could not evaluate U-shaped association of sleep duration with incident NAFLD, as only 70 participants reported sleep duration more than 8.5 h in our study. Therefore, we could not accurately evaluate incident NAFLD in the long sleep duration group. Even when we excluded the participants in whom sleep duration was more than 8.5 h, the shortest sleep duration group was a significant risk for incident NAFLD, compared to the longest sleep duration group (men; hazard ratio [HR] 1.40, 95% confidence interval [95%CI] 1.13-1.73, p=0.002, women; HR 1.36, 95%CI 1.08-1.73, p=0.010). Fifth, we do not have data regarding insulin resistance, which plays a key role in fatty liver disease [57, 58]. If we could have evaluated insulin resistance, we could have more accurately evaluated the relationship between sleep duration and insulin resistance in the development of NAFLD. Sixth, we had a limited ability to examine different levels of physical activity on incident NAFLD. If we can evaluate the frequency and intensity of exercise, more accurate analysis becomes possible. Lastly, the generalizability of our study to non-Japanese populations is uncertain.

CONCLUSION

Short sleep duration (<5 h) presented a risk of incident NAFLD. For the prevention of incident NAFLD, we should encourage patients to have appropriate sleep duration.

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Authors' contributions: T.O. contributed to data research and analyses and wrote the manuscript. Y.H. originated and designed the study, analyzed data and reviewed the manuscript for intellectual content. M.H. contributed to the manuscript organization and reviewed and edited the manuscript. A.O. and T.K. originated the study, analyzed the data and contributed to the discussion. M.F. analyzed the data and reviewed and edited the manuscript. H.M. is guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors approved the manuscript's final version.

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