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Association between Wakeup Frequency at Night and Atherogenic Dyslipidemia: Evidence for Sex Differences

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Aim: This study aimed to determine whether sleep disturbance, defined as the wakeup frequency at night, is associated with atherogenic dyslipidemia and to explore possible sex differences.

Methods: A total of 1,368 adults aged 19–70 years were included in the study of lifestyles and atherogenic dyslipidemia at the National Taiwan University Hospital in the period of 2008–2012. They completed a questionnaire regarding lifestyle information and sleep quality, including sleep hour duration, use of sleeping pills, and wakeup frequency during nighttime sleep. The measured lipid profiles included total cholesterol, triglycerides, low- and high-density lipoprotein cholesterol (LDL-C and HDL-C, respectively), non-HDL-C, and small dense LDL-C (sdLDL-C). Multivariate logistic regression was performed to determine habitual interrupted sleep and the odds ratio of atherogenic dyslipidemia following adjustment for conventional risk factors and for sex-based subgroup analysis.

Results: A wakeup frequency ≥ 3 times per night was independently associated with an increased risk [odds ratio (95% confidence interval)] of dyslipidemia was 1.96 (1.17–3.28), and non-HDL-C ≥ 160 mg/dL was 1.78 (1.09–2.89). A higher wakeup frequency was associated with increased atherogenic dyslipidemia in women than in men. The multivariate adjusted relative risks for non-HDL ≥ 160 mg/dL and cholesterol ≥ 200 mg/dL were 3.05 (1.27–7.34) and 4.01(1.29-12.45) for female individuals with insomnia and those with a wakeup frequency ≥ 2 times per night, respectively.

Conclusion: A higher wakeup frequency was associated with atherogenic dyslipidemia in Taiwanese adults, particularly in women. This study also provided another evidence of increasing cardiovascular diseases in subjects with habitual interrupted sleep.

Key words: Wakeup frequency, Dyslipidemia, Sex, Sleep disturbance

Introduction

Sleep is a complex process crucial to our health, and in the past few decades, sleep habits and their impact on various health issues have been investigated. Multiple cohort studies have concluded that inadequate sleep and insomnia severity both relate to increased cardiovascular risks in the general population. A Taiwanese cohort consisting of 3,430 adults exhibited a 2.07-fold higher risk of cardiovascular disease (CVD) in participants reporting frequent insomnia and sleep duration ≥ 9 h. A 1.78-fold higher CVD risk was observed in patients experiencing insomnia nearly every day. The study concluded that the optimal sleep duration is 7–8 h[1)]. An increase in the incidence of coronary heart disease

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(CHD) was also observed. A 10-year follow-up study of 71,617 middle-aged US female health professionals concluded that the relative risks (RRs) of CHD for individuals sleeping ≤ 5 h and ≥ 9 h were 1.45 and 1.38, respectively[2]. Another Taiwanese case–control study of 322 middle-aged male workers with CHD compared with 644 non-CHD workers concluded that sleep duration <6 h was related to 2.7-fold higher odds of CHD than sleep duration 6–9 h. The odds of acute myocardial infarction were even higher, with a 2.9-fold increased risk for those with a short sleep duration <6 h[3].

Furthermore, patients with existing CHD also carry this risk. In a prospective study of the Emory Cardiovascular Biobank database including 2,846 patients with documented CHD with a median follow-up of 2.8 years, a short sleep duration (<6.5 h) and a long sleep duration (≥ 7.5 h) were both associated with higher all-cause mortality (hazard ratio: 1.44 [1.10–1.89] and 1.41 [1.08–1.85]). A short sleep duration, but not a long sleep duration, was associated with higher cardiovascular mortality (HR: 1.48 [1.05–2.09])[4].

To date, the association between sleep duration and the cardiovascular system has not been well explained and requires more evidence. Previous evidence has already proven that self-reported short sleep duration is associated with cardiometabolic disorders, including diabetes, obesity, hypertension, and hyperlipidemia[5]. Further evidence has demonstrated that a short sleep duration and interrupted sleep contribute to increased incidence of atherosclerosis. A US study involving 3,974 patients from the PESA project found that self-reported short sleep duration correlated with increased volume and affected territories of atherosclerotic plaques in the carotid and femoral arteries. Objectively measured sleep fragmentation also correlated with increased affected territories[6].

Dyslipidemia is a known risk factor for atherosclerosis. We hypothesized that sleep disorders increase cardiovascular risks by increasing the incidence of dyslipidemia. Studies have focused on lipid profiles, including LDL-C and triglycerides (TGs). However, limited studies have reported findings regarding small dense LDL-C (sdLDL-C) or non-high-density lipoprotein cholesterol (non-HDL-C). SdLDL-C is a subclass of LDL-C with a higher atherogenic potency than other LDL-C subfractions[7]. Both hypertriglyceridemia and non-HDL-C are also considered as strong predictors of CVDs[8].

Sex hormones can also affect sleep, according to a previous hypothesis[9]. We assume that different sexes are exposed to different risks when the sleep quality is poor. However, in real-world human data, the results are mixed. There are few studies on sex affecting sleep-associated cardiovascular risks. In the Augsburg cohort study of 6896 adults, women sleeping ≤ 5 h had a 2.98 times higher risk of developing myocardial infarction within an average of 10.1 years compared with those sleeping 8 h. In men, the hazard ratio was 1.13 (95% CI, 0.66–1.92)[10]. In the Whitehall II study, a British cohort study of 10308 participants, sex differences were also reported. A short sleep duration (≤ 5 h per night) was associated with a higher risk of hypertension compared with a sleep duration of 7 h. The odds ratio was 2.01 (1.13 to 3.58); no association was detected in men[11]. Few studies have mentioned sex differences in insomnia in the past. Some have concluded that women are more susceptible to cardiovascular accidents than men, but they did not clearly explain the underlying mechanism. Our study provided a sex-based subgroup analysis on lipid profiles that may explain the sex difference in cardiovascular risk.

Previous studies have used sleep duration or insomnia symptoms to define the severity of insomnia. However, most healthy adults tend to over-report their sleep duration, and patients who report poor-quality sleep were more likely to underestimate their sleep duration[12]. Short and long sleep durations were both found to correlate with cardiovascular risk, and patients with very long sleep durations have fragmented sleep[6]. We hypothesize that self-reported wakeup frequency is potentially an indicator of sleep disturbance and can be associated with atherogenic dyslipidemia.

Methods

Study Design and Population

In total, 1368 adults (953 males and 415 females) aged 19–70 years who were under regular outpatient follow-up at the cardiovascular department were voluntarily enrolled as study subjects at the National Taiwan University Hospital (NTUH) in the period of 2008–2012. Individuals with physician-diagnosed CHD, heart failure, or cerebrovascular disease were excluded. This study was approved by the Institutional Review Board of the NTUH. Informed consent was obtained from each subject before enrollment.

Patients with secondary hyperlipidemia, such as hypothyroid disease, nephrotic syndrome, chronic kidney disease with a creatinine level of 3, obstructive liver disease, and malignant disease, and those who pregnant or taking drugs were known to influence

lipid metabolism were excluded. In addition, all participants had to have no history of receiving lipid-lowering therapy or had to discontinue using lipid-lowering agents for at least 1 month before being enrolled in the study. All subjects with diabetes mellitus (DM) or histories of receiving hypertension medication were recorded for further investigation. All the participants fasted for 10–14, and venous blood samples were collected in the morning for the measurement of serum lipid profile.

CVD Risk Factors and Anthropometric Assessments

Blood pressure (BP) measurements were performed using a mercury sphygmomanometer in a standardized fashion. Two measurements were taken after 5 min of rest in the sitting position. Subjects with SBP (systolic BP) ≥ 140 mmHg or DBP (diastolic BP) ≥ 90 mmHg were considered hypertensive[13]. Lifestyle information, such as alcohol consumption, smoking, and exercise, was collected from the self-report questionnaires. Smokers were considered as those who regularly smoked tobacco and alcohol drinkers as those who had two or more alcoholic beverages per week. The demographic and anthropometric data of patients were obtained from the medical record archive of the NTUH. The body mass index (BMI) of the participants was calculated by dividing the body weight in kilograms by height in meters squared. Waist circumference was measured in centimeters.

Lipid, Lipoprotein, and Oral Glucose Tolerance Test (OGTT) Measurements

The concentrations of lipids, including total cholesterol (TCHO), TG, HDL-C, and LDL-C, were analyzed using a homogeneous enzymatic method [coefficient of variation (CV) of 2%] with reagent kits. The serum TCHO and TG levels were measured using automated enzymatic methods and a CV of 3%. The plasma sdLDL-C levels were determined using the sdLDL-EX "SEIKEN" method[14]. The fasting plasma glucose concentrations were measured using a hexokinase assay kit. The aforementioned analyses were all conducted using a Toshiba FR-200 automatic chemistry analyzer (Toshiba, Tokyo, Japan).

After collecting the fasting blood sample, all subjects without evident DM underwent an OGTT with 75 g of glucose loading, in accordance with the World Health Organization standards[15]. Next, venous blood samples were collected every 30 min until 2 h following the OGTT, and the results were classified according to the American Diabetes Association criteria: those with 200 mg/dL (11.11 mmol/L) of 2-h blood glucose levels were considered diabetic[15]. The plasma glucose concentration was determined using reagent kits and the hexokinase method and analyzed using a Toshiba FR-120 automatic chemistry analyzer (Toshiba). The CV for plasma glucose was less than 3%.

Questionnaire Definition of Sleep Quality

We obtained self-reported sleep quality data using a structured self-administered questionnaire, and the participants underwent a health examination for cardiovascular health at the NTUH. In addition to traditional questionnaires on sleep duration, sleeping pills, and insomnia severity, we investigated the self-reported wakeup frequency during nighttime sleep, such as 0, 1, 2, 3, and greater than 2 or 3 times, as novel markers of sleep disturbance. Self-reported insomnia severity was defined as follows: mild, once per week or less; moderate, 2–3 days per week; and severe, over 4 days per week. If self-reported moderate or severe insomnia was defined as positive insomnia.

Statistical Analysis

Continuous variables, such as the serum levels of TCHO, TGs, glucose, HDL-C, and LDL-C, were expressed as means ± standard deviation, and categorical data, such as the number of smokers and number of alcohol drinkers, were expressed as percentages. Multivariate logistic regression analysis was employed to estimate the odds ratios (95% confidence intervals) of the wakeup frequency at night and the RRs of different definitions of atherogenic dyslipidemia. The atherogenic dyslipidemia phenotypes included TG ≥ 200 mg/dL, sdLDL-C ≥ 75th percentile, non-HDL-C ≥ 160 mg/dL, TCHO ≥ 200 mg/dL, TCHO to HDL-C ratio > 5, and dyslipidemia defined as a lipid pattern with either TGs ≥ 150 mg/dL or HDL-C < 40 mg/dL in male and < 50 mg/dL in female subjects. All analyses were conducted using SAS 9.1 (SAS Institute Inc., Cary, NC). $P < 0.05$ was considered statistically significant.

Results

Characteristics of the Participants and their Association with the Wakeup Frequency

All 1,368 participants were categorized into groups according to their wakeup frequency, and their basic characteristics are listed in **Table 1**. Our results indicated that participants with a higher wakeup frequency were more likely to be female and older. A higher wakeup frequency was significantly associated with increased BP levels, clinical hypertension, self-reported diabetes, newly diagnosed diabetes by laboratory results in the present study, and metabolic

Table 1. Basic characteristics of the participants stratified by wakeup frequency

	Wakeup frequency				
	0 N=467	1 N=542	2 N=230	≥ 3 N=129	*P* for trend
Age, year	43.51 ± 11.78	46.53 ± 10.29	48.68 ± 10.25	50.29 ± 12.26	<.001
Male, %	76.02	70.48	62.17	56.59	<.001
Body mass index, kg/m^2	25.18 ± 3.92	24.85 ± 3.74	24.51 ± 3.37	25.12 ± 3.26	0.107
Waist length, cm	85.16 ± 10.42	84.77 ± 10.37	85.05 ± 10.20	84.6 ± 9.44	0.679
Metabolic syndrome, %	26.55	32.23	35.22	41.98	<.001
Hypertension, %	27.19	28.91	30.87	38.17	0.022
Systolic BP, mmHg	123.24 ± 14.34	124.95 ± 14.33	126.61 ± 16.25	129.34 ± 17.38	<.001
Diastolic BP, mmHg	76.58 ± 10.17	77.81 ± 9.8	78.72 ± 10.53	78.9 ± 10.35	0.002
HbA1C, %	5.68 ± 0.73	5.77 ± 0.96	5.84 ± 0.85	5.85 ± 0.92	0.008
Diabetes %	13.49	16.76	20.43	25.19	0.001
Diabetes history, %	2.41	4.55	4.46	6.3	0.036
Insomnia severity, %	2.78	6.63	12.61	38.93	<.001
Sleep hours <6, %	33.83	33.15	39.57	47.33	0.004
Sleep pills, %	71.73	76.98	68.26	71.76	0.591
Smoking, %	20.13	19.15	15.65	19.08	0.352
Alcohol, %	14.35	15.65	20	19.08	0.053
Exercise habit, %	38.33	35.36	33.04	35.11	0.233
Education ≥12 years, %	76.21	77.46	67.7	62.6	0.001
Family income, %	88.44	88.21	82.61	83.97	0.036

N=1368

Metabolic syndrome: In this study, the classification of MS according to the modified ATP III criteria for Asians required meeting at least three of the following component risk factors: (1) a waist circumference >90 cm for men and >80 cm for women; (2) TG ≥ 150 mg/dL; (3) HDL-C <40 mg/dL for men and <50 mg/dL for women; (4) systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg or the current use of antihypertensive drugs; (5) FPG ≥ 110 mg/dL or the current use of antihyperglycemic drugs.

Hypertension: Previously diagnosed hypertension or SBP ≥ 140 mmHg or DBP ≥ 90 mmHg.

Diabetes: Previously diagnosed diabetes or fasting glucose ≥ 126 mg dL or HbA1c ≥ 6.5%.

syndrome. No significant differences were observed in the BMI or waist length.

Higher educational attainment was significantly associated with a lower wakeup frequency. No significant differences were observed in lifestyle factors, such as smoking, alcohol consumption, and exercise habits, across different wakeup frequencies.

As presented in **Supplemental Table 1**, significantly higher levels of fasting glucose and post-challenge glucose levels; summation of glucose levels on OGTT, in terms of glucose levels under the curve (glucose AUC); and diagnosis of diabetes were also noted in participants with a higher wakeup frequency.

Association between the Wakeup Frequency and Dyslipidemia

A higher wakeup frequency was associated with increased levels of TCHO, sdLDL-C, and non-HDL-C **(Table 2)**. No significant differences were observed in TG, HDL-C, LDL-C, or the cholesterol ratio (TCHO/HDL-C) across different wakeup frequencies. The serum TG level was not significantly associated with the wakeup frequency.

The average TCHO level was 230.49 mg/dL in participants with a wakeup frequency ≥ 3 times per night and 214.51 mg/dL with 0 wakeup per night. The average sdLDL-C was 40.4 mg/dL in participants with a wakeup frequency ≥ 3 times per night and 35.4 mg/dL with 0 wakeup per night. The average non-HDL-C was 180.7 mg/dL in participants with a wakeup frequency ≥ 3 times per night and 164.07 mg/dL with 0 wakeup per night. The percentage of participants with non-HDL-C ≥ 160 mg/dL was 61.07% with a wakeup frequency ≥ 3 times and 45.82% with a wakeup frequency ≥ 3 times. Among those with TG levels ≥ 200 mg/dL, 35.88% had a wakeup frequency more than or equal to 3 times, and 22.91% had 0 wakeup.

Sex-based Subgroup Analysis in Sleep Disturbance-Associated Dyslipidemia

To evaluate sex differences in sleep disturbance-

Table 2. Distribution of the different lipid profiles according to the wakeup frequency (n=1368)

	Wakeup frequency				
	0 N=467	1 N=542	2 N=230	≥ 3 N=129	*P* for trend
TCHO, mg/dL	214.51 ± 46.08	218.76 ± 53.52	222.87 ± 52.76	230.49 ± 61.81	0.002
TCHO ≥ 200 mg/dL, %	57.39	61.88	63.04	69.47	0.010
TCHO ≥ 240 mg/dL, %	23.55	26.52	30	29.77	0.048
TG, mg/dL	187.4 ± 296.23	200.28 ± 266.26	196.33 ± 212.5	239.37 ± 274.84	0.117
TG ≥ 200 mg/dL, %	22.91	26.7	26.96	35.88	0.006
LDL-C, mg/dL	124.64 ± 41.36	125.89 ± 45.09	125.19 ± 43.58	128.4 ± 51.77	0.521
sdLDL-C, mg/dL	35.4 ± 18.5	37.89 ± 26.0	38.92 ± 22.02	40.4 ± 21.41	0.014
HDL-C, mg/dL	50.44 ± 13.89	50.79 ± 14.73	52.05 ± 15.36	49.79 ± 15.75	0.548
TCHO/HDL	4.52 ± 1.48	4.77 ± 3.87	4.59 ± 1.66	5.16 ± 3.66	0.133
TCHO/HDL ≥ 5, %	30.41	29.83	35.65	35.88	0.108
Non-HDL-C, mg/dL	164.07 ± 46.91	167.97 ± 53.41	170.82 ± 52.02	180.7 ± 58.75	0.003
Non-HDL-C ≥ 160 mg/dL,%	45.82	51.2	52.17	61.07	0.002

Table 3. Distribution of different lipid profiles according to the wakeup frequency in female participants

	Wakeup frequency				
	0 N=112	1 N=160	2 N=87	≥ 3 N=56	*P* for trend
TCHO, mg/dL	212.6 ± 48.1	227.3 ± 71.9	232.0 ± 63.5	254.4 ± 73.5	<.001
TCHO ≥ 200 mg/dL, %	53.57	65	71.26	87.5	<.001
TCHO ≥ 240 mg/dL, %	23.21	32.5	36.78	46.43	0.002
TG, mg/d L	150.0 ± 266.9	144.1 ± 128.5	210.3 ± 284.5	239.9 ± 295.6	0.002
TG ≥ 200 mg/dL, %	15.18	17.5	22.99	33.93	0.004
LDL-C, mg/dL	116.1 ± 44.2	129.8 ± 52.2	128.1 ± 55.2	139.9 ± 63.6	0.009
sdLDL-C, mg/dL	32.32 ± 20.66	38.12 ± 38.29	39.84 ± 28.73	44.0 ± 25.73	0.028
HDL-C, mg/dL	60.83 ± 18.32	59.97 ± 17.4	60.05 ± 16.35	54.74 ± 17.13	0.099
TCHO/HDL-C	3.78 ± 1.46	4.33 ± 5.54	4.27 ± 1.88	5.49 ± 5.18	0.045
TCHO/HDL-C ≥ 5, %	17.86	17.5	28.74	33.93	0.005
Non-HDL-C, mg/dL	151.8 ± 47.9	167.3 ± 72.1	176.2 ± 64.4	199.7 ± 72.3	<.001
Non-HDL-C ≥ 160 mg/dL,%	32.14	48.75	50.57	71.43	<.001

associated dyslipidemia, we also conducted sex-based subgroup analysis. The results among male and female participants are presented in **Tables 3 and 4**, respectively. Significantly increased lipid parameters were observed with a higher wakeup frequency, particularly among the female group. Despite the much smaller sample size of female participants (n=415) than male participants (n=953), we still noticed a net effect on dyslipidemia when analyzing all participants (n=1,368). This finding indicates a strong sex impact on sleep disturbance-associated dyslipidemia.

In female participants, we noticed significantly increased serum T-CHO and non-HDL-C levels and significantly increased serum TG, LDL-C, sdLDL-C, and TCHO/HDL-C levels with higher wakeup frequencies **(Table 3)**.

Among male participants with TG levels ≥ 200 mg/dL, 38.36% had a wakeup frequency more than or equal to 3 times, and 25.35% had 0 wakeup. There were inconsistent and non-statistically significant findings with regard to the serum levels of TCHO, TG, LDL-C, sdLDL-C, HDL-C, TCHO/HDL-C, and non-HDL-C **(Table 4)**.

Multivariate Logistic Regression Analysis for the RRs of Dyslipidemia

Multiple lipid factors were included in the logistic regression analysis to evaluate the RRs of dyslipidemia with a higher wakeup frequency than

Table 4. Distribution of different lipid profiles according to the wakeup frequency in male participants

	Wakeup frequency				
	0 N=355	1 N=382	2 N=143	≥ 3 N=73	*P* for trend
TCHO, mg/dL	215.12 ± 45.49	215.21 ± 43.23	214.76 ± 43.22	212.14 ± 43.41	0.751
TCHO ≥ 200 mg/dL, %	58.59	60.73	58.04	57.53	0.892
TCHO ≥ 240 mg/dL, %	23.66	24.08	25.87	17.81	0.654
TG, mg/dL	199.2 ± 304.3	223.7 ± 302.9	187.9 ± 153.7	238.9 ± 259.9	0.544
TG ≥ 200 mg/dL, %	25.35	30.63	29.37	38.36	0.033
LDL-C, mg/dL	127.34 ± 40.13	124.26 ± 41.77	123.4 ± 34.74	119.34 ± 38.18	0.110
sdLDL-C, mg/dL	36.27 ± 17.78	37.79 ± 18.87	38.37 ± 16.93	37.66 ± 17.11	0.222
HDL-C, mg/dL	47.16 ± 10.18	46.97 ± 11.5	47.18 ± 12.47	46.0 ± 13.54	0.642
TCHO/HDL-C	4.75 ± 1.41	4.95 ± 2.89	4.79 ± 1.48	4.91 ± 1.76	0.550
TCHO/HDL-C ≥ 5, %	34.37	35.08	39.86	38.36	0.277
Non-HDL-C, mg/dL	167.96 ± 45.99	168.24 ± 43.41	167.57 ± 42.73	166.14 ± 40.57	0.843
Non-HDL-C ≥ 160 mg/dL,%	50.14	52.36	53.15	54.79	0.383

Table 5. Relative risks and 95% confidence interval values of various unfavorable lipid profiles according to different wakeup frequencies (all participants)

	N	sdLDL-C ≥ 75th percentile *n*=345	TCHO/HDL-C ≥ 5 *n*=461	Non-HDL-C ≥ 160 mg/dL *n*=735	TG ≥ 200 mg/dL *n*=381	TCHO ≥ 200 mg/dL *n*=838	Dyslipidemia *n*=699
Wakeup frequency							
0	467	1	1	1	1	1	1
1	542	1.23 (0.86, 1.77)	0.86 (0.61, 1.21)	1.15 (0.85, 1.56)	1.22 (0.86, 1.75)	1.22 (0.90, 1.66)	1.47 (1.06, 2.04)
2	230	1.99 (1.28, 3.12)	1.77 (1.14, 2.73)	1.50 (1.00, 2.24)	1.49 (0.94, 2.36)	1.25 (0.83, 1.88)	1.68 (1.09, 2.58)
≥ 3	129	1.34 (0.78, 2.29)	1.17 (0.70, 1.97)	1.78 (1.09, 2.89)	1.68 (0.99, 2.86)	1.54 (0.93, 2.54)	1.96 (1.17, 3.28)
P for trend		0.114	0.147	0.010	0.038	0.098	0.009

Adjusted variables: Age, sex, fasting sugar, body mass index, systolic blood pressure, habits of smoking, drinking alcohol, exercise, and use of sleeping pills. Dyslipidemia was defined as TG ≥ 150 mg/dL or HDL < 40 mg/dL in male participants or < 50 mg/dL in female participants.

with 0 wakeup. The analysis was adjusted for covariates such as age, sex, fasting glucose, BMI, SBP, smoking habit, drinking habit, exercise habit, and use of sleeping pills **(Table 5)**[16]. The subgroup analysis according to sex is presented in **Supplemental Tables 2 and 3**.

Our results indicated that a higher wakeup frequency (≥ 3 times/night) was independently associated with dyslipidemia (OR: 1.96; 95% CI: 1.17–3.28) and non-HDL ≥ 160 mg/dL (OR: 1.78; 95% CI: 1.09–2.89). The highest odds of sdLDL-C over the 75th percentile (OR: 1.99; 95% CI: 1.28–3.12) and a higher cholesterol ratio (TCHO/HDL-C ≥ 5) (OR: 1.77; 95% CI: 1.14–2.73) appeared in participants with wakeup twice a night. A linear trend with the wakeup frequency was observed in non-HDL ≥ 160 mg/dL (*P*=0.010), TG ≥ 200 mg/dL (*P*=0.038), and dyslipidemia (*P*=0.009) for all participants, dyslipidemia (*P*=0.040) in male participants, and non-HDL ≥ 160 mg/dL (*P*=0.003) and TCHO ≥ 200 mg/dL (*P*=0.005) in female participants.

We also conducted logistic regression with interaction analysis between self-reported wakeup frequency (≥ 2 times per night/< 2 times per night) and insomnia (yes/no) to estimate the synergistic effects of these two subjective complaints of sleep disturbance in **Table 6**. An interaction term (wakeup frequency * Insomnia) was included to estimate multiplicative interaction. Relative excess risk due to interaction (RERI) and attributable proportion (AP) were calculated to evaluate additive interaction. RERI is defined as OR (Insomnia: Yes, Wakeup frequency ≥ 2) − OR (Insomnia: Yes, Wakeup frequency < 2) − OR (Insomnia: No, Wakeup frequency ≥ 2) +1, whereas AP equals to RERI/OR (Insomnia: Yes, Wakeup frequency ≥ 2). Additive interaction is absent if 0 falls under the

Table 6. Logistic regression analysis for the odds ratio (95% C.I.) of atherogenic dyslipidemia

	Wakeup frequency	Insomnia		Wakeup frequency * Insomnia	Relative excess risk due to interaction (RERI)	Attributable proportion (AP)
		No	Yes			
All participants						
sdLDL-C ≥ 75th percent	< 2	1	1.04 (0.49, 2.18)	1.03 (0.40.2.67)	0.06 (-1.16, 1.28)	0.04 (-0.72, 0.79)
	≥ 2	1.50 (1.03, 2.19)	1.59 (0.91, 2.80)			
TCHO/HDL-C ≥ 5	< 2	1	0.44 (0.20, 1.01)	2.45 (0.88, 6.77)	0.69 (-0.38, 1.76)	0.41 (-0.07, 0.90)
	≥ 2	1.53 (1.05, 2.21)	1.66 (0.94, 2.92)			
Non-HDL-C ≥ 160 mg/dL	< 2	1	1.07 (0.57, 2.00)	2.15 (0.89.5.21)	1.50 (-0.21, 3.21)	0.54 (0.17, 0.91)
	≥ 2	1.21 (0.86, 1.70)	2.78 (1.55, 4.98)			
TG ≥ 200 mg/dL	< 2	1	1.45 (0.72, 2.92)	0.91 (0.36, 2.32)	-0.02 (-1.45, 1.40)	-0.01 (-0.83, 0.80)
	≥ 2	1.33 (0.90, 1.96)	1.76 (0.99, 3.12)			
TCHO ≥ 200 mg/dL	< 2	1	1.20 (0.63, 2.30)	2.18 (0.85, 5.55)	1.39 (-0.39, 3.17)	0.54 (0.11, 0.97)
	≥ 2	0.98 (0.70, 1.39)	2.58 (1.36, 4.90)			
Dyslipidemia	< 2	1	1.15 (0.58, 2.27)	1.69 (0.66, 4.28)	1.00 (-0.58, 2.58)	0.42 (-0.07, 0.91)
	≥ 2	1.22 (0.85, 1.77)	2.37 (1.31, 4.30)			
Male						
sdLDL-C ≥ 75th percent	< 2	1	1.25 (0.52, 3.02)	1.06 (0.31, 3.56)	0.17 (-1.57, 1.90)	0.10 (-0.87, 1.07)
	≥ 2	1.29 (0.81, 2.07)	1.71 (0.78, 3.72)			
TCHO/HDL-C ≥ 5	< 2	1	0.53 (0.21, 1.31)	3.43 (1.01, 11.66)	1.40 (-0.25, 3.04)	0.68 (0.27, 1.08)
	≥ 2	1.14 (0.73, 1.78)	2.06 (0.96, 4.43)			
Non-HDL-C ≥ 160 mg/dL	< 2	1	0.87 (0.40, 1.89)	3.33 (1.05, 10.54)	2.05 (-0.37, 4.46)	0.70 (0.35, 1.05)
	≥ 2	1.01 (0.67, 1.52)	2.93 (1.31, 6.55)			
TG ≥ 200 mg/dL	< 2	1	1.80 (0.77, 4.17)	0.68 (0.20, 2.27)	-0.53 (-2.46, 1.40)	-0.36 (-1.85, 1.12)
	≥ 2	1.20 (0.75, 1.93)	1.47 (0.66, 3.27)			
TCHO ≥ 200 mg/dL	< 2	1	1.04 (0.48, 2.25)	2.43 (0.77, 7.65)	1.28 (-0.61, 3.17)	0.59 (0.08, 1.09)
	≥ 2	0.87 (0.58, 1.30)	2.19 (0.98, 4.87)			
Dyslipidemia	< 2	1	1.16 (0.50, 2.68)	1.92 (0.56, 6.61)	1.28 (-1.11, 3.68)	0.49 (-0.09, 1.07)
	≥ 2	1.18 (0.76, 1.83)	2.62 (1.12, 6.11)			
Female						
sdLDL-C ≥ 75th percent	< 2	1	0.77 (0.19, 3.12)	0.99 (0.19, 5.26)	-0.26 (-2.24, 1.72)	-0.17 (-1.50, 1.16)
	≥ 2	2.06 (1.05, 4.02)	1.56 (0.67, 3.65)			
TCHO/HDL-C ≥ 5	< 2	1	0.22 (0.02, 2.00)	1.82 (0.17, 19.93)	-1.22 (-3.71, 1.27)	-0.89 (-3.06, 1.28)
	≥ 2	3.36 (1.64, 6.91)	1.37 (0.54, 3.48)			
Non-HDL-C ≥ 160 mg/dL	< 2	1	1.54 (0.49, 4.87)	0.96 (0.21, 4.33)	0.44 (-2.76, 3.65)	0.14 (-0.82, 1.11)
	≥ 2	2.06 (1.06, 4.01)	3.05 (1.27, 7.34)			
TG ≥ 200 mg/dL	< 2	1	1.10 (0.26, 4.56)	1.18 (0.21, 6.53)	0.39 (-2.01, 2.80)	0.18 (-0.87, 1.23)
	≥ 2	1.65 (0.80, 3.40)	2.14 (0.90, 5.09)			
TCHO ≥ 200 mg/dL	< 2	1	1.58 (0.44, 5.68)	1.69 (0.28, 10.07)	1.93 (-2.91, 6.77)	0.48 (-0.30, 1.26)
	≥ 2	1.50 (0.74, 3.06)	4.01 (1.29, 12.45)			
Dyslipidemia	< 2	1	1.28 (0.38, 4.31)	1.25 (0.27, 5.80)	0.53 (-1.83, 2.90)	0.24 (-0.73, 1.22)
	≥ 2	1.37 (0.70, 2.68)	2.18 (0.93, 5.13)			

Adjusted variables: Age, sex, fasting glucose, body mass index, systolic blood pressure, habits of smoking, alcohol consumption, exercise, and use of sleeping pills.
Definition: Insomnia was defined as self-reported moderate (2-3 times per week) to severe (≥ 4 days per week) insomnia.
Relative excess risk due to interaction (RERI) = OR (Insomnia: Yes, Wakeup frequency ≥ 2) –OR (Insomnia: Yes, Wakeup frequency <2) - OR (Insomnia: No, Wakeup frequency ≥ 2) + 1; Attributable proportion (AP) = RERI / OR (Insomnia: Yes, Wakeup frequency ≥ 2)

95% CI of RERI and AP. Positive additive interaction exists if RERI >0 or AP >0 [17-18].

As compared with those with a low wakeup frequency (<2 times/night) and without insomnia, those with both high wakeup frequency (≥ 2 times/night) and self-reported insomnia have a significantly increased RR and 95% CI of 2.78 (1.55–4.98) for non-HDL ≥ 160 mg/dL, 2.58 (1.36–4.90) for TCHO ≥ 200 mg/dL, and 2.37 (1.31–4.30) for dyslipidemia in all participants. Positive multiplicative interaction between wakeup frequency and insomnia was observed in TCHO/HDL-C ≥ 5 (RR for interaction term: 3.43; 95% CI: 1.01–11.66) and non-HDL ≥ 160 mg/dL (RR for interaction term: 3.33; 95% CI: 1.05–10.54) for male participants. In addition, positive additive interaction exists in all participants with AP and 95% CI of 0.54 (0.17–0.91) for non-HDL ≥ 160 mg/dL and 0.54 (0.11–0.97) for TCHO ≥ 200 mg/dL and in male participants with AP and 95% CI of 0.68 (0.27–1.08) for TCHO/HDL-C ≥ 5, 0.70 (0.35–1.05) for non-HDL ≥ 160 mg/dL and 0.59 (0.08–1.09) for TCHO ≥ 200 mg/dL.

Discussion

To the best of our knowledge, our study is the first to use the wakeup frequency at night as a quantifiable marker of sleep disturbance and link its relationship with atherogenic dyslipidemia. Our results indicated that wakeup frequency at night is significantly associated with atherogenic dyslipidemia, and a significant synergistic effect when both the wakeup frequency and subjective insomnia were concurrently taken into account. In addition, it is associated with atherogenic dyslipidemia, including higher levels of cholesterol, TG, sdLDL-C, and non-HDL-C as well as low HDL-C levels, a complex picture of disarrangement of TG-rich lipoproteins[8]. A novel finding of our study is that women are more susceptible to increased TG, low HDL-C, and sdLDL-C. This may partially explain why previous studies suspect that female participants with insomnia are more susceptible to CVDs.

Hyperlipidemia is considered to be one of the most important risk factors for CHD, particularly sdLDL-C and non-HDL-C[19-23]. The study findings of increased risk of higher sdLDL-C levels in subjects with higher wakeup frequency at night also gained support from our recent report that sdLDL-C is positively associated with biomarkers of thrombosis, prediabetes, and inflammation in non-diabetic adults[17]. In the ARIC study in the US had shown that higher sdLDL-C a more important predictor than large-buoyant LDL-C for future cardiovascular events[19]. Higher levels of non-HDL-C and ApoB have been proven as more important predictors for CHD in ethnic Chinese after 13.6 years of follow-up in the Chin-Shan Cardiovascular Cohort Study[20]. Furthermore, the HRs of cardiovascular events were more evident if higher cholesterol ratio and higher ApoB levels are combined. Thus, the metabolic disarrangement on atherogenic dyslipidemia, such as higher sdLDL-C, non-HDL-C, hypercholesterolemia, and dyslipidemia in this study, may subsequently lead to a higher risk of cardiovascular diseases if the status of higher wakeup frequency persisted.

Supporting evidence on lowering hyperlipidemia to prevent atherogenesis has emerged largely in recent decades, and multiple modifiable lifestyle factors have been investigated to evaluate their possible correlation with dyslipidemia. As a common complaint by the general population, insomnia has been extensively studied for its impact on health, particularly on CVDs[21]. However, limited evidence has been studied between sleep disturbance and atherogenic dyslipidemia. Sleep disturbance, defined as wakeup frequency at night in this study, may shed a new light into further elucidation of the underlying mechanism of cardiovascular effects of sleep disorders.

The causes of wakeup at night can be summarized as follows: (1) pain, especially from arthritis, heart failure, sickle cell anemia, or cancer; (2) breathing trouble from asthma, bronchitis, or another lung disease; (3) digestive problems, especially pain and cough from acid reflux or symptoms of irritable bowel syndrome; (4) effects of period or menopause, such as hot flashes and night sweats, in women; (5) neurologic diseases, including Alzheimer's and Parkinson's; (6) frequent urination due to drinking a lot of fluids during the day or due to chronic disease, diabetes, heart disease, kidney disease, or bladder inflammation (prostate hypertrophy or urinary tract infection); (7) stress and other mental disorders, including anxiety disorders, bipolar disorder, depression, or schizophrenia; (8) consumption of food with diuretic effects at dinner or before sleep, such as coffee, tea, alcohol, or fruits[25, 26]; (9) and environmental factors, such as very hot or cold weather, humid or noisy environment, poor air quality, and too dark or bright environment[27]. Thus, wakeup frequency at night indicated a common phenomenon of many underlying diseases or physiological status, lifestyle habits, or disturbed sleep environment, which may subsequently lead to atherogenic dyslipidemia. Furthermore, the aforementioned factors can cause sleep disturbance and increase sympathetic activity and venous endothelial dysfunction and may also help explain the

association between short sleep and increased cardiovascular risk in epidemiological studies[28]. Hence, this study provided another mechanistic evidence of increased incidence of CVDs in subjects with habitual interrupted sleep at night.

To date, the evidence of insomnia-related dyslipidemia seems to be mixed. Both long and short sleep durations were associated with a moderately increased risk of CHD among Chinese[29], Caucasian[2, 30], African-American, and Hispanic ethnicities[30] In a large cohort study of healthy Taiwanese adults (n=162,121), only short sleep duration was associated with metabolic syndrome[31] Self-reported severe insomnia and long and short sleep durations were all demonstrated to be associated with increased TCHO and TG levels, as well as cardiovascular events and all-cause mortality in a Taiwanese prospective cohort study (n=3,430)[1]. More conflicting evidence was reported on this issue. A large cross-sectional study involving 9798 participants in the USA suggested that insomnia symptoms were not associated with the LDL-C, HDL-C, or TG levels[16]. Instead, they found that the use of sleeping pills was associated with increased LDL-C, but they did not provide a mechanism behind this finding.

In contrast to self-reported sleep duration, insomnia symptoms, or subjectively measured sleep quality, the present study used the wakeup frequency at night as a marker of sleep disturbance. This marker is associated with two advantages. First, it is a simpler and more quantifiable index to evaluate sleep disturbance. Second, it causes less recall bias than recording sleep duration. Third, it represents a physiological response to a broader range of underlying diseases or disturbed physical status.

Another novel finding in our study was sex differences. We noticed that female participants were more susceptible to hyperlipidemia if they had self-reported insomnia or increased wakeup frequency. Previous studies have demonstrated that women were more prone to experiencing insomnia than men[32] and that women with insomnia may be more prone to dyslipidemia[10, 16, 33]. However, the evidence supporting this finding is limited. Only a few studies have investigated this subject, and they could not provide a thorough explanation. In a German cohort study of 3,508 healthy middle-aged men and women, short sleep duration and difficulty maintaining sleep were associated with an increased risk of myocardial infarction in middle-aged women but not men[10]. Another US cohort of over 9,000 participants concluded that after adjustment for basic characteristics, women with insomnia symptoms had significantly increased TG levels but not LDL-C or HDL-C levels. No significant increase in dyslipidemia was observed in men[16]. In a Chinese cohort of more than 10,000 healthy adults, a 25% increased odds of elevated TCHO levels was found among women with insomnia ≥ three times per week compared with those women without insomnia[33].

The present study confirms previous evidence concluding that an inadequate sleep duration is associated with dyslipidemia in women. A Chinese cohort of 8,574 adults showed that short and long sleep durations were associated with TCHO, LDL-C, and ApoB in women but not in men[34].

The present study demonstrated that interrupted sleep correlated with increased sdLDL-C and traditional atherogenic dyslipidemia. Inflammation might participate in this process, as a previous study found increased inflammation markers in sleep-restricted participants[35] and that sdLDL-C is increased in patients with chronic inflammatory diseases[36]. Experimental studies in mice have demonstrated that sustained sleep fragmentation is a potent inducer of increased food intake and leptin resistance, which led to obesity and insulin resistance[37, 38]. The possible mechanisms for the sex difference in sleep disorder-related dyslipidemia include insulin insensitivity[39]. and increased inflammation[40]. In an animal study, chronic sleep fragmentation especially activates the TNF-alpha-dependent inflammation pathway[41]. Sleep deprivation elevates stress hormones, such as cortisol and catecholamine, which lead to decreased insulin sensitivity[42]. Women with insomnia are more likely to have obesity[43] and decreased insulin sensitivity. Our results support that hyperglycemia is also increased in the high-wakeup-frequency groups **(Supplemental Table 1)**. A 5-year follow-up study in the USA demonstrated that women with poor subjective sleep quality at the beginning have a significantly higher risk of increased interleukin-6, C-reactive protein, and fibrinogen. Insulin resistance causes increased TG and sdLDL-C and decreased HDL-C, both independent CHD risk factors[44]. Women have much higher risks of insomnia after puberty[45], suggesting that sexual hormones may affect sleep[9].

Our study has the following strengths. First, the use of the wakeup frequency as a novel indicator of sleep disturbance can be corroborated by the significant trend of increasing sleep duration < 6 h and insomnia severity with an increasing wakeup frequency in **Table 1**. Second, the wakeup frequency also increased with the fasting glucose and glucose levels at different times after glucose challenge, as well as with the glucose AUC and OGTT diagnosis of DM. This finding indicates that wakeup frequency is

positively associated with not only atherogenic dyslipidemia but also impaired glucose metabolism. These findings also support that wakeup frequency is a good and novel indicator of dysglycemia and dyslipidemia. Third, we identified synergistic effects of the wakeup frequency combined with insomnia severity to predict the RR of dyslipidemia. This finding also indicates that wakeup frequency can be a novel indicator independent of the traditional definition of subjective insomnia.

Limitations

Our study has several limitations. First, this study was retrospective; thus, we cannot establish a clear cause–effect relationship between sleep quality and dyslipidemia. Second, we did not record the medical histories of urologic diseases that affect sleep quality, such as benign prostate hypertrophy in men. We did not record the frequency of nocturia or severe urinary symptoms. Furthermore, we did not include a history of sleep apnea, which may affect sleep quality and is an independent predictive factor of increased cholesterol and TGs[46]. However, our data revealed no significant association between the use of sleeping pill and wakeup frequency. Thus, further multivariate analysis did not include this adjustment factor. The concept of "wakeup frequency" applied in our research overlaps with the traditional definition of "insomnia" but differs in the measuring method. For example, in our questionnaire, we did not focus on the collection of information on the participants' daytime symptoms, which was considered as a key factor of clinically significant insomnia. We only included wakeup frequency in the questionnaire, because the main purpose of our research was to determine if interrupted sleep is related to dyslipidemia.

Lastly, we did not provide information on the menopausal status of the female participants. Previous evidence demonstrated that due to loss of estrogen protection, hyperlipidemia is significantly increased in postmenopausal women. Also, sleep disorder is frequently complained among peri-menopausal women. It is arguable that menopausal status might confound the wakeup frequency and hyperlipidemia rate, considering that most participants were middle-aged. However, after age adjustment, there is still correlation between wakeup frequency and lipid factors.

Conclusion

In summary, this study indicates that wakeup frequency might be used as a novel indicator of metabolic disarrangement, particularly in atherogenic dyslipidemia independent of the traditional definition of subjective insomnia. Self-reported higher wakeup frequency is associated with increased atherogenic dyslipidemia, particularly in women. This study also provides a novel risk marker for the proactive prevention of cardiovascular disease and evidence for public health implication.

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Conflicts of Interests

None.

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Supplemental Table 1. Oral glucose tolerance test at 0, 30, 60, 90, and 120 minutes and glucose area under the curve (AUC), according to the wakeup frequencies

	Wakeup frequency				
	0 N = 467	1 N = 542	2 N = 230	≥ 3 N = 129	*P*-value
Diabetes by OGTT, %	5.52	5.49	9.00	9.57	0.042
OGTT 0', mg/dL	97.29 ± 22.92	99.71 ± 27.26	99.17 ± 21.41	103.26 ± 30.26	0.034
OGTT 30', mg/dL	159.63 ± 39.25	159.41 ± 38.31	164.96 ± 42.15	169.11 ± 43.64	0.030
OGTT 60', mg/dL	159.99 ± 56.47	159.8 ± 58.88	170.93 ± 56.15	169.12 ± 66.37	0.037
OGTT 90', mg/dL	141.41 ± 58.08	143.94 ± 59.86	152.82 ± 58.94	151.04 ± 68.90	0.025
OGTT 120', mg/dL	125.35 ± 54.03	126.61 ± 55.47	131.53 ± 53.59	132.72 ± 60.52	0.130
Glu AUC, mg/dL	557.75 ± 189.1	562.28 ± 177.03	604.2 ± 192.79	596.96 ± 218.4	0.013

Supplemental Table 2. Multivariate logistic regression analysis for the odds ratios (95% CIs) of higher sdLDL-C levels (≥ 75th percentile) and dyslipidemia according to different wakeup frequencies in male subjects

Male	N	sdLDL-C ≥ 75th percentile N = 235	TCHO/HDL-C ≥ 5 N = 330	Non-HDL-C ≥ 160 mg/dL N = 517	TG ≥ 200 mg/dL *n* = 293	TCHO ≥ 200 mg/dL *n* = 538	Dyslipidemia *n* = 508
Wakeup frequency							
0	355	1	1	1	1	1	1
1	382	1.23 (0.80, 1.80)	0.94 (0.64, 1.38)	1.12 (0.79, 1.59)	1.47 (0.97, 2.22)	1.18 (0.83, 1.67)	1.58 (1.08, 2.30)
2	143	1.70 (0.98, 2.93)	1.54 (0.92, 2.59)	1.33 (0.82, 2.14)	1.42 (0.81, 2.48)	1.19 (0.74, 1.91)	1.60 (0.96, 2.70)
≥ 3	73	1.23 (0.61, 2.49)	0.95 (0.49, 1.84)	1.36 (0.75, 2.49)	1.64 (0.83, 3.25)	1.04 (0.58, 1.89)	2.02 (1.05, 3.90)
P for trend		0.392	0.745	0.248	0.178	0.887	0.040

Dyslipidemia was defined as TG ≥ 150 mg/dL or HDL-C < 40 mg/dL in male participants or < 50 mg/dL in female participants. The data are shown after controlling for age, sex, fasting sugar, body mass index, systolic blood pressure, habits of smoking, drinking alcohol, exercise, and use of sleeping pills.

Supplemental Table 3. Relative risks and 95% CI values of higher non-HDL and hypercholesterolemia according to different wakeup frequencies in female subjects

Female	N	sdLDL-C ≥ 75th percentile *n* = 110	TCHO/HDL-C ≥ 5 *n* = 131	Non-HDL ≥ 160 mg/dL *n* = 218	TG ≥ 200 mg/dL *n* = 88	TCHO ≥ 200 mg/dL *n* = 300	Dyslipidemia *n* = 191
Wakeup frequency							
0	112	1	1	1	1	1	1
1	160	1.33 (0.64, 2.78)	0.69 (0.31, 1.53)	1.44 (0.76, 2.72)	0.72 (0.33, 1.57)	1.56 (0.81, 3.01)	1.30 (0.66, 2.54)
2	87	2.95 (1.28, 6.78)	2.67 (1.13, 6.29)	2.43 (1.11, 5.30)	1.48 (0.63, 3.50)	1.73 (0.76, 3.92)	1.83 (0.83, 4.05)
≥ 3	56	1.64 (0.67, 4.05)	1.68 (0.66, 4.29)	3.49 (1.43, 8.50)	1.50 (0.60, 3.74)	4.78 (1.61, 14.25)	1.87 (0.77, 4.53)
P for trend		0.112	0.053	0.003	0.185	0.005	0.115

The data are shown after controlling for age, sex, fasting sugar, BMI, SBP, habits of smoking, drinking alcohol, exercise, and use of sleeping pills.