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Association of Sleep Duration and Quality with Subclinical Atherosclerosis

Fernando Domínguez, MD, PhD;[1,2,3] Valentín Fuster, MD, PhD;[1,4 *] Juan Miguel Fernández-Alvira, PhD;[1] Leticia Fernández-Friera, MD, PhD;[1,3,5] Beatriz López Melgar, MD, PhD;[1,3,5] Ruth Blanco-Rojo, PhD;[6] Antonio Fernández-Ortiz;[1,3,7,8] Pablo García-Pavía, MD, PhD;[2,3,9] Javier Sanz, MD;[1,4] Jose M Mendiguren, MD;[10] Borja Ibañez, MD, PhD;[1,3,11] Héctor Bueno, MD, PhD;[1,12,13] Enrique Lara-Pezzi, PhD;[1,3] José M. Ordovás, PhD;[1,6,14 *]

[1]Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain; [2] Heart Failure and Inherited Cardiac Diseases Unit. Department of Cardiology. Hospital Universitario Puerta de Hierro, Madrid, Spain; [3]Centro de Investigacion Biomedica en Red en Enfermedades Cardiovasculares (CIBERCV); [4] Zena and Michael A. Wiener Cardiovascular Institute/Marie-Josée and Henry R. Kravis Center for Cardiovascular Health, Icahn School of Medicine at Mount Sinai, New York, New York; [5] HM Hospitales-Centro Integral de Enfermedades Cardiovasculares HM-CIEC, Madrid, Spain; [6] IMDEA Food Institute, CEI UAM + CSIC, Madrid, Spain; [7] Universidad Complutense, Madrid, Spain; [8] Cardiovascular Institute, IDSSC, Hospital Clínico San Carlos, Madrid, Spain; [9] Faculty of Health Sciences, University Francisco de Vitoria (UFV), Pozuelo de Alarcon, Madrid, Spain; [10] Banco de Santander, Madrid, Spain [11] IIS-Fundación Jiménez Díaz Hospital, Madrid, Spain; [12] Cardiology Department, Hospital Universitario 12 de Octubre, and Instituto de Investigación Sanitaria Hospital 12 de Octubre, Madrid, Spain; [13] Facultad de Medicina, Universidad Complutense de Madrid, Spain; [14] U.S. Department of Agriculture Human Nutrition Research Center on Aging, Tufts University, Boston, Massachusetts

Brief title: Association of sleep characteristics with subclinical atherosclerosis

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Correspondence:
José M. Ordovás
Director Nutrition and Genomics, Professor Nutrition and Genetics
JM-USDA-HNRCA at Tufts University
711 Washington St.
Boston, Massachusetts 02111
Telephone: 617-556-3102
Fax: 617-556-3103
E-mail: jose.ordovas@tufts.edu
Twitter: @jordovas56

Valentin Fuster, MD, PhD
Director of Zena and Michael A. Wiener Cardiovascular Institute

Medicine Professor, Cardiology
The Mount Sinai Hospital
1190 5th Avenue
New York, NY 10029
Telephone: 212-241-7911
Fax: 212-423-9488
E-mail: vfuster@cnic.es
Twitter: @CNIC_Cardio | @MountSinaiNYC

Abstract
Background. Sleep duration and quality have been associated with increased cardiovascular risk. However, large studies linking objectively measured sleep and subclinical atherosclerosis assessed in multiple vascular sites are lacking.
Objectives. To evaluate the association of actigraphy-measured sleep parameters with subclinical atherosclerosis in an asymptomatic middle-aged population, and investigate interactions between sleep, conventional risk factors, psychosocial factors, dietary habits, and inflammation.
Methods. Seven days actigraphic recording was performed in 3974 participants (age 45.8±4.3; 62.6% men) from the Progression of Early Subclinical Atherosclerosis (PESA) study. Four groups were defined: Very-short sleep duration (VSSD) <6 hours, short sleep duration (SSD): 6-7 hours; reference sleep duration: 7-8 hours and long sleep duration: >8 hours. Sleep fragmentation index was defined as the sum of the movement index and FI. Carotid and femoral 3D vascular ultrasound (VUS) and cardiac computed tomography were performed to quantify noncoronary atherosclerosis and coronary calcification.
Results. When adjusted for conventional risk factors, VSSD was independently associated with a higher atherosclerotic burden with 3D VUS compared to the reference group (OR: 1.27; 95%CI, 1.06-1.52; p=0.008). Participants within the highest quintile of sleep fragmentation presented a higher prevalence of multiple affected noncoronary territories (OR:1.34; 95% CI:1.09-1.64; p=0.006). No differences were observed regarding coronary artery calcification score in the different sleep groups.
Conclusions. Lower sleeping times and fragmented sleep are independently associated with an increased risk of subclinical multi-territory atherosclerosis. These results highlight the importance of healthy sleep habits for the prevention of cardiovascular disease.

Condensed abstract: Large studies evaluating links between objectively measured sleep parameters and subclinical atherosclerosis are lacking. We sought to evaluate the association of sleep duration and quality with subclinical atherosclerosis using 3D vascular ultrasound (3DVUS) and coronary computed tomography. When adjusted for conventional risk factors, very short sleep duration (<6h), was independently associated with a higher noncoronary atherosclerotic burden measured with 3DVUS compared to the reference group (7-8h). Likewise, participants with more fragmented sleep presented a higher prevalence of multiple noncoronary affected territories. Thus, these results highlight the association between poor sleep habits and subclinical atherosclerosis.

Key words: Subclinical atherosclerosis, sleep, actigraph, 3D vascular ultrasound, cardiac computed tomography.

Abbreviations
3D VUS: 3D vascular ultrasound
CT: Computed tomography
LSD: Long sleep duration
MetS: Metabolic syndrome
PESA: Progression of Early Subclinical Atherosclerosis
RSD: Reference sleep duration
SSD: Short sleep duration

SFI: Sleep fragmentation index
VSSD: Very short sleep duration

Introduction

Sleep is an essential physiological process that protects our physical and mental health. Sleep deficiency is highly prevalent in Western societies, and epidemiological studies suggest that not only short but also long sleep duration (LSD) is related to an increased cardiovascular risk (1, 2).

Several studies and meta-analyses have reported associations between short sleep duration (SSD) and hypertension (3,4), with some showing a relationship with incident hypertension in subjects <65 years old (4,5). No such association has been found with LSD (5). Recent meta-analyses have shown a 30% increased risk of diabetes mellitus among subjects sleeping less than 5-6 hours per day, as well as in those who sleep >8 hours per day (6). In addition, SSD has been reported to influence food intake and obesity (7).

Although sleep quality and duration have been associated with the risk of coronary heart disease, stroke (1) and subclinical atherosclerosis (8), most studies rely on self-reported questionnaires of sleep evaluation (8, 9). The value of previous actigraphy-based studies evaluating atherosclerotic burden is limited because these were mostly small studies (10–12) and focused on patients with sleep disorders, such as obstructive sleep apnea (OSA) (13, 14). Previous studies relying on objectively-assessed sleep have shown that shorter sleep duration is associated with greater carotid intima media thickness in men (15), and longer sleep duration is associated with a lower coronary calcification incidence, which is related to subclinical atherosclerosis (16). However, studies using newer and more reliable imaging techniques for measuring atherosclerosis are lacking. Moreover, the association of multiterritory atherosclerosis and sleep has not been yet assessed.

The aim of this study was to evaluate the association between actigraphy-measured sleep parameters and subclinical atherosclerosis, investigating possible interactions between sleep parameters, risk factors, dietary habits and inflammatory markers.

Methods

Study population

PESA-CNIC-Santander is an observational prospective cohort study (PESA stands for "Progression of Early Subclinical Atherosclerosis") that recruited 4184 male and female employees of Santander Bank in Madrid from 40 to 54 years of age (17). All participants were free of known cardiovascular disease (CVD). The baseline visit included a fasting blood test, urine sample and a 12-lead ECG. Patients with a history of OSA at baseline (n=77) and those without actigraphic recording (n=133) were excluded from the study. The final sample available for this analysis consisted of 3974 participants. Of those, 3,804 were examined at baseline by 3D vascular ultrasound (170 were excluded due to incomplete 3DVUS studies and incomplete clinical data necessary for the adjusted models) and 3899 with non-contrast cardiac computed tomography (CT) (75 were excluded because of incomplete clinical data necessary for the adjusted models).

Assessment of CVD risk factors, metabolic syndrome and dietary intake

The participants' medical history, traditional CVD risk factors, lifestyle features, (18) and physical examination including anthropometric characteristics were recorded. Obesity was defined as a body mass index (BMI) ≥30 kg/m^2. Metabolic syndrome (MetS) was defined according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria (19), which requires three or more of the following characteristics: a) abdominal obesity, defined as a waist circumference in men ≥102 cm and women ≥88 cm, b) serum triglycerides ≥150 mg/dL or drug treatment for elevated triglycerides, c) serum high density lipoprotein (HDL) cholesterol <40 mg/dL in men and <50 mg/dL in women or drug treatment for low HDL cholesterol, d) blood pressure ≥130/85 mmHg or drug treatment for elevated blood pressure and e) fasting plasma glucose (FPG) ≥100 mg/dL or drug treatment for high blood glucose. The Framingham 10 year and 30-year scores, as well as the Fuster-BEWAT (20) score, were calculated in all study participants. Additionally, to avoid the potential confounding factor of underdiagnosed OSA, a modified STOP-BANG (mSTOP-BANG) questionnaire score was calculated and results were adjusted for this variable. The STOP-BANG questionnaire (acronym stands for Snoring, Tired, Observed apnea, high blood Pressure, BMI >35 kg/m^2, Age >50, Neck circumference >43 cm in males, >41 cm in females and male gender) evaluates the risk of sleep apnea and has been proved to be a practical tool to screen for OSA (21). The clinical data from the PESA study included all the necessary parameters to calculate the STOP-BANG score, except for the neck circumference, and therefore the results of a mSTOP-BANG score were incorporated into our study.

All participants underwent a survey to complete the computerized dietary history adapted to the Spanish population, which was initially developed and validated in the EPIC-Spain study (22, 23). The survey is based on a computer application and is structured according to the episodes of intake throughout the day (breakfast, mid-morning, lunch, snack, dinner, and ingestion between meals) (24,25). Once the survey was completed, the software provided information on foods consumed, caloric intake, macro and micronutrient intake and different forms of food preparation, as well as specific eating habits. Regarding caffeinated drinks, participants were asked to report their daily intake in grams (g) including coffee, tea, cola drinks and energy drinks. Assuming an approximate density of 1 g/cm^3, results were converted to milliliters (ml).

Assessment of quantity and quality of sleep

Actigraphic and self-reported sleep durations were firstly analyzed as continuous variables and then divided into multiple categories to achieve groups of adequate sample sizes to reflect the possible non-linear (U- or J- shaped) associations between sleep duration and risk outcomes. The quantity of sleep was assessed by triaxial accelerometry, using Acti Trainers accelerometers (Actigraph, Pensacola, Florida) placed on the participant's waist for 7 days. Based on the last scientific statement from the American Heart Association regarding the impact of sleep duration on cardiometabolic health, a sleep duration of 7 to 8 hours was considered normal and participants within that range were considered as the reference sleep duration (RSD) group (26). The remaining groups included participants with very short sleep duration (VSSD, <6 hours), short sleep duration (SSD, 6-7 hours) and participants with long sleep duration (LSD, >8 hours).

Sleep quality was assessed by the total sleep Fragmentation Index (SFI), which is defined as the sum of the movement index (MI) and the fragmentation index (FI). MI is the percentage of epochs with y-axis counts greater than zero in the sleep period. FI is the percentage of one-minute periods of sleep vs. all periods of sleep during the sleep period (27). Study participants were divided into quintiles according to sleep fragmentation and those with less fragmented sleep (1st quintile) were considered to be the reference group. Additionally, participants completed the Sleep Habits Questionnaire, developed and validated by the Sleep Heart Health Study (28).

Psychosocial evaluation

The presence of depressive symptoms was evaluated by the Center for Epidemiological Studies-Depression (CES-D) scale. It has been translated and validated in the Spanish population (29) and has demonstrated high sensitivity and specificity for the identification of depressive symptoms in epidemiological studies. Participants also completed the Perceived Stress Scale (PSS) (30), which is widely used for measuring nonspecific perceived stress.

Assessment of subclinical atherosclerosis

PESA participants underwent 3DVUS studies using a volumetric-linear array transducer to evaluate plaque burden in the bilateral carotid and femoral arteries. As there is currently no standard definition for plaque presence using 3DVUS, noncoronary atherosclerosis was defined as plaque presence using the Manheim criteria for 2DVUS (31), and the number of affected territories (1 to 4) was also recorded. Cumulative plaque volume (burden, in mm^3) was quantified and divided into tertiles to classify atherosclerosis as mild, moderate or severe (32). This 3DVUS method has already proven to be accurate measuring plaque volumes in-vitro and in vivo (33). Moreover, atherosclerosis assessed by 3DVUS in the PESA cohort correlates with classic cardiovascular risk factors, especially for femoral arteries (32). A 16-slice CT scan was used to quantify the Agatston coronary calcium score (CACS), which was categorized as 0,<1, 1-100, 101-400, and >400 (34).

All performed imaging tests were blind, and the Centro Nacional de Investigaciones Cardiovasculares (CNIC) conducted the analysis.

Assessment of inflammatory biomarkers

The assessed inflammatory biomarkers included neutrophil count, P selectin, high sensitivity C- reactive protein (hs-CRP) and vascular cell adhesion molecule (VCAM).

Statistical analysis

Statistical analyses were performed with SPSS software version 21.0. The population baseline characteristics of the study are presented as percentages for categorical variables and as the mean and standard deviation (SD) for continuous variables. Bonferroni (analysis of variance with multiple-testing correction) was used for continuous variables, including p for trend values for the general comparison of groups and specific p values when compared to the reference group. Chi-square test was used for categorical variables.

The degree of agreement between actigraphic and self-reported sleep duration was quantified by computing a concordance correlation coefficient. Associations between actigraphic sleep parameters (fragmentation index and sleep duration) and cardiometabolic risk outcomes or inflammation markers were evaluated by multivariable ordinal regression models adjusted for age, gender, physical activity, BMI, smoking status, alcohol consumption, systolic blood pressure, education level, fasting glucose, total cholesterol, total kcal per day marital status, CES-D/PSS, and mSTOP-BANG questionnaire scores. Moreover, to obtain p values for the overall adjusted association between sleep parameters and atherosclerosis variables we performed a likelihood-ratio test comparing the final models and the model including all variables other than the predictive variable in each analysis.

As gender has been reported to modify the association between sleep and cardiometabolic parameters (35), associations were additionally explored separately in men and women when a test for significance of effect modification by sex showed a p value <0.05.

Results

A total of 2488 men (62.6%) and 1486 women (37.4%) underwent actigraphic analysis to evaluate sleep duration and quality. The proportion of participants with RSD was 30.7%, whereas SSD or VSSD accounted for 65.3% of cases. Only 4% of participants presented LSD

(**Table 1**). Regarding sleep fragmentation, each of the quintiles comprised 774-786 participants (Online Table 1).

There was a significant but weak correlation between actigraphic and self-reported sleep duration among the study participants (Pearson correlation coefficient: 0.35; $p<0.001$). Moreover, the 3899 subjects who answered the questionnaires overestimated their sleep duration as compared to the accelerometer results (Online Figure 1).

Clinical profile and sleep parameters

Baseline characteristics according to actigraphy-measured sleep duration are presented in **Table 1**. Increased age, higher systolic and diastolic blood pressure hypertension, BMI, lower HDL cholesterol, and MetS were significantly more prevalent in participants with VSSD or SSD compared to RSD (7-8h) (**Table 1**). Similar to VSSD and SSD, participants in the higher quintile for SFI were significantly older and had an increased prevalence of smoking and hypertension (**Online Table 1**).

The Framingham risk score for 10 and 30 years estimated a significantly higher cardiovascular risk in participants with VSSD or SSD compared to RSD as well as in those included in the three higher quintiles of sleep fragmentation. The same findings were observed with the recently described Fuster-BEWAT score (20) (**Table 2**). No differences were found regarding psychosocial characteristics according to sleep duration and fragmentation (**Online Table 2**).

Association of sleep duration and quality with subclinical atherosclerosis

3DVUS in carotid and femoral territories was available for analysis in 3804 participants (**Online Table 3**). When adjusted for age, gender, moderate to vigorous physical activity (MVPA), BMI, smoking status, alcohol consumption, systolic blood pressure, education level, fasting glucose, total cholesterol, total kcal per day, marital status, CES-D, PSS and mSTOP-BANG questionnaire scores, VSSD was independently associated with an increased plaque burden compared to the reference group (sleep duration 7-8h) (OR of being in the highest tertile of plaque burden: 1.27; 95%CI, 1.06-1.52; p=0.008, **Table 3, Central Illustration**). The ordinal regression analysis considering tertiles for 3D plaque burden and number of diseased territories also showed that participants who slept <6 hours presented a trend towards a more extensive atherosclerosis with a higher number of affected vascular territories, but the differences were not significant (OR of presenting more affected territories 1.21; 95% CI, 1.02-1.45; p=0.03, but overall test p value = 0.18). The association between subclinical atherosclerosis and sleep duration was also investigated using the Sleep Habits Questionnaire. In this case, no statistically significant differences in plaque burden or CAC score were observed between the different sleep groups in the overall association tests (**Table 3**), further highlighting the importance of objective sleep data for these kinds of studies. The same analysis was performed to evaluate the impact of sleep fragmentation on plaque burden. We found that the whole cohort and more specifically male participants with a more fragmented sleep (5th quintile) presented a higher number of affected territories compared to the reference group (1st quintile) (**Central Illustration**). A sub-analysis excluding subjects with a mSTOP-BANG score ≥3 (n = 450, 11.8% of study participants with available 3DVUS) was conducted and showed similar results regarding VSSD and non-coronary atherosclerosis (**Online Table 4**). Moreover, in this case participants with LSD showed a higher non-coronary plaque burden, although this difference was specifically observed in women (OR: 1.95, CI 95%: 1.20-3.19, p:0.007 vs. OR: 1.07, CI 95%: 0.64-1.80, p: 0.80 in men).

Next, we investigated the relationship between sleep patterns and CACS. Coronary CT was available for analysis in 3899 participants. Patients in the VSSD and SSD groups, as well as those with more disrupted sleep (5^{th} quintile) were associated with a higher CACS (**Online Table 5**). Adjusting for the aforementioned confounding factors, CACS was not significantly higher in SSD, VSSD or LSD participants regardless of the method used for the evaluation of sleep habits (Online Table 4). Similarly, no significant association was observed between sleep quality and CACS in the variable-adjusted analysis or after excluding subjects with a mSTOP-BANG score ≥3 (**Figure 4; Online Table 6**).

Association of dietary intake and inflammation with sleep parameters

No differences were observed between the different sleep duration groups when the quantity of nutrients was adjusted to grams in 2000 kcal per day (Online Table 7). However, participants in the VSSD group presented a higher daily intake of alcohol and those included in the VSSD and SSD groups presented a higher intake of caffeine compared to participants in the RSD group (Online Table 7). Similarly, participants in the higher quintile of SFI presented a higher intake of alcohol and caffeine compared to the reference group, and also an increased total energy intake (Online Table 8).

We next investigated the association between inflammation markers and sleep patterns. P-selectin and hs-CRP were significantly higher in VSSD participants (**Table 1**) and hs-CRP values were significantly higher in the higher quintile of FI as compared with the lower quintile (Online Table 1). However, neither VSSD nor SSD were associated with a higher level of inflammatory markers in the adjusted model (Online Table 9).

Discussion

Our study shows that objectively assessed sleep duration and sleep fragmentation are independently associated with subclinical atherosclerosis after adjusting for cardiovascular risk factors and OSA risk. Unlike previous studies, the atherosclerotic plaque burden was accurately assessed by two imaging techniques including 3DVUS and coronary CT. Since various vascular territories were evaluated, this is the first study to report the impact of actigraphic sleep parameters on multi-territorial atherosclerosis and counts with the biggest cohort with objectively measured sleep in this regard published to date. Furthermore, subjects diagnosed with obstructive sleep OSA were excluded and the results were adjusted for the potential presence of OSA based on a mSTOP-BANG questionnaire score, thereby avoiding the confounding effect of this sleep disorder on our analysis.

We observed that participants sleeping <6 hours/night (VSSD group) present a higher burden of noncoronary atherosclerosis and those with most fragmented sleep (5^{th} FI quintile) show in addition a higher number of affected territories measured by 3DVUS, independently of the presence of conventional CVD risk factors. Furthermore, LSD was related to a higher atherosclerotic burden specifically in women. However, we did not find that sleep duration and quality had an impact on CACS or inflammation biomarkers in our study population.

In our study, participants with SSD or VSSD presented a higher prevalence of classical cardiovascular risk factors, which is consistent with previous findings reported in the literature (3,36–39). Consequently, individuals with poorer sleep presented higher scores in various cardiovascular risk scales (**Table 2**). Moreover, participants with VSSD, SSD and a more fragmented sleep (4^{th} and 5^{th} quintiles) were more overweight (>26 kg/m^2) and those with LSD and non-fragmented sleep presented the lower BMI values (**Table 1, Online Table 1**).

The link between short sleep duration and higher energy intake has already been suggested (40), but the most extensive related study published to date relied on self-reported sleep measurements (41) and the only available actigraphy-based study was restricted to women (42). In our study, only subjects with the most disrupted sleep (quintile 5) presented a higher energy intake, but the fats, carbohydrates or proteins did not differ significantly between sleep groups. Only alcohol and caffeine consumption were higher in participants with short and disrupted sleep (Tables 5S and 6S). Lately, coffee intake has been associated with better cardiovascular outcomes and lower mortality (43), but in our study a higher caffeine intake seems to be related to unhealthier sleep patterns in groups with more cardiovascular risk factors.

These results support that subjects with poor sleep hygiene are clustered into groups apparently less engaged in healthy cardiovascular behaviors. However, apart from confirming the direct association of sleep duration and quality with cardiovascular risk factors and dietary habits, the current study is the first to show that objectively measured sleep is independently associated with subclinical multi-territory atherosclerosis. The fact that sleep duration and quality was objectively measured is relevant, as we have observed that self-reported sleep duration may not be reliable. In our study, while 27.1% of participants slept less than 6 hours per day according to the accelerometer, only 10.7% reported less than 6 hours sleep in the Sleep Habits Questionnaire. Consequently, the association of self-reported sleep with noncoronary atherosclerosis in our study is different to that seen with actigraphy, and only the subgroup of men who slept 6-7 hours showed a trend towards a higher risk of noncoronary atherosclerosis (**Table 3**). This could be explained by the fact that these participants actually present VSSD rather than SSD.

The larger study to date that analyzed the impact of sleep on atherosclerosis with objectively measured sleep parameters (n = 1,844) included subjects with obstructive sleep apnea and the clinical endpoint was peripheral artery disease measured with ankle-brachial index(14). Another study with 1,465 participants showed that poorer sleep was associated with greater coronary artery calcification (13). However, the impact of sleep patterns on subclinical atherosclerosis in a population without sleep-related disorders has not been adequately studied until now. In the present study, roughly 3,800 participants underwent coronary CT and 3D VUS in different territories. An association with CACS was discarded after adjusting for confounders, but the association with higher noncoronary atherosclerotic burden was significant. This may be explained by the fact that 3D VUS is a more sensitive technique than CACS to measure early atherosclerosis (32).

Regarding LSD, despite the result of a previous meta-analysis that found a correlation with cardiovascular outcomes (1), its impact on subclinical atherosclerosis remains unresolved due to variable results in previous studies (8). We have observed that LSD is not associated with plaque burden or CACs in the general cohort. However, the subgroup of women who slept >8h presented a higher burden of subclinical atherosclerosis, and similar results were observed in the general cohort after excluding participants with a mSTOP-BANG score of ≥3 (Online Table 4). As the number of patients with LSD was limited, these results need validation in a bigger cohort. In any case, these findings suggest that too long sleep duration may not be healthy either and recommendations should be restricted to 7-8 hours of sleep.

Overall, our findings support the potential role of healthy sleeping in protecting against atherosclerosis. Thus, recommending a good sleep hygiene should be part of the lifestyle modifications provided in our daily clinical practice.

Limitations

The PESA study cohort is relatively homogeneous and may not be representative of the general population, as it includes only middle-aged subjects with generally low cardiovascular risk and a characteristic occupation and lifestyle. Although subjects with an established OSA diagnosis were excluded, this condition is usually underdiagnosed in the general population. Thus, the results were adjusted for a mSTOP-BANG questionnaire score with the available clinical data in the PESA study. The original STOP-BANG questionnaire could not be used due to the lack of neck circumference data. A definite OSA evaluation would need a polysomnography study in all study participants, which was not available. In addition, whereas wrist actigraphy might be the preferred method for sleep evaluation in this study we used waist actigraphy. However, previous studies have shown a good correlation between polysomnography and waist actigraphy data (44).

Furthermore, the LSD group represents only 4% of the total cohort and results are not as generalizable as in the other groups. Finally, as follow-up data is not included in the study, the effects of sleep patterns on subclinical atherosclerosis over time are not addressed.

On the other hand, the proportion of women participants is higher than in other studies and sleep and clinical data are well characterized in this cohort, which are both strengths of this study.

Conclusions

Sleep is an important factor influencing cardiovascular health and could have a role as a marker of subclinical atherosclerosis. Objectively measured short sleeping times and fragmented sleep are associated with an increased risk of subclinical atherosclerosis. These results highlight the importance of healthy sleep habits for the prevention of CVD.

CLINICAL PERSPECTIVES

Competency in Medical Knowledge: Metabolic syndrome is associated with abnormal sleep patterns. Short and fragmented sleep patterns are independently associated with increased atherosclerotic plaque burden in middle-aged individuals.

Translational Outlook: Prospective studies are needed to assess the impact of specific interventions that improve sleep hygiene on clinical ischemic events.

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FIGURE LEGENDS

Sleep duration and quality vs. Subclinical atherosclerotic burden. Forest plots showing the odds ratios and confidence intervals of total plaque burden measured by 3D echo (carotid and femoral territories) in the different groups according to actigraphic sleep duration and fragmentation compared to the reference group (7-8 hours of sleep, quintile 1). Ordinal regression model adjusted for age, gender, MVPA, BMI, smoking status, alcohol consumption, systolic blood pressure, education level, fasting glucose, total cholesterol, total kcal per day marital status, CES-D, PSS and mSTOP-BANG questionnaire scores. The p values for the overall adjusted association between sleep parameters and atherosclerosis variables correspond to: Sleep duration vs. non-coronary plaque burden: **p=0.045** (men: p=0.32, women: **p=0.02**), sleep fragmentation vs. non-coronary plaque burden: **p=0.004** (men: **p=0.02**, women: p=0.09). Total plaque burden divided in no plaque and tertiles (Men: 0, 1.09-31.98 mm^3, 31.99-105.62 mm^3, 106.53-1241.98 mm^3; Women: 0 mm^3, 1.19-14.83 mm^3, 15.02-38.89 mm^3, 40.69- 536.34 mm^3). Sleep fragmentation index values 5th quintile: 7.39-43.43, 4th quintile: 5.29-7.38, 3rd quintile: 4.04-5.29, 2nd quintile: 2.88-4.04, 1st quintile (reference group): 0.23-2.88.

Figure 1: **Coronary artery calcium score vs. actigraphic sleep duration and fragmentation index.** Forest plot showing the odds ratios and confidence intervals of CAC scores in the different groups according to sleep duration fragmentation compared to the reference groups (7-8 hours and 1st quintile, respectively). CAC score was divided into the following groups according to Agatston score: <1, 1-100, 100-400, >400. Ordinal regression model adjusted for age, gender, MVPA, BMI, smoking status, alcohol consumption, systolic blood pressure, education level, fasting glucose, total cholesterol, total kcal per day marital status, CES-D, PSS and mSTOP-BANG questionnaire scores. The p values for the overall adjusted association between sleep

parameters and CAC score correspond to: Sleep duration vs. CAC score ≥ 1: p=0.44 (men: p=0.47, women: p=0.70), sleep fragmentation vs. CAC score ≥ : **p=0.02** men: p=0.12, women: p=0.08). CAC: Coronary artery calcium, CI: Confidence Interval, OR: Odds ratio. Total plaque burden divided in no plaque and tertiles (Men: 0, 1.09-33.70 mm^3, 33.71-107.69 mm^3, 108.03-1241.98 mm^3, > 1241.98 mm^3; Women: 0 mm^3, 1.19-16.32 mm^3, 16.56-45,24 mm^3, 45.49-536.34 mm^3, >536.34 mm^3). Sleep fragmentation index values 5th quintile: 7.39-43.43, 4th quintile: 5.29-7.38, 3rd quintile: 4.04-5.29, 2nd quintile: 2.88-4.04, 1st quintile (reference group): 0.23-2.88.

Table 1: Baseline characteristics and sleep duration measured by Actigraph

TOTAL SLEEP TIME (Actigraph)	Total	VSSD <6h	SLD 6 to <7h	RSD 7 to <8h (ref)	LSD ≥8h	p value[1]
Number of participants (%)	3974	1071 (27.0)	1521 (38.3)	1222 (30.7)	160 (4.0)	
Age, y (mean ± SD)	45.8 ± 4.3	46.6 ±4.3 *	45.8 ± 4.4 *	45.1 ± 4.0	44.5 ± 4.0	**<0.001**
Men, %	62.6	74	65.2	52	43.1	**<0.001**
Smoking status, %						**0.02**
Never	39.4	33.9	41.2	41.7	41.5	
Former	32.4	35.1	30.8	32.9	26.4	
Social	7.5	7.3	7.4	7.4	10.1	
Current	20.7	23.7	20.6	18	22	
Alcohol intake, g/day (median (Q1,Q3))	5.8 (1.5-12.6)	7.2 (1.7-14.1)	6.3 (1.8-13.2)	4.7 (1.0-11.0)	4.1 (0.9-10.4)	**<0.001**
MVPA, min/day (mean ± SD)	46.8 ± 20.7	47.9 ±22.0	47.2 ± 20.8	46.3 ± 19.6	40.6 ±17.0*	0.13
Married, %	75.9	74.6	76.4	77	71.1	0.06
BMI, kg/m2 (mean ± SD)	26.1 ± 3.79	26.9 ± 3.9*	26.2 ± 3.9*	25.5 ± 3.8	24.5 ± 3.6*	**<0.001**
Systolic BP, mm Hg (mean ± SD)	116.2 ± 12.5	117.9 ± 12.3 *	116.6 ± 12.2 *	114.3 ± 12.6	114.0 ± 13.7	**<0.001**
Diastolic BP, mm Hg (mean ± SD)	72.4 ± 9.4	73.6 ± 9.5 *	72.5 ± 9.4 *	71.4 ± 9.1	71.7 ± 9.6	**<0.001**
Hypertension, %	12.1	13.9	12.5	10	10	**0.03**
Antihypertensive drugs, %	7.3	9.2	7.2	6	5	**0.02**
Fasting glucose, mg/dL (mean ± SD)	90.4 ± 13.7	92.1 ± 13.1*	90.6 ± 15.7*	88.9 ± 10.2	89.3 ± 17.3	**<0.001**
Diabetes mellitus, %	1.7	2.5	1.5	1.2	1.3	0.1
Anti-diabetic drugs, %	1.5	2.1	1.2	1.2	1.2	0.31

Total cholesterol, mg/dL (mean ± SD)	200 ± 34.3	202± 34.3	200 ± 32.6	200 ± 33.3	203± 33.0	0.443
HDL cholesterol, mg/dL (mean ± SD)	49.2 ± 12.2	47.2 ± 11.8*	49.0 ± 11.7*	50.9 ± 12.8	50.8 ± 13.4	**<0.001**
LDL cholesterol, mg/dL (mean ± SD)	132± 29.8	134 ± 30.6	132± 28.8	131 ± 29.9	133 ± 31.9	0.135
Triglycerides, mg/dL (mean ± SD)	94.5 ± 57.3	100.7 ± 61.1*	94.4 ± 55.0	89.1 ± 50.7	95.5 ± 88.3	**<0.001**
Lipid-lowering drugs, %	6.9	8.3	8.0	4.7	4.4	**0.001**
Metabolic syndrome[2], %	9.5	12.6	8.8	8.0	6.9	**<0.001**
STOP-BANG score[3] (mean ± SD)	1.3 ± 1.1	1.6 ± 1.1*	1.3 ± 1.02*	1.1 ± 0.9	1.0 ± 1.0	**<0.001**
STOP-BANG score[3] ≥ 3, %	13.2	18.9	13.2	9.0	6.2	<0.001
CRP, mg/dL (median (Q1,Q3))	0.10 (0.05-0.19)	0.11 (0.05-0.20)	0.09 (0.05-0.18)	0.09 (0.05-0.19)	0.09 (0.05-0.17)	<0.07
VCAM, ng/ml (median (Q1,Q3))	617 (490-765)	593 (476-747)	621 (494-769)	627 (498-772)	630 (488-774)	**0.006**
P-selectin, ng/ml (median (Q1,Q3))	129 (104-154)	133 (108-157)	129 (104-154)	127 (102-151)	122 (96-140)	**0.002**
Neutrophil % (median (Q1,Q3))	57.6 (52.5-62.8)	57.8 (52.8-63.4)	57.3 (52.4-62.2)	57.6 (52.3-62.8)	58.3 (52.2-64.1)	0.23
Use of benzodiazepines and its derivatives, %	6.8	6.2	6.8	**6.5**	13.8	**0.012**

BMI: Body mass index, **CRP:** C-reactive protein, **LSD**: Long sleep duration,, **MVPA:** Moderate to vigorous physical activity, **RSD**: reference sleep duration**, SSD:** Short sleep duration, **VCAM:** Vascular cell adhesion molecule, VSSD: Very short sleep duration.
*: p<0.05 vs. RSD (reference group)
[1]: p values for continuous variables in this column reflect p for trend.
[2] : National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria
[3]: Modified score. Neck circumference not available.

Table 2: Cardiovascular risk scales according to sleep duration and fragmentation.

Total sleep time (Actigraph)	Total	VSSD <6h	SSD 6 to <7h	RSD 7 to <8h (ref)	LSD ≥8h	p value[1]
CVD risk scales						
FRS-10 % (SD)	5.9 (4.4)	6.9 (4.8) *	5.9 (4.3) *	5.0 (3.8)	5.1 (4.6)	**<0.001**
FRS-30 %, (SD)	17.7 (11.8)	20.9 (12.7) *	17.7 (11.4) *	15.2 (10.6)	15.6 (12.3)	**<0.001**
Fuster-BEWAT score						
Poor (%)	6.5	9.3	5.6	5.5	4.4	**<0.001**
Intermediate (%)	60.1	63.4	62.2	55.6	51.6	
Ideal (%)	33.4	27.3	32.2	38.9	44	
Sleep fragmentation Index (Actigraph)	**Quintile 1 (ref)** 0.23 - 2.88	**Quintile 2** 2.88 - 4.04	**Quintile 3** 4.04 - 5.29	**Quintile 4** 5.29 – 7.38	**Quintile 5** 7.39 – 43.43	
CVD risk scales						
FRS-10 %, (SD)	5.0 (3.8)	5.4 (3.9)	5.9 (4.4) *	6.3 (4.9) *	6.8 (4.7) *	**<0.001**
FRS-30 %, (SD)	15.2 (10.3)	16.6 (10.9)	17.5 (11.4) *	18.8 (12.6) *	20.4 (12.8) *	**<0.001**
Fuster-BEWAT score						
Poor (%)	4.5	5.8	6.2	7.2	9	**<0.001**
Intermediate (%)	57.8	59.8	59.7	58.3	64.8	
Ideal (%)	37.7	34.4	34.1	34.5	26.2	

FRS: Framingham risk score, RSD: Reference sleep duration. Post-Hoc Bonferroni analysis
[1]: p values for continuous variables in this column reflect p for trend. * $p<0.05$ compared to reference group

Table 3. Atherosclerotic plaque burden and number of affected territories measured by 3D-echocardiography and sleep duration: Comparison between self-reported sleep and actigraphy.

	ACTIGRAPH							SLEEP HABITS QUESTIONNAIRE					
	NONCORONARY PLAQUE BURDEN			NUMBER OF TERRITORIES AFFECTED (1-4)				NONCORONARY PLAQUE BURDEN (mm3)			NUMBER TERRITORIES AFFECTED (1-4)		
	OR	95% CI	P-value	OR	95% CI	P-value		OR	95% CI	P-value	OR	95% CI	P-value
TOTAL							**TOTAL**						
Sleep duration			**0.045***			0.18*	Sleep duration			0.33*			0.20*
<6h	1.27	1.06-1.52	**0.008**	1.21	1.02-1-45	0.03	**<6h**	0.99	0.79-1.24	0.92	0.92	0.74-1.16	0.50
6-7h	1.10	0.94-1.30	0.25	1.07	0.90-1.26	0.40	**6-7h**	1.13	0.98-1.31	0.10	1.13	0.98-1.31	0.09
7-8h	1.00	(reference)		1.00	(reference)		**7-8h**	1.00	(reference)		1.00	(reference)	
>8h	1.31	0.92-1.85	0.13	1.13	0.79-1.13	0.50	**>8h**	0.91	0.78-1.34	0.50	1.03	0.78-1.34	0.86
MEN							**MEN**						
Sleep duration			0.32*			0.41*	Sleep duration			0.27*			0.22*
<6h	1.21	0.98-1.49	0.08	1.14	0.93-1.41	0.21	**<6h**	1.15	0.88-1.50	0.32	1.06	0.81-1.39	0.69
6-7h	1.13	0.93-1.38	0.23	1.04	0.85-1.27	0.71	**6-7h**	1.23	1.03-1.46	0.02	1.21	1.02-1.44	0.03
7-8h	1.00	(reference)		1.00	(reference)		**7-8h**	1.00	(reference)		1.00	(reference)	
>8h	0.96	0.59-1.56	0.87	1.13	0.79-1.13	0.44	**>8h**	0.99	0.69-1.43	0.96	1.08	0.75-1.55	0.68
WOMEN							**WOMEN**						
Sleep duration			**0.02***			0.11*	Sleep duration			0.37*			0.33*
<6h	1.48	1.06-2.07	**0.02**	1.38	1.00-1.93	0.053	**<6h**	0.69	0.44-1.07	0.10	0.68	0.44-1.05	0.08

6-7h	1.01	0.76-1.36	0.90	1.04	0.85-1.27	0.44	**6-7h**	0.94	0.72-1.23	0.66	0.97	0.74-1.26	0.81
7-8h	1.00	(reference)		1.00	(reference)		**7-8h**	1.00	(reference)		1.00	(reference)	
>8h	1.83	1.12-3.01	**0.02**	1.65	1.01-2.72	0.05	**>8h**	0.77	0.51-1.18	0.23	0.89	0.59-1.35	0.59

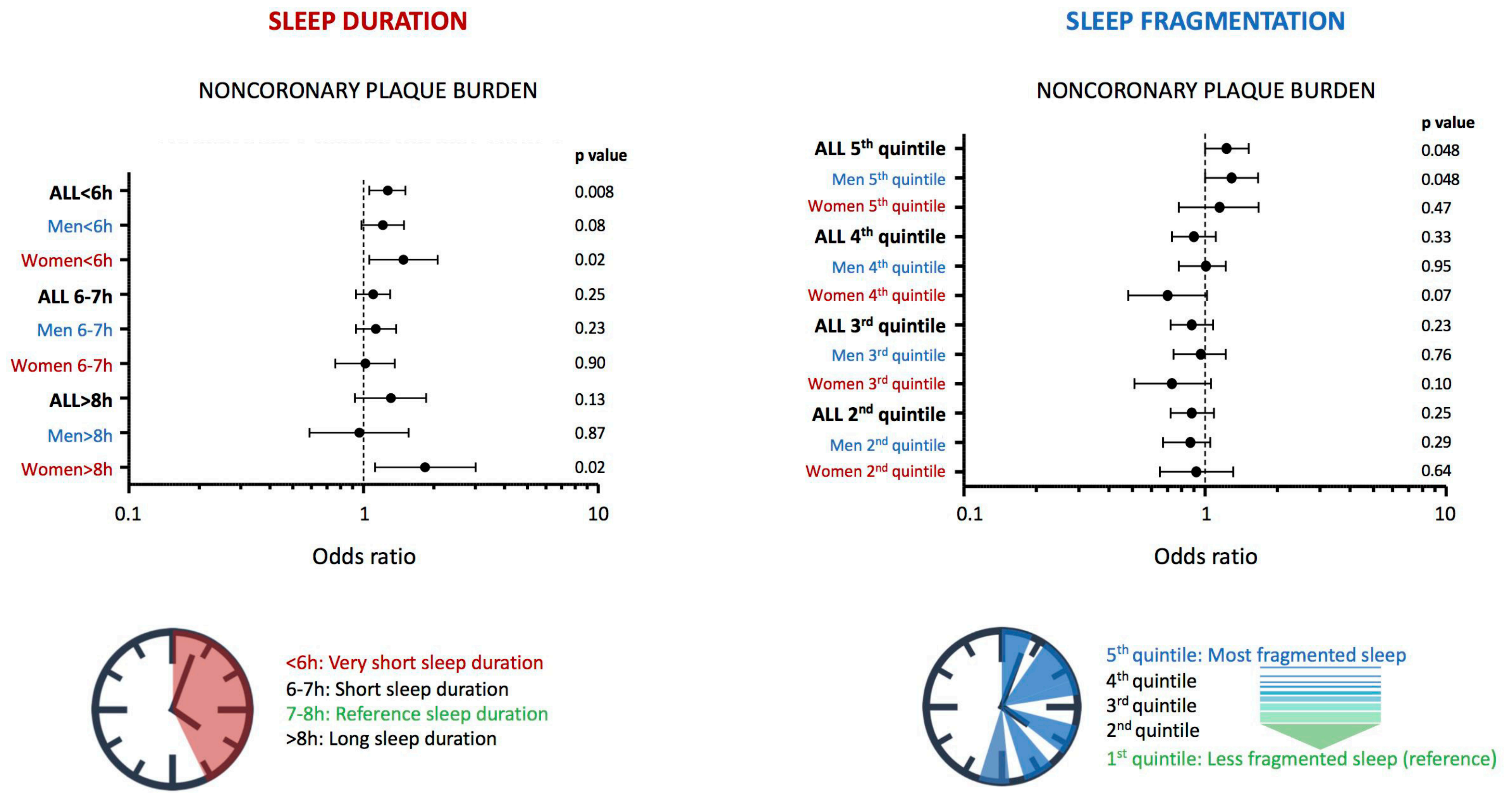
Odds ratios and confidence intervals of total plaque burden measured by 3D echo (carotid and femoral territories) and affected territories in the different groups according to sleep duration compared to the reference group (7-8 hours of sleep).

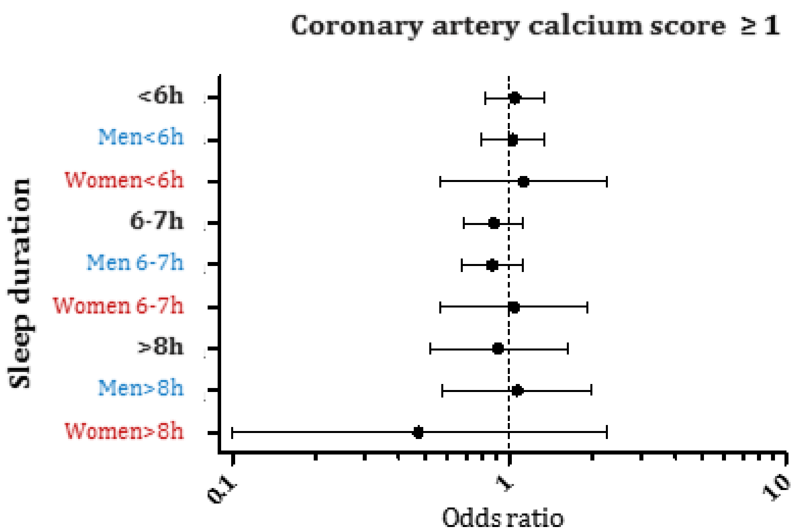
Ordinal regression model adjusted for age, gender, MVPA, BMI, smoking status, alcohol consumption, systolic blood pressure, education level, fasting glucose, total cholesterol, total kcal per day marital status, CES-D ,PSS and modified STOP-BANG questionnaire scores. CAC: Coronary artery calcium, CI: Confidence Interval, OR: Odds ratio.

Total plaque burden divided in no plaque and tertiles (Men: 0, 1.09-31.98 mm^3, 31.99-105.62 mm^3, 106.53-1241.98 mm^3; Women: 0 mm^3, 1.19-14.83 mm^3 , 15.02-38.89 mm^3, 40.69- 536.34 mm^3).

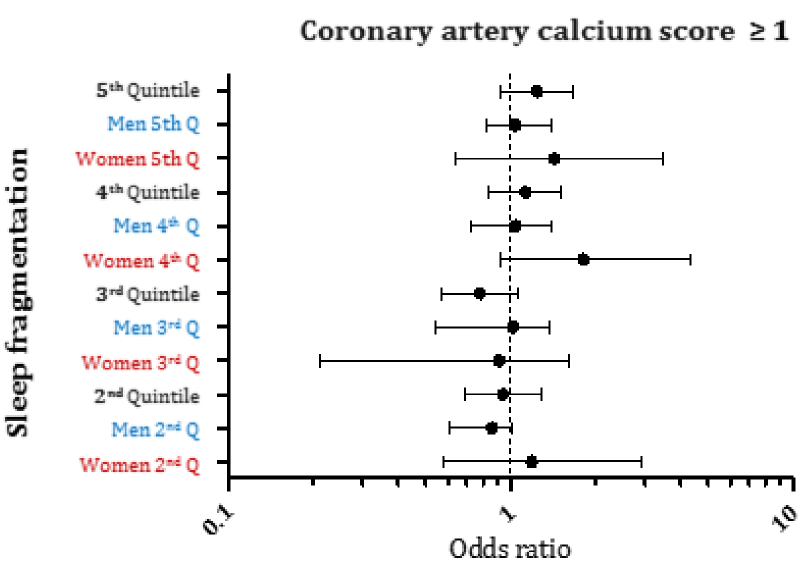
*: p values for the overall adjusted associations for each analysis (likelihood-ratio test). The other p values correspond to pairwise comparisons with the reference group.

SLEEP DURATION AND QUALITY AND SUBCLINICAL ATHEROSCLEROSIS





	OR (CI 95%)	p value
<6h	1.05 (0.82-1.35)	0.67
Men<6h	1.03 (0.79-1.34)	0.82
Women<6h	1.13 (0.56-2.27)	0.73
6-7h	0.88 (0.69-1.12)	0.30
Men 6-7h	0.87 (0.67-1.12)	0.28
Women 6-7h	1.04 (0.56-1.92)	0.91
>8h	0.92 (0.52-1.62)	0.77
Men>8h	1.07 (0.57-1.99)	0.83
Women>8h	0.47 (0.01-2.25)	0.35



	OR (CI 95%)	p value
5th Quintile	1.24 (0.92-1.66)	0.16
Men 5th Q	1.13 (0.82-1.55)	0.45
Women 5th Q	1.50 (065-3.50)	0.35
4th Quintile	1.13(0.84-1.53)	0.42
Men 4th Q	0.99 (0.72-1.38)	0.98
Women 4th Q	1.99 (0.92-4.31)	0.08
3rd Quintile	0.78 (0.57-1.07)	0.12
Men 3rd Q	0.76 (0.54-1.07)	0.12
Women 3rd Q	0.58 (0.21-1.60)	0.30
2nd Quintile	0.94 (0.69-1.29)	0.71
Men 2nd Q	0.86 (0.61-1.22)	0.40
Women 2nd Q	1.30 (0.58-2.90)	0.52

Supplemental material

Online Table 1: Baseline characteristics and sleep fragmentation measured by Actigraph

SLEEP FRAGMENTATION INDEX (Actigraph)	Quintile 1 (ref) FI: 0.23 - 2.88	Quintile 2 FI: 2.88- 4.04	Quintile 3 FI: 4.04 - 5.29	Quintile 4 FI: 5.79 – 5.29	Quintile 5 FI: 7.39 – 43.43	p values[1]
Number of participants (%)	786	774	787	774	778	
Age, y (mean ± SD)	46.5 ± 4.1	45.9 ± 4.3	46.3 ± 4.2	46.5 ± 4.3	46.6 ± 4.5	**0.014**
Men, %	53.1	58.8	63.9	64.7	72.5	**<0.001**
Smoking status, %						**0.04**
Never	40.6	40.2	40.7	40.3	35.2	
Former	33.5	31.9	31.8	31.4	33.5	
Social	6	8.3	9.5	6.6	7.1	
Current	20	19.6	18	21.7	24.2	
Alcohol intake, g/day (median (Q1,Q3))	4.9 (13-11.2)	5.6 (1.3-12.0)	6.5 (1.6-13.1)	5.6 (1.6-12.0)	7.2 (1.9-15.6)	**<0.001**
MVPA, min/day (mean ± SD)	45.7 ± 19.8	46.6 ± 20.3	46.2 ± 19.9	47.6 ± 21.9	48.0 ± 21.5	**0.15**
Married, %	77.4	74.7	78.3	74.7	74.4	0.08
BMI, kg/m2 (mean ± SD)	25.1 ± 3.4	25.6 ± 3.5	26.0 ± 3.6 *	26.5 ± 4.0 *	27.3 ± 4.0 *	**<0.001**
Systolic BP, mm Hg (mean ± SD)	114 ± 12.4	115 ± 12.6	116 ±12.1	117 ± 12.6*	118 ± 12.4*	**<0.001**
Diastolic BP, mm Hg (mean ± SD)	71.1 ± 9.3	71.6 ± 9.2	72.4 ± 9.0	73.2 ± 9.7*	73.8 ± 9.4*	**<0.001**
Hypertension, %	9.4	10.3	11.1	14.2	15.3	**0.01**
Antihypertensive drugs, %	5	6.2	5.7	9.8	9.7	**<0.001**
Fasting glucose, mg/dL (mean ± SD)	89.1 ± 11.0	89.6 ± 11.0	89.4 ± 9.4	91.1 ± 14.8*	92.9± 19.4*	**<0.001**
Diabetes mellitus, %	1.0	1.8	1.3	1.9	2.3	0.27
Anti-diabetic drugs, %	1.0	1.3	1.1	1.7	2.3	0.22
Total cholesterol, mg/dL (mean± SD)	198 ± 31.8	201 ± 32.9	201 ± 32.9	201 ± 33.8	202 ± 34.8	0.29
HDL cholesterol, mg/dL (mean± SD)	51.1 ± 12.0	49.9 ± 12.2	49.2 ± 12.3*	48.4 ± 12.4*	47.1 ± 11.6*	**<0.001**
LDL cholesterol, mg/dL (mean± SD)	130.2 ± 28.7	132.2 ± 28.9	132.5 ± 30.7*	133.0 ± 29.9*	133.5 ± 30.6*	0.24

Triglycerides, mg/dL (mean± SD)	84.0 ± 40.9	91.1 ± 49.7	94.8 ± 66.7*	97.5 ± 64.4*	105 ± 64.4*	<**0.001**
Lipid-lowering drugs, %	6.1	5.4	7.9	5.8	9.2	**0.001**
Metabolic syndrome2, %	6.0	8.0	11.5	14.7	9.5	<**0.001**
STOP-BANG score[3] (mean ± SD)	1.1 ± 1.0	1.2 ± 1.0	1.3 ± 1.0*	1.4 ± 1.0	1.6 ± 1.1*	<**0.001**
STOP-BANG score[3] ≥3, %	19.5	15.6	11.6	9.5	9.6	<**0.001**
CRP, mg/dL (median (Q1,Q3))	0.08 (0.04-0.16)	0.09 (0.05-0.18)	0.10 (0.05-0.19)	0.10 (0.05-0.20)	0.11 (0.06-0.22)	<**0.001**
VCAM, ng/ml (median (Q1,Q3))	620 (496-770)	607 (485-760)	620 (492-620)	619 (494-769)	615 (479-754)	0.59
P-selectin, ng/ml (median (Q1,Q3))	126 (103-126)	129 (104-153)	127 (103-153)	130 (107-156)	130 (105-156)	0.24
Neutrophil % (median (Q1,Q3))	57.9 (52.7-62.9)	57.8 (52.5-63.1)	57.4 (52.5-63.1)	57.0 (52.3-62.3)	57.5 (52.5-62.9)	0.22
Use of benzodiazepines and its derivatives, %	6.8	6.6	5.4	7.5	7.8	0.34

BMI: Body mass index, **CRP:** C-reactive protein**, FI:** Fragmentation index **MVPA:** Moderate to vigorous physical activity, **VCAM**: Vascular cell adhesion molecule

*: p<0.05 vs. Quintile 1 (reference group)

[1]: p values for continuous variables in this column reflect p for trend.

[2] : National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria

[3]: Modified score. Neck circumference not available.

Online Table 2: Psychosocial characteristics according to sleep duration and fragmentation.

Total sleep time (Actigraph)	**Total**	**VSSD <6h**	**SSD 6 to <7h**	**RSD 7 to <8h**	**LSD ≥8h**
Perceived stress questionnaire score (SD)	17.4 (6.1)	17.3 (6.9)	17.4 (7.0)	17.5 (7.3)	18.3 (7.7)
Depression questionnaire score (SD)	5.7 (5.8)	5.9 (5.9)	5.6 (5.8)	5.6 (5.6)	6.7 (7.3)
Sleep fragmentation Index (Actigraph)	**Quintile 1 (ref)** 0.23 - 2.88	**Quintile 2** 2.88 - 4.04	**Quintile 3** 4.04 - 5.29	**Quintile 4** 5.29 - 7.38	**Quintile 5** 7.39 - 43.43
Perceived stress questionnaire score (SD)	17.3 (7.1)	18.0 (7.1)	17.7 (7.1)	17.3 (7.3)	16.9 (6.8)
Depression questionnaire score (SD)	5.7 (5.7)	5.9 (6.3)	5.7 (5.5)	5.7 (5.9)	5.6 (5.6)

LSD: Long sleep duration, RSD: reference sleep duration, SSD: Short sleep duration, VSSD: Very short sleep duration.

Online Table 3: Atherosclerotic plaque burden measured by coronary artery calcium score and 3D vascular echography and vs. actigraphic sleep duration.

TOTAL SLEEP TIME	**Total**	**VSSD <6h**	**SSD 6 to <7h**	**RSD 7 to <8h (ref)**	**LSD ≥8h**	**p value**
Coronary calcium (Agatston score), n (%)						
CACs <1	3210 (82.3)	801 (75.9)	1242 (83.2)	1027 (86.1)	140 (88.1)	**<0.001**
CACs 1-99	555 (14.2)	197 (18.7)	205 (13.7)	136 (11.4)	17 (10.7)	
CACs 100-399	106 (2.7)	43 (4.1)	35 (2.3)	26 (2.2)	2 (1.3)	
CACs >400	28 (0.7)	14 (1.3)	10 (0.7)	4 (0.3)	0 (0)	
3D VUS: Territories affected, n (%)*						
0	2108 (55.4)	481 (47.5)	806 (55.2)	728 (62)	93 (59.2)	**<0.001**
1	845 (22.2)	239 (23.6)	334 (22.9)	235 (20.0)	37 (23.6)	
2	530 (13.9)	165 (16.3)	195 (13.3)	150 (12.8)	20 (12.7)	
3	192 (5.0)	68 (6.7)	84 (5.7)	34 (2.9)	6 (3.8)	
4	129 (3.4)	59 (5.8)	42 (2.9)	27 (2.3)	1 (0.6)	
SLEEP FRAGMENTATION INDEX	**Quintile 1 0.23 - 2.88** (ref)	**Quintile 2 2.88 - 4.04**	**Quintile 3 4.04 - 5.29**	**Quintile 4 5.29 - 7.38**	**Quintile 5 7.39 - 43.43**	**p value**
Coronary calcium (Agatston score), n (%)						
CACs <1 (n=3210)	675 (85.9)	655 (84.6)	669 (85)	620 (80.1)	591 (76)	**<0.001**
CACs 1-99 (n=555)	85 (10.8)	103 (13.3)	96 (12.2)	123 (15.9)	148 (19)	

CACs 100-399 (n=106)	21 (2.7)	16 (2.1)	18 (2.3)	21 (2.7)	30 (3.9)	
CACs >400 (n=28)	5 (0.6)	0 (0)	4 (0.5)	10 (1.3)	9 (1.2)	
3D VUS: Territories affected, n (%)*						
0	455 (59.1)	437 (58.5)	440 (57.4)	431 (57.0)	345 (45.2)	**<0.001**
1	175 (22.7)	165 (22.1)	165 (21.5)	146 (19.3)	194 (25.4)	
2	91 (11.8)	94 (12.6)	109 (14.2)	96 (12.7)	140 (18.3)	
3	27 (3.5)	32 (4.3)	37 (4.8)	50 (6.6)	46 (6.0)	
4	22 (2.9)	19 (2.5)	16 (2.1)	33 (4.4)	39 (5.1)	

This analysis was performed with Chi square test. Unadjusted results.

LSD: Long sleep duration, RSD: reference sleep duration, SSD: Short sleep duration, VSSD: Very short sleep duration.

*Carotid and femoral right and left territories.

Online Table 4. Atherosclerotic plaque burden measured by 3D-echocardiography vs. actigraphic sleep duration and fragmentation excluding subjects with modified STOP-BANG score of ≥ 3

	NONCORONARY PLAQUE BURDEN			NUMBER OF TERRITORIES AFFECTED (1-4)		
	OR	95% CI	P-value	OR	95% CI	P-value
Sleep duration			0.04*			0.11*
<6h	1.24	1.02-1.50	0.03	1.24	1.02-1-50	0.03
6-7h	1.12	0.94-1.33	0.21	1.07	0.90-1.27	0.43
7-8h	1.00	(reference)		1.00	(reference)	
>8h	1.47	1.03-2.11	0.03	1.28	0.89-1.84	0.18
Sleep fragmentation			0.01*			0.001*
5th quintile	1.20	0.98-1.49	0.11	1.35	1.08-1.68	0.01
4th quintile	0.86	0.69-1.07	0.18	0.90	0.72-1-13	0.38
3rd quintile	0.86	0.69-1.07	0.18	0.89	0.71-1.11	0.30
2nd quintile	0.86	0.69-1.06	0.17	0.94	0.75-1.17	0.58
1st quintile	1.00	(reference)		1.00	(reference)	

Odds ratios and confidence intervals of total plaque burden measured by 3D echo (carotid and femoral territories) and affected territories in the different groups according to sleep duration compared to the reference group (7-8 hours of sleep) excluding subjects with modified STOP-Bang score ≥ 3.
Ordinal regression model adjusted for age, gender, MVPA, BMI, smoking status, alcohol consumption, systolic blood pressure, education level, fasting glucose, total cholesterol, total kcal per day marital status, CES-D ,PSS and modified STOP-BANG questionnaire scores.
*: p values for the overall adjusted associations for each analysis (likelihood-ratio test). The other p values correspond to pairwise comparisons with the reference group.

CAC: Coronary artery calcium, CI: Confidence Interval, OR: Odds ratio.
Total plaque burden divided in no plaque and tertiles (Men: 0, 1.09-31.98 mm^3, 31.99-105.62 mm^3, 106.53-1241.98 mm^3; Women: 0 mm^3, 1.19-14.83 mm^3 , 15.02-38.89 mm^3, 40.69- 536.34 mm^3).

Online Table 5. Coronary artery calcium score and sleep duration: Comparison between self-reported sleep duration and actigraphy

	ACTIGRAPH				**SLEEP HABITS QUESTIONNAIRE**		
	CORONARY ARTERY CALCIUM SCORE ≥ 1				**CORONARY ARTERY CALCIUM SCORE ≥ 1**		
	OR	95% CI	P-value		OR	95% CI	P-value
TOTAL				**TOTAL**			
Sleep duration			0.02*	Sleep duration			0.27*
<6h	1.05	0.82-1.35	0.67	**<6h**	1.28	0.95-1.74	0.11
6-7h	0.88	0.69-1.12	0.30	**6-7h**	1.17	0.95-1.44	0.13
7-8h	1.00	(reference)		**7-8h**	1.00	(reference)	
>8h	0.92	0.52-1.62	0.77	**>8h**	0.90	0.58-1.41	0.66
MEN				**MEN**			
Sleep duration			0.12*	Sleep duration			0.48*
<6h	1.03	0.79-1.34	0.82	**<6h**	1.27	0.91-1.76	0.16
6-7h	0.87	0.67-1.12	0.28	**6-7h**	1.16	0.93-1.45	0.19
7-8h	1.00	(reference)		**7-8h**	1.00	(reference)	
>8h	1.07	0.57-1.99	0.83	**>8h**	0.98	0.60-1.59	0.92
WOMEN				**WOMEN**			
Sleep duration			0.08*	Sleep duration			0.41*
<6h	1.13	0.56-2-27	0.73	**<6h**	1.36	0.58-3.21	0.45
6-7h	1.04	0.56-1.92	0.91	**6-7h**	1.31	0.73-2.36	0.36
7-8h	1.00	(reference)		**7-8h**	1.00	(reference)	
>8h	0.47	0.01-2.25	0.35	**>8h**	0.56	0.16-1.92	0.36

Odds ratios and confidence intervals of CAC scores in the different groups according to sleep duration fragmentation compared to the reference groups (7-8 hours). CAC score was divided into the following groups according to Agatston score: <1, 1-100, 100-400, >400. Ordinal regression model adjusted for age, gender, MVPA, BMI, smoking status, alcohol consumption, systolic blood pressure, education level, fasting glucose, total cholesterol, total kcal per day marital status, CES-D, PSS and modified STOP-BANG questionnaire scores.
*: p values for the overall adjusted associations for each analysis (likelihood-ratio test). The other p values correspond to pairwise comparisons

with the reference group.

CAC: Coronary artery calcium, CI: Confidence Interval, OR: Odds ratio. Total plaque burden divided in no plaque and tertiles (Men: 0, 1.09-33.70 mm^3, 33.71-107.69 mm^3, 108.03-1241.98 mm^3, > 1241.98 mm^3; Women: 0 mm^3, 1.19-16.32 mm^3, 16.56-45,24 mm^3, 45.49- 536.34 mm^3 , >536.34 mm^3).

Online Table 6. Coronary artery calcium score vs. actigraphic sleep duration and fragmentation excluding subjects with modified STOP-BANG score of ≥ 3

	CORONARY ARTERY CALCIUM SCORE ≥ 1		
	OR	95% CI	P-value
Sleep duration			0.12*
<6h	1.08	0.82-1.42	0.61
6-7h	0.99	0.77-1.29	0.96
7-8h	1.00	(reference)	
>8h	1.06	0.58-1.91	0.85
Sleep fragmentation			0.22*
5th quintile	1.04	0.75-1.44	0.82
4th quintile	1.02	0.73-1.41	0.93
3rd quintile	0.78	0.56-1.00	0.16
2nd quintile	0.87	0.62-1.22	0.43
1st quintile	1.00	(reference)	

Odds ratios and confidence intervals of CAC scores in the different groups according to sleep duration fragmentation compared to the reference groups (7-8 hours) and excluding subjects with modified STOP-BANG score ≥ 3. CAC score was divided into the following groups according to Agatston score: <1, 1-100, 100-400, >400. Ordinal regression model adjusted for age, gender, MVPA, BMI, smoking status, alcohol consumption, systolic blood pressure, education level, fasting glucose, total cholesterol, total kcal per day marital status, CES-D, PSS and modified STOP-BANG questionnaire scores.
*: p values for the overall adjusted associations for each analysis (likelihood-ratio test). The other p values correspond to pairwise comparisons with the reference group.

CAC: Coronary artery calcium, CI: Confidence Interval, OR: Odds ratio. Total plaque burden divided in no plaque and tertiles (Men: 0, 1.09-33.70 mm^3, 33.71-107.69 mm^3, 108.03-1241.98 mm^3, > 1241.98 mm^3; Women: 0 mm^3, 1.19-16.32 mm^3, 16.56-45,24 mm^3, 45.49- 536.34 mm^3 , >536.34 mm^3).

Online Table 7. Dietary intake vs. actigraphic sleep duration

Sleep duration (Actigraph)	Total	VSSD <6h	SSD 6-7h	RSD 7-8h (ref)	LSD >8h	p value [1]
Number of participants	3863	1045	1478	1182	158	
Total energy, kcal /day ± SD	2309 ± 466	2380 ± 480	2326 ± 461	2243 ± 451	2148 ± 417	0.11
Proteins/2000 kcal, g/day ± SD	87.5 ± 11.8	87.6 ± 12.6	87.3 ± 11.5	87.6 ± 11.5	87.2 ± 11.5	0.31
Fat/2000 kcal, g/day ± SD	92.7 ± 10.5	91.9 ± 11.0	92.3 ± 10.3	93.6 ± 10.4	93.8 ± 10.1	0.21
MUFA/2000 kcal, g/day ± SD	42.5 ± 6.8	42.3 ± 6.8	42.3 ± 6.5	43.1 ± 7.1	42.9 ± 7.0	0.48
PUFA/2000 kcal, g/day ± SD	15.1 ± 3.5	15.0 ± 3.5	15.1 ± 3.5	15.2 ± 3.5	15.1 ± 3.5	0.63
SFA/ 2000 kcal, g/day ± SD	27.2 ± 5.0	26.8 ± 5.0	27.1 ± 5.0	27.4 ± 5.0	28.00 ± 4.6	0.14
CH/2000 kcal/, g/day ± SD	189 ± 30	188 ± 31	189 ± 30	188 ± 30	189 ± 29	0.88
Fibre/2000 kcal, g/day ± SD	18.2 ± 4.8	18.0 ± 4.7	18.2 ± 4.9	18.4 ± 4.8	18.4 ± 4.8	0.96
Alcohol/2000 kcal, g/day ± SD	8.5 ± 9.2	9.7 ±10.1 *	8.9 ±9.2	7.4 ± 8.3	6.7 ± 7.3	**0.03**
Caffeinated drinks (ml/day) ± SD	229 ± 211	240 ± 206 *	232 ± 222*	218 ± 206	217 ± 175	**<0.001**

CH: Carbohydrates, FI: Fragmentation index, LSD: Long sleep duration, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, RSD: reference sleep duration, SFA: Saturated fatty acids , SSD: Short sleep duration, VSSD: Very short sleep duration. Post Hoc Bonferroni analysis. Results adjusted for age, sex, marital status, educational level, moderate to vigorous physical activity (MVPA). [1]: p values for continuous variables in this column reflect p for trend. * p<0.05 compared to reference group

SLEEP FRAGMENTATION INDEX (Actigraph)	Quintile 1 (ref) FI: 0.23 - 2.88	Quintile 2 FI: 2.89 - 4.04	Quintile 3 FI: 4.05 - 5.29	Quintile 4 FI: 5.30 - 7.38	Quintile 5 FI: 7.39 - 43.43	p value [1]
Number of participants	779	770	777	766	771	
Total energy, kcal /day ± SD	2226 ± 437	2255 ± 435	2293 ± 454	2360 ± 469 *	2410 ± 508*	**<0.001**

Online Table 8. Dietary intake vs. actigraphic sleep fragmentation

Proteins/2000 kcal, g/day ± SD	87.5 ± 11.8	87.8 ± 11.9	87.5 ± 11.8	86.6 ± 11.4	87.8 ± 12.1	0.14
Fat/2000 kcal, g/day ± SD	92.9 ± 10.5	92.9 ± 10.7	93.1 ± 10.6	92.5 ± 10.5	92.1 ± 10.5	0.61
MUFA/2000 kcal, g/day ± SD	42.6 ± 6.8	42.6 ± 6.9	42.9 ± 7.0	42.3 ± 6.7	42.5 ± 6.5	0.36
PUFA/2000 kcal, g/day ± SD	15.0 ± 3.5	15.3 ± 3.7	15.1 ± 3.4	15.2 ± 3.6	14.9 ± 3.2	0.11
SFA/ 2000 kcal, g/day ± SD	27.4 ± 5.0	27.1 ± 5.1	27.4 ± 5.0	27.2 ± 4.8	26.9 ± 4.9	0.32
CH/2000 kcal/, g/day ± SD	190 ± 30	189 ± 30	187 ± 31	190 ± 30	187 ± 31	0.08
Fiber/2000 kcal, g/day ± SD	18.4 ± 4.8	18.3 ± 4.9	18.2 ± 4.6	18.3 ± 4.6	18.0 ± 5.0	0.98
Alcohol/2000 kcal, g/day ±SD	7.5 ± 8.3	8.1 ± 8.8	8.9 ± 9.5	8.3 ± 9.1	9.9 ± 10.0 *	**0.01**
Caffeinated drinks (ml/day)	220 ± 190	217 ± 200	225 ± 217	237 ± 225	249 ± 223*	**0.02**

CH: Carbohydrates, FI: Fragmentation index, LSD: Long sleep duration, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, RSD: reference sleep duration, SFA: Saturated fatty acids , SSD: Short sleep duration, VSSD: Very short sleep duration. Post Hoc Bonferroni analysis. Results adjusted for age, sex, marital status, educational level, moderate to vigorous physical activity (MVPA). 1: p values for continuous variables in this column reflect p for trend. * p<0.05 compared to reference group.

Online Table 9. Inflammatory markers and total sleep time measured by Actigraph

TOTAL SLEEP TIME (Actigraph)	VSSD <6h (n=1048) OR (95%CI)	p value	SSD 6 to <7h (n=1489) OR (95%CI)	p value	RSD 7 to <8h (n=1189) (ref)	LSD ≥8h (n=159) OR (95%CI)	p value
hs C-reactive protein (≥1 mg/dL)	1.19 (0.32-2.29)	0.60	0.92 (0.48-1.75)	0.79	-	1.19 (0.34-4.19)	0.784
VCAM (>2 SD)	0.86 (0.44-1.68)	0.65	0.43 (0.21-0.90)	**0.03**	-	0.74 (0.17-3.24)	0.69
P selectin (>2 SD)	0.97 (0.61-1.55)	0.91	1.02 (0.66-1.58)	0.92	-	0.38 (0.09-1.59)	0.19
Neutrophil count (≥7.5 x 10^3 /mm^3)	1.13 (0.55-1.60)	0.73	0.92 (0.45-1.86)	0.81	-	0.41 (0.05-3.16)	0.39

Ordinal regression model adjusted for age, gender, MVPA, BMI, smoking status, alcohol consumption, systolic blood pressure, education level, fasting glucose, total cholesterol, marital status, CES-D score and PSS. Reference group corresponds to 7-8 hours of sleep.
Mean and SD of inflammatory markers: hs C-reactive protein: 0.18 mg/dL ± 0.37, VCAM: 670.69 ± 490.96, P selectin: 131.21 ng/mL ± 39.83, neutrophil count: 5883.50/ mm^3 ± 15.
CI: Confidence Interval, hs: high sensitivity, LSD: Long sleep duration OR: Odds ratio, RSD: reference sleep duration, SD: Standard deviation, SSD: Short sleep duration, VCAM: Vascular cell adhesion molecule, VSSD: Very short sleep duration

Online Figure 1. **Comparison between self-reported sleep duration and actigraphy**