

# Accepted Manuscript

Sleep duration and subclinical atherosclerosis: The Aragon Workers' Health Study

Elena Blasco-Colmenares, Belén Moreno-Franco, Montserrat León Latre, Eusebio Mur-Vispe, Miguel Pocovi, Estíbaliz Jarauta, Fernando Civeira, Martín Laclaustra, José A. Casasnovas, Eliseo Guallar



PII: S0021-9150(18)30230-2

DOI: [10.1016/j.atherosclerosis.2018.05.003](https://doi.org/10.1016/j.atherosclerosis.2018.05.003)

Reference: ATH 15498

To appear in: *Atherosclerosis*

Received Date: 3 January 2018

Revised Date: 16 April 2018

Accepted Date: 1 May 2018

Please cite this article as: Blasco-Colmenares E, Moreno-Franco Belé, Latre MontserratLeó, Mur-Vispe E, Pocovi M, Jarauta Estí, Civeira F, Laclaustra Martí, Casasnovas JoséA, Guallar E, Sleep duration and subclinical atherosclerosis: The Aragon Workers' Health Study, *Atherosclerosis* (2018), doi: 10.1016/j.atherosclerosis.2018.05.003.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

**Sleep duration and subclinical atherosclerosis:****The Aragon Workers' Health Study**

Elena Blasco-Colmenares <sup>a</sup>, Belén Moreno-Franco <sup>b\*</sup>, Montserrat León Latre <sup>b,c</sup>,  
Eusebio Mur-Vispe <sup>d</sup>, Miguel Pocovi <sup>b,c</sup>, Estíbaliz Jarauta <sup>b</sup>, Fernando Civeira <sup>b,c</sup>, Martín  
Laclaustra <sup>b</sup>, José A. Casasnovas <sup>b,c</sup>, Eliseo Guallar <sup>a,e</sup>

<sup>a</sup> Division of Internal Medicine and Welch Center for Prevention, Epidemiology and Clinical Research, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

<sup>b</sup> Instituto de Investigación Sanitaria Aragón. Hospital Universitario Miguel Servet. CIBER Cardiovascular, Zaragoza, Spain.

<sup>c</sup> Universidad de Zaragoza, Zaragoza, Spain.

<sup>d</sup> Prevention Department, Opel Spain, Figueruelas, Spain.

<sup>e</sup> Department of Epidemiology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, USA.

\* **Corresponding author:** Belén Moreno-Franco. Unidad de Prevención Cardiovascular. Hospital Universitario Miguel Servet. Paseo Isabel La Católica 1-3, 50009, , Zaragoza, Spain.

Email: [mbmoreno@unizar.es](mailto:mbmoreno@unizar.es)

**Abstract**

*Background and aims:* Few studies have evaluated the association of sleep duration with subclinical atherosclerosis, and with heterogeneous findings. We evaluated the association of sleep duration with the presence of coronary, carotid, and femoral subclinical atherosclerosis in healthy middle-age men with low prevalence of clinical comorbidities.

*Methods:* We performed a cross-sectional analysis of 1,968 men, 40–60 years of age, participating in the Aragon Workers' Health Study (AWHS). Duration of sleep during a typical work week was assessed by questionnaire. Coronary artery calcium scores (CACs) was assessed by computed tomography and the presence of carotid plaque and femoral plaque by ultrasound.

*Results:* In fully adjusted models, the odds ratios (95% CI) for CACS >0 comparing sleep durations of ≤5, 6, and ≥8 hours with 7 hours were 1.34 (0.98-1.85), 1.35 (1.08–1.69) and 1.21 (0.90-1.62), respectively ( $p=0.04$ ). A similar U-shaped association was observed for CACS ≥100 and for CACS. The corresponding odds ratios for the presence of at least one carotid plaque were ≤5, 6, and ≥8 hours with 7 hours were 1.23 (0.88–1.72), 1.09 (0.86–1.38), and 0.86 (0.63-1.17), respectively ( $p=0.31$ ), and for the presence of at least one femoral plaque were 1.25 (0.87–1.80), 1.19 (0.93–1.51) and 1.17 (0.86–1.61), respectively ( $p=0.39$ ).

*Conclusions:* Middle-aged men reporting 7 hours of sleep duration had the lowest prevalence of subclinical coronary atherosclerosis as assessed by CAC. Our results

support that men with very short or very long sleep durations are at increased risk of atherosclerosis.

**Keywords:** sleep duration, self-reported sleep duration, subclinical atherosclerosis, coronary artery calcification, carotid artery disease, femoral artery disease.

ACCEPTED MANUSCRIPT

## Introduction

Adequate duration and quality of sleep play a key role in good health and quality of life [1]. For healthy individuals, appropriate sleep duration is usually considered between 7 to 8 hours [2], but there is substantial inter-individual variability. Work schedules, shift work, social commitments, and leisure time activities may result in a shorter sleep duration, a phenomenon that has been described as a global sleep crisis [3]. In the US, for instance, 31% of adults reported sleeping an average of less than 6 hours per night in 2010 [4]. On the other hand, long sleep duration, defined as sleep duration  $\geq 9$  hours, has decreased over time and was reported by only 8% of subjects in 2009 [5].

Compared to subjects with normal average sleep duration, those with chronic short or long sleep duration had an increased risk of clinical cardiovascular disease (CVD) outcomes [6]. A common limitation of these studies, however, is that extreme sleep duration may be the consequence of comorbidities, introducing reverse causation bias. It is thus important to assess the association between sleep duration and subclinical atherosclerosis in relatively young and apparently healthy populations, in which reverse causation is less likely to explain an association between sleep duration and subclinical findings.

Few studies have evaluated the association of sleep duration with coronary calcium with heterogeneous results [7-12]. Three cross-sectional studies did not find any association [8,9,12], while a small prospective study found that longer sleep duration was associated with lower incidence of calcification [10] and a large cross-sectional study found that extreme sleep duration and poor subjective sleep quality

were associated with increased prevalence of coronary artery calcium (CAC) with a U-shaped relationship [7]. To our knowledge, no study has evaluated the association of sleep duration with the presence of carotid or femoral plaque, although some studies have evaluated the association of sleep duration with carotid intima-media thickness (CIMT) [11]. Thus, we evaluated the association of sleep duration with the presence of coronary, carotid, and femoral atherosclerosis in the Aragon Workers' Health Study (AWHS), a study designed to identify risk factors for the development of atherosclerosis in an apparently healthy middle-age population with low prevalence of clinical comorbidities.

## **Patients and methods**

### Study design and population

AWHS is an ongoing prospective longitudinal cohort study designed to characterize the association of traditional and emergent CVD risk factors with the prevalence and progression of subclinical atherosclerosis in apparently healthy middle-aged workers in an automobile assembly plant in Spain [13,14]. AWHS included noninvasive imaging of subclinical atherosclerosis of study participants 40 to 60 years of age during 2011 to 2013. Due to the small number of women, the present report is restricted to men (N = 2,033). We excluded participants with missing data on sleeping time, CVD outcomes or relevant covariates (N = 65). The final sample was composed of 1,968 men. The study was approved by the Clinical Research Ethics Committee of Aragon (CEICA). All participants provided written informed consent.

### Data collection

Demographic information included age, sex, marital status (married, divorced/separated, single, widow, and other), education level (middle school, high school, professional training and college), type of job (manual vs office work), and work shift. Work shifts included two rotatory shifts (a morning-evening shift with morning sessions from 6 AM to 2 PM and evening sessions from 2 to 10 PM, and a morning-evening-night shift with morning sessions from 6 AM to 2 PM, evening sessions from 2 to 10 PM, and night sessions from 10 PM to 6 AM) and two fixed shifts (a central shift with work sessions from 8 AM to 4 PM, and a night shift with work sessions from 10 PM to 6 AM). Smoking habits were categorized as current smoking if the participant reported having smoked in the last year, former smoking if the participant had smoked at least 50 cigarettes in his lifetime, but not last year, and never smoking.

Information on diet and physical activity were obtained using standardized questionnaires administered by trained interviewers. Usual diet over the preceding year was assessed using a 136-item semi-quantitative food frequency questionnaire (FFQ) previously validated in Spain [15], considering seasonal variations and differences between weekday and weekend patterns. Duration of sleep was obtained from a question about the usual time dedicated each day to sleeping during a typical work week during the past year. The questionnaire included 12 possible response categories, from “never” to “9+ hours/day”.

Leisure time physical activity and time spent in sedentary activities was assessed using the Spanish validated version of the Nurses' Health Study [16] and Health Professionals' Follow-up physical activity questionnaires [17]. Participants were asked

about the time devoted to 17 different sports during the preceding year and we estimated overall metabolic equivalents of task (MET). Leisure-time energy expenditure in physical activity was expressed as METs-h/week. A detail description of clinical, biochemical and subclinical atherosclerosis measurements has been described previously [14,19] and are described in the Supplementary material.

### Statistical methods

The study outcomes were the presence of coronary calcium (CACS >0), the presence of at least one plaque in the carotid territory, and the presence of at least one plaque in the femoral territory. For CACS, we also categorized study participants into 3 groups (0, 1–99, and  $\geq 100$ ) and used  $\log(\text{CACS} + 1)$  as a continuous variable in secondary analysis. For the main analyses, we calculated odds ratios (OR) and 95% confidence intervals (CI) for the presence of coronary, carotid, or femoral atherosclerosis comparing each category of sleep duration to the reference category (7 hours). For secondary analyses, we used multinomial logistic regression to estimate the prevalence ratios (PR) with 95% CIs for CACS 1–99 and  $\geq 100$  using CACS = 0 as the base category comparing each category of sleep duration to the reference category. In addition, we used multiple linear regression to estimate the average ratio of CACS comparing each category of sleep duration to the reference category.

For all analyses, we used three models with progressive adjustment for covariates. Model 1 was adjusted for age (continuous), civil status (married, divorced/separated, single, widow, and other), education (middle school, high school, professional training and college), work shift (rotatory morning/evening, rotatory



morning/evening/night, central, and night), and work type (manual vs office work).

Model 2 further adjusted for lifestyle factors: smoking (never, former and current smoker), total energy intake, intake of saturated fat, *trans* fats, n-3 long chain polyunsaturated fatty acids (fish oils), cholesterol, and total fiber, alcohol use, and physical activity. Model 3 further adjusted for cardiometabolic intermediate variables: total cholesterol, HDL cholesterol, systolic blood pressure, BMI and diabetes. A  $p < 0.05$  was considered statistically significant. All analyses were performed with Stata software version 15.

## Results

The mean (SD) age of participants was 51.5 (3.7) years (**Table 1**). The majority of study participants worked in a rotatory morning/evening shift (63.2%) and had a manual type of work (88.9%). The proportion of participants reporting sleep durations of  $\leq 5$ , 6, 7 and  $\geq 8$  hours/day was 10.9, 30.4, 44.7 and 14.0%, respectively. Compared to participants with an average sleep duration of 7 h, those sleeping  $\leq 5$  hours/day were on average older, had a higher BMI, a higher prevalence of hypertension, and a higher average level of leisure time physical activity, while those sleeping  $\geq 8$  hours/day were on average older, had a higher BMI, a higher prevalence of hypertension and dyslipidemia, and a lower average level of leisure time physical activity.

The proportion of participants with CACS  $> 0$ , with at least one carotid plaque, and with at least one femoral plaque were 39.6, 38.3, and 57.8%, respectively.

Traditional CVD risk factors were generally associated with the presence of subclinical disease in each territory (**Supplemental Table 1**).

The proportion of participants with CACS >0 among those reporting sleep durations of  $\leq 5$ , 6, 7, and  $\geq 8$  hours were 45.8, 42.9, 35.0, and 42.4%, respectively ( $p=0.002$ ; **Table 2 and Figure 1**). In fully adjusted models, the OR (95% CI) for the presence of coronary calcium comparing sleep durations of  $\leq 5$ , 6, and  $\geq 8$  hours with 7 hours were 1.34 (0.98–1.85), 1.35 (1.08–1.69) and 1.21 (0.90–1.62), respectively ( $p=0.04$ ; **Table 2**). The association between short sleep duration and the prevalence of coronary atherosclerosis was stronger for participants with more advanced disease (CACS  $\geq 100$ ; **Table 3**). A similar U-shaped association was observed when  $\log(\text{CACS} + 1)$  was analyzed as a continuous outcome (**Table 4**).

The proportion of participants with at least one carotid plaque among those reporting sleep durations of  $\leq 5$ , 6, 7, and  $\geq 8$  hours was 44.9, 39.8, 36.3, and 36.1%, respectively ( $p=0.11$ ; **Table 2 and Fig. 1**). In fully adjusted models, the OR (95% CI) for the presence of at least one carotid plaque comparing sleep durations of  $\leq 5$ , 6, and  $\geq 8$  hours with 7 hours were 1.23 (0.88–1.72), 1.09 (0.86–1.38), and 0.86 (0.63–1.17), respectively ( $p=0.31$ ; **Table 2**).

The proportion of participants with at least one femoral plaque among those reporting sleep durations of  $\leq 5$ , 6, 7, and  $\geq 8$  hours were 64.6, 59.5, 54.0 and 61.3%, respectively ( $p=0.01$ ); **Table 2 and Fig. 1**) In fully adjusted models, the OR (95% CI) for the presence of at least one femoral plaque comparing sleep durations of  $\leq 5$ , 6, and  $\geq 8$  hours with 7 hours were 1.25 (0.87–1.80), 1.19 (0.93–1.51) and 1.17 (0.86–1.61), respectively ( $p=0.39$ ; **Table 2**).

## Discussion

In this study of young and middle-age asymptomatic workers, men reporting 7 hours of sleep duration had the lowest prevalence of subclinical atherosclerosis. Men with both shorter and longer sleep durations had increased prevalence of atherosclerotic disease, although the differences were statistically significant only for coronary atherosclerosis. These differences persisted after adjusting for several demographic, work-related, lifestyle and cardiometabolic factors that can potentially confound the association.

For coronary calcium, three cross-sectional studies (512, 224 and 1,093 participants, respectively) did not find any association [8,9,12]. A small prospective study (495 participants) found that longer sleep duration was associated with lower incidence of calcification [10]. However, the Kangbuk Samsung Health Study, a large cross-sectional study of 22,203 participants, with an average age of 40 years, found that extreme sleep duration and poor subjective sleep quality were associated with increased prevalence of CAC with a U-shaped relationship [7]. Our study corroborates these findings and adds to the evidence that subjects with extreme duration of sleep have a higher prevalence of coronary atherosclerosis. In the Kangbuk Samsung Health Study, poor sleep quality was associated with an increased prevalence of CAC in women. In a recent systematic review [11], the association between self-reported sleep quality and CAC showed mixed results. Unfortunately, we did not collect data on sleep quality, and further research is needed to establish the association between sleep quality and coronary atherosclerosis.

In a systematic review of eight studies of sleep duration and CIMT and a posterior cross-sectional study [11,20], short sleep duration was generally associated with increased CIMT compared with the reference category (6–8 hours/day), but the association with longer sleep duration was inconsistent. In our study, we used the presence of carotid plaque as outcome instead of CIMT, as carotid plaque may be a better predictor of CVD risk than intima-media thickness [21]. To our knowledge, no other studies have evaluated sleep duration with the presence of carotid plaque [11].

Consistent with CIMT studies, we found that men with short sleep duration had a higher prevalence of carotid plaque compared to men with sleep duration of 7 hours/day, although the differences were not statistically significant. In our study, however, men with  $\geq 8$  hours/day of sleep had virtually the same prevalence of carotid plaque compared to those with 7 hours of sleep. The lack of statistical significance in our analysis of carotid plaque compared to coronary calcium scores could be due to the smaller number of men with available carotid ultrasound data compared to coronary calcium score data, or to variability and measurement error for detecting atherosclerosis using ultrasound [22,23].

Two large cross-sectional studies that evaluated the association between sleep duration and peripheral artery disease measured by the ankle-brachial index (ABI) also found U-shaped associations, with the lowest prevalence in subjects with a duration of sleep of 7 hours/day [7,24]. Instead of ABI, we used the presence of femoral plaque assessed by ultrasound as a marker of atherosclerosis [14]. ABI may detect more advanced obstructive atherosclerosis, while femoral ultrasound may detect earlier stages of the disease. The association of sleep duration and femoral plaque in our study

was not statistically significant, although it showed a similar U-shaped as reported in prior studies using ABI.

Several mechanisms may underlie the U-shaped association between sleep and subclinical atherosclerosis. In several epidemiological studies and meta-analyses, short sleep duration was associated with obesity [25] and hypertension [26,27], and short [28,29] and long [28] sleep duration were associated with incident type 2 diabetes [28,29]. Indeed, sleep restriction may induce insulin resistance and cardiometabolic abnormalities [30]. Short sleep duration may also be associated with increased energy expenditure, increased food intake, increased consumption of high density foods and irregular eating habits [31]. In our analysis, sleep duration was associated with subclinical atherosclerosis even after adjusting for cardiometabolic risk factors and for macronutrient intake, suggesting that additional mechanisms may be implicated in this association.

Several limitations should be acknowledged in our study. First, with our cross-sectional design we cannot establish a firm causal relation between sleep duration and subclinical atherosclerosis. Second, sleep duration was assessed by self-report. Self-reported sleep duration is moderately associated with objective measures of sleep duration, but the associated measurement error in subjective sleep duration is likely to attenuate the observed associations with the prevalence of subclinical atherosclerosis [32]. Third, extreme sleep duration may be a marker of other comorbidities. Our study population, however, was relatively young and free of clinical CVD and other major diseases, and reverse causation may be less of a concern in this cohort compared to cohorts of older subjects. The major strengths of our study were the use of high quality

data collection methods to obtain information on subclinical atherosclerosis outcomes and potential confounders, and the use of a healthy population free of CVD.

In conclusion, we found that extreme sleep duration was associated with higher prevalence of coronary atherosclerosis. Men with short sleep duration also had a high prevalence of carotid and femoral plaque, and those with long sleep duration had a high prevalence of femoral plaque, although the associations between sleep duration and carotid or femoral plaque did not reach statistical significance. Our results support a growing body of data suggesting that men with very short or very long sleep durations are at increased risk of atherosclerosis and CVD.

## **Conflicts of interest**

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

## **Financial support**

This study was supported in part by grants from the Carlos III Research Institute: CIBER CV (co-supported by the European Regional Development Fund (ERDF) which is allocated by the European Union and IIS16/0114).

## **Author contributions**

Blasco-Colmenares: data analysis and interpretation, writing of manuscript, and critical revision of manuscript for important intellectual content.

Moreno-Franco: acquisition of data, data interpretation, and critical revision of manuscript for important intellectual content.

León Latre: study concept and design, acquisition of data, data analysis and interpretation, and critical revision of manuscript for important intellectual content.

Mur-Vispe: acquisition of data, data interpretation, and critical revision of manuscript for important intellectual content.

Pocovi: study concept and design, acquisition of data, data interpretation, and critical revision of manuscript for important intellectual content.

Estíbaliz Jarauta: acquisition of data, data interpretation, and critical revision of manuscript for important intellectual content.

Civeira: study concept and design, acquisition of data, data interpretation, and critical revision of manuscript for important intellectual content.

Laclaustra: data interpretation, and critical revision of manuscript for important intellectual content.

Casasnovas: study concept and design, acquisition of data, data interpretation, and critical revision of manuscript for important intellectual content.

Guallar: study concept and design, data analysis and interpretation, writing of manuscript, and critical revision of manuscript for important intellectual content.

### **Acknowledgements**

The authors thank the study participants, doctors, and health care professionals at the Opel factory. The authors also thank the AWHS technical staff: Angeles Bamala, Raquel Langarita, Marta Ledesma, Elisabeth Penón, Natalia Sánchez, Zoraida Soriano, and Rosa Villa; and Dr. Juan J. Soriano, who supervised CT studies.



## References

- [1] Luyster FS, Strollo PJ, Jr., Zee PC, Walsh JK, Boards of Directors of the American Academy of Sleep M, the Sleep Research S. Sleep: a health imperative. *Sleep* 35 (2012) 727-34.
- [2] Consensus Conference P, Watson NF, Badr MS, et al. Joint Consensus Statement of the American Academy of Sleep Medicine and Sleep Research Society on the Recommended Amount of Sleep for a Healthy Adult: Methodology and Discussion. *Sleep* 38 (2015) 1161-83.
- [3] Walch OJ, Cochran A, Forger DB. A global quantification of "normal" sleep schedules using smartphone data. *Sci. Adv.* 2 (2016) 1501705.
- [4] Liu Y, Wheaton AG, Chapman DP, Croft JB. Sleep duration and chronic diseases among U.S. adults age 45 years and older: evidence from the 2010 Behavioral Risk Factor Surveillance System. *Sleep* 36 (2013)1421-7.
- [5] Jean-Louis G, Williams NJ, Sarpong D, et al. Associations between inadequate sleep and obesity in the US adult population: analysis of the national health interview survey (1977-2009). *BMC Public Health* 14 (2014) 290.
- [6] Cappuccio FP, Cooper D, D'Elia L, Strazzullo P, Miller MA. Sleep duration predicts cardiovascular outcomes: a systematic review and meta-analysis of prospective studies. *Eur. Heart J.* 32 (2011) 1484-92.
- [7] Kim CW, Chang Y, Zhao D, et al. Sleep Duration, Sleep Quality, and Markers of Subclinical Arterial Disease in Healthy Men and Women. *Arterioscler. Thromb. Vasc. Biol.* 35 (2015) 2238-45.

- [8] Matthews KA, Everson-Rose SA, Kravitz HM, Lee L, Janssen I, Sutton-Tyrrell K. Do reports of sleep disturbance relate to coronary and aortic calcification in healthy middle-aged women?: Study of women's health across the nation. *Sleep Med.* 14 (2013) 282-7.
- [9] Matthews KA, Strollo PJ, Jr., Hall M, et al. Associations of Framingham risk score profile and coronary artery calcification with sleep characteristics in middle-aged men and women: Pittsburgh SleepSCORE study. *Sleep* 34 (2011) 711-6.
- [10] King CR, Knutson KL, Rathouz PJ, Sidney S, Liu K, Lauderdale DS. Short sleep duration and incident coronary artery calcification. *JAMA* 300 (2008) 2859-66.
- [11] Aziz M, Ali SS, Das S, et al. Association of Subjective and Objective Sleep Duration as well as Sleep Quality with Non-Invasive Markers of Sub-Clinical Cardiovascular Disease (CVD): A Systematic Review. *J. Atheroscler. Thromb.* 24 (2017) 208-26.
- [12] Suzuki S, Arima H, Miyazaki S, et al. Self-reported Sleep Duration and Subclinical Atherosclerosis in a General Population of Japanese Men. *J. Atheroscler. Thromb.* 2017. [Epub ahead of print]
- [13] Casasnovas JA, Alcaide V, Civeira F, et al. Aragon workers' health study--design and cohort description. *BMC Cardiovasc. Disord.* 12 (2012) 45.
- [14] Laclaustra M, Casasnovas JA, Fernandez-Ortiz A, et al. Femoral and Carotid Subclinical Atherosclerosis Association With Risk Factors and Coronary Calcium: The AWHS Study. *J. Am. Coll. Cardiol.* 67 (2016) 1263-74.
- [15] Martin-Moreno JM, Boyle P, Gorgojo L, et al. Development and validation of a food frequency questionnaire in Spain. *Int. J. Epidemiol.* 22 (1993) 512-9.
- [16] Martinez-Gonzalez MA, Lopez-Fontana C, Varo JJ, Sanchez-Villegas A, Martinez JA. Validation of the Spanish version of the physical activity questionnaire used in the

Nurses' Health Study and the Health Professionals' Follow-up Study. *Public Health Nutr.* 8 (2005) 920-7.

[17] Chasan-Taber S, Rimm EB, Stampfer MJ, et al. Reproducibility and validity of a self-administered physical activity questionnaire for male health professionals. *Epidemiology* 7 (1996) 81-6.

[18] Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Jr., Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J. Am. Coll. Cardiol.* 15 (1990) 827-32.

[19] Junyent M, Gilabert R, Zambon D, et al. Femoral atherosclerosis in heterozygous familial hypercholesterolemia: influence of the genetic defect. *Arterioscler. Thromb. Vasc. Biol.* 28 (2008) 580-6.

[20] Zonoozi S, Ramsay SE, Papacosta O, et al. Self-reported sleep duration and napping, cardiac risk factors and markers of subclinical vascular disease: cross-sectional study in older men. *BMJ Open* 7 (2017) e016396.

[21] Inaba Y, Chen JA, Bergmann SR. Carotid plaque, compared with carotid intima-media thickness, more accurately predicts coronary artery disease events: a meta-analysis. *Atherosclerosis* 220 (2012) 128-33.

[22] Polak JF, Funk LC, O'Leary DH. Inter-reader differences in common carotid artery intima-media thickness: implications for cardiovascular risk assessment and vascular age determination. *J. Ultrasound Med.* 30 (2011) 915-20.

[23] Saba L, Montisci R, Molinari F, et al. Comparison between manual and automated analysis for the quantification of carotid wall by using sonography. A validation study with CT. *Eur. J. Radiol.* 81 (2012) 911-8.

- [24] Nagayoshi M, Lutsey PL, Benkeser D, et al. Association of sleep apnea and sleep duration with peripheral artery disease: The Multi-Ethnic Study of Atherosclerosis (MESA). *Atherosclerosis* 251 (2016) 467-75.
- [25] Cappuccio FP, Taggart FM, Kandala NB, et al. Meta-analysis of short sleep duration and obesity in children and adults. *Sleep* 31 (2008) 619-26.
- [26] Meng L, Zheng Y, Hui R. The relationship of sleep duration and insomnia to risk of hypertension incidence: a meta-analysis of prospective cohort studies. *Hypertens. Res.* 36 (2013) 985-95.
- [27] Guo X, Zheng L, Wang J, et al. Epidemiological evidence for the link between sleep duration and high blood pressure: a systematic review and meta-analysis. *Sleep Med.* 14 (2013) 324-32.
- [28] Cappuccio FP, D'Elia L, Strazzullo P, Miller MA. Quantity and quality of sleep and incidence of type 2 diabetes: a systematic review and meta-analysis. *Diabetes Care* 33 (2010) 414-20.
- [29] Shan Z, Ma H, Xie M, et al. Sleep duration and risk of type 2 diabetes: a meta-analysis of prospective studies. *Diabetes Care* 38 (2015) 529-37.
- [30] Broussard JL, Ehrmann DA, Van Cauter E, Tasali E, Brady MJ. Impaired insulin signaling in human adipocytes after experimental sleep restriction: a randomized, crossover study. *Ann. Intern. Med.* 157 (2012) 549-57.
- [31] Dashti HS, Scheer FA, Jacques PF, Lamon-Fava S, Ordovas JM. Short sleep duration and dietary intake: epidemiologic evidence, mechanisms, and health implications. *Adv. Nutr.* 6 (2015) 648-59.

[32] Lauderdale DS, Knutson KL, Yan LL, Liu K, Rathouz PJ. Self-reported and measured sleep duration: how similar are they? *Epidemiology* 19 (2008) 838-45.

ACCEPTED MANUSCRIPT

## Captions of figures

**Figure 1.** Adjusted prevalences (95% CI) for the presence of coronary calcium, carotid plaque, and femoral plaque by sleep duration category.

Adjusted prevalences were calculated as marginally adjusted proportions in logistic regression models adjusted for age, civil status, education, work shift, work type, smoking, intakes of total calories, saturated fat, *trans* fat, fish oils, cholesterol, total fiber, and alcohol, and leisure time physical activity.

**Table 1.** Characteristics of study participants by sleep duration category (N = 1,968).

	Overall	Sleep duration category, h				p-value
		≤ 5	6	7	≥ 8	
<b>Number</b>	1,969	214 (10.9)	599 (30.4)	879 (44.7)	276 (14.0)	
<b>Age, years</b>	51.5 (3.7)	52.3 (3.3)	51.6 (3.8)	51.3 (3.8)	51.7 (3.6)	0.004
<b>Work shift</b>						0.18
Rotatory M-E	1245 (63.2)	129 (60.3)	384 (64.1)	552 (62.8)	180 (65.2)	
Rotatory M-E-N	382 (19.4)	51 (23.8)	121 (20.2)	165 (18.8)	45 (16.3)	
Central	141 (7.2)	11 (5.1)	31 (5.2)	74 (8.4)	25 (9.1)	
Night	200 (10.2)	23 (10.8)	63 (10.5)	88 (10.0)	26 (9.4)	
<b>Manual work</b>	1750 (88.9)	196 (91.6)	542 (90.5)	773 (87.9)	239 (86.6)	0.14
<b>Married</b>	1689 (85.8)	179 (83.6)	525 (87.7)	749 (85.1)	236 (85.5)	0.34
<b>Highest education degree</b>						0.15
Middle school	1,034 (52.5)	124 (57.9)	312 (52.1)	436 (49.6)	162 (58.7)	
High school	218 (11.1)	24 (11.2)	66 (11.0)	106 (12.1)	22 (8.0)	
Professional training	636 (32.3)	60 (28.0)	196 (32.7)	302 (34.4)	78 (28.3)	

College	80 (4.1)	6 (2.8)	25 (4.2)	35 (4.0)	14 (5.1)	
<b>Smoking status</b>						0.12
Never	450 (22.9)	41 (19.2)	133 (22.2)	223 (25.4)	53 (19.2)	
Former	706 (35.9)	85 (39.7)	204 (34.1)	306 (34.8)	111 (40.2)	
Current	812 (41.3)	88 (41.1)	262 (43.7)	350 (39.8)	112 (40.6)	
<b>BMI, kg/m<sup>2</sup></b>	27.8 (3.4)	28.2 (3.9)	28.0 (3.5)	27.6 (3.2)	27.9 (3.4)	0.04
<b>Hypertension</b>	421 (21.4)	60 (28.0)	118 (19.7)	179 (20.3)	64 (23.2)	0.05
<b>Diabetes</b>	74 (3.8)	10 (4.7)	21 (3.5)	32 (3.6)	11 (4.0)	0.88
<b>Dyslipidemia</b>	369 (18.8)	38 (17.8)	93 (15.5)	174 (19.8)	64 (23.2)	0.04
<b>Physical activity, MET-h/wk</b>	32.8 (22.6)	37.4 (23.5)	35.1 (23.5)	31.7 (22.1)	27.7 (20.1)	<0.001
<b>Energy intake, Kcal/day</b>	2935.7 (739.9)	2958.0 (773.9)	2916.9 (736.3)	2939.5 (736.8)	2947.1 (733.6)	0.88
<b>Saturated fat intake, %En</b>	10.0 (2.1)	10.1 (2.2)	9.9 (2.1)	10.0 (2.1)	10.0 (1.9)	0.47
<b>Trans fat intake, %En</b>	0.3 (0.1)	0.3 (0.1)	0.3 (0.1)	0.3 (0.1)	0.3 (0.1)	0.95
<b>Fish oil intake, %En</b>	0.2 (0.1)	0.2 (0.1)	0.2 (0.2)	0.2 (0.1)	0.2 (0.1)	0.19
<b>Cholesterol intake, mg/day</b>	470.8 (161.2)	466.2 (152.3)	465.3 (169.7)	474.4 (158.6)	474.5 (157.5)	0.69
<b>Total fiber intake, g/day</b>	25.6 (7.9)	25.6 (8.3)	25.5 (7.7)	25.6 (8.0)	25.6 (8.0)	0.99
<b>Alcohol, g/day</b>	21.9 (20.7)	23.2 (23.2)	22.9 (21.2)	21.5 (19.7)	20.3 (20.3)	0.26



<b>Total CAC score</b>	46.6 (178.1)	78.5 (255.4)	54.5 (224.9)	34.5 (120.2)	43.7 (137.0)	0.007
------------------------	--------------	--------------	--------------	--------------	--------------	-------

---

BMI: body mass index; CAC: coronary artery calcium; %En: percentage of energy intake.  
Values in the table are mean (SD) or number (%).

ACCEPTED MANUSCRIPT

**Table 2.** Odds ratios (95% CI) for the presence of coronary calcium (coronary calcium score > 0), femoral plaque, and carotid plaque by sleep duration category.

	Sleep duration, h				p-value
	≤ 5	6	7	≥ 8	
<b>CAC Score &gt;0</b>					
Number with CAC > 0 / Total	98 / 214	257 / 599	308 / 879	117 / 276	0.002
Model 1	1.38 (1.01 - 1.89)	1.37 (1.10 - 1.71)	1.00 Reference	1.29 (0.97 - 1.72)	0.02
Model 2	1.34 (0.98 - 1.84)	1.36 (1.09 - 1.70)	1.00 Reference	1.24 (0.94 - 1.68)	0.03
Model 3	1.34 (0.98 - 1.85)	1.35 (1.08 - 1.69)	1.00 Reference	1.21 (0.90 - 1.62)	0.04
<b>Carotid plaque</b>					
Number with carotid plaque / Total	88 / 196	221 / 556	292 / 804	93 / 258	0.11
Model 1	1.27 (0.92 - 1.76)	1.12 (0.89 - 1.41)	1.00 Reference	0.92 (0.68 - 1.24)	0.31
Model 2	1.23 (0.89 - 1.71)	1.10 (0.87 - 1.39)	1.00 Reference	0.89 (0.66 - 1.21)	0.36

Model 3	1.23 (0.88 – 1.72)	1.09 (0.86 – 1.38)	1.00 Reference	0.86 (0.63 – 1.17)	0.31
<b>Femoral plaque</b>					
Number with femoral plaque / Total	122 / 189	326 / 548	443 / 821	157 / 256	0.01
Model 1	1.39 (0.99 – 1.94)	1.20 (0.96 – 1.50)	1.00 Reference	1.30 (0.97 – 1.74)	0.11
Model 2	1.25 (0.88 – 1.78)	1.18 (0.93 – 1.50)	1.00 Reference	1.21 (0.89 – 1.66)	0.35
Model 3	1.25 (0.87 – 1.80)	1.19 (0.93 – 1.51)	1.00 Reference	1.17 (0.86 – 1.61)	0.39

Model 1: adjusted for age, civil status, education, work shift, and work type.

Model 2: further adjusted for lifestyle factors: smoking, intakes of total calories, saturated fat, *trans* fat, fish oils, cholesterol, total fiber, and alcohol, and leisure time physical activity.

Model 3: further adjusted for cardiometabolic intermediate variables: total cholesterol, HDL cholesterol, systolic blood pressure, body mass index and diabetes.

**Table 3.** Prevalence ratios (95% CI) for the presence of coronary calcium (CACS 1 – 99, and  $\geq 100$ ) by sleep duration category.

	Sleep duration, h				p-value
	$\leq 5$	6	7	$\geq 8$	
<b>CAC Score 1–99</b>					
Model 1	1.29 (0.91 – 1.82)	1.40 (1.10 – 1.78)	1.00 Reference	1.33 (0.98 – 1.81)	0.03
Model 2	1.26 (0.89 – 1.79)	1.40 (1.10 – 1.78)	1.00 Reference	1.29 (0.95 – 1.76)	0.04
Model 3	1.27 (0.90 – 1.81)	1.39 (1.09 – 1.77)	1.00 Reference	1.25 (0.92 – 1.71)	0.05
<b>CAC Score <math>\geq 100</math></b>					
Model 1	1.65 (1.02 – 2.67)	1.26 (0.86 – 1.83)	1.00 Reference	1.18 (0.73 – 1.91)	0.22
Model 2	1.57 (0.96 – 2.56)	1.22 (0.83 – 1.79)	1.00 Reference	1.14 (0.70 – 1.85)	0.33
Model 3	1.57 (0.96 – 2.58)	1.22 (0.82 – 1.79)	1.00 Reference	1.08 (0.66 – 1.78)	0.33

CAC = 0 was used as the base group in multinomial logistic regression models.

Model 1: adjusted for age, civil status, education, work shift, and work type.

Model 2: further adjusted for lifestyle factors: smoking, intakes of total calories, saturated fat, *trans* fat, fish oils, cholesterol, total fiber, and alcohol, and leisure time physical activity.

Model 3: further adjusted for cardiometabolic intermediate variables: total cholesterol, HDL cholesterol, systolic blood pressure, body mass index and diabetes.

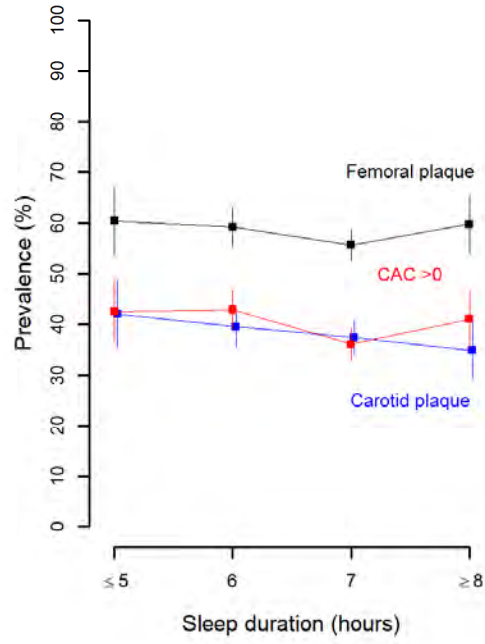
**Table 4.** Average ratio of CAC scores (95% CI) by sleep duration category.

	Sleep duration, h				p-value
	≤ 5	6	7	≥ 8	
Average CAC score	78.5	54.5	34.4	43.7	
Model 1	1.52 (1.14 – 2.03)	1.33 (1.09 – 1.62)	Reference	1.24 (0.96 – 1.61)	0.05
Model 2	1.47 (1.10 – 1.95)	1.31 (1.08 – 1.60)	Reference	1.21 (0.93 – 1.56)	0.01
Model 3	1.45 (1.09 – 1.92)	1.31 (1.07 – 1.59)	Reference	1.18 (0.91 – 1.52)	0.01

Model 1: adjusted for age, civil status, education, work shift, and work type.

Model 2: further adjusted for lifestyle factors: smoking, calories, saturated fat, trans fat, fish oils, cholesterol, total fiber, and alcohol use and metabolic equivalents of task.

Model 3: further adjusted for cardiometabolic intermediate variables: total cholesterol, HDL cholesterol, systolic blood pressure, body mass index and diabetes.



**Sleep duration and subclinical atherosclerosis:****The Aragon Workers' Health Study****Highlights**

- Duration and quality of sleep play a key role in good health and quality of life
- Few studies have evaluated the association of sleep duration with atherosclerosis
- Compared to sleeping 7 hours/day, shorter and longer sleep duration was associated with a higher prevalence of coronary calcium
- The association between sleep duration and carotid or femoral plaques was not statistically significant
- These findings support that subjects with short or long sleep are at increased risk of CVD