Arteriosclerosis, Thrombosis, and Vascular Biology

CLINICAL AND POPULATION STUDIES

Obstructive Sleep Apnea, Sleep Duration, and Associated Mediators With Carotid Intima-Media Thickness

The ELSA-Brasil Study

Silvana P. Souza, Ronaldo B. Santos, Itamar S. Santos, Barbara K. Parise, Soraya Giatti, Aline N. Aielo, Lorenna F. Cunha, Wagner A. Silva, Luiz A. Bortolotto, Geraldo Lorenzi-Filho, Paulo A. Lotufo, Isabela M. Bensenor, Luciano F. Drager

OBJECTIVE: To elucidate the independent associations of obstructive sleep apnea (OSA) and sleep duration (SD) as well as the potential inflammatory and metabolic mediators on carotid intima-media thickness (CIMT) in a large cohort of adults.

APPROACH AND RESULTS: Consecutive participants from the ELSA-Brasil performed a clinical evaluation, sleep study, 1-week actigraphy for defining SD and CIMT using standard techniques. Gamma regression models were used to explore the association between OSA and SD with CIMT. Mediation analysis was performed using the mediation R package. A total of 2009 participants were included in the main analysis. As compared with no OSA (apnea-hypopnea index [AHI] <5 events/hour; n=613), patients with mild (AHI, 5–14.9; n=741), moderate (AHI, 15–29.9; n=389), and severe OSA (AHI ≥30 events/hour; n=266) presented a progressive CIMT increase (0.690 [0.610–0.790], 0.760 [0.650–0.890], 0.810 [0.700–0.940], and 0.820 [0.720–0.958] mm; $P<0.001$). In contrast, CIMTs were similar for those with SD <6 hours (0.760 [0.650–0.888]), 6 to 8 hours (0.750 [0.640–0.880]) and >8 hours (0.740 [0.670–0.900]). All forms of OSA were independently associated with CIMT (mild: β: 0.019, SE 0.008; $P=0.022$; moderate: β: 0.025, SE 0.011; $P=0.022$; severe OSA: β: 0.040, SE 0.013; $P=0.002$). Moreover, the association of AHI with CIMT was mediated by increased C-reactive protein and triglycerides ($P<0.01$). SD did not interact with OSA in the association with CIMT.

CONCLUSIONS: OSA is independently associated with increased CIMT in a dose-response relationship. This association is partially mediated by inflammation and dyslipidemia. In contrast, SD is not associated nor interacted with OSA to increase CIMT.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: actigraphy ■ adult ■ atherosclerosis ■ inflammation ■ intima-media thickness ■ sleep apnea ■ sleep duration

Carotid intima-media thickness (CIMT) measurement by ultrasound has been considered a surrogate marker of cardiovascular events.[1–3] Increased CIMT represent an adaptive mechanism to increased arterial load and a response to an inflammatory milieu, which could promote smooth muscle cell proliferation and collagen deposition.[4] Traditional cardiovascular risk factors have been shown to be determinants of CIMT in the general population[1] but a previous evidence from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) showed that >60% of CIMT variance were not explained by demographic and traditional cardiovascular risk factors, which underscore the need of exploring additional clinical conditions with biological relevance as risk factors for cardiovascular disease.[5]

There is growing evidence that obstructive sleep apnea (OSA) and extremes of sleep duration (SD) may be important cardiovascular risk factors.[6] OSA is characterized by recurrent obstructions in the upper airway, intermittent hypoxia, and fragmented sleep.[6] A previous meta-analysis comprising small observational studies

Correspondence to: Luciano F. Drager, MD, PhD, Center of Clinical and Epidemiologic Research (CPCE), Av. Prof. Lineu Prestes, 2565 – 4° andar, Cidade Universitária, São Paulo, SP 05508-000, Brazil. Email luciano.drager@incor.usp.br

The Data Supplement is available with this article at https://www.ahajournals.org/doi/suppl/10.1161/ATVBAHA.120.315644.

For Sources of Funding and Disclosures, see page 1556.

Arterioscler Thromb Vasc Biol is available at www.ahajournals.org/journal/atvb

Nonstandard Abbreviations and Acronyms

AHI	apnea-hypopnea index
BP	blood pressure
CIMT	carotid intima-media thickness
HDL	high-density lipoprotein
LDL	low-density lipoprotein
OSA	obstructive sleep apnea
SD	sleep duration

showed that OSA is associated with increased CIMT, but most of the evidence were related to severe forms of OSA.[7] Moreover, one of the largest studies in this area to date (n=985)[8] found that positive associations between OSA and subclinical atherosclerosis were attributed to the presence of cardiovascular risk factors, which emphasize the need of additional data.

SD has also gained attention not only by the epidemic sleep deprivation worldwide but also for the intriguing and consistent evidence showing that long SD is not an innocent bystander but associated with cardiovascular and cerebrovascular events.[9,10] Previous evidence showed that short and long SD were inconsistently associated with CIMT.[11,12] However, the vast majority of previous studies evaluated SD in a subjective way, which may not reflect the real sleep time.[13,14] It is worth noting that most studies on OSA did not account for SD or vice versa. Based on the aforementioned gaps, this large study aimed to analyze not only the associations of mild, moderate, and severe OSA but also objective SD with CIMT. We also sought to explore potential mediators in these associations. To the best of our knowledge, no previous study explored the association of OSA and SD with CIMT in the same sample, as well as their potential interactions between these 2 sleep conditions. We made the hypothesis that OSA and extremes of SD will be independently associated with CIMT.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request. Please see the Major Resources Table in the Data Supplement.

As previously described, this investigation is ancillary to the ELSA-Brasil Study, the cohort profile and routines of which were previously reported.[15,16] The sleep evaluation was performed in the Sao Paulo center of the ELSA-Brasil cohort.[17] The local ethical committee approved the study (1166/11), and all the participants provided signed informed consent. We performed the following evaluations.

Highlights

- As compared with participants without obstructive sleep apnea, patients with mild, moderate, and severe obstructive sleep apnea presented a progressive carotid intima-media thickness (CIMT) increase.
- All forms of obstructive sleep apnea were independently associated with CIMT.
- The association of apnea-hypopnea index with CIMT was mediated by increased C-reactive protein and triglycerides.
- In contrast, CIMTs were similar for those with objective sleep duration <6 hours, 6 to 8 hours, and >8 hours.
- Sleep duration did not interact with obstructive sleep apnea in the association with CIMT.

ELSA-Brasil Main Study Protocol

Each participant was interviewed in their workplace and visited the research center for clinical examination according to standard protocols. Trained personnel conducted interviews and examinations under strict quality control.[16] The procedures for anthropometric and blood pressure (BP) measurements as well as definitions for chronic conditions were previously described[17] but also reported in the Data Supplement.

Overnight Home Sleep Study

The sleep studies were performed using the Embletta Gold (Natus Medical, Inc, Ontario, Canada), a standardized level-3 portable diagnostic device that was previously described and validated in the ELSA-Brasil.[18] All the studies were manually scored by an expert in sleep medicine according to the American Academy of Sleep Medicine 2012 criteria.[19] The sum of the apnea and hypopneas per hour determined the apnea-hypopnea index (AHI). We excluded participants with predominantly (>50%) central sleep apnea. In this study, the severity of OSA was classified into mild OSA: AHI 5 to 14.9; moderate OSA: 15 to 29.9, and severe OSA: ≥30 events/hour. Otherwise, AHI<5 was considered as a normal result, no OSA.

Wrist Actigraphy

The SD was measured using an Actiwatch model 2 (Philips Respironics) as previously described.[17] Participants were instructed to fill a sleep diary and wear the actigraph continuously over a period of 7 consecutive days and nights on the nondominant wrist during a typical week. The participants were asked to press the event marker button on the actigraph when they began trying to fall asleep and again when they woke up (including for naps). Short SD was defined when the mean SD was <6 hours, as defined by the National Sleep Foundation as inappropriate for adults.[20] Usual SD was defined by 6 to 8 hours and long SD was set at >8 hours.[17,21]

CIMT Measurement

A detailed protocol was published earlier.[22,23] Briefly, CIMT was measured using a Toshiba (Aplio XG) with a 7.5 MHz linear

transducer. CIMT was calculated in the outer wall of a predefined carotid segment of 1 cm in length from 1 cm below carotid bifurcation, during 3 cardiac cycles.[22,23] All images underwent strict quality control, and only images with adequate visualization of anatomic guides and vascular interfaces were included. We used MIA software to standardize the reading and interpretation of carotid scans. In this article, CIMT is defined as the average of the highest IMT value between left and right maximum thickness.[22,23]

Statistical Analysis

We used the software R 3.6.0 (R Core Team, 2019) for all analyses. The graphics were built using the ggplot2 package.[24] For comparisons of categorical variables, the χ^2 test was performed. Normally distributed continuous variables were compared using 1-way ANOVA and were presented as the means and SD (mean±SD). For skewed variables, medians and interquartile ranges were reported and Kruskal-Wallis tests were performed. We used Spearman correlation coefficients for continuous variables. Gamma regression models[25] were used to explore the association between OSA severity and SD with CIMT. Three models we progressively tested independently for OSA and SD: Model 1: unadjusted; model 2: adjusted for age, sex and race; model 3: model 2 plus obesity (body mass index ≥30 kg/m^2), smoking, diabetes, hypertension, and use of statins. An interaction term between AHI and SD was introduced for testing the combinations of theses sleep conditions on CIMT. For the analysis of potential mediators in the AHI and CIMT, the mediation package was used.[26] A mediation analysis was performed using data from the model 3 and the following variables with potential relevance for OSA and CIMT: C-reactive protein, systolic BP, LDL (low-density lipoprotein), HDL (high-density lipoprotein), triglycerides, and glycated hemoglobin (HbA1c). The tests for the significance of the mediating effects were performed with the bootstrap procedure, using 5000 samples. For all statistical tests, a significance level of 5% was adopted.

RESULTS

We invited 2463 consecutive participants to perform sleep evaluations during the 2-year study recruitment. After the exclusion of refusals, technical issues during sleep monitoring, predominant central sleep apnea, previous OSA treatment, nocturnal/shift workers, medications that directly interfere with the SD and unavailable CIMT measurements, a total of 2009 participants were included in the final analysis.

Association of OSA With CIMT

The characteristics of the studied population according to OSA severity are presented in Table 1. As expected, there was a progressive increase in age, proportion of males, markers of adiposity, and traditional comorbidities such as hypertension, diabetes, and dyslipidemia (and related BP, glucose, and lipid levels) in parallel to the increase in OSA severity. Ultrasensitive C-reactive protein also increased in parallel to OSA severity. In contrast, we observed a progressive decrease of objective SD and HDL cholesterol levels in parallel to OSA severity. Figure 1 shows the progressive increase in CMIT in parallel with the increase in OSA severity. We observed a significant but modest correlations between AHI (r=0.33; $P<0.001$), lowest oxygen (O_2) saturation (r=–0.27; $P<0.001$), and total time with O_2 saturation levels <90% (r=0.28; $P<0.001$) with CIMT (Figure 2A through 2C). Table 2 showed that mild, moderate, and severe OSA remained independently associated with CIMT after adjustments for potential confounders. Additional analysis using lowest oxygen (O_2) saturation and total time with O_2 saturation levels <90% instead of AHI were presented on Tables I and II in the Data Supplement. Interestingly, log C-reactive protein mediated the association of AHI with CIMT with an estimated proportion of 15.6% (Figure 3A). In our study, systolic BP did not mediate the association of AHI with CIMT although it has a direct effect on this vascular parameter (Figure 3B). Regarding the metabolic parameters, log triglyceride mediated the association of AHI with CIMT in a proportion of 11.3% (Figure 3C). In contrast, LDL cholesterol, HDL cholesterol, and log HbA1C did not mediate the association of AHI and CIMT in our study (Figure 3D through 3F).

Association of SD With CIMT

Table III in the Data Supplement depicted the characteristics of the studied population according to the objective SD stratification: short SD (<6 hours), reference group (SD, 6–8 hours), and long SD (>8 hours). Overall, participants who slept <6 hours were younger, had higher proportion of men, blacks, higher values of adiposity, severity OSA but not comorbidities such as hypertension, dyslipidemia, diabetes, and smoking. Of note, there are no differences in CIMT among the 3 groups. We observed a significant but very low correlation between SD (r=–0.05; $P=0.025$) with CIMT (Figure 2D). Table IV in the Data Supplement showed that short and long SD were not independently associated with CIMT.

Interactions Between OSA and SD With CIMT

Figure I in the Data Supplement showed a progressive increase in the CIMT regardless of SD classification. Interactions of AHI×SD with CIMT were not significant.

DISCUSSION

Our study revealed that all degrees of OSA (from mild to severe) were independently associated with increased CIMT in a large cohort of adults. Our analysis addressing objective SD did not find significant associations with CIMT. Moreover, we explored potential mediators for these associations beyond a direct effect of the respiratory events on CIMT. Log C-reactive protein and log-triglyceride mediated this association with an estimated

Table 1. Characteristics of the Studied Population According to the OSA Severity

	No OSA (n=622)	Mild OSA (n=760)	Moderate OSA (n=401)	Severe OSA (n=277)	*P* value*
Age, y	46.0 (41.0–50.0)	48.0 (44.0–54.0)	50.0 (45.0–56.0)	50.0 (45.0–55.0)	0.001
Male, %	28.7%	41.7%	52.2%	62.4%	<0.001
Race/ethnicity					
White participant	64.4%	61.4%	57.2%	57.8%	0.051
Black participant	11.0%	14.6%	13.1%	9.5%	
Brown participant	19.8%	18.4%	23.5%	25.1%	
Others	4.8%	5.7%	6.3%	7.6%	
BMI, kg/m^2	24.1 (21.9–26.7)	26.3 (24.0–29.4)	28.1 (25.5–31.4)	29.5 (26.1–32.6)	0.001
Neck circumference, cm	33.4 (31.6–36.1)	35.5 (33.4–38.2)	36.9 (34.6–39.5)	38.3 (35.5–41.0)	0.001
Waist circumference, cm	80.6 (74.3–87.3)	87.8 (80.8–95.8)	93.6 (86.7–100.4)	97.2 (88.8–105.9)	0.001
Hypertension, %	14.8%	23.9%	35.7%	43.6%	<0.001
Antihypertensive treatment, %	11.3%	18.9%	28.0%	32.3%	<0.001
Systolic BP, mm Hg	112 (104–122)	115 (107–125)	120 (111–131)	122 (112–133)	0.001
Diastolic BP, mm Hg	71 (65–78)	73 (68–80)	76 (70–83)	79 (72–86)	0.001
Dyslipidemia, %	44.9%	56.0%	60.9%	65.0%	<0.001
Statins, %	4.9%	11.1%	12.3%	12.5%	<0.001
Total cholesterol, mg/dL	203 (181–228)	210 (185–233)	213 (188–243)	215 (191–242)	0.001
HDL cholesterol, mg/dL	57 (48–68)	53 (46–62)	52 (45–61)	51 (44–60)	0.001
LDL cholesterol, mg/dL	122 (103.0–143.0)	129 (109–148)	131 (110–154)	134 (110–156)	0.001
TG, mg/dL	92 (68–130)	112 (79–159)	124 (90–182)	138 (100–187)	0.001
C-reactive protein, mg/dL	1.0 (0.5–2.4)	1.4 (0.7–3.1)	1.7 (0.9–3.4)	2.1 (1.0–4.3)	0.001
Diabetes, %	7.5%	14.3%	23.9%	24.4%	<0.001
Glucose, mg/dL	101 (95–107)	104 (98–111)	107 (100–115)	108 (102–117)	0.001
HbA1C, %	5.2 (4.9–5.6)	5.3 (5.0–5.8)	5.4 (5.0–5.9)	5.4 (5.0–6.0)	0.001
Smoking, %					
Never	63.0%	52.1%	50.4%	54.9%	<0.001
Former	23.3%	32.9%	35.5%	32.0%	
Current	13.7%	15.0%	14.1%	13.2%	
Sleep duration, h	6.7±0.9	6.6±0.9	6.4±1.0	6.3±1.1	0.001†
Sleep duration category					<0.001
<6 h	21.9%	24.3%	32.4%	39.8%	
6–8 h	70.8%	68.7%	62.7%	53.8%	
>8 h	7.3%	7.0%	4.9%	6.4%	
AHI, events/h	2.9 (1.7–4.1)	9.5 (7.1–12.1)	20.7 (17.8–24.4)	41.0 (34.7–51.6)	0.001
Baseline Spo_2, %	95.4 (94.5–96.2)	94.4 (93.4–95.3)	93.9 (92.8–94.8)	92.8 (91.4–93.9)	0.001
Lowest Spo_2, %	90.0 (88.0–92.0)	86.0 (83.0–89.0)	83.0 (79.0–85.0)	78.0 (73.0–82.0)	0.001
Total time Spo_2 <90%	0.0 (0.0–0.1)	0.3 (0.1–1.4)	1.9 (0.7–4.7)	7.7 (2.9–17.6)	0.001
CIMT, mm	0.690 (0.610–0.790)	0.760 (0.650–0.890)	0.810 (0.700–0.940)	0.820 (0.720–0.958)	0.001

AHI indicates apnea-hypopnea index; BP, blood pressure; CIMT, carotid intima-media thickness; HbA1C, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and TG, triglyceride.

*Mann-Whitney *U* test for continuous variables, and χ^2 test for categorical variables.

†Analyses of variance.

proportion of 15.6% and 11.3%, respectively. SD did not interact with OSA in the association with CIMT. Together, these findings reinforce the role of OSA as a potential nontraditional risk factor for increased CIMT. Our data also underscore the potential role of subclinical inflammation and triglyceride increase, thereby conferring increased risk for future cardiovascular events.

Despite the extensive literature already addressing the impact of OSA on CIMT, the vast majority of studies comprised small sample of patients (usually n<100) presenting one or more traditional comorbidities (please see a review and a recent meta-analysis for details).[7,27] Only recently, data from MESA exploring carotid atherosclerosis in 1615 participants (mean age of 68 years)

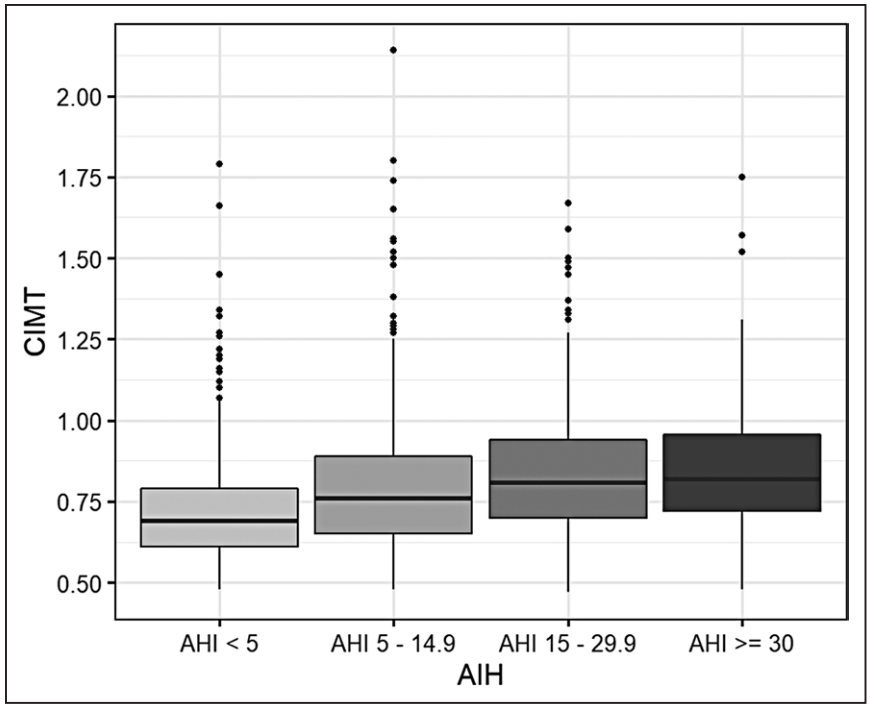

Figure 1. Carotid intima-media thickness (CIMT) boxplots for each combination of apnea-hypopnea index (AHI) and sleep duration.

showed an independent association between moderate to severe OSA with CIMT in younger but not in older individuals (>68 years).[28] The ELSA-Brasil is a younger cohort (mean age: 48 years) and our study significantly add to the literature by showing an independent association of OSA with CIMT in all degrees of OSA severity, including mild OSA. Mild OSA is by far the most common form of OSA (in our study mild OSA represented 38% of the whole sample or 55% of all patients with OSA), but the cardiovascular impact of this mild form is unknown underscoring the need of additional research as suggested by a recent statement from the American Thoracic Society.[29] Therefore, our data underscore the need of future well-designed studies addressing the potential cardiovascular consequences of mild OSA.

Another important piece of new information was the analysis of potential pathways involved in the association of OSA with CIMT. The pathogenesis of cardiovascular complications in OSA is not completely understood, but given the complexity of this sleep disordered breathing, a multifactorial cause is likely.[30] OSA has often a metabolic syndrome phenotype, which is associated with vascular inflammation.[31,32] Inflammatory processes have emerged as critical in the pathogenesis of atherosclerosis in general and also important for OSA. In our study, there is a parallel increase in C-reactive protein with the severity of OSA. Mediation analysis revealed that log C-reactive protein was positively related to the AHI-CIMT association. Several studies have shown that patients with OSA have elevated markers of proinflammatory mediators and inflammatory markers with proatherogenic properties such as TNF-α (tumor necrosis factor alpha), interleukins, C-reactive protein, leukotriene B4, and adhesion molecules.[30] Basic and translational evidence suggest that OSA and its components have a crucial role of NF-κB (nuclear factor kappa B) activation with the downstream consequences of production of inflammatory genes in response inflammatory pathways.[33] Recent experimental studies have pointed to the importance of inflammation in the vascular dysfunction and atherogenesis induced by OSA.[34,35] Log triglyceride also mediated the AHI-CIMT association in our study. Although the impact of triglyceride is still debatable, there is growing evidence pointing triglyceride as a potential atherosclerotic risk factor.[36] Particularly, triglyceride-rich lipoprotein degradation and uptake into macrophage foam cells in the arterial intima seems to contribute to ischemic heart disease, stroke, and cardiovascular mortality independent of other lipoproteins.[37–39] Previous translational studies suggested that intermittent hypoxia was associated with dyslipidemia by upregulating lipid biosynthesis in the liver and inhibiting lipoprotein clearance of triglyceride-rich lipoproteins and contributing to atherosclerosis.[40–42] The consistent findings from this epidemiological study underscore the need of continuing exploring subclinical inflammation and triglyceride in the cardiovascular pathogenesis of OSA.

Our study also carefully evaluated the association of SD with CIMT. Contrary to our hypothesis, we found no independent associations of objective SD with CIMT as previously observed in studies using subjective data.[11,12] To the best of our knowledge, the only study exploring objective SD using actigraphy and markers of subclinical atherosclerosis (such as coronary calcium score) was an observational cohort in a healthy middle-aged population of 495 participants from the Coronary Artery Risk Development in Young Adults.[43] Long SD was

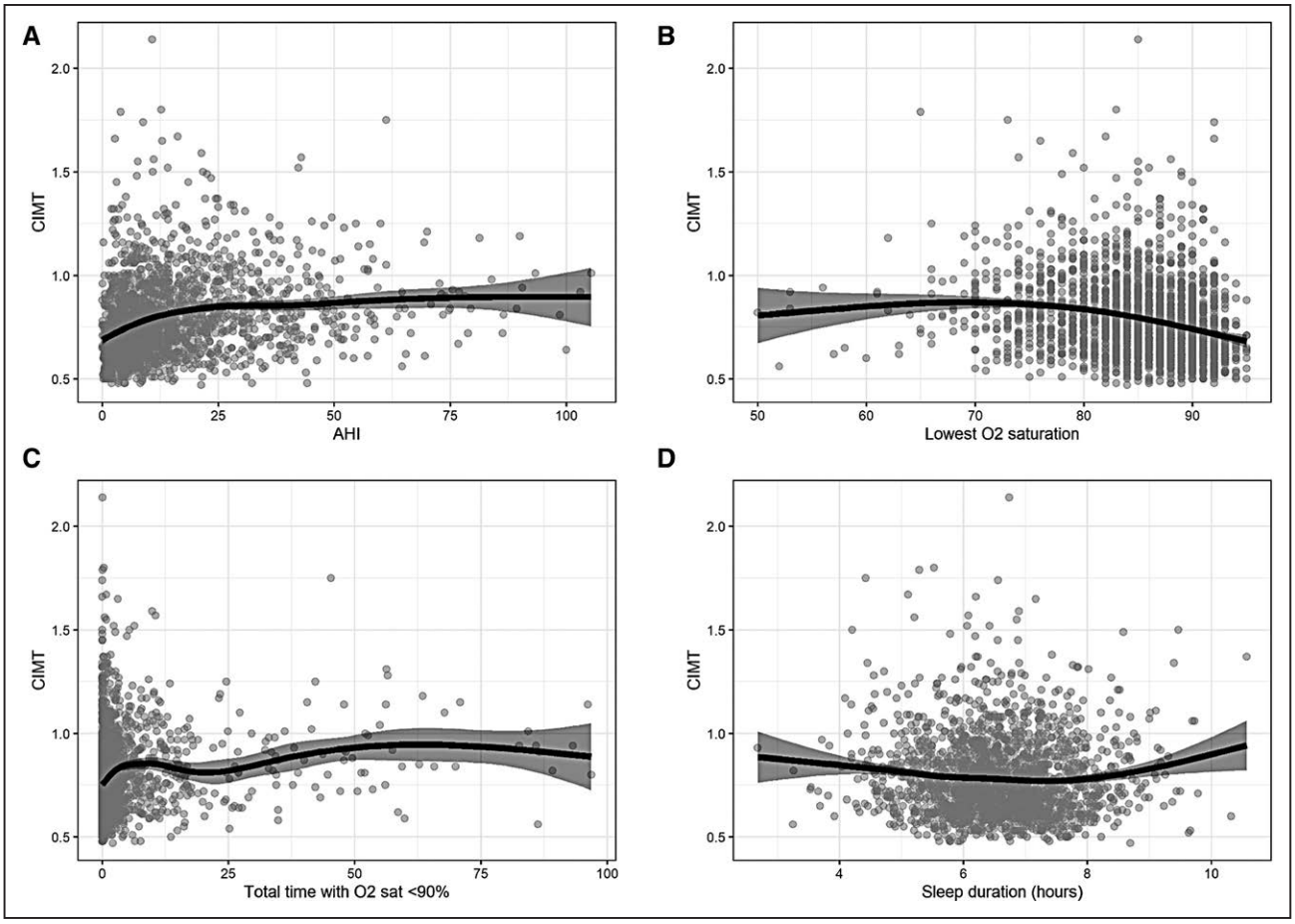

Figure 2. Spearman correlations for carotid intima-media thickness (CIMT).
A, Apnea-hypopnea index (AHI). **B**, Lowest oxygen (O2) saturation. **C**, Total time with O2 saturation <90%. **D**, Sleep duration. CIMT indicates carotid intima-media thickness.

significantly associated with reduced calcification incidence over a 5-year follow-up. In that important study, formal sleep studies for detecting OSA were not performed.[43] Beyond the potential differences in the vascular coronary and carotid territories, the current findings emphasizes the need of exploring OSA in studies addressing SD. In our study, we found that participants sleeping <6 hours had higher frequency of moderate to severe OSA than the other SD categories. The lack of SD interactions with AHI on CIMT highlights the relevance of OSA for cardiovascular disease.

The current investigation has strengths such a huge sample size, standard protocols for all measurements, and objective SD data, but it has limitations to be addressed. A first limitation is that our analyses were constrained due to its cross-sectional design, which precludes definitive assessment of directionality of associations. However, our previous preliminary data showing significant effects of effective treatment with OSA on CIMT underscore the biological plausibility of this association.[44] Second, it is important to note that while the indirect mediation pathways were statistically significant, the effect sizes of the pairwise associations were small. Despite the independent association between OSA and CIMT, the interfaces between this sleep-disordered breathing with age, BP, local adiposity, and comorbidities are complex

Table 2. Generalized Linear Model With Gamma Distribution Testing the Association of the 4 Categories of OSA Severity With CIMT

	n	Model 1			Model 2			Model 3		
		Coefficient	SD error	*P* value	Coefficient	SD error	*P* value	Coefficient	SD error	*P* value
AHI, 5–14.9 (mild OSA)	741	0.072	0.010	<0.001	0.039	0.009	<0.001	0.025	0.008	0.003
AHI, 15–29.9 (moderate OSA)	389	0.117	0.012	<0.001	0.061	0.011	<0.001	0.032	0.011	0.003
AHI≥30 (severe OSA)	266	0.137	0.014	<0.001	0.090	0.013	<0.001	0.049	0.013	<0.001

No OSA (AHI<5) as the reference group; model 1: crude; model 2: adjusted for age, sex, and race; model 3: model 2 plus obesity, smoking, diabetes, hypertension, and use of statins. AHI indicates apnea-hypopnea index; CIMT, carotid intima-media thickness; and OSA, obstructive sleep apnea

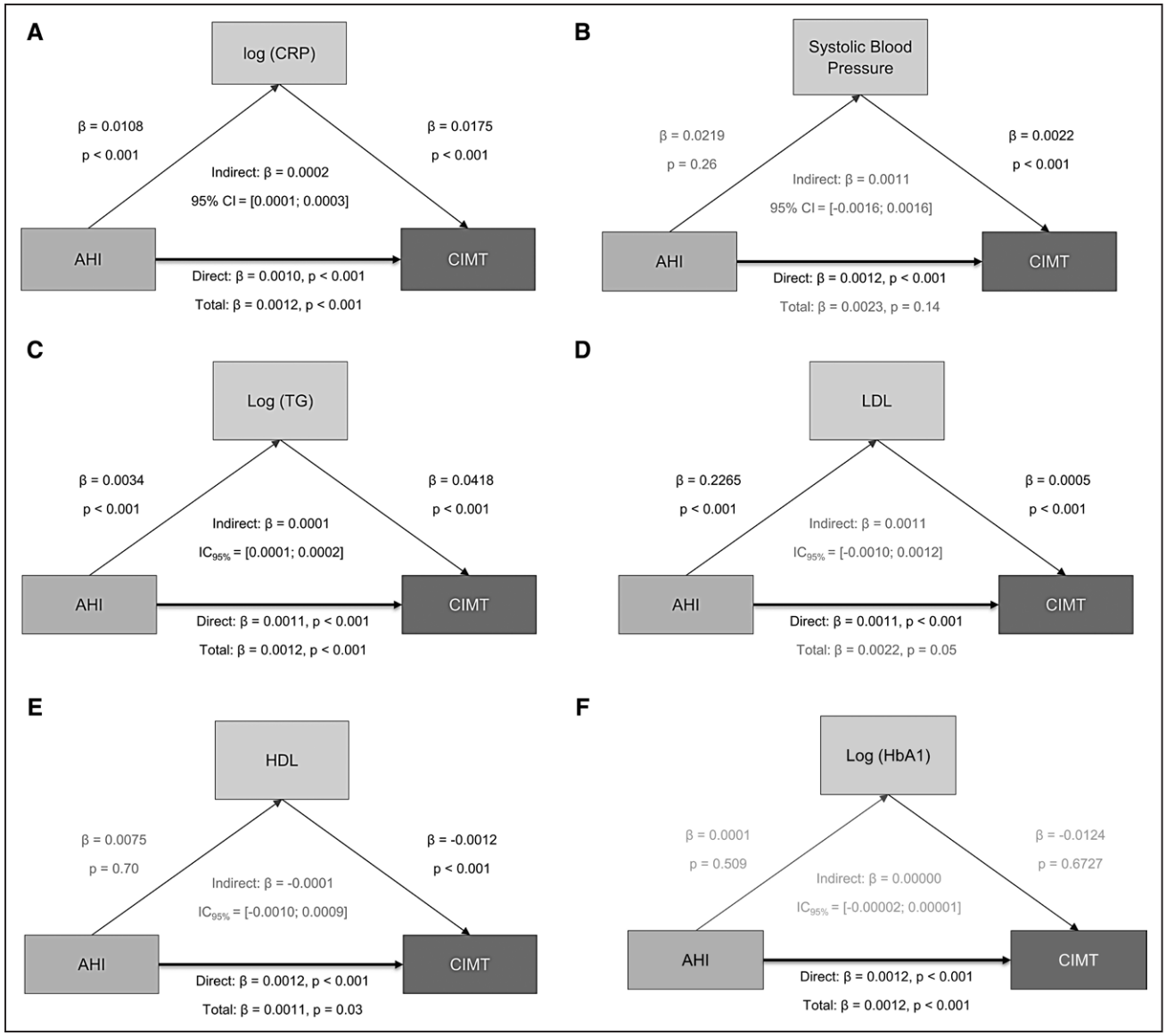

Figure 3. Mediation analysis between apnea-hypopnea index (AHI) with carotid intima-media thickness (CIMT) using CRP (C-reactive protein CRP; A), systolic blood pressure (B), and metabolic markers (C–F).
HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; and TG, triglyceride.

and multifaceted. Therefore, we cannot exclude potential residual confounders. It is conceivable that effects of OSA upon the large artery structure are mediated by the multiple hemodynamic and metabolic changes that were partially addressed in our mediation analysis. Other pathways include altered autonomic nervous system activity, increased oxidative stress, and endothelial dysfunction were not explored. Third, our cohort is relatively young which preclude us for having enough power for exploring carotid plaques. We strong think that this fact did not mitigate our findings considering the relevance of CMIT in predicting cardiovascular events reinforced by a recent meta-analysis of 119 clinical trials involving >100 000 patients.[45] Fourth, the AHI was the OSA variable with higher correlation with CIMT in our study, but the unavailability of a formal hypoxia burden marker[46] preclude us to define which the best OSA-related marker for atherosclerosis is. Finally, because we use a portable, although validated sleep monitor,[18] important sleep components were not captured. A recent study found that sleep fragmentation was associated with coronary calcium score mediated by inflammatory-associated neutrophil and monocyte count.[47] However, the observed associations were not significant when adjusting for the AHI suggesting that OSA is the main drive for causing sleep fragmentation.

In conclusion, all forms of OSA were associated with increased CIMT. The association of AHI and CIMT is partially mediated by metabolic and inflammatory parameters. SD was not associated nor interacted with OSA to increase CIMT, which had not mitigated the relevance of extremes of SD on several domains but

highlighted the need of studying comorbid sleep conditions such as OSA.[48]

ARTICLE INFORMATION

Received November 18, 2020; accepted January 19, 2021.

Affiliations

Center of Clinical and Epidemiologic Research (CPCE) (S.P.S., R.B.S., I.S.S., B.K.P., S.G., A.N.A., L.F.C., W.A.S., P.A.L., I.M.B., L.F.D.), Hypertension Unit, Heart Institute (InCor) (S.P.S., R.B.S., W.A.S., L.A.B., L.F.D.), Hypertension Unit, Renal Division (B.K.P., S.G., A.N.A., L.F.C., L.F.D.), and Sleep Laboratory, Pulmonary Division (G.L.-F.), University of Sao Paulo, Brazil.

Acknowledgments

We thank the research team of the ELSA-Brasil baseline study for their contribution, all participants included in this study, the ELSA-Brasil headquarters in Sao Paulo.

Sources of Funding

L.F.D. received funding from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) 2019/23496-8.

Disclosures

None.

REFERENCES

1. Bauer M, Caviezel S, Teynor A, Erbel R, Mahabadi AA, Schmidt-Trucksäss A. Carotid intima-media thickness as a biomarker of subclinical atherosclerosis. *Swiss Med Wkly.* 2012;142:w13705. doi: 10.4414/smw.2012.13705
2. Simon A, Megnien JL, Chironi G. The value of carotid intima-media thickness for predicting cardiovascular risk. *Arterioscler Thromb Vasc Biol.* 2010;30:182–185. doi: 10.1161/ATVBAHA.109.196980
3. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation.* 2007;115:459–467. doi: 10.1161/CIRCULATIONAHA.106.628875
4. Kozakova M, Morizzo C, Bianchi C, Di Filippi M, Miccoli R, Paterni M, Di Bello V, Palombo C. Glucose-related arterial stiffness and carotid artery remodeling: a study in normal subjects and type 2 diabetes patients. *J Clin Endocrinol Metab.* 2014;99:E2362–E2366. doi: 10.1210/jc.2014-2028
5. Santos IS, Alencar AP, Rundek T, Goulart AC, Barreto SM, Pereira AC, Benseñor IM, Lotufo PA. Low impact of traditional risk factors on carotid intima-media thickness: the ELSA-Brasil Cohort. *Arterioscler Thromb Vasc Biol.* 2015;35:2054–2059. doi: 10.1161/ATVBAHA.115.305765
6. Drager LF, Togeiro SM, Polotsky VY, Lorenzi-Filho G. Obstructive sleep apnea: a cardiometabolic risk in obesity and the metabolic syndrome. *J Am Coll Cardiol.* 2013;62:569–576. doi: 10.1016/j.jacc.2013.05.045
7. Zhou M, Guo B, Wang Y, Yan D, Lin C, Shi Z. The association between obstructive sleep apnea and carotid intima-media thickness: a systematic review and meta-analysis. *Angiology.* 2017;68:575–583. doi: 10.1177/0003319716665985
8. Wattanakit K, Boland L, Punjabi NM, Shahar E. Relation of sleep-disordered breathing to carotid plaque and intima-media thickness. *Atherosclerosis.* 2008;197:125–131. doi: 10.1016/j.atherosclerosis.2007.02.029
9. 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.* 2011;32:1484–1492. doi: 10.1093/eurheartj/ehr007
10. Wang YH, Wang J, Chen SH, Li JQ, Lu QD, Vitiello MV, Wang F, Tang XD, Shi J, Lu L, et al. Association of longitudinal patterns of habitual sleep duration with risk of cardiovascular events and all-cause mortality. *JAMA Netw Open.* 2020;3:e205246. doi: 10.1001/jamanetworkopen.2020.5246
11. Suzuki S, Arima H, Miyazaki S, Fujiyoshi A, Kadota A, Takashima N, Hisamatsu T, Kadowaki S, Zaid M, Torii S, et al; SESSA Research Group. Self-reported sleep duration and subclinical atherosclerosis in a general population of Japanese men. *J Atheroscler Thromb.* 2018;25:186–198. doi: 10.5551/jat.40527
12. Aziz M, Ali SS, Das S, Younus A, Malik R, Latif MA, Humayun C, Anugula D, Abbas G, Salami J, et al. Association of subjective and objective sleep duration as well as sleep quality with non-invasive markers of subclinical cardiovascular disease (CVD): a systematic review. *J Atheroscler Thromb.* 2017;24:208–226. doi: 10.5551/jat.36194
13. Lauderdale DS, Knutson KL, Yan LL, Liu K, Rathouz PJ. Self-reported and measured sleep duration: how similar are they? *Epidemiology.* 2008;19:838–845. doi: 10.1097/EDE.0b013e318187a7b0
14. Cespedes EM, Hu FB, Redline S, Rosner B, Alcantara C, Cai J, Hall MH, Loredo JS, Mossavar-Rahmani Y, Ramos AR, et al. Comparison of self-reported sleep duration with actigraphy: results from the Hispanic Community Health Study/Study of Latinos Sueño Ancillary Study. *Am J Epidemiol.* 2016;183:561–573. doi: 10.1093/aje/kwv251
15. Aquino EM, Barreto SM, Bensenor IM, Carvalho MS, Chor D, Duncan BB, Lotufo PA, Mill JG, Molina Mdel C, Mota EL, et al. Brazilian Longitudinal Study of Adult Health (ELSA-Brasil): objectives and design. *Am J Epidemiol.* 2012;175:315–324. doi: 10.1093/aje/kwr294
16. Bensenor IM, Griep RH, Pinto KA, Faria CP, Felisbino-Mendes M, Caetano EI, Albuquerque Lda S, Schmidt MI. Rotinas de organizacao de exames e entrevistas no centro de investigacao ELSA-Brasil. *Revista de Saúde Pública.* 2013;47(suppl 2):37–47.
17. Drager LF, Santos RB, Silva WA, Parise BK, Giatti S, Aielo AN, Souza SP, Furlan SF, Lorenzi-Filho G, Lotufo PA, et al. OSA, short sleep duration, and their interactions with sleepiness and cardiometabolic risk factors in adults: the ELSA-Brasil Study. *Chest.* 2019;155:1190–1198. doi: 10.1016/j.chest.2018.12.003
18. Aielo AN, Santos RB, Silva WA, Parise BK, Souza SP, Cunha LF, Giatti S, Lotufo PA, Bensenor IM, Drager LF. Pragmatic validation of home portable sleep monitor for diagnosing obstructive sleep apnea in a non-referred population: the ELSA-Brasil study. *Sleep Sci.* 2019;12:65–71. doi: 10.5935/1984-0063.20190072
19. Berry RB, Budhiraja R, Gottlieb DJ, Gozal D, Iber C, Kapur VK, Marcus CL, Mehra R, Parthasarathy S, Quan SF, et al; American Academy of Sleep Medicine. Rules for scoring respiratory events in sleep: update of the 2007 AASM manual for the scoring of sleep and associated events. *J Clin Sleep Med.* 2012;8:597–619. doi: 10.5664/jcsm.2172
20. Hirshkowitz M, Whiton K, Albert SM, Alessi C, Bruni O, DonCarlos L, Hazen N, Herman J, Adams Hillard PJ, Katz ES, et al. National Sleep Foundation's updated sleep duration recommendations: final report. *Sleep Health.* 2015;1:233–243. doi: 10.1016/j.sleh.2015.10.004
21. Chen X, Wang R, Zee P, Lutsey PL, Javaheri S, Alcántara C, Jackson CL, Williams MA, Redline S. Racial/ethnic differences in sleep disturbances: the Multi-Ethnic Study of Atherosclerosis (MESA). *Sleep.* 2015;38:877–888. doi: 10.5665/sleep.4732
22. Santos IS, Bittencourt MS, Oliveira IR, Souza AG, Meireles DP, Rundek T, Foppa M, Bezerra DC, Freire CM, Roelke LH, et al. Carotid intima-media thickness value distributions in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). *Atherosclerosis.* 2014;237:227–235. doi: 10.1016/j.atherosclerosis.2014.09.004
23. Mill JG, Pinto K, Griep RH, Goulart A, Foppa M, Lotufo PA, Maestri MK, Ribeiro AL, Andreão RV, Dantas EM, et al. [Medical assessments and measurements in ELSA-Brasil]. *Rev Saude Publica.* 2013;47(suppl 2):54–62. doi: 10.1590/s0034-8910.2013047003851
24. Wickham H. *ggplot2: Elegant Graphics for Data Analysis.* 3rd ed. Springer; 2009:221.
25. McCullagh P, Nelder JA. *Generalized Linear Models.* Chapman & Hall / CRC; 1989.
26. Tingley J, Yamamoto T, Hirose K, Keele L, Imai K. Mediation: R package for causal mediation analysis. *J Stat Softw.* 2014;59:1–38.
27. Drager LF, Polotsky VY, Lorenzi-Filho G. Obstructive sleep apnea: an emerging risk factor for atherosclerosis. *Chest.* 2011;140:534–542. doi: 10.1378/chest.10-2223
28. Zhao YY, Javaheri S, Wang R, Guo N, Koo BB, Stein JH, Korcarz CE, Redline S. Associations between sleep apnea and subclinical carotid atherosclerosis: the Multi-Ethnic Study of Atherosclerosis. *Stroke.* 2019;50:3340–3346. doi: 10.1161/STROKEAHA.118.022184
29. Chowdhuri S, Quan SF, Almeida F, Ayappa I, Batool-Anwar S, Budhiraja R, Cruse PE, Drager LF, Griss B, Marshall N, et al; ATS Ad Hoc Committee on Mild Obstructive Sleep Apnea. An official American Thoracic Society research statement: impact of mild obstructive sleep apnea in adults. *Am J Respir Crit Care Med.* 2016;193:e37–e54. doi: 10.1164/rccm.201602-0361ST
30. Drager LF, Polotsky VY, O'Donnell CP, Cravo SL, Lorenzi-Filho G, Machado BH. Translational approaches to understanding metabolic dysfunction and cardiovascular consequences of obstructive sleep apnea. *Am J Physiol Heart Circ Physiol.* 2015;309:H1101–H1111. doi: 10.1152/ajpheart.00094.2015

31. Drager LF, Lopes HF, Maki-Nunes C, Trombetta IC, Toschi-Dias E, Alves MJ, Fraga RF, Jun JC, Negrão CE, Krieger EM, et al. The impact of obstructive sleep apnea on metabolic and inflammatory markers in consecutive patients with metabolic syndrome. *PLoS One.* 2010;5:e12065. doi: 10.1371/journal.pone.0012065
32. Hirotsu C, Haba-Rubio J, Togeiro SM, Marques-Vidal P, Drager LF, Vollenweider P, Waeber G, Bittencourt L, Tufik S, Heinzer R. Obstructive sleep apnoea as a risk factor for incident metabolic syndrome: a joined Episono and HypnoLaus prospective cohorts study. *Eur Respir J.* 2018;52:1801150.
33. Ryan S, Taylor CT, McNicholas WT. Selective activation of inflammatory pathways by intermittent hypoxia in obstructive sleep apnea syndrome. *Circulation.* 2005;112:2660–2667. doi: 10.1161/CIRCULATIONAHA.105.556746
34. Li RC, Haribabu B, Mathis SP, Kim J, Gozal D. Leukotriene B4 receptor-1 mediates intermittent hypoxia-induced atherogenesis. *Am J Respir Crit Care Med.* 2011;184:124–131. doi: 10.1164/rccm.201012-2039OC
35. Song D, Fang G, Mao SZ, Ye X, Liu G, Gong Y, Liu SF. Chronic intermittent hypoxia induces atherosclerosis by NF-κB-dependent mechanisms. *Biochim Biophys Acta.* 2012;1822:1650–1659. doi: 10.1016/j.bbadis.2012.07.010
36. Nordestgaard BG. Triglyceride-rich lipoproteins and atherosclerotic cardiovascular disease: new insights from epidemiology, genetics, and biology. *Circ Res.* 2016;118:547–563. doi: 10.1161/CIRCRESAHA.115.306249
37. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA.* 2007;298:299–308. doi: 10.1001/jama.298.3.299
38. Freiberg JJ, Tybjaerg-Hansen A, Jensen JS, Nordestgaard BG. Nonfasting triglycerides and risk of ischemic stroke in the general population. *JAMA.* 2008;300:2142–2152. doi: 10.1001/jama.2008.621
39. Varbo A, Benn M, Tybjærg-Hansen A, Jørgensen AB, Frikke-Schmidt R, Nordestgaard BG. Remnant cholesterol as a causal risk factor for ischemic heart disease. *J Am Coll Cardiol.* 2013;61:427–436. doi: 10.1016/j.jacc.2012.08.1026
40. Drager LF, Li J, Shin MK, Reinke C, Aggarwal NR, Jun JC, Bevans-Fonti S, Sztalryd C, O'Byrne SM, Kroupa O, et al. Intermittent hypoxia inhibits clearance of triglyceride-rich lipoproteins and inactivates adipose lipoprotein lipase in a mouse model of sleep apnoea. *Eur Heart J.* 2012;33:783–790. doi: 10.1093/eurheartj/ehr097
41. Drager LF, Yao Q, Hernandez KL, Shin MK, Bevans-Fonti S, Gay J, Sussan TE, Jun JC, Myers AC, Olivecrona G, et al. Chronic intermittent hypoxia induces atherosclerosis via activation of adipose angiopoietin-like 4. *Am J Respir Crit Care Med.* 2013;188:240–248. doi: 10.1164/rccm.201209-1688OC
42. Drager LF, Tavoni TM, Silva VM, Santos RD, Pedrosa RP, Bortolotto LA, Vinagre CG, Polotsky VY, Lorenzi-Filho G, Maranhao RC. Obstructive sleep apnea and effects of continuous positive airway pressure on triglyceride-rich lipoprotein metabolism. *J Lipid Res.* 2018;59:1027–1033. doi: 10.1194/jlr.M083436
43. King CR, Knutson KL, Rathouz PJ, Sidney S, Liu K, Lauderdale DS. Short sleep duration and incident coronary artery calcification. *JAMA.* 2008;300:2859–2866. doi: 10.1001/jama.2008.867
44. Drager LF, Bortolotto LA, Figueiredo AC, Krieger EM, Lorenzi GF. Effects of continuous positive airway pressure on early signs of atherosclerosis in obstructive sleep apnea. *Am J Respir Crit Care Med.* 2007;176:706–712. doi: 10.1164/rccm.200703-500OC
45. Willeit P, Tschiderer L, Allara E, Reuber K, Seekircher L, Gao L, Liao X, Lonn E, Gerstein HC, Yusuf S, et al; PROG-IMT and the Proof-ATHERO Study Groups. Carotid intima-media thickness progression as surrogate marker for cardiovascular risk: meta-analysis of 119 clinical trials involving 100 667 patients. *Circulation.* 2020;142:621–642. doi: 10.1161/CIRCULATIONAHA.120.046361
46. Azarbarzin A, Sands SA, Stone KL, Taranto-Montemurro L, Messineo L, Terrill PI, Ancoli-Israel S, Ensrud K, Purcell S, White DP, et al. The hypoxic burden of sleep apnoea predicts cardiovascular disease-related mortality: the Osteoporotic Fractures in Men Study and the Sleep Heart Health Study. *Eur Heart J.* 2019;40:1149–1157. doi: 10.1093/eurheartj/ehy624
47. Vallat R, Shah VD, Redline S, Attia P, Walker MP. Broken sleep predicts hardened blood vessels. *PLoS Biol.* 2020;18:e3000726. doi: 10.1371/journal.pbio.3000726
48. Drager LF, Santos RB. Struggling with comorbid sleep disturbances: insights from the ELSA-Brasil study. *Sleep Sci.* 2020;13:94–96. doi: 10.5935/1984-0063.20200028