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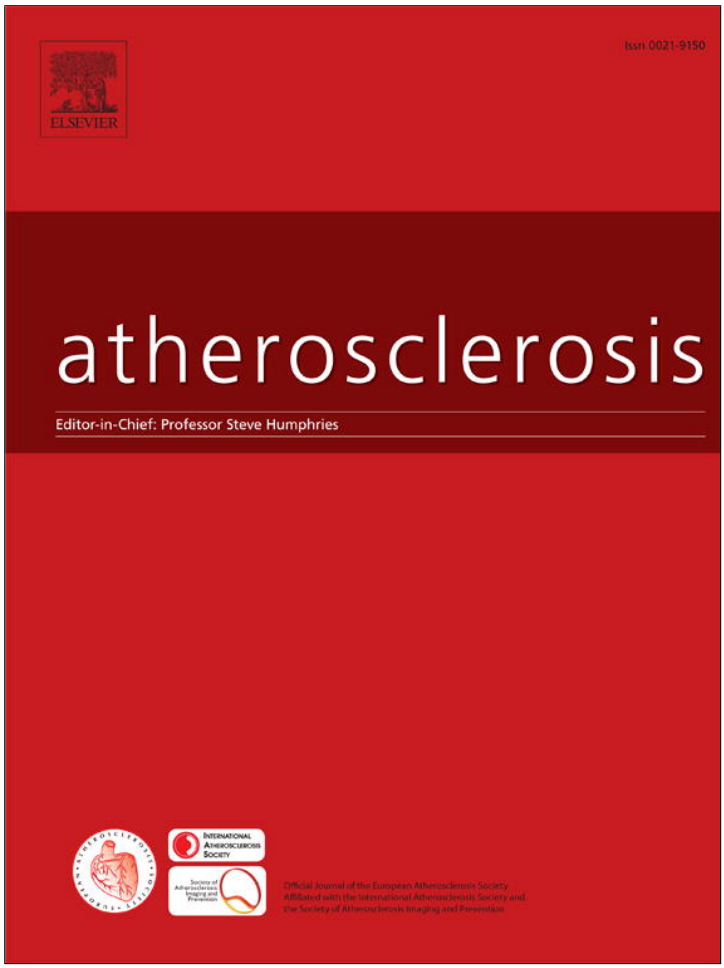

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Sedentary behaviour patterns and carotid intima-media thickness in Spanish healthy adult population

Antonio García-Hermoso [a], Vicente Martínez-Vizcaíno [b, *], José Ignacio Recio-Rodríguez [c], Mairena Sánchez-López [b], Manuel Ángel Gómez-Marcos [d], Luis García-Ortiz [d], the EVIDENT Group[1]

[a] Laboratorio de Ciencias de la Actividad Física, el Deporte y la Salud, Facultad de Ciencias Médicas, Universidad de Santiago de Chile, USACH, Chile
[b] Social and Health Care Research Center, University of Castilla-La Mancha, Cuenca, Spain
[c] School of Education, University of Castilla-La Mancha, Ciudad Real, Spain
[d] The Alamedilla Health Center, Castilla y León Health Service–SACYL, USAL, IBSAL, Salamanca, Spain

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ABSTRACT

Objective: The aim of this study was to analyze the association between sedentary behaviour, as assessed by an accelerometer, and mean carotid intima-media thickness (IMT).
Methods: The study included 263 healthy subjects belonging to the EVIDENT study (59.3% women). Carotid IMT was measured by carotid ultrasonography. Sedentary behaviour was measured objectively over 7 days using ActiGraph accelerometers. Thresholds of 10 consecutive minutes were used to establish sedentary bouts, and assess the number (n/day), and length ≥10 min (min/day).
Results: Total sedentary time and sedentary time in bouts ≥10 min was higher in participants with a larger mean carotid IMT (>P75). Otherwise, this sedentary time in bouts ≥10 min parameter was weakly associated with augmented carotid IMT injury in the logistic regression model.
Conclusion: Total sedentary time and sedentary time in bouts ≥10 min, as assessed by accelerometer, was positively but weakly associated with carotid IMT. Equally, this sedentary time in bouts ≥10 min was associated with carotid injury, but disappears after adjusting for potential confounders. These findings support that reducing sedentary time and increasing breaks in bouts of sedentary time might represent a useful additional strategy in the cardiovascular disease prevention.
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1. Introduction

Carotid intima-media thickness (IMT) is an intermediate phenotype for early atherosclerosis [1]. Because it can be measured relatively simply and noninvasively, it is frequently used in population-based studies. Several longitudinal studies have found a relationship between IMT and future cardiovascular events, particularly cardiac events (myocardial infarction, angina pectoris, coronary intervention) and cerebrovascular events (stroke or transient ischemic attack) [2].

Sedentary behaviour is one of the major risk factors for cardiovascular disease (CVD) [3]. It is defined as any waking behaviour characterized by an energy expenditure ≤1.5 metabolic equivalents while in a sitting or reclining posture [4]. Previous studies have shown that the proportion of sedentary time was associated to metabolic risk and it is also considered a major risk factor for clinical atherosclerosis [5], independent of physical activity [6]. However, evidence to date suggests that interventions aimed to reduce sedentary behaviour are needed to provide a clearer understanding of its impact on health outcomes [7].

Besides measuring total sedentary time, it is important to examine the way in which it is accumulated, especially considering that technological and social factors promote prolonged sitting during working, domestic, and recreational time [8]. It has been suggested that bouts and breaks of sedentary time, independent of

* Corresponding author. Universidad de Castilla-La Mancha, Edificio Melchor Cano, Centro de Estudios Socio-Sanitarios, Santa Teresa Jornet s/n, 16071 Cuenca, Spain.
E-mail address: Vicente.Martinez@uclm.es (V. Martínez-Vizcaíno).
[1] EVIDENT Group. redIAPP: Red de Investigación en Actividades Preventivas y Promoción de la Salud (Research Network on Preventive Activities and Health Promotion), Spain.

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total sedentary time and moderate-vigorous physical activity (MVPA), were not associated with metabolic syndrome and its parameters [9]. In contrast, three cross-sectional studies have shown that adults with a lesser daily number of interruptions in sedentary time (thus, higher length of bouts of sedentary time) have a worse cardiometabolic risk profile, specifically on metabolic syndrome parameters [6,8] and inflammation [10]. These studies illustrate the potential importance of studying patterns of sedentary periods.

To date, few studies have investigated the independent associations of sedentary behaviour with carotid IMT in adult populations and have not reported conclusive evidence [11,12]. Thus while one study has reported that the time spent on sedentary activities (watching television) is not associated with the carotid IMT [11], another study has found a positive relationship between carotid IMT and total sedentary time, independently of age and several atherosclerotic risk factors [12]. A systematic review published by Kadoglou et al. [13] concluded that physical inactivity is associated with an increased IMT, and that it remains questionable whether exercise over the long term is able to slow the development of carotid atherosclerosis. However, it is unclear whether the total sedentary time, the number of bouts of sedentary time or the length of sedentary bouts (e.g. more than 10 min) can exert similar adverse effects on carotid IMT when adjusting for potential confounders.

The objective of our study was to examine the association between the characteristics of sedentary behaviour (number of bouts of sedentary time, mean length of them), as assessed by an accelerometer, and mean carotid IMT.

2. Methods

2.1. Study design

The EVIDENT study is a cross-sectional, multicentre study with participation by six study groups distributed throughout Spain with the purpose of increasing the sample size to increase the external validity of the study (NCTT01083082) [14]. This study aimed to analyze the relationship of physical activity and dietary pattern to the circadian pattern of blood pressure, central and peripheral blood pressure, pulse wave velocity, carotid IMT, and biological markers of endothelial dysfunction in active and sedentary individuals without arteriosclerotic disease.

2.2. Study population

Subjects aged 20–80 years were selected through random sampling from the offices of general practitioners in six primary care centers from six different Spanish provinces (response rate ranged from 65% to 85%, depending on each center). The exclusion criteria were: known coronary or cerebrovascular atherosclerotic disease; heart failure; moderate or severe chronic obstructive pulmonary disease; walking-limiting musculoskeletal disease; advanced respiratory, renal, or hepatic disease; severe mental disease; treated oncological disease diagnosed in the past 5 years; status as a terminal patient; and pregnancy. These criteria were confirmed by the general practitioner based on electronic clinical records and information reported by the patients. From the 1553 subjects included in the EVIDENT study, 263 subjects were recruited from Salamanca, the only center where carotid intima media thickness was measured; this subsample was similar in demographics and biological characteristics to the whole sample. The 263 participants with carotid IMT assessment were estimated to be enough to detect a mean difference of 20 min/day in sedentary time between two of the three categories of IMT in a two-sided test, assuming a common standard deviation (SD) of 45 min/day with a significance level of 0.05 and a statistical power of 80%. The study was approved by an independent Ethics Committee of Salamanca University Hospital (Spain), and all participants gave written informed consent according to the general recommendations of the Declaration of Helsinki [15].

2.3. Measurements

A detailed description has been published elsewhere regarding how the clinical data were collected, the anthropometric measurements were made and the analytical parameters were obtained [14].

Body weight was determined twice using a homologated electronic scale (Seca 770) following calibration (precision ±0.1 kg), with the patient wearing light clothing and no shoes. Height was measured twice with a portable system (Seca 222). The average of the two readings of weight and height was used to calculate body mass index (BMI) as weight (kg) divided by height squared (m^2). A BMI $\geq$30 kg/m^2 was taken to define obesity. Finally, waist circumference was measured using a flexible graduated measuring tape with the patient in the standing position without clothing.

Venous blood sampling was performed between 08:00 and 09:00 h, after the individuals had fasted and abstained from smoking and the consumption of alcohol and caffeinated beverages for the previous 12 h. Blood samples were collected in the respective health centres, and were analyzed at the hospital of the city participating in external quality assurance programmes of the Spanish Society of Clinical Chemistry and Molecular Pathology. Fasting plasma glucose, serum total cholesterol, HDL-cholesterol and triglyceride concentrations were measured using standard enzymatic automated methods. LDL-cholesterol was estimated by the Friedewald equation when the direct parameter was not available. High sensitive C-reactive protein levels and fibrinogen concentrations were determined by immunoturbidimetric assay.

Blood pressure was determined at the clinical setting, with three measurements of systolic blood pressure (SBP) and diastolic blood pressure (DBP), taking the average of the last two. This was performed using a validated OMRON model M10-IT sphygmomanometer (Omron Health Care, Kyoto, Japan) and following the recommendations of the European Society of Hypertension [16]. We calculated the mean arterial blood pressure (MAP) as [(2 * DBP) + SBP]/3.

Carotid ultrasonography to assess carotid IMT was performed by two investigators, blinded to clinical information, trained for this purpose before starting the study. Reliability was evaluated before the study began, using the intraclass correlation coefficient, which showed values of 0.97 (95% CI: 0.94–0.99) for intra-observer agreement on repeated measurements in 20 subjects, and 0.90 (95% CI: 0.74–0.96) for inter-observer agreement. Moreover, in the Bland-Altman analysis, the limit of inter-observer agreement was 0.02 (95% CI: −0.05–0.10) and the limit of intra-observer agreement was 0.01 (95% CI: −0.03–0.06). A Sonosite Micromax ultrasound device paired with a 5–10 MHz multifrequency high-resolution linear transducer with Sonocal software was used for performing automatic measurements of carotid IMT; this technology obtained automatically 120 values, 10 measurements in each of the 12 projections, in order to optimize reproducibility. Measurements were made of the common carotid artery after the examination of a longitudinal section of 10 mm at a distance of 1 cm from the bifurcation, performing measurements in the near wall, and in the far wall in the lateral (90°), anterior (45°) and posterior (135°) projections, following an axis perpendicular to the artery to discriminate two lines, one for the intima–blood interface and the other for the media-adventitia interface, which was synchronized

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with the R-wave peaks on the ECG to avoid possible errors resulting from variable arterial compliance. A total of six measurements were obtained of the right carotid and another six of the left carotid, using mean average values (mean carotid IMT) and maximum average values (maximum carotid IMT) calculated automatically by the software [17]. The measurements were obtained with the subject lying down, with the head extended and slightly turned opposite to the carotid examined, following the recommendations of the Manheim Carotid Intima-Media Thickness Consensus [18]. The mean carotid IMT was considered abnormal if it measured 0.90 mm, or if there were atherosclerotic plaques with a diameter of 1.5 mm or a focal increase of 0.5 mm or 50% of the adjacent IMT [19].

Physical activity (PA) and sedentary behaviour were measured by an ActiGraph GT3X accelerometer (ActiGraph, Shalimar, FL, USA) which has been previously validated [20,21]. ActiGraph is a monitor that uses a piezoelectric acceleration sensor to filter and convert the signals produced from the sensor in samples collected at a preset frequency in hertz. The samples are summed over a user-specified time sampling interval, called an "epoch". Output from the ActiGraph is in the form of activity "counts," where one count is equivalent to 16 milli-g per second, and where g is equal to 9.825 m s^{-2}, the acceleration of gravity). Activity "counts" are recorded to the internal memory of accelerometers by converting acceleration units over a given epoch [22].

Participants wore the accelerometer fastened with an elastic band to the right side of the waist for seven consecutive days with habitual PA, except for bathing and performing activities in the water. All subjects were verbally instructed on how to use the accelerometer. The accelerometer was set to record PA data every minute. The MAHUFFE software (http://www.mrc-epid.cam.ac.uk/research/resources/materials-transfer-disclaimer/physical-activity-downloads/) was used to analyze these data. Sequences of 10 or more consecutive zero counts were considered non-wearing time and excluded from the analyses. Inclusion criteria were a minimum of 4 days of recording, including at least 1 weekend day and at least 600 registered min per day. The main outcome variable from the activity monitor was the average intensity of PA (counts min), calculated with equal weighting given to each day (regardless of registered time per day). The intensity of PA was categorized according to the cut-off points proposed by Freedson [23]: sedentary (<100 counts min), light (100–1952 counts min), moderate (1952–5724 counts min), vigorous (>5724 counts min) and very vigorous (>9498 counts min). Moderate-vigorous activity was considered activity accumulated from all bouts lasting for at least 1 min.

Based on the 10 min bout threshold [24] and to identify specific sedentary activity parameters, four variables of interest were included in this study: (i) total duration of sedentary time (sedentary min valid per day); (ii) sedentary time in bouts ≥10 min (calculated as the sum of all bouts of sedentary time lasting more than 10 or more consecutive min per day); (iii) number of sedentary behaviour bouts (calculated as the sum of all bouts of sedentary behaviour that were performed in 10 or more consecutive min per day).

Smoking history was assessed through questions on smoking status (current smokers or non-smokers) and alcohol consumption in the last week (g per week).

3. Statistical analysis

All statistical analyses were performed using IBM SPSS statistical software (Software, v.22.0 SPSS Inc., Chicago, IL, USA), and the level of significance was set at $\alpha < 0.05$. Descriptive characteristics are shown as mean ± standard deviation, and frequency distribution for categorical data. Variables were checked for normality of distribution using both graphical (normal probability plots) and statistical (Kolmogorov–Smirnov test) procedures. Due to their skewed distribution, BMI, high-density lipoprotein cholesterol, triglycerides, and fasting plasma glucose were log-transformed before to be included in the models as covariates. No missing data were found.

ANCOVA models were estimated to test differences in the mean of several indicators of sedentary time (total sedentary time, sedentary time in bouts ≥10 min, and number of bouts of sedentary behaviour) by categories of carotid IMT (low = quartile 1; medium = quartiles 2 and 3; high = quartile 4). Three sets of variables were successively entered in the multivariate models: i) sociodemographic and physical activity data (model 1); ii) lifestyle variables and cardiovascular risk factors (model 2); and iii) inflammatory process-related variables (model 3). First, we examined the crude differences and then we estimated three models adjusting for age, sex, MVPA, time accelerometer worn, and total sedentary time when number of bouts of sedentary behaviour was the dependent variable in a first step (model 1); also including smoking habit, drinking habit, MAP, heart rate, BMI, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, and baseline blood glucose in a second step (model 2); and finally adding C-reactive protein, and plasma fibrinogen in model 3. Pairwise post-hoc comparisons were examined using the Bonferroni test. Effect sizes (ES) were calculated and interpreted using Cohen's categories as follows: small = $0 \leq |d| \leq 0.5$; medium = $0.5 < |d| \leq 0.8$; and large = $|d| > 0.8$ [25].

Finally, logistic regression models were estimated using a carotid IMT injury (no = 0; yes = 1) as the dependent variable, sedentary behaviour variables as independent variables in a similar way to that shown in the above four models. The data are presented as odds ratio (OR) with 95% confidence intervals (CI). The Hosmer–Lemeshow goodness-of-fit test was used to evaluate how well the observed event rates match expected event rates in subgroups of the model population.

4. Results

We studied 263 subjects with a mean age of 55.8 ± 12.2 years, of whom 59.3% were female. Table 1 shows the demographic and clinical characteristics of the study participants. Both mean carotid IMT and mean sedentary time were significantly higher in men than in women.

Mean differences in sedentary time variables according to carotid IMT categories are shown in Table 2. Participants in the first carotid IMT category accumulated less total sedentary time (ES = −0.26 to −0.87) and sedentary time in bouts ≥10 min (minute/day) than participants in the high category (ES = −0.36 to −0.60), and statistical significance remained in all models.

Logistic regression models were tested adjusting for age, sex, MVPA, and time accelerometer worn, in a first step (model 1); also including smoking habit, drinking habit, MAP, heart rate, BMI, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, and baseline blood glucose in a second step (model 2); and finally adding C-reactive protein, and plasma fibrinogen in model 3. These are shown in Table 3. Only sedentary time in bouts ≥10 min was associated with presence of augmented carotid IMT injury in the first model (OR = 1.010, p = 0.010), but the statistical significance disappeared when additional covariates were added in the subsequent models. The Hosmer–Lemeshow test provides a p-value of 0.928 ($\chi^2 = 3.169$), showing no evidence of lack of fit.

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Table 1
Cardiovascular risk profile and physical activity patterns of the sample, by sex.

	Total (n = 263)	Men (n = 107)	Women (n = 156)	*p*
Age, mean (SD), years	55.8 (12.2)	55.5 (12.5)	56.1 (12.0)	0.933
Covariates				
Smokers, n (%)	53 (20.1)	23 (21.5)	30 (19.2)	0.757
Drinkers, n (%)	18 (6.8)	11 (10.3)	7 (4.5)	0.134
Mean arterial pressure, mmHg	92.5 (12.5)	97.8 (10.9)	88.8 (12.2)	<0.001
Heart rate, bpm	68.7 (10.7)	66.9 (11.9)	69.9 (9.7)	0.068
BMI, kg/m^2	27.3 (4.3)	27.8 (3.9)	26.9 (4.5)	0.220
LDL-C, mg/dL	132.3 (33.5)	133.2 (35.3)	132.9 (32.2)	0.857
HDL-C, mg/dL	58.9 (15.0)	50.3 (11.1)	64.1 (15.9)	<0.001
Triglycerides, mg/dL	110.4 (69.8)	1333.2 (89.1)	94.9 (47.3)	0.857
Glucose, mg/dL	88.1 (14.9)	88.7 (14.7)	87.7 (15.1)	0.936
Insulin, μU/mL	6.2 (6.3)	5.9 (4.8)	6.3 (7.0)	0.342
Waist circumference, cm	93.5 (11.5)	98.3 (10.9)	90.3 (10.7)	<0.001
C-reactive protein, mg/dL	0.3 (0.5)	0.2 (0.2)	0.4 (0.6)	0.056
Plasma fibrinogen, mg/dL	352.8 (70.4)	338.8 (69.6)	361.7 (69.8)	0.225
Common carotid artery ultrasound				
Carotid IMT, mm	0.7 (0.1)	0.7 (0.1)	0.6 (0.1)	0.011
Carotid injury[a], n (%)	22 (8.4)	16 (14.9)	6 (3.8)	0.116
Accelerometer variables				
Time accelerometer worn, hours	156.7 (30.9)	159.4 (24.7)	154.7 (34.3)	<0.001
MVPA, min/day	50.4 (32.3)	59.3 (34.6)	44.7 (29.6)	0.005
Total sedentary time, min/day	507.6 (120.8)	548.9 (118.5)	481.5 (115.3)	<0.001
Sedentary time in bouts ≥10 min, min/day	390.0 (151.6)	387.7 (145.6)	391.4 (155.9)	0.881
Bouts in Sedentary time (≥10 min), n/day	14.4 (4.8)	14.2 (3.9)	14.5 (5.3)	0.628

Values are means (SD) except when indicated.
BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; IMT, intima-media thickness; LDL-C, low-density lipoprotein cholesterol; MVPA, moderate-vigorous physical activity; VPA, vigorous physical activity; SD, standard deviation.
[a] Carotid injury: if the mean carotid IMT was >0.9 mm and/or there were arteriosclerotic plaques (carotid IMT ≥1.5 mm).

Table 3
Sedentary behaviour and increased carotid injury thickness (if the mean carotid IMT was >0.9 mm and there were atherosclerotic plaques [carotid IMT ≥ 1.5 mm]) in non-conditional logistic regression models.

	Or	IC 95%	*p*
Model 1			
Total sedentary time, min/day	1.002	0.997 to 1.008	0.056
Sedentary time in bouts ≥10 min, min/day	1.010	1.002 to 1.019	0.010
Bouts in Sedentary time (≥10 min), n/day[#]	1.096	0.924 to 1.299	0.292
Model 2			
Total sedentary time, min/day	1.005	0.995 to 1.041	0.366
Sedentary time in bouts ≥10 min, min/day	1.001	0.991 to 1.009	0.462
Bouts in Sedentary time (≥10 min), n/day[#]	1.046	0.921 to 1.172	0.363
Model 3			
Total sedentary time, min/day	1.003	0.989 to 1.062	0.121
Sedentary time in bouts ≥10 min, min/day	1.002	0.993 to 1.014	0.705
Bouts in Sedentary time (≥10 min), n/day[#]	1.014	0.941 to 1.049	0.741

Model 1: adjusted for age, sex, moderate-vigorous physical activity, time accelerometer worn, and [#]total sedentary time when number of bouts in sedentary time was the dependent variable. Model 2: adjusted for the same covariates as Model 1 and smoking habit, drinking habit, mean arterial pressure, heart rate, body mass index, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, and baseline blood glucose. Model 3: adjusted for the same covariates as Model 1 and 2, C-reactive protein and plasma fibrinogen.

5. Discussion

There is convincing evidence about an inverse relationship between time spent performing PA and carotid IMT [26–28], but evidence about the association between sedentary behaviour and this early marker of atherosclerotic disease is scarce. In this cross-sectional study, accumulated daily total sedentary time and sedentary time in bouts ≥10 min were positively and weakly associated with carotid IMT. However, when controlling for potential confounders like MVPA and inflammatory markers, sedentary time in bouts ≥10 min was no longer associated with the presence of carotid injury. Our results are in line with other studies suggesting that not only is the amount of total sedentary time associated to carotid IMT, but also the manner in which it is accumulated [6,8].

The evidence for an independent effect of sedentary behaviour

Table 2
Mean differences in sedentary time variables by carotid intima-media thickness (IMT) categories controlling for potential confounders.

	Carotid IMT				Effect size[a]		
	Low (<0.61 mm) n = 69	Medium (0.61–0.74 mm) n = 124	High (>0.74 mm) n = 70	P	Low-medium	Low-high	Medium-high
Crude data							
Total sedentary time, min/day	451.0 ± 114.9	498.8 ± 120.4	546.9 ± 112.3	0.003	−0.41	−0.87**	−0.41
Sedentary time in bouts ≥10 min, min/day	350.4 ± 151.8	398.1 ± 151.7	434.4 ± 125.1	0.007	−0.31	−0.60*	−0.25
Bouts in Sedentary time (≥10 min), n/day	13.4 ± 4.5	14.6 ± 5.0	16.1 ± 4.2	0.132	−0.25	−0.62	−0.32
Model 1							
Total sedentary time, min/day	468.3 ± 105.4	501.5 ± 118.1	564.4 ± 117.7	0.006	−0.29	−0.85**	−0.53
Sedentary time in bouts ≥10 min, min/day	354.9 ± 153.3	382.5 ± 151.3	435.3 ± 122.8	0.011	−0.19	−0.59*	−0.38
Bouts in Sedentary time (≥10 min), n/day[#]	13.36 ± 4.64	14.3 ± 4.99	15.8 ± 4.19	0.221	−0.19	−0.55	−0.32
Model 2							
Total sedentary time, min/day	465.5 ± 100.6	501.8 ± 122.2	548.8 ± 119.8	0.010	−0.31	−0.75*	−0.39
Sedentary time in bouts ≥10 min, min/day	349.5 ± 160.1	388.9 ± 152.4	417.7 ± 120.2	0.013	−0.25	−0.48*	−0.21
Bouts in Sedentary time (≥10 min), n/day[#]	13.2 ± 5.09	14.5 ± 5.31	15.4 ± 4.23	0.315	−0.25	−0.47	−0.18
Model 3							
Total sedentary time, min/day	466.2 ± 130.5	468.2 ± 138.0	495.3 ± 89.4	0.045	−0.01	−0.26*	−0.22
Sedentary time in bouts ≥10 min, min/day	351.1 ± 161.3	390.2 ± 153.9	402.1 ± 121.8	0.041	−0.25	−0.36*	−0.09
Bouts in Sedentary time (≥10 min), n/day[#]	13.7 ± 4.85	14.4 ± 5.03	15.1 ± 4.26	0.568	−0.14	−0.31	−0.15

Values are means (SD). *p < 0.05; **p < 0.01.
Model 1: adjusted for age, sex, moderate-vigorous physical activity, and time accelerometer worn, and [#]total sedentary time when number of bouts in sedentary time was the dependent variable. Model 2: adjusted for the same covariates as Model 1 and smoking habit, drinking habit, mean arterial pressure, heart rate, body mass index, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, and baseline blood glucose. Model 3: adjusted for the same covariates as Model 1 and 2, C-reactive protein and plasma fibrinogen.
[a] Pairwise comparisons by using Bonferroni's post hoc hypothesis tests for multiple comparisons.

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is not fully convincing [7], so intrinsic mechanisms linking the absence of body movement with chronic disease risk factors is far from being elucidated [29]. In the same way, the relationship between sedentary time and carotid IMT is a controversial issue, since while one study reported a positive association, independent of age [12], another found no association [11]. Overall, our data show that total sedentary time is weakly associated with carotid IMT, supporting the findings of RISC study [12]. Indeed, other sedentary time related variables such as accumulated sedentary time in bouts $\geq$10 min are associated with this early marker of atherosclerosis. Even though the effect size of the differences among categories of IMT decreased, the associations after adjustment for several potential confounders including traditional cardiovascular risk factors and MVPA remained significant, but attenuated.

Little is known about how sedentary behaviour impacts on deterioration in the carotid IMT and the metabolic risk associated. Thus, some studies have reported that breaks in sedentary time were associated with some components of metabolic syndrome (i.e. waist circumference or triglycerides levels) [6,8], and inflammatory markers [30], after adjusting for total sedentary time and/or PA. In contrast, other studies did not find any association between sedentary behaviour (total sedentary time, bouts and breaks) and metabolic syndrome and its factors after adjusting for time spent in MVPA [9]. Another prospective study has reported that time spent at MVPA is associated with indicators of insulin resistance independent of time spent sedentary [31]. Our data show that sedentary time in bouts $\geq$10 min is faintly associated with carotid IMT injury (if the mean carotid IMT was >0.9 mm and there were atherosclerotic plaques [carotid IMT $\geq$ 1.5 mm]). The importance of this analysis is due to several reports have documented an association between carotid IMT and/or plaque with stroke, transient ischemic attacks, and other clinical manifestations of CVD [1,32]. Therefore, our study examines if the manner in which sedentary time is accumulated is relevant when analyzing the association between sedentariness and atherosclerotic process. However, this association disappears after adjusting for sociodemographic, lifestyle and other potential confounders. This could be due to the high correlation between inflammation markers and the presence of carotid injury [33]. Therefore, these inflammatory markers could be an intermediate variable between sedentary variables and carotid injury. These findings, along with those of other studies, suggest the importance of avoiding prolonged uninterrupted periods of sedentary time to prevent atherosclerotic progression, not forgetting the MVPA.

In conclusion, this study, in a small-sized population-based sample, provides preliminary evidence that total sedentary time and sedentary time in bouts $\geq$10 min, as assessed by accelerometer, was positively but weakly associated with carotid IMT. Equally, this sedentary time in bouts $\geq$10 min was associated with carotid injury, but disappears after adjusting for MVPA and other potential confounders. Our findings have methodological importance, in providing a better understanding of the components of sedentary behaviour that are associated with atherosclerotic progression. Likewise, these results suggest the importance of avoiding prolonged uninterrupted periods of sedentary time, and highlight the need for new public health recommendations regarding breaking up sedentary time that should be complementary to those for PA.

5.1. Limitations

This study has several limitations. First, the cross-sectional design prevents us from establishing a causal relationship between sedentary behaviour and carotid IMT. Further, the sample size was too small to control for the large number of potential confounders measured in the whole EVIDENT sample. Second, the carotid IMT and the presence of plaques were evaluated by carotid ultrasonography; however three-dimensional ultrasound use may provide more accurate measurements of both area and volume of the plaque [34,35]. Third, because of the limited sample size, other behavioural variables such as diet have not been considered for this cross-sectional analysis. However, as far as we know, this is the first study to relate several objectively measured sedentary associated variables and carotid IMT, controlling for classical and emergent atherosclerotic risk factors; moreover, in the logistic regression models this weakness is much more evident, since although the sample size was also 263 participants, only 22 of them showed carotid IMT injury. Fourth, the short interruption period due to non-wear time (i.e. 10 min) was likely to underestimate sedentary time [36]. Usually, most Spanish people are used to napping for 20–40 min, and therefore this sedentary time could be not considered when a greater non wear-time (e.g. 60 min) was set-up during data reduction. Finally, despite the lack of consensus on the required amount of sedentary time needed to negatively influence the health of adults, we decided to take into account only sedentary bouts of at least 10 min, in a similar way to recommendations about physical activity.

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