Original Article (short paper)

Relationship between carotid intima-media thickness, physical activity, sleep quality, metabolic/inflammatory profile, body fatness, smoking and alcohol consumption in young adults

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Abstract — Aim: The aim of this longitudinal study was to analyze the relationship between sleep disorder and intima-media thickness. Method: Baseline measurements included carotid intima-media thickness, assessed by an ultrasound device; questionnaires about sleep and other behavioral variables; physical activity was measured by pedometer; body fatness was estimated by Dual-Energy X-ray Absorptiometry; fasting glucose, lipid profile and C-reactive protein were collected. Results: The occurrence rate of sleep-related disorders was 47% (95%CI= 37.2%-56.7%). Carotid intima-media thickness was related to symptoms of insomnia (r= 0.328 [0.141 to 0.493]) and, after adjustments for potential confounders, the relationship between carotid intima-media thickness and insomnia remained statistically significant (β= 0.121 [95%CI= 0.017; 0.225]). Conclusions: In young adults, sleep disorder was significantly related to premature increase in carotid intima-media thickness.

Keywords: Atherosclerosis, insulin resistance, lipid profile, diabetes mellitus.

Introduction

Sleep is a fundamental condition into the circadian rhythm, especially for the restoration of physiological systems1. Scientific literature generally states that humans spend about one-third of their time sleeping2. On the other hand, studies have shown that sleep time has decreased in different countries3-5 and insomnia is the most common sleep disorder in young and middle-aged adults6.

Recently, studies have shown that sleep disorders in adults have been associated with a large variety of diseases, such as obesity, arterial hypertension, dyslipidemia, and diabetes mellitus (DM)7-9, as well as increased cardiovascular morbidity/mortality10,11. Nowadays, the harmful effects associated with sleep disorders happen among several age groups, besides the elderly12. Recent studies have consistently demonstrated a close relationship between sleep disorder and cardiovascular diseases in different age groups, including adolescents4,5,12.

In clinical practice, intima-media thickness, mainly carotid (CIMT), has been used as a marker of subclinical atherosclerosis13. In fact, increased CIMT has been associated with aging, but also with sleep disorders, such as obstructive sleep apnea, short sleep and long sleep13,14. However, the actual burden of sleep disorders over CIMT in young adults remains unclear because its progression is affected by other potential confounders, such as sex, age, and dyslipidemia13. Moreover, the effect of other behavioral variables, like physical activity, alcohol consumption and smoking, on the possible relationship between CIMT and insomnia / hypersomnia is not very well explored.

Thus, the aim of this study was to analyze the relationship between sleep disorders (insomnia and hypersomnia), behavioral variables and intima-media thickness in young adults.

Methods

Subjects

The present study was part of the longitudinal study titled “Maintenance of physical activity throughout life and vascular stiffness in adults: cross-sectional analysis and cohort of 12 months”. The present data are from the baseline measurements, which happened during the second half of 2013, and involved a sample of 100 adults of both genders, aged 30-50 years old. Inclusion criteria were defined as: (i) age between 30-50 years old; (ii) no previous history of stroke or myocardial infarction; (iii) no amputation or visual problems related to diabetes mellitus; (iv) no physical limitation that could affect physical activity engagement; (v) either presence or absence of sports participate on in both childhood and adolescence; (vi) sign consent form to participate.
The sample was composed of university employees (faculty, office, and general services staff) and people engaged in sports clubs of the city. The university staff composed 50% of all participants, were randomly selected and invited to participate in the study. Initially, 122 subjects were accepted to participate in the present study, but after a face-to-face interview and carefully checking the inclusion criteria, the final sample was composed of 100 subjects of both genders (54 men and 46 women). Experimental procedures were approved by the Ethical Research Group of the Universidade Estadual Paulista (UNESP), Presidente Prudente, Brazil (CAAE: 07770112.3.0000.5402).

Dependent variable

Carotid intima-media thickness measurements

A Doppler ultrasound device (Toshiba Xario, SSA-660A) was used to assess CIMT. All tests were performed at the hospital during the morning, from 8:00 – 11:00 am, and the analyses were performed by a medical doctor specialized in diagnostic for imaging. The procedures adopted for the exams followed the recommendations of the Brazilian Society of Cardiology. Prior to the tests, all subjects were resting in a supine position in a quiet and acclimatized room. To measure CIMT, the neck was positioned in hyperextension and slightly inclined at 45°. The measurements were carried out in the posterior wall, farthest from the transducer, manually and with the caliper method. Three measurements were obtained in the stretch of 15 mm focal free plates, where the pattern is clearly observed. For analysis of the results, we considered mean values in millimeters (mm).

Independent variable

Quality of sleep

The quality of sleep was assessed by the Mini-Sleep Questionnaire (MSQ), previously validated for Brazilian Portuguese. The questionnaire consisted of 10 questions with seven possible answers (never = 1, very rarely = 2, rarely = 3, sometimes = 4, often = 5, very often = 6 and always = 7). The sum of the 10 responses generated a numerical score, which was organized into four categories: good sleep (score 10 to 24 points), moderately altered sleep (score between 25 and 30 points) and very altered sleep (score above 30 points), slightly altered sleep (score between 25 and 27 points), moderately altered sleep (score between 28 and 30 points) and very altered sleep (score above 30 points). Symptoms of insomnia were analyzed by four questions (difficulty in falling asleep, having trouble sleeping, being restless during sleep and feeling tired after awakening). Furthermore, the numerical score was analyzed in three ways: overall (sum of the four questions related to insomnia) and hypersomnia (sum of the six questions related to insomnia).

Potential confounders

General variables

Body fat (in percentage) was assessed using a dual-energy x-ray absorptiometry (DXA) scanner (Lunar DPX-NT; General Electric Healthcare, Little Chalfont, Buckinghamshire, UK) with GE Medical System Lunar software (version 4.7). DXA measures were performed during the morning after a light breakfast, and the scanner quality was tested by a trained researcher before each day of measurement, following the manufacturer’s recommendations. The participants wore light clothing, without shoes and remained in the supine position on the machine (approximately 15 min).

Chronicological age, sex, and race were registered. Systolic and diastolic blood pressures (SBP and DBP) were measured indirectly by auscultation using a sphygmomanometer (Bic®) and stethoscope (Classic II SE, Littmann®) at the laboratory in a controlled temperature room according to standardized protocols. The number of cardiovascular diseases/events (e.g. arterial hypertension, diabetes mellitus, stroke and heart attack) in the family (father, mother, brothers) was registered and used as a proxy of family history of cardiovascular diseases.

Metabolic/Inflammatory variables

Blood samples were collected and biochemical analyses were performed in a private laboratory, which reaches the quality control adopted by the Brazilian Health Ministry. Blood samples were collected after 12-hour fasting. High sensitive C-reactive protein (hsCRP) was determined using an enzyme-linked immunoturbidimetric assay kit (Millipore, St Charles, MO [inter- and inter-assay coefficients of variation were 4.6 and 6.0%, respectively]). Fasting glucose was managed by an enzymatic colorimetric kit and processed in a unit Autohumalyzer A5+. Blood levels of insulin were estimated by chemiluminescence according to Burtis, Ashwood, Bruns. HOMA-IR was obtained considering fasting blood glucose (mmol/L) and fasting blood insulin (IU/mL), using the following formula: (glucose* insulin) ÷ 22.5. Glycated hemoglobin (HbA1C) was obtained from blood samples and stored in vacuum sealed tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant lyophilized. The determination of the glycated hemoglobin was performed in a primary tube by high-performance liquid chromatography (HPLC) equipment in D10 - Hemoglobin A1C Testing System (Bio-Rad®, France).

Behavioral variables

Current physical activity was assessed using pedometers (Digi-Walker Yamax, SW200), which were fixed laterally at the hip.
Participants were instructed to remove the device while sleeping, showering and performing aquatic activities. The pedometer was used for seven consecutive days. At the end of each day, the participants recorded the number of steps taken throughout the day. Each morning, the “reset” button was pushed to zero out the device and start collecting new data. The average number of steps for the week was assigned as the level of habitual physical activity.

Smoking was analyzed according to the number of cigarettes smoked per day (cigarettes/day). Alcohol consumption was assessed by self-report of the frequency of alcohol consumption in a common week (never = 1, sometimes = 2, often = 3, very often = 4 and always = 5). Skipping meals was analyzed through the frequency of eating meals like breakfast, dinner, and lunch.

Statistical analysis

Mean and standard deviation (SD) were used as descriptive statistics. Independent sample t-tests were used to compare participants according to sleep quality (good and disturbed). Measurements of effect size were provided by the Cohen’s d test (Effect size: Small ≥0.20, Medium ≥0.50, Large ≥0.80 and Very Large ≥1.30). Pearson correlation was used to analyze the relationship between CIMT, MSQ scores, and all independent variables. Finally, the linear regression model was used to analyze the relationship between CIMT, MSQ scores, and all independent variables. The variables significantly related to CIMT in the Pearson correlation were inserted simultaneously in the multivariate model. In this final multivariate model, CMIT and insomnia were both logarithm transformed. All analyses were performed by the statistical software BioEstat (release 5.0 [BioEstat, Tefé, Amazonas]) and statistical significance was set at 5%.

Results

In the present study, the occurrence of sleep-related disorders was 47% (95%CI= 37.2% - 56.7%) (Table 1). Participants reporting sleep-related disorders had significantly higher values of HOMA-IR (Medium effect size), body fat (Medium effect size) and CIMT (Small effect size) than those without sleep disorders. HbA1c was marginally significant (p-value= 0.090), but presented very large effect size. Moreover, the current physical activity level was 25% significantly higher in adults without sleep-related disorders than those with sleep-related disorders (Medium effect size).

Table 1. General characteristics of the sample stratified by sleep quality among young adults.

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>Good Sleep (n= 53)</th>
<th>Disturbed Sleep (n= 47)</th>
<th>Cohen’s d (effect size)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>30 / 23</td>
<td>24 / 23</td>
<td>---</td>
</tr>
<tr>
<td>Age (years)</td>
<td>38.7 (3.8)</td>
<td>39.7 (5.8)</td>
<td>-0.026 Small</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>90.9 (9.1)</td>
<td>98.2 (32.6)</td>
<td>-0.313 Small</td>
</tr>
<tr>
<td>Insulin</td>
<td>5.6 (3.2)</td>
<td>7.6 (4.6)*</td>
<td>-0.510 Medium</td>
</tr>
<tr>
<td>hsCRP (mg/dL)</td>
<td>3.1 (3.9)</td>
<td>3.1 (3.9)</td>
<td>-0.225 Small</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.29 (0.84)</td>
<td>1.92 (1.51)*</td>
<td>-0.524 Medium</td>
</tr>
<tr>
<td>Steps/day</td>
<td>9731.4 (3746.3)</td>
<td>7743.9 (3181.1)*</td>
<td>0.569 Medium</td>
</tr>
<tr>
<td>BF (%)</td>
<td>29.4 (11.3)</td>
<td>34.8 (9.9)*</td>
<td>-0.506 Medium</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>110.7 (12.9)</td>
<td>111.6 (10.6)</td>
<td>-0.076 Trivial</td>
</tr>
<tr>
<td>CMIT (mm)</td>
<td>0.63 (0.15)</td>
<td>0.70 (0.14)*</td>
<td>-0.481 Small</td>
</tr>
</tbody>
</table>

*= Student t test with p-value < 0.05; CIMT= carotid intima media thickness; HOMA-IR= homeostasis model assessment – insulin resistance; BF= body fatness; hsCRP= high sensitivity C-reactive protein; SBP= systolic blood pressure; DBP= diastolic blood pressure.

CIMT was positively related to smoking habit, alcohol consumption, age and SBP (correlations ranging from r=0.19 to r=0.34 [Table 2]). Body fat and HOMA-IR were related to insomnia. Higher physical activity level was significantly related to better quality of sleep (r= -0.29; p-value= 0.001) and lower symptoms of hypersonia (snoring, feeling tired after awakening, excessive daytime sleepiness, and restless sleep [r= -0.30; p-value= 0.002]), while higher age was related to increased symptoms of insomnia (difficulty in falling asleep, mid-sleep awakenings, early awakening, and use of sleep medication [r= 0.19; p-value= 0.047]).

Table 2. Relationship between intima-media thickness, sleep and metabolic, inflammatory, behavioral variables among young adults.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>CIMT r (95%CI)</th>
<th>MSQ overall r (95%CI)</th>
<th>MSQ Insomnia r (95%CI)</th>
<th>MSQ Hypersonia r (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behavioral variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical activity (mean steps/day)</td>
<td>-0.09 (-0.28 to 0.10)</td>
<td>-0.29 (-0.46 to -0.09)*</td>
<td>-0.19 (-0.37 to -0.01)</td>
<td>-0.31 (-0.46 to -0.11)*</td>
</tr>
<tr>
<td>Smoking (cigarettes/day)</td>
<td>0.34 (0.15 to 0.50)*</td>
<td>0.13 (-0.06 to 0.31)</td>
<td>0.08 (-0.11 to 0.27)</td>
<td>0.14 (-0.05 to 0.32)</td>
</tr>
<tr>
<td>Alcohol (days/week)</td>
<td>0.20 (0.01 to 0.38)*</td>
<td>0.07 (-0.12 to 0.26)</td>
<td>0.17 (-0.02 to 0.35)</td>
<td>0.01 (-0.18 to 0.20)</td>
</tr>
</tbody>
</table>
Control variables

<table>
<thead>
<tr>
<th>Indicator</th>
<th>β</th>
<th>(β 95%CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA-IR</td>
<td>0.20</td>
<td>(0.01 to 0.38)*</td>
<td>0.20 (0.01 to 0.38)*</td>
</tr>
<tr>
<td>hsCRP (mg/dL)</td>
<td>-0.02</td>
<td>(-0.22 to 0.16)</td>
<td>0.15 (-0.04 to 0.33)</td>
</tr>
<tr>
<td>BF (%)</td>
<td>0.20</td>
<td>(0.01 to 0.38)*</td>
<td>0.20 (0.01 to 0.38)*</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.01</td>
<td>(-0.20 to 0.18)</td>
<td>0.31 (0.12 to 0.47)*</td>
</tr>
<tr>
<td>Age (yes)</td>
<td>0.26</td>
<td>(0.06 to 0.43)*</td>
<td>0.04 (-0.15 to 0.23)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>0.19</td>
<td>(0.01 to 0.38)*</td>
<td>0.10 (-0.10 to 0.09)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>0.18</td>
<td>(-0.01 to 0.36)</td>
<td>0.10 (-0.09 to 0.29)</td>
</tr>
</tbody>
</table>

Sleep variables

<table>
<thead>
<tr>
<th>Indicator</th>
<th>β</th>
<th>(β 95%CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSQ overall</td>
<td>0.20</td>
<td>(0.01 to 0.38)*</td>
<td>---</td>
</tr>
<tr>
<td>MSQ insomnia</td>
<td>0.30</td>
<td>(0.11 to 0.46)*</td>
<td>---</td>
</tr>
<tr>
<td>MSQ hypersomnia</td>
<td>0.10</td>
<td>(-0.09 to 0.29)</td>
<td>---</td>
</tr>
</tbody>
</table>

CIMT was positively related with bad sleep quality (r= 0.20 [0.01 to 0.38]) and increased symptoms of insomnia (r= 0.30 [0.11 to 0.46]). On the other hand, in the multivariate model (after adjustments), only the relationship between CIMT and insomnia remained statistically significant (Table 3). Similarly, smoking and cluster of behaviors still remained related to CIMT.

### Discussion

The main results of the present study show that insomnia in young adults is significantly related to higher carotid intima-media thickness. There are compelling evidences supporting that over the last decade, sleep time has decreased in both sexes independently of age\(^23\). Moreover, more than 30% of the American population reports sleep disorders\(^23\). The disorders related to sleep do not allow adequate biological rest and have consequences in the daily energy demands. Insomnia, hypersomnia and somnambulism are some of the examples that can cause this type of disorders\(^a\). This alarming background is also observed in developing nations, such as Brazil. In Sao Paulo, the most industrialized Brazilian city, the number of adults reporting sleep-related disorder increased significantly between 1987 and 2007\(^24\). In addition, Bittencourt, Santos-Silva, Taddei, Andersen, de Mello, Tufik\(^25\) described that 35% of the Brazilian adults have insomnia. Findings of the present study are consistent with the previous research claiming effective strategies to promote healthy sleep habits in developing countries.

Cumulative evidence suggests an important link between sleep disorders and cardiovascular diseases\(^26-28\). Patients with sleep disorders have high cardiovascular risk profile, given the higher prevalence of conventional risk factors including obesity, diabetes mellitus, and hypertension\(^29,30\). Fox and colleagues found that patients with sleep disorders but free of cardiovascular diseases and conventional risk factors, such as body mass index, age and sex, presented a significant correlation when compared to controls. These findings prove that confounders, such as age, sleep disturbances, conventional risk factors (unhealthy behaviors [e.g. alcohol consumption and smoking] and higher food intake) and cardiovascular diseases, are notably linked, being able to favor the development of some pathological condition that affects health of the people.

Regarding physical activity, there was no correlation with sleep disturbances. However, this variable is considered...
a protective factor for several pathological conditions, as evidenced by Søren Spörndly-Nees and colleagues. The authors found that women who performed physical activity at moderate to high intensity during 10 years showed a protective factor against insomnia, independently of psychological stress, age, body mass index, smoking, alcohol consumption or educational level.

Results of the present study revealed that SBP and age were positively related to CMIT, while age and insomnia were also positively related to each other. Insomnia is defined as sleep difficulties and aging is commonly associated with insomnia diagnosis. At the same time, insomnia is linked to cardiovascular disorders and depressive symptoms, mainly in elderly. The autonomic nervous system stimulates blood pressure according to the metabolic demand of the movement aiming vasoconstriction and acceleration of cardio functions of the sympathetic nervous system, producing a reciprocal inhibition of the parasympathetic vagal signals on the parasympathetic vascular effects. Thus, when both effects are combined they generate an increase in blood pressure. Studies have demonstrated how sympathetic system activities, such as catecholamines and the period of cardiac pre-ejection, decrease with the sleep progression, whereas in the parasympathetic measurements variables, such as the RR interval, change as early as two hours before sleep time.

The biological processes during aging affect the circadian rhythms and, hence, body temperature and secretion of melatonin, which affect sleep quality. Moreover, older people present increased sympathetic activity and decreased parasympathetic activity, accompanied by weight gain, which are relevant risk factors for high blood pressure and increased CIMT. In agreement with other studies, our sample had age ranging from 30-50 years old, and insomnia and CIMT were significantly related. This finding identifies that insomnia seems to affect cardiovascular health not only in elderly, but also among young adults suggesting that these symptoms occur from early adulthood to older ages, and thus, preventive actions targeting improvement of sleep quality in young populations seem to be relevant to prevent diseases among elders in the future.

Among young adults, smoking and alcohol consumption were behaviors related to higher CIMT. Cigarette smoking has been linked to the development of atherosclerosis due to its harmful action in different pathways related to the deposition of lipids in the arterial wall and increases the production of reactive oxygen species, which is closely linked to insulin resistance, significant decreases bioavailability of nitric oxide, and increases lipid oxidation and recruitment of adhesion molecules. Moreover, this habit seems to intensify the natural effect of aging in the vascular wall. The physiological mechanism linking alcohol consumption and atherosclerosis is similar to smoking and depends on the amount of alcohol ingested. While low alcohol ingestion is related to protective cardiovascular effect mainly red wine ingestion, high alcohol ingestion is related to insulin resistance and subsequent oxidative stress.

Separately, cigarette smoking and alcohol consumption were not related to insomnia and in the final multivariate model both variables did not demonstrate statistical significance, but when grouped together, there was significant relationship with insomnia (r = 0.199 [0.003 to 0.381]) and the relationship with CIMT increased slightly (r = 0.242 [0.048 to 0.419]). Therefore, our findings seem to identify that clustering of unhealthy behavioral constitutes relevant risk factor related to insomnia and atherosclerosis in young adults, which should be avoided in this population due to its characteristics of maintenance throughout life.

The present study revealed a significant relationship between insomnia and CMIT independently of age, SBP, cigarette smoking and alcohol consumption, but also identified that insomnia was related to body fat and insulin resistance. There is limited data about insomnia, but it is well documented that other sleep disturbances and shorter duration of sleep are factors associated with higher weight gain and dyslipidemia. Magee, Huang, Iverson, Caputi identified three possible pathways by which sleep and obesity are linked: i) alterations in neuroendocrine and metabolic functioning (sleep deprivation affects cortisol levels, reduces leptin and increases ghrelin in bloodstream), ii) alteration in glucose regulation (sleep deprivation leads to insulin resistance and, hence, dyslipidemia) and iii) modifications in waking behaviors (during waking time, there is sedentary behavior [e.g. watching TV and computer usage] and higher food intake). However, it is unclear the causality between obesity and development of sleep insomnia and, therefore, further research is needed, particularly with a prospective design.

Diabetes mellitus (DM) is a progressive and complex disease that is tightly associated with heterogeneous metabolic disorders, particularly in glucose and lipid metabolism. In patients with DM, the production of insulin is limited and the cells do not respond adequately to the stimuli, exhibiting insulin resistance, which causes a dangerous high concentration of glucose in the blood and can trigger metabolic deficits that are harmful to health. Regarding our findings from the HOMA-IR and HbA1, it has been evidenced that the results can be directly related to the genotype and daily behavioral habits. These two components increase the chances of developing a metabolic disorder, such as DM. Eun-Jung Rhee and colleagues analyzed the association between baseline lipoprotein and DM risk after four years of follow-up, and found that low baseline lipoprotein levels led to a greater predisposition to develop DM. Despite the results of current studies, it is still sought to clarify how sleep disorders can directly or indirectly influence the development of pathological conditions.

Limitations should be recognized. Despite being based on the hypothesis with consistent biologic plausibility, the main limitation of the study is its cross-sectional design, disabling cause-effect relationships. Moreover, although pedometer constitutes an interesting tool to measure physical activity, the device does not provide data about intensity, which could affect the relationship between physical activity and sleep quality. On the other hand, neck circumference was not measured, which may have significant relevance concerning sleep disturbances. Thus, we emphasize the need for future research with clinical factors and signs that can give a more precise response to the correlation of sleep disturbances with potential confounders.

In conclusion, the present study revealed that insomnia in young adults is significantly related to higher CIMT. In addition, the presence of certain behavioral habits, such as poor sleep or...
diet, may cause increased body fat, resulting in an inadequate production and receptivity of insulin.

References


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