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Title: Poor Sleep Quality and Lipid Profile in a Rural Cohort (The Baependi Heart Study)

Article Type: Original Article

Keywords: obstructive sleep apnea; lipids; Pittsburgh sleep quality index; PSQI; cholesterol; VLDL cholesterol; very-low density lipoprotein; triglycerides; subjective sleep quality; cardiometabolic; Baependi

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Abstract:
Aim: To test the association between cardiometabolic risk factors and subjective sleep quality assessed by the Pittsburgh sleep quality index (PSQI), independent of obstructive sleep apnea (OSA) and sleep duration.
Methods: 573 participants from the Baependi Heart Study, a rural cohort from Brazil that completed sleep questionnaires and underwent polygraphy for OSA evaluation. Multivariable linear regression analysis tested the association between cardiovascular risk factors (outcome variables) and sleep quality measured by PSQI, adjusting for OSA and other potential confounders (age, sex, race, salary/wage, education, marital status, alcohol intake, obesity, smoking, hypertension, and sleep duration).
Results: The sample mean age was of 43±16y, 66% were female, and mean body mass index (BMI) was 26±5 kg/m2. Only 20% were classified as obese (BMI ≥30). Fifty percent of participants reported poor sleep quality as defined by a PSQI score ≥5. A high PSQI score was significantly associated with higher very-low density lipoprotein (VLDL) cholesterol levels (beta=0.392, p=0.012) and higher triglyceride levels (beta=0.017, p=0.006), even after adjustments, including the apnea-hypopnea index. Further adjustments accounting for marital status, alcohol intake, and medication use did not change these findings. No significant association was observed between PSQI scores and glucose or blood pressure. According to PSQI components, sleep disturbances (beta=1.976, p=0.027), sleep medication use (beta=1.121, p=0.019), and daytime dysfunction (beta=1.290, p=0.024) were significantly associated with higher VLDL serum levels. Only the daytime dysfunction domain of the PSQI components was significantly associated with higher triglyceride levels (beta=0.066, p=0.004).
Conclusion: Poorer lipid profile was independently associated with poor sleep quality, assessed by the PSQI questionnaire, regardless of a normal sleep duration and accounting for OSA and socio-economic status.

August 14th, 2018

Sudhansu Chokroverty, MD, FRCP
Editor-In-Chief
Sleep Medicine Journal

Dear Dr Sudhansu Chokroverty,

Please find enclosed the revised manuscript entitled: "Poor Sleep Quality and Lipid Profile in a Rural Cohort (The Baependi Heart Study)" to be considered for publication in your prestigious Journal.

Thank you for considering this manuscript for publication and for the opportunity for improvements and clarification. All comments and suggestions were addressed and we have provided point-by-point replies to reviewers' queries and suggestions.

All authors have read and approved the manuscript that is not being considered for publication elsewhere in whole or part except as an abstract.

Sincerely,

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Answer to Reviewers' comments:

Ref.: No. SLEEP-D-18-00185
Poor Sleep Quality and Lipid Profile in a Rural Cohort (The Baependi Heart Study)

Dear Editor and Reviewer,

Thank you for considering this manuscript for publication and for the opportunity for improvements and clarification. All comments and suggestions were addressed and below we provide point-by-point replies to reviewers' queries and suggestions.

Reviewer #1:

This manuscript by Reis Geovanni and coworkers reports on an association between poor sleep quality and deranged lipid profile in a rural family based cohort (the Baependi Heart Study). This cohort was designed to address genetic and environmental influences on CVD risk factors. In brief, a high PSQI score was associated with higher VLDL cholesterol and TGs. Glucose and blood pressure were not related with PSQI. The PSQI components sleep disturabnces, sleep medication use and daytime dysfunction were signicantly associated with higher VLDL.

1-The study reports on an interesting association between lipid metabolism and sleep disturbances. However, the main critisism relates to the preselected study cohort which evidently fails to represent the population at large. This limitation needs to be better disussed. Another important limitation is the failure to convincingly address the potential confounding influence of socioeconomic factors on sleep disturbances (see below).

Thank you for bringing this into consideration. In Table 1 and Table 2, we showed characteristics of the full sample (n= 1,801). As shown, participants included in the analytical sample (n= 573) were not so different from participants from the full sample according to many characteristics. The test for differences for each covariate showed statistical significance for some variables that were included in adjustments. In addition, in order to account for confounding factors, we have not only further adjusted for medication, alcohol intake, marital status (supplemental data), but also added education level to adjustments, as suggested. Thus, our findings now adjust for key socioeconomic and demographic factors, including wage/salary and education level, which are two commonly used indicators of socioeconomic status.

2-The authors assume that the Baependi cohort is well known but this is unfortunately not the case. Hence, the cohort needs to be much better described in the current method section. The cohort was previously used to study associations related to cardiovascular disease. What about the lipid analysis in that context? Were there associations with other relevant CVD risk factors?

Thank you again for highlighting the importance of a detailed description of the full sample. We added further information in the Methods section (pages 3 and 4). Also, Table 1 and Table 2 have addressed this issue with newer detailed information on demographics, cardiometabolic factors, and sleep. Table 3 presents the regression models and each row represents a unique regression model for several CVD risk factors. Outcome variables were lipid profile (LDL, HDL, total cholesterol, triglycerides, and cholesterol ratio), glucose (glycated hemoglobin and fasting glucose), hypertension (systolic and diastolic blood pressure continuous variables), and obesity.

3-A subgroup of 573 participants were addressed in the current study. Which factors determined the selection of this group? Was the investigated subgroup representative of the whole study group at large? Mean age was below 50 (range unclear), two thirds female but more than 50% reporting a poor sleep quality. If there is a possibility that the selection process has influenced the outcome that needs to be discussed.

We only studied individuals with complete sleep questionnaire data AND sleep polygraphy. Our sample size is contingent on the fact that the sleep polygraphy protocol was only performed in a subset of participants. There was no specific inclusion criteria for the polygraphy protocol and individuals where randomly sampled from the total sample. This is described in the revised Methods section (Methods section, study cohort, pages 3 and 4). In order to provide readers a comparison between the overall sample and the analyzed subset, we added in Table 1 and Table 2 characteristics of the full sample (n=1,801).

4-What about socioeconomic factors and CVD? Poor socioeconomical conditions have been identified as a very strong risk factor for sleep disturbances in other epidemiological

cohort studies. What was the rational for the salary cut-offs applied in the current study? Also, salaries could be given in USD. It is noted that the salary distribution is indeed very skewed. Could you consider an analysis based on e.g. salary quartiles or Quintiles? Moreover, do the reported findings only reflect salary? What about family income? Approximately one third of participants lived as singles. Has an analysis related to educational level been considered? It remains that socioeconomy may be one of the strongest predictors in the current analysis and the possibility for this needs to be carefully evaluated.

We agree that socioeconomic status (SES) is an important potential confounder. In our analyses, we have adjusted for two commonly-used indicators of SES, wage/salary and education level, in addition to other sociodemographic variables, such as age, sex, race, smoking status, alcohol intake, marital status, and medication. Then, after your suggestion regarding education level, we further adjusted our models for education with no changes in findings. Wage/salary is a variable gathered by questionnaire. Additionally, we also used wage as quartiles or quintiles as suggested. Finally, we have added all categories (as shown in table 1), by minimum wage, into regression models (Table 3 footnotes), without changes in findings. We do not believe using a dollar conversion rule can be helpful to the overall economic understanding of this sample since, over the last decade, it has fluctuated between 1.5 and 4 Real/Dollar.

5-A total of 11% were on lipid lowering agents and this was statistically adjusted for according to the text. How was that adjustment made? Would you consider excluding those on medication? Also, hypnotic medication may be better clarified. How was this classified? Was an assessemnt of sleep quality made with or without correcting sleep medication?

Thank you for the suggestions. After your suggestion, we added Table 5 in the main manuscript showing the restricted analysis, which excludes those taking lipid-lowering medications. The findings remained similar. We did not adjust for sleep medication because it is already included in PSQI, preventing model inflation due to the collinearity between variables. We did not assess

hypnotic medication due to its complexity and overlapping uses with other diseases. We wrote this as a limitation of the current analysis (page 11).

6-Finally, the authors are tempted to discuss a causal relationship at several places in the manuscript. Clearly, this study design would not allow for such conclusions to be drawn.

We agree. We have revised our text to avoid suggesting causality and we have discussed this as a limitation of this observational study on page 11. In the conclusion, we have added a sentence stressing the need for caution and consideration of potential residual confounding.

HIGHLIGHTS

- Poor sleep quality was independently associated with a worse lipid profile, specifically higher VLDL and triglycerides, independently of OSA and sleep duration.
- The PSQI component most significantly associated with both high VLDL and triglycerides blood levels was daytime dysfunction.
- Lipid alterations may be early markers of metabolic dysfunction induced by poor sleep quality.
- Poor sleep quality may be an epiphenomenon of other sleep disturbances, beyond OSA and sleep duration.

Poor Sleep Quality and Lipid Profile in a Rural Cohort (The Baependi Heart Study)

Running title: Sleep quality and cardiometabolic disturbances

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Supplemental material-appendix A: 1
Highlights: 1

ABSTRACT

Aim: To test the association between cardiometabolic risk factors and subjective sleep quality assessed by the Pittsburgh sleep quality index (PSQI), independent of obstructive sleep apnea (OSA) and sleep duration.

Methods: 573 participants from the Baependi Heart Study, a rural cohort from Brazil that completed sleep questionnaires and underwent polygraphy for OSA evaluation. Multivariable linear regression analysis tested the association between cardiovascular risk factors (outcome variables) and sleep quality measured by PSQI, adjusting for OSA and other potential confounders (age, sex, race, salary/wage, education, marital status, alcohol intake, obesity, smoking, hypertension, and sleep duration).

Results: The sample mean age was of 43±16y, 66% were female, and mean body mass index (BMI) was 26±5 kg/m^2. Only 20% were classified as obese (BMI ≥30). Fifty percent of participants reported poor sleep quality as defined by a PSQI score ≥5. A high PSQI score was significantly associated with higher very-low density lipoprotein (VLDL) cholesterol levels (beta=0.392, p=0.012) and higher triglyceride levels (beta=0.017, p=0.006), even after adjustments, including the apnea-hypopnea index. Further adjustments accounting for marital status, alcohol intake, and medication use did not change these findings. No significant association was observed between PSQI scores and glucose or blood pressure. According to PSQI components, sleep disturbances (beta=1.976, p=0.027), sleep medication use (beta=1.121, p=0.019), and daytime dysfunction (beta=1.290, p=0.024) were significantly associated with higher VLDL serum levels. Only the daytime dysfunction domain of the PSQI components was significantly associated with higher triglyceride levels (beta=0.066, p=0.004).

Conclusion: Poorer lipid profile was independently associated with poor sleep quality, assessed by the PSQI questionnaire, regardless of a normal sleep duration and accounting for OSA and socio-economic status.

Keywords: lipids; sleep quality; PSQI; Pittsburgh Sleep Quality Index; very-low density lipoprotein cholesterol; low-density lipoprotein cholesterol; obstructive sleep apnea

1.0 INTRODUCTION

Sleep disturbances have been associated with metabolic dysregulation and may contribute to weight gain, obesity, and diabetes. [1-6] Obstructive sleep apnea (OSA) is known to explain part of this association, but other factors besides OSA are thought to also play a role.[7-13] For example, poor sleep quality has been associated with obesity, diabetes and weight gain, [8, 9, 11, 12] but few studies have shown these associations in the absence of OSA. [8, 11] In addition, there is still controversy about the association between poor sleep quality and the lipid profile, once a poorer lipid profile has been initially associated only with sleep duration, but not with self-reported sleep quality. [13] However, a worse lipid profile was also independently associated with poor subjective sleep quality scores. [12]

The aim of the current study is to test whether there is an independent association between subjective sleep quality, assessed by the Pittsburgh sleep quality index (PSQI) questionnaire, and cardiometabolic factors, while adjusting for OSA in a sample from the general population.

2.0 METHODS

2.1 Study cohort

The Baependi Heart Study, now in its 12th year, is a family-based cohort study aimed at evaluating genetic and environmental influences on cardiovascular risk factors. [14, 15] Baependi is a small rural town in the state of Minas Gerais, Brazil, with very limited inward migration. At baseline, 1691 individuals across 95 families were evaluated. Cross-sectional data have been collected for 2,239 participants. [15] The current study analyzed a subset of participants at the second evaluation period (from 2010 to 2014).

All participants in this subset had completed sleep questionnaires (the PSQI and Epworth sleepiness scale-ESS) (n=1,801), and underwent OSA evaluation by overnight polygraphy (n= 573). We only studied individuals with complete sleep questionnaire data and sleep polygraphy. Our sample size is contingent on the fact that the sleep polygraphy protocol was only performed in a subset of participants. There was no specific inclusion criteria for the polygraphy protocol and all individuals from the total sample were invited to participate in the sleep polygraphy protocol.

The study protocol was approved by the ethics committee of the *Hospital das Clinicas*, University of São Paulo, Brazil, and each subject provided informed written consent before participation.

2.2 Covariates

Anthropometric measures, such as height, weight, waist, hip, and neck circumferences, were measured following standardized procedures as previously described.[16] Body mass index (BMI) was calculated from height and weight (kg/m^2). Smoking status was defined by asking the question: "Have you smoked cigarettes...?" Being possible answers: (1) Yes, in the past, but not currently; (2) Yes, and I still smoke; (3) I have never smoked. The three choices were thus characterized as (1) former, (2) current, and (3) non-smokers, respectively. [17] Seated systolic blood pressure (SBP) and diastolic blood pressure (DBP) were assessed by one trained examiner. The mean out of three measures was used to describe blood pressure at rest. Medication use, alcohol use, salary, education level, and marital status were assessed by questionnaires.

2.3 Sleep questionnaires

The PSQI is a self-rated questionnaire which assesses sleep quality over the past month. Nineteen individual items generate seven component scores: subjective sleep quality, habitual sleep efficiency, sleep latency, sleep duration, sleep disturbances, use of sleeping medication, and daytime dysfunction. A global PSQI score equal or greater than five has been proposed to distinguish between good and poor sleepers. [18, 19]

The ESS is a self-administered questionnaire that provides a measurement of daytime sleepiness. The ESS score (the sum of 8 item scores, 0-3) can range from 0 to 24. Excessive daytime sleepiness is defined as a score >10. [20]

2.4 Other sleep measures

The diagnosis of OSA was made based on a portable type III study (Stardust II; Philips, Eindhoven, Netherlands). The overnight sleep monitor was installed by a technician at the patient's home. Briefly, the portable monitor used pulse oximetry, airflow detection through nasal cannula pressure, position sensor, heart rate recorded by pulse oximetry, and respiratory effort (chest-strap piezoelectric detection). Apneas were classified as central (absence of airflow or ≥90% reduction of baseline for ≥10s associated with the absence of thoracic and abdominal effort), obstructive (absence of airflow or ≥90% reduction of baseline for ≥10s associated with the presence of thoracic and abdominal effort), or mixed (absence of airflow or ≥90% reduction of baseline for ≥10s in the presence of thoracic and abdominal effort at the end of the event). [21, 22] Hypopneas were classified as a 30% or more drop in airflow for ≥10s with a 4% decrease in oxygen desaturation. [21, 22] The apnea hypopnea index (AHI) was assessed by the number of respiratory events (apneas and hypopneas) divided by the total sleep recording time. The OSA severity was determined according to standard cutoffs as mild (AHI 5-14),

moderate (AHI 15-29) or severe (AHI ≥30). [21, 22] More details about the polygraphy exam were previously described. [23]

Bedtime and sleep duration were self-reported on the PSQI. Sleep efficiency information was extracted from the PSQI questionnaire. Data from sleep duration of this cohort has been previously published.[14]

2.5 Metabolic risk factors

Fasting blood glucose, glycated hemoglobin (HbA1c), total cholesterol, lipoprotein fractions, and triglycerides were assayed by standard techniques in 12-hour fasting blood samples. Serum samples were stored at -80°C prior to analysis. We also tested the cholesterol ratio (total cholesterol divided by high-density lipoprotein (HDL) cholesterol). [24, 25]

2.6 Statistical Analysis

Descriptive analyses are shown as mean ±SD for continuous and percentage for categorical variables. Natural log transformation (ln) was used in cases of departure from normality. Because triglycerides showed high kurtosis we log transformed this variable (ln) while running linear regression analysis. Multivariable linear regression analysis was used to test the independent relationship between cardiovascular risk factors as outcome variables (lipid/glucose blood levels and blood pressure) and sleep quality as a main predictor (assessed by the PSQI), adjusting for age, sex, race, salary (monthly), education level, smoking status (never, former, current), BMI, SBP, daytime sleepiness (by ESS-continuous score), AHI continuous score, sleep duration, and bedtime. Further adjustments were also made for lipid-lowering medication use, blood glucose levels, alcohol use, and marital status. We have also run sensitivity analysis

excluding those with OSA (AHI ≥15) in order to explore the association between cardiometabolic factors and sleep quality independent of OSA. Similarly, we performed a restricted analysis for participants who did not use lipid-lowering medication. Sleep quality by the PSQI was used as a continuous score, a dichotomous category (cutoff 5), and by its seven domains. The final sample size for the current analysis was 573 participants, who underwent overnight polygraphy and had completed sleep questionnaires. We show descriptive data from the full sample (n=1,801) and from those used in the present analysis (n=573). We tested whether those included in our analytic sample differed significantly from those who did not participate in the polygraphy examinations using independent t tests for continuous variables or chi squared tests for categorical variables. Missing data were excluded while running regression analysis. Data were analyzed by the IBM SPSS statistics software version 18. The alpha level of significance was set as <0.05.

3.0 RESULTS

Table 1 shows descriptive data from the total and the analytical sample for demographics and cardiovascular risk factors. The analytical sample is predominantly female (66%), mean age was 43±16y, age range from 18 to 81yo, and mean BMI of 26±5kg/m^2. Overweight (BMI between 25 and 30) participants were 30% and only 20% were classified as obese subjects (BMI ≥30). Most reported never having smoked and not using alcohol. Almost one quarter of the women in the sample was post-menopausal and almost 30% of the sample used anti-hypertensive medication. In general, cardiovascular risk factors were within normal ranges. The analytical sample showed a similar distribution of variables (demographic and cardiometabolic factors) compared to the full sample, except for age, sex, race, smoking status, and blood pressure levels.

Table 2 shows sleep characteristics according to questionnaires and polygraphy. Fifty percent classified their sleep quality as poor by the PSQI, and 34% reported excessive daytime sleepiness. OSA, by polygraphy, was shown in 18% of the study sample. Sleep efficiency was considered to be normal (>85%) and self-reported sleep duration indicated that the majority of them slept more than 6h/night. Self-reported bedtime showed a mean bedtime of 22:40. The analytical sample showed a slightly higher percentage of participants with poor sleep quality by the PSQI compared to full sample participants (50 vs 46%, respectively). Likewise, the analytical sample showed higher percentage of participants with excessive daytime sleepiness compared to participants from full sample (34 vs 30%, respectively). After testing for independent associations between sleep quality and cardiovascular risk factors, only VLDL cholesterol and triglyceride levels remained significantly associated with higher PSQI scores (Table 3). To explore other potential confounding factors relating poor sleep quality with the lipid profile, we further adjusted for lipid-lowering medication use, fasting glucose levels, alcohol use, and marital status. Results remained similar (supplemental material-appendix A1). To test the association between sleep quality and cardiometabolic risk variables, independently of OSA, we also performed an analysis only for those with an AHI<15, that is, excluding those with moderate-to-severe OSA. The results remained statistically significant (Table 4). In addition, we have also run a restricted analysis excluding those taking lipid-lowering medication. The findings remained significant (Table 5).

Furthermore, we evaluated the PSQI by components as main predictors. Supplemental material shows all multiple regression analyses testing PSQI components association with cardiovascular risk factors. According to PSQI components, we have first modeled them as continuous scores, and then, if statistically significant, they were

modeled within strata. Higher scores in the daytime dysfunction domain (showed overall and within strata) were significantly associated with higher ln (triglycerides) levels (p=0.004) (Appendix A2). The sleep disturbances domain (p=0.027), sleep medication use domain (p=0.019), and daytime dysfunction domain (p=0.024) were significantly associated with higher VLDL blood levels (Appendix A3).

4.0 DISCUSSION

Prior evidence supports that sleep duration and quality may be a potentially modifiable risk factor for cardiovascular and metabolic disease. We tested for associations between cardiometabolic variables and poor sleep quality, assessed by the PSQI, controlling for sleep duration and severity of OSA. Our findings showed that (1) 50% of this rural-based population reported poor sleep quality, even within a normal sleep duration range; (2) higher VLDL cholesterol and triglycerides levels were associated with higher PSQI score (i.e. worse sleep quality), even after adjusting for potential confounders; and (3) the PSQI component most significantly associated with both high VLDL and triglyceride blood levels was daytime dysfunction. Importantly, our results appear to be independent of OSA as we have not only adjusted for AHI (continuous score), but also tested for these associations excluding those with moderate-to-severe OSA (AHI ≥15) and the results remained statistically significant. Our results are in line with the hypothesis that lipid alterations may be early markers of metabolic dysfunction induced by poor sleep quality.

There are complex mechanisms behind the association between sleep duration and cardiovascular risk factors, since both short [3, 10, 26, 27] and long sleep duration [12, 13] have been associated with cardiometabolic alterations. Studies have shown that obesity is associated with poor sleep quality, sleep fragmentation, and sleep duration.[3, 4, 7]

However, only few of these studies tested this association in the absence of OSA. [8, 9, 11] A higher PSQI score has been associated with larger waist circumferences, BMI, and percentage of body fat, as well as with higher serum levels of insulin and glucose. [28] Nonetheless, associations between PSQI and lipids (HDL cholesterol and triglycerides) have been inconsistent, being reported to be negative, [28] and positive. [29]

In the scenario of the controversy about the association between lipid profile and sleep quality, [28, 29] the CARDIA study showed that a higher PSQI score was not significantly associated with a poorer lipid profile. [13] The poorer lipid profile was statistically associated only with sleep duration. [13] However, VLDL cholesterol levels were not reported in the CARDIA study, as we explored in this current study. In addition, there are several other mechanisms, besides sleep duration, that have been enrolled in the association between sleep disturbances and poor lipid profile. [30, 31] For instance, sleep fragmentation, classified as the number of microarousals during sleep, has been associated with increased levels of lipids, cortisol, and blood pressure. [30] A reasonable hypothesis to be considered is that poor sleep quality may be an epiphenomenon of other sleep-related metabolic changes, such as sleep fragmentation.[30] Our results are consistent with these findings. Our findings demonstrated that other sleep disturbances besides OSA [5-7, 13, 32-36] and sleep duration, [12, 13, 26, 27] specifically sleep quality, may be potential risk factors for poor lipid profiles. A clear understanding of the biological mechanisms linking these sleep disturbances and higher VLDL cholesterol levels remains to be identified.

PSQI is a commonly used subjective sleep quality instrument.[18, 19, 37] Despite the limitations due to its subjective nature, it has also been considered a tool that added significant contributions to the general quality of life (QoL) evaluation. [37] Our findings showed that the daytime dysfunction domain significantly associated with high levels of

both VLDL cholesterol and triglycerides, so it seems to be in line with previous report about the idea that the sleep may have significant impact on QoL, even in the absence of sleep disordered breathing or other health problems, such as lipid alterations. [38]

Our study has some limitations. First, the cross-sectional nature does not allow causal inference. Second, although we adjust for major confounders, there may still be residual confounding due to incomplete adjustments and unknown confounders. Sleep medication use was analyzed separately, as it formed a component of PSQI questionnaire, and is shown in the supplemental data. We did not ascertain type of sleep medication or duration of use. Similarly, hypnotic medication was not taken into account separately in the current analysis. In addition, the subjective nature of the questionnaires should be carefully evaluated. Finally, overnight polygraphy was performed through a type III portable sleep study, which does not allow sleep duration to be objectively measured. Thus, sleep duration in the current study was self-reported. Moreover, portable overnight polygraphy may underdiagnose OSA, [39, 40] since there are no brain channels to assess respiratory events related to arousals. Therefore, our results should be interpreted with caution.

5.0 CONCLUSION

Our findings revealed that poor sleep quality was independently associated with poorer lipid profile, specifically higher VLDL and triglycerides. Poor sleep quality may reflect an unhealthy lifestyle, but also may be an epiphenomenon of other sleep disturbances, beyond OSA and sleep duration. Our findings were robust even after adjusting for socio-economic status, but possible residual confounding caused by unknown factors should be considered in an observational study. An objectively

measured sleep evaluation, by a full polysomnography, may shed light the mechanistic pathways by which poor sleep quality may lead to elevated lipid blood levels.

6.0 ACKNOWLEDGMENTS

The authors wish to thank the Municipal Council of Baependi for logistical support and assistance with field work, the dedicated staff at the field station and the participants of the study.

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Disclosures: This was not an industry supported study. The authors have indicated no financial conflicts of interest.

Author contributions

Concept and design: GRG, GLF, and ACP

Analysis and interpretation of data: GRG, ACP, KLK, and MvS

Drafting the article or revising it critically for important intellectual content: GRG, GLF, de Paula LK, CMO, ROA, FB, ABN, MvS, KLK, JEK, and ACP

Final approval: all

ABBREVIATIONS

AHI= apnea hypopnea index

BMI= body mass index

DBP= diastolic blood pressure

ESS= Epworth sleepiness scale

HDL= high-density lipoprotein

LDL= low-density lipoprotein

OSA= obstructive sleep apnea

PSQI= Pittsburgh sleep quality index

SBP= systolic blood pressure

VLDL= very-low-density lipoprotein

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Table 1. Univariate Analysis of All Complete Sleep Questionnaires (Full Sample) and Those Who Also Underwent Portable Sleep Study (Overnight Polygraphy; Analytical Sample)

Demographics	Full sample (n= 1,801)	Analytical sample (n= 573)	*P*-value
Age, years	45 ±17	43 ±16	**0.023**
Sex (male), %	42	34	**<0.001**
Self-reported race, %			
White	76	80	**0.005**
Black	6	4	
Mixed	13	16	
Smoking status, %			
Never	66	72	**<0.001**
Former	22	20	
Current	12	8	
Alcohol use, %			
None	70	73	0.260
<Once/month	6	7	
Once to 3 times/week	10	9	
Up to 3 times/month	11	9	
Daily	1	1	
Other	2	1	
Salary, %			
Up to 1 minimum wage (MW)	17	15	0.280
From 1 to 5 MW	76	78	
From 5 to 10 MW	3.5	4.0	
From 10 to 20 MW	1.0	0.5	
>20 MW	0.5	0.5	
No answer	2.0	2.0	
Marital status, %			0.122

Married **Single** **Divorced** **Widowed**	62 26 6 6	62 27 5 6	
Education level,% **Illiterate** **High school** **Technical training** **Graduation**	 3 82 3 12	 2 81 4 13	0.564
BMI, kg/m^2	26 ±5	26 ±5	0.740
Waist circumference, cm	91 ±12	91 ±12	0.580
Hip circumference, cm	98 ±10	98 ±10	0.670
Neck circumference, cm	36 ±8	36 ±12	0.790
Medication use, % **Anti-hypertensive** **Hypoglicemic** **Lipid-lowering** **Oral contraceptive**	 28 5 8 15	 28 6 11 18	 0.700 0.210 **0.004** 0.965
Post-menopausal status, %	22	24	0.830
Cardiometabolic risk factors			
VLDL-cholesterol, mg/dL	25 ±12	25 ±11	0.190
LDL-cholesterol, mg/dL	125 ±35	123 ±35	0.093
HDL-cholesterol, mg/dL	47 ±12	48 ±11	0.490
Total cholesterol, mg/dL	198 ±41	195 ±41	0.075
Cholesterol ratio	4.40 ±1.30	4.30 ±1.30	0.052
Triglycerides, mg/dL*	131 ±75	126 ±60	0.190
Glucose, mg/dL	93 ±20	94 ±19	0.106
HbA1c, %	5.7 ±0.8	5.7 ±0.7	0.084
Systolic BP, mmHg	123 ±11	121 ±11	**0.001**
Diastolic BP, mmHg	78 ±9	77 ±8	**0.017**

Data are shown as mean ±SD for continuous and percentage for categorical variables. *P*-value is by independent t-test for continuous and normally distributed variables (the test is independent for each covariate) (*P* value indicates comparison of those included in analytic sample to those excluded) and by chi-square for categorical variables. BMI= body mass index; VLDL=very-low density lipoprotein; LDL= low-density lipoprotein; HDL= high-density lipoprotein; cholesterol ratio= total cholesterol/HDL; HbA1c= glycated hemoglobin; BP= blood pressure.

*Since triglycerides was skewed, the *P*-value is by Mann-Whitney, a non-parametric test. The median and IQR (25^{th}, 75^{th}) for triglycerides in the Full sample is 116 (85, 158) mg/dL and in the Analytical sample is 112 (87, 154) mg/dL.

Table 2. Univariate Analysis of All Complete Sleep Questionnaires (Full Sample) and Those Who Also Underwent Portable Sleep Study (Overnight Polygraphy; Analytical Sample)

Sleep characteristics	Full sample (n= 1,801)	Analytical Sample (n= 573)	*P*-value
AHI, events/h	-	10 ±10	-
ODI, events/h	-	10 ±11	-
Nadir SpO_2,	-	82 ±10	-
Basal SpO_2	-	95 ±5	-
AHI ≥ 5, %	-	58	-
AHI ≥ 15, %	-	18	-
AHI ≥ 30, %	-	5	-
ESS continuous score	7 ±5	7±5	0.094
ESS >10, %	30	34	**0.009**
PSQI total score	5 ±3	5±3	0.095
PSQI ≥ 5, %	46	50	**0.018**
Sleep duration (self-reported) ≥ 6h,%	94	93	0.180
Sleep duration (self-reported), min	472 ±106	461 ±87	**0.002**
Sleep efficiency, %	92 ±34	93 ±16	0.200
Bed time (hours: minutes)*	22:44 ±01:60	22:40 ± 01:40	**<0.001**

Data are shown as mean ±SD for continuous and percentage for categorical variables. *P*-value is by independent t-test for continuous and normally distributed variables (the test is independent for each covariate) (*P* value indicates comparison of those included in analytic sample to those excluded) and by chi-square for categorical variables. OSA= obstructive sleep apnea; BMI= body mass index; AHI= apnea-hypopnea index; ODI= oxygen desaturation index (with 4% drop in oxygen saturation); SpO_2= oxygen saturation; AHI ≥5= mild OSA; AHI ≥15= moderate OSA; AHI ≥30= severe OSA; ESS= Epworth sleepiness scale; ESS >10= excessive daytime sleepiness; PSQI= Pittsburgh sleep quality index; PSQI ≥5= poor sleep quality;

*Bedtime = median and IQR 25^{th}, 75^{th} (22:50 and 21:50, 23:50) for the full sample. Bedtime = median and IQR 25^{th}, 75^{th} (22:50 and 21:50, 23:50) for the analytical sample. Bedtime was skewed, so *P*-value by Mann-Whitney, a non-parametric test.

Those left blank were not measured by polygraphy.

Table 3. Multivariable Linear Regression Analysis Testing Association of CV Risk Factors (Outcome) and PSQI (main Predictor)

	PSQI total score			PSQI <5 vs ≥ 5		
Outcome variable	**Beta**	**Se**	***P*-value**	**Beta**	**se**	***P*-value**
VLDL-cholesterol, mg/dL	0.392	0.156	**0.012**	1.763	0.980	0.072
ln Triglycerides, mg/dL	0.017	0.006	**0.006**	0.071	0.040	0.071
HDL-cholesterol, mg/dL	-0.194	0.155	0.208	-0.897	0.973	0.356
LDL-cholesterol, mg/dL	0.082	0.500	0.870	1.886	3.120	0.546
Total cholesterol, mg/dL	0.495	0.561	0.377	2.932	3.532	0.407
Cholesterol ratio	0.033	0.017	0.060	0.195	0.110	0.077
Glucose, mg/dL	0.180	0.247	0.468	-0.601	1.560	0.700
HbA1c, %	-0.015	0.009	0.102	-0.066	0.056	0.238
Systolic BP, mmHg*	0.073	0.152	0.634	0.516	0.961	0.613
Diastolic BP, mmHg*	0.060	0.116	0.602	0.290	0.731	0.693

CV= cardiovascular; VLDL= very-low density lipoprotein; ln= natural logarithm transformation; HDL= high-density lipoprotein; LDL= low-density lipoprotein; cholesterol ratio= total cholesterol/HDL; HbA1c= glycated hemoglobin; BP= blood pressure; PSQI= Pittsburgh sleep quality index; PSQI ≥5= poor sleep quality; BMI= body mass index; ESS= Epworth sleepiness scale; OSA= obstructive sleep apnea; AHI= apnea hypopnea index (number of events per hour of sleep); se= standard error. Each line represents a unique regression model. Adjustments for age, sex (female= ref), race (White vs others), Wage categories (up to 5 MW vs >5MW and >5MW as ref), Education categories (illiterate vs high school vs technical/graduation and illiterate as ref), smoking status (never as ref), systolic BP, BMI, sleep duration (minutes); ESS continuous score, bedtime (in hours), and OSA by AHI continuous score. Missing data were excluded (all complete for regression analysis= 554).
*not included systolic BP in adjustments.

Table 4. Restricted Analysis for those Without OSA (AHI<15), Testing the Association Between Lipid Components (Outcome) and Sleep Quality (n= 472)

	VLDL-cholesterol, mg/dL			ln Triglycerides, mg/dL		
	Beta	**se**	***P*-value**	**Beta**	**se**	***P*-value**
PSQI total score	0.446	0.170	**0.009**	0.018	0.007	**0.010**
PSQI cutoff 5	2.188	1.037	**0.035**	0.084	0.426	**0.048**

OSA= obstructive sleep apnea; AHI= apnea hypopnea index; VLDL= very-low density lipoprotein cholesterol; ln= natural logarithm transformation; se= standard error; PSQI= Pittsburgh sleep quality index; PSQI ≥5= poor sleep quality; BMI= body mass index; ESS= Epworth sleepiness scale; BP= blood pressure. Adjustments: age, sex (female= ref), race (White vs others), Wage categories (up to 5 MW vs >5MW and >5MW as ref), Education categories (illiterate vs high school vs technical/graduation and illiterate as ref), smoking status (never as reference), BMI, ESS continuous score, sleep duration (minutes), bedtime (in hours), and daytime systolic BP. Missing data were excluded. All complete for regression analysis (n=423).

Table 5. Restricted Analysis for those Without Lipid-lowering Medication (n= 510) Testing the Association Between Lipid Components (Outcome) and Sleep Quality

	VLDL-cholesterol, mg/dL			ln Triglycerides, mg/dL		
	Beta	**se**	***P*-value**	**Beta**	**se**	***P*-value**
PSQI total score	0.426	0.161	**0.008**	0.019	0.006	**0.004**
PSQI cutoff 5	1.681	1.013	0.097	0.080	0.040	**0.047**

VLDL= very-low density lipoprotein cholesterol; ln= natural logarithm transformation; se= standard error; PSQI= Pittsburgh sleep quality index; PSQI ≥5= poor sleep quality; BMI= body mass index; ESS= Epworth sleepiness scale; BP= blood pressure, AHI= apnea hypopnea index. Adjustments: age, sex (female=ref), race (White vs others), Wage categories (up to 5 MW vs >5MW and >5MW as ref), Education categories (illiterate vs high school vs technical/graduation and illiterate as ref), smoking status (never as reference), BMI, ESS continuous score, sleep duration (minutes), bedtime (in hours), AHI, and daytime systolic BP. Missing data were excluded. All complete for regression analysis (n= 462).

Poor Sleep Quality and Lipid Profile in a Rural Cohort (The Baependi Heart Study)

Running title: Sleep quality and cardiometabolic disturbances

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ABSTRACT

Aim: To test the association between cardiometabolic risk factors and subjective sleep quality assessed by the Pittsburgh sleep quality index (PSQI), independent of obstructive sleep apnea (OSA) and sleep duration.

Methods: 573 participants from the Baependi Heart Study, a rural cohort from Brazil that completed sleep questionnaires and underwent polygraphy for OSA evaluation. Multivariable linear regression analysis tested the association between cardiovascular risk factors (outcome variables) and sleep quality measured by PSQI, adjusting for OSA and other potential confounders (age, sex, race, salary/wage, education, marital status, alcohol intake, obesity, smoking, hypertension, and sleep duration).

Results: The sample mean age was of 43±16y, 66% were female, and mean body mass index (BMI) was 26±5 kg/m^2. Only 20% were classified as obese (BMI ≥30). Fifty percent of participants reported poor sleep quality as defined by a PSQI score ≥5. A high PSQI score was significantly associated with higher very-low density lipoprotein (VLDL) cholesterol levels (beta=0.392, p=0.012) and higher triglyceride levels (beta=0.017, p=0.006), even after adjustments, including the apnea-hypopnea index. Further adjustments accounting for marital status, alcohol intake, and medication use did not change these findings. No significant association was observed between PSQI scores and glucose or blood pressure. According to PSQI components, sleep disturbances (beta=1.976, p=0.027), sleep medication use (beta=1.121, p=0.019), and daytime dysfunction (beta=1.290, p=0.024) were significantly associated with higher VLDL serum levels. Only the daytime dysfunction domain of the PSQI components was significantly associated with higher triglyceride levels (beta=0.066, p=0.004).

Conclusion: Poorer lipid profile was independently associated with poor sleep quality, assessed by the PSQI questionnaire, regardless of a normal sleep duration and accounting for OSA and socio-economic status.

Keywords: lipids; sleep quality; PSQI; Pittsburgh Sleep Quality Index; very-low density lipoprotein cholesterol; low-density lipoprotein cholesterol; obstructive sleep apnea

1.0 INTRODUCTION

Sleep disturbances have been associated with metabolic dysregulation and may contribute to weight gain, obesity, and diabetes. [1-6] Obstructive sleep apnea (OSA) is known to explain part of this association, but other factors besides OSA are thought to also play a role.[7-13] For example, poor sleep quality has been associated with obesity, diabetes and weight gain, [8, 9, 11, 12] but few studies have shown these associations in the absence of OSA. [8, 11] In addition, there is still controversy about the association between poor sleep quality and the lipid profile, once a poorer lipid profile has been initially associated only with sleep duration, but not with self-reported sleep quality. [13] However, a worse lipid profile was also independently associated with poor subjective sleep quality scores. [12]

The aim of the current study is to test whether there is an independent association between subjective sleep quality, assessed by the Pittsburgh sleep quality index (PSQI) questionnaire, and cardiometabolic factors, while adjusting for OSA in a sample from the general population.

2.0 METHODS

2.1 Study cohort

The Baependi Heart Study, now in its 12th year, is a family-based cohort study aimed at evaluating genetic and environmental influences on cardiovascular risk factors. [14, 15] Baependi is a small rural town in the state of Minas Gerais, Brazil, with very limited inward migration. At baseline, 1691 individuals across 95 families were evaluated. Cross-sectional data have been collected for 2,239 participants. [15] The current study analyzed a subset of participants at the second evaluation period (from 2010 to 2014).

All participants in this subset had completed sleep questionnaires (the PSQI and Epworth sleepiness scale-ESS) (n=1,801), and underwent OSA evaluation by overnight polygraphy (n= 573). We only studied individuals with complete sleep questionnaire data and sleep polygraphy. Our sample size is contingent on the fact that the sleep polygraphy protocol was only performed in a subset of participants. There was no specific inclusion criteria for the polygraphy protocol and all individuals from the total sample were invited to participate in the sleep polygraphy protocol.

The study protocol was approved by the ethics committee of the *Hospital das Clinicas*, University of São Paulo, Brazil, and each subject provided informed written consent before participation.

2.2 Covariates

Anthropometric measures, such as height, weight, waist, hip, and neck circumferences, were measured following standardized procedures as previously described.[16] Body mass index (BMI) was calculated from height and weight (kg/m^2). Smoking status was defined by asking the question: "Have you smoked cigarettes...?" Being possible answers: (1) Yes, in the past, but not currently; (2) Yes, and I still smoke; (3) I have never smoked. The three choices were thus characterized as (1) former, (2) current, and (3) non-smokers, respectively. [17] Seated systolic blood pressure (SBP) and diastolic blood pressure (DBP) were assessed by one trained examiner. The mean out of three measures was used to describe blood pressure at rest. Medication use, alcohol use, salary, education level, and marital status were assessed by questionnaires.

2.3 Sleep questionnaires

The PSQI is a self-rated questionnaire which assesses sleep quality over the past month. Nineteen individual items generate seven component scores: subjective sleep quality, habitual sleep efficiency, sleep latency, sleep duration, sleep disturbances, use of sleeping medication, and daytime dysfunction. A global PSQI score equal or greater than five has been proposed to distinguish between good and poor sleepers. [18, 19]

The ESS is a self-administered questionnaire that provides a measurement of daytime sleepiness. The ESS score (the sum of 8 item scores, 0-3) can range from 0 to 24. Excessive daytime sleepiness is defined as a score >10. [20]

2.4 Other sleep measures

The diagnosis of OSA was made based on a portable type III study (Stardust II; Philips, Eindhoven, Netherlands). The overnight sleep monitor was installed by a technician at the patient's home. Briefly, the portable monitor used pulse oximetry, airflow detection through nasal cannula pressure, position sensor, heart rate recorded by pulse oximetry, and respiratory effort (chest-strap piezoelectric detection). Apneas were classified as central (absence of airflow or ≥90% reduction of baseline for ≥10s associated with the absence of thoracic and abdominal effort), obstructive (absence of airflow or ≥90% reduction of baseline for ≥10s associated with the presence of thoracic and abdominal effort), or mixed (absence of airflow or ≥90% reduction of baseline for ≥10s in the presence of thoracic and abdominal effort at the end of the event). [21, 22] Hypopneas were classified as a 30% or more drop in airflow for ≥10s with a 4% decrease in oxygen desaturation. [21, 22] The apnea hypopnea index (AHI) was assessed by the number of respiratory events (apneas and hypopneas) divided by the total sleep recording time. The OSA severity was determined according to standard cutoffs as mild (AHI 5-14),

moderate (AHI 15-29) or severe (AHI ≥30). [21, 22] More details about the polygraphy exam were previously described. [23]

Bedtime and sleep duration were self-reported on the PSQI. Sleep efficiency information was extracted from the PSQI questionnaire. Data from sleep duration of this cohort has been previously published.[14]

2.5 Metabolic risk factors

Fasting blood glucose, glycated hemoglobin (HbA1c), total cholesterol, lipoprotein fractions, and triglycerides were assayed by standard techniques in 12-hour fasting blood samples. Serum samples were stored at -80°C prior to analysis. We also tested the cholesterol ratio (total cholesterol divided by high-density lipoprotein (HDL) cholesterol). [24, 25]

2.6 Statistical Analysis

Descriptive analyses are shown as mean ±SD for continuous and percentage for categorical variables. Natural log transformation (ln) was used in cases of departure from normality. Because triglycerides showed high kurtosis we log transformed this variable (ln) while running linear regression analysis. Multivariable linear regression analysis was used to test the independent relationship between cardiovascular risk factors as outcome variables (lipid/glucose blood levels and blood pressure) and sleep quality as a main predictor (assessed by the PSQI), adjusting for age, sex, race, salary (monthly), education level, smoking status (never, former, current), BMI, SBP, daytime sleepiness (by ESS-continuous score), AHI continuous score, sleep duration, and bedtime. Further adjustments were also made for lipid-lowering medication use, blood glucose levels, alcohol use, and marital status. We have also run sensitivity analysis

excluding those with OSA (AHI ≥15) in order to explore the association between cardiometabolic factors and sleep quality independent of OSA. Similarly, we performed a restricted analysis for participants who did not use lipid-lowering medication. Sleep quality by the PSQI was used as a continuous score, a dichotomous category (cutoff 5), and by its seven domains. The final sample size for the current analysis was 573 participants, who underwent overnight polygraphy and had completed sleep questionnaires. We show descriptive data from the full sample (n=1,801) and from those used in the present analysis (n=573). We tested whether those included in our analytic sample differed significantly from those who did not participate in the polygraphy examinations using independent t tests for continuous variables or chi squared tests for categorical variables. Missing data were excluded while running regression analysis. Data were analyzed by the IBM SPSS statistics software version 18. The alpha level of significance was set as <0.05.

3.0 RESULTS

Table 1 shows descriptive data from the total and the analytical sample for demographics and cardiovascular risk factors. The analytical sample is predominantly female (66%), mean age was 43±16y, age range from 18 to 81yo, and mean BMI of 26±5kg/m^2. Overweight (BMI between 25 and 30) participants were 30% and only 20% were classified as obese subjects (BMI ≥30). Most reported never having smoked and not using alcohol. Almost one quarter of the women in the sample was post-menopausal and almost 30% of the sample used anti-hypertensive medication. In general, cardiovascular risk factors were within normal ranges. The analytical sample showed a similar distribution of variables (demographic and cardiometabolic factors) compared to the full sample, except for age, sex, race, smoking status, and blood pressure levels.

Table 2 shows sleep characteristics according to questionnaires and polygraphy. Fifty percent classified their sleep quality as poor by the PSQI, and 34% reported excessive daytime sleepiness. OSA, by polygraphy, was shown in 18% of the study sample. Sleep efficiency was considered to be normal (>85%) and self-reported sleep duration indicated that the majority of them slept more than 6h/night. Self-reported bedtime showed a mean bedtime of 22:40. The analytical sample showed a slightly higher percentage of participants with poor sleep quality by the PSQI compared to full sample participants (50 vs 46%, respectively). Likewise, the analytical sample showed higher percentage of participants with excessive daytime sleepiness compared to participants from full sample (34 vs 30%, respectively). After testing for independent associations between sleep quality and cardiovascular risk factors, only VLDL cholesterol and triglyceride levels remained significantly associated with higher PSQI scores (Table 3). To explore other potential confounding factors relating poor sleep quality with the lipid profile, we further adjusted for lipid-lowering medication use, fasting glucose levels, alcohol use, and marital status. Results remained similar (supplemental material-appendix A1). To test the association between sleep quality and cardiometabolic risk variables, independently of OSA, we also performed an analysis only for those with an AHI<15, that is, excluding those with moderate-to-severe OSA. The results remained statistically significant (Table 4). In addition, we have also run a restricted analysis excluding those taking lipid-lowering medication. The findings remained significant (Table 5).

Furthermore, we evaluated the PSQI by components as main predictors. Supplemental material shows all multiple regression analyses testing PSQI components association with cardiovascular risk factors. According to PSQI components, we have first modeled them as continuous scores, and then, if statistically significant, they were

modeled within strata. Higher scores in the daytime dysfunction domain (showed overall and within strata) were significantly associated with higher ln (triglycerides) levels (p=0.004) (Appendix A2). The sleep disturbances domain (p=0.027), sleep medication use domain (p=0.019), and daytime dysfunction domain (p=0.024) were significantly associated with higher VLDL blood levels (Appendix A3).

4.0 DISCUSSION

Prior evidence supports that sleep duration and quality may be a potentially modifiable risk factor for cardiovascular and metabolic disease. We tested for associations between cardiometabolic variables and poor sleep quality, assessed by the PSQI, controlling for sleep duration and severity of OSA. Our findings showed that (1) 50% of this rural-based population reported poor sleep quality, even within a normal sleep duration range; (2) higher VLDL cholesterol and triglycerides levels were associated with higher PSQI score (i.e. worse sleep quality), even after adjusting for potential confounders; and (3) the PSQI component most significantly associated with both high VLDL and triglyceride blood levels was daytime dysfunction. Importantly, our results appear to be independent of OSA as we have not only adjusted for AHI (continuous score), but also tested for these associations excluding those with moderate-to-severe OSA (AHI ≥15) and the results remained statistically significant. Our results are in line with the hypothesis that lipid alterations may be early markers of metabolic dysfunction induced by poor sleep quality.

There are complex mechanisms behind the association between sleep duration and cardiovascular risk factors, since both short [3, 10, 26, 27] and long sleep duration [12, 13] have been associated with cardiometabolic alterations. Studies have shown that obesity is associated with poor sleep quality, sleep fragmentation, and sleep duration.[3, 4, 7]

However, only few of these studies tested this association in the absence of OSA. [8, 9, 11] A higher PSQI score has been associated with larger waist circumferences, BMI, and percentage of body fat, as well as with higher serum levels of insulin and glucose. [28] Nonetheless, associations between PSQI and lipids (HDL cholesterol and triglycerides) have been inconsistent, being reported to be negative, [28] and positive. [29]

In the scenario of the controversy about the association between lipid profile and sleep quality, [28, 29] the CARDIA study showed that a higher PSQI score was not significantly associated with a poorer lipid profile. [13] The poorer lipid profile was statistically associated only with sleep duration. [13] However, VLDL cholesterol levels were not reported in the CARDIA study, as we explored in this current study. In addition, there are several other mechanisms, besides sleep duration, that have been enrolled in the association between sleep disturbances and poor lipid profile. [30, 31] For instance, sleep fragmentation, classified as the number of microarousals during sleep, has been associated with increased levels of lipids, cortisol, and blood pressure. [30] A reasonable hypothesis to be considered is that poor sleep quality may be an epiphenomenon of other sleep-related metabolic changes, such as sleep fragmentation.[30] Our results are consistent with these findings. Our findings demonstrated that other sleep disturbances besides OSA [5-7, 13, 32-36] and sleep duration, [12, 13, 26, 27] specifically sleep quality, may be potential risk factors for poor lipid profiles. A clear understanding of the biological mechanisms linking these sleep disturbances and higher VLDL cholesterol levels remains to be identified.

PSQI is a commonly used subjective sleep quality instrument.[18, 19, 37] Despite the limitations due to its subjective nature, it has also been considered a tool that added significant contributions to the general quality of life (QoL) evaluation. [37] Our findings showed that the daytime dysfunction domain significantly associated with high levels of

both VLDL cholesterol and triglycerides, so it seems to be in line with previous report about the idea that the sleep may have significant impact on QoL, even in the absence of sleep disordered breathing or other health problems, such as lipid alterations. [38]

Our study has some limitations. First, the cross-sectional nature does not allow causal inference. Second, although we adjust for major confounders, there may still be residual confounding due to incomplete adjustments and unknown confounders. Sleep medication use was analyzed separately, as it formed a component of PSQI questionnaire, and is shown in the supplemental data. We did not ascertain type of sleep medication or duration of use. Similarly, hypnotic medication was not taken into account separately in the current analysis. In addition, the subjective nature of the questionnaires should be carefully evaluated. Finally, overnight polygraphy was performed through a type III portable sleep study, which does not allow sleep duration to be objectively measured. Thus, sleep duration in the current study was self-reported. Moreover, portable overnight polygraphy may underdiagnose OSA, [39, 40] since there are no brain channels to assess respiratory events related to arousals. Therefore, our results should be interpreted with caution.

5.0 CONCLUSION

Our findings revealed that poor sleep quality was independently associated with poorer lipid profile, specifically higher VLDL and triglycerides. Poor sleep quality may reflect an unhealthy lifestyle, but also may be an epiphenomenon of other sleep disturbances, beyond OSA and sleep duration. Our findings were robust even after adjusting for socio-economic status, but possible residual confounding caused by unknown factors should be considered in an observational study. An objectively

measured sleep evaluation, by a full polysomnography, may shed light the mechanistic pathways by which poor sleep quality may lead to elevated lipid blood levels.

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Final approval: all

ABBREVIATIONS

AHI= apnea hypopnea index

BMI= body mass index

DBP= diastolic blood pressure

ESS= Epworth sleepiness scale

HDL= high-density lipoprotein

LDL= low-density lipoprotein

OSA= obstructive sleep apnea

PSQI= Pittsburgh sleep quality index

SBP= systolic blood pressure

VLDL= very-low-density lipoprotein

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Table 1. Univariate Analysis of All Complete Sleep Questionnaires (Full Sample) and Those Who Also Underwent Portable Sleep Study (Overnight Polygraphy; Analytical Sample)

Demographics	Full sample (n= 1,801)	Analytical sample (n= 573)	*P*-value
Age, years	45 ±17	43 ±16	**0.023**
Sex (male), %	42	34	**<0.001**
Self-reported race, %			
White	76	80	**0.005**
Black	6	4	
Mixed	13	16	
Smoking status, %			
Never	66	72	**<0.001**
Former	22	20	
Current	12	8	
Alcohol use, %			
None	70	73	0.260
<Once/month	6	7	
Once to 3 times/week	10	9	
Up to 3 times/month	11	9	
Daily	1	1	
Other	2	1	
Salary, %			
Up to 1 minimum wage (MW)	17	15	0.280
From 1 to 5 MW	76	78	
From 5 to 10 MW	3.5	4.0	
From 10 to 20 MW	1.0	0.5	
>20 MW	0.5	0.5	
No answer	2.0	2.0	
Marital status, %			0.122

Married **Single** **Divorced** **Widowed**	62 26 6 6	62 27 5 6	
Education level,% **Illiterate** **High school** **Technical training** **Graduation**	 3 82 3 12	 2 81 4 13	0.564
BMI, kg/m^2	26 ±5	26 ±5	0.740
Waist circumference, cm	91 ±12	91 ±12	0.580
Hip circumference, cm	98 ±10	98 ±10	0.670
Neck circumference, cm	36 ±8	36 ±12	0.790
Medication use, % **Anti-hypertensive** **Hypoglicemic** **Lipid-lowering** **Oral contraceptive**	 28 5 8 15	 28 6 11 18	 0.700 0.210 **0.004** 0.965
Post-menopausal status, %	22	24	0.830
Cardiometabolic risk factors			
VLDL-cholesterol, mg/dL	25 ±12	25 ±11	0.190
LDL-cholesterol, mg/dL	125 ±35	123 ±35	0.093
HDL-cholesterol, mg/dL	47 ±12	48 ±11	0.490
Total cholesterol, mg/dL	198 ±41	195 ±41	0.075
Cholesterol ratio	4.40 ±1.30	4.30 ±1.30	0.052
Triglycerides, mg/dL*	131 ±75	126 ±60	0.190
Glucose, mg/dL	93 ±20	94 ±19	0.106
HbA1c, %	5.7 ±0.8	5.7 ±0.7	0.084
Systolic BP, mmHg	123 ±11	121 ±11	**0.001**
Diastolic BP, mmHg	78 ±9	77 ±8	**0.017**

Data are shown as mean ±SD for continuous and percentage for categorical variables. *P*-value is by independent t-test for continuous and normally distributed variables (the test is independent for each covariate) (*P* value indicates comparison of those included in analytic sample to those excluded) and by chi-square for categorical variables. BMI= body mass index; VLDL=very-low density lipoprotein; LDL= low-density lipoprotein; HDL= high-density lipoprotein; cholesterol ratio= total cholesterol/HDL; HbA1c= glycated hemoglobin; BP= blood pressure.

*Since triglycerides was skewed, the *P*-value is by Mann-Whitney, a non-parametric test. The median and IQR (25^{th}, 75^{th}) for triglycerides in the Full sample is 116 (85, 158) mg/dL and in the Analytical sample is 112 (87, 154) mg/dL.

Table 2. Univariate Analysis of All Complete Sleep Questionnaires (Full Sample) and Those Who Also Underwent Portable Sleep Study (Overnight Polygraphy; Analytical Sample)

Sleep characteristics	Full sample (n= 1,801)	Analytical Sample (n= 573)	*P*-value
AHI, events/h	-	10 ±10	-
ODI, events/h	-	10 ±11	-
Nadir SpO_2,	-	82 ±10	-
Basal SpO_2	-	95 ±5	-
AHI ≥ 5, %	-	58	-
AHI ≥ 15, %	-	18	-
AHI ≥ 30, %	-	5	-
ESS continuous score	7 ±5	7±5	0.094
ESS >10, %	30	34	**0.009**
PSQI total score	5 ±3	5±3	0.095
PSQI ≥ 5, %	46	50	**0.018**
Sleep duration (self-reported) ≥ 6h,%	94	93	0.180
Sleep duration (self-reported), min	472 ±106	461 ±87	**0.002**
Sleep efficiency, %	92 ±34	93 ±16	0.200
Bed time (hours: minutes)*	22:44 ±01:60	22:40 ± 01:40	**<0.001**

Data are shown as mean ±SD for continuous and percentage for categorical variables. *P*-value is by independent t-test for continuous and normally distributed variables (the test is independent for each covariate) (*P* value indicates comparison of those included in analytic sample to those excluded) and by chi-square for categorical variables. OSA= obstructive sleep apnea; BMI= body mass index; AHI= apnea-hypopnea index; ODI= oxygen desaturation index (with 4% drop in oxygen saturation); SpO_2= oxygen saturation; AHI ≥5= mild OSA; AHI ≥15= moderate OSA; AHI ≥30= severe OSA; ESS= Epworth sleepiness scale; ESS >10= excessive daytime sleepiness; PSQI= Pittsburgh sleep quality index; PSQI ≥5= poor sleep quality;

*Bedtime = median and IQR 25^{th}, 75^{th} (22:50 and 21:50, 23:50) for the full sample. Bedtime = median and IQR 25^{th}, 75^{th} (22:50 and 21:50, 23:50) for the analytical sample. Bedtime was skewed, so *P*-value by Mann-Whitney, a non-parametric test.

Those left blank were not measured by polygraphy.

Table 3. Multivariable Linear Regression Analysis Testing Association of CV Risk Factors (Outcome) and PSQI (main Predictor)

	PSQI total score			PSQI <5 vs ≥ 5		
Outcome variable	**Beta**	**Se**	***P*-value**	**Beta**	**se**	***P*-value**
VLDL-cholesterol, mg/dL	0.392	0.156	**0.012**	1.763	0.980	0.072
ln Triglycerides, mg/dL	0.017	0.006	**0.006**	0.071	0.040	0.071
HDL-cholesterol, mg/dL	-0.194	0.155	0.208	-0.897	0.973	0.356
LDL-cholesterol, mg/dL	0.082	0.500	0.870	1.886	3.120	0.546
Total cholesterol, mg/dL	0.495	0.561	0.377	2.932	3.532	0.407
Cholesterol ratio	0.033	0.017	0.060	0.195	0.110	0.077
Glucose, mg/dL	0.180	0.247	0.468	-0.601	1.560	0.700
HbA1c, %	-0.015	0.009	0.102	-0.066	0.056	0.238
Systolic BP, mmHg*	0.073	0.152	0.634	0.516	0.961	0.613
Diastolic BP, mmHg*	0.060	0.116	0.602	0.290	0.731	0.693

CV= cardiovascular; VLDL= very-low density lipoprotein; ln= natural logarithm transformation; HDL= high-density lipoprotein; LDL= low-density lipoprotein; cholesterol ratio= total cholesterol/HDL; HbA1c= glycated hemoglobin; BP= blood pressure; PSQI= Pittsburgh sleep quality index; PSQI ≥5= poor sleep quality; BMI= body mass index; ESS= Epworth sleepiness scale; OSA= obstructive sleep apnea; AHI= apnea hypopnea index (number of events per hour of sleep); se= standard error. Each line represents a unique regression model. Adjustments for age, sex (female= ref), race (White vs others), Wage categories (up to 5 MW vs >5MW and >5MW as ref), Education categories (illiterate vs high school vs technical/graduation and illiterate as ref), smoking status (never as ref), systolic BP, BMI, sleep duration (minutes); ESS continuous score, bedtime (in hours), and OSA by AHI continuous score. Missing data were excluded (all complete for regression analysis= 554).
*not included systolic BP in adjustments.

Table 4. Restricted Analysis for those Without OSA (AHI<15), Testing the Association Between Lipid Components (Outcome) and Sleep Quality (n= 472)

	VLDL-cholesterol, mg/dL			ln Triglycerides, mg/dL		
	Beta	**se**	***P*-value**	**Beta**	**se**	***P*-value**
PSQI total score	0.446	0.170	**0.009**	0.018	0.007	**0.010**
PSQI cutoff 5	2.188	1.037	**0.035**	0.084	0.426	**0.048**

OSA= obstructive sleep apnea; AHI= apnea hypopnea index; VLDL= very-low density lipoprotein cholesterol; ln= natural logarithm transformation; se= standard error; PSQI= Pittsburgh sleep quality index; PSQI ≥5= poor sleep quality; BMI= body mass index; ESS= Epworth sleepiness scale; BP= blood pressure. Adjustments: age, sex (female= ref), race (White vs others), Wage categories (up to 5 MW vs >5MW and >5MW as ref), Education categories (illiterate vs high school vs technical/graduation and illiterate as ref), smoking status (never as reference), BMI, ESS continuous score, sleep duration (minutes), bedtime (in hours), and daytime systolic BP. Missing data were excluded. All complete for regression analysis (n=423).

Table 5. Restricted Analysis for those Without Lipid-lowering Medication (n= 510) Testing the Association Between Lipid Components (Outcome) and Sleep Quality

	VLDL-cholesterol, mg/dL			ln Triglycerides, mg/dL		
	Beta	**se**	***P*-value**	**Beta**	**se**	***P*-value**
PSQI total score	0.426	0.161	**0.008**	0.019	0.006	**0.004**
PSQI cutoff 5	1.681	1.013	0.097	0.080	0.040	**0.047**

VLDL= very-low density lipoprotein cholesterol; ln= natural logarithm transformation; se= standard error; PSQI= Pittsburgh sleep quality index; PSQI ≥5= poor sleep quality; BMI= body mass index; ESS= Epworth sleepiness scale; BP= blood pressure, AHI= apnea hypopnea index. Adjustments: age, sex (female=ref), race (White vs others), Wage categories (up to 5 MW vs >5MW and >5MW as ref), Education categories (illiterate vs high school vs technical/graduation and illiterate as ref), smoking status (never as reference), BMI, ESS continuous score, sleep duration (minutes), bedtime (in hours), AHI, and daytime systolic BP. Missing data were excluded. All complete for regression analysis (n= 462).

Optional online-only supplementary files

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