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## Genetic and environmental determinants of cardiometabolic health

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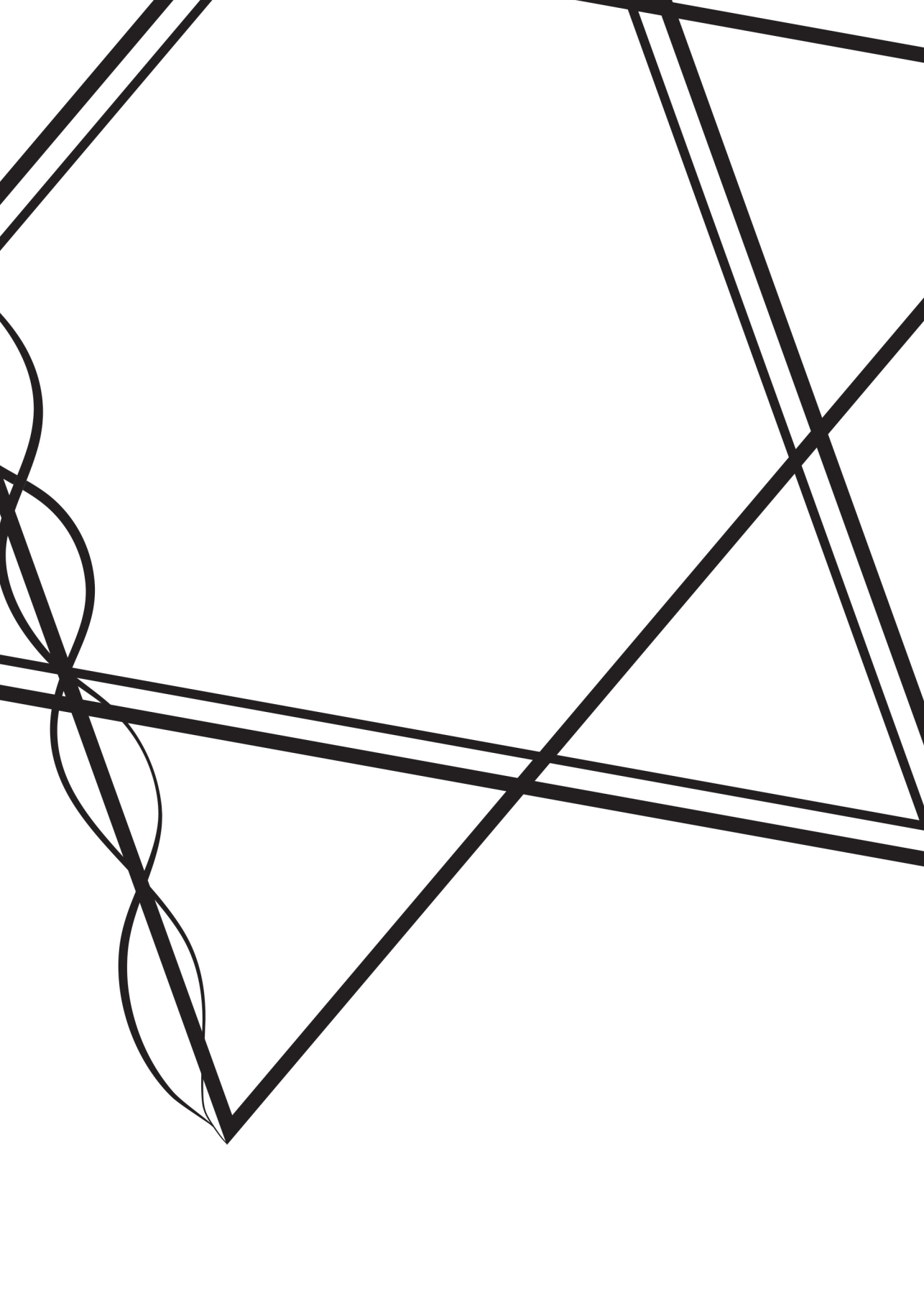




# PART II

Sleep







# CHAPTER 3.1

Associations between sleep duration and quality with serum and hepatic lipids: the Netherlands Epidemiology of Obesity Study.

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## ABSTRACT

Short and long sleep duration and poor sleep quality may affect serum and hepatic lipid content, but available evidence is inconsistent. Therefore, we aimed to investigate the associations between sleep duration and quality with serum and hepatic lipid content in a large population-based cohort of middle-aged individuals. The present cross-sectional study was embedded in the Netherlands Epidemiology of Obesity (NEO) study and consisted of 4,260 participants (mean age: 55 years, proportion men: 46%) not using lipid-lowering agents. Self-reported sleep duration and quality were assessed using the Pittsburgh Sleep Quality Index questionnaire (PSQI). Outcomes of this study were fasting lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides), postprandial triglyceride (response) levels, and hepatic triglyceride content as measured with magnetic resonance spectroscopy. We performed multivariable linear regression analyses, adjusted for confounders, and additionally for measures that link to adiposity (e.g., BMI, sleep apnea). We observed that relative to the group with median sleep duration ( $\approx 7.0$  hours of sleep), the group with shortest sleep ( $\approx 5.0$  hours of sleep) had 1.5 fold higher hepatic triglyceride content (95% confidence interval (CI): 1.0;2.2). The group with PSQI score  $\geq 10$  had a 1.1 (95%CI: 1.0;1.2) fold higher serum triglyceride level compared with the group with PSQI  $\leq 5$ . However, these associations disappeared after adjustment for BMI and sleep apnea. Therefore, we concluded that previously observed associations between shorter sleep duration and poorer sleep quality with an adverse lipid profile, may be explained by BMI and sleep apnea, rather than by a direct effect of sleep on the lipid profile.

## INTRODUCTION

Cardiovascular disease (CVD) is one of the leading causes of death worldwide and is responsible for 17.7 million deaths in 2015<sup>1</sup>. Several lifestyle factors have been associated with a deleterious CVD risk profile and a higher risk of cardiovascular mortality, for example smoking and alcohol intake<sup>2</sup>. Multiple epidemiological studies, have shown that sleep duration is another important risk factor for the development of CVD<sup>3-11</sup>. However, in some studies both extremes (long and short sleep duration) are associated with an increased risk of CVD<sup>3,4</sup>, while in other studies only short sleep duration or long sleep duration has been associated with an increased risk of CVD and with CVD risk factors (notably obesity and metabolic syndrome)<sup>5-13</sup>.

Besides sleep duration, poor sleep quality has also been associated with an increased risk of metabolic syndrome<sup>14,15</sup>, yet not in all studies<sup>11</sup>. Several factors could contribute to the discrepant findings regarding the associations between sleep duration and sleep quality with cardiovascular risk factors. Body mass index (BMI) and sleep apnea are associated with alterations in sleep and with high circulating lipids and incidence of coronary heart disease<sup>16,17</sup>. In the study of Petrov *et al.* (2013) poor sleep quality was associated with a poor lipid profile, however, after adjustment for covariates including BMI and obstructive sleep apnea (OSA) risk, this association disappeared<sup>11</sup>. Importantly, previous studies generally adjusted for BMI, however, most studies did not adjust for sleep apnea<sup>5-13</sup>. Therefore, the question remains to what extent the previously described associations between sleep duration and sleep quality with CVD were confounded by BMI and also OSA. Moreover, both BMI and OSA are risk factors for non-alcoholic liver disease (NAFLD)<sup>18-20</sup>, which is a risk factor for myocardial dysfunction<sup>21</sup>. To the best of our knowledge hepatic triglyceride content (HTGC) has not been studied in relation to sleep duration and/or sleep quality.

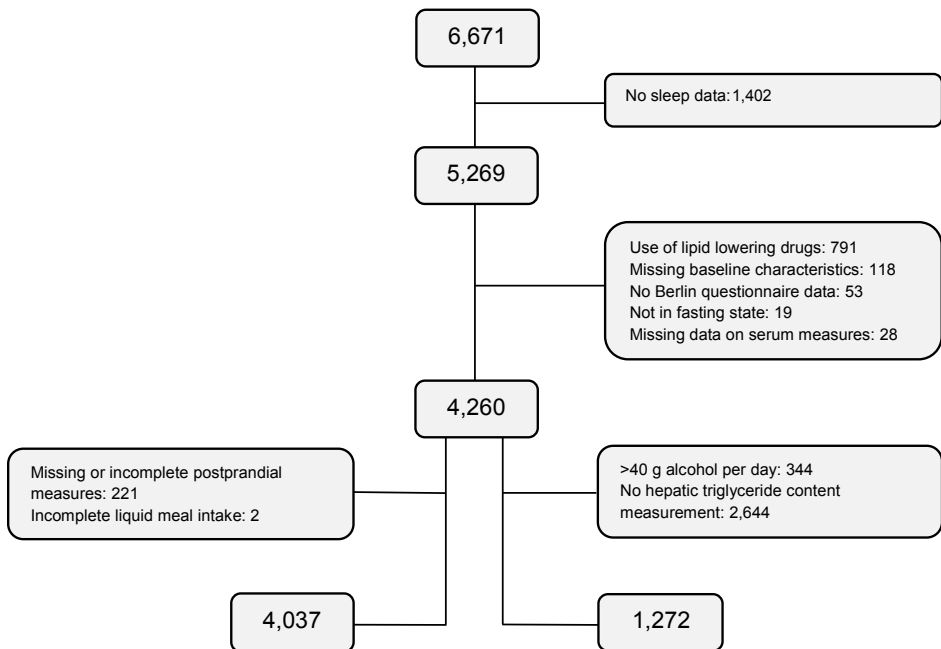
Based on previous studies, we hypothesized that both short and long sleep duration and poor sleep quality are associated with an adverse serum and hepatic lipid profile. However, we hypothesize that after adjustment for BMI and the risk of sleep apnea, these associations may decrease. In the present study we aim to assess these associations in a large population-based cohort of middle-aged adults from the Netherlands, considering all important confounding factors (including BMI and sleep apnea).



## METHODS

### *Study design and study population*

The present study is a cross-sectional analysis of baseline measurements of the Netherlands Epidemiology of Obesity (NEO) study, a cohort of 6,671 individuals with an oversampling of individuals with overweight or obesity. Between September 2008 and September 2012, men and women aged between 45 and 65 years with a self-reported body mass index (BMI) of 27 kg/m<sup>2</sup> or higher living in the greater area of Leiden were invited to participate in the NEO study. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited irrespective of their BMI, allowing for a reference distribution of BMI. Baseline data were collected at the NEO study center of the Leiden University Medical Center (LUMC). Prior to the NEO study visit, participants completed a questionnaire about demographic and clinical information and fasted for at least 10 hours. Participants came to the research site in the morning to undergo several baseline measurements including anthropometric measurements and fasting and postprandial blood sampling. At the study site, a screening form was completed by all participants asking about anything that might create a health risk or interfere with MRI imaging (most notably metallic devices, claustrophobia, and a body circumference of more than 1.70 m). Of the participants who were eligible for MRI, approximately 35% of the total study population were randomly selected to undergo direct assessment of VAT. A medication inventory was performed to collect data on medication use during the month preceding the visit to the study center. More detailed information on the study design and data collection was described elsewhere<sup>22</sup>. This study was approved by the medical ethics committee of the Leiden University Medical Center (LUMC) (and the NEO board) and all participants gave written informed consent. As demonstrated in **Figure 1**, we excluded participants with missing data on the PSQI questionnaire (N=1,402). The PSQI questionnaire was only added to the baseline questionnaire after July 2009 and therefore participants entering the study before this date have missing data on this questionnaire. Moreover, we excluded participants who used lipid lowering drugs (N=791), had missing baseline characteristics (N=118), missing data of the Berlin questionnaire (N=53), were not in a fasting state during the hospital visit (N=19), or missed data on serum triglycerides (N=27) or cholesterol (N=1). We additionally excluded participants who drank >40 g alcohol per day (N=344) from the analyses on hepatic triglyceride content (HTGC). For the analyses on postprandial triglyceride levels, we excluded participants with missing or incomplete postprandial serum triglyceride concentrations (N=221) or who had no or incomplete liquid meal intake (N=2).



**Figure 1.** Flowchart of participant inclusion

### *Sleep characteristics*

To assess habitual sleep duration and quality, we used the Pittsburgh sleep quality index (PSQI)<sup>23</sup>, which is a self-rated questionnaire to retrospectively measure sleep parameters over a one month time interval. Total sleep duration was derived from the question "On an average day, how much sleep do you get?". To obtain a classification of short and long total sleep duration, we calculated the age- and sex- adjusted residuals with linear regression analysis for total sleep duration with age and sex and determined subgroups on the basis of these residuals. We used the 5<sup>th</sup> lowest percentile of the age- and sex-adjusted residuals to define shortest sleep, the 5<sup>th</sup> till 20<sup>th</sup> percentile to define short sleep, the 20<sup>th</sup> till 80<sup>th</sup> to define medium sleep, the 80<sup>th</sup> till 95<sup>th</sup> to define long sleep and the 95<sup>th</sup> till 100<sup>th</sup> percentile to define longest sleep. Sleep quality was assessed using the total score of the PSQI questionnaire. The questionnaire consists of seven components of which an overall score can be calculated. The global score ranges from 0 to 21, in which a higher score indicates a poorer sleep quality<sup>23</sup>. For sleep quality, we formed three sleep quality groups, in which we used the good sleep quality group (PSQI total score  $\leq 5$ ) as a reference group in linear regression analyses. The poor sleep quality group was defined by a PSQI total score between 5 and 10, and worst sleep quality as PSQI total score  $\geq 10$ .

### *Serum lipid profile and hepatic triglyceride content*

After an overnight fast of at least 10 h, fasting blood samples were taken at the study center. Within 5 min after the first blood sample was taken, participants drank a liquid mixed meal (400 mL) with an energy content of 600 kcal, with 16% of energy derived (En%) from protein, 50 En% from carbohydrates and 34 En% from fat. Postprandial blood samples were taken 30 and 150 minutes after ingestion of the meal. Serum triglyceride concentrations were determined at the 3 time points. Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric methods (Roche Modular Analytics P800, Roche Diagnostics, Mannheim, Germany; CV < 5%) and HDL-cholesterol with homogenous HDLc method, 3<sup>rd</sup> generation (Roche Modular Analytics P800, Roche Diagnostics, Mannheim, Germany; CV < 5%). Low-density lipoprotein (LDL) cholesterol concentration was estimated using Friedewald's formula<sup>24</sup>. All measures were performed in the central clinical chemistry laboratory of the Leiden University Medical Center. The area under the curve (AUC) for postprandial serum triglyceride levels was calculated using the Trapezoid Rule as  $(15 \cdot \text{fasting concentration} + 75 \cdot \text{concentration}_{30\text{min}} + 60 \cdot \text{concentration}_{150\text{min}}) / 150$ <sup>25</sup>. Hepatic <sup>1</sup>H magnetic resonance (MR) spectra were obtained in a random subset of 1,207 participants with data on habitual sleep. In short, an 8-mL voxel was positioned in the right lobe of the liver. A point-resolved spectroscopy sequence was used to acquire spectroscopic data during continuous breathing with automated shimming. Spectra were obtained with and without water suppression. Spectral data were fitted by using Java-based MR user interface software (jMRUI, version 3.0; developed by A. van den Boogaart, Katholieke Universiteit Leuven, Leuven, Belgium)<sup>26</sup>. Mean line widths of the spectra were calculated. The resonances that were fitted and used for calculation of the triglycerides were methylene (peak at 1.3 ppm,  $[\text{CH}_2]_n$ ) and methyl (peak at 0.9 ppm,  $\text{CH}_3$ ). The HTGC relative to water was calculated with the following formula:  $(\text{signal amplitude of methylene} + \text{methyl}) / (\text{signal amplitude of water}) \times 100$ .

### *Covariates*

A semi-quantitative food frequency questionnaire (FFQ)<sup>27</sup> questionnaire was used to assess energy intake. Energy intake was estimated from the FFQ with the 2011 version of the Dutch food composition table (NEVO-2011). Participants reported the frequency and duration of their physical activity in leisure time using the Short Questionnaire to Assess Health-enhancing physical activity (SQUASH)<sup>28</sup>, which was expressed in hours per week of metabolic equivalents (MET-h/week). Body weight was measured at the study center without shoes and one kilogram (kg) was subtracted to correct for the weight of clothing. BMI was calculated by dividing the weight in kilograms by the height in meters squared. The risk for the presence of obstructive sleep apnea syndrome was assessed using the Berlin questionnaire<sup>29</sup>. This questionnaire consists of 10 questions



that form three categories (snoring (category 1), daytime somnolence (category 2) and hypertension and BMI (category 3)) related to the likelihood of the presence of sleep apnea. Individuals can be classified as either having a high (2 or more categories with a positive score) or low likelihood of sleep apnea (only 1 or no categories with a positive score).

### *Statistical Analysis*

Because individuals with a BMI of 27 kg/m<sup>2</sup> or higher were oversampled in the NEO study population, adjustments were made to correctly represent associations in the general population<sup>30-32</sup>. This was done by weighting individuals towards to the BMI distribution of participants from the Leiderdorp municipality, whose BMI distribution was similar to the BMI distribution of the general Dutch population<sup>22</sup>. Consequently, all presented results are based on weighted analyses and apply to a population-based study without oversampling of participants with a BMI of 27 kg/m<sup>2</sup> or higher. Characteristics of the study population were expressed as mean (with standard deviation, SD) for normally distributed measures, median with inter-quartile ranges for non-normally distributed measures, and proportions for categorical variables. We performed all statistical analyses using Stata version 12.1 (Stata, College Station, Texas, USA) software.

Not normally distributed outcomes were log transformed to approximate a normal distribution (notably serum triglycerides, HTGC and AUC of serum triglycerides). However, in order to present the results with a similar interpretation, we log transformed normally distributed outcomes (notably serum HDL-cholesterol, LDL-cholesterol, total cholesterol) as well. We performed linear regression analyses using the medium sleep category (characterized by 20<sup>th</sup> till 80<sup>th</sup> percentile of sleep duration residuals) as reference group. The subsequent beta regression coefficients were back-transformed and expressed as a ratio with accompanying 95% confidence interval (95% CI), which can be interpreted as the relative change in outcome compared to the reference group. The initial model for linear regression analyses was adjusted for age and sex (Model 1). In addition to age and sex, we adjusted in Model 2 for ethnicity (white/other), education level (high/other), smoking (never/former/current), alcohol consumption, energy intake, physical activity and sleep medication (yes/no). In Model 3 we additionally adjusted for BMI and sleep apnea. In the analyses for sleep quality we did not adjust for sleep medication in Models 2 and 3, as this is a component of the PSQI total score.

## RESULTS

### *Characteristics of the study population*

In total, after exclusion of non-eligible participants, this study comprised 4,260 participants with a mean age of 55 (SD 6.0) years, of whom 46% were men. As compared with the medium sleep group (40%), there were more men in both the shortest (45%) and the longest (51%) sleep group (**Table 1**). Less individuals had higher education in both the shortest sleep group (39%) and the longest sleep group (36%) than in the medium sleep group (51%). More participants used sleep medication in the shortest (14%) and longest sleep group (7%) as compared with the medium sleep group (4%). HTGC was higher both in the shortest sleep group (6% [2.5;10.5]) and in the longest sleep group (4% [2.0;7.4]) than the medium sleep group (2%, [1.2;5.1]). All other studied characteristics were similar between the groups.

### *Associations between sleep duration and fasted and postprandial lipids*

In the analyses adjusted for age and sex (Model 1), shortest sleep duration was associated with a 1.52 (95%CI: 1.04-2.24) fold higher HTGC as compared with the medium sleep group (**Figure 2 and Online Supplementary Table 1**). This association persisted after adjustment for potential confounding factors (Model 2). However, the association between shortest sleep and higher HTGC disappeared after we additionally adjusted for BMI and sleep apnea in Model 3 (ratio of 1.00 (95%CI: 0.68-1.45)). There were no associations between short and long sleep duration and total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides or AUC of triglycerides.

### *Associations between sleep quality and fasting and postprandial lipids*

A poor sleep quality (PSQI total score 5-10; **Figure 3 and Online Supplementary Table 2**) was associated with 1.07 (95%CI: 1.01;1.13) fold increased serum triglyceride level in the analyses (age and sex adjusted) as compared with good sleep quality (PSQI score  $\leq 5$ ). Adjustment for potential confounding factors (Model 2) did not materially change the results (ratio 1.06 (95%CI: 1.00;1.11)), but when we additionally adjusted for BMI and sleep apnea (Model 3), the association between poor sleep quality and serum triglycerides disappeared (ratio 1.04 (95%CI: 0.99;1.09)). Poor sleep quality was associated with a 1.24 (95% CI: 1.04;1.49) fold increased HTGC as compared with good sleep quality in Model 1, which persisted in Model 2 (ratio 1.21 (95%CI: 1.01;1.45)). However, after additional adjustment for BMI and sleep apnea (Model 3) the association disappeared (ratio 1.08 (95%CI: 0.91;1.27)). Worst sleep quality (PSQI  $\geq 10$ ) was associated with a 1.10 (95% CI: 1.02;1.18) fold increased fasting serum triglyceride level as compared with good sleep quality in Model 1.

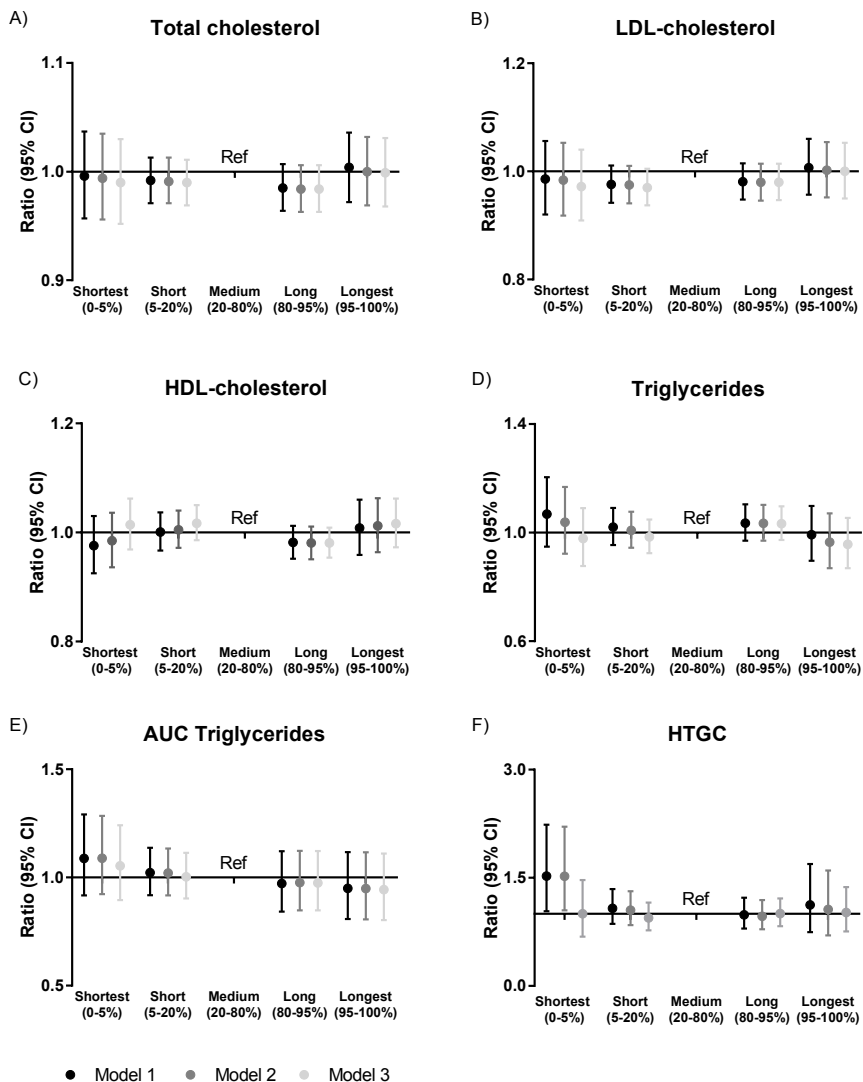
This association persisted in Model 2 (ratio 1.08 (95%CI: 1.00;1.16), but the association disappeared in Model 3 (ratio 1.01 (95%CI: 0.99;1.09). There were no associations between sleep quality and total cholesterol, LDL-cholesterol, HDL-cholesterol or AUC of triglycerides.

**Table 1.** Characteristics of participants in the Netherlands Epidemiology of Obesity study, stratified by sleep duration (N=4,260)

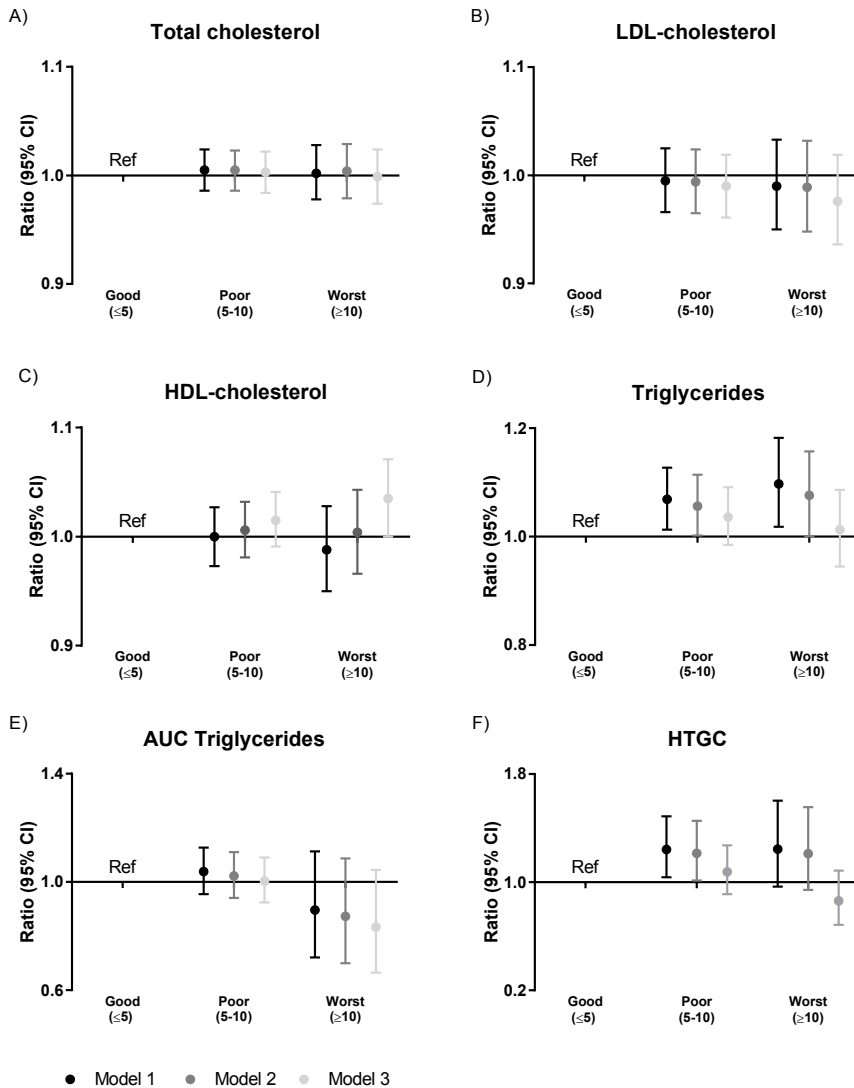
Sleep duration	Shortest	Short	Medium	Long	Longest
	0-5%	5-20%	20-80%	80-95%	95-100%
Age (years)	57 (5)	57 (5)	55 (6)	54 (6)	57 (6)
Sex (% men)	45	47	40	41	51
BMI (kg/m <sup>2</sup> )	27 (5)	26 (5)	26 (4)	26 (4)	26 (5)
Ethnicity (% white)	90	93	96	96	93
Education (% high)	39	43	51	48	36
Smoking (% current)	19	14	16	17	19
Sleep medication (%)	14	9	4	5	7
Alcohol consumption (g/day)	12 (3;22)	10 (3;22)	10 (3;21)	9 (2;21)	9 (0;21)
Physical activity (MET-h/week)	25 (12;44)	30 (16;47)	30 (17;50)	32 (16;52)	30 (15;51)
Sleep duration (h/day)	5 (4;5)	6 (6;6)	7 (7;8)	8 (8;8)	9 (9;9)
PSQI (total score)	11 (9;13)	7 (5;10)	4 (3;6)	3 (2;4)	3 (2;5)
Sleep apnea (%)	33	26	16	16	22
Fasting total cholesterol (mmol/L)	6 (1)	6 (1)	6 (1)	6 (1)	6 (1)
Fasting LDL-cholesterol (mmol/L)	4 (1)	4 (1)	4 (1)	4 (1)	4 (1)
Fasting HDL-cholesterol (mmol/L)	2 (1)	2 (1)	2 (1)	2 (0)	2 (0)
Fasting triglycerides (mmol/L)	1 (1;2)	1 (1;2)	1 (1;1)	1 (1;2)	1 (1;1)
Triglycerides 30 min (mmol/L)	1 (1;2)	1 (1;2)	1 (1;2)	1 (1;2)	1 (1;2)
Triglycerides 120 min (mmol/L)	2 (1;3)	2 (1;2)	2 (1;2)	2 (1;2)	2 (1;2)
AUC Triglycerides±	48 (28;77)	48 (26;73)	46 (26;66)	46 (27;68)	44 (26;68)
Hepatic triglyceride content (%)*	6 (3;11)	3 (2;6)	2 (1;5)	2 (1;6)	4 (2;8)

Abbreviations: AUC, area under the curve; BMI, body mass index; HDL, high-density lipoprotein; kJ, kilojoule; LDL, low-density lipoprotein; MET, metabolic equivalents of task; NEO, Netherlands Epidemiology of Obesity; PSQI, Pittsburgh Sleep Questionnaire Index. Results were based on analyses weighted towards the BMI distribution of the general Dutch population. Data presented as mean ± standard deviation (SD); proportion (%); median (25<sup>th</sup>-75<sup>th</sup> percentile). ±, N=4,037; \*, N=1,272.





**Figure 2.** Associations between sleep duration and A) TC, B) LDL-cholesterol, C) HDL-cholesterol, D) TG, E) AUC of TG and F) hepatic triglyceride content (HTGC). The medium sleep duration group is used as reference category in linear regression analyses. Results are presented as ratios with accompanying 95% confidence intervals, linear regression coefficients of the log transformed outcomes were back transformed in order to present ratios. The ratio reflects the relative change to provide an indication of the fold change of the outcome as compared to the reference category. Results were based on analyses weighted towards the BMI distribution of the general Dutch population. Model 1: adjusted for age and sex; Model 2: adjusted for age, sex, ethnicity, education level, smoking, alcohol intake, caloric intake and physical activity; Model 3: adjusted for Model 2 + sleep apnea and BMI. Abbreviations: AUC, area under the curve; CI, confidence interval; HDL, high-density lipoprotein; HTGC, hepatic triglyceride content; LDL, low-density lipoprotein; Ref, reference category.



**Figure 3.** Associations between sleep quality and A) TC, B) LDL-cholesterol, C) HDL-cholesterol, D) TG, E) AUC of TG and F) HTGC. The good sleep quality group is used as reference category in linear regression analyses. Results are presented as ratios with accompanying 95% confidence intervals, linear regression coefficients of the log transformed outcomes were back transformed in order to present ratios. The ratio reflects the relative change to provide an indication of the fold change of the outcome as compared to the reference category. Results were based on analyses weighted towards the BMI distribution of the general Dutch population. Model 1: adjusted for age and sex; Model 2: adjusted for age, sex, ethnicity, education level, smoking, alcohol intake, caloric intake and physical activity; Model 3: adjusted for Model 2 + sleep apnea and BMI. AUC, area under the curve; CI, confidence interval; HDL, high-density lipoprotein; HTGC, hepatic triglyceride content; LDL, low-density lipoprotein; Ref, reference category.

## DISCUSSION

The present study aimed to address the associations between sleep duration and quality with hepatic triglyceride content (HTGC) and serum lipid levels in a cohort of 4,260 middle-aged individuals. When analyses were adjusted for age and sex, we observed an association between shortest sleep duration with higher HTGC. Moreover, poor sleep quality was associated with higher fasting serum triglyceride levels and higher HTGC than good sleep quality. However, all observed associations disappeared after additional adjustment for BMI and sleep apnea.

In previous research it has been shown that both short and long sleep duration, or only short or long sleep duration were associated with cardiovascular risk and cardiovascular risk factors<sup>3-11</sup>. We hypothesized that these discrepant findings could be explained by differences in the considered confounding factors, which includes the adjustment for the confounding factors BMI and sleep apnea<sup>16, 17</sup>. In agreement, in our study, when adjusted for age and sex, short sleep duration was associated with higher serum triglyceride levels. When we additionally adjusted for ethnicity, education level, smoking, alcohol intake, caloric intake and physical activity, this association remained.. However, this association disappeared after additional adjustment for BMI and sleep apnea. Similar, we observed an association between poor sleep quality and higher serum triglyceride levels and HTGC after adjustment for classical confounders, however, again these associations disappeared after additional adjustment for BMI and sleep apnea. In agreement, in a cohort study comprising 503 adults, it has been observed that there was no association between poor sleep quality and lipid profile after adjustment for covariates including BMI and sleep apnea risk<sup>11</sup>.

In contrast to our findings, a study of Anujuo *et al.*<sup>33</sup> did not observe an association between short sleep duration and higher triglyceride levels in neither of their analyses (adjusted for only age and sex, or other confounding factors including BMI) in a population consisting of 2,146 participants of Dutch origin. One of the explanations for these discrepant findings could be the different cut-off point used for determining short sleep duration, which was <7 hours based on the small number of individuals with very short sleep duration. Therefore, the group defined as "short sleep" might not be sleeping sufficiently short to observe clinical relevant associations with lipid levels.

Questions regarding the direction of the observed associations remain to be elucidated. In this study we considered BMI and sleep apnea as potential confounding factors, suggesting that BMI and sleep apnea are a common cause of alterations in sleep and in lipid metabolism. Alternatively, sleep duration might have a causal effect on BMI and



sleep apnea meaning that BMI and sleep apnea may mediate the association between sleep and cardiovascular risk factors. In this case the observed associations between sleep duration/quality and serum and hepatic lipid profile in the present study may be underestimated.

There are several biological pathways that could link sleep duration and quality to CVD. For example, a short sleep duration might have an adverse effect on cardiovascular health via increased cortisol levels and/or inflammatory mediators through higher sympathetic nervous system activity<sup>34</sup>. In addition, a genome-wide association study on total sleep duration suggests that there is also a shared genetic component between insomnia symptoms and a higher BMI, waist circumference and insulin resistance, which have all been associated with a higher risk of developing CVD<sup>35</sup>. Moreover, a shorter sleep duration has been associated with altered levels of leptin and ghrelin and a higher craving for carbohydrate-rich foods<sup>36, 37</sup>. These are possible mechanisms via which alteration in sleep could lead to obesity and obesity-related disorders. Both obesity and sleep apnea are shown to be associated with high circulating lipids and a higher incidence of coronary heart disease<sup>16, 17</sup>. Moreover, obesity is a risk factor for non-alcoholic liver disease (NAFLD)<sup>18</sup>. It was shown that a higher HTGC was associated with a higher risk of myocardial dysfunction, as characterized by a lower diastolic function in the NEO study population, however, only in obese individuals<sup>21</sup>. Also, it was shown that obstructive sleep apnea (OSA) is prevalent in 60% of NAFLD patients, and that OSA may contribute to progression of NAFLD<sup>19, 20</sup>. OSA is thought to exert an effect via different mechanisms (e.g. inflammation, oxidative stress and intermittent hypoxia)<sup>37</sup> and can be treated with continuous positive airway pressure (CPAP)<sup>38</sup>. This supports our hypothesis that sleep apnea and BMI affect the relation between short sleep duration and poor sleep quality with CVD. Our findings support the idea of weight loss and sleep apnea screening in individuals with short sleep duration and poor sleep quality in order to contribute to prevention of cardiovascular disease onset. Nevertheless, the individual contributions of sleep, sleep quality, circadian rhythm, and other lifestyle adaptations and their interrelations are complex and difficult to assess separately and at least require prospective analyses in large populations.

A strength of this study is the use of residuals as a determinant for sleep duration. By using the residuals we obtained a classification of sleep duration which is independent of age and sex. One of the other strengths of this study is the extensive phenotyping of the NEO study which enables us to correct for a broad range of possible confounding factors (e.g. ethnicity, physical activity and risk of sleep apnea). Moreover, previous studies used different questionnaires to assess sleep duration and sleep quality, which may result in inconclusive results. Therefore, we have used the PSQI questionnaire, which is a widely

used and validated too to assess sleep disturbances <sup>39</sup>. However, the present study also has some limitations. First, inherent to the observational cross-sectional design, whereby we are not able to exclude reverse causation or residual confounding in this study. Second, we used subjective sleep questionnaires to assess sleep duration and sleep quality and the risk of sleep apnea. Self-reported data is subject to recall bias, whereby participants may either under- or over-report their sleep duration and their quality of sleep. There may be a measurement error which results in non-differential misclassification for the exposure. However, the Pittsburgh sleep quality index was shown to be a reliable and validated tool to assess sleep dysfunction <sup>39</sup>. Moreover, although the risk of sleep apnea is self-reported using the Berlin questionnaire, this tool has also been validated in several populations and showed the highest specificity to detect mild and severe OSA in patients from sleep clinics, as compared to other OSA screening questionnaires<sup>40</sup>.

In conclusion, we observed that shorter sleep duration and poorer sleep quality were associated with an adverse lipid profile. However, all observed associations disappeared after additional adjustment for BMI and sleep apnea, indicating that BMI and risk of sleep apnea, likely confound previously observed associations and should therefore be considered in future studies.

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### **Conflict of interest**

All authors declare to have no conflict of interest.

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