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(AASM), and Sleep Research Society (SRS), which recommend 7 to 9 hours as the basal sleep need for healthy adults. [25] We focused on short sleep only because in our previous study, we demonstrated that short sleep was the major problem for the ethnic minority groups, [21] and because previous studies found that short sleep was more consistently related to CVD risk factors compared to long sleep. [26-28]

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The prevalence of cardiovascular disease was assessed using the Rose questionnaire on angina pectoris, intermittent claudication and possible myocardial infarction. [29]

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Weight (kg) and height (cm) were measured in duplicate in barefoot subjects wearing light clothes only. Waist circumference (cm) was measured twice using a tape measure at the level midway between the lowest rib margin and the iliac crest, and hip circumference (cm) was measured twice at the widest level over the trochanter major. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2), and waist-to-hip ratio (WHR) was calculated as waist circumference divided by hip circumference.

Blood pressure (BP) was measured in duplicate using a semi-automatic sphygmomanometer (Microlife WatchBP Home, Microlife AG, Switzerland) on the left arm in a seated position after the participant had seated for at least 5 minutes. Hypertension was defined as systolic BP ≥ 140 mmHg, or diastolic BP ≥ 90 mmHg, or being on BP lowering medication, or self-reported hypertension.

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Fasting blood samples were taken to determine the concentration of glucose by spectrophotometry, using hexokinase as primary enzyme (Roche Diagnostics, Japan). Total cholesterol, triglycerides and high-density lipoprotein (HDL) cholesterol were determined by colorimetric spectrophotometry (Roche Diagnostics, Japan). Low-density lipoprotein cholesterol (LDL) was calculated according to the Friedewald formula. [30] Type 2 diabetes was defined as increased fasting glucose ≥7mmol/L or current use of glucose-lowering medication or self-reported diabetes. Dyslipidemia was defined as total cholesterol (TC) >6.22mmol/L, or high density lipoprotein cholesterol (HDL-C) <1.04mmol/L, or low density lipoprotein cholesterol (LDL-C) >4.14mmol/L, or triglyceride (TG) >1.69mmol/L, [31] or use of lipid-lowering medication.

Educational level was determined using participant's highest level of education obtained (either in the Netherlands or in the country of origin). Participants were categorized into those who have never been to school or had elementary schooling only (1st category), those with lower vocational schooling or lower secondary schooling (2nd category), those with intermediate vocational schooling or intermediate/higher secondary education schooling (3rd category), and those with higher vocational schooling or university (4th category). For the current analyses, the first two categories were combined because of small numbers.

Alcohol intake in the past 12 months (yes/no) and smoking status (yes/no/ex-smoker) were obtained by questionnaire. Habitual physical activity was measured using the SQUASH questionnaire. [32] The SQUASH questions about multiple activities referring to a normal week in the past months. We categorized participants according to the Dutch guideline for physical activity by summing up the number of days per week for each moderate and high intensity activity lasting at least 30 minutes. A total of 5 days or more resulted in participants being categorized as achieving the Dutch norm for physical activity. [32]

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Baseline data were expressed as percentages, means or median. The analyses were performed in two steps. In the first step, the association of short sleep duration with prevalent CVD within each ethnic group was analysed using prevalence ratio(s) (PR) with 95% confidence interval (CI), with adjustments for potential confounders (age, gender and conventional CVD risk factors). In the second step, comparisons of prevalent CVD between ethnic groups was performed with adjustments for age and gender (model 1). In order to assess the contribution of short sleep and conventional CVD risk factors to the ethnic differences in CVD, we subsequently added short sleep (model 2), CVD risk factor variables (hypertension, diabetes, BMI, WHR, dyslipidaemia, smoking, alcohol, and physical activity; model 3), or both short sleep and CVD risk factors (model 4) to the regression models. The change in PR (percentage) before and after inclusion was used to assess the relative total contribution of short sleep and CVD risk factors to ethnic differences in CVD. By subtracting the changes in PR (percentage) of model 4 and 3, the contribution of short sleep independent of CVD risk factors was calculated for each ethnic minority group. All analyses were performed using STATA 11.0 (Stata Corp, College Station, TX). A p-value of <0.05 was considered as statistically significant.

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Table 1 shows the characteristics of the study population by ethnic group. Moroccans and Turks were younger, had higher prevalence of CVD, lower educational levels, consumed less

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alcohol, less often achieved the physical activity norm, and had a lower prevalence of hypertension as compared with the other ethnic origin groups. Similar to Turkish participants, South-Asian Surinamese also had higher prevalence of CVD than the other groups. Ghanaians also had lower educational levels than Dutch. South-Asian Surinamese, African Surinamese, and Ghanaian participants had a lower mean sleep duration and higher prevalence of short sleep than Dutch, Turks and Moroccans. Dutch and South-Asian Surinamese had a lower mean BMI than the other ethnic groups, whereas mean WHR was higher in South-Asian Surinamese, Ghanaian and Turkish participants as compared with the other ethnic groups. The prevalence of hypertension and diabetes was higher in all ethnic minority groups, while lipid profile was more favourable for the ethnic minority groups, as compared with Dutch.

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Figure 2 shows the crude association between short sleep duration and the prevalence of CVD by ethnicity. The prevalence of CVD was consistently higher in short sleepers than among healthy sleepers in all ethnic groups. This association remained significant after adjustment for potential confounders and conventional CVD risk factors (Table 2).

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Table 3 shows the ethnic differences in CVD, adjusting for short sleep and CVD risk factors separately and simultaneously. Model 1 shows the PR for each of the ethnic groups adjusting for confounders (age and sex) only. For instance, South-Asian Surinamese were 3.69 times more likely than Dutch to have a CVD. Adjusting for short sleep as can be seen in model 2, the PR reduced to 3.3. This implies that 14 % of the increased prevalence can be explained by short sleep (model 2 compared to model 1: 3.69-3.31/3.69-1 * 100%). The contribution of short sleep (model 2) was lower than that of the all conventional CVD risk factors combined (24%, model 3). This contribution of short sleep is the sum of the contribution through conventional risk factors and its independent contribution (figure 1). The contribution of short sleep independent of CVD risk factors is indicated by the difference in PR reduction between model 3 and model 4. That is, 10% (calculated as 34% - 24%) is contributed by sleep independently of CVD risk factors, while in total, this was 14% (model 2). This implies that two-third of the total contribution of short sleep was independent of conventional CVD risk factors, and one-third through these risk factors. Similarly, this applies to the other ethnic minority groups as shown in table 3. The independent contribution of short sleep was higher in African Surinamese (15%) and Ghanaians (15%) compared to other ethnic groups ranging from 5% in Turkish to 10% in South-Asian Surinamese.

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In this study, we investigated the contribution of short sleep to ethnic inequalities in CVD prevalence, and to what extent short sleep affects these inequalities, independent of conventional CVD risk factors. Our study findings indicate that, compared to healthy sleep, short sleep duration was consistently associated with an increased prevalence of CVD in all ethnic groups. Short sleep contributed substantially to ethnic differences in prevalence of CVD. In African Surinamese and Ghanaians, the contribution of short sleep was almost comparable to the contribution of all conventional CVD risk factors combined. In addition, short sleep contributed mostly independent of these risk factors to ethnic inequalities in CVD. The independent contribution and total contribution of sleep was higher in African Surinamese and Ghanaians than in South-Asian Surinamese, Turks and Moroccans.

Our study indicated that short sleep was consistently associated with CVD in all ethnic groups after adjustment for age, gender, and also after conventional CVD risk factors had been controlled for. Amagi et al. (2010) [12] and Meisinger et al. (2007) [13] also demonstrated a higher risk of myocardial infarction in short sleepers compared to healthy sleepers. The MORGEN study, investigating sleep duration and sleep quality in relation to 12-year CVD incidence, [14] alongside other studies, [15, 16] also found that short sleep was associated with CVD. Our cross-sectional study results are thus consistent with these findings. In addition, our result concurs with a recent multi-ethnicity study, which found that short sleep was associated with prevalent CVD (angina, MI and stroke) in Hispanics, African Americans, and other ethnicities in the U.S. [19] The influence of sleep on CVD is partly mediated through conventional risk factors, such as obesity. In particular, biological mechanisms involving endocrinological and metabolic functions have been proposed for the association between short sleep and CVD. Sleep deprivation may trigger inflammatory processes and activation of the sympathetic

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nervous system, increasing BP and cortisol levels, and impaired glucose tolerance, [33-36] and therefore may lead to an increased risk of CVD. It has also been suggested that short sleep promotes increased levels of ghrelin and reduced leptin levels, which are hormones involved in appetite and satiety regulation. Imbalance in these hormones increases food ingestion, leading to obesity. [37]

In the second part of our study, that examined the contribution of short sleep to the ethnic differences in prevalence of CVD, we found that short sleep significantly accounted for ethnic differences in CVD prevalence, with most of the contribution being independent of known CVD risk factors. The independent contribution of short sleep was particularly marked in African Surinamese, Ghanaian, and South-Asian Surinamese (15%, 15% and 10%) compared with Turks and Moroccans (5% and 5%). The reason that short sleep contributed more in African Surinamese, Ghanaian, and South-Asian Surinamese than in Turks and Moroccans is likely to be due to higher prevalence of short sleep in these groups and its accompanying negative consequences compared to Turks and Moroccans. We noticed that large proportions of the difference in CVD between Dutch and ethnic minority groups were not explained by conventional CVD risk factors and short sleep, suggesting that other unmeasured factors may be at play.

The mechanism underlying the contribution of short sleep to differences in CVD prevalence independently of conventional CVD risk factors is not clear. However, the mechanism may be through allostatic load, involving the stress response system. [11] Short sleep results in the activation of the neuroendocrine stress response leading to increased sympathetic activity. [38] that involves sympathoadrenal system and the hypothalamic-pituitary-adrenal (HPA) axis, which might also directly influence the risk of CVD. (Although this is partly through CVD risk factors, it may also be independent of these risk factors). This activation results in

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increased levels of glucocorticoids, notably cortisol and catecholamine which play an important role in regulation of energy balance and in cardiovascular function. Catecholamines can accelerate disease process through allostatic load when the activation lasts over a long period of time. According to this mechanism, disease manifest as a price, which the body pays for being forced to adapt to adverse psychosocial or physical situations. Allostatic load, represents either the presence of too much stress, or the inefficient operation of the stress hormone response system to adjust properly after the occurrence of the stressful event. [11] Future studies should further investigate other factors that may influence the mechanism of allostatic load in the contribution of short sleep to ethnic differences in CVD.

The strength of our study lies in utilization of large sample sizes, permitting more reliable estimations. Also, multiple ethnic groups living in one city were investigated together using the same methodology. In addition, three CVD endpoints were combined ensuring more reliable results. A limitation of the study is that we only used self-reported data on CVD and sleep, which may be subject to recall bias; hence the participants may have under -or over-reported short sleep durations and CVD. Also, the conventional CVD risk factors (health behavior) used in the study were measured only once, and therefore may not have been measured accurately. Stringhini et al. (2010) [39] have demonstrated a larger contribution of health behavior in explaining inequalities when health behaviors were measured longitudinally. Hence, once the measurements are followed up, a greater percentage of CVD may be explained. Being a cross-sectional study, causal associations between short sleep and CVD could not be established. Because important associations of short sleep and CVD were observed among the ethnic groups, further longitudinal studies are required among these populations. Furthermore, information on sleep quality and on daytime sleepiness, which may

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affect sleep duration, was lacking and should be considered in future studies. We also note that inaccurate measures of conventional behaviour risk factors of CVD may affect the obtained results. In other words, residual confounding cannot be excluded and might have led to overestimating the independent contribution of sleep.

In conclusion, our study showed that short sleep duration was related to the prevalence of CVD across all ethnic groups, and short sleep, independent of conventional CVD risk factors contributed significantly to ethnic differences in CVD. The findings might suggest that reducing sleep deprivation may be a relevant entry point for reducing increased CVD risks among the various ethnic minority groups.

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We are most grateful to the participants of the HELIUS study and the management team, research nurses, interviewers, research assistants and other staff who have taken part in gathering the data of this study.

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KS, MBS, RJP, CA, BJV contributed to acquisition of data. KA, KS, CA and GJL contributed to analysis and interpretation of data. All authors critically reviewed the manuscript, gave final approval and agree to be accountable for all work ensuring integrity and accuracy.

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The authors declare that they have no conflict of interest.

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The study protocols were approved by the AMC Ethical Review Board. All participants provided written informed consent.

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Data are available from the HELIUS study, a third party, Dr Snijder, Dr Stronks, and Dr Peters are affiliated with the HELIUS research cohort and are co-authors in this paper in accordance with HELIUS requirements for collaboration. Dr Snijder is the Scientific Coordinator of HELIUS and may be contacted with further questions (m.b.snijder@amc.uva.nl). Additionally, researchers interested in further collaboration with HELIUS may see the following URL: http://www.heliusstudy.nl/nl/researchers/collaboration.

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	Dutch	South-Asian Surinamese	African Surinamese	Ghanaians	Turks	Moroccans
Age						
Men (%)						
Sleep duration (h)						
Short sleep (% yes)						
Prevalence CVD (%)						
Hypertension (% yes)						
Diabetes (% yes)						
BMI (kg/m2)						
WHR						
Dyslipidaemia (% yes)						
Cholesterol (Total) >6.22mmol/l						
HDL cholesterol <1.04 mmol/L						
LDL cholesterol >4.14mmol/L						

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Triglyceride (TG) <1.69 mmol/L	
Education	
1st and 2nd category (%)	
3rd category (%)	
4th category (%)	
Smoking status	
Never smoked (% yes)	
Ex-smoker (% yes)	
Current smoker (%yes)	
Achieving Dutch norm for physical activity (%yes)	
Alcohol intake (%yes)	

Data are presented as meand and percentages with 95% CI

CVD, cardiovascular disease; BMI, body mass index; WHR, waist to hip ratio; HDL, high density lipoprotein; LDL, low density lipoproptein; TG, triglyceride; CI, confidence interval.

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	Dutch n = 4495	South-Asian Surinamese n = 2933	African Surinamese n = 4039	Ghanaians n = 2181	Turks n = 3395	Moroccans n = 3687
	PR (95%CI)	PR (95%CI)	PR (95%CI)	PR (95%CI)	PR (95%CI)	PR (95%CI)
Short sleep						
Crude						
Model 1						
Model 2						

Model 1: adjusted for age and sex Model 2: adjusted for model 1 plus BMI, WHR, hypertension, diabetes, dyslipidaemia, smoking, alcohol consumption and physical activity (*: p < 0.001, **: p < 0.05)

PR, prevalence ratio; CI, confidence interval.

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Table 3: Prevalence ratio(s) for ethnic differences in prevalence CVD, adjusting for short sleep and CVD risk factors separately, and simultaneously.

Confounders (model 1)		Confounders + short sleep (model 2)		Confounders + CVD risk factors (model 3)		Confounders + short sleep, CVD risk factors (model 4)		Difference in (%) reduction between model 4 and model 3
Ethnic group	PR (95% CI)	PR (95% CI)	Reduction PR total short sleep (%)[X]	PR (95% CI)	Reduction PR total CVD risk factors (%)[J]	PR (95% CI)	Reduction PR total short sleep, total CVD risk factors (%)[Y]	Difference in reduction (%)[Z]
	1.00	1.00		1.00		1.00		
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CVD, cardiovascular disease; CI, confidence interval.

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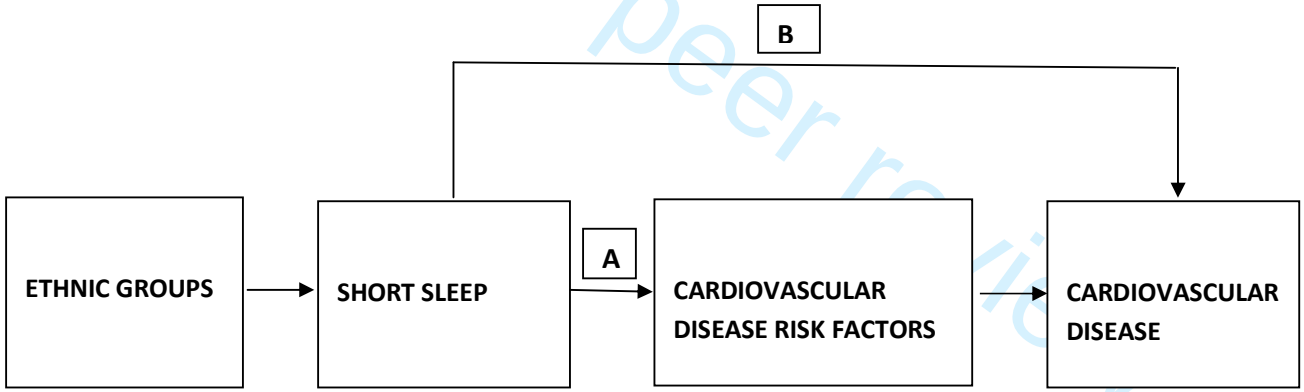

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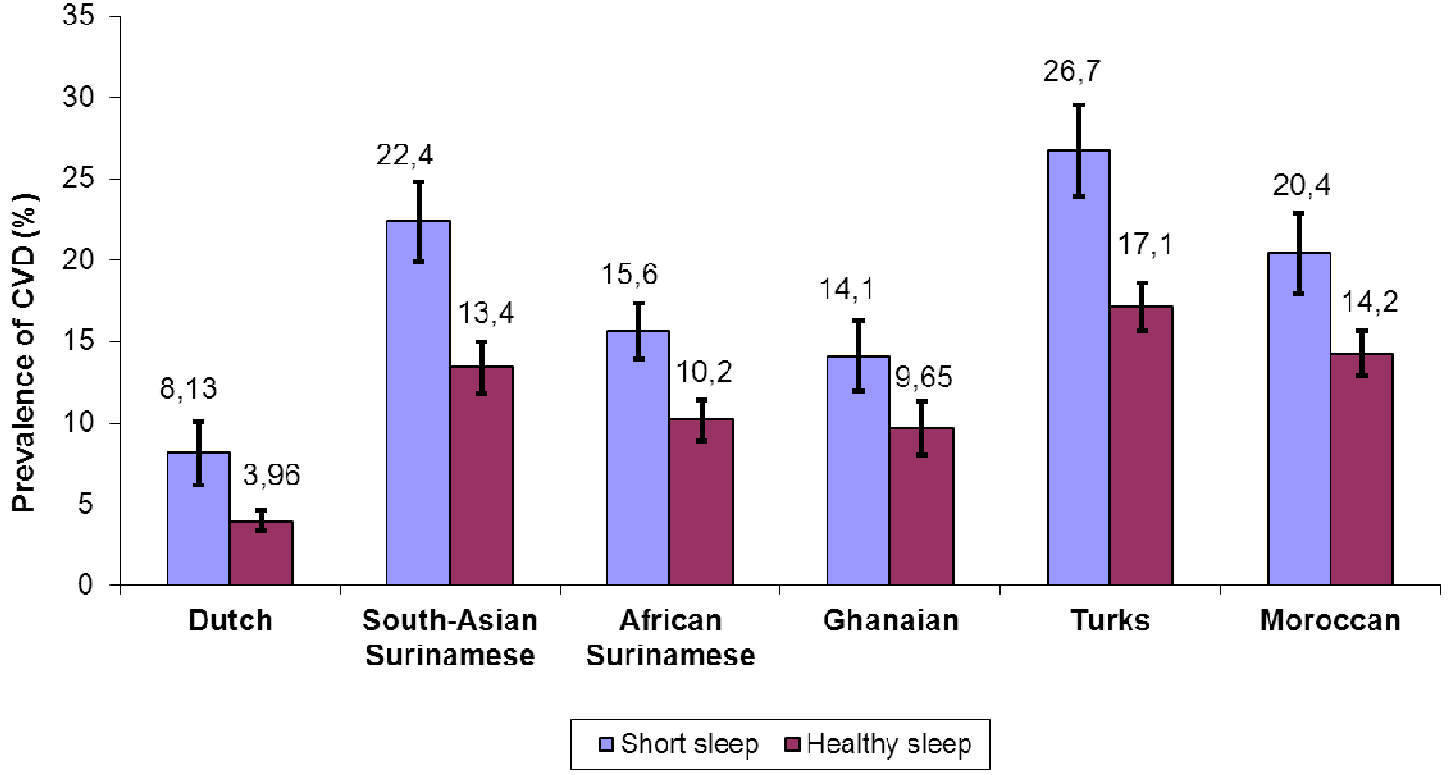
Fig. 2: Crude association between sleep duration and prevalence of cardiovascular disease among ethnic groups in Amsterdam



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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract *Also well described in the method section*
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (X) p.1
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (X) p.2-3
Objectives	3	State specific objectives, including any prespecified hypotheses (X) p.1, 4
Methods		
Study design	4	Present key elements of study design early in the paper (X) p.4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (X) p.4-7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants (X) p. 4-7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (X) p.5-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (X) p.4-8
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at (X) p.4-5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (X) p.8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (X) p.8
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed (X) p.5
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

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Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (X) p.4-5
		(b) Give reasons for non-participation at each stage (X) p.5
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (X) p.8-9, 22-23
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures (X) p.22-23
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (X) p.8, 24
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives (X) p.11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. (X) p.13-14 Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (X) p.11-14
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (X) p.15

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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THE CONTRIBUTION OF SHORT SLEEP DURATION TO ETHNIC DIFFERENCES IN CARDIOVASCULAR DISEASE: RESULTS OF THE HELIUS STUDY

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Abstract

Objectives: We analysed association between short sleep duration and prevalence of cardiovascular disease (CVD) in a multi-ethnic population living in the Netherlands, and the contribution of short sleep to the observed ethnic differences in the prevalence of CVD, independent of CVD risk factors.

Methods: 20730 participants (aged 18-71 years) of the HELIUS study were investigated. Self-reported sleep duration was classified as: short (<7 hours/night), and healthy (7-9 hours/night). Prevalence of CVD was assessed using the Rose questionnaire on angina pectoris, intermittent claudication and possible myocardial infarction. Association of short sleep duration with prevalent CVD, and the contribution of short sleep to the observed ethnic differences in the prevalence of CVD were analysed using adjusted prevalence ratio(s) (PR) with 95% confidence interval (CI).

Results: Results indicate that Short sleep was associated with CVD among all ethnic groups with PRs ranging from 1.41 (95%CI 1.21-1.65) in Moroccans to 1.62 (**95%CI** 1.20-2.18) in Dutch after adjustment for age, sex and conventional CVD risk factors. The independent contributions of short sleep (in percentage) to ethnic differences in CVD compared to Dutch were 10%, 15%, 15%, 5%, and 5% in South-Asian Surinamese, African Surinamese, Ghanaian, Turkish and Moroccan respectively.

Conclusion: Short sleep contributed to ethnic differences in CVD independent of well-known CVD risk factors particularly in Surinamese and Ghanaian groups. Reducing sleep deprivation may be a relevant entry point for reducing increased CVD risks among the various ethnic minority groups.

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Keywords: Cardiovascular disease, sleep duration, ethnicity, minority group, HELIUS study.

Strengths and limitations of this study

- This study utilized large sample size which permits more reliable estimations.
- Multiple ethnic groups living in one city were investigated together using the same methodology.
- Three cardiovascular disease endpoints were combined ensuring more reliable results.
- This study used self-reported data on CVD and sleep, which may be subject to recall bias.
- Being a cross-sectional study, causal associations between short sleep and CVD could not be established.

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INTRODUCTION

Cardiovascular disease (CVD) ranks first as the leading cause of mortality across the globe. Previous studies have shown that CVD is higher in ethnic minority groups compared to host populations. [1-3] It is evident that known conventional risk factors of CVD do not entirely explain ethnic differences in prevalence CVD. [4] Therefore, it becomes necessary to identify novel modifiable risk factors, which may play a mediatory role in ethnic differences in CVD, and which may reduce the likely surge in prevalence of CVD among various ethnic groups.

Several studies have shown that sleep may play a significant role in the pathogenesis and progression of cardiac and vascular diseases. [5] Studies conducted in Europe and United States have demonstrated that aberrant sleep duration was associated with increased risk of CVD risk factors including obesity, [6] diabetes, [7] hypertension, [8] and dyslipidaemia. [9,10] Sleep may influence CVD through these risk factors and other factors. [11]

However, results from existing studies on the association between short sleep duration and CVD are contradictory. [12-19] For instance, while previous studies found that short sleep was associated with CVD such as stroke, myocardial infarction, and coronary heart disease (CHD); [12-16] other studies indicate that short sleep was not associated with CHD, [17] and stroke. [18] Only one of these studies extended their investigation by including several ethnic groups, and the results suggested that short sleep was associated with CVD in Hispanic **and** African Americans and other ethnicities. [19]

In Europe, data on sleep duration and CVD are lacking, and have not yet been explored among ethnic minority groups. Literature has shown that CVD risk factors are more common in these groups. [20] We have previously shown that in the Netherlands, several ethnic minority groups also experience shorter sleep duration compared to their host majority populations, [21]

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and the association between short sleep duration and CVD risk factors differed among the various ethnic groups. [22] **Ethnic minority groups experience short sleep duration because they more frequently have a low socioeconomic status (education and occupation), are more frequently engaged in shift work, [21] or because of adverse living conditions such as crowding and stressful neighbourhood conditions.** [23] Therefore, investigating the relationship between short sleep duration and CVD among various ethnic groups is relevant to provide additional insight on how short sleep is related to ethnic differences in cardiovascular disease. This might be first, through well-known CVD risk factors, second, short sleep may also independently contribute to CVD, and as such could be a novel target for prevention of CVD among various ethnic groups (see research model presented in Figure 1).

Methods

Study population

The current study was based on baseline data from the HELIUS (Healthy Life in an Urban Setting) study. The aims and design of the HELIUS study have been described elsewhere. [24] In brief, HELIUS is a large-scale cohort study on health and health care among different ethnic groups living in Amsterdam. The study includes individuals aged 18-70 years from the six major ethnic groups in Amsterdam (African-Surinamese, South-Asian Surinamese, Turkish, Moroccan, Ghanaian, and Dutch origin), and focuses on three major disease categories: cardiovascular disease, mental health and infectious diseases. Participants were randomly sampled from the municipal registers, stratified by ethnicity. The study protocols

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were approved by the AMC Ethical Review Board. All participants provided written informed consent.

Baseline data collection took place in 2011-2015. Data from both questionnaire and the physical examination were available in 22165 participants. For current analyses, individual with no data on sleep duration (n=345), as well as those sleeping more than 9 hours per night (n=560) were excluded from the analysis. This resulted in a dataset of 21260 participants, including 4495 Dutch, 2933 South-Asian Surinamese, 4039 African Surinamese, 2181 Ghanaians, 3395 Turks, 3687 Moroccans, 228 Javanese Surinamese origin, 255 other/unknown Surinamese origin and 47 other/unknown origin. Because of small sample size, the last three groups were excluded resulting in a final dataset of 20730 participants.

Ethnicity

Participant's ethnicity was defined according to the country of birth of the participant as well as that of his/her parents and self-report. Specifically, a participant was considered as of non-Dutch ethnic origin if he/she fulfils either of the following criteria: 1) he or she was born abroad and has at least one parent born abroad (first generation); or 2) he or she was born in the Netherlands but both his/her parents born abroad (second generation).[25] Of the Surinamese immigrants in the Netherlands, approximately 80% are either African or South-Asian origin. Both subgroups were classified according to self-reported ethnic origin. Participants were considered as of Dutch origin if the person and both parents were born in the Netherlands.

Sleep duration

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Participants were asked to provide information on the average number of hours they usually sleep at night. Sleep duration was assessed using the item, "How many hours do you sleep on average per night?". Short sleep was defined as having less than 7 hours of sleep per night, according to National Sleep Foundation (NSF), American Academy of Sleep Medicine (AASM), and Sleep Research Society (SRS), which recommend 7 to 9 hours as the basal sleep need for healthy adults. [26] We focused on short sleep only because in our previous study, we demonstrated that short sleep was the major problem for the ethnic minority groups, [21] and because previous studies found that short sleep was more consistently related to CVD risk factors compared to long sleep. [27-29]

Cardiovascular disease

The prevalence of cardiovascular disease was assessed using the Rose questionnaire on angina pectoris, intermittent claudication and possible myocardial infarction. **The Rose questionnaire has three parts. Part A: include questions of experience of pain or discomfort in the chest during exercise, or walking fast or climbing stairs, and whether the pain stops or as the exercise or walking/running stops; and how long (less or more than 10 minutes), and the part of the body where pain was experienced. Part B: include questions of experience of severe chest pain lasting for half an hour or more, and Part C: include questions of experience of pains on either legs while walking uphill, or at ordinary pace, while standing still or sitting, and exact location of the pain (calf/calves), and whether the pain disappear when stopped walking, and how long (less or more than 10 minutes). Participants were classified as having angina pectoris, or possible myocardial infarction, or intermittent claudication based on their responses to these questions according to Rose et al 1977.** [30]

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Although Rose questionnaire was not specifically validated for the ethnic groups in this study, it has been shown to work well in other validated studies with similar ethnic group as in our study. [31]

Other measurements

Weight (kg) and height (cm) were measured in duplicate in barefoot subjects wearing light clothes only. Waist circumference (cm) was measured twice using a tape measure at the level midway between the lowest rib margin and the iliac crest, and hip circumference (cm) was measured twice at the widest level over the trochanter major. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2), and waist-to-hip ratio (WHR) was calculated as waist circumference divided by hip circumference.

Blood pressure (BP) was measured in duplicate using a semi-automatic sphygmomanometer (Microlife WatchBP Home, Microlife AG, Switzerland) on the left arm in a seated position after the participant had seated for at least 5 minutes. Hypertension was defined as systolic BP ≥ 140 mmHg, or diastolic BP ≥ 90 mmHg, or being on BP lowering medication, or self-reported hypertension.

Fasting blood samples were taken to determine the concentration of glucose by spectrophotometry, using hexokinase as primary enzyme (Roche Diagnostics, Japan). Total cholesterol, triglycerides and high-density lipoprotein (HDL) cholesterol were determined by colorimetric spectrophotometry (Roche Diagnostics, Japan). Low-density lipoprotein cholesterol (LDL) was calculated according to the Friedewald formula. [32] Type 2 diabetes was defined as increased fasting glucose ≥7mmol/L or current use of glucose-lowering medication or self-reported diabetes. Dyslipidemia was defined as total cholesterol (TC) >6.22mmol/L, or

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high density lipoprotein cholesterol (HDL-C) <1.04mmol/L, or low density lipoprotein cholesterol (LDL-C) >4.14mmol/L, or triglyceride (TG) >1.69mmol/L, [33] or use of lipid-lowering medication.

Educational level was determined using participant's highest level of education obtained (either in the Netherlands or in the country of origin). Participants were categorized into those who have never been to school or had elementary schooling only (1st category), those with lower vocational schooling or lower secondary schooling (2nd category), those with intermediate vocational schooling or intermediate/higher secondary education schooling (3rd category), and those with higher vocational schooling or university (4th category). For the current analyses, the first two categories were combined because of small numbers. **Occupation was categorised into: elementary, lower, medium, higher and scientific levels depending on the type of work. Shift work was assessed with the item "do you work irregular hours including services during night hours" (yes/no).**

Alcohol intake in the past 12 months (yes/no) and smoking status (yes/no/ex-smoker) were obtained by questionnaire. Habitual physical activity was measured using the SQUASH questionnaire. [34] The SQUASH questions about multiple activities referring to a normal week in the past months. We categorized participants according to the Dutch guideline for physical activity by summing up the number of days per week for each moderate and high intensity activity lasting at least 30 minutes. A total of 5 days or more resulted in participants being categorized as achieving the Dutch norm for physical activity. [34]

Data analysis

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Baseline data were expressed as percentages, means or median. **For the association of short sleep with CVD, we assessed interaction between short sleep and CVD. There was no significant interaction between short sleep and ethnicity.** The analyses were performed in two steps. In the first step, the association of short sleep duration with prevalent CVD within each ethnic group was analysed using prevalence ratio(s) (PR) with 95% confidence interval (CI), with adjustments for potential confounders (age, gender) and**, additionally**, conventional CVD risk factors. **Although we considered the inclusion of other potential confounders such as depressive symptoms and socio-economic status, we decided not to include these in the multivariate model, because of the risk of overadjustment. For depressive symptoms could also be considered as an intermediary factor in the causal pathway between sleep and CVD**, **whereas factors such as socioeconomic status and shift work are factors that drive the pattern of short sleep among ethnic groups.** In the second step, comparisons of prevalent CVD between ethnic groups was performed with adjustments for age and gender (model 1). In order to assess the contribution of short sleep and conventional CVD risk factors to the ethnic differences in CVD, we subsequently added short sleep (model 2), CVD risk factor variables (hypertension, diabetes, BMI, WHR, dyslipidaemia, smoking, alcohol, and physical activity model 3), or both short sleep and CVD risk factors (model 4) to the regression models. The change in PR (percentage) before and after inclusion was used to assess the relative total contribution of short sleep and CVD risk factors to ethnic differences in CVD. By subtracting the changes in PR (percentage) of model 4 and 3, the contribution of short sleep independent of CVD risk factors was calculated for each ethnic minority group. **This method of calculation has been used in previous study.** [35] All analyses were performed using STATA 11.0 (Stata Corp, College Station, TX). A p-value of <0.05 was considered as statistically significant.

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Results

Characteristics of study population

Table 1 shows the characteristics of the study population by ethnic group. Moroccans and Turks were younger, had higher prevalence of CVD, lower educational levels, consumed less alcohol, less often achieved the physical activity norm, and had a lower prevalence of hypertension as compared with the other ethnic origin groups. Similar to Turkish participants, South-Asian Surinamese also had higher prevalence of CVD than the other groups. Ghanaians also had lower educational levels than Dutch. **All ethnic minority groups have lower occupational levels compared with Dutch. African Surinamese and South-Asian Surinamese have higher prevalence of shift work compared with other ethnic groups**. South-Asian Surinamese, African Surinamese, and Ghanaian participants had a lower mean sleep duration and higher prevalence of short sleep than Dutch, Turks and Moroccans. Dutch and South-Asian Surinamese had a lower mean BMI than the other ethnic groups, whereas mean WHR was higher in South-Asian Surinamese, Ghanaian and Turkish participants as compared with the other ethnic groups. **The prevalence of depressive symptoms was higher in Turks, Moroccans and South-Asian Surinamese compared with other ethnic groups**. The prevalence of hypertension and diabetes was higher in all ethnic minority groups, while lipid profile was more favourable for the ethnic minority groups, as compared with Dutch.

Association between short sleep duration and cardiovascular disease

Figure 2 shows the crude association between short sleep duration and the prevalence of CVD by ethnicity. The prevalence of CVD was consistently higher in short sleepers than among

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healthy sleepers in all ethnic groups. This association remained significant after adjustment for potential confounders and conventional CVD risk factors (Table 2).

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Contributions of short sleep duration to ethnic differences in the prevalence of CVD

Table 3 shows the ethnic differences in CVD, adjusting for short sleep and CVD risk factors separately and simultaneously. Model 1 shows the PR for each of the ethnic groups adjusting for confounders (age and sex) only. For instance, South-Asian Surinamese were 3.69 times more likely than Dutch to have a CVD. Adjusting for short sleep as can be seen in model 2, the PR reduced to 3.3. This implies that 14 % of the increased prevalence can be explained by short sleep (model 2 compared to model 1: 3.69-3.31/3.69-1 * 100%). The contribution of short sleep (model 2) was lower than that of the all conventional CVD risk factors (24%, model 3). This contribution of short sleep is the sum of the contribution through conventional risk factors and its independent contribution (figure 1). The contribution of short sleep independent of CVD risk factors is indicated by the difference in PR reduction between model 3 and model 4. That is**,** 10% (calculated as 34% - 24%) is contributed by sleep independently of CVD risk factors, while in total, this was 14% (model 2). This implies that two-third of the total contribution of short sleep was independent of conventional CVD risk factors, and one-third through these risk factors. Similarly, this applies to the other ethnic minority groups as shown in table 3. The independent contribution of short sleep was higher in African Surinamese (15%) and Ghanaians (15%) compared to other ethnic groups ranging from 5% in Turkish to 10% in South-Asian Surinamese.

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Discussion

In this study, we investigated the contribution of short sleep to ethnic inequalities in CVD prevalence, and to what extent short sleep affects these inequalities, independent of conventional CVD risk factors. Our study findings indicate that, compared to healthy sleep, short sleep duration was consistently associated with an increased prevalence of CVD in all ethnic groups. Short sleep contributed substantially to ethnic differences in prevalence of CVD. In African Surinamese and Ghanaians, the contribution of short sleep was almost comparable to the contribution of all conventional CVD risk factors combined. In addition, short sleep contributed mostly independent of these risk factors to ethnic inequalities in CVD. The independent contribution and total contribution of sleep was higher in African Surinamese and Ghanaians than in South-Asian Surinamese, Turks and Moroccans.

Our study indicated that short sleep was consistently associated with CVD in all ethnic groups after adjustment for age, gender, and also after conventional CVD risk factors had been controlled for. Amagi et al. (2010) [12] and Meisinger et al. (2007) [13] also demonstrated a higher risk of myocardial infarction in short sleepers compared to healthy sleepers. The MORGEN study, investigating sleep duration and sleep quality in relation to 12-year CVD incidence, [14] alongside other studies, [15, 16] also found that short sleep was associated with CVD. Our cross-sectional study results are thus consistent with these findings. In addition, our result concurs with a recent multi-ethnicity study, which found that short sleep was associated with prevalent CVD (angina, MI and stroke) in Hispanics, African Americans, and other ethnicities in the U.S. [19] The influence of sleep on CVD is partly mediated through conventional risk factors, such as obesity. In particular, biological mechanisms involving endocrinological and metabolic functions have been proposed for the association between short sleep and CVD. Sleep deprivation may trigger inflammatory processes and activation of the sympathetic

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nervous system, increasing BP and cortisol levels, and impaired glucose tolerance, [36-40] and therefore may lead to an increased risk of CVD. It has also been suggested that short sleep promotes increased levels of ghrelin and reduced leptin levels, which are hormones involved in appetite and satiety regulation. Imbalance in these hormones increases food ingestion, leading to obesity. [41]

In the second part of our study, that examined the contribution of short sleep to the ethnic differences in prevalence of CVD, we found that short sleep significantly accounted for ethnic differences in CVD prevalence, with most of the contribution being independent of known CVD risk factors. The independent contribution of short sleep was particularly marked in African Surinamese, Ghanaian, and South-Asian Surinamese (15%, 15% and 10%) compared with Turks and Moroccans (5% and 5%). The reason that short sleep contributed more in African Surinamese, Ghanaian, and South-Asian Surinamese than in Turks and Moroccans is likely to be due to higher prevalence of short sleep in these groups and its accompanying negative consequences compared to Turks and Moroccans. We noticed that large proportions of the difference in CVD between Dutch and ethnic minority groups were not explained by conventional CVD risk factors and short sleep, suggesting that other unmeasured factors may be at play.

The mechanism underlying the contribution of short sleep to differences in CVD prevalence independently of conventional CVD risk factors is not clear. However, the mechanism may be through allostatic load, involving the stress response system, [11] **or through depressive symptoms**. Short sleep results in the activation of the neuroendocrine stress response leading to increased sympathetic activity. [42] that involves sympathoadrenal system and the hypothalamic-pituitary-adrenal (HPA) axis, which might also directly influence the risk of CVD. Although this is partly through CVD risk factors, it may also be independent of these

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risk factors. This activation results in increased levels of glucocorticoids, notably cortisol and catecholamine which play an important role in regulation of energy balance and in cardiovascular function. Catecholamines can accelerate disease process through allostatic load when the activation lasts over a long period of time. According to this mechanism, disease manifest as a price, which the body pays for being forced to adapt to adverse psychosocial or physical situations. Allostatic load, represents either the presence of too much stress, or the inefficient operation of the stress hormone response system to adjust properly after the occurrence of the stressful event. [11] Future studies should further investigate other factors that may influence the mechanism of allostatic load in the contribution of short sleep to ethnic differences in CVD.

The strength of our study lies in utilization of large sample sizes, permitting more reliable estimations. Also, multiple ethnic groups living in one city were investigated together using the same methodology. In addition, three CVD endpoints were combined ensuring more reliable results. A limitation of the study is that we only used self-reported data on CVD and sleep, which may be subject to recall bias; hence the participants may have under -or over-reported short sleep durations and CVD. Also, the conventional CVD risk factors (health behavior) used in the study were measured only once, and therefore may not have been measured accurately. Stringhini et al. (2010) [43] have demonstrated a larger contribution of health behavior in explaining inequalities when health behaviors were measured longitudinally. Hence, once the measurements are followed up, a greater percentage of CVD may be explained. Being a cross-sectional study, causal associations between short sleep and CVD could not be established. Because important associations of short sleep and CVD were observed among the ethnic groups, further longitudinal studies are required among these populations. Furthermore, information on sleep quality, **sleep hours during weekends** and on

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daytime sleepiness, **use of sleep hypnotics, antidepressant medications and insomnia,** which may affect sleep duration, were lacking and should be considered in future studies. **Also, sleep apnea has been shown to be associated with CVD and cardiorespiratory problems. [44,45] and causes hemodynamic changes and significant sleep disturbances. [46,47] Sleep apnea may confound the association between sleep duration and CVD. However, we did not investigate sleep apnea in this study as we do not have the information in our dataset.** We also note that inaccurate measures of conventional behaviour risk factors of CVD may affect the obtained results. In other words, residual confounding cannot be excluded and might have led to overestimating the independent contribution of sleep. **Another important factor which may play a role in the association of sleep duration with CVD include genetics. [48] However, information on genetics was not available in our dataset, and was not investigated. Despite the lack of data on these factors, we were still able to answer our key research question.**

In conclusion, our study showed that short sleep duration was related to the prevalence of CVD across all ethnic groups, and short sleep, independent of conventional CVD risk factors contributed significantly to ethnic differences in CVD. The findings might suggest that reducing sleep deprivation may be a relevant entry point for reducing increased CVD risks among the various ethnic minority groups.

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Author contribution statement

Karien Stronks contributed to acquisition of data, analysis and interpretation of data, critically reviewed the manuscript and gave final approval of the submission. Marieke B Snijder contributed to acquisition of data, analysis and interpretation of data, critically reviewed the manuscript and gave final approval for submission. Ron J Peters contributed to acquisition of data, critically reviewed the manuscript and gave final approval for submission. Charles Agyemang contributed to acquisition of data, analysis and interpretation of data, critically reviewed the manuscript and gave final approval for submission, Bert-jan van den Born contributed to acquisition of data, critically reviewed the manuscript and gave final approval for submission. Kenneth Anujuo contributed to analysis and interpretation of data, critically reviewed the manuscript and gave final approval for submission. Girardin Jean-Louis contributed to analysis and interpretation of data, critically reviewed the manuscript and gave final approval for submission. All authors agree to be accountable for all work ensuring integrity and accuracy.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

The study protocols were approved by the AMC Ethical Review Board. All participants provided written informed consent.

Data sharing statement

Data are available from the HELIUS study, a third party, Dr Snijder, Dr Stronks, and Dr Peters are affiliated with the HELIUS research cohort and are co-authors in this paper in accordance with HELIUS requirements for collaboration. Dr Snijder is the Scientific Coordinator of HELIUS and may be contacted with further questions (m.b.snijder@amc.uva.nl). Additionally, researchers interested in further collaboration with HELIUS may see the following URL: http://www.heliusstudy.nl/nl/researchers/collaboration.

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48. **Grandner MA, Sands-Lincoln MR, Pak VM,** ***et al.*** **Sleep duration, cardiovascular disease, and proinflammatory biomarkers.** ***Nat Sci Sleep*****. 2013; 5: 93–107.**

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Table 1: Characteristics of study population by ethnicity

	Dutch	South-Asian Surinamese	African Surinamese	Ghanaians	Turks	Moroccans
Age						
Men (%)						
Sleep duration (h)						
Short sleep (% yes)						
Angina (% yes)						
Myocardial infarction (% yes)						
Intermittent claudication (% yes)						
Prevalence CVD (%)						
Hypertension (% yes)						
Diabetes (% yes)						
BMI (kg/m2)						
WHR						
Dyslipidaemia (% yes)						

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Cholesterol (Total) >6.22mmol/l
HDL cholesterol <1.04 mmol/L
LDL cholesterol >4.14mmol/L
Triglyceride (TG) <1.69 mmol/L
Depressive symptoms (%)
Education
1st and 2nd category (%)
3rd category (%)
4th category (%)
Occupation
Elementary (%)
Lower (%)
Medium (%)
Higher (%)
Scientific (%)
Shift work (%)
Smoking status
Never smoked (% yes)
Ex-smoker (% yes)

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Current smoker (%yes)	
Achieving Dutch norm for physical activity (%yes)	
Alcohol intake (%yes)	

Data are presented as means and percentages with 95% CI

CVD, cardiovascular disease; BMI, body mass index; WHR, waist to hip ratio; HDL, high density lipoprotein; LDL, low density lipoproptein; TG, triglyceride; CI, confidence interval.

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Table 2: Prevalence ratio(s) for the relationship between sleep duration (short sleep versus healthy sleep) and the prevalence of cardiovascular disease by ethnicity

	Dutch n = 4495	South-Asian Surinamese n = 2933	African Surinamese n = 4039	Ghanaians n = 2181	Turks n = 3395	Moroccans n = 3687
	PR (95%CI)	PR (95%CI)	PR (95%CI)	PR (95%CI)	PR (95%CI)	PR (95%CI)
Short sleep						
Crude						
Model 1						
Model 2						

Model 1: adjusted for age and sex Model 2: adjusted for model 1 plus BMI, WHR, hypertension, diabetes, dyslipidaemia, smoking, alcohol consumption and physical activity (*: p < 0.001, **: p < 0.05)

PR, prevalence ratio; CI, confidence interval.

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Table 3: Prevalence ratio(s) for ethnic differences in prevalence CVD, adjusting for short sleep and CVD risk factors separately, and simultaneously.

Confounders (model 1)		Confounders + short sleep (model 2)		Confounders + CVD risk factors (model 3)		Confounders + short sleep, CVD risk factors (model 4)		Difference in (%) reduction between model 4 and model 3
Ethnic group	PR (95% CI)	PR (95% CI)	Reduction PR total short sleep (%)[X]	PR (95% CI)	Reduction PR total CVD risk factors (%)[J]	PR (95% CI)	Reduction PR total short sleep, total CVD risk factors (%)[Y]	Difference in reduction (%)[Z]
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,! '! *. % " / (!!- **(*: p < 0.001)**

CVD, cardiovascular disease; CI, confidence interval.

X: Contribution of short sleep: Reduction in PR (%) between model 1 and model 2

J: Contribution of CVD risk factors: Reduction in PR (%) between model 1 and model 3

Y: Contribution of both CVD risk factors and short sleep: Reduction in PR (%) between model 1and model 4

Z: contribution of short sleep independent of CVD risk factors; Difference in PR reduction (%) between model 3 and model 4

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Figure legends

Fig. 1: Research model by which short sleep and CVD risk factors may lead to CVD in various ethnic groups.

Fig. 2: Crude association between sleep duration and prevalence of cardiovascular disease among ethnic groups in Amsterdam.

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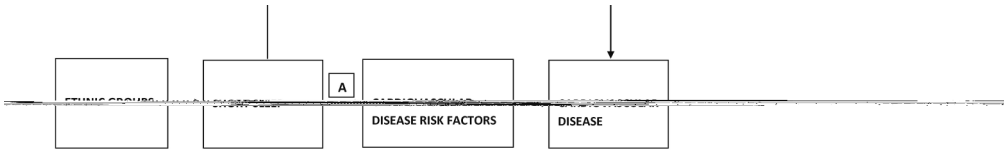

Research model by which short sleep and CVD risk factors may lead to CVD in various ethnic groups.

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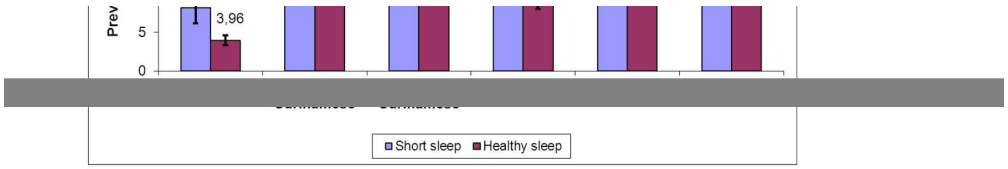

Crude association between sleep duration and prevalence of cardiovascular disease among ethnic groups in Amsterdam

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract *Also well described in the method section*
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (X) p.1
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (X) p.2-3
Objectives	3	State specific objectives, including any prespecified hypotheses (X) p.1, 4
Methods		
Study design	4	Present key elements of study design early in the paper (X) p.4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (X) p.4-7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants (X) p. 4-7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (X) p.5-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (X) p.4-8
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at (X) p.4-5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (X) p.8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (X) p.8
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed (X) p.5
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

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Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (X) p.4-5
		(b) Give reasons for non-participation at each stage (X) p.5
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (X) p.8-9, 22-23
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures (X) p.22-23
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (X) p.8, 24
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives (X) p.11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. (X) p.13-14 Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (X) p.11-14
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (X) p.15

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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