SLEEP AND RISK OF NEURODEGENERATIVE DISEASE

A population-based approach

Thom S. Lysen

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Een populatie-gebonden aanpak

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Paranimfen	Silvan Licher
	Frank J. Wolters

For dreamers

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Sleep that knits up the raveled sleave of care,
The death of each day's life, sore labor's bath,
Balm of hurt minds, great nature's second course,
Chief nourisher in life's feast.

William Shakespeare. Macbeth (1623).

1 GENERAL INTRODUCTION

"Birds do it, bees do it, even educated flees do it…" – Cole Porter

Sleep is an integral part of life.[1] Everyone will agree that humans need sleep, judging from what happens when you do not get it for a night. Yet, sleep is poorly understood compared to other basic things you do every day, such as eating, drinking or breathing. We largely lack insight into what brings about sleep, what are its underlying biological mechanisms, and why we sleep. Even defining sleep can be difficult and may require long, sometimes sleep-inducing descriptions. One definition describes sleep as "*a recurring, reversible neuro-behavioral state of relative perceptual disengagement from and unresponsiveness to the environment, typically accompanied by postural recumbence, behavioral quiescence, and closed eyes*".[2] Essentially, sleep differs from 'chilling out' by sensory disconnection that cannot be achieved voluntarily.[3] Yet, this neuro-behavioral state, in which nothing much seems to be happening, involves unique patterns of brain activity, and seems preserved across all animal species.[1] Assuming that '*if sleep does not serve an absolutely vital function, it is the biggest mistake evolution ever made*' (Allan Rechtschaffen, University of Chicago Sleep Laboratory, Smithsonian Institute, 1978), sleep research is thus empowered. Answers to questions on the mechanisms and functions of sleep will surely provide essential biological insights into one of the key behavioral experiences of everyday life. These insights are especially relevant to learn about sleep's role in health and disease.

As sleep is primarily 'by the brain, for the brain',[4] its role in brain health and disorders is specifically interesting. Neurodegenerative diseases in the aged, such as Alzheimer's disease, other forms of dementia, and Parkinson's disease are common and highly burdensome diseases.[5-7] The societal impact of these diseases, in terms of healthy years lost and healthcare costs, is enormous.[8,9] Treatment aimed at modifying these diseases are currently thought ineffective as too much brain damage has already accumulated by the time recognizable symptoms emerge. This helped fuel the search for factors that identify the disease earlier, or that causally contribute to its development or progression. In the search for such factors, sleep has gained increasing attention.[10-13] Recent studies into the 'nightlife' of neurons and astrocytes have helped understand the potential functions of sleep, most of which are highly relevant to the study of neurodegenerative processes and diseases. Non-mutually exclusive hypotheses on sleep's function include the synaptic homeostasis hypothesis,[14,15] which states that sleep '*is the price the brain pays for synaptic plasticity*'.[3] During wakefulness, synapses – the connections that allow neurons to communicate – are on average strengthened while the brain is continuously processing information, or learning.[3] Information from experiences is continuously materialized in synaptic strength, and sleep allows going offline from the environment to reduce synaptic strength, sorting out the most salient information collected along the way. This reduction also decreases expenditure of cellular supplies and energy on costly

synapses.[3] Another hypothesis posits that sleep is necessary for 'housekeeping' in the brain, as it drives fluid exchange in the brain which circulates signaling molecules and clears metabolic waste.[16-18]

Both aforementioned hypotheses imply that disturbed sleep, if severe or chronic enough, may harm the brain by dysfunction of aforementioned homeostatic processes. Importantly, these processes overlap with the key pathological features found in neurodegenerative diseases in the aged, e.g. synaptic dysfunction[19] or a detrimental accumulation of proteins.[20] Against this background, observing associations of sleep disturbances, i.e. sleep disorders or otherwise abnormal sleep, with a higher risk of cognitive decline or neurodegenerative diseases in humans suggests an etiological, causal role of sleep disturbances.[13,21] As sleep disturbances are common in the aged,[22] and have been hypothesized to be modifiable,[11,23] this supposed causal relation may harbor a large preventive potential for these conditions. It is therefore important we try to further substantiate the etiological role of sleep disturbances in these diseases.

This thesis is rooted in epidemiology,[24] a scientific discipline concerned with quantifying (biomedical) relations through comparing groups of individuals, aimed at controlling health problems.[25] Its principles and methods are applied by many if not all researchers in the biomedical field seeking to answer causal questions. This thesis uses observational data from the population-based, prospective Rotterdam Study cohort of middle-aged and elderly individuals, designed to investigate risk factors of common chronic diseases. The Rotterdam Study focuses among others on neurodegenerative diseases such as dementia, including Alzheimer's disease, and Parkinson's disease. Study participants routinely undergo measurements relevant to these conditions such as cognitive tests, locomotor screening, blood sampling or a brain MRI. Also, virtually all participants consented to provide access to their medical records, allowing continuous ascertainment of any neurodegenerative diseases for which any care was given. The study incorporated sleep measurements since 2002 and leverages over a decade of follow-up for neurodegenerative disease.

The aim of this thesis is to investigate the etiological role of sleep in neurodegenerative diseases, specifically dementia and Parkinson's disease, and related neurobiological correlates measures in middle-aged and elderly persons. First, in chapter 2, we describe sleep in the general population, using individual-level data from 36 national sleep cohorts, as well as objective and subjective sleep data from different countries. We also review recent studies investigating the 24-hour activity rhythm in relation to common age-related diseases in older adults. The 24-hour activity rhythm is a behavioral reflection of functioning of the circadian timing system, a key determinant of the sleep-wake cycle. In chapter 3, we investigate the relation of sleep characteristics with incident dementia including Alzheimer's disease, and Parkinson's disease. In chapter 4, we investigate associations of sleep characteristics with related aspects of brain aging: Neuronal

damage indicated by neurofilament light chain in plasma, brain waste clearance indicated by the structural appearance of perivascular spaces on brain magnetic resonance imaging, and brain functional connectivity measured with resting state functional MRI. Lastly, the main discussion in chapter 5 synthesizes results of chapters 2-4, discusses key methodological considerations in appraising these findings, and discusses implications for current clinical and public health practices, and future research.

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We have 24 hours a day. We sleep 6 hours a day, so that gives you still 18 hours. Now there is probably someone shaking their head out there, saying "I don't sleep 6 hours I sleep 8 hours!", right? Well..... just sleep faster!

Arnold Schwarzenegger. The Speech That Broke the Internet.

2 SLEEP AND 24-HOUR ACTIVITY RHYTHM REVIEWS

2.1 SLEEP CHARACTERISTICS SYSTEMATIC REVIEW AND INDIVIDUAL PARTICIPANT META-ANALYSIS

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Submitted

ABSTRACT

The study has three main objectives: 1) to obtain reliable reference charts for sleep duration; 2) estimate the prevalence of sleep complaints in the general population across the lifespan; and 3) to identify risk indicators of poor sleep.

We identified studies identified through systematic literature search in Embase, Medline and Web of Science (August 9th 2019), and through personal contacts with colleagues in the UK and US. Studies were eligible if published between 2000 and 2017 with data on sleep characteristics assessed with questionnaires, sampling at least 100 participants from the general population of the Netherlands. Large population-based studies/surveys from UK and US were included for comparisons. For IPD analysis, data were obtained for 36 out of 47 eligible studies. Two researchers independently coded sleep variables: (time in bed (TIB), sleep duration (Total Sleep Time, TST), sleep efficiency (TST/TIB*100)), self/caregiver-reported sleep quality, insomnia symptoms and other sleep complaints, as well as socio-demographic characteristics (sex, age, education, ethnic origin, employment and partnership status) and health risk indicators (smoking and body mass index). All variables were coded following a standardized protocol. For comparison, complementary sleep data from the UK Biobank and the National Health Interview Survey in the USA were included. Where available, actigraphic sleep estimates were obtained using validated algorithms.

We assembled IPD from 200,358 persons (age range 1-100 years, 55% female) from the Netherlands, 471,759 persons (40 to 69 years old, 55.5% female) from the UK, and 409,617 persons (≥18 years, 55.8% female) from the US. Age-specific percentile curves for TST demonstrate that overall 24.5% of the studied population slept less than age-specific recommendations, but only 5.8% slept outside of the "acceptable range" for sleep duration. Short sleep duration was most prevalent in teenagers, as 51.5% reported TST less than the recommended 8-10 hours and 18% report daytime sleepiness. In adults (≥18yrs), poor sleep quality (13.3%) and insomnia symptoms (9.6-19.4%) were more prevalent than short sleep duration (6.5% with TST<6 hours). Insomnia symptoms were least frequent in 26-to-40-year-olds and most frequent in persons aged >65 years, and those spending 9 or more hours in bed. Poor sleep quality was most common in those spending <6 hours in bed. Women, persons of non-European origin, overweight persons and smokers were more prone to poor sleep. While habitual TST was similar in the different countries, insomnia symptoms were between 1.5 to 2.9 times higher in USA than in the Netherlands. Women (41+) reported sleeping shorter or less efficient than men, which was opposite to actigraphy estimates where women were estimated to sleep longer and more efficiently than man, both in the UK and in the Netherlands.

In conclusion, we provide age- and sex-specific population reference charts for sleep duration and efficiency which can be used in research, clinical and preventive in indus-

trialized countries. More people report poor sleep quality than short sleep duration. Thus, whereas most available guidelines address optimal sleep duration, our findings highlight the importance of also targeting sleep quality.

INTRODUCTION

Poor sleep is common and increasingly recognized as a potentially modifiable risk factor for various physical and mental health problems.[1,2] Yet, sleep has received little attention from a public health perspective. This may partly be due to the lack of valid descriptions of typical sleep patterns in the general population. Estimating reference ranges for sleep duration can help compare an individual's sleep characteristics with that of men or women of the same age in the general population and quantify the prevalence of insufficient sleep at a population level.

The widely used sleep duration recommendations issued by the American National Sleep Foundation (NSF),[3,4] synthesize relevant empirical studies but partly rely on expert opinion, thus may differ from data-driven descriptions of sleep in the general population.[5] In addition, these recommendations target healthy populations, whereas the general population represents the continuum between health and disease. It is also unclear how the three categories of sleep duration (recommended, acceptable, not recommended) relate to sleep quality or other sleep complaints. Ideally, recommendations for sleep duration in the general population should be described over multiple physiologically and clinically relevant aspects, including age, sex, demographics, or lifestyle. We described variations in sleep duration and estimated the proportion that falls outside of the recommendations, and studied factors related to suboptimal sleep.

Few epidemiological studies have systematically summarized sleep characteristics in the general population. The studies conducted to date have either collected data via mobile devices[6] or online surveys,[7,8] have focused on a particular age group such as children[9,10] or older adults,[11,12] or studied a single sleep problem such as short sleep,[13] long sleep or insomnia.[14,15]

We summarized available information in the general population by jointly investigating multiple sleep variables across the lifespan. Importantly, as opposed to previous meta-analytical efforts,[16-18] also of similar sample sizes,[19] we assembled individual participant data (IPD) from 200,358 persons aged 1 to 100 years, from 36 population-based studies from the Netherlands. This allowed us to explore sleep characteristics in various subgroups as well as interrelations between sleep indices. In addition, we compared the available estimates with those from two large population-based adult samples from the UK (n=498,320) and USA (n=409,617).

This study provides reliable estimates of self-reported sleep duration, sleep timing, sleep efficiency, but also perceived sleep quality, insomnia symptoms and other sleep complaints (non-restorative sleep, sleepiness, snoring and use of sleep medication) in the general population. In order to obtain valuable population percentile curves and reference values we described sleep duration, time in bed and sleep efficiency across age and sex. We also explored educational level, ethnic origin, partnership and employ-

ment status, as well as BMI and smoking, as potential risk indicators associated with these sleep variables. Where data was available, we complemented subjective data with objectively estimated sleep variables. Moreover, we evaluated consistency and differences in sleep parameters across populations from the Netherlands, UK and USA.

METHODS

Search strategy, eligibility and selection criteria

We conducted a systematic literature search to identify population-based cohorts from the Netherlands assessing sleep characteristics via questionnaires. We searched Embase, Medline Ovid, and Web of Science Core Collection on August 9th 2019 with a search strategy developed by a biomedical information specialist (WB; Supplementary Text). Inclusion criteria were: i) population-based sample from the Netherlands; ii) inclusion of at least 100 participants older than 1 year; iii) assessment of sleep with questionnaires; iv) publication in a peer-reviewed journal after the year 2000. Exclusion criteria and steps are outlined in a detailed flowchart (Supplementary Figure 1a and 1b). All 5,750 identified abstracts were checked for eligibility by two independent reviewers (DK and either TSL, YX, MEKV or ID, references were split randomly), after which DK assessed 381 full-text articles for eligibility, and TSL again assessed the excluded articles. From 142 publications that met our inclusion criteria, we identified 43 non-overlapping study populations. We additionally added 4 studies identified by personal contacts, but sought IPD from 47 studies (IPD was not requested from 3 studies that were published after data collection had been completed in early 2017), of which 36 agreed (response 81%). From studies with repeated measurements, the baseline measurement was used for this IPD as it comprised the largest sample size.

All studies included in the meta-analysis (Supplementary Table 1) were approved by the ethics committee of the local university, institute or organization. Written informed consent was obtained in the original studies from all participants or caregivers (see publications in Supplementary Table 1). The first and corresponding authors obtained legal rights for access to anonymized datasets. This article follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement for Individual Patient Data reporting guidelines, Supplementary Text 2.[20]

To evaluate consistency of sleep characteristics across countries, we included two large population-based datasets from adults in the UK Biobank (n=498,320) and the National Health Interview Survey (NHIS) from the USA (n=409,617). These samples were not meta-analytically pooled with the data from the Netherlands, as this would further increase heterogeneity, thereby resulting in estimates that cannot be generalized to any population

Patient and Public Involvement

This research is a response to public interest. In April 2015, residents of the Netherlands were asked to indicate which scientific questions should be addressed in the next decade. Requests of 11,700 people laid the foundation for the National Science Agenda (https://wetenschapsagenda.nl/). Text analysis revealed that attention for sleep-related issues was requested 423 times; hence the current research question can be considered relevant by the general population. However, participants were not invited to comment on the study design or interpretation of the results. Participants did not contribute to the writing or editing of this document for readability or accuracy.

Individual Participant Data coding

To maximize internal validity, we harmonized the datasets in a three step procedure: 1) we agreed upon definitions for each sleep variable (described in the Coding Steps and Protocol, see Supplementary Text), also socio-demographic variables were classified in line with Statistics Netherlands[21,22]; 2) two independent coders (DK and TSL) coded all datasets according to the standardized protocol (reliability statistics reported in Coding Steps and Protocol); and 3) coding disagreements were resolved by consensus supervised by a senior sleep researcher (HT).

Sleep variables

We distinguished the following 10 sleep variables:

- *Time in bed (TIB, hours)* was calculated as the difference between *bedtime* and *wake up time* in hours, for weekdays and weekends separately. Bedtimes between 12:00 and 17:00, and wake up times between 17:00 and 02:00 were excluded (N=97).
- *Sleep duration (Total Sleep Time, TST, hours)* was self- or caregiver-reported, values ≤2h or ≥20h were excluded (N=81).
- *Sleep efficiency (SE, %)* was calculated as (TST/TIB)*100. Note that TST and bedtimes and wake times were assessed separately, which may result in implausible values, e.g. TST of 7.5, and TIB between 11pm and 7am results in implausible SE, but likely represents high SE. To balance bias in estimates with loss of precision: values between 100% and 110% were recoded to 100% (mainly errors in reporting times, n=7,630, 8.8%), values above 110% were excluded (n=2,597, 2.9%, most from the largest cohort, Lifelines Study).
- *Daytime napping* was defined as reporting 'regularly' or 'frequently' sleeping ≥30min during the day (yes/no).
- *Insomnia symptoms* (yes/no) included difficulty initiating sleep (DIS), defined as trouble falling asleep (≥30 minutes); difficulty maintaining sleep (DMS), defined as trouble falling asleep again after nocturnal awakening; and early morning awakening (EMA), defined as waking up earlier than desired and not being able to fall asleep

anymore. Insomnia symptoms were present if symptoms were reported to occur often, frequently, or ≥3 times per week.[23]

- *Sleep medication* was defined as the reported use of any medication to aid sleep at least once a week (yes/no).
- *Non-restorative sleep* was defined as not feeling rested when waking up in the morning, reported at least 'often' or ≥3 times per week (yes/no).
- *Sleepiness* was defined as 'feeling sleepy' during the day, reported at least 'often' or ≥3 times per week (yes/no).
- *Snoring* was present if snoring was reported at least once a week (yes/no).
- *Poor sleep quality* was present if any questions on how individuals perceived or judged their habitual sleep were answered with "bad", "unsatisfactory", "insufficient", or similar qualifications (yes/no).

Socio-demographic variables

Ethnic origin was based on self-report on the country of birth of the participant and his/her parent[24] and categorized into European origin - Dutch, European origin – other, and non-European origin.[22] Educational level was based on self-reported highest education and categorized into low (lower vocational training, or ≤3 years at general secondary), medium (>3 years general secondary school, intermediate vocational training or first year of higher vocational training), or high (university degree, higher vocational training).[21] Having paid employment and having a partner (including non-cohabiting) were self-reported and classified as yes/no.

Health risk indicators and lifestyle variables

Smoking was self-reported and categorized into: never, former, or current smoker. BMI (kg/m^2) was calculated based on self-reported or measured weight and height. BMI from 18.5 kg/m^2 to 25 kg/m^2 was defined as normal weight. Underweight was defined as BMI below 18.5kg/m^2, overweight as BMI above 25 kg/m^2 and obese above 30 kg/m^2. These variables were only defined for adults.

Complementary objective sleep estimates

In two cohorts from the Netherlands, subjective sleep reports were collected simultaneously with sleep diaries and actigraphy. In the Generation R Study children aged 10-15 years (n=1386) wore Geneactiv watches during 9 days.[25] In the Rotterdam Study participants aged 45-98 years (n=1940) wore Actigraphy watches during 7 days.[26] Actigraphic sleep variables were estimated with validated algorithms. Actigraphy and diary sleep estimates were averaged across days. The actigraphic sleep variables were complemented by those of 85,499 participants from the UK Biobank (UKBB).[27]

International comparisons

To evaluate consistency across countries, the IPD analyses were complemented by data from international cohorts. First, the UK Biobank (UKBB) (www.ukbiobank.ac.uk) is a large population-based cohort study aimed at improving prevention, diagnosis and treatments of various illnesses. Between 2006 and 2010, approximately 9.2 million people aged 40-69 years were invited. Second, US data were obtained from the National Health Interview Survey (NHIS, https://www.cdc.gov/sleep), harmonized by Integrated Public Use Microdata Series (https://nhis.ipums.org/nhis/), a nationally representative survey of non-institutionalized American adults surveyed annually (2004-2017). We included adults aged 18-84 years with non-missing responses for the respective sleep measures.

In the UKBB, adults reported on TST by answering the question *"About how many hours sleep do you get in every 24 hours? (please include naps)".* We excluded participants reporting usual daytime napping from the UKBB (n=26,561). NHIS participants answered the question "*On average, how many hours of sleep do you get in a 24-hour period?",* with responses in hour increments. Symptoms of insomnia in the UKBB were assessed by the question: "*Do you have trouble falling asleep at night or do you wake up in the middle of the night?*", which did not map on any of our individual insomnia constructs, thus was not further analyzed. NHIS participants reported DIS and DMS using two questions: "*In the past week, how many times did you have trouble falling asleep?*" and "*In the past week, how many times did you have trouble staying asleep?",* respectively. Participants that reported having these symptoms "*usually*" in the UKBB, and *"≥3 times per week*" in the NHIS were coded as "yes". These estimates were compared to the pooled IPD meta-analysis sample.

Statistical analyses

We explored whether the population in the meta-analysis was representative of the general population of the Netherlands by comparing the distributions of age, sex, and education with the last Dutch Census in 2011.[28] For descriptive purposes, we pooled the data across studies, with different studies contributing data for different sleep variables, according to what data had been collected.

First, age and sex specific means and prevalence of sleep variables were computed based on systematically coded variables to reduce between-study heterogeneity (see Coding Protocol in Supplementary Text). Age categories were aligned to those of NSF: toddlers (1-2 years), preschoolers (3-5 years), school-aged children (6-13 years), teenagers (14-17 years), young adults (18-25 years), adults (26-40 years), middle-aged adults (41-64 years), and older adults (65+ years).

Second, variations in TST, SE, and TIB were plotted using age-specific percentiles (10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th}). To facilitate comparison, TST was also plotted against the NSF sleep duration recommendations: 11-14h for toddlers, 10-13 hours for preschoolers, 9-11 hours for school-aged children, 8-10 hours for teenagers, 7-9 hours for adults 26-64

years old, and 7-8 hours for older adults.[3] To explore detailed age-related changes in TST, SE and TIB we also estimated percentile curves against continuous age between 1 and 100 years using *gamlss* R package.

Third, we examined associations of sleep duration, sleep efficiency and insomnia symptoms with socio-demographic and health indicators using one step approach. We used linear mixed models, with a random intercept for each study to account for between study heterogeneity. The random effects for study were significant in all models. In these analyses, we only included participants aged 18 years and older as sleep characteristics change rapidly during childhood and adolescence.[9] Three models were constructed: a "demographic determinants model" where we studied the association of mutually adjusted age (continuous), sex, educational level and ethnic origin with sleep variables, a "social determinants model" where we studied the association of employment status and partnership on sleep variables adjusted for demographic determinants, and a "health indicators model" where we studied the association of smoking and BMI with sleep variables adjusted for demographic determinants.

As more sophisticated imputation methods cannot account for within-study clustering, missing values on age (0.3%) were imputed with the study-specific mean, and a missing category was used to account for missing values in categorical variables (education=0.6%, ethnic origin=26.6, employment=7.4%, partner=62.2%, smoking=15.0%, BMI=13.3%). Ethnicity was not assessed in 8 studies, whereas of the studies in adult populations five did not assess employment and three did not assess smoking.

Missing or implausible values on sleep variables were not imputed. Data were analyzed using *SPSS Statistics,* version 21 (IBM Corp., Armonk, NY) and R version 3.4.1.

RESULTS

We included 34 studies, identified by systematic review, including 200,358 participants from the Netherlands between the age of 1 and 100 years. Additionally, 471,759 persons (40 to 69 years old, 55.5% female) from the UK, and 409,617 persons (≥18 years, 55.8% female) from the US were included. Population characteristics of the studies identified in the systematic review are presented in Supplementary Table 1. Compared to data of the 2011 Dutch Census,[28] females in age groups between 10 to 80 years were slightly over-represented (ranging from a 1% to 9% difference).

Persons in both the high (29.9% vs, 29.0%, p=0.013) and the middle (37.3% vs. 34.4%, p<0.001) educational level were slightly overrepresented in our sample, compared to the population described in the Dutch Census of 2011. Study specific sleep estimates are provided in Supplementary Table 2.

Time in bed, sleep duration and sleep efficiency

Adults (≥18 years) reported a mean±SD TIB of 7.8±0.9 hours, a TST of 7.1±1.0 hours, and a SE of 89±9% (Table 1). Short sleep duration (TST<6 hours) was reported by 6.5% of this population, whereas 25.8% reported a TST of <7 hours. Population percentile curves of TST and SE across age categories defined by NSF recommendations are shown in Figure 1, and in Supplementary Figure 2 for age (continuous). Although 24.5% of the population sleeps less than the recommended sleep duration for age, only 5.6% fall outside of the "acceptable" ranges (see Supplementary Table 3). More than half (51.5%) of 14-to-17-year-olds reported sleeping less than recommended 8-10 hours per night; those in the 25th percentile sleep 54 minutes less, whereas those in the 10th percentile sleep 96 minutes less than recommended. In all other age groups, even the 5% and 95% percentile groups, sleep duration was in the "acceptable range" as defined by the NSF [3]. SE decreases from mean±SD= 97±5% in childhood to 91±8% in teenage years. This SE decline continues into adulthood, however 25% of >65-year-olds reported sleeping over 95% of their TIB.

Sex difference were observed from adulthood onwards (Table 1). Adult women reported a longer TST (B=0.14 hours, 95%CI: 0.18;0.21, p<0.001), but a marginally lower SE (B=-0.02%, 95%CI: -0.03;-0.02, p<0.001) than men (Supplementary Table 4). For example, women between 41 and 65 years of age sleep on average 7.1±1.1 hours, whereas at the same age men sleep on average 6.9±1.0 hours per night. However, the women sleep 89±10% of the TIB, whereas men sleep 92±9% of the TIB. From about 14 years onwards, the between-person variation in TIB increases substantially, more so for men than for women (Figure 2). Sex-specific TIB percentiles using age (continuous) are shown in Supplementary Figure 3. From 14 years onwards bedtime is gradually delayed, whereas wake time remains stable around 7:00h across the lifespan (Figure 3). Poor sleep quality is most prevalent in persons (≥18 years) spending <6 hours in bed, whereas difficulty initiating sleep is most commonly reported by those spending ≥9 hours in bed (Figure 4).

We found that TIB is longer on weekend days than on weekdays only for age groups that go to school or work. In young children and older adults, the TIB on week- and weekend days is roughly equal. The weekday-weekend difference increases as children start going to school (median difference of 30 minutes), peaks in teenagers (median difference of 75 minutes), and is around 60 minutes in working adults.

Daytime Napping

As expected, most children nap in the first 3 years (80% of 1-2 year-olds, 65% of 3 years-old). Napping is less common during school age (12.7% of 6-13 year-olds nap) and adulthood (13.7% of people between 26 and 64 years nap regularly), than in persons aged >65 years (27%).

Table 1. Time in bed, total sleep time and sleep efficiency, stratified by age and sex

Strata by age and sex	Time in bed, hours		Total sleep time, hours		Sleep efficiency, %	
	20 studies (198-2013)		15 Studies 1993 to 2015		15 Studies 2002 to 2013	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
1-2 years						
Total	3,240	11.7 ± 0.72	-	-	-	-
Male	1,594	11.6 ± 0.73	-	-	-	-
Female	1,646	11.7 ± 0.70	-	-	-	-
3-5 years						
Total	6,421	11.5 ± 0.6	1,266	11.6 ±0.6	1,183	99 ± 2
Male	3,241	11.4 ± 0.6	653	11.5 ± 0.6	604	99 ± 2
Female	3,180	11.5 ± 0.6	613	11.6 ± 0.6	579	99 ± 3
6-13 years						
Total	18,905	10.8 ± 0.9	8,377	10.6 ± 1.0	6,931	97 ± 5
Male	9,477	10.7 ± 0.8	4,185	10.5 ± 0.9	3,461	97 ± 5
Female	9,420	10.8 ± 0.9	4,189	10.6 ± 1.1	3,468	97 ± 5
14-17 years						
Total	3,747	8.8 ± 0.8	513	7.7 ± 1.1	509	91 ± 8
Male	1,745	8.7 ± 0.8	189	7.9 ± 1.0	186	92 ± 7
Female	2,000	8.8 ± 0.8	324	7.6 ± 1.1	323	91 ± 8
18-25 years						
Total	1,174	8.3 ± 1.2	5,192	7.5 ± 1.1	-	-
Male	588	8.0 ± 1.2	2,049	7.4 ± 1.1	-	-
Female	606	8.5 ± 1.1	3,143	7.6 ± 1.0	-	-
26-40 years						
Total	23,896	8.0 ± 0.9	38,635	7.2 ± 0.9	21,204	89 ± 9
Male	9,938	7.7 ± 0.9	16,182	7.1 ± 0.9	8,678	90±8
Female	13,931	8.1 ± 0.8	22,453	7.3 ± 1.0	12,526	89±10
41-65 years						
Total	51,086	7.8 ± 0.9	93,837	7.0 ± 1.1	49,513	90 ± 10
Male	21,235	7.5 ± 0.9	40,603	6.9 ± 1.0	20,570	92 ± 9
Female	29,851	7.9 ± 0.9	53,234	7.1 ± 1.1	28,943	89 ± 10
65+ years						
Total	5,480	7.9 ± 1.1	8,195	7.0 ± 1.3	4,922	88 ± 13
Male	2,288	7.9 ± 1.1	3,504	7.2 ± 1.2	2,021	90 ± 11
Female	3,192	7.8 ± 1.1	4,691	6.8 ± 1.4	2,901	86 ± 14

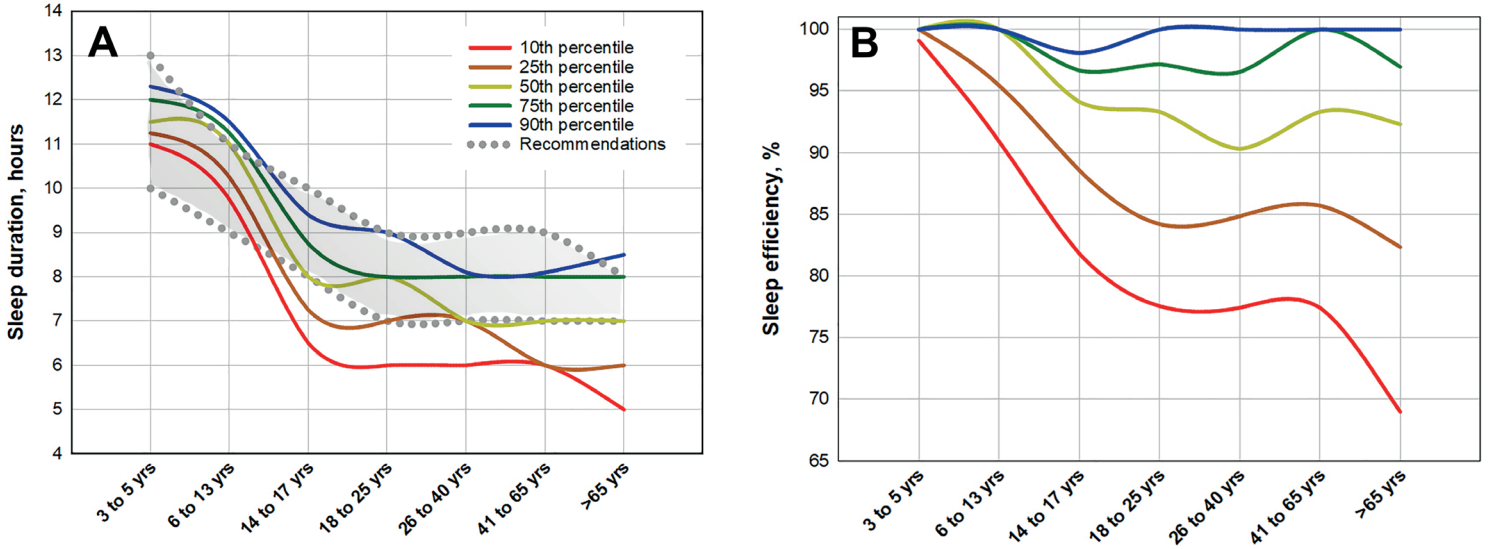

Figure 1. Age-specific percentile curves of total sleep time (N=164,069) and sleep efficiency (N=76,746)
Panel A represents percentiles of sleep duration per age group, where the gray area represent the NSF recommended sleep duration. Panel B represents percentiles of sleep efficiency (% of sleep within time in bed: (TST/TIB)*100) per age group.

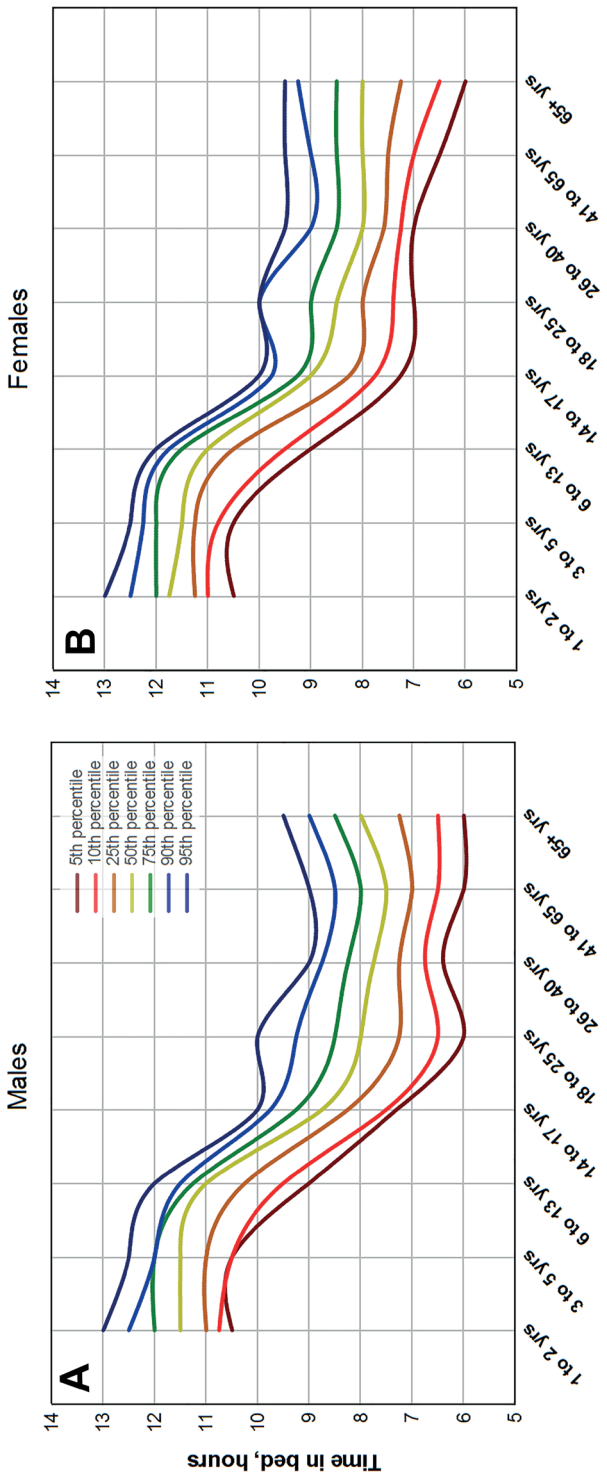

Figure 2. Age-specific percentile curves for time in bed, stratified by sex (N=106,282, 56% females)
Panel A represents percentiles of time in bed per age group in males, and panel B represents percentiles of time in bed per age group in females.

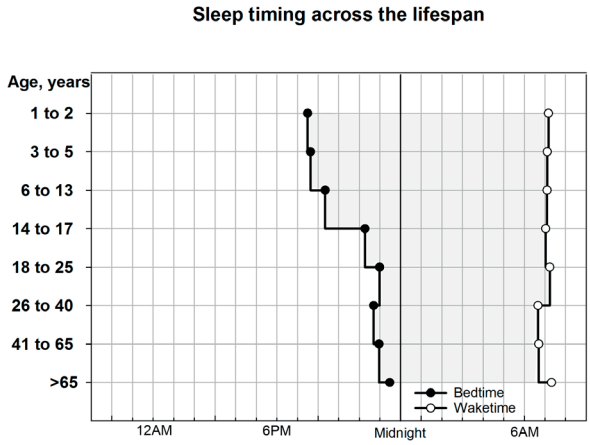

Figure 3. Nighttime sleep timing across the lifespan (N=106,282)

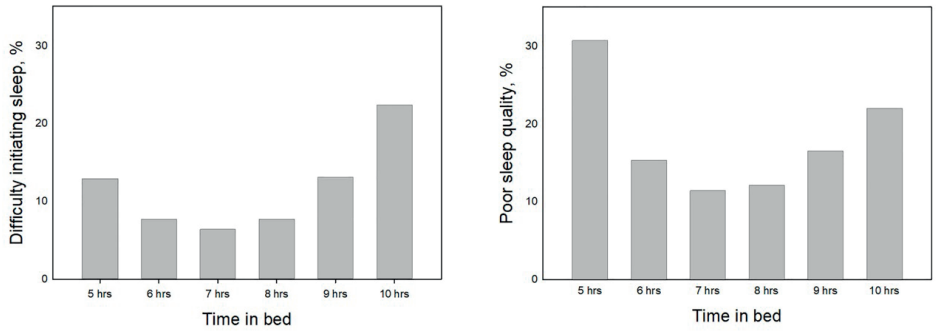

Figure 4. Prevalence of having difficulty initiating sleep (N=95,603) and 'poor' sleep quality (N=77,854), across different durations of time in bed
Note: Data on both difficulty maintaining sleep and early morning awakenings and time in bed were not available

Insomnia symptoms

Symptoms of insomnia increase from childhood (3 to 5 year-olds: 4% DIS, 6% DMS) into adolescence (6-13 year-olds: 13% DIS, 9% DMS). In adulthood, insomnia symptoms are least frequent in 26 to 40-year-olds and most frequent in >65-year-olds. DIS is most prevalent in 18 to 25-year-olds (22.6%), whereas DMS (23.2%) and EMA (23.5%) are most prevalent in the >65-year-olds. Sex difference in insomnia symptoms become evident only in puberty (i.e. for 14 to 17 year olds, Males vs. Females: 12% vs. 19% DIS, 16% vs. 28% DMS). In adults, women are at increased odds for DIS (OR=2.26, 95% CI 2.16;2.36), DMS (OR=2.05, 95% CI 1.91;2.19), or EMA (OR=1.49, 95% CI 1.37;1.62; Supplementary Table 5) compared to men after adjusting for demographic factors.

Table 2. Prevalence of insomnia symptoms, stratified by age and sex

Strata by age and sex	Difficulty initiating sleep		Difficulty maintaining sleep		Early morning awakenings	
	22 studies (1997-2015)		15 studies (1998-2015)		9 studie (1997-2015)	
	N	%	N	%	N	%
1-2 years						
Total	1,336	4.5	-	-	-	-
Male	655	4.9	-	-	-	-
Female	681	4.1	-	-	-	-
3-5 years						
Total	5,484	4.0	1,678	6.1	ND	ND
Male	2,778	4.2	834	6.6	ND	ND
Female	2,704	3.8	844	5.7	ND	ND
6-13 years						
Total	13,227	13.2	7,210	9.1	ND	ND
Male	6,697	12.3	3,601	7.9	ND	ND
Female	6,570	14.0	3,602	10.2	ND	ND
14-17 years						
Total	1,631	16.5	1,175	23.2	-	-
Male	719	12.9	501	16.8	-	-
Female	910	19.3	672	28.1	-	-
18-25 years						
Total	2,227	22.6	1,961	9.4	2,023	10.3
Male	969	19.4	856	8.6	892	9.2
Female	1,252	25.1	1,105	10.0	1,131	11.2
26-40 years						
Total	26,264	7.2	3,795	11.3	1,636	14.1
Male	10,850	5.5	1,550	7.4	722	12.0
Female	15,413	8.3	2,244	13.9	913	15.7
41-65 years						
Total	73,648	9.3	19,056	15.7	8,417	21.0
Male	31,637	5.4	8,640	10.5	3,904	17.5
Female	41,975	12.3	10,416	20.1	4,513	24.0
65+ years						
Total	8,869	14.9	3,255	20.2	3,376	23.5
Male	3,841	8.0	1,527	14.5	1,579	18.3
Female	5,028	20.2	1,728	25.3	1,797	28.0

Note: Prevalence rates were not calculated if <200 participants in a cell. Abbreviations: ND=Not defined if inapplicable for the age group

Other sleep complaints

Sleepiness is most prevalent in teenagers (20.4%; Supplementary Table 6). Although there are no clear sex difference in sleepiness, non-restorative sleep is more prevalent in women than in men. Women also use sleep medication more often (8.6% vs. 5.2% in 26 to 40-year-olds, to 17.5% vs. 6.3% in >65-year-olds). Snoring is more commonly reported in adult men than in women (40.2% vs. 23.2%), although this difference becomes less pronounced at older ages (Supplementary Table 6).

Associations of socio-demographics with sleep characteristics in adults

Adults with a low educational level did not differ in TST (B=-0.01 hours, 95%CI -0.02;0.00, p=0.191) compared to highly educated adults, but reported a slightly lower SE (B=-0.01%, 95% CI -0.03;-0.00, p<0.001). In addition, persons with a non-European ethnic origin sleep shorter (B=-0.30 hours, 95%CI: -0.34;-0.30, p <0.001), and less efficiently (B=-0.03%, 95%CI: -0.03;-0.02, p<0.001) compared to persons with Dutch ethnic origin. Similarly, both low education and non-European ethnic origin were risk indicators for insomnia symptoms (Supplementary Table 5). Having paid employment and a partner were both associated with longer sleep duration and less insomnia symptoms, independent of demographics (Supplementary Table 4 & 5).

Association of health risk indicators with sleep characteristics in adults

In adults, we observed shorter TST for overweight (2.4 minutes, 95% CI: 3.6;1.8) and obese persons (6.6 minutes, 95% CI: 7.2; 5.4), compared to persons with normal weight. Obese, but not overweight persons, had a marginally lower SE (B=-0.004%, 95%CI: -0.01; -0.00) and experienced more DIS (OR=1.08, 95%CI: 1.02; 1.17; Supplementary Table 4). Both former and current smokers reported sleeping shorter relative to non-smokers, and current smokers also reported a lower SE. Current smokers experienced more DIS, but experienced less DMS (Supplementary Table 5).

Complementing subjective with objective sleep data

TIB and TST were between 0.4-1.9 hours shorter when estimated with actigraphy as compared to sleep diary reports of the same nights (Supplementary Table 7). Similarly, actigraphic SE estimates were lower compared to diary estimates, averaging to 9.7±7% difference in the Generation R sample, and 9.6±9% difference in the Rotterdam Study sample. The sleep diary SE estimates were also lower than those computed from the pooled IPD, except for the group of teenagers where SE based on pooled IPD was estimated to be 91±8%, as compared to 95.6±4% estimated by sleep diary.

According to actigraphic TST estimates, more than 80% of the population, sleeps less than the US recommendations (Supplementary Table 8). The proportion of persons sleeping less than the "acceptable" TST ranged between 16.3%-38.7% in the pediatric

cohort, and between 9.4%-47.3% in the older adults, as measured with actigraphy. Actigraphic sleep parameters of the adults from the Netherlands were compared with respective values from adults in the UK (Supplementary Table 9). Both TIB and TST were ≥1 hour longer in the UK cohort regardless of age and sex, however SE differences were small (1.6% to 2.1%). Women (41+ years) reported sleeping shorter and/or less efficiently than men both in sleep diaries and sleep questionnaires, whereas actigraphy estimates indicate the opposite: women sleep longer and slightly more efficiently than men of similar age (Supplementary Table 7). This was also found in the UKBB cohort.

International comparisons

Average self-reported TST as well as sex difference in TST were similar in the adult Dutch, UK and US populations (Supplement Table 10). The proportion adults reporting TST shorter than recommended for age was the highest in the US (30.3%), compared to 24.5% in the Netherlands, and 25.0% in the UK. The proportion of adults sleeping less than the "acceptable" values were below 10% in all three countries. The prevalence of insomnia symptoms (Supplementary Table 11) was 1.5 to 2.9 times higher in the US sample (for DIS and DMS, across adult ages with the exception of 18-25 year olds) than in the Netherlands. Sex and age differences in insomnia symptoms were similar across populations: DIS reduced and DMS increased with advanced age, whereas women reported insomnia symptoms more commonly irrespective of age.

DISCUSSION

Our results suggest that: i) the population of the Netherlands reported sleeping within "acceptable" sleep duration range at all ages, but more than half of teenagers slept almost an hour less than recommendations; ii) actigraphic sleep duration and efficiency are consistently lower than self-reported estimates, which limits the applicability of current recommendations to objective sleep variables, iii) insomnia symptoms were least frequent in 26 to 40-year-olds and most frequent in persons aged >65 years, and those spending 9 or more hours in bed; iv) self-reported TST did not differ substantially between adults from the Netherlands and from the UK and US, but insomnia symptoms were 1.5 to 2.9 times more prevalent in the US than in the Netherlands, v) poor sleep quality and insomnia symptoms were more prevalent than short sleep duration; vi) women, persons of non-European origin, overweight persons and smokers were particularly prone to experiencing poor sleep.

Strengths and weaknesses

Our study is the largest descriptive sleep study to date. However, several methodological issues must be discussed. First, variables such as sleep timing and duration may be more objectively assessed with actigraphy or polysomnography.[29,30] However, subjective complaints are clinically relevant, and highly related to daily functioning. Moreover, the implementation of measures such as polysomnography in large-scale population-based studies is currently limited. In this study we were able to complement subjective data with objective sleep parameters in teenagers and older adults. These are the two age groups with the highest prevalence of insufficient sleep duration. Sleep duration estimates differ by method of assessment, but habitual sleep duration is reasonably stable within individuals.[31,32] Thus, the inter-individual differences in sleep duration can reliably be compared when assessed with the same method only. Moreover, absolute numbers should be interpreted with caution because age or reporter could influence sleep estimates (e.g. parents may underreport their children's sleep onset latency and wake time during the night, resulting in higher SE estimates). Second, heterogeneity between studies could have introduced misclassification bias (e.g. different definitions of bedtimes and wake times can influence TIB estimates). However, access to IPD improves data quality through standardization of definitions. Third, we could not assess how our estimates, and differences between meta-analyzed studies, were influenced by underlying sleep disorders (e.g. sleep apnea), psychiatric disorders, chronic medical conditions, or environmental or occupational factors such as screen exposure, noise, and shift work. Fourth, although we studied a representative large population sample of the Netherlands, and compared sleep estimates to other populations from developed countries, findings may not be generalizable to populations with different sociodemographic or cultural characteristics. These international comparisons were possible for some sleep parameters only. However, all studies sampled participants from the general population, which reduces the chance of selection bias, and increases the interpretability of the comparisons. Fifth, multivariable adjusted models indicated that the reported differences in sleep patterns across socio-demographic groups were small, thus their clinical implications may be limited.

Comparison with other studies

In our study, 25% of the adult population reported sleeping less than the recommended 7-9 hours, whereas the Centers for Disease Control and Prevention has estimated up to 44.1% of the US population aged ≥18 years slept less than 7-9 hours.[33] We showed that the average self-reported sleep duration does not differ between the Netherlands, US and UK, but the prevalence of sleeping below the recommended TST was higher in the USA population (30%), than in the European populations (24-25%). We also showed that the recommendations are only applicable to subjective sleep reports. Specifically, 80%

of participants above 40 years, have an actigraphic TST less than the "recommended" 7 hours TST. It is important to note that a portion of this population still falls within the "acceptable" range of 6 to 11 hours developed by the NSF expert panel.[3,4] The pooled IPD data show that 6.8% of the adult population report sleeping less than the "acceptable" 6 hours, but this increased to 25% at an older age. Using actigraphic TST estimates up to 47% adults were estimated to sleep less than the "acceptable" values.

Based on an online questionnaire, Kerkhof has reported a higher percentage (30.4%) of <6 hours of sleep in an adult population from the Netherlands.[7] Studies included in our meta-analysis have shown that participants aged 18-65 years old sleeping both less than 6 hours[34] and less than 7 hours[35] per night have higher cardiovascular risk as compared to those sleeping 7 to 8 hours per night. A Time Use Survey Panel in industrialized countries in Europe and North America[36] has also shown that older adults sleeping <7 hours have lower self-reported health, although the "acceptable" sleep duration for this age group can be as short as 5 hours per night. It thus remains unclear what the appropriate amount of self-reported sleep duration is for preserving health, and reference values for objective sleep duration are merely unknown.

Despite the premise that 'optimal' sleep duration likely differs per outcome, individual and circumstances, providing reference values for sleep length can be useful in clinical or prevention practice. This way it is possible to estimate the extent of the problem (i.e. the proportion that falls outside of recommended values) which could guide public health policies for improving sleep in the general population. Therefore, we estimated sleep duration percentile curves, which to date have been estimated only in children and adolescents.[9,10,37] Healthcare professionals can easily assess sleep characteristics by interviews or questionnaires, but with increased use of accelerometers in research and daily settings, reference curves for actigraphic sleep variables should also be estimated.

Several previous observational studies have estimated the prevalence of insomnia in European populations.[7,11,14,15,38] Our study estimates (7 to 23% depending on insomnia symptom and age group) largely correspond with those reported in telephone interviews by 25,579 persons from seven European countries in the 90's.[14] The prevalence of DIS and DMS in the Netherlands, however, was substantially lower than in the US. Our study, adds age-specific information on the prevalence of insomnia symptoms across the lifespan, and shows which insomnia symptoms are most common in each age group. We also show that these age related changes in insomnia symptoms are similar in the USA. This information could be used to improve sleep on a population level, i.e. young adults would likely benefit from interventions tackling difficulty initiating sleep, whereas older adults might need help with sleep maintenance or early morning awakenings. We also show that spending 7 to 8 hours in bed is associated with better sleep quality and fewest insomnia symptoms, similar to a general-population study in Norwich, UK.[11]

In line with previous reports based on smaller samples, we found using pooled IPD data that women report longer sleep duration but lower sleep efficiency.[7,11] For example, a 28-year-old woman reporting to spend 9 hours in bed is in the 90th percentile of the female population of similar age, whereas, a 28-year-old man with the same TIB, would be in the 95th percentile of the male population of similar age. When measured with actigraphy, however, women's sleep was slightly longer and more efficient than that of men in the Netherlands and in the UK. Women experience more insomnia problems than men in all three countries. This commonly reported difference[7,14,38,39] emerges during puberty, suggesting sex hormones, among other social factors such as stress or parenting, might play a role in the development of insomnia problems. Interestingly, women do not report daytime sleepiness more often, despite experiencing more insomnia problems and using more sleep medication than men.

Relevance of the study

The estimated population reference charts for sleep timing, sleep duration and efficiency across the lifespan, will help guide personalized advice on sleep. However, current recommendations are applicable only to self-reported average sleep duration. Given that poor sleep (i.e. low sleep quality or insomnia symptoms) is more common than short sleep (i.e. TST below "acceptable" values) in Europe and in the US, recommendations for improving sleep might need to focus on sleep quality. Importantly, we identified subgroups that are prone to short or inefficient sleep, such as teenagers, women, persons of non-European origin, obese and smokers. These population strata could be used as sampling schemes when developing interventions to improve sleep at a population level. We also show that the lowest prevalence of poor sleep in the general population occurs in those spending 7 to 8 hours in bed. This finding, taken together with the relatively high prevalence of poor sleep despite close to appropriate sleep duration, warrants towards defining new targets for sleep hygiene advice. In other words, by recommending optimal sleep duration we are unlikely to accomplish better sleep at a population level.

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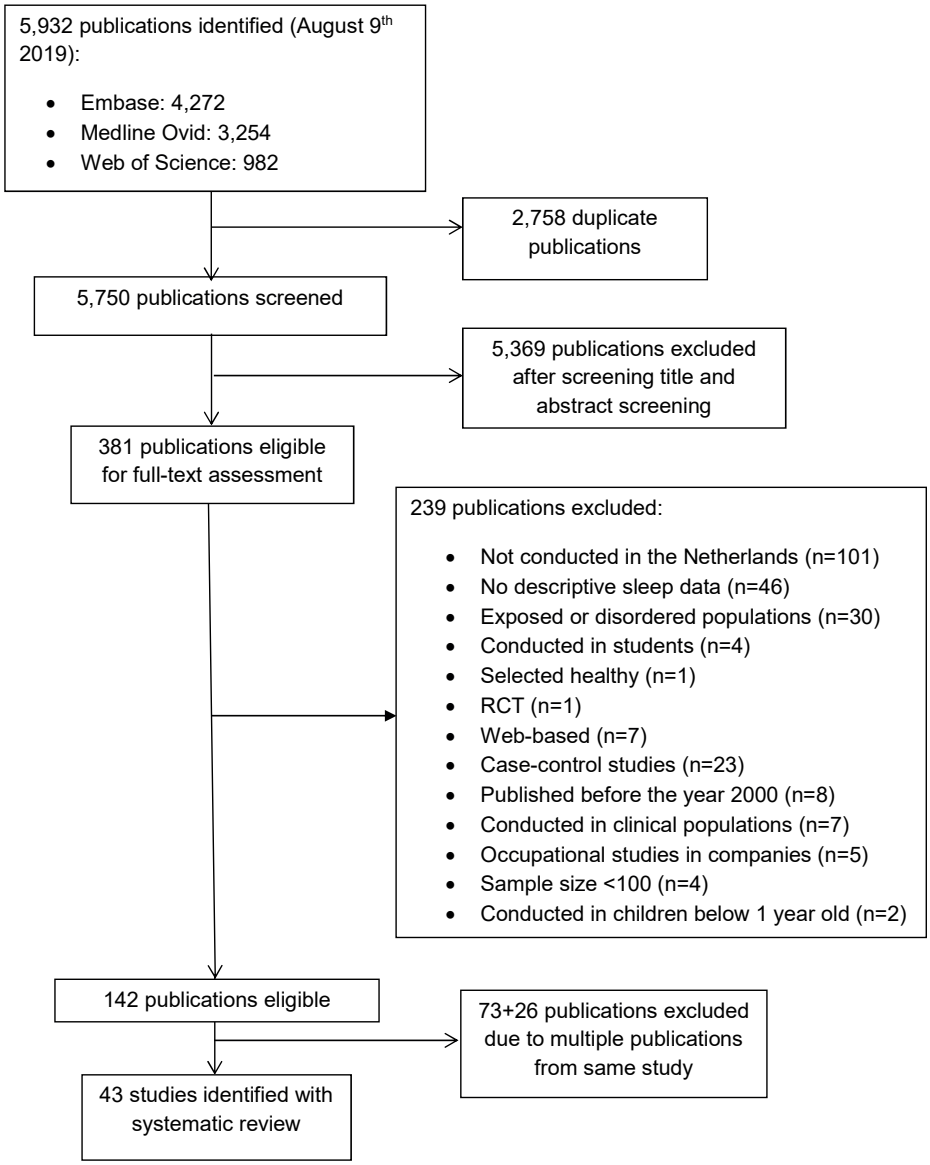
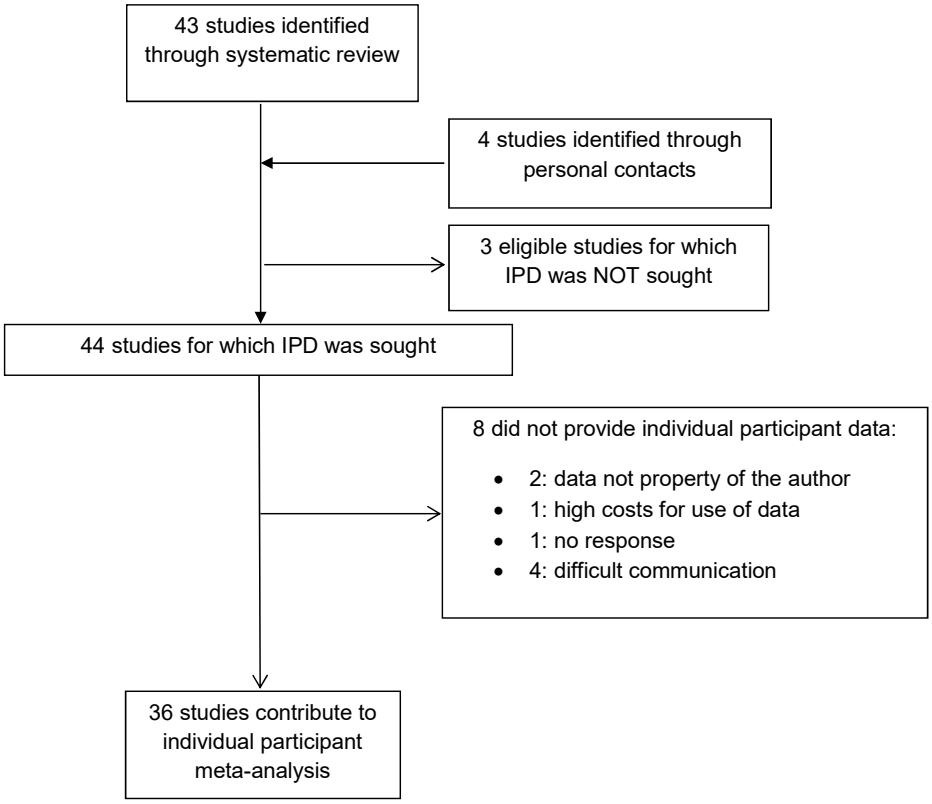
SUPPLEMENTARY MATERIAL



Supplementary Figure 1a. Flowchart of systematic literature review and study inclusion



Supplementary Figure 1b. Flowchart of individual participant data (IPD) attainment

Supplementary Table 1. Characteristics of the Dutch studies included in the individual participant meta-analysis

Study Name	N	Year of data-collection	Age	Sex		Education			Ethnic Origin			Employment	
			mean ± SD	male	female	low	middle	high	Dutch	Other Europe	Non Europe	No	Yes
ABCD[1]	3,444	2011	7.4 ± 0.4	51.1	48.9	9.6	19.2	71.2	71.1	12.1	16.9	-	-
AGHLS[2]	340	2006	42.1 ± 0.7	47.6	52.4	17.6	42.8	39.5	-	-	-	7.6	92.4
AMIGO3	14,670	2011-2012	50.7 ± 9.4	44.2	55.8	30.6	31.2	38.2	91.1	6.6	2.3	28.2	71.8
ChecKid[4,5]	10,362	2006;2009; 2012	7.9 ± 2.3	50.1	49.9	10.9	21.2	67.9	94.4	3.3	2.3	-	-
CSHQ[6,7]	1,504	2006-2007	8.5 ± 2.7	52	48	11.6	31.7	56.6	93.9	2.9	3.2	-	-
deBruin[8]	308	2011-2014	14.5 ± 1. 3	41.6	58.4	3.8	14.6	81.6	95.1	-	-	-	-
deVrijer[9]	300	2003	15.9 ± 0.7	46	54	-	-	-	52.7	0	47.3	-	-
Dewald[10]	954	2011	14.7 ± 1.7	41.3	58.7	10.6	41.2	48.2	-	-	-	-	-
Doetinchem[11]	4,014	2007-2013	59.9 ± 9.7	47.3	52.7	46.1	29.9	24	-	-	-	51.7	48.3
ENERGY[12-14]	396	2010	11.7 ± 0.8	48.5	51.5	69.9	25	5.1	-	-	-	-	-
Enschede[15-17]	806	2001-2002	44.5 ± 15.8	46.1	53.9	53.2	29.6	17.2	84.9	-	-	-	-
EPHE[18,19]	127	2013	7.8 ± 1.0.	47.2	52.8	9.4	38.7	51.9	-	-	-	-	-
GECKO[20,21]	1,922	2009	2.1 ± 0.2	49.4	50.6	-	-	37.9	94.5	1.8	3.6	-	-
Generation R[22-28]	5,012	2005-2009	3.1 ± 0.1	50.2	49.8	15.1	27.3	57.6	65.2	9.3	25.5	-	-
GLOBE[29,30]	4,122	1997	48.9 ± 14.8	48.5	51.5	60.3	22.3	17.4	95.2	-	-	-	-
Heijden[31]	269	2010	10 ± 1.5	44.2	55.8	14.8	34.6	50.6	-	-	-	-	-
HELIUS[32,33]	23,563	2011-2015	43.8 ± 13.4	42.6	57.4	44.1	29.9	26	19.6	-	80.2	39.9	60.1
INPACT[34]	1,914	2008-2009	8.7 ± 0.5	49.8	50	23.1	43.8	33.1	90.1	3.2	6.7	-	-
LASA[35]	1,535	2008-2009	72.3 ± 8.3	44.8	55.2	46.6	34.1	19.3	95.4	0.3	4.4	83.5	16.5
Lifelines	63,446	-	43.0 ± 7.5	41.1	58.9	24.9	41.6	33.3	97.1	1.5	1.5	11.1	88.1
Meijer[36]	446449	1998	10.9 ± 0..7	51.1	48.9	-	-	-	-	-	-	-	-
Meijer[37,38]	638	2000	13.4 ± 0.6.	49.5	50.5	8.7	53	38.3	76.8	-	17.1	-	-

Supplementary Table 1. Characteristics of the Dutch studies included in the individual participant meta-analysis (*continued*)

Study Name	N	Year of data-collection	Age	Sex		Education			Ethnic Origin			Employment	
			mean ± SD	male	female	low	middle	high	Dutch	Other Europe	Non Europe	No	Yes
MORGEN39,40	22,847	1993-1997	42.7 ± 11.2.	45.2	54.8	48.9	27.5	23.6	-	-	-	39	61
NEMESIS-2[41]	4,618	2013-2015	47.7 ± 13.1.	49.8	50.2	6.9	63.5	29.6	84.8	6	9.2	28.2	71.8
NEO[42]	5,808	2011	55.8 ± 6.0	46.9	53.1	25.1	45.5	29.5	-	-	-	35.4	64.6
NESDA Controls[43-49]	601	2008	42.9 ± 14.6	38.9	61.1	13.6	36.2	50.2	94.5	1.8	3.8	-	-
New Hoorn[50,51]	1,678	2013-2015	60.8 ± 6.4	47.4	52.6	37.6	32.1	30.3	99.1	0	0.9	-	-
PIAMA[52,53]	2,969	2007	11.3 ± 0.3	50.7	49.3	20.3	41.4	38.3	92.7	4.3	3	-	-
Pieters[54,55]	545	2008	14 ± 0.8	47.5	52.5	28.8	60.9	10.3	90.4	0.4	9.6	-	-
Reijneveld[56]	4,394	2005-2006	27.5 ± 8.5	48.7	51.3	36.1	37.8	26.2	90.9	1.5	7.6	-	-
Rotterdam Study[57-65]	9,818	2002-2008	65.8 ± 10.6	41.7	58.3	12.1	69.5	18.4	-	-	-	72.6	27.4
Schalkwijk[66]	354	-	14.6 ± 0.7	55.2	44.8	14.7	32	53.3	96	-	-	-	-
TRAILS[67-70]	1,668	2008	19 ± 0.6	44.9	55.1	1.4	86.3	12.3	89.4	2.8	7.8	30.2	69.8
Vermeulen[71]	100	2012	10.5 ± 0.8	42	58	10	38	52	99	-	-	-	-
Wolbeek[72]	3,445	-	14.6 ± 1.4	49.5	50.5	-	-	-	-	-	-	-	-
Zuid Holland[73]	1,706	2003	12.7 ± 3.8	49.1	50.9	39.2	36.2	24.6	84.3	3.1	12.6	-	-
TOTAL	200,358	1993-2015	39.3 ± 18.5	44.4	55.6	29.2	38.3	31.8	62.2	1.9	11.9	26.8	65.8

Footnote: numbers do not add up to 100% due to missing values. Parental education is reported for pediatric cohorts. References are provided on the following page.

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Supplementary Table 2. Study-specific sleep estimates

Study Name	N	TST, hrs	Bedtime	Wake time	TIB, hrs	DIS	DMS	EMA	NA	Med	NRS	Sleepy	Snore	PSQ
ABCD	3,444	10.6±0.7	20.0±0.5	7.1±0.4	11.2±0.6	11.4	-	-	8.0	-	6.8	4.5	18.8	2.7
AGHLS	340	7.7±1.1	-	-	-	4.7	13.9	14.2	13.3	-	9.7	10.0	-	-
AMIGO	14,670	7.0±1.0	-	-	-	13.3	15.4	-	8.9	8.4	11.6	11.2	25.3	11.6
ChecKid	10,362	11.2±0.6	19.8±0.6	7.0±0.4	11.2±0.6	10.5	4.1	29.3	-	-	-	1.3	-	-
CSHQ	1,504	10.6±0.9	20.0±0.7	7.1±0.4	11.1±0.7	7.3	-	-	10.4	-	7.2	2.5	20.7	-
deBruin	308	-	22.2±0.8	7.1±0.4	8.9±0.8	18.4	29.2	-	3.9	3.2	36.8	12.4	-	7.2
deVrijer	300	-	22.5±0.9	7.1±0.6	8.5±0.8	14.5	18.7	-	7.3	-	26.1	16.0	-	7.0
Dewald	954	7.9±1.2	22.2±0.9	6.8±0.5	8.6±0.9	-	26.1	-	10.0	7.2	25.6	22.4	-	6.1
Doetinchem	4,014	-	-	-	-	15.0	15.0	16.7	34.7	-	-	-	-	-
ENERGY	396	9.6±0.9	-	-	-	-	-	-	34.9	-	-	-	-	-
Enschede	806	-	-	-	-	11.7	25.6	4.8	-	-	68.2	-	-	18.1
EPHE	127	10.1±1.0	19.9±6.0	7.0±0.4	11.1±0.6	-	-	-	-	-	-	-	-	-
GECKO	1,922	-	19.3±0.5	7.1±0.6	11.9±0.6	-	-	-	-	-	-	-	-	-
Generation R	5,012	-	19.9±0.7	7.3±0.7	11.4±0.7	4.1	-	-	3.5	-	-	-	-	-
GLOBE	4,122	-	-	-	-	11.2	-	32.6	8.2	7.5	-	-	-	15.0
Heijden	269	-	21.1±0.7	7.2±0.4	10.1±0.7	-	-	-	-	-	-	-	-	-
HELIUS	23,563	6.9±1.3	-	-	-	-	-	-	-	-	-	-	-	-
INPACT	1,914	-	20.1±0.4	7.2±0.3	11.1±0.5	-	-	-	-	-	-	-	-	-
LASA	1,535	7.5±1.3	23.7±0.9	-	-	17.2	25.3	24.6	6.8	-	-	-	-	15.8
Lifelines	63,446	7.1±0.9	22.8±0.7	6.6±0.8	7.8±8.4	7.1	-	-	21.8	9.3	-	5.6	28.3	13.0
Meijer	446	-	20.9±0.5	7.2±0.5	10.3±0.7	20.3	33.6	-	18.7	-	43.8	25.0		
Meijer	638	-	21.8±0.7	6.8±0.5	9.0±0.8	15.4	23.7	-	8.4	-	26.8	8.4		
MORGEN	22,847	7.3±0.9	-	-	-	-	-	-	-	-	-	-		
NEMESIS-2	4,618	7.0±1.1	-	-	-	8.2	12.7	20.8	31.2	-	-	-		

Supplementary Table 2. Study-specific sleep estimates (*continued*)

Study Name	N	TST, hrs	Bedtime	Wake time	TIB, hrs	DIS	DMS	EMA	NA	Med	NRS	Sleepy	Snore	PSQ
NEO	5,808	6.9±1.1	23.3±0.9	7.3±1.2	8.0±1.1	14.0	-	-	32.7	28.8	-	10.5		
NESDA Controls	601	7.4±0.9	23.2±0.9	7.1±1.1	8.1±1.0	5.7	7.8	15.7	29.3	-	-	-		
New Hoorn	1,678	-	23.2±0.9	7.4±1.1	8.2±1.1	-	-	-	48.8	4.5	-	4.2		
PIAMA	2,969	-	20.7±0.5	7.2±0.4	10.5±0.5	16.0	7.9	-	4.3	-	18.1	5.0		
Pieters	545	-	-	-	-	21.6	-	-	-	-	-	-		
Reijneveld	4,394	-	22.8±1.1	6.9±1.0	8.2±1.2	-	-	-	-	-	-	-		
Rotterdam Study	9,818	6.8±-1.3	23.5±0.9	7.1±1.1	7.8±1.1	13.3	-	-	17.2	9.8	-	0.7		
Schalkwijk	354	-	-	-	-	-	-	-	-	-	32.7	12.8		
TRAILS	1,668	-	-	-	-	27.9	-	10.3	-	2.0	-	-		
Vermeulen	100	-	-	-	9.9±0.6	-	-	-	-	-	-	-		
Wolbeek	3,445	-	21.9±0.8	7.0±0.4	9.0±0.8	-	-	-	-	-	53.7	-		
Zuid Holland	1,706	-	-	-	-	-	9.5	-	-	-	-	-		

Numbers represent mean ± standard deviation, or percentages.

Abbreviations: Total Sleep Time, TST, Time in Bed (TIB), hours difficulty initiating sleep (DIS), difficulty maintaining sleep (DMS), early morning awakening (EMA), night-time awakenings (NA), sleep medication use (Med), Non-restorative sleep (NSR, Sleepiness (Sleepy), Poor Sleep Quality (PSQ)

Supplementary Text

Search Strategies

1. Embase

(sleep/de OR 'sleep medicine'/de OR 'night sleep'/de OR 'nonREM sleep'/de OR 'REM sleep'/de OR 'sleep pattern'/de OR 'sleep quality'/de OR 'sleep stage'/de OR 'sleep time'/de OR 'sleep waking cycle'/de OR 'slow wave sleep'/de OR 'sleep induction'/de OR 'circadian rhythm'/de OR wakefulness/de OR insomnia/de OR 'sleep disorder'/de OR 'sleep parameters'/de OR 'sleep deprivation'/de OR 'polysomnography'/de OR ((sleep* NEAR/10 (night OR nonrem OR rem OR pattern* OR qualit* OR quantit* OR stage* OR time OR cycle* OR induc* OR registrat* OR measure* OR habit* OR distur* OR durat* OR architect* OR consolidate* OR parameter* OR disrupt* OR efficien* OR nocturn* OR medicat* OR drug* OR assess* OR lack OR problem* OR poor)) OR ((circadian* OR diurnal* OR ultradian* OR Nyctohemer*) NEAR/3 rhythm*) OR wakeful* OR insomnia* OR Dyssomnia* OR polysomnogra*):ab,ti) AND (Netherlands/de OR (Netherland* OR dutch*):ab,ti,ca,ta,cy,ad) AND ('cohort analysis'/de OR 'longitudinal study'/de OR 'population research'/de OR population/de OR 'epidemiological data'/de OR 'prospective study'/de OR 'retrospective study'/de OR prevalence/de OR questionnaire/de OR psychometry/de OR (cohort* OR longitudinal* OR prospectiv* OR retrospective* OR (population NEAR/3 (general* OR based* OR research* OR healthy)) OR (epidemiolog* NEAR/3 data) OR prevalen* OR questionnaire* OR psychometr*):ab,ti) NOT ([animals]/lim NOT [humans]/lim) NOT (hospital/exp OR 'hospital care'/exp OR (hospital* OR 'intensive care' OR ward OR icu):ab,ti) NOT (('disorders of higher cerebral function'/exp OR (patient* OR case*):ab,ti) NOT ('controlled study'/exp OR (control* OR group*):ab,ti)) NOT ([Conference Abstract]/lim OR [Letter]/lim OR [Note]/lim OR [Editorial]/lim)

2. Medline ovid

(exp Sleep/ OR "Sleep Medicine Specialty"/ OR exp "Sleep Wake Disorders"/ OR "Circadian Rhythm"/ OR wakefulness/ OR Polysomnography/ OR ((sleep* ADJ10 (night OR nonrem OR rem OR pattern* OR qualit* OR quantit* OR stage* OR time OR cycle* OR induc* OR registrat* OR measure* OR habit* OR distur* OR durat* OR architect* OR consolidate* OR parameter* OR disrupt* OR efficien* OR nocturn* OR medicat* OR drug* OR assess* OR lack OR problem* OR poor)) OR ((circadian* OR diurnal* OR ultradian* OR Nyctohemer*) ADJ3 rhythm*) OR wakeful* OR insomnia* OR Dyssomnia* OR polysomnogra*).ab,ti.) AND (Netherlands/ OR (Netherland* OR dutch*).ab,ti,jn,cp,in.) AND (exp "Cohort Studies"/ OR population/ OR "Epidemiologic Studies"/ OR prevalence/ OR questionnaires/ OR Psychometrics/ OR (cohort* OR longitudinal* OR prospectiv* OR retrospective* OR (population ADJ3 (general* OR based* OR research* OR healthy)) OR (epidemiolog* ADJ3 data) OR prevalen* OR questionnaire* OR psychometr*).ab,ti.) NOT (exp animals/ NOT humans/) NOT (exp hospitals/ OR exp "Hospital Units"/ OR (hospital* OR "intensive care" OR ward OR icu).ab,ti.) NOT ((exp "Mental Disorders"/ OR (patient* OR case*).ab,ti.) NOT (exp "Controlled Clinical Trial"/ OR (control* OR group*).ab,ti.)) NOT (letter OR news OR comment OR editorial OR congresses OR abstracts).pt.

3. Web of science

TS=((((sleep* NEAR/10 (night OR nonrem OR rem OR pattern* OR qualit* OR quantit* OR stage* OR time OR cycle* OR induc* OR registrat* OR measure* OR habit* OR distur* OR durat* OR architect* OR consolidate* OR parameter* OR disrupt* OR efficien* OR nocturn* OR medicat* OR drug* OR assess* OR lack OR problem* OR poor)) OR ((circadian* OR diurnal* OR ultradian* OR Nyctohemer*) NEAR/2 rhythm*) OR wakeful* OR insomnia* OR Dyssomnia* OR polysomnogra*)) AND ((cohort* OR longitudinal* OR prospectiv* OR retrospective* OR (population NEAR/2 (general* OR based* OR research* OR healthy)) OR (epidemiolog* NEAR/2 data) OR prevalen* OR questionnaire* OR psychometr*)) NOT ((animal* OR rat OR rats OR mouse OR mice OR murine) NOT (human*)) NOT ((hospital* OR "intensive care" OR ward OR icu)) NOT (((patient* OR case*)) NOT ((control* OR group*)))) AND DT=(article) AND (TS=((Netherland* OR dutch*)) OR AD=((Netherland* OR dutch*))

Coding steps and protocol

First, the sleep constructs were defined based on expert knowledge and exploration of the datasets included in our study. Two raters (D.K. and T.S.L.) coded all datasets in two phases. We first trained using the coding protocol on datasets from three adult cohorts (Rotterdam Study, NEMESIS, LASA) and three pediatric cohorts (Generation R, ChecKid, PIAMA), for which interrater performance was monitored and calculated by a third rater (M.P.C.M.L.). Agreement on the selection of variables measuring a certain construct (sleep characteristic) over these 6 cohorts was excellent (kappa coefficient 0.83). Interrater reliability of the coded values (e.g. recoding categories) per construct was good to excellent (for continuous/ordinal variable: median intraclass correlation coefficient 1.00 [interquartile range=0.92-1.00]; for nominal variables: median kappa coefficient 1.00 [interquartile range 0.52-1.00], median percentage of agreement 88% [interquartile range=75-98%]). Consensus for disagreements on which constructs to code, and how to code them, was achieved by discussion and final decision of a third rater. No changes to the coding protocol were made after training.

Interrater reliability of coding in the next phase was further assessed for 25 random cohorts. Agreement on which constructs to code in this stage was excellent (kappa coefficient= 0.98). Disagreement of how the agreed-upon constructs had to be coded (74 out of 475) were discussed between raters after coding all datasets. Consensus was reached for all disagreements without a third rater decision.

Coding protocol

General instructions

- Always recode into different variables, with consistent variable names, and keep the original variables provided by the cohorts.
- Keep the original question in the variable label.
- Try to code each construct using one, best fitting variable that measures this symptom/construct. When discrepancies in the choice of question happen, we will solve them with consensus.
- Compute a meta-analysis participant ID.
- Compute a meta-analysis study ID

Socio-demographic data

1. Sex:
   - ✓ Variable name sex_studyname
   - ✓ Variable labels 0="male", 1="female"

2. Age: exact age in years
   - ✓ Variable name age_studyname

3. Ethnic origin is based on country of birth indicator or self-report
- o Dutch
- o European origin – other (e.g. other European, USA, Canada, Australia)
- o non-European origin (e.g. Dutch Antilles, Suriname, Turkey, Morocco, Indonesia, Ghana)

*Children's ethnic origin can be based on parent's country of birth:
- ➢ If one parent is Dutch, the child is Dutch.
- ➢ If two parents are of non-Dutch origin, the ethnicity of the mother is used.

- ✓ Variable name ethnicity_studyname
- ✓ Variable labels 0="Dutch", 1="other Western", 2=" nonWestern"

4. Education will be based on the highest educational level finished for persons (>18 years old) or parents (persons <18 years old). The categorization is:
- o High: university degree, higher vocational training
- o Medium: more than 3 years general secondary school, intermediate vocational training or first year of higher vocational training
- o Low: no education, primary school, lower vocational training, intermediate general school or 3 years or less at general secondary school

- ✓ Variable name education_studyname
- ✓ Variable labels 0="high", 1="medium", 2="low"

5. Employment: does the participant have a paid job (yes/no).
- ✓ Variable name employment_studyname
- ✓ Variable labels 0="no", 1="yes"

Sleep variables

1. TST: self- or caregiver-reported hours of sleep.
- o Minutes are coded as decimals.
- o When reported in categories, we will code it as numerical values to the closest quarter-hour.
- o Values ≤2h or ≥20h are considered implausible and are excluded

- ✓ Variable name: TST_studyname

2. Clock bedtime: scheduled/usual time to go to bed.
- o Code it as a numerical value where minutes are coded as decimals.
- o When bedtime is reported in categories, we will code it as numerical values to the closest quarter-hour.
- o Values <17:00h and >12:00h are considered implausible, and are excluded. Add 24 to values after midnight (e.g. 1AM=25h).

- When weekend and weekday data is available, reports for weekdays are used.
- Code a separate variable for weekend times (BedtimeWE_studyname).

✓ Variable name: Bedtime_studyname
✓ Variable name: BedtimeWE_studyname

3. Wake up time: scheduled/usual wake up time

- Code it as a numerical value where minutes are coded as decimals.
- When wake time is reported in categories, we will code it as numerical values to the closest quarter-hour.
- Values before 01:00 and after 17:00 are considered implausible, and are excluded.
- When weekend and weekday data is available, reports for weekdays are used.

a. Code a separate variable for weekend times

✓ Variable name: Waketime_studyname
✓ Variable name: WaketimeWE_studyname

4. TIB: time between usual bedtime and wake up time

- Numerical value in hours.
- When reported in categories, we will code it as numerical values to the closest quarter-hour.
- When weekend and weekday data is available, reports for weekdays are used.
- Code a separate variable for the weekend times.

✓ Variable name: TIB_studyname
✓ Variable name: TIBwe_studyname

5. Daytime napping: sleeping during the day

- Adults:
  - Does the participant usually nap (no/yes)?
  - Regular napping for ≥30min per day.
  - Frequent napping
    ✓ Variable name: Nap_studyname: 0="no", 1="yes"

6. Sleep consolidation: nighttime awakenings (yes/no)

- YES= "Frequently"/"Often"/" ≥3x per week" awakes at night
- YES= ≥2x awakening/night (>2x/night for children below 6 years old)
  ✓ Variable name: AWAKE_studyname
  ✓ Variable label "AWAKE_studyname": 0="no", 1="yes"

Insomnia

A) Presence of insomnia symptoms: These symptoms will be coded as yes/no. Frequency-coded answer categories will fall into yes if the symptom occurs often/frequently/≥3 times per week.

1. Difficulty initiating sleep: difficulty falling asleep
   - ✓ Variable name: INS1_studyname,
   - ✓ Variable label "INS1_studyname": 0="no", 1="yes"
2. Difficulty maintaining sleep: Participant wakes up at night and has trouble going back to sleep (e.g. "Last month problems waking up at night and not going back to sleep", "Regulary waking up and cannot go back to sleep")
   - ✓ Variable name: INS2_studyname
   - ✓ Variable label "INS2_studyname": 0="no", 1="yes"
3. Early morning awakenings
   - ✓ Variable name: INS3_studyname
   - ✓ Variable label "INS3_studyname": 0="no", 1="yes"

Other sleep quality

1. "Poor quality": Question, or answer categories, containing words like: "quality", "satisfaction", "good", "bad", or an opinion on sleep otherwise.
   - ✓ Variable name: SQbad_studyname
   - ✓ Variable label: SQbad_studyname": 0="no", 1="yes"

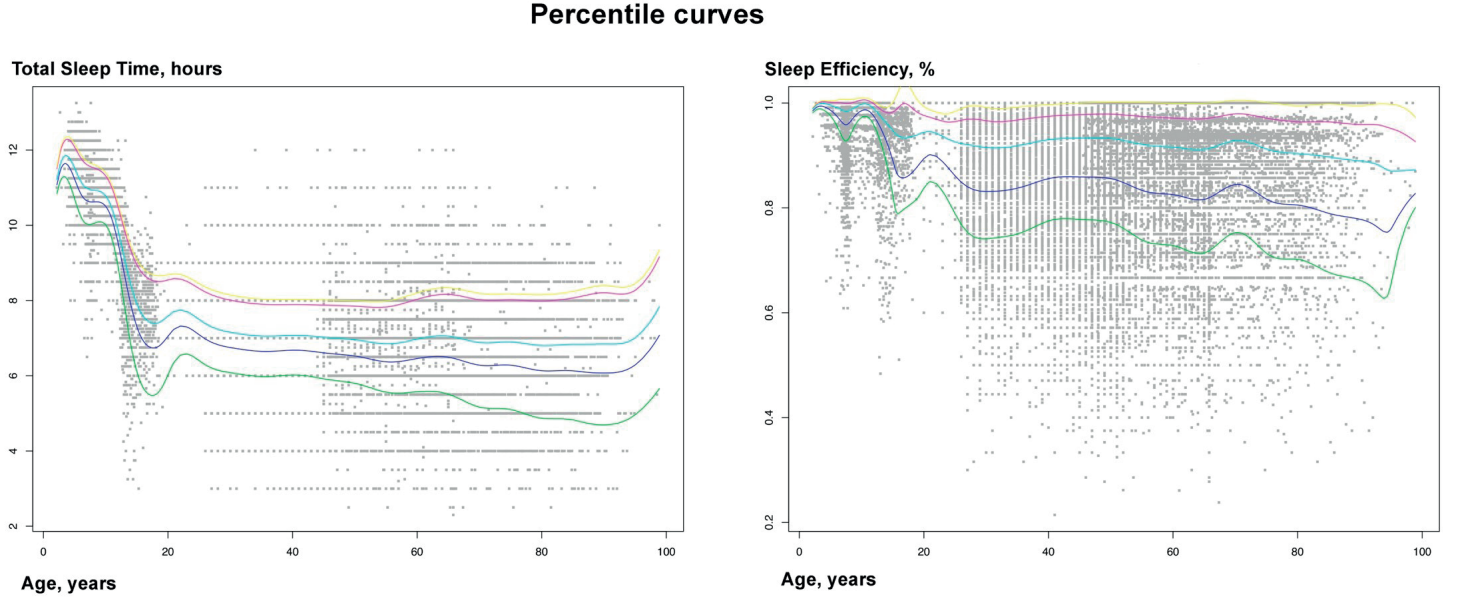
2. Non-Restorative sleep, "yes" indicates "non-restorative" sleep, e.g.:
   - o Get enough sleep to feel rested upon waking in the morning (code reversed)
   - o Feel non-rested after a normal night
   - o Needs long in the morning to wake up
   - o Not rested when waking up
     - ✓ Variable name: rest_studyname
     - ✓ Variable label: rest_studyname": 0="no", 1="yes"

3. Feeling sleepy during the day
   - o How often during the past month did you have difficulty staying awake during car rides, eating or social activities?
   - o Sleepy during watching television
   - o Feel drowsy or sleepy during the day
     - ✓ Variable name: sleepy_studyname
     - ✓ Variable label: sleepy_studyname": 0="no", 1="yes"

4. Habitual snoring: Snore=1 is considered if the participant snores at least once a week. Here we are more lenient on coding 'yes' if the frequency of snoring is described over a different time frame (e.g. sometimes=yes)
    - ✓ Variable name: Snore_studyname
    - ✓ Variable label: Snore_studyname": 0="no", 1="yes"

5. Sleep medication: A user (Med=1) is considered if the participant uses sleep medication at least once a week. Here we are more lenient on coding 'yes' if the frequency of medication use is described over a different time frame (e.g. sometimes=yes)
    - ✓ Variable name: MED_studyname

Variable label: MED_studyname": 0="no", 1="yes



Supplementary Figure 2. Percentile curves of total sleep time and sleep efficiency for continous age between 1 and 100 years

Note: Panels show the relations of self-reported sleep duration (left), and sleep efficiency (right), with continuously modeled age. Data points are provided in gray, and percentile curves per age are plotted in coloured lines (modeled with the *gamlss* R package). Percentile curves depict the 10th (green), 25th (dark blue), 50th (light blue), 75th (pink), and 90th (yellow) percentiles.

Supplementary Table 3. Sleep duration according to recommendations

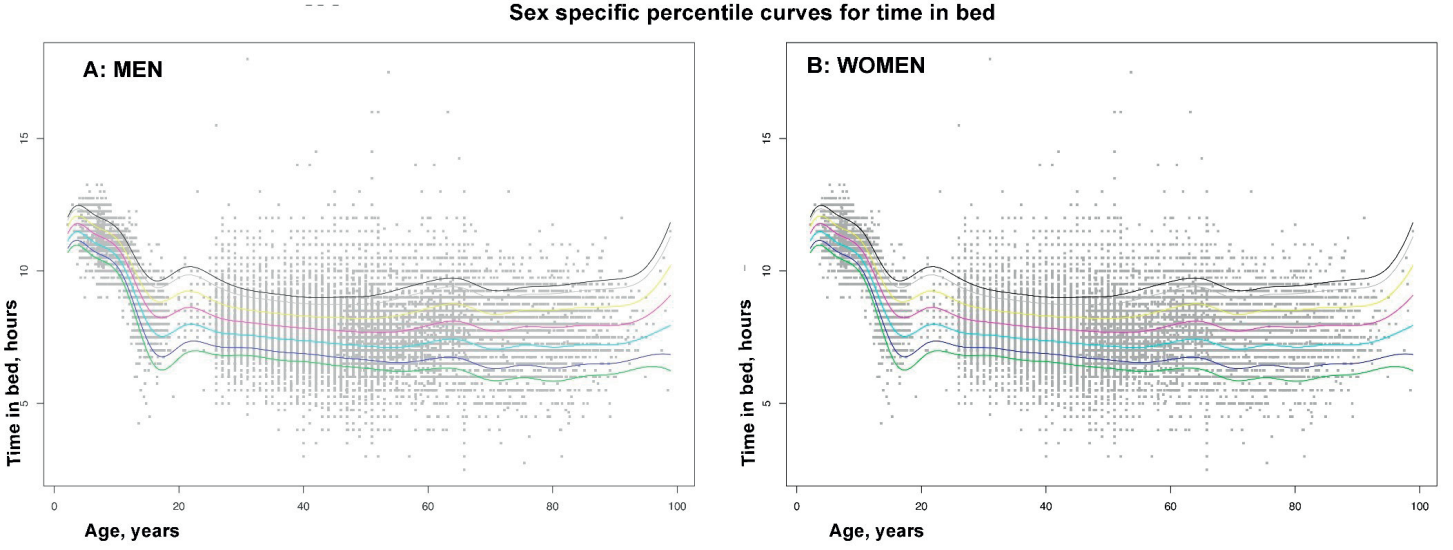
Age group	N	Recommended	Sleeping less, %	Sleeping more, %	Acceptable	Sleeping less, %	Sleeping more, %
1-2 yrs	9	11-14 hrs	-	-	9-16 hrs	-	-
3-5 yrs	1,266	10-13 hrs	1.0	0.1	8-14 hrs	0	0
6-13 yrs	8,377	9-11 hrs	5.4	24.3	7-12 hrs	0.6	1.3
14-17 yrs	513	8-10 hrs	51.5	0.4	7-11 hrs	17.9	0
18-25 yrs	5,192	7-9 hrs	14.3	3.1	6-11 hrs	2.8	0.3
26-40 yrs	38,635	7-9 hrs	20.1	0.7	6-10 hrs	3.7	0.1
41-64 yrs	93,837	7-9 hrs	27.8	0.8	6-10 hrs	7.1	0.1
>65 yrs	8,195	7-8 hrs	35.4	10.6	5-9 hrs	4.7	2.0
Total	156,025	-	24.5	2.6	-	5.6	0.2

Note: Recommended and acceptable durations were derived from the US National Sleep Foundation (Hirshkowitz M, Whiton K, Albert SM, et al. National Sleep Foundation's updated sleep duration recommendations: final report. *Sleep Health*. 2015;1(4):233-243).

Supplementary Table 4. Determinants of sleep duration and sleep efficiency in persons ≥18 years old

	Self-reported sleep duration, hrs			Sleep efficiency, %		
	N=145,858 from 11 studies			N=75,752 from 5 studies		
	B	95% CI	P	B	95% CI	P
Demographic determinants model						
Age, years	-0.01	-0.0008;-0.007	<0.001	0.0003	-0.0001;-0.0004	<0,001
Sex						
Male	reference			reference		
Female	0.14	0.18; 0.21	<0.001	-0.02	-0.03;-0.02	<0.001
Education						
High	reference			reference		
Middle	-0.02	-0.04; -0,01	0.001	-0.001	-0.003;-0.001	0.207
Low	-0.01	-0.02; 0.004	0.191	-0.01	-0.03;-0.003	<0.001
Ethnic origin						
European origin - Dutch	reference			reference		
European origin - other	-0.07	-0.12;-0.03	0.001	-0.01	-0.01;0.001	0.022
non-European origin	-0.30	-0.34;-0.30	<0.001	-0.03	-0.03;-0.02	<0.001
Social determinants model						
Employment						
Yes	reference			reference		
No	0.24	0.23;0.25	<0.001	-0.02	-0.03;-0.02	<0,001
Has a partner						
Yes	reference			reference		
No	-0.06	-0.08;-0.05	<0.001	-0.01	-0.03;-0.02	0.005
Health risk indicators model						
BMI						
Underweight	0.08	-0.02;0.14	0.010	-0.01	-0.02;-0.01	0.002
Normal weight	reference			reference		
Overweight	-0.04	-0.06;-0.03	0.002	-0.001	-0.003;-0.001	0.229
Obese	-0.11	-0.12;-0.09	<0.001	-0.004	-0.01;-0.002	<0.001
Smoking						
nonsmoker	reference			reference		
former smoker	-0.03	-0.04;-0.02	<0.001	-0.003	-0.01;-0.001	<0.001
current smoker	-0.06	-0.07;-0.04	<0.001	-0.001	-0.004;0.0001	0.078

Note: All estimates are adjusted for age, sex, education and ethnic origin. Betas are estimated using a linear mixed model with random effects for study



Supplementary Figure 3. Sex-specific percentile curves for time in bed

Note: Panel A represents percentiles of time in bed per age group in males. Panel B represents percentiles of time in bed per age group in females. The 5th (green), 10th (dark blue), 25th (light blue), 50th (pink), 75th (yellow), 90th (grey), and 95th (black) percentiles are plotted.

Supplementary Table 5. Determinants of insomnia symptoms in persons ≥18 years old

	Difficulty Initiating Sleep			Difficulty Maintaining Sleep			Early Morning Awakenings		
	N=108,447 from 15 studies			N=28,051 from 11 studies			N=15,436 from 8 studies		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
Demographic determinants model									
Age	1.01	1.01;1.01	<0.001	1.02	1.01;1.02	<0.001	1.02	1.01;1.02	<0.001
Sex									
Male	Reference			Reference				Reference	
Female	2.26	2.16;2.36	<0.001	2.05	1.91;2.19	<0.001	1.49	1.37;1.62	<0.001
Education									
High	Reference			Reference				Reference	
Middle	1.38	1.31;1.46	<0.001	1.07	0.99;1.17	0.077	1.10	0.99;1.23	0.069
Low	2.01	1.90;2.13	<0.001	1.32	1.21;1.44	<0.001	1.30	1.37;1.75	<0.001
Origin/ethnicity									
European origin - Dutch	Reference			Reference				Reference	
European origin - other	1.37	1.21;2.56	<0.001	1.17	1.003;1.37	0.045	1.24	0.92;1.66	0.160
Non-European origin	1.75	1.53;2.02	<0.001	1.35	1.12;1.63	0.002	1.09	0.87;1.37	0.436
Social determinants model, additionally adjusted									
Employment									
Yes	Reference			Reference				Reference	
No	1.77	1.68;1.86	<0.001	1.22	1.13;1.33	<0.001	1.01	0.91;1.14	0.801
Partner									
Yes	Reference			Reference				Reference	
No	1.37	1.24;1.51	<0.001	1.11	0.98;1.24	0.078	1.20	1.09;1.32	<0.001
Health risk indicators model									
BMI									
Underweight	1.47	1.14;1.90	0.003	1.53	0.86;2.70	0.144	1.14	0.72;1.81	0.587
Normal weight	Reference			Reference			Reference		
Overweight	0.97	0.92;1.02	0.281	0.97	0.85;1.09	0.598	1.02	0.93;1.13	0.610
Obese	1.09	1.02;1.17	0.008	1.01	0.86;1.17	0.981	1.13	0.99;1.29	0.056
Smoking									
Non-smoker	Reference			Reference			Reference		
Former smoker	1.03	0.97;1.09	0.289	1.02	0.91;1.16	0.671	1.11	1.01;1.23	0.036
Smoker	1.32	1.25;1.41	<0.001	0.79	0.66;0.95	0.012	0.95	0.82;1.11	0.511

Note: Odds ratios are estimated using a linear mixed model adjusted for age, sex, education and ethnic origin, using random effects for study. BMI=body mass index; CI=confidence interval; N=total number of participants; OR=odds ratio.

Supplementary Table 6. Prevalence of sleep complaints

	Nighttime awakenings 20 studies 1998 to 2013				**Sleep medication** 7 studies 1997 to 2013		**Non-Restorative Sleep** 14 studies 1998 to 2013		**Sleepiness** 14 studies 1998 to 2012		**Snoring** 5 studies 2002 to 2011		**Poor sleep quality** 14 studies 1997 to 2011	
	N	% Never	% Sometimes	% Often	N	%	N	%	N	%	N	%	N	%
3-5 years														
Total	3,854	76.1	20.5	3.4	-	-	288	2.8	1,962	2.3	286	25.9	-	-
Male	1,964	75.4	20.5	4.1	-	-	157	3.8	993	3.0	160	21.9	-	-
Female	1,890	76.8	20.5	2.6	-	-	131	1.5	969	1.5	126	31.0	-	-
6-13 years														
Total	7,535	72.3	24.5	3.1	466	7.3	9,864	18.3	13,068	4.4	4,468	19.0	4,790	3.3
Male	3,817	73.6	23.3	3.1	216	7.4	4,961	15.7	6,592	4.3	2,293	19.4	2,411	3.4
Female	3,712	71.1	25.8	3.1	250	7.2	4,893	20.8	6,466	4.5	2,172	18.6	2,374	3.3
14-17 years														
Total	472	27.3	66.3	6.4	671	5.5	3,608	47.9	1,417	17.8	-	-	1,211	6.4
Male	213	35.2	61.5	3.3	256	4.3	1,720	40.0	641	14.5	-	-	519	4.0
Female	259	20.8	70.3	8.9	415	6.3	1,885	54.8	773	20.4	-	-	690	8.1
18-25 years														
Total	209	43.5	43.1	13.4	2,040	1.9	-	-	-	-	-	-	2,005	13.2
Male	77	54.5	39.0	6.5	913	1.2	-	-	-	-	-	-	878	9.3
Female	132	37.1	45.5	17.4	1,127	2.5	-	-	-	-	-	-	1,127	16.2
26-40 years														
Total	25,573	30.1	51.5	18.4	25,160	7.2	2,724	21.1	24,403	7.1	21,814	20.8	25,177	13.4
Male	10,514	34.9	52.1	15.5	10,396	5.2	1,092	19.3	10,031	6.2	8,891	29.3	10,369	10.0
Female	15,059	26.7	51.2	22.1	14,764	8.6	1,631	22.2	14,372	7.8	12,923	14.9	14,807	15.8

Supplementary Table 6. Prevalence of sleep complaints (*continued*)

	Nighttime awakenings 20 studies 1998 to 2013				**Sleep medication** 7 studies 1997 to 2013		**Non-Restorative Sleep** 14 studies 1998 to 2013		**Sleepiness** 14 studies 1998 to 2012		**Snoring** 5 studies 2002 to 2011		**Poor sleep quality** 14 studies 1997 to 2011	
	N	% Never	% Sometimes	% Often	N	%	N	%	N	%	N	%	N	%
41-65 years														
Total	72,049	30.7	47.7	21.7	65,716	10.2	15,494	12.5	61,530	6.0	58,020	33.6	64,128	12.9
Male	30,897	3.0	47.1	15.9	27,638	6.2	7,035	11.2	26,010	6.3	25,221	43.6	27,126	9.1
Female	41,152	26.0	48.0	26.0	38,078	13.0	8,459	13.7	35,520	5.8	32,799	25.9	37,002	15.7
>65 years														
Total	8,557	46.1	32.1	21.8	6,556	12.8	1,437	14.3	5,975	1.2	4,755	39.2	7,467	15.0
Male	3,689	53.3	29.4	17.2	2,789	6.3	731	11.9	2,518	1.4	2,105	48.5	3,197	8.8
Female	4,868	40.7	34.1	25.2	3,767	17.6	706	16.7	3,457	1.0	2,650	31.7	4,270	19.6

Supplementary Table 7. Objective and subjective time in bed, total sleep time and sleep efficiency, stratified by age and sex

Strata by age and sex	Time in bed, hours			Total sleep time, hours			Sleep efficiency, %		
	N	Actigraphy Mean ± SD	Diary Mean ± SD	N	Actigraphy Mean ± SD	Diary Mean ± SD	N	Actigraphy Mean ± SD	Diary Mean ± SD
6-13 years									
Total	900	9.2±0.7	10.2±0.6	900	7.7±0.7	9.6±0.7	900	83.8±4	93.5±5
Male	427	9.2±0.7	10.2±0.6	427	7.6±0.7	9.6±0.7	427	82.7±5	93.6±5
Female	473	9.2±0.7	10.2±0.6	473	7.8±0.7	9.6±0.8	473	84.7±4	93.5±5
14-17 years									
Total	486	8.4±0.8	8.9±0.8	486	7.2±0.9	8.5±0.8	486	85.9±7	95.6±4
Male	221	8.3±0.8	8.8±0.8	221	7.0±0.8	8.5±0.7	221	84.6±6	95.9±4
Female	265	8.4±0.8	8.9±0.8	265	7.3±0.9	8.4±0.8	265	87.1±8	95.2±4
41-64 years									
Total	1,270	-	8.1±0.9	1,270	6.0±0.9	6.8±0.9	1,270	74.4±9	84.6±10
Male	557	-	7.8±0.8	557	5.7±0.9	6.8±0.9	557	73.5±9	86.9±9
Female	713	-	8.3±0.8	713	6.2±0.8	6.8±0.9	713	75.1±8	82.8±10
≥65 years									
Total	668	-	8.4±0.8	668	6.2±0.9	6.9±1.0	668	73.9±8	82.3±11
Male	319	-	8.3±0.8	319	6.1±0.9	7.0±1.0	319	73.3±9	84.4±11
Female	349	-	8.4±0.8	349	6.3±0.9	6.7±1.0	349	74.4±8	80.3±10

Note: Prevalence was not calculated if <200 participants in a cell. Abbreviations: N=sample size; SD=standard deviation.

Supplementary Table 8. Sleep diary reported and actigraphically estimated sleep duration contrasted to recommendations

Strata of age	N	Recommended	Actigraphy		Diary		Acceptable	Actigraphy		Diary	
			Sleeping less, %	Sleeping more, %	Sleeping less, %	Sleeping more, %		Sleeping less, %	Sleeping more, %	Sleeping less, %	Sleeping more, %
6-13 yrs	900	9-11 hrs	98.1	0	18.1	1.6	7-12 hrs	16.3	0	0.4	0.2
14-17 yrs	486	8-10 hrs	87.7	0.6	24.2	2.8	7-11 hrs	38.7	0.6	3.7	0.2
41-64 yrs	93,837	7-9 hrs	89.2	0.1	54.7	1.2	6-10 hrs	47.3	0	14.7	0.2
>65 yrs	8,195	7-8 hrs	81.7	2.1	51.1	9.5	5-9 hrs	9.4	0.1	2.6	96.5

Supplementary Table 9. Actigraphically estimated time in bed, total sleep time and sleep efficiency in the Netherlands and the UK, stratified by age and sex

Strata by age and sex	Time in bed, hours				Total sleep time, hours				Sleep efficiency, %			
	Rott Study N	Rott Study Mean ± SD	UKBB N	UKBB Mean ± SD	Rott Study N	Rott Study Mean ± SD	UKBB N	UKBB Mean ± SD	Rott Study N	Rott Study Mean ± SD	UKBB N	UKBB Mean ± SD
41-64 years												
Total	1,270	8.1±0.9	47,726	9.6±1.0	1,270	6.0±0.9	47,726	7.2±0.9	1,270	74.4±9	47,726	76 ±7
Male	557	7.8±0.8	19,244	9.7±1.0	557	5.7±0.9	19,244	7.1±0.10	557	73.5±9	19,244	74 ±7
Female	713	8.3±0.8	28,482	9.6±0.9	713	6.2±0.8	28,482	7.4±0.9	713	75.1±8	28,482	78 ±7
≥65 years												
Total	668	8.4±0.8	37,773	9.8±0.9	1,270	6.2±0.9	37,773	7.4±0.9	1,270	73.9±8	37,773	76 ±7
Male	319	8.3±0.8	18,167	9.9±0.9	319	6.1±0.9	18,167	7.3±0.9	319	73.3±9	18,167	75 ±7
Female	349	8.4±0.8	19,606	9.7±0.9	349	6.3±0.9	19,606	7.4±0.8	349	74.4±8	19,606	78 ±7

Note: Prevalence was not calculated if <200 participants in a cell. Abbreviations: N=sample size; SD=standard deviation. Rott=Rotterdam Study, UKBB=UK Biobank

Supplementary Table 10. International comparisons of total sleep time, stratified by age and sex

Strata by age and sex	Total sleep time, hours		Total sleep time, hours		Total sleep time, hours	
	15 Studies Netherlands 1993 to 2015		UK Biobank* 2006-2010		USA NHIS 2004-2017	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
18-25 years						
Total	5,192	7.5 ± 1.1	-	-	47,123	7.3 ± 1.4
Male	2,049	7.4 ± 1.1	-	-	22,034	7.3 ± 1.3
Female	3,143	7.6 ± 1.0	-	-	25,089	7.3 ± 1.4
26-40 years						
Total	38,635	7.2 ± 0.9	-	-	108,332	7.0 ± 1.3
Male	16,182	7.1 ± 0.9	-	-	48,561	6.9 ± 1.2
Female	22,453	7.3 ± 1.0	-	-	59,771	7.1 ± 1.3
41-64 years						
Total	93,837	7.0 ± 1.1	405,331	7.1 ± 1.1	164,834	6.9 ± 1.4
Male	40,603	6.9 ± 1.0	178,456	7.0 ± 1.0	75,666	6.9 ± 1.3
Female	53,234	7.1 ± 1.1	226,875	7.1 ± 1.1	89,168	7.0 ± 1.4
≥65 years						
Total	8,195	7.0 ± 1.3	66,428	7.3 ± 1.1	89,328	7.5 ± 1.6
Male	3,504	7.2 ± 1.2	31,593	7.4 ± 1.1	36,109	7.6 ± 1.6
Female	4,691	6.8 ± 1.4	34,835	7.2 ± 1.2	53,219	7.4 ± 1.6

Note: Prevalence was not calculated if <200 participants in a cell. Abbreviations: N=sample size; SD=standard deviation, NHIS=National Health Interview Survey

*only persons that did not report to take naps 'usually' were selected

Supplementary Table 11. International comparisons of the prevalence of insomnia symptoms, stratified by age and sex

Strata by age and sex	Difficulty initiating sleep				Difficulty maintaining sleep			
	Netherlands 22 Studies 1997 to 2015		USA NHIS 2013-2017		Netherlands 15 Studies 1998 to 2015		USA NHIS 2013-2017	
	N	%	N	%	N	%	N	%
18-25 years								
Total	2,227	22.6	17,107	20.6	1,961	9.4	17,100	16.3
Male	969	19.4	8,238	16.6	856	8.6	8,235	11.4
Female	1,252	25.1	8,869	24.2	1,105	10.0	8,865	20.9
26-40 years								
Total	26,264	7.2	39,605	21.1	3,795	11.3	39,595	22.9
Male	10,850	5.5	17,811	17.3	1,550	7.4	17,801	17.9
Female	15,413	8.3	21,794	24.2	2,244	13.9	21,794	27.1
41-64 years								
Total	73,648	9.3	63,299	23.1	19,056	15.7	63,265	32.3
Male	31,637	5.4	29,368	18.4	8,640	10.5	29,353	27.8
Female	41,975	12.3	33,931	27.2	10,416	20.1	33,912	36.3
≥65 years								
Total	8,869	14.9	39,679	18.1	3,255	20.2	39,640	29.9
Male	3,841	8.0	16,392	14.1	1,527	14.5	16,380	28.3
Female	5,028	20.2	23,287	21.0	1,728	25.3	23,260	31.1

Note: Prevalence rates were not calculated if <200 participants in a cell. Abbreviations: ND=not defined if inapplicable for the age group. NHIS=National Health Interview Survey

2.2 | 24-HOUR ACTIVITY RHYTHMS REVIEW

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24-hour activity rhythms and health in older adults. Current Sleep Medicine Reviews 2020

ABSTRACT

Circadian rhythms, including 24-hour activity rhythms, change with age. Disturbances in these 24-hour activity rhythm at older age have also been implied in various diseases. This review evaluates recent findings on 24-hour activity rhythms and disease in older adults.

Growing evidence supports that 24-hour activity rhythm disturbances at older age are related to the presence and/or progression of disease. Longitudinal and genetic work even suggests a potential causal contribution of disturbed 24-hour activity rhythms to disease development. Interventional studies targeting circadian and 24-hour activity rhythms demonstrate that 24-hour rhythmicity can be improved, but the effect of improving 24-hour rhythmicity on disease risk or progression remains to be shown.

Increasing evidence suggests 24-hour activity rhythms are involved in age-related diseases. Further studies are needed to assess causality, underlying mechanisms, and the effects of treating disturbed 24-hour activity rhythms on age-related disease.

INTRODUCTION

The circadian rhythm is integral to physiological processes throughout the body.[1] These approximately 24-hour rhythms are regulated by the master clock located in the brain's suprachiasmatic nucleus (SCN),[2] and are shaped using endogenous and exogenous cues. Together, this ensures that our physiological functioning can be optimized and adapted to changing environmental conditions and social demands.[3] Circadian rhythms can be observed in a range of physiological and behavioral processes throughout the body, for example fluctuations in clock gene expression, hormone levels, body temperature, and cognitive processes.[1] Although many of these fluctuations are valid and precise markers of the circadian rhythm, they are often less feasible to study when the circadian rhythm needs to be assessed over longer periods of time or in large populations. An accessible, affordable and unobtrusive alternative to measure 24-hour rhythmicity is actigraphy, also known as accelerometry. Actigraphy can measure activity continuously over multiple days, weeks or even months. Naturally, activity is under voluntary control and may therefore misrepresent some of the underlying endogenous rhythms. Yet, measuring 24-hour activity rhythms with actigraphy has been demonstrated to be a valid method to estimate circadian rhythmicity, in both patients as well as healthy adults.[1,4] With ever increasing recording quality, better storage capacities, longer recording lengths, and the availability of open source algorithms, actigraphy has become a mainstay for studying circadian rhythms in research and clinical practice.[5,6]

Circadian rhythms, and associated 24-hour activity rhythms, are altered with increasing age.[1,7] Older age is also accompanied by an increase in the prevalence of non-communicable diseases.[8] It has been hypothesized that changes in 24-hour activity rhythms might indicate poor health or even pose a risk factor for poor health, not the least because fragmented 24-hour activity rhythms have been associated with an increased risk of mortality.[9] As modern 24/7 society puts a widespread strain on our rhythms, for example through artificial lighting and shift work, it is crucial to better understand the role of 24-hour activity rhythm disturbance in the development of age-related disease. In this review, we will briefly discuss the measurement of 24-hour activity rhythms, give a short overview of age-related changes in 24-hour activity rhythms, and discuss recent findings around 24-hour activity rhythms and some of the most common diseases in older adults.

MEASURING THE 24-HOUR ACTIVITY RHYTHM

A range of scientific grade actigraphs, typically worn around the wrist, has become available over the past decades. Starting with relatively simple devices measuring activity on one axis, nowadays almost all actigraphs measure movement on three axes and

are equipped with additional sensors measuring temperature, light and/or heart rate. Typically, only the activity data has been used to estimate the 24-hour rhythm. Multiple commercial wearables that measure activity have also become available, their value in assessing 24-hour activity rhythms remains to be determined.[10]

Several methods have been developed to derive 24-hour rhythmicity estimates from actigraphy,[4,11,12] the most used methods are based on adapted cosinor models[4,11] and non-parametric models.[12] From these models a range of 24-hour activity rhythms estimates is calculated, which are correlated to some extent.[13] Both methods come with their own set of advantages and disadvantages. For example, non-parametric measures have been suggested to better reflect 24-hour rhythms in elderly persons, because rhythms are generally less cosine shaped in older adults.[12] In contrast, cosinor measures seem to be associated more consistently with outcomes such as cognitive functioning.[14] A description of the most commonly used cosinor and non-parametric estimates can be found in Table 1.

Table 1. Commonly used cosinor and non-parametric 24-hour activity rhythm estimates.[4,11,12]

Variable	Explanation
Cosinor	
Acrophase	Timing of maximum activity (clock time)
Amplitude	Difference between maximum and minimum value of activity (score)
Mesor	Average activity (counts/min)
Period	Time interval over which cycles repeat (hours)
Pseudo-F	Fit of activity data to the cosine function, indicating rhythm 'robustness' (score)
Non-parametric	
Interdaily Variability	Fragmentation of the rhythm relative to its 24-h amplitude (score)
Intradaily Stability	Stability of activity profiles over days (score)
Relative Amplitude	Normalized difference between most active 10 hours and least active 5 hours (score)
M10 onset	Onset of most active 10-hour period (clock time)
L5 onset	Onset of least active 5-hour period (clock time)

24-HOUR ACTIVITY RHYTHMS AND AGING

Old age is accompanied by multiple changes in the 24-hour activity rhythm, including a well-described phase advance.[13] In recent work a lower amplitude, lower mesor, earlier acrophase, and more fragmented rhythm have been described in older adults.[6,9,15-17] Daytime activity levels are also lower at old age,[18] but nighttime activity levels do not necessarily change in old age.[19] The stability of the 24-hour activity rhythm seems to remain similar across ages,[16] and has even been suggested to be higher in old age.[20] A recent study in 91,105 individuals suggested that age was not associated with relative

amplitude,[21] but this study only included persons aged 73 or younger. This fits previous suggestions that 24-hour activity rhythm disturbances are most pronounced in those aged 80 years or older.[9,16]

Older and more recent studies thus both demonstrate that middle-aged and elderly persons have 'dampened' and less robust 24-hour activity rhythms,[6] similar to alterations seen in endogenous measures of the circadian rhythm at older age.[1] It is largely unknown to what extent these changes may be attributed to the aging process per se. They could also be caused by environmental changes that accompany older age, such as retirement, less physical activity, or the emergence of age-related diseases. Probably, a combination of endogenous and exogenous factors play a role in age-related changes in the 24-hour activity rhythm.[1]

24-HOUR ACTIVITY RHYTHMS AND NEURODEGENERATIVE DISEASE

Over the last 3 years neurodegenerative disease has been the most studied disease in relation to 24-hour activity rhythms. Indeed, circadian disturbances, including disrupted 24-hour activity rhythms, are common in neurodegenerative disease.[5,22,23] These disturbances are potentially attributable to neurodegenerative processes directly affecting circadian regulatory circuits,[2,24] or indirectly through behavioral symptoms impairing daily functioning and inadequate exposure to exogenous cues. Vice versa, disturbed circadian rhythms have also been hypothesized to contribute to neurodegenerative processes.[5] In the next paragraphs, we will focus on recent findings on the role of 24-hour activity rhythm disturbances in Alzheimer's disease and other dementias, and Parkinson's disease.

Alzheimer's disease and Dementia

Dementia, of which Alzheimer's disease is the most common subtype, is characterized by progressive cognitive decline and impairment in activities of daily living.[25] Disruptions of 24-hour activity rhythms in these patients were first recorded over two decades ago,[26] and have been reviewed recently.[5,14,27] These disruptions predominantly include fragmentation and a reduced amplitude of the 24-hour activity rhythm, and behaviors such as 'sun-downing'[27] and frequent daytime napping.[28] Recent cross-sectional studies report a lower amplitude,[28-30] a lower stability,[29-31] and more fragmentation[29] in patients with dementia. More fragmented 24-hour activity rhythms were also found in persons with early-onset dementia.[32] Together these disturbances substantially impair quality of life of patients and caregivers[33,34] and are thought to be an important determinant for the institutionalization of patients.[27]

Research increasingly focuses on the pre-diagnostic phase of dementia to investigate the potential etiological or predictive role that 24-hour activity rhythm disturbances may have in dementia. Two recent studies investigated persons with potential prodromal symptoms of dementia, but no evidence was found for an association of phase with subjective cognitive complaints[35] or of amplitude with mild cognitive impairment.[30] This was even though the latter was found to be disturbed in those with Alzheimer's disease.[30] In contrast, some earlier studies have reported a phase advance in persons with mild cognitive impairment compared to healthy controls[36,37] and a higher fragmentation and lower mesor in those with preclinical AD.[16] Data from prospective cohorts provide some further insight into the temporality of the association of 24-hour activity rhythms with dementia. An advanced acrophase was associated with an increased risk of cognitive decline in elderly men,[15] whereas in elderly women a phase delay and lower robustness of the rhythm were associated with an increased risk of dementia and mild cognitive impairment.[38] A higher fragmentation was also related with a decline in cognition measured over the prior 12 years.[39]

Associations of 24-hour activity rhythms with biomarkers of neurodegeneration in non-demented individuals have been investigated to shed further light on the link between 24-hour activity rhythms and dementia. First, fragmentation was most strongly related to a cerebrospinal fluid biomarker profile indicative of Alzheimer's disease, when compared to other disturbances.[16] Additionally, fragmentation was related to temporal lobe atrophy in cognitively unimpaired persons[29] and to loss of gray matter in parietal regions specific to early accumulation of Alzheimer's pathology.[39] Further research remains needed to determine whether 24-hour activity rhythm estimates could also serve as a valid biomarker for dementia.

Parkinson's disease

In Parkinson's disease, which has a notable association with REM sleep behavior disorder,[5] 24-hour activity rhythms disturbances have been hypothesized to occur early in the disease process and to potentially contribute to various symptoms and pathological processes specific to Parkinson's disease.[40] Patients have a higher fragmentation, lower stability, lower amplitude, and lower mesor than healthy controls.[40-42] A low stability is also associated with poorer cognitive performance in Parkinson's disease.[42] It is unclear to what extent the 24-hour activity rhythm estimates are affected by impaired motor functioning associated with the Parkinson's disease diagnosis or dopaminergic treatments for Parkinson's disease.[5]

The longitudinal relation of 24-hour activity rhythms with incident Parkinson's disease has received limited attention so far, but a recent study with 11 years of follow-up showed that daytime actigraphy-estimated inactivity, indicative of 'napping', was associated with increased risk of Parkinson's disease.[43] Longitudinal studies assessing 24-hour

activity rhythms in relation to Parkinson's disease in particular, and neurodegenerative disease more broadly, are therefore highly needed.

24-HOUR ACTIVITY RHYTHMS AND LATE-LIFE PSYCHIATRIC DISEASE

Disturbances in 24-hour activity rhythms are related to a range of psychiatric disorders such as depression, anxiety, psychosis and schizophrenia,[44,45] of which some are also common in old age. Depression is of specific interest in the context of this review as a second peak in the prevalence of depression starts around the age of 60 years. Depression, characterized by a depressed mood or a loss of pleasure as a key symptom, and additionally symptoms such as weight change, changes in sleep, psychomotor agitation/retardation, fatigue, worthlessness, cognitive complaints or suicidality,[46] has a major impact on global health.[47]

Depression

Patients diagnosed with Major Depressive Disorder have a tendency to eveningness, delayed 24-hour activity rhythms, a dampened amplitude and a lower mesor.[17,48-51] Disturbed 24-hour activity rhythms are also related with the severity of depressive symptoms, even when symptoms are of a subclinical level, which is common in elderly persons. A cross-sectional population-based study of middle-aged and elderly persons found an association of a lower stability and higher fragmentation of the 24-hour activity rhythm with more depressive symptoms.[20,52] A preference for eveningness and a phase delay were also associated with severity of depressive symptoms.[53,54] Potentially, the association of 24-hour activity rhythms and depressive symptoms differs between men and women; in one study associations of disturbed 24-hour activity rhythms with depressive symptoms were found in men but not in women.[55]

Increasing evidence supports that disturbances in the 24-hour activity rhythm are not only apparent during the depressive episode, but might also precede depressive episodes or may persist afterwards.[49] A recent UK biobank study suggested a lower relative amplitude in those with a retrospectively determined lifetime incidence of major depressive disorder, bipolar disorder and mood instability.[21] A longitudinal study in elderly men reported that both a late acrophase alone and the combination of an early acrophase with a dampened 24-hour activity rhythm amplitude were associated with a faster increase in depressive symptoms over time.[56] Additionally, a Genome-Wide Association Study (GWAS) including 71,500 participants reported a possible association between genetic risk of a low relative amplitude and mood disorders.[45]

It remains unclear to what extent associations between disturbances of 24-hour activity rhythms and mental health are due to medication use.[44,57] There is evidence that these

associations are independent of medication use,[56] but we also know that some 24-hour activity rhythm disturbances are related to medication use. For example, eveningness and phase delay potentially hamper the efficacy of antidepressants,[58,59] and ketamine might improve 24-hour activity rhythms, independent of its effect on mood.[60]

Other psychiatric disorders in late life

Bipolar disorders, in which the depressive episodes are accompanied by manic episodes, have also been related to disturbances in the 24-hour activity rhythm. Associations seem to be state-dependent, with depressive episodes being accompanied by a phase delay and manic episodes being accompanied by a phase advance.[61,62] However, the disturbances in the 24-hour activity rhythms might not only be a state marker as the phase advance of 24-hour activity rhythms and lower mesor may persist after successful treatment of bipolar disorder.[44]

For anxiety, relatively common at older age, it was shown that more fragmented 24-hour activity rhythms were associated with higher odds of having an anxiety disorder,[52] and that lower activity levels and a lower mesor were associated with current anxiety.[48] Additionally, disrupted 24-hour activity rhythms have also been linked to more suicidal behaviors,[63,64] but the causality of this association remains to be determined. Although actigraphy has been used in patients witch schizophrenia, 24-hour rhythmicity has not often been assessed.[65] Only one study assessed 24-hour activity rhythms and did not find an association of 24-hour rhythm estimates with positive or negative symptoms of schizophrenia.[66]

24-HOUR ACTIVITY RHYTHMS AND OTHER AGE-RELATED DISEASES

Aging is accompanied by an increase in other non-communicable diseases such as type 2 diabetes, cardiovascular disease, lung disease, and cancer,[8] which have also been suggested to involve circadian and 24-hour activity rhythm disturbances.[1,50]

Cardio-metabolic disease

Cross-sectional studies reported prolonged napping,[67] a lower amplitude,[68] less stable,[20,68,69] and more fragmented[20] 24-hour activity rhythms in middle-aged and elderly persons with a higher Body Mass Index, a well-known risk factor for cardio-metabolic disease. Additionally, less stable 24-hour activity rhythms were associated with increased odds of metabolic syndrome and hypertension in elderly women.[69] Longitudinal work suggests that 24-hour activity rhythm disturbances might already be apparent early on. A longitudinal population-based study reported that a lower robustness of the rhythm and a lower amplitude were associated with an increased risk of overall cardiovascular

disease, and that a lower mesor was associated with an increased risk of coronary heart disease.[70] It has also been repeatedly shown that shift work is a risk factor for cardiometabolic disease, such as type 2 diabetes, obesity and coronary artery disease,[50,71,72] the 24-hour activity rhythm has however not been assessed in these studies. Most of these findings have been based on observational studies, making it difficult to determine the underlying mechanisms. Experimental studies in humans do however suggest that short-term circadian misalignment already affects biomarkers for metabolic disease, such as systolic blood pressure and preclinical states of diabetes.[73,74]

Cancer

Disturbed 24-hour activity rhythms are also seen in those suffering from cancer. A study in palliative cancer care reported that 24-hour activity rhythms are more disrupted towards the end of life.[75] Several recent studies also reported an association between 24-hour activity rhythm disturbance and cancer-related mortality, most prominently in lung cancer patients, where early mortality risks were up to 4 times higher in patients with disrupted 24-hour activity rhythms compared to those with robust rhythms.[76,77] More disturbed 24-hour activity rhythms were also associated with a shorter survival time in patients suffering from head and neck cancers[78] and patients receiving palliative cancer care.[75] Cancer treatment also seems to affect the 24-hour activity rhythm, a recent longitudinal study showed that several 24-hour activity rhythm estimates, including amplitude, worsen with each cycle of chemotherapy in women with breast cancer.[79]

RESEARCH AGENDA

Collectively, these studies suggest that with older age, 24-hour activity rhythms are dampened, more fragmented, and more advanced. Presence of 24-hour activity rhythm disturbances are associated with various age-related diseases. Most evidence is available for dementia and depression but these have also been the most studied diseases in relation to 24-hour activity rhythms. Associations between the 24-hour activity rhythm and disease also differ per 24-hour activity rhythm estimate, which creates a complex picture. Only a minority of studies has investigated the association of 24-hour activity rhythms and health longitudinally, which limits our knowledge on the temporality of associations between 24-hour rhythm disturbances and age-related disease. Although there is some evidence for a possible causal role of 24-hour activity rhythms in disease at old age, more definitive evidence needs to be generated with sophisticated analyses methods in prospective cohort studies and intervention studies.

Studies specifically intervening on circadian rhythms that take into account the 24-hour activity rhythm remain scarce. A number of studies have reported reduced

circadian disruption after bright-light therapy in patients with dementia,[80] Parkinson's Disease,[81] depression,[82] cardiovascular disease,[83] and cancer.[84] However, it is largely unknown to what extent intervening on circadian factors and, subsequently 24-hour activity rhythms, improves relevant clinical outcomes such as disease progression or mortality. So far, we do know that interventions focusing on advancing circadian timing, such as early morning bright light therapy have a positive effect on mood. Bright light therapy decreases depression severity in depressed patients, and 8 weeks of dawn-dusk stimulation improved mood and reduced anxiety in elderly persons living in a care home.[85] It remains to be determined if improvement in 24-hour activity rhythms is a mediating factor.

Together, we feel that three items are essential to add to the research agenda to improve our understanding of the role of 24-hour activity rhythms in health at older age. First, implementation of actigraphy in prospective cohort studies has not only been proven feasible, it is also needed to investigate temporality. It particularly creates a unique opportunity if the 24-hour activity rhythm disturbances can be studied before the diagnosis of the disease in population-based cohorts. These studies should ideally include repeated measurements of both disease-related constructs and actigraphy to gain more insight in potentially bi-directional associations. Second, studies have reported successful improvement of circadian rhythms and mental health after interventions focused on the 24-hour rhythm, but effects on somatic conditions are largely unclear. Well-controlled intervention trials that integrate actigraphy and have longer follow-up periods will be needed to assess whether treatment of disturbed 24-hour activity rhythms can reduce disease burden or even alter disease progression or incidence. This could also provide information for any potential preventive effects of targeting 24-hour activity rhythm disturbances. Lastly, as new studies become available with high speed, well-executed meta-analyses will be needed to direct the field. For this aim, a standardized approach using the same estimates and methods between studies will be highly beneficial.

CONCLUSION

The 24-hour activity rhythm is disturbed in a broad range of age-related diseases. In neurodegenerative disease, psychiatric disease, cardio-metabolic disease and cancer, patients have more phase shifts, lower amplitudes, more fragmented and less stable 24-hour activity rhythms. An increasing number of longitudinal studies suggest that these disturbed 24-hour activity rhythms may also precede disease, but causality remains to be determined. The need for longitudinal observational studies remains substantial, as well as the need for investigating promising interventions for those diseases where

circadian disruption could be involved in disease etiology, symptom maintenance or impaired quality of life.

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Je moet niet wakker liggen, om wat je niet weet.

Based on: Acda en de Munnik. Laat Me Slapen. Naar Huis (1998).

3 RISK OF CLINICAL OUTCOMES

3.1 DEMENTIA – SUBJECTIVE SLEEP QUALITY

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Subjective sleep quality is not associated with incident dementia: The Rotterdam Study. Journal of Alzheimer's disease 2018

ABSTRACT

Poor sleep is related to higher dementia risk, but this association is more equivocal for subjective sleep quality specifically. This study investigates the link between subjective sleep quality and dementia risk in the general population. We studied the role of subjective sleep quality in the risk of dementia in the general population.

In the prospective population-based Rotterdam Study, 4,835 persons (mean age 72 years, 58% women) underwent a home interview (2002-2006) that included the validated Pittsburgh Sleep Quality Index (PSQI) to assess sleep quality. Participants were followed until 2015 for incident dementia, through in-person screening and continuous monitoring of medical records. We used Cox regression models to associate sleep quality with dementia risk, adjusting for age, sex, education, smoking, employment, coffee consumption, alcohol consumption, activities of daily living, cardiovascular risk factors, anxiety, depressive symptoms, cognition and snoring.

During 41,385 person-years (8.5 years mean), 420 participants developed dementia, of whom 320 Alzheimer's disease (AD). Poorer subjective sleep quality was not associated with the risk of all-cause dementia (hazard ratio [HR] per SD increase in PSQI score: 0.91, 95% CI 0.82-1.02) or AD (HR 0.92, 95% CI 0.81-1.05). Similarly, individual components of the PSQI were also not associated with dementia. Several sensitivity analyses, i.e. excluding last years of the follow-up time duration or restricting to those with best MMSE scores at baseline, did not reveal subgroups with increased risks.

In this study, we found no association of poor subjective sleep quality with higher risk of dementia.

INTRODUCTION

Sleep problems are highly frequent in the elderly and sleep deprivation is known to acutely affect cognitive performance. Emerging evidence suggests that chronic sleep problems might increase the risk of cognitive decline,[1] and possibly dementia.[2-4] These associations are supported biologically as undisturbed sleep has been implicated in the clearance of amyloid-β, a pathological hallmark of Alzheimer's disease (AD).[5,6] Other important mechanisms through which sleep may affect dementia risk is through regulating synaptic homeostasis,[7] affecting levels of neuro-inflammation in hippocampal areas,[8] or hypoxia-related increased activity in inflammatory or oxidative pathways occurring in sleep-disordered breathing.[1]

Two recent meta-analyses substantiated this link of poor sleep and higher AD[2] or dementia[4] risk in the general population, for both quantitative and qualitative aspects of sleep. However, authors suggest distinct roles for different sleep aspects in risk for different dementia types,[4] or even caution that short follow-up duration of studies, and use of heterogeneous, and thus incomparable, measures of sleep aspects hamper interpreting results.[2] Specifically for the general construct of subjective sleep quality, included studies used different measures, such as sleep disturbances or daytime problems, which only partly represent the construct. Also, sleep quality was measured with objective measures such as actigraphy, which, while important for accurate measurement of basic sleep parameters or insight in biological processes, might not fully capture the qualitative experience of sleep, which inherently involves a subjective component.[9-11]

Studies using validated instruments that take the qualitative experience of sleep into account, such as the Pittsburgh Sleep Quality Index (PSQI), are more equivocal about the relation of subjective sleep quality with dementia. They have shown a link with imaging[12,13] or CSF[14,15] markers of neurodegeneration, but results with cognitive decline have been inconclusive,[1,16-19] and risk of dementia was hardly investigated. The few studies that did, interestingly, failed to find an association.[20-22]

It is important to study the relation of subjective sleep quality to dementia risk, as subjective sleep quality seems to be an independent aspect of sleep[11] that has been ill-characterized in longitudinal studies in dementia risk so far. Also, in the context of identifying potentially modifiable risk factors for dementia, subjective measures are inexpensive and easily administered, and subjective sleep quality can be modified (e.g. through cognitive behavioral therapy, the recommended treatment approach for insomnia[23]). Studies investigating the relation of subjective sleep quality and dementia risk, with sufficient follow-up time to account for reverse causation, are needed. In this population-based cohort study we aim to investigate the association of subjective sleep quality, measured by the PSQI, and dementia risk over a 13-year follow-up period.

METHODS

Study setting

The Rotterdam Study (RS) is an ongoing prospective population-based cohort study, starting in 1990, of inhabitants of the Ommoord district in Rotterdam aged 55 years or over, details of which have been described previously.[24] The RS has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the Population Studies Act: Rotterdam Study. All participants provided written consent to participate in the study and share information from their treating physicians.

In brief, inhabitants willing to participate underwent examination rounds, consisting of a home interview and two subsequent center visits, which were repeated every 4-5 years. In between, incident disease is assessed through continuous linkage of the study database and medical records of general practitioners (GPs) which, in the Netherlands, also holds summaries of medical records from all specialist and inpatient care. Also, regular checks of nursing home medical records were performed. In 2000, the cohort was extended with new invitees from the same district and inclusion age. The current study includes all persons participating in the fourth wave of the original cohort (RS-I-4; 2002-2004) and the second wave of the second cohort (RS-II-2; 2003-2006) when the PSQI was introduced. They were followed up from this baseline measurement until study ending at January 1st of 2014 (RS-I-4) or 2015 (RS-II-2).

Study population

Of 6052 individuals who were scheduled for a home interview, 145 did not complete the PSQI due to withdrawal of consent, calling in sick or logistic reasons. Another 140 participants were excluded from the current analyses because they missed more than one of the seven component scores of the PSQI. We excluded an additional 119 participants with prevalent dementia at baseline, and 74 persons for not having any follow-up available for dementia, which left 5574 participants. Lastly, we included only participants with a Mini Mental State Examination (MMSE) score >25 at baseline to a final sample of 4835 participants (82% of eligible).

Sleep quality

Sleep quality was measured with a Dutch version of the PSQI, which was filled out with the help of a research nurse. The PSQI assesses sleep quality and behavior in the past month using questions about bedtimes and multiple sleep problems.[25] It was designed to distinguish 'poor' sleepers from 'good' sleepers in a clinical setting, and also has good test-retest reliability and validity when tested in a non-clinical sample of older adults.[26] Answer scores are combined in seven component scores (range: 0 – 3): quality, latency,

duration, efficiency, disturbances, medication and daytime dysfunction. These are summed to provide a global sleep quality score (range: 0 – 21). Higher scores indicate poorer sleep, with scores >5.0 indicating 'poor' versus 'good' sleepers. We calculated weighted component scores for participants that had one component score missing (330 out of 4835 participants [7%]) by multiplying six-component sum scores by 7/6. Mainly the components latency (n=148) and efficiency (n=138) were missing.

Dementia screening and surveillance

Participants were screened for dementia at baseline and subsequent center visits with the Mini-Mental State Examination[27] and the Geriatric Mental Schedule organic level.[28] Those with a Mini-Mental State Examination score <26 or Geriatric Mental Schedule score >0 underwent further investigation and informant interview, including the Cambridge Examination for Mental Disorders of the Elderly[29]. All participants also underwent routine cognitive assessment. In addition, the entire cohort was continuously under surveillance for dementia through electronic linkage of the study database with medical records from general practitioners and the regional institute for outpatient mental health care. Available information on cognitive testing and clinical neuroimaging was used when required for diagnosis of dementia subtype. A consensus panel led by a consultant neurologist established the final diagnosis according to standard criteria for dementia (DSM-III-R), Alzheimer's disease (NINCDS–ADRDA) and vascular dementia (NINDS-AIREN).

Follow-up until end of the study was nearly complete (96.7% of potential person-time). Participants were censored starting at date of dementia diagnosis, death, loss to follow-up, or study ending, whichever occurred first.

Covariates

Analyses were adjusted for potential confounders measured at baseline; selection was based on relevant publications.[2,20-22,30-39] Smoking habits were assessed by interview and categorized as never, former or current smoking. Educational attainment was assessed by interview and categorized as primary, secondary/lower vocational, intermediate vocational and higher vocational/university. Having current paid employment was self-reported. Activities of Daily Living (ADL) were assessed by a Dutch version of the Stanford Health Assessment Questionnaire and measured in a 'disability index'.[40] Coffee consumption was categorized in 0-1, 2-3 or >3 cups/day. Habitual alcohol consumption was self-reported with a validated Dutch version of the Food Frequency Questionnaire,[41] harmonized over use of different types of preparations and expressed in gr/day intake. Body mass index (BMI) was calculated from measured weight and height (kg/m^2). Hypertension was defined as elevated systolic (≥160) or diastolic (≥100 mm Hg) blood pressure (averaged from two right-arm measurements, sitting up, using a random-zero sphygmanometer), or self-reported use of antihypertensive medication.

Diabetes mellitus was defined as a fasting serum glucose level ≥7.0 mmol/L and/or self-reported use of anti-diabetic medication. Self-reported history of coronary heart disease (CHD) and cerebrovascular disease were confirmed via medical records. Total and high-density lipoprotein-cholesterol and glucose levels in serum were processed through an automated enzymatic procedure (Boehringer Mannheim System). Depressive symptoms were assessed with the validated Dutch version of the Centre for Epidemiological Studies Depression Scale.[42] Cognitive status was assessed with the MMSE. Presence of one or more 12-month prevalent DSM-IV anxiety disorders was assessed by an adapted version of the Munich Composite International Diagnostic Interview. Loud snoring was reported by participants and/or bedpartners in categories of frequency per week. *APOE*-genotype was determined by either polymerase chain reaction on coded DNA samples in RS-I-4 or bi-allelic Taqman assays (rs7412 and rs429358) in RS-II-2, and classified by number of ε4-alleles.

Statistical analysis

We first explored the association of individual covariates with global PSQI score at baseline using age- and sex-adjusted linear regression. For our main analysis, we used Cox proportional hazard models to determine the association of global PSQI scores with incident dementia, with follow-up time as timescale. We constructed three incremental models. Model 1 was adjusted for age at baseline, sex, education, smoking, employment, coffee consumption, alcohol consumption and activities of daily living. Model 2 was additionally adjusted for cardiovascular risk factors. Model 3 further incorporated MMSE-score, CES-D score, prevalent anxiety disorders, and snoring.

We performed several additional analyses. First, we studied the seven components of the PSQI separately as well as dichotomized global PSQI score in 'poor' vs. 'good' sleep quality. Second, we studied potential effect-modification by age, sex and clinically relevant depressive symptoms by stratification and formally testing for multiplicative interaction in the fully adjusted model.[42] Third, to examine how the relation of sleep quality and dementia was modified by baseline cognitive status, beyond including only participants with an MMSE>25, we incrementally restricted our sample to participants with highest MMSE-scores, per point, as high as MMSE>28.Fourth, to aid comparison with other studies that use shorter follow-up times, we performed analyses after restricting follow-up duration by two incremental year intervals, i.e. end-date at 2, 4, 6, 8, and 10 years after baseline. Fifth, we studied AD separately. Finally, we additionally adjusted the main analysis for number of *APOE*-ε4 alleles.

Missing data on covariates (≤13%) before including only participants with an MMSE >25 were imputed using 5 multiple imputation based on all variables used in our analyses. We plotted Schoenfeld residuals of all variables against time, using the free and open 'R' software[43] (package: 'survival'): no violations of proportionality were identified.

Statistical testing was performed two-sided at $p<0.05$. Data were analysed using SPSS Statistics, version 21 (IBM Corp., Armonk, NY).

RESULTS

Baseline characteristics are summarized in Table 1. The median PSQI score was 3 and 30% of participants had poor sleep quality. At baseline, higher depressive symptoms, presence of anxiety disorders, female sex, higher age, less coffee consumption, worse scores on ADL, presence of hypertension or CHD, and less snoring were associated with

Table 1. Baseline characteristics of the study population.

Characteristic (unit)	N=4,835
Global PSQI score	3 (2-6)
Age in years	71.9 ± 7.4
Women	2791 (58%)
Medium or higher education	2264 (47%)
Never smoker	1483 (31%)
Currently employed	325 (7%)
Coffee consumption >3 cups/day	2489 (52%)
Alcohol consumption (gr/day)	7 (1-20)
Disability index	0.4 (0.1-0.7)
Body mass index (kg/m^2)	27.6 ± 4.0
Hypertension	1732 (36%)
Total cholesterol (mmol/l)	5.6 ± 1.0
HDL-cholesterol (mmol/l)	1.5 ± 0.4
Diabetes mellitus	716 (15%)
History of CHD	291 (6%)
History of TIA or stroke	284 (7%)
CES-D	4 (2-8)
MMSE-score	28 (27-29)
Anxiety disorder	357 (8%)
Snoring ≥ 1/week	2009 (31.6%)
≥1 *APOE-ε4* allele(s)	1211 (27%)
Missing	288 (6%)
Incident all-cause dementia	420 (9%)
Of which Alzheimer's disease	320 (76%)

Values include imputed missing values and indicate frequency (%), or median (interquartile range) for categorical variables, and mean ± standard deviation for continuous variables, unless specified otherwise. Abbreviations: PSQI=Pittsburgh Sleep Quality Index; IQR= interquartile range; HDL= high-density lipoprotein; CES-D=Center for Epidemiologic Studies – Depression Scale; MMSE=Mini Mental State Examination; CHD=Coronary heart disease.

worse sleep quality (Supplementary Table 1). During 13 years of follow-up (mean 8.5 years), we observed 420 incident dementia cases, of which 320 had AD (76%).

We found no association of subjective sleep quality with the risk of dementia (hazard ratio [HR] per SD increase 0.91, 95% CI 0.81-1.02, Table 2). Additional adjustment did not substantially alter effect sizes. We found no association between worse scores on the separate PSQI-components and dementia risk with results consistent across components (Table 3). After dichotomizing the global PSQI score, we found that poor sleep quality was not associated with a higher risk of dementia than good sleep quality (HR 0.93, 95% CI 0.74-1.16).

Table 2. Association of subjective sleep quality and dementia risk

Global PSQI score (per SD increase)	Hazard ratio (95% CI)
Model 1	0.96 (0.86 - 1.06)
Model 2	0.95 (0.86 - 1.06)
Model 3	0.91 (0.81 - 1.02)

Estimates obtained from models with cases/N: 420/4835. Model 1: adjusted for age, sex, education, smoking, employment, coffee consumption, alcohol consumption, activities of daily living. Model 2: Model 1 + cardiovascular risk factors. Model 3: Model 2 + MMSE-score, depressive symptoms, anxiety and snoring. Abbreviations: CI=Confidence Interval; PSQI=Pittsburgh Sleep Quality Index; SD=Standard Deviation.

Table 3. Association of PSQI component scores and dementia risk

PSQI-components	Cases/N	HR (95% CI)
Quality	420/4806	0.88 (0.76-1.02)
Latency	407/4660	1.00 (0.89-1.10)
Duration	420/4808	0.96 (0.88-1.06)
Efficiency	411/4669	0.93 (0.83-1.03)
Disturbances	417/4780	0.85 (0.70-1.03)
Medication	420/4808	0.93 (0.83-1.04)
Daytime dysfunction	418/4795	0.96 (0.80-1.14)

Hazard ratios adjusted for age, sex, education, smoking, employment, coffee consumption, alcohol consumption, activities of daily living, cardiovascular risk factors, MMSE-score, depressive symptoms, anxiety and snoring, calculated per point increase for every component score. Abbreviations: CI=Confidence Interval; HR=hazard ratio; PSQI=Pittsburgh Sleep Quality Index.

Incrementally restricting analyses to participants with higher MMSE-scores at baseline resulted in hazard ratio estimates that were even closer to the null-value (Figure 1). We observed no significant interaction of PSQI with age, sex and CES-D, although a difference between men and women was suggested in the stratified analysis (Supplementary Table 2). Results were similar when restricting the follow-up time to 2, 4, 6, 8 or 10 years (Figure 2). Finally, additionally adjusting for *APOE*-ε4 allele status (HR 0.91, 95% CI 0.79-1.30) or studying AD separately (0.92, 95% CI 0.81-1.05) did not change results from the main analysis.

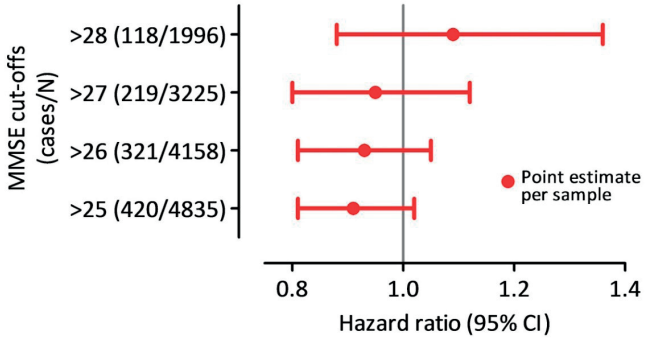

Figure 1. Association of subjective sleep quality and dementia risk, analyzed by incrementally restricting the sample to higher cognitive status.
Number of dementia cases per analyzed sample size is shown on the Y-axis. Hazard ratios adjusted for age, sex, education, smoking, employment, coffee consumption, alcohol consumption, activities of daily living, cardiovascular risk factors, MMSE-score, depressive symptoms, anxiety and snoring, calculated for samples incrementally restricted to higher MMSE scores. Abbreviations: CI=Confidence Interval; MMSE=Mini Mental State Examination.

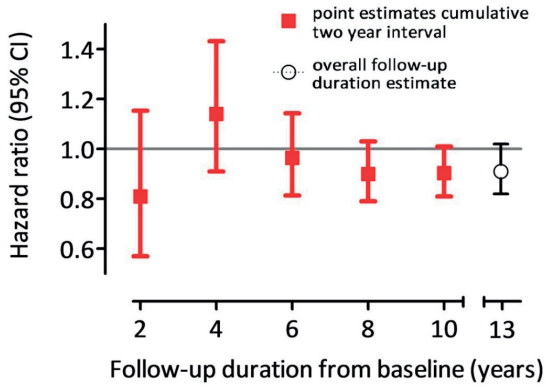

Figure 2. Association of subjective sleep quality and dementia risk, analyzed for cumulative 2-year follow-up intervals from baseline.
Hazard ratios adjusted for age, sex, education, smoking, employment, coffee consumption, alcohol consumption, activities of daily living, cardiovascular risk factors, MMSE-score, depressive symptoms, anxiety and snoring, calculated per standard deviation increase of global PSQI score. Estimates for each interval were obtained by censoring of all participants still at risk at year 2, 4, 6, 8 and 10 after baseline. Abbreviations: CI=Confidence Interval.

DISCUSSION

In this population-based cohort study with up to 13 years of follow-up, we did not find any association of subjective sleep quality, measured by PSQI, and the risk of all-cause dementia. All separate PSQI components were also not associated to incident dementia.

Some methodological considerations deserve mention. We lacked non-selectively repeated measures of the PSQI to assess the association of time-varying subjective sleep quality exposure on dementia risk, which could have accounted for intra-individual variability of subjective sleep quality over time. Yet, PSQI scores have been shown to remain relatively stable in elderly persons over 3 years.[44] Also, we could not assess the effect of time-varying covariates on our outcome. Next, baseline sleep quality in our study was relatively good compared to other population-based studies,[17,18,26] which might have precluded us finding an effect as the contrast between participants was small. Also, we could not assess to what extent associations of common sleep disorder (i.e. insomnia and sleep apnea), or excessive daytime sleepiness with our exposure and outcome might have influenced our results, although this cannot easily explain the lack of an association. Lastly, ethnic and socioeconomic differences in sleep (behavior) limit generalizability of findings to persons of European descent with middle or high income.

We did not find an association of the subjective experience of sleep with dementia risk. Such null associations have been reported before in similarly designed studies that, besides their main analysis, also specifically included sleep quality measures. A Finnish study with a median follow-up duration of 22.5 years found no association between a single sleep quality question ("Do you usually sleep well?") and AD.[20] A large registry-based study in Swedish twins[21] used a validated sleep quality index of four questions and reported a similar risk estimate (HR 0.93, 95% CI 0.85-1.01) to our study over a 17 year study period, just like the French Three City Study which studied the association of self-reported 'poor' sleep quality (rated 'poor', 'average' or 'good'; compared to answering 'good') with risk of dementia (OR 0.85, 95% CI 0.65-1.13) across 8 years.[22] Noteworthy in our study is that individual PSQI-components were also not associated to dementia risk, which range from very subjective experience (e.g. the 'quality' component) to components based on estimation of time. The components measure aspects of sleep quality that considerably overlap with sleep constructs that have been associated with dementia risk in previous prospective population-based studies, for instance self-reported sleep duration,[20,21,30-33] insomnia symptoms,[34,35] or self-reported sleep disturbances.[38,39]

There are several possible explanations for these discrepancies across studies, including ours. First, the predominantly positive associations in literature may indicate a role of sleep in dementia, but could equally well indicate publication bias. Indeed, in aforementioned meta-analysis of sleep and dementia, the funnel plot showed significant asymmetry, which is indicative of publication bias.[2] Another factor contributing to this predominance is the use of slightly differing sleep measures between studies that have been associated with dementia risk, caused by a lack of standardized measures of sleep feasible for use in large observational studies.[2,26] Use of validated or well-known sleep instruments might increase the reproducibility of reported findings.

Second, subjective reporting of sleep quality by persons that are at risk for dementia could introduce misclassification of sleep exposure that has to be taken into account in prospective studies. Intact cognition is a necessity for accurately recalling, reflecting on, and reporting past month's sleep, judging its 'quality'[9] using questionnaires.[45] As self-reported sleep is misclassified in the presence of cognitive impairment, even for measures as straightforward as sleep duration,[46] we only included participants with MMSE>25 to minimize misclassification bias of sleep quality. Noteworthy, persons with mild cognitive impairment with a low-normal MMSE score may still be included in our sample, for which we incrementally restricted the sample on higher MMSE scores. If not accounted for, such bias causes some aspects of sleep that deteriorate simultaneously with cognitive status before dementia diagnosis, or subjective sleep measures in general, to be falsely related to dementia. Importantly, such a restriction of the study population based on baseline cognition is not likely to have prevented us finding an increased dementia risk, as further incremental restrictions to higher MMSE-scores drove the hazard ratio estimate upwards: including participants with cognitive impairment might have further decreased the effect estimate towards an association of worse sleep quality and lower dementia risk, not higher dementia risk. This is supported by observations of better subjective sleep quality in early-stage AD-patients compared to controls, while actigraphic measures revealed their sleep to be worse than controls.[47]

Third, choice of follow-up duration influences the reported hazard ratios obtained from a Cox regression model, as it averages hazards over time into a single metric.[48] We accounted for this by analysing risks in shorter follow-up durations from baseline and found no association of poor sleep and higher dementia risk. We cannot exclude that poor sleep quality relates to increased dementia risk after 13 years, which might be biologically plausible considering that preclinical AD pathology is presumably present more than a decade[49] before diagnosis. However, this might be unlikely as absence of associations on the short term show that reverse causation, or the effect of preclinical neurodegenerative pathology on sleep quality, did not materially influence our results. Additionally, significant differences in strength of associations between studies with short and long follow-up duration were not found in a recent meta-analysis.[2]

Fourth, methodological concerns were identified in previous studies on subjective sleep measures and dementia risk[2]: the relative dearth of studies of sufficient quality, need for long-term follow-up to better study temporality and controlling for comorbid disorders. These concerns were well addressed by using data from the Rotterdam Study, which has sensitive case-finding which will in general increase the effect size of any association found between exposure and outcome, minimal loss to follow-up, adequate follow-up duration, and elaborate work-up to extensively control for confounding.

Currently, qualitative assessments of sleep are less preferred in dementia research in comparison to more quantitative or objective measures of sleep, as they may provide

more unbiased measures.[45] However, despite current efforts[10] to capture for instance sleep quality objectively, measures obtained with polysomnography, the gold standard sleep measurement, cannot sufficiently explain differences in perceived quality of sleep.[11] Therefore, a more quantitative assessment is not the same as a 'less biased qualitative assessment', as they seem to measure different constructs. Moreover, subjective assessments are important as perception of sleep will likely guide the diagnostic work-up in clinical practice. This underwrites the potential importance of studying sleep in dementia research with subjective assessments, also as great value is attributed to a person's appraisal of their own health status, such as in patient-reported outcomes, in medicine at large.[26]

This study indicates that the value of subjective sleep quality as a potentially modifiable risk factor, or marker, of dementia is limited. Compared to the recent meta-analyses, our study shows that the relation between sleep and dementia risk differs depending on the aspect of sleep studied. Also, it emphasizes that negative results should be published to not artificially inflate conclusions on the role of sleep in dementia risk.

Future studies may want to confirm that subjective sleep quality is not related to dementia risk, or investigate related topics, such as the association of subjective sleep quality with cognitive decline, or the role of sleep quality as a marker or risk factor for incident dementia or cognitive decline in vulnerable subgroups such as persons with subjective memory complaints, or *APOE-ε4* allele carriers.

Lastly, we reported determinants of sleep quality at baseline, most of which were related to sleep quality in the expected direction. Surprisingly, less coffee consumption and less snoring were related to worse sleep quality. An association of less coffee consumption and worse sleep quality may be explained by individuals cutting back on coffee after experiencing worse sleep quality[50] or may indicate a 'healthy coffee drinker'-effect.[51] The association with less snoring is not readily explained, and may be due to unreliability in self-reporting.

In conclusion, in this study we found that subjective sleep quality measured by the PSQI is not associated with risk of dementia, nor are the separate PSQI components.

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SUPPLEMENTARY TABLES

Supplementary Table 1. Cross-sectional ssociations of baseline characteristics with subjective sleep quality

Characteristic (unit or category)	Beta (95% CI)[a]	P-value
Age (per SD increase)	0.43 (0.33; 0.53)*	<0.001
Sex (women vs. men)	2.18 (1.98; 2.39)*	<0.001
Education (per higher level)	-0.05 (-0.17; 0.07)	0.430
Smoking status (ever vs. never)	0.02 (-0.14; 0.18)	0.819
Paid employment (present vs. absent)	-0.15 (-0.56; 0.27)	0.490
Coffee consumption (per category increase)	-0.21 (-0.38; -0.05)*	0.011
Alcohol consumption (per SD increase)	-0.10 (-0.20; 0.01)	0.070
Disability index (per SD increase)	0.68 (0.57; 0.79)*	<0.001
Body Mass Index (per SD increase)	-0.06 (-0.16; 0.04)	0.262
Hypertension (present vs. absent)	0.37 (0.16; 0.58)*	0.001
Total cholesterol (per SD increase)	-0.02 (-0.13; 0.08)	0.664
HDL-cholesterol (per SD increase)	0.04 (-0.06; 0.15)	0.438
Diabetes mellitus (present vs. absent)	0.02 (-0.26; 0.30)	0.895
History of coronary heart disease (present vs. absent)	0.67 (0.25; 1.09)*	0.002
History of TIA or stroke (present vs. absent)	0.26 (-0.17; 0.69)	0.235
CES-D (per SD increase)	1.46 (1.37; 1.55)*	<0.001
MMSE-score (per point increase)	-0.02 (-0.10; 0.06)	0.572
Anxiety disorder (present vs. absent)	1.74 (1.36; 2.12)*	<0.001
Snoring ≥ 1/week (present vs. absent)	-0.13 (-0.21; -0.04)*	0.003
APOE- ε4 alleles (per allele increase)	-0.17 (-0.38; 0.04)	0.111

Estimates from linear regression model adjusted for age and sex, if applicable, calculated per unit increase of the determinant, with global PSQI score as dependent variable, and marked for significance at P<0.05. Analysis for *APOE* in n=4,521.
Abbreviations: CI=Confidence Interval; PSQI=Pittsburgh Sleep Quality Index; IQR= interquartile range; HDL= high-density lipoprotein; CES-D=Center for Epidemiologic Studies – Depression Scale; MMSE=Mini Mental State Examination.

Supplementary Table 2. Association of subjective sleep quality and dementia risk, stratified by age, sex and depressive symptoms

Effect-modifier	Cases/N	HR (95% CI)	P-value interaction term
Age			0.780
≤ 71,0 years	97/2,550	0.95 (0.73-1.23)	
> 71,0 years	323/2,254	0.90 (0.80-1.02)	
Sex			0.342
Male	144/2,030	1.03 (0.82-1.30)	
Female	276/2,773	0.87 (0.76-0.98)	
Depressive symptoms			0.464
CES-D < 16	371/4,344	0.90 (0.79-1.02)	
CES-D ≥ 16	49/463	0.92 (0.70-1.23)	

Hazard ratios adjusted for age, sex, education, smoking, employment, coffee consumption, alcohol consumption, activities of daily living, cardiovascular risk factors, MMSE-score, depressive symptoms, anxiety and snoring (excluding stratified variable), calculated per standard deviation increase of global PSQI score. Age is split at the median of the sample.

Abbreviations: CI=Confidence Interval; HR=Hazard Ratio; CES-D=Center for Epidemiological Studies – Depression Scale.

3.2 DEMENTIA – ACTIGRAPHY-ESTIMATED SLEEP AND 24-HOUR ACTIVITY RHYTHMS

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ABSTRACT

We investigated and compared associations of objective estimates of sleep and 24-hour activity rhythms using actigraphy with risk of dementia.

We included 1,322 non-demented participants from the prospective, population-based Rotterdam Study cohort with valid actigraphy data (mean age 66±8 years, 53% women), and followed them for up to 11.2 years to determine incident dementia.

During follow-up, 60 individuals developed dementia, of which 49 had Alzheimer's disease. Poor sleep as indicated by longer sleep latency, wake after sleep onset, and time in bed and lower sleep efficiency, as well as an earlier 'lights out' time, were associated with increased risk of dementia, especially Alzheimer's disease. We found no associations of 24-hour activity rhythms with dementia risk.

Poor sleep, but not 24-hour activity rhythm disturbance, is associated with increased risk of dementia. Actigraphy-estimated nighttime wakefulness may be further targeted in etiologic or risk prediction studies.

INTRODUCTION

Sleep is essential to the brain as it supports learning and memory, regulates synaptic plasticity, and enhances waste clearance from the brain.[1,2] Conversely, disturbed sleep may harm the brain through increased neuro-inflammation[3] or atherosclerosis,[4] or by accumulation of detrimental proteins involved in Alzheimer's disease pathology.[1,5] Against this background, sleep disturbances have been associated with incident dementia[6,7] and as such may be regarded as a potential risk factor, a prodromal disease feature, or as signaling presence of preclinical brain pathology.

Sleep is closely related to the circadian timing system,[8] functioning of which is reflected behaviorally in 24-hour rhythms of physical activity. Disturbed 24-hour activity rhythms have also been linked to dementia risk.[9-11] Yet, it remains unknown how sleep and 24-hour activity rhythms compare with respect to dementia risk, and to what extent these aspects contribute to risk independent from each other.[12] Also, we need to consider relevant interactions, such as that of sleep disturbances with presence of the Apolipoprotein E ε4 (*APOE* ε4) allele on risk of Alzheimer's disease.[13] Lastly, only a minority of population-based studies studied objectively measured sleep in relation to dementia risk, while most studies[6,7,14] measured sleep using self-report measures as these are feasible to obtain in large study populations. Although important for evaluating sleep,[15] self-report measures may hamper attributing associations to sleep per se as they rely on cognitive and affective factors that determine the subjective appraisal of sleep.[16]

Sleep and 24-hour activity rhythms may be independently inferred from physical activity measurements over multiple days using actigraphy. In this study, we investigated associations of actigraphy-derived sleep and 24-hour activity rhythm parameters with the risk of dementia, using over 11 years of follow-up data from the population-based Rotterdam Study cohort. We compared sleep and 24-hour activity rhythm parameters using mutually adjusted models, and investigated effect-modification by *APOE* ε4 status.

METHODS

Study setting and population

This study is embedded in the Rotterdam Study, a prospective population-based cohort in a Dutch suburban district starting in 1990.[17] Examination rounds are repeated every 4-5 years. Incident disease is assessed continuously with electronic linkage between the study database and medical records. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC. All participants provided written informed consent for participation and to have medical information obtained from their treating physicians.

Between 2004 and 2007 (baseline of the current study), 2063 participants (78% of 2632 invited) aged 62.4±9.4 years wore an actigraph for ≥4 days and also completed a daily sleep diary. We excluded participants with: i) Actigraph malfunctioning (n=197); ii) Less than 96 hours of consecutive recording (n=109); iii) Measurements during daylight savings (n=23); iv) Missing information on dementia status (n=54). Lastly, we excluded persons aged <55 years at baseline, as those were considered not at risk for dementia in a population-based setting (n=358).[18] The 1,322 included individuals were on average 2.5 years younger, 8% less likely to be female, and had a 0.4 higher Mini-mental status examination score, but did not differ in questionnaire-assessed sleep or bedtimes compared to invited persons aged >55 who did not participate (n=768). Included participants were followed for 11,630 person-years (95% of possible total if no loss to follow-up[19]) until onset of dementia, loss to follow-up, death, or 1 January 2016.

Sleep and 24-hour activity rhythms

Participants wore an actigraph (ActiWatch model AW4, Cambridge Technology Ltd) for 138 ± 14 hours (median=144) and completed a sleep diary during the same time period.[20] Participants pressed a marker button on the device to denote 'lights out' time and getting up time. Missing marker times (21% of all time values) were imputed from the sleep diary, or estimated by inspecting actigraphy recordings when sleep diaries were missing. Within the defined time in bed, total sleep time and wakefulness were estimated using a validated algorithm with a threshold of 20 activity counts.[21] We defined 'sleep onset' as the midpoint of the first immobile period lasting ≥10 minutes after 'lights out' with ≤one epoch of movement. Sleep onset latency was calculated as the time from 'lights out' to sleep onset, and wake after sleep onset was calculated as the wakefulness after sleep onset. Sleep efficiency was calculated as total sleep time / time in bed * 100%.

We calculated the following indicators of the 24-hour activity rhythm[22,23]: Intradaily variability which quantifies the amount of alterations of activity-inactivity, interdaily stability which quantifies how activity profiles across days resemble each other, and the average time of day when the least active 5 consecutive hours started (L5 onset) indicating phase of most inactivity.

Correlations amongst sleep and 24-hour activity rhythm parameters at baseline in a similar study population have been reported previously.[24]

Dementia

Diagnosing dementia involved cognitive screening for all participants visiting the research center. We further assessed individuals scoring a Mini-mental state examination<26 or Geriatric Mental Schedule organic level>0 with the Cambridge Mental Disorders of the Elderly Examination, including a spouse or informant interview. Simultaneously, for all participants we surveilled medical records of general practitioners and the

regional institute for outpatient mental health care for dementia. A consensus panel adjudicated diagnoses according to standard criteria. In this study, we considered the outcomes of all-cause dementia (DSM-III-R; hereafter: dementia), and Alzheimer's disease (NINCDS–ADRDA).

Covariates

Using the disjunctive cause criterion,[25] we considered age, sex, education (categorized as primary, secondary/lower vocational, intermediate vocational and higher vocational/university), paid employment, self-reported physical activity,[26,27] habitual alcohol consumption, body mass index, positive history of cardiovascular disease (TIA, stroke, heart disease), smoking status, presence of hypertension, and presence of diabetes mellitus as potential confounders or appropriate proxies for unmeasured confounders.[25] Measurements took place during home interviews or at research center visits and are described in detail elsewhere.[28] For the sensitivity analyses we assessed depressive symptoms[29] (Centre for Epidemiological Studies - Depression Scale [CES-D]), possible sleep apnea (2 questions of the Pittsburgh Sleep Quality Index,[30] napping (napping per day during daytime and evenings according to the sleep diary), and number of *APOE* ε4 alleles.[28]

Statistical analysis

We used Cox proportional hazards regression models to associate sleep and 24-hour activity rhythm parameters with incident dementia and Alzheimer's disease, adjusted for age/sex and additionally for abovementioned confounders. We also investigated non-linearity in associations for total sleep time and time in bed by modeling a quadratic term. We additionally adjusted all associations of sleep and bedtime parameters observed in the main analysis for the 24-hour activity rhythm variables, to evaluate their independence. In sensitivity analysis, we separately adjusted analyses for possible sleep apnea, napping, and number of *APOE* ε4 alleles, and restricted analyses to persons without clinically relevant depressive symptoms (CES-D ≤16).

Also, we presented stratified results for all parameters by *APOE* ε4 genotype (≥1 ε4-allele versus no ε4-alleles), age (≤75 versus >75), and sex on risk of dementia, and formally tested multiplicative interaction by modeling a product term. We evaluated statistical significance of interaction terms at P<.0016, defined by applying a Bonferroni correction for testing 10 parameters across 3 stratifications (P=.05/30).

Lastly, we explored whether associations depended on follow-up time to provide some insight into possible reverse causation.[31] We performed analyses in increasingly longer epochs of follow-up time from baseline (e.g. baseline to 2 years, baseline to 4 years, etc.), using Firth's penalized Cox regression to account for the smaller number of events.[32]

Testing the proportional hazards assumption of the main analyses using Schoenfeld residuals indicated a violation for L5 onset. Please note that this non-proportionality was not removed, but made insightful with aforementioned analysis.[31]

Sleep variables were winsorized (i.e. values of outliers changed towards the mean) to 3 SD and subsequently standardized to facilitate comparison. Missing values on covariates (except *APOE*-genotype) were imputed using five multiple imputations, performed with IBM SPSS Statistics version 24 (IBM Corp, Armonk, NY). Statistical analyses were performed with R software (packages: survival, coxphf).

RESULTS

We included 1,322 participants at baseline (Table 1) aged 66.1±7.6 years. During 11.2 years of follow-up (median=9.5), 60 individuals developed dementia, including 47 with Alzheimer's disease.

Longer sleep onset latency (hazard ratio [HR] per standard deviation [SD] increase 1.44, 95% confidence interval [CI] 1.13-1.83) and longer time in bed (HR 1.40, 95% CI 1.04-1.88) were associated with an increased risk of dementia. A higher sleep efficiency (HR 0.72, 95% CI 0.55-0.93) and later 'lights out' time were associated with decreased dementia risk (HR 0.56, 95% CI 0.41-0.76). For Alzheimer's disease, aforementioned associations were stronger, including an association for longer wake after sleep onset (Table 2). In contrast, total sleep time was not associated with the risk of dementia (HR 0.97, 95% CI 0.74-1.29) or Alzheimer's disease (HR 0.92, 95% CI 0.68-1.26, Table 2). Estimates were not meaningfully different when only adjusted for age and sex (Table 2).

We found no statistically significant non-linearity after fitting quadratic terms for the associations of total sleep time (P value=.95) or time in bed (P value=.27) with dementia risk, nor with Alzheimer's disease risk (P value=.44; P value=.30, respectively).

The 24-hour activity rhythms were not associated with dementia risk (Table 2). Aforementioned associations of sleep parameters with dementia risk were also not affected by further adjustment for 24-hour activity rhythm parameters (Table 3).

Estimates remained similar after separate further adjustment for possible sleep apnea, number of naps, or number of *APOE* ε4 alleles (Supplementary Table 1). Also, restricting analyses to persons without clinically relevant depressive symptoms did not substantially affect estimates (Supplementary Table 2).

Stratifying by *APOE* ε4 suggested that associations of sleep parameters with increased risk of dementia were present only in ε4-negative individuals (Table 4), but when formally tested no sleep-by-*APOE* interaction term survived multiple testing.

Table 1. Characteristics of study population at baseline

Characteristic (unit)	Values (N=1,322)
Age at baseline (years)	66.1 ± 7.6
Female	699 (53%)
Educational level	
Primary education	109 (8%)
Lower/intermediate or lower vocational	585 (44%)
Higher or intermediate vocational	390 (30%)
Higher vocational or university	238 (18%)
Paid employment	274 (21%)
Physical activity (MET-hours/week)	62 (19-96)
Alcohol consumption (grams/day)	9 (1-20)
Smoking status	
Never	413 (31%)
Former	695 (53%)
Current	214 (16%)
Body mass index (kg/m^2)	28.0 ± 4.0
History of cardiovascular disease	1.1 (0.3 – 32.0)
Presence of hypertension	888 (67%)
Presence of diabetes mellitus	104 (8%)
Depressive symptoms (CES-D score)	3 (1-7)
Possible sleep apnea	369 (28%)
Napping (number of naps)	1 (0-3)
Presence of ≥1 *APOE* ε4 allele*	346 (26%)
Total sleep time (hours)	6.4 ± 0.9
Sleep efficiency (%)	79 (74-83)
Wake after sleep onset (hours)	1.1 (0.9-1.4)
Sleep latency (minutes)	13 (7-22)
Time in bed (hours)	8.2 ± 0.9
Bedtime ('lights out') (hh:mm)	23:50 ± 00:50
Time getting up (hh:mm)	08:05 ± 00:50
Intradaily variability (score)	0.40 (0.33-0.49)
Interdaily stability (score)	0.83 (0.76-0.88)
Onset least active consecutive 5 hours (hh:mm)	01:50 ± 01:08

Values are expressed as No. (%) for categorical variables and mean ± standard deviation or median (1st quartile – 3rd quartile) for continuous variables, unless specified otherwise. Includes imputed values for covariates.

*Missing 71 participants, including 3 persons with incident Alzheimer's disease

Abbreviations: CES-D=Center for Epidemiological Studies – Depression Scale; MET=Metabolic equivalent of task; N=sample size.

Table 2. Associations of sleep, bedtime and 24-hour activity rhythm parameters with incident dementia and Alzheimer's disease

Determinant (per SD increase)	Dementia HR (95% CI)		Alzheimer's disease HR (95% CI)	
	Cases/N=60/1322		Cases/N=49/1322	
	Model 1	Model 2	Model 1	Model 2
Sleep				
Total sleep time	0.99 (0.76-1.30)	0.97 (0.74-1.29)	0.95 (0.70-1.28)	0.92 (0.68-1.26)
Sleep onset latency	1.38 (1.10-1.74)	1.44 (1.13-1.83)	1.42 (1.11-1.83)	1.45 (1.11-1.89)
Wake after sleep onset	1.17 (0.92-1.51)	1.23 (0.95-1.59)	1.30 (1.00-1.70)	1.38 (1.05-1.81)
Time in bed	1.34 (1.00-1.80)	1.40 (1.04-1.88)	1.40 (1.01-1.95)	1.49 (1.06-2.10)
Sleep efficiency	0.78 (0.60-1.00)	0.72 (0.55-0.93)	0.72 (0.54-0.94)	0.66 (0.50-0.87)
Bedtimes				
Time 'lights out'	0.57 (0.42-0.76)	0.56 (0.41-0.76)	0.55 (0.40-0.76)	0.53 (0.37-0.74)
Time getting up	0.79 (0.59-1.06)	0.79 (0.58-1.08)	0.81 (0.58-1.13)	0.79 (0.56-1.13)
24-hour activity rhythm				
Intradaily variability	1.06 (0.82-1.38)	1.07 (0.82-1.40)	1.04 (0.78-1.40)	1.05 (0.78-1.41)
Interdaily stability	0.93 (0.71-1.22)	0.92 (0.70-1.20)	0.90 (0.67-1.21)	0.87 (0.65-1.17)
L5 onset	0.88 (0.69-1.13)	0.92 (0.72-1.17)	0.85 (0.65-1.12)	0.88 (0.67-1.16)

Hazard ratios were obtained with Cox regression models. Model 1 is adjusted for age and sex. Model 2 is additionally adjusted for educational level, employment status, physical activity, alcohol consumption, body mass index, smoking status, history of cardiovascular disease, presence of hypertension, and presence of diabetes mellitus. Abbreviations: CI=Confidence interval; HR=Hazard ratio; L5=Least active consecutive 5 hours of the day; N=sample size; SD=Standard deviation

Table 3. Associations of sleep parameters with incident dementia and Alzheimer's disease, additionally adjusted for 24-hour activity rhythm parameters

Determinant (per SD increase)	Dementia HR (95% CI)	Alzheimer's disease HR (95% CI)
	Cases/N=60/1322	Cases/N=49/1322
Sleep		
Total sleep time	1.00 (0.74-1.34)	0.93 (0.67-1.29)
Sleep onset latency	1.52 (1.17-1.97)	1.53 (1.14-2.05)
Wake after sleep onset	1.25 (0.95-1.64)	1.42 (1.07-1.90)
Time in bed	1.44 (1.06-1.95)	1.52 (1.07-2.15)
Sleep efficiency	0.70 (0.52-0.93)	0.63 (0.46-0.86)

Hazard ratios were obtained with Cox regression models, adjusted for main analysis confounder and additionally for intradaily variability, interdaily stability, and time of onset of the least active consecutive 5 hours of the day. Confounders included age, sex, educational level, employment status, physical activity, alcohol consumption, body mass index, smoking status, history of cardiovascular disease, presence of hypertension, and presence of diabetes mellitus. In all models, no 24-hour activity rhythm parameter was statistically significant at P value<0.05. We observed no multicollinearity: All variance inflation factors were lower than 2. Abbreviations: CI=Confidence interval; HR=Hazard ratio; N=sample size; SD=Standard deviation

Table 4. Effect-modification of associations of sleep, bedtime and 24-hour activity rhythm parameters with risk of dementia by *APOE* ε4

Determinant (per SD increase)	Dementia HR (95% CI), *APOE*-stratified		Interaction
	ε4 carriers	ε4 non-carriers	
	Cases/N*=21/346	Cases/N*=36/905	P value
Sleep			
Total sleep time	1.04 (0.65-1.66)	0.96 (0.68-1.35)	0.89
Sleep onset latency	1.28 (0.82-2.01)	1.51 (1.10-2.06)	0.38
Wake after sleep onset	0.83 (0.49-1.39)	1.63 (1.19-2.25)	0.01
Time in bed	1.14 (0.72-1.82)	1.73 (1.16-2.57)	0.02
Sleep efficiency	0.95 (0.57-1.56)	0.61 (0.44-0.84)	0.04
Bedtimes			
'Lights out' time	0.52 (0.31-0.87)	0.42 (0.27-0.65)	0.12
Getting up time	0.55 (0.31-0.98)	0.84 (0.56-1.26)	0.20
24-hour activity rhythm			
Intradaily variability	0.75 (0.41-1.36)	1.16 (0.85-1.60)	0.03
Interdaily stability	1.36 (0.75-2.47)	0.85 (0.62-1.18)	0.07
L5 onset	0.70 (0.42-1.16)	0.94 (0.69-1.29)	0.48

Hazard ratios were obtained from Cox regression models, adjusted for age and sex (if applicable), and educational level, employment status, physical activity, alcohol consumption, body mass index, smoking status, history of cardiovascular disease, presence of hypertension, and presence of diabetes mellitus. We tested interaction through modeling a product term of the unstandardized determinant with the number of ε4-alleles. *Missing data on *APOE* ε4 genotype for 71 individuals in total, of whom 3 had incident Alzheimer's disease. Abbreviations: *APOE*=Apolipoprotein E gene; CI=Confidence interval; L5=Least active consecutive 5 hours of the day; N=sample size

Age-stratified analyses did not show a consistent pattern of differences in associations across age, and we found no statistically significant multiplicative interactions with age (Supplementary Table 3).

Sex-stratified analyses showed shorter total sleep time was associated with lower dementia risk in women, opposite to the direction of the point estimate in men. Vice versa, longer time in bed was associated with increased dementia risk only in men (Supplementary Table 3). Yet, we found no statistically significant interactions with sex.

For the sleep parameters, hazard ratio estimates remained mostly similar over increasing follow-up time (Figure 1A). The strong association of later 'lights out' with lower dementia risk in the first 2 years of follow-up (HR 0.27, 95% CI 0.10-0.73) attenuated with increasing follow-up time (Figure 1B). Later L5 onset was associated with lower dementia risk in the first 2 years of follow-up only (HR 0.23, 95% CI 0.09-0.61) (Figure 1C). Incident cases in this period all had Alzheimer's disease. Overall, findings were similar for Alzheimer's disease.

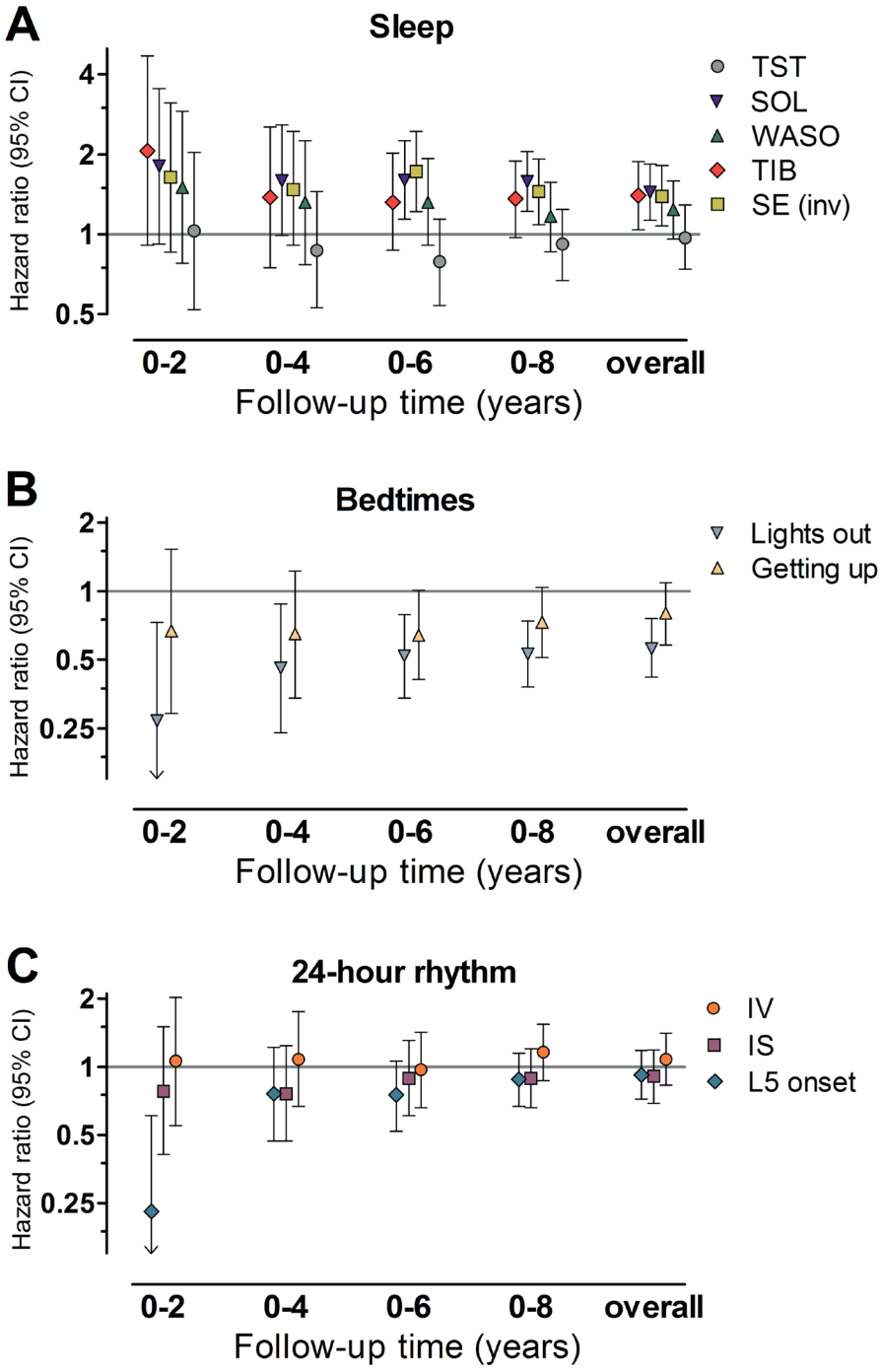

Figure 1. Associations of sleep, bedtime and 24-hour activity rhythm parameters with incident dementia, over increasing epochs of follow-up time

Associations of (A) sleep, (B) bedtimes and (C) 24-hour activity rhythm parameters with risk of dementia are shown for increasing epochs of follow-up time within the study timeframe. Hazard ratios for epochs in shorter follow-up time were obtained using multivariate Firth's penalized Cox regression models. We obtained estimates after censoring all participants still at risk at 2 years (8 incident dementia cases), 4 years (15 cases), 6 years (28 cases), 8 years (47 cases), and after the total follow-up of 11.2 years after baseline (60 cases). Hazard ratios are adjusted for age, sex, educational level, employment status, physical activity, alcohol consumption, body mass index, smoking status, history of cardiovascular disease, presence of hypertension, and presence of diabetes mellitus, are expressed per standard deviation increase in the parameter, and plotted at a log2-scale. Please note that estimates obtained for sleep efficiency were inversed (transformed as 1/estimate depicting sleep 'inefficiency') for graphical comparison of effect sizes of sleep parameters.
Abbreviations: CI=Confidence interval; IS=Interdaily stability; IV=Intradaily variability; L5=Least active consecutive 5 hours of the day; SE=Sleep efficiency; SOL=Sleep onset latency; TIB=Time in bed; TST=Total sleep time; WASO=Wake after sleep onset

DISCUSSION

In the general population, actigraphy-estimated longer sleep onset latency, longer wake after sleep onset, longer time in bed, and lower sleep efficiency, as well as earlier 'lights out' time, were associated with a higher risk of dementia. In contrast, 24-hour activity rhythm fragmentation or stability did not influence dementia risk.

Several methodological considerations should be mentioned. Actigraphy-derived behavioral rhythms do not necessarily equate to the endogenous circadian rhythm. Additionally, the gold standard for measuring sleep is polysomnography. Potential misclassification of sleep and circadian rhythms, and the low number of incident cases in this study, may have reduced our power to detect small effect sizes. Also, we could not assess the extent to which preclinical amyloid β (Aβ) or tau pathology, which may affect sleep-wake regulating brainstem regions[33] years before dementia diagnosis,[34,35] confounded associations with dementia risk. Lastly, selection bias may have influenced our findings, although characteristics of included and non-included participants were largely similar.

Our study adds to previous actigraphy-based studies[9,11,13,36-38] by showing that disturbed sleep is more predictive of developing dementia than disrupted 24-hour activity rhythms. Instead of total sleep time, it was rather an increased amount of wakefulness when in bed, in line with previous findings,[9,36] and an advanced 'lights out' time that determined dementia risk. We speculate that this indicates that a reduced capability to sleep when in bed drives dementia risk, rather than for example deliberate lifestyle choices to curtail sleep. Our findings suggest that individuals may have tried to adapt to such an 'incapability' to sleep by increasing time in bed, mainly by advancing 'lights out' time, to maintain a sufficient amount of sleep. Several mechanisms could underlie this incapability to sleep.

First, associations may indicate presence of an underlying disease process that both increases dementia risk and impairs sleep, for which accumulation of Alzheimer's disease pathology[39] in the brain seems to be a likely[40] substrate. Such confounding, however, is not in line with finding that associations for poor sleep seemed restricted to *APOE* ε4 non-carriers, and not ε4-carriers who are at increased risk of having more brain Aβ deposition at this age.[40] Also, a previous study found that high intradaily variability was related strongest to an increased cerebrospinal fluid biomarker profile suggestive of preclinical Alzheimer's disease.[41] Yet, intradaily variability was unrelated to incident dementia or Alzheimer's disease in our study. Also arguing against confounding by preclinical pathology are the time-stratified analyses, showing that poor sleep was not associated substantially stronger with dementia risk in short versus longer follow-up durations, in contrast to early 'lights out' and early L5 onset. Second, the slowly progressing dementia process may impair sleep not directly but through emergence of prodromal features such as behavioral or neuropsychiatric symptoms. This mechanism may be less likely as associations were also present in persons without depressive symptoms, and independent of napping. Third, sleep disorders, particularly the presence of sleep-disordered breathing, may underlie some of the associations of poor sleep with dementia risk.[42] Sleep-disordered breathing may instigate neurodegenerative processes through intermittent hypoxia and oxidative stress, or through cardiovascular or proteostatic mechanisms.[43] We could only account for such effects by adjusting for a self-reported proxy of sleep-disordered breathing, which might have been insufficient. Further research to disentangle the specific roles of actigraphy-estimated nighttime wakefulness and sleep-disordered breathing in neurodegenerative or Alzheimer's disease pathologies remains needed.

Another remark regarding our *APOE*-stratified findings is that, interestingly, associations of sleep with dementia risk seemed restricted to *APOE* ε4 non-carriers, although we found no statistically significant interactions after correcting for multiple testing. Possibly, disturbed sleep and carrying *APOE* ε4 impact dementia risk similarly, e.g. through protein misfolding,[39] synaptic[44] or hematopoietic effects.[4] The damage accumulated by carrying ε4 throughout life then marginalizes potential harmful effects disturbed sleep, or what underlies it, may have on dementia risk. The discrepancy of our findings with previous work,[13] reporting that sleep fragmentation increases risk of Alzheimer's disease only in *APOE* ε4-carriers, is not readily explained. Possibly, survival bias in this previous study[13] through including old (mean age >80) ε4-carriers,[45,46] or modeling poor sleep differently may have played a role.

We could not confirm the hypothesis that circadian disturbances, reflected by variability and stability of activity rhythms, are implicated[47] in dementia etiology. Yet, the association of earlier L5 onset with increased dementia risk in the next 2 years suggests a phase advance of nighttime inactivity as a prodromal feature of dementia and

Alzheimer's disease. Heterogeneity of activity rhythm findings in dementia risk, including ours, with regard to the direction of a prodromal phase shift[10] and use of different modeling strategies[11,48] should be further investigated.

In conclusion, actigraphy-estimated nighttime wakefulness indicating an incapability to sleep is associated with an increased risk of dementia, especially Alzheimer's disease. At the same time, circadian disturbances as reflected in 24-hour activity rhythms played a limited role in dementia risk in this population of middle-aged and elderly persons.

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SUPPLEMENTARY TABLES

Supplementary Table 1. Associations of sleep, bedtime and 24-hour activity rhythm parameters with risk of dementia, additionally adjusted for sleep apnea, napping and *APOE*-ε4 genotype

Determinant (per SD increase)	Dementia HR (95% CI), after additionally adjusting for:		
	Possible sleep apnea	Napping	No. of *APOE*-ε4 allele(s)
	Cases/N=60/1,322	Cases/N=60/1,322	Cases/N=57/1,251
Sleep			
Total sleep time	1.01 (0.76-1.35)	1.03 (0.77-1.37)	0.95 (0.72-1.27)
Sleep onset latency	1.44 (1.12-1.85)	1.44 (1.13-1.84)	1.45 (1.14-1.84)
Wake after sleep onset	1.23 (0.94-1.61)	1.23 (0.95-1.60)	1.24 (0.96-1.60)
Time in bed	1.43 (1.06-1.93)	1.50 (1.11-2.04)	1.39 (1.03-1.87)
Sleep efficiency	0.73 (0.55-0.96)	0.72 (0.55-0.94)	0.71 (0.55-0.92)
Bedtimes			
'Lights out' time	0.56 (0.41-0.76)	0.54 (0.40-0.73)	0.56 (0.41-0.76)
Getting up time	0.79 (0.57-1.10)	0.82 (0.60-1.13)	0.79 (0.58-1.08)
24-hour activity rhythm			
Intradaily variability	1.05 (0.79-1.40)	0.98 (0.73-1.33)	1.08 (0.82-1.41)
Interdaily stability	0.93 (0.70-1.23)	0.95 (0.72-1.25)	0.90 (0.68-1.19)
L5 onset	0.92 (0.72-1.19)	0.92 (0.72-1.19)	0.92 (0.72-1.18)

Hazard ratios were obtained from Cox regression models, adjusted for age, sex, educational level, employment status, physical activity, alcohol consumption, body mass index, smoking status, history of cardiovascular disease, presence of hypertension, and presence of diabetes mellitus.
Abbreviations: *APOE*= Apolipoprotein E gene; CI=Confidence interval; HR=Hazard ratio; N=sample size.

Supplementary Table 2. Associations of sleep, bedtime and 24-hour activity rhythm parameters with risk of dementia, in persons without clinically relevant depressive symptoms

Determinant (per SD increase)	Dementia HR (95% CI)
	Cases/N=56/1,209
Sleep	
Total sleep time	0.95 (0.72-1.25)
Sleep onset latency	1.46 (1.14-1.88)
Wake after sleep onset	1.28 (0.99-1.64)
Time in bed	1.39 (1.03-1.88)
Sleep efficiency	0.70 (0.54-0.91)
Bedtimes	
'Lights out' time	0.55 (0.40-0.74)
Getting up time	0.77 (0.56-1.06)
24-hour activity rhythm	
Intradaily variability	1.00 (0.76-1.33)
Interdaily stability	0.95 (0.72-1.26)
L5 onset	0.82 (0.63-1.07)

Clinically relevant depressive symptoms were defined as a score ≥16 on the Center for Epidemiologic Studies – Depression Scale. Hazard ratios were obtained from Cox regression models, adjusted for age, sex, educational level, employment status, physical activity, alcohol consumption, body mass index, smoking status, history of cardiovascular disease, presence of hypertension, and presence of diabetes mellitus.
Abbreviations: CI=Confidence interval; N=sample size

Supplementary Table 3. Effect-modification of associations of sleep, bedtime and 24-hour activity rhythm parameters with risk of dementia by age and sex

Determinant (per SD increase)	Dementia HR (95% CI), age-stratified			Dementia HR (95% CI), sex-stratified		
	Age ≤ 75 years	Age > 75 years		Men	Women	
	Cases/ N=29/1,129	Cases/ N=31/193	P_{INT}	Cases/ N=29/623	Cases/ N=31/699	P_{INT}
Sleep						
Total sleep time	1.18 (0.80-1.75)	0.74 (0.49-1.11)	0.14	1.39 (0.94-2.04)	0.66 (0.44-1.00)	0.02
Sleep onset latency	1.22 (0.83-1.78)	1.54 (1.11-2.16)	0.16	1.28 (0.85-1.92)	1.57 (1.16-2.14)	0.22
Wake after sleep onset	1.34 (0.97-1.84)	1.03 (0.66-1.62)	0.77	1.24 (0.87-1.75)	1.21 (0.82-1.79)	0.81
Time in bed	1.63 (1.09-2.44)	1.00 (0.62-1.63)	0.28	2.07 (1.33-3.22)	0.99 (0.65-1.50)	0.02
Sleep efficiency	0.75 (0.52-1.07)	0.68 (0.45-1.01)	0.40	0.83 (0.57-1.23)	0.64 (0.44-0.91)	0.29
Bedtimes						
'Lights out' time	0.60 (0.40-0.89)	0.48 (0.28-0.83)	0.50	0.53 (0.36-0.79)	0.61 (0.38-0.98)	0.61
Getting up time	0.97 (0.63-1.49)	0.61 (0.37-0.99)	0.77	0.93 (0.59-1.45)	0.68 (0.43-1.06)	0.46
24-hour activity rhythm						
Intradaily variability	1.46 (0.99-2.14)	0.93 (0.64-1.35)	0.12	0.79 (0.52-1.21)	1.35 (0.93-1.96)	0.08
Interdaily stability	0.88 (0.61-1.26)	0.94 (0.61-1.43)	0.27	1.13 (0.73-1.75)	0.82 (0.57-1.18)	0.42
L5 onset	0.89 (0.59-1.33)	0.87 (0.62-1.21)	0.34	0.69 (0.47-1.02)	1.10 (0.77-1.55)	0.06

Hazard ratios were obtained from Cox regression models, adjusted for age and sex (if applicable), and educational level, employment status, physical activity, alcohol consumption, body mass index, smoking status, history of cardiovascular disease, presence of hypertension, and presence of diabetes mellitus. The age cut-off of 75 years was chosen to balance dementia incidence between strata. We tested interaction through modeling a product term of the unstandardized determinant with age or sex.
Abbreviations: CI=Confidence interval; N=sample size; $P_{INT=}$ P-value for multiplicative interaction

3.3 PARKINSON'S DISEASE

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Sleep and the risk of parkinsonism and Parkinson's disease: a population-based study. Brain 2019

ABSTRACT

Sleep disturbances may signal presence of prodromal parkinsonism, including Parkinson's disease. Whether general sleep quality or duration in otherwise healthy individuals is related to the risk of parkinsonism remains unclear. We hypothesized that both worse self-reported sleep quality and duration, as well as a longitudinal deterioration in these measures, are associated with the risk of parkinsonism, including Parkinson's disease.

In the prospective population-based Rotterdam Study, we assessed sleep quality and duration with the Pittsburgh Sleep Quality Index in 7,726 persons (mean age 65 years, 57% women) between 2002-2008, and again in 5,450 persons between 2009-2014. Participants were followed until 2015 for a diagnosis of parkinsonism and Parkinson's disease. Outcomes were assessed using multiple modalities: interviews, physical examination, and continuous monitoring of pharmacy records and medical records of general practitioners. We used Cox regression to associate sleep, and changes in sleep over time, with incident parkinsonism and Parkinson's disease, adjusting for age, sex, education and smoking status.

Over 64,855 person-years in 13 years of follow-up (mean: 8.4 years), 75 participants developed parkinsonism, of whom 47 developed Parkinson's disease. We showed that within the first 2 years of follow-up, worse sleep quality (hazard ratio 2.38 per standard deviation increase (95% confidence interval 0.91-6.23)) and shorter sleep duration (hazard ratio 0.61 per standard deviation increase (0.31-1.21)) related to a higher risk of parkinsonism. Associations of worse sleep quality (hazard ratio 3.86 (1.19-12.47)) and shorter sleep duration (hazard ratio 0.48 (0.23-0.99)) with Parkinson's disease were more pronounced, and statistically significant, compared to parkinsonism. This increased risk disappeared with longer follow-up duration. Worsening of sleep quality (hazard ratio 1.76 per standard deviation increase (95% confidence interval 1.12-2.78)), as well as shortening of sleep duration (hazard ratio 1.72 per standard deviation decrease (1.08-2.72)), were related to Parkinson's disease risk in the subsequent 6 years. Therefore we argue that, in the general population, deterioration of sleep quality and duration are markers of the prodromal phase of parkinsonism, including Parkinson's disease.

INTRODUCTION

Parkinson's disease is primarily characterized by motor disturbances,[1] but also includes non-motor features. Sleep-wake disturbances are a common non-motor feature of Parkinson's disease[2-8] and related synucleinopathies.[9-11] Sleep-wake disturbances are also reported to precede a diagnosis of parkinsonism in prodromal Parkinson's disease.[12] Objectively measured increases in sleep fragmentation have also been related to increased Parkinson's disease pathology at brain autopsy in old individuals without Parkinson's disease.[13] Sleep-wake disturbances may be a risk factor for Parkinson's disease, or indicate presence of disease in a prodromal phase. [14,15]

Several sleep disorders have been reported to precede Parkinson's disease or related synucleinopathies,[12] including rapid eye movement (REM) sleep behavior disorder[1,16,17] and obstructive sleep apnea.[18-21] These seem to represent, however, only the 'tip of the iceberg' of various sleep-wake disturbances in prodromal Parkinson's disease.[22-27] Subclinical impairments in sleep, such as poor sleep quality and short sleep duration, are more common in the general population and may well capture aforementioned sleep-wake disturbances. These impairments are particularly important as they are often investigated and easily determinable aspects of sleep in any healthcare setting. To date, however, only few studies investigated if sleep duration reflects prodromal Parkinson's disease,[27,28] and none studied sleep quality. Furthermore, it is unknown if long-term changes in sleep duration and quality relate to subsequent risk of parkinsonism, including Parkinson's disease.

We studied the association of subjectively assessed sleep quality and duration with parkinsonism, including Parkinson's disease. We hypothesized that i) worse sleep quality, and shorter sleep duration, are associated with the risk of parkinsonism, including Parkinson's disease; and ii) deterioration in sleep quality and duration over time is associated with the subsequent risk of parkinsonism. We tested these hypotheses in a prospective, population-based study, using the Pittsburgh Sleep Quality Index to (repeatedly) measure sleep quality and duration, with up to 13 years of follow-up for incident parkinsonism.

METHODS

The study was embedded in the Rotterdam Study, a large, prospective, population-based study in a suburban district in the city of Rotterdam, the Netherlands, details of which are described elsewhere.[29] The study was set up to investigate the frequency, risk factors and natural history of common chronic diseases in the elderly, including neurodegenerative diseases such as Parkinson's disease. The first cohort was initiated in

1990 and included 7,983 persons aged ≥55 years, and was expanded with 3,011 persons aged ≥55 years in 2000, and 3,932 persons aged ≥45 years in 2006. Examination rounds consisted of a home interview and visits to our dedicated research center, including a wide range of questionnaires and physical measurements. Visits are repeated every 4-5 years. Measurements are kept similar across inclusion rounds and time. In between examination rounds, incident disease is assessed with continuous linkage of the study database and medical records of general practitioners, which also holds summaries from all specialist and inpatient care.

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study Personal Registration Data collection is filed with the Erasmus MC Data Protection Officer under registration number EMC1712001. This study is registered with the Netherlands National Trial Register and WHO International Clinical Trials Registry Platform under the shared catalogue number NTR6831. All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

Study population

We included participants from all three inclusion rounds when a sleep questionnaire, the Pittsburgh Sleep Quality Index (PSQI), was first introduced. At this baseline visit (between 2002-2008), we included 7,726 individuals who had valid data on sleep quality or sleep duration, did not have prevalent parkinsonism or Parkinson's disease,[30] and were not cognitively impaired based on a Mini-mental state examination (MMSE) score > 25. We followed the remaining participants until the first of: onset of parkinsonism or Parkinson's disease, 1 January 2015, or death. Study follow-up for incident parkinsonism was nearly complete (64,855 person-years [98.1%]).[31]

For analyses of changes in sleep over time, we similarly included 5,450 individuals at the follow-up visit (between 2009-2014) and started follow-up time for parkinsonism and Parkinson's disease after this visit. See Supplementary Fig. 1 for a detailed flowchart of included participants for analyses at baseline and the follow-up visit.

Assessment of sleep

Subjective aspects of sleep were measured with a Dutch version of the PSQI, which assesses past month's average sleep quality. The PSQI[32] has a good test-retest reliability and validity in a non-clinical sample of older adults.[33] Answers can be categorized, scored and combined into seven component scores ranging from 0 (not problematic) to 3 (very problematic), labeled 'quality', 'latency', 'duration', 'efficiency', 'disturbances', 'sleep medication', and 'daytime dysfunction'. These scores are summed to provide the global

PSQI score (range: 0 – 21) of subjectively assessed sleep quality (hereafter: 'sleep quality'). Higher scores indicate poorer sleep, and scores > 5 indicate a 'poor' sleep quality.

For participants with more than one PSQI component missing, the global PSQI score was not calculated (n=156, 2%). To minimize loss of participants, we calculated weighted component scores for participants who missed one component score (n=1,099, 13%) by multiplying the six-component sum scores by 7/6. Most of these participants missed information on sleep disturbances (n=847) due to introducing a skip rule in PSQI items on disturbances (5a-5j[32]) in a subset of participants, to limit participant burden. If answers to items 5a-5b were both negative ('not in the last month'), items 5c-5j were skipped. Weighting scores minimized any effect of the skip rule on global PSQI scores, as in persons who answered items 5a-5b negatively, weighted scores were not different between those who followed the skip rule versus those who did not (data not shown). Analogously, at follow-up we did not calculate the global PSQI score for 484 (8%) due to missing more than one PSQI component at the follow-up visit (and excluded participants who missed global PSQI score at the baseline visit so that changes could not be calculated [n=203]). We weighted scores for 252 participants (5%) who mostly missed data on efficiency (n=190; see flowchart in Supplementary Fig. 1).

Assessment of parkinsonism and Parkinson's disease

A detailed description of parkinsonism and Parkinson's disease assessment methods used in this study has previously been published.[34] In short, we used four overlapping modalities to collect information on parkinsonism and Parkinson's disease: in-person interviews, examinations, use of antiparkinsonian medication, and continuous monitoring of medical records. Examinations included standardized screening assessments of parkinsonian signs (i.e. tremors, hypo- and bradykinesia, cogwheel rigidity, and postural reflex) by a trained research nurse during center visits. If one or more signs were present, individuals were subsequently invited for a structured physical examination by a trained research physician.

Parkinsonism was defined by presence of hypo- or bradykinesia in combination with ≥1 cardinal sign (resting tremor, rigidity or postural imbalance) observed by any physician, or a clinical diagnosis of parkinsonism by a neurologist or geriatrician (if motor examination details were unavailable). Within those individuals, we diagnosed Parkinson's disease in presence of a clinical Parkinson's disease diagnosis by a neurologist or geriatrician, or a documented positive response to dopaminergic treatment in persons who did not have evidence for a secondary cause (e.g. preexistent dementia diagnosis, use of anti-dopaminergic drugs, cerebrovascular disease). We classified individuals with 'unspecified parkinsonism' if they had multiple possible causes or lacked a clear underlying cause of parkinsonism.

Potential confounders and effect-modifiers

Analyses were adjusted for potential confounders measured at baseline, selected based on relevant publications[1,22,35]: age, sex, education and smoking history. Educational attainment was assessed by interview and categorized as primary, secondary/lower vocational, intermediate vocational, and higher vocational or university. Smoking habits were assessed by interview and categorized as never, former or current smoking. We also examined potential effect-modification by depressive symptoms and anxiety disorders. Depressive symptoms were assessed with the validated Dutch version[36] of the Centre for Epidemiological Studies Depression Scale.[37] Presence of an anxiety disorder was assessed by an adapted version of the Munich Composite International Diagnostic Interview.[38]

Statistical analysis

A detailed explanation of our statistical methods is provided in the Supplementary Text. In short, we first used Cox proportional hazards regression models to associate both sleep quality and duration at baseline with both incident parkinsonism and Parkinson's disease. As we found that the Cox model assumption of proportionality was violated in some analyses, we also examined how aforementioned associations changed over follow-up time by performing analyses in incremental epochs of follow-up time from baseline (extending follow-up time e.g. baseline to 2 years, baseline to 4 years, etc.)[39], or using a stratified Cox model to obtain period-specific hazards (e.g. baseline to 2 years, 2 to 4 years, etc.). We furthermore looked at the effect of other PSQI components separately. As sensitivity analyses, we restricted analyses to persons without comorbid depression and anxiety. We also investigated potential effect-modification by age, sex, and presence versus absence of any of four parkinsonian signs. Second, we related changes in sleep quality and duration between the baseline and the follow-up visit with incident parkinsonism and Parkinson's disease after the follow-up visit.

Variables were standardized and, when right-skewed, log-transformed before standardization. Missing values on covariates were imputed using five multiple imputations.

RESULTS

Characteristics of the study sample at baseline are summarized in Table 1. Median global PSQI score was 3, and 2,115 participants (27%) scored over 5 indicating poor sleep quality. Global PSQI score and sleep duration were moderately correlated (Spearman's $r = -0.69$; $P<0.01$)). During 13.0 years of follow-up (mean 8.4 years), we observed 75 incident parkinsonism cases, of which 47 (63%) with Parkinson's disease (Supplementary Table 1).

Table 1. Characteristics of study population at baseline

Characteristic (unit)	Total sample	Incident PS	No incident PS
	N = 7,726	N = 75	N = 7,651
Age at baseline (years)	65.4 ± 10.3	71.6 ± 8.4	65.4 ± 10.3
Female	4,396 (57%)	33 (44%)	4,365 (57%)
Educational level			
Primary education	708 (9%)	8 (11%)	700 (9%)
Lower/intermediate or lower vocational	3,088 (40%)	29 (39%)	3,060 (40%)
Higher or intermediate vocational	2,371 (31%)	24 (32%)	2,347 (31%)
Higher vocational or university	1,559 (20%)	14 (19%)	1,545 (20%)
Smoking status			
Never	3,416 (44%)	34 (45%)	3,383 (44%)
Former	3,549 (46%)	33 (44%)	3,516 (46%)
Current	761 (10%)	8 (11%)	753 (10%)
Cognitive functioning (MMSE score)	28 (27-29)	28 (27-29)	28 (27-29)
Depressive symptoms (CES-D score)	3 (1-8)	4 (1-8)	3 (1-8)
Presence of any anxiety disorder	588 (8%)	8 (11%)	580 (8%)
Presence of any parkinsonian signs	807 (10%)	16 (21%)	792 (10%)
Sleep quality (global PSQI score)	3 (2-6)	3 (1-6)	3 (2-6)
Missing	46 (1%)	0 (0%)	46 (1%)
Sleep duration (hours)	6.8 ± 1.2	7.1 ± 1.3	6.8 ± 1.2

Characteristics of study population at baseline. Values are expressed as frequency (%) for categorical variables and mean ± standard deviation or median (interquartile range) for continuous variables, unless specified otherwise. Includes imputed values for covariates.
Abbreviations: CES-D=Center for Epidemiological Studies – Depression Scale; MMSE=Mini-mental state examination; N=sample size; PS=parkinsonism; PSQI=Pittsburgh Sleep Quality Index

Sleep quality was not associated with the risk of parkinsonism (hazard ratio [HR] per standard deviation [SD] increase in global PSQI score: 0.95 (95% confidence interval (CI) 0.76-1.20)) or Parkinson's disease (HR per SD increase 0.87 (95% CI 0.65-1.16)). We observed similar estimates when analyzing categorized poor (versus good) sleep quality: HR 0.97 (95% CI 0.57-1.66) for parkinsonism, and HR 0.79 (95% CI 0.39-1.59) for Parkinson's disease (Supplementary Table 2).

Longer sleep duration was not associated with the risk of parkinsonism (HR per SD increase 1.21, 95% CI 0.95-1.54) and PD (HR 1.24, 95% CI 0.92-1.69). After categorizing sleep duration, we did not observe a significant increase in risk with increasing categories of sleep duration (Supplementary Table 2).

In aforementioned analyses for Parkinson's disease risk, but not for parkinsonism, the proportionality assumption for both sleep quality and duration was significantly violated.

We found that worse sleep quality related to an increased risk of parkinsonism (HR 2.38, 95% CI 0.91-6.23) in the first 2 years of follow-up, which disappeared when increasing follow-up time from baseline (Fig. 1A). In these 2 years, associations were more pronounced, and statistically significant, for Parkinson's disease (HR 3.86, 95% CI 1.19-12.47) compared to parkinsonism. Results for sleep duration were analogous (Fig. 1B): short sleep duration was associated with an increased risk of parkinsonism (HR 0.61, 95% CI 0.31-1.21) and Parkinson's disease (HR 0.48, 95% CI 0.23-0.99). Additionally, analysis of period-specific hazard ratios using a stratified Cox model suggested that associations of worse sleep quality, and shorter sleep duration, with an increased risk of parkinsonism and Parkinson's disease are confined to the first 2 years of follow-up (Supplementary Fig. 2).

Most PSQI components showed a similar pattern of associations with cumulative increasing follow-up duration, except for sleep medication (Fig. 2A-F). We observed noteworthy changes in effect sizes from short to long follow-up for sleep efficiency, and to a lesser extent for sleep quality, latency and daytime dysfunction (Fig. 2A-C; Fig. 2F; Supplementary Table 3). Also, for daytime dysfunction, the direction of hazard ratio estimates changed over increasing epochs of follow-up (Supplementary Table 3).

We further restricted the sample to persons without clinically relevant depressive symptoms and without any anxiety disorder, leaving 6,605 individuals of which 61 cases of parkinsonism, including 39 cases with Parkinson's disease. Associations over cumulatively increasing follow-up duration were similar to the total sample (Supplementary Fig. 3). For the association of sleep duration with Parkinson's disease, all hazard ratios shifted to higher values. As a result, longer sleep duration was now associated with increased Parkinson's disease risk in the overall follow-up (HR 1.47, 95% CI 1.02-2.11), for which proportionality was not violated.

Analyses stratified at median age did not reach statistical significance. We observed hazard ratio estimates suggesting associations of worse sleep quality with a lower risk of parkinsonism and Parkinson's disease in younger persons, while hazard ratios in older persons were close to the null. Similarly, estimates also suggested associations of longer sleep duration with a higher risk of both outcomes in younger persons. Case numbers in separate strata were small. Also, there were no significant interactions between age and sleep quality or duration on the risk of either outcome (Supplementary Table 4).

We observed a similar relation between sleep quality and duration and disease risk in persons without parkinsonian signs at baseline. Statistically testing these interactions on a multiplicative scale showed significant interactions of sleep quality with presence of parkinsonian signs on the risk of both parkinsonism and Parkinson's disease (Supplementary Table 4).

Characteristics of the study population at the follow-up visit are provided in Supplementary Table 5. Changes in sleep between the baseline and follow-up visit were

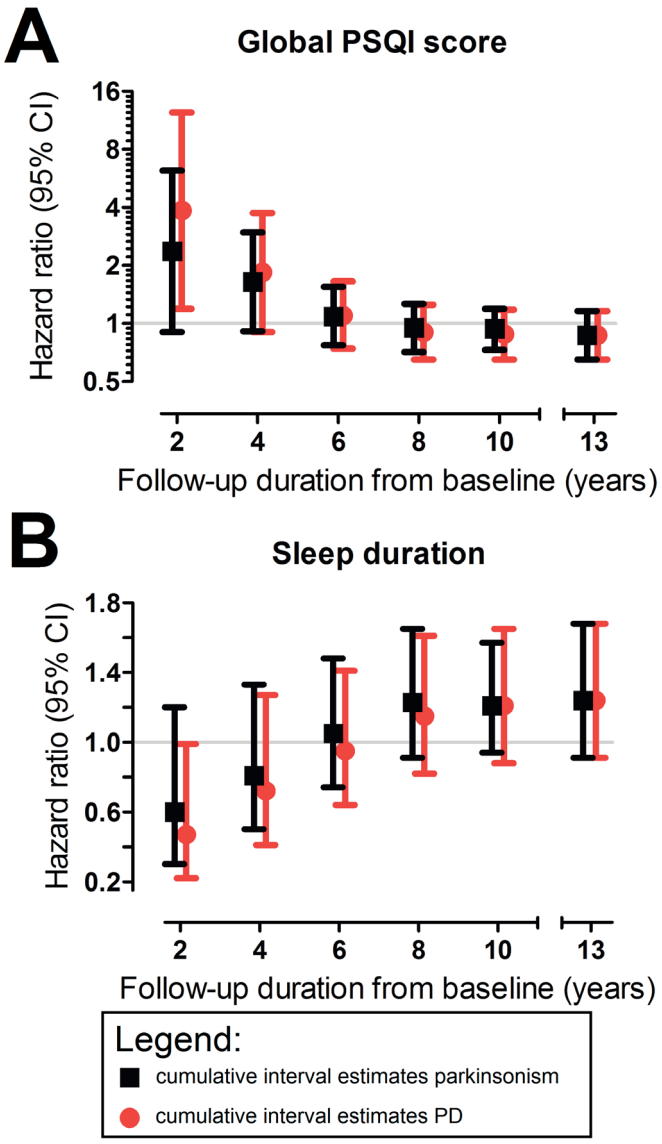

Figure 1. Associations of sleep quality and duration with risk of parkinsonism and Parkinson's disease, per cumulatively increasing duration of follow-up

The associations of (A) sleep quality and (B) sleep duration with incident parkinsonism and Parkinson's disease are shown for cumulatively increasing follow-up duration within the study timeframe. Hazard ratio estimates were obtained from multivariate Firth's penalized Cox regression models by censoring all participants still at risk at year 2, 4, 6, 8 and 10 after baseline, and after the total follow-up of 13 years. Hazard ratio estimates were adjusted for age at baseline, sex, educational level and smoking status, are expressed per standard deviation increase of (A) worse sleep quality, or (B) longer sleep duration, and are plotted at a (A) logarithmic (base 2) scale and (B) a linear scale. Abbreviations: CI=Confidence Interval; PD=Parkinson's disease; PSQI=Pittsburgh Sleep Quality Index

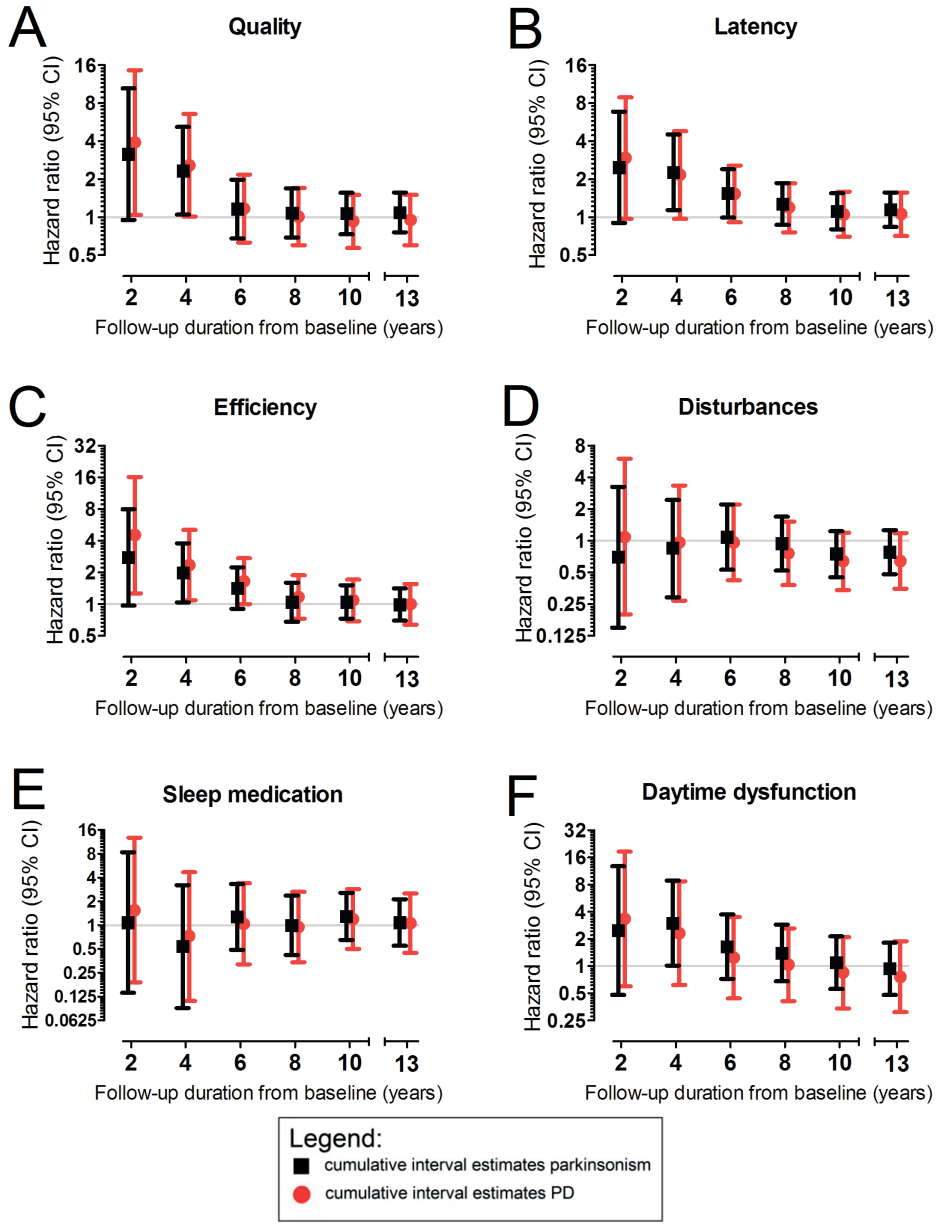

Figure 2. Associations of Pittsburgh Sleep Quality Index component scores with risk of parkinsonism and Parkinson's disease, per cumulatively increasing duration of follow-up

The associations of the PSQI components (A) quality, (B) latency, (C) efficiency, (D) disturbances, (E) sleep medication, and (F) daytime dysfunction with incident parkinsonism and Parkinson's disease are shown for cumulatively increasing follow-up duration within the study timeframe. Hazard ratio estimates were obtained from multivariate Firth's penalized Cox regression models by censoring all participants still at risk at year 2, 4, 6, 8 and 10 after baseline, and after the total follow-up of 13 years. Estimates are adjusted for age at baseline, sex, educational level, and smoking status, are expressed per category increase in component

score, and are plotted at different logarithmic (base 2) scales per component. For parkinsonism analyses, we included following numbers of participants: (A) 7716, (B) 7718, (C) 7473, (D) 6840, (E) 7725, (F) 7689 (samples were 5 to 7 participants smaller for analyses on Parkinson's disease). To ensure sufficient (>10%) observations in each category, we combined scores 2 and 3 for components quality, latency and efficiency, and scores 1, 2 and 3 for components disturbances, medication and daytime dysfunction.
Abbreviations: CI=Confidence interval; HR=Hazard ratio; N=Sample size; PD=Parkinson's disease; PSQI=Pittsburgh Sleep Quality Index

measured over 10.9 years (on average 6.0 years) in all participants. In the subsequent 6.0 years (average follow-up: 2.9) after the follow-up visit, we observed 25 incident parkinsonism cases, of which 17 with Parkinson's disease.

Worsening of sleep quality was related to a subsequent increase in Parkinson's disease risk (HR per SD increase 1.76, 95% CI 1.12-2.78), as was a shortening of sleep duration (HR per SD increase 1.72, 95% CI 1.08-2.72; Table 2). Results were independent of the absolute average level of sleep quality or duration (Table 2). Also, additional adjustment for depressive symptoms at baseline did not attenuate results. Associations of sleep quality (HR 1.23, 95% CI 0.83-1.83) and sleep duration (HR 1.45, 95% CI 0.99-2.13) with incident parkinsonism were less pronounced. When examining hazard ratios over increasing epochs of follow-up time measured from the follow-up visit, we found that worsening of sleep quality, and shortening of sleep duration, were associated with parkinsonism on the short term, but not the longer term (Supplementary Fig. 4). For both sleep parameters, risk of Parkinson's disease was also slightly higher on the short than on the long term.

Table 2. Association of changes in sleep quality and duration between the baseline and follow-up visit, and risk of parkinsonism and Parkinson's disease

Determinant (unit)	Parkinsonism		Parkinson's disease	
	Cases/N	HR (95% CI)	Cases/N	HR (95% CI)
Change sleep quality (worse sleep)	25/5,206	1.23 (0.83-1.83)	17/5,244	**1.76 (1.12-2.78)**
Change sleep duration (shorter sleep)	25/5,244	1.45 (0.99-2.13)	17/5,238	**1.72 (1.08-2.72)**

Changes in sleep quality were modeled per standard deviation increase ('worsening') of global Pittsburgh Sleep Quality Index score, and changes for sleep duration were modeled as standard deviation decrease ('shortening') of sleep duration from the baseline visit to the follow-up visit. Hazard ratio estimates are adjusted for age at baseline, sex, educational level, smoking status and time interval between measurements. Additional adjustment for depressive symptoms at baseline minimally changed point and interval estimates (data not shown). After additional adjustment for the average level of sleep quality or sleep duration of the two measurements, point and interval estimates for the relation with parkinsonism barely changed. Estimates for associations of change in sleep quality (HR 1.87, 95% CI 1.12-3.10) and change in sleep duration (HR 1.85, 95% CI 1.14-2.98) with risk of Parkinson's disease increased. **Bold** estimates indicate statistically significant results at $p<0.05$. Abbreviations: CI=Confidence interval; HR=Hazard ratio; N=Sample size.

DISCUSSION

In the general population, baseline sleep quality and duration within the next 2 years relate to incident parkinsonism, and specifically to Parkinson's disease. Similarly, deterioration over 6 years in these parameters is associated with incident parkinsonism and Parkinson's disease.

Several methodological considerations should be mentioned. First, our study focused on subjectively measured sleep, which does not necessarily reflect similar constructs as objective measurements. While the first incorporates the experience of sleep, objective measurements indicate physiological sleep. Therefore, subjective measures cannot provide similar insight in underlying biological processes as objective measures (e.g. polysomnography). Second, we did not include individuals with cognitive impairment to minimize information bias of sleep quality[40,41] and duration[40,42], but these individuals are at increased risk of having prodromal parkinsonism[43] which could bias our associations. In addition, persons with cognitive impairment are also predisposed to develop REM sleep behavior disorder,[44] which has been suggested to be associated to a longer sleep duration in the general population.[45] This could lead to an underestimation of associations of sleep duration with parkinsonism and Parkinson's disease. Third, although the PSQI is used in patients with Parkinson's disease,[46] it may miss Parkinson's disease-specific sleep disturbances.[47,48] Patients with prodromal disease may thus underreport sleep problems, or overstate their sleep quality. If so, we have even underestimated especially short-term effect estimates of worse sleep quality with increased risk of parkinsonism and Parkinson's disease risk. Fourth, the number of parkinsonism and Parkinson's disease cases in our study is small, which may have unpowered us to detect small effects. Fifth, subjective assessment of sleep may be more prone to measurement error than objective methods. This lack of precision may have precluded us from detecting small effect sizes. Sixth, we cannot exclude any residual confounding of medication use beyond those questioned in the PSQI in our estimates.

We found associations of poor sleep quality and short sleep duration with increased risk of parkinsonism, and especially Parkinson's disease, in the first 2 years of follow-up, attenuating with incremental follow-up. Our study adds to the previous findings by showing that associations evidently change with incremental follow-up time. This is in line with findings of large registry-based studies in general practice that show increases in insomnia diagnoses 2 years,[22,49] but not 5 and 10 years before diagnosis of Parkinson's disease [22]. Such results suggest that sleep disturbances occur as prodromal features rather than as causes of Parkinson's disease and related synucleinopathies, as sleep is measured closer to the diagnosis of an incident case when follow-up is short. Our measurements of sleep likely represent common, subclinical sleep problems as well as

those severe enough to diagnose a sleep disorder, and therefore fit well with the variety of sleep disturbances preceding Parkinson's disease.[12]

Mechanisms behind sleep disturbances marking prodromal Parkinson's disease remain speculative. Sleep may be disturbed by early-stage dysfunction of serotonergic neurons in the dorsal raphe nuclei and sleep-promoting areas in the basal forebrain.[50] Such dysfunction may also negatively impact switching between sleep and wake.[51] Additionally, early spread of pathology to the coeruleus/subcoeruleus complex may disturb REM sleep independent of REM sleep behavior disorder.[15] Sleep may also be impaired via circadian dysfunction occurring around the time of diagnosis,[2] via hypothalamic neuron loss,[52,53] or via the loss of dopaminergic modulation.[54]

Of note, results do not exclude that sleep disturbances may cause Parkinson's disease. An effect of sleep disturbance on neurodegenerative disease is plausible, as sleep deprivation has been shown to increase levels of beta-amyloid, a pathological hallmark of Alzheimer's disease. Mechanisms include decreased clearance,[55] or activity-dependent increased production, of beta-amyloid. The sleep wake cycle has also been shown to regulate tau levels, and sleep deprivation can increase extracellular levels of tau and, interestingly, alpha-synuclein.[56] A recent study importantly showed that increased actigraphy-derived sleep fragmentation in old individuals without Parkinson's disease was associated with an increased burden of Parkinson's disease pathology at brain autopsy.[13] This indicates that objective disturbances, besides subjectively impaired sleep, relates to Parkinson's disease pathology. Unfortunately, the cross-sectional design does not allow inference on temporality of the association. Authors speculate that potential pathways between sleep fragmentation and disease risk may include increased oxidative stress, or reduced clearance of metabolic waste including extracellular α-synuclein.[13]

Analyses of changes in sleep quality and duration suggest that sleep in prodromal Parkinson's disease already deteriorates over 2 years prior to diagnosis in the general population, independent from baseline depressive symptoms, and the absolute levels over which the changes occurred. To our knowledge, the only study investigating changes in sleep has been performed in patients with REM sleep behavior disorder.[57] This study, however, reported opposite findings: improving insomnia symptoms and increasing self-reported sleep duration increased the risk for conversion to Parkinson's disease and dementia with Lewy bodies. Differences in findings could results from their selection of patients prone to develop a severe, cognitively more impaired subtype of prodromal Parkinson's disease,[58] but differences may additionally be explained by non-recognition of sleep problems due to including subjects with subclinical cognitive deficits.[59-61] Their study not only had a high incidence (50%) of Lewy body dementia patients, but also showed underreporting (reporting increased sleep duration and quality discrepant from objective decreases in total sleep time) in those developing neurodegenerative disease.[57]

If aforementioned changes in sleep were driven by a specific sleep disorder, REM sleep behavior disorder may not be a likely candidate: Persons with REM sleep behavior disorder in a population-based polysomnography study had a similar sleep quality, and even longer sleep duration, than others.[45] REM sleep behavior disorder patients also did not perceive their sleep as worse, or shorter, than healthy controls.[57]

After excluding persons with comorbid depressive symptoms or anxiety disorders, results remained mostly similar. Noteworthy was that hazard ratio estimates of the relation of sleep duration with Parkinson's disease risk were all slightly higher. This resulted in an association of longer sleep duration with increased Parkinson's disease risk in the overall follow-up. Given the number of associations investigated in our sensitivity analyses, and the small number of cases when restricting the sample, this result may be a spurious finding and should be interpreted with caution.

A methodological explanation is that in these sensitivity analyses persons in a late prodromal phase of Parkinson's disease may have been selectively excluded, as depression and anxiety are both part of the prodromal phase and considered predominantly late features.[22,62,63] This could have resulted in selective exclusion of susceptible individuals[39] resulting in a decreased long-term risk of Parkinson's disease in those remaining individuals with short sleep duration. It is also possible that short sleep duration is merely symptomatic of (prodromal emergence of) depression, which explains why exclusion of persons with depression resulted in an inverse association of sleep duration with Parkinson's disease. Nevertheless, we re-emphasize the small number of cases in our analyses, which may have compromised the robustness of these findings.

Analogous to aforementioned sensitivity analysis, stratified analyses on the presence of parkinsonian signs might also select participants based on either a more advanced stage of an underlying neurodegenerative process, or its absence. A statistical interaction with sleep quality could guide future investigations of identifying high risk groups for parkinsonism or Parkinson's disease risk.

Patterns of associations between separate PSQI components and Parkinson's disease risk over time indicate that, aside from sleep duration, efficiency may mark prodromal disease. This applies to sleep quality, latency and daytime dysfunction to a lesser extent. Although these aspects of sleep may correlate well to known markers of prodromal Parkinson's disease such as pain or autonomic failure,[22] or excessive daytime sleepiness,[12,64] results also warrant further investigation of these easily measured aspects of sleep in etiological or risk prediction efforts. Future studies on prodromal Parkinson's disease are needed to investigate associations with objective measures of sleep, and to assess the predictive value of (perceived) shortening or worsening of sleep over known (sleep) markers of prodromal parkinsonism.

In conclusion, poor sleep quality and short sleep duration increased the risk of parkinsonism and Parkinson's disease in the next 2 years. Moreover, sleep quality and dura-

tion change for the worse over 2 years prior to a diagnosis of parkinsonism, especially Parkinson's disease. Both are congruent with presence of prodromal Parkinson's disease progressively deteriorating sleep.

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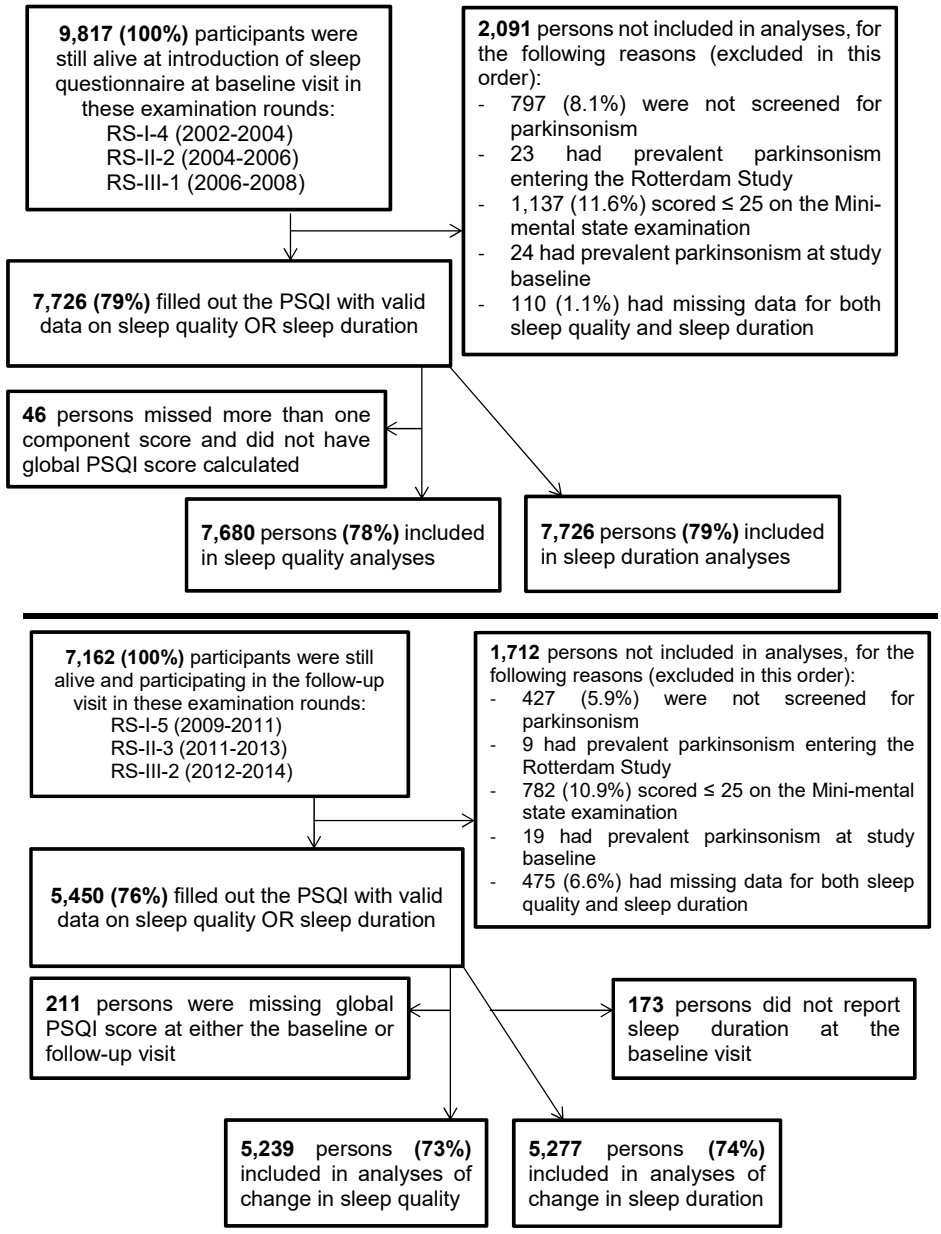
SUPPLEMENTARY TABLES AND FIGURES



Supplementary Figure 1. Flowchart inclusion of study participants at the baseline and follow-up visits

Supplementary Text

Statistical analysis

For our main analyses, we used Cox proportional hazard models to determine the association of sleep quality, measured with the global PSQI score, and sleep duration at baseline with incident parkinsonism and Parkinson's disease, using follow-up time as timescale. We repeated the main analysis after categorizing sleep quality and duration (sleep duration according to international recommendations for elderly individuals[65]).

When investigating the assumption of proportional hazards of the Cox model through visually examining and statistically testing the scaled *Schoenfeld* residuals, we found slight ($0.01<p<0.05$) violations of proportionality for both sleep determinants in the main analyses on Parkinson's disease. This indicates that the hazard ratio, which provides an average of the relative risk over the included follow-up time, is a poor representation of the changes occurring over time within the study timeframe.[39] To examine these changes over time, we repeated the main analyses for both outcomes after restricting follow-up to shorter study duration by censoring participants at 2, 4, 6, 8, and 10 years after baseline, using Firth's penalized Cox regression analysis to account for low cumulative incidences of outcomes after short follow-up.[66,67] Such an approach shows how the choice of follow-up time from baseline affects the hazard ratio.[39] Of note, proportionality was not violated for analyses of 6 years after baseline or shorter (for the association of sleep duration and Parkinson's disease), or at 2 years after baseline (for the association of sleep quality and Parkinson's disease). To further examine hazard ratio changes over time, we used a stratified Cox model by stratifying by follow-up time intervals of 0-2, 2-4, 4-6, 6-8, 8-10, and 10-13 years. Hazard ratios were obtained by modeling the interaction of the determinant with a term of categorized follow-up time, and combining the coefficients of point and interval estimation for the determinant and that stratum.

Next, to investigate if any associations found for global PSQI score were driven by specific components, we also investigated the relation between PSQI component scores (quality, latency, efficiency, disturbances, medication and daytime dysfunction) and incident parkinsonism or Parkinson's disease, in overall and shorter follow-up durations similarly as described above. As sleep duration was already investigated separately, we did not additionally investigate the duration component of the PSQI (which categorizes reported sleep duration[32]). Also, we performed the main analyses in persons without any comorbid clinically relevant depressive symptoms (CES-D score ≥16) and without any anxiety disorders. Furthermore, we studied possible effect modification in the main analyses by median age, sex, and presence versus absence of any of four parkinsonian signs (scoring details published previously[62]), by performing stratified analyses and formally testing for multiplicative interaction. Proportionality was not violated in the tests of multiplicative interaction.

We also examined how changes in sleep quality and duration between the baseline and follow-up visit were related to subsequent risk of parkinsonism and Parkinson's disease. Follow-up time was calculated from the follow-up visit, and analyses were additionally adjusted for the time interval between the baseline and follow-up visit. A change in sleep quality was modeled by subtracting the baseline global PQSI score from the score at the follow-up visit, so that positive values indicated worsening of sleep quality over time. Change in sleep duration was modeled as shorter sleep duration, by subtracting self-reported sleep duration at the follow-up visit from that at baseline. We repeated analyses after i) additionally adjusting for averaged global PSQI score, or sleep duration, over baseline and follow-up visits to examine if effects were dependent on absolute levels (i.e. if decreases in e.g. sleep duration from 9 to 7 hours would be different from decreases from 7 to 5 hours); ii) adjusting for depressive symptoms at baseline to see if sleep changes were driven by depression. As we also observed non-proportionality of hazard ratios ($0.01<p<0.05$) in the analyses of changes in sleep quality and duration between the baseline and follow-up visit on the risk of parkinsonism, we also obtained period-specific hazard ratios for these relations.

To obtain normally distributed values and minimize the effect of outliers, we log-transformed (ln(variable + 1)) right-skewed variables (global PSQI score) and subsequently winsorized (i.e. transformed towards the mean) outliers to three standard deviations from the mean (1.3% of observations for global PSQI score, 0.8% for sleep duration). Both variables were then standardized (subtracting the mean and dividing by the standard deviation) to facilitate comparison of effect sizes.

Missing data on covariates (missing values in covariates at baseline: median=1.6%, maximum=29.7% (smoking status)) were imputed using five multiple imputation based on all variables used in our analyses. Statistical testing was performed two-sided at $p<0.05$. Data were analysed using *SPSS Statistics*, version 21 (IBM Corp., Armonk, NY), and with the open *R* software (packages: 'survival', 'coxphf').

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Supplementary Table 1. Overview of incident parkinsonism diagnoses

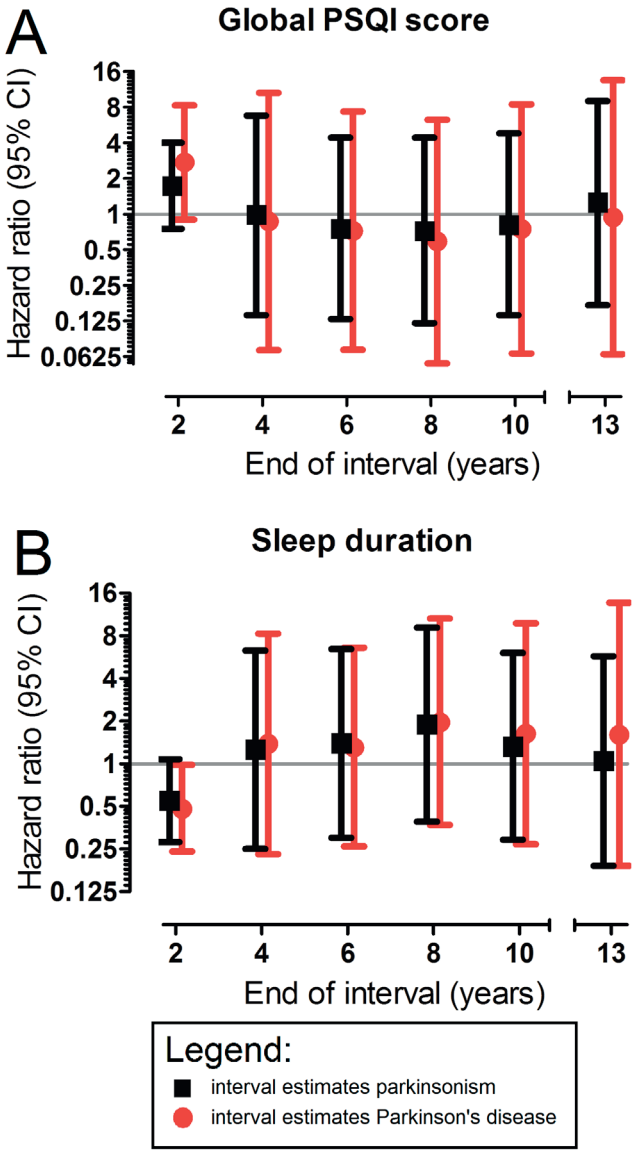
Clinical diagnosis	After the baseline visit (N=7,726)	After the follow-up visit (N=5,450)
Probable Parkinson's disease	47 (63%)	17 (68%)
Vascular parkinsonism	3 (4%)	1 (4%)
Medication-induced parkinsonism	5 (7%)	1 (4%)
Progressive supra-nuclear palsy	1 (1%)	0
Multiple system atrophy	0	0
Corticobasal degeneration	1 (1%)	1 (4%)
Lewy body dementia	2 (3%)	0
Parkinsonism with dementia – not Lewy body type	2 (3%)	0
Unspecified parkinsonism*	14 (19%)	5 (20%)
All parkinsonism diagnoses	75 (100%)	25 (100%)

Number of diagnoses expressed as frequency (%), for the samples used in analyses at the baseline and follow-up visits.
*Denotes parkinsonism patients that did not have any of the above clinical diagnoses

Supplementary Table 2. Association between categories of sleep quality or duration and risk of incident parkinsonism or Parkinson's disease

Determinant	Categories	Parkinsonism		Parkinson's disease	
		Cases/N	HR (95% CI)	Cases/N	HR (95% CI)
Global PSQI score	≤5 ('good' quality)	55/5,565	1.00 (reference)	36/5,562	1.00 (reference)
	>5 ('poor' quality)	20/2,115	0.97 (0.57-1.66)	11/2,112	0.79 (0.39-1.59)
Sleep duration	<7 hours	21/3,155	1.00 (reference)	13/3,150	1.00 (reference)
	≥7 - ≤8 hours	45/4,033	1.61 (0.95-2.71)	28/4,031	1.65 (0.86-3.21)
	>8 hours	9/538	2.19 (1.00-4.81)	6/537	2.54 (0.96-6.72)

Hazard ratios were obtained from Cox regression models, adjusted for age at baseline, sex, educational level, and smoking status, expressed in reference to the lowest global PSQI score, or sleep duration, category. Categorization of sleep duration in three categories is based on the US National Sleep Foundation recommended sleep duration for elderly persons.[65]
Abbreviations: HR=Hazard ratio; N=sample size; PSQI=Pittsburgh Sleep Quality Index.



Supplementary Figure 2. Associations of sleep quality and duration with risk of parkinsonism and Parkinson's disease, over separate intervals of follow-up time

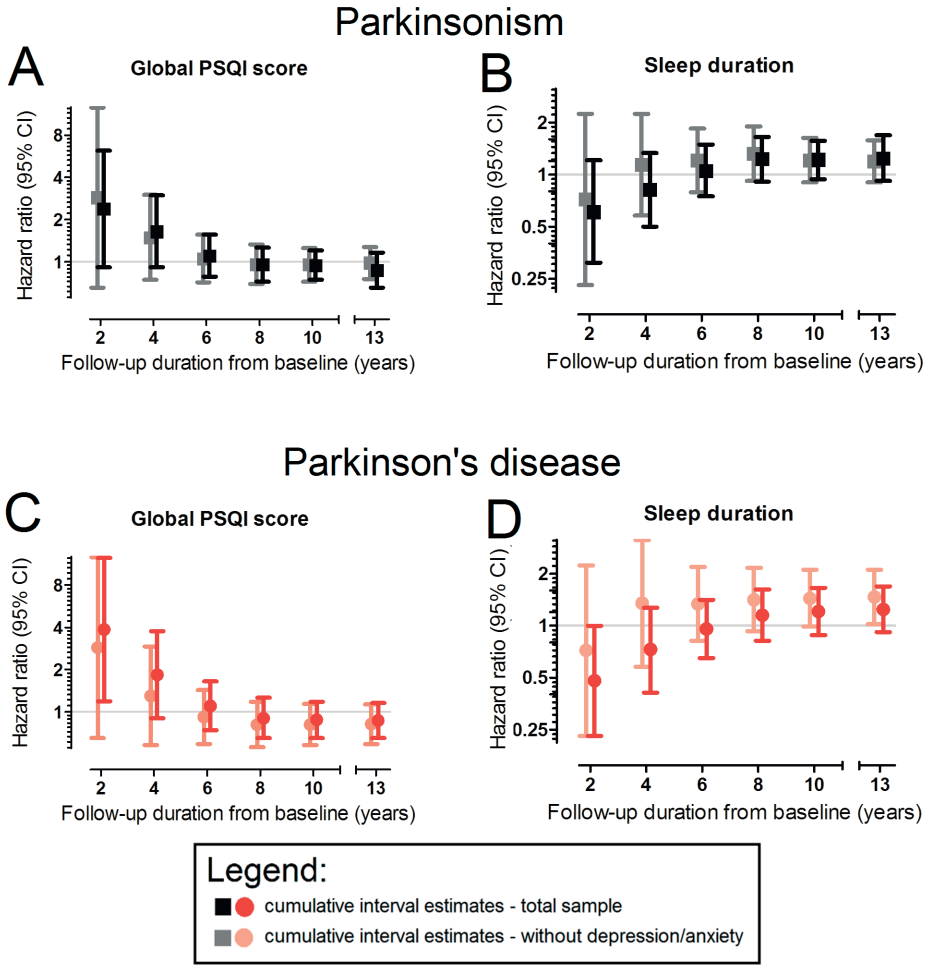
The associations of (A) sleep quality and (B) sleep duration with incident parkinsonism and Parkinson's disease are shown for separate intervals of follow-up duration within the study timeframe, using a stratified Cox model. Hazard ratio were estimated for the intervals 0-2 years, 2-4 years, 4-6 years, 6-8 years, 8-10 years, and 10-13 years (end of follow-up) and obtained from modeling the interaction of the determinants with follow-up time strata. Hazard ratio estimates were adjusted for age at baseline, sex, educational level and smoking status, are expressed per standard deviation increase of (A) worse sleep quality, or (B) longer sleep duration, and are plotted at a logarithmic (base 2) scale. Abbreviations: CI=Confidence Interval; PD=Parkinson's disease; PSQI=Pittsburgh Sleep Quality Index

Supplementary Table 3. Associations of Pittsburgh Sleep Quality Index component scores with risk of parkinsonism and Parkinson's disease, per cumulatively increasing duration of follow-up

PSQI component	Outcome	N	Duration of follow-up time in years					
			≤2	≤4	≤6	≤8	≤10	Overall
			Hazard ratio per point increase (worse sleep) on the component score (95% CI)					
Quality	PS	7716	3.16 (0.95; 10.46)	2.33 (1.05; 5.17)	1.16 (0.68; 1.98)	1.08 (0.69; 1.69)	1.07 (0.73; 1.56)	1.09 (0.76; 1.57)
	PD	7709	3.90 (1.04; 14.58)	2.57 (1.01; 6.57)	1.17 (0.63; 2.18)	1.01 (0.60; 1.70)	0.93 (0.57; 1.50)	0.95 (0.60; 1.50)
Latency	PS	7718	2.48 (0.90; 6.83)	2.27 (1.14; 4.51)	1.54 (0.99; 2.40)	1.27 (0.87; 1.86)	1.11 (0.80; 1.55)	1.15 (0.84; 1.57)
	PD	7712	2.94 (0.97; 8.87)	2.16 (0.97; 4.79)	1.53 (0.91; 2.56)	1.19 (0.76; 1.85)	1.05 (0.70; 1.59)	1.06 (0.71; 1.57)
Efficiency	PS	7473	2.78 (0.97; 7.98)	1.98 (1.04; 3.77)	1.42 (0.90; 2.24)	1.05 (0.68; 1.60)	1.05 (0.73; 1.51)	0.99 (0.70; 1.41)
	PD	7466	4.54 (1.27; 16.18)	2.35 (1.09; 5.07)	1.65 (1.00; 2.73)	1.17 (0.73; 1.88)	1.09 (0.69; 1.71)	1.00 (0.64; 1.56)
Disturbances	PS	6840	0.70 (0.15; 3.25)	0.85 (0.29; 2.44)	1.08 (0.53; 2.21)	0.94 (0.52; 1.69)	0.75 (0.45; 1.23)	0.78 (0.48; 1.26)
	PD	6835	1.08 (0.20; 6.01)	0.96 (0.27; 3.34)	0.96 (0.42; 2.21)	0.76 (0.38; 1.52)	0.64 (0.34; 1.19)	0.64 (0.35; 1.18)
Sleep medication	PS	7725	1.08 (0.14; 8.32)	0.54 (0.09; 3.23)	1.28 (0.49; 3.33)	1.00 (0.42; 2.37)	1.29 (0.65; 2.56)	1.08 (0.55; 2.14)
	PD	7718	1.54 (0.19; 12.76)	0.73 (0.11; 4.68)	1.04 (0.32; 3.41)	0.95 (0.34; 2.66)	1.19 (0.50; 2.85)	1.06 (0.45; 2.52)
Daytime dysfunction	PS	7689	2.49 (0.48; 12.81)	3.00 (1.01; 8.89)	1.64 (0.72; 3.74)	1.40 (0.68; 2.87)	1.10 (0.56; 2.14)	0.94 (0.48; 1.82)
	PD	7684	3.34 (0.60; 18.55)	2.31 (0.62; 8.71)	1.24 (0.44; 3.51)	1.04 (0.41; 2.63)	0.85 (0.34; 2.10)	0.76 (0.31; 1.89)

The associations of the PSQI components with incident parkinsonism and Parkinson's disease are provided for cumulatively increasing follow-up duration within the study timeframe. Hazard ratio estimates were obtained from multivariate Firth's penalized Cox regression models by censoring all participants still at risk at year 2, 4, 6, 8 and 10 after baseline, and after the total follow-up of 13 years. Estimates are adjusted for age at baseline, sex, educational level, and smoking status, and are expressed per category increase in component score. To ensure sufficient (>10%) observations in each category, we combined scores 2 and 3 for components quality, latency and efficiency, and scores 1, 2 and 3 for components disturbances, medication and daytime dysfunction.

Abbreviations: CI=Confidence interval; N=Sample size; PD=Parkinson's disease; PS=parkinsonism; PSQI=Pittsburgh Sleep Quality Index.



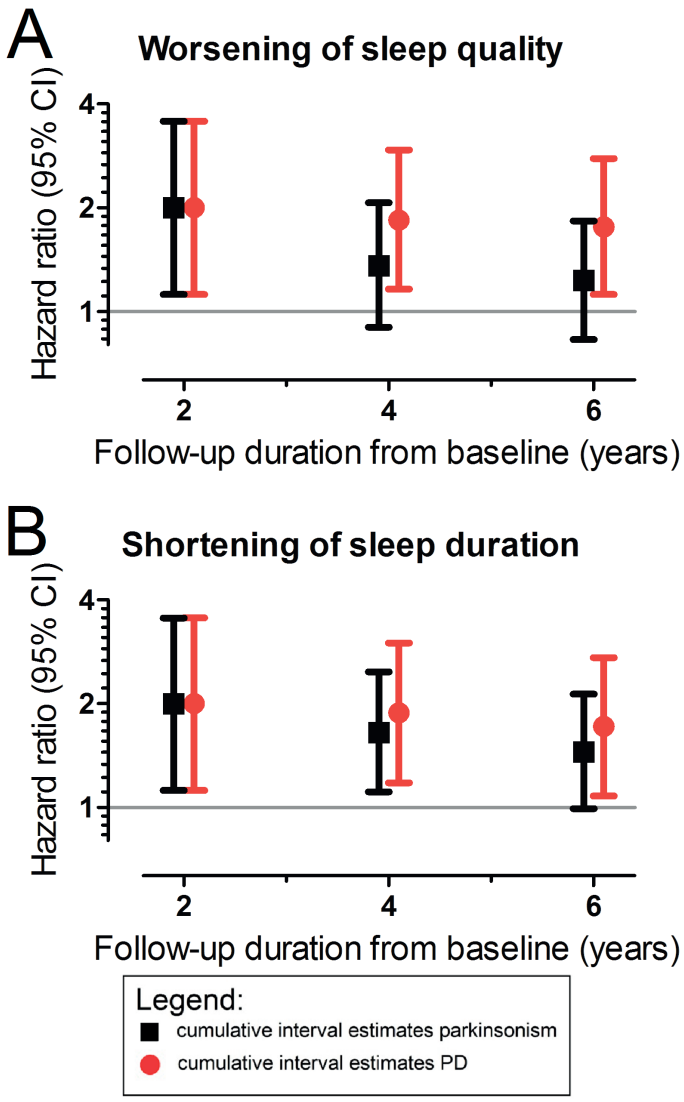
Supplementary Figure 3. Associations of sleep quality and duration with parkinsonism and Parkinson's disease in persons without comorbid depression and anxiety, per cumulatively increasing duration of follow-up

Associations of (A) sleep quality and (B) sleep duration with incident parkinsonism, and (C) sleep quality and (D) sleep duration with incident Parkinson's disease, analyzed both in the total sample and in persons without clinically relevant depressive symptoms and anxiety disorders. Associations are depicted for cumulatively increasing follow-up duration within the study timeframe. Hazard ratio estimates were obtained from multivariate Firth's penalized Cox regression models by censoring all participants still at risk at year 2, 4, 6, 8 and 10 after baseline, and after the total follow-up of 13 years. Estimates were adjusted for age at baseline, sex, educational level and smoking status, are expressed per standard deviation increase of PSQI score or sleep duration, and are plotted at a logarithmic (base 2) scale. Abbreviations: CI=Confidence Interval; PSQI=Pittsburgh Sleep Quality Index

Supplementary Table 4. Association of sleep quality or duration and risk of incident Parkinson's disease or parkinsonism, stratified by potential effect-modifiers

Effect-modifier	Strata	Cases/N	Parkinsonism HR per SD increase (95% CI)	P_{int}	Cases/N	Parkinson's dis. HR per SD increase (95% CI)	P_{int}
Sleep quality							
Age	≤67.5 y.	12/384	0.56 (0.31 – 1.00)	0.816	10/3837	0.62 (0.32 - 1.19)	0.683
	>67.5 y.	63/3,840	1.07 (0.82 - 1.38)		37/3837	0.95 (0.69 - 1.32)	
Sex	Male	42/3,305	1.01 (0.74 - 1.38)	0.289	24/3300	0.81 (0.53 - 1.22)	0.479
	Female	33/4,375	0.89 (0.63 - 1.25)		23/4374	0.93 (0.62 - 1.41)	
Park. signs	Present	16/804	**1.96 (1.07 - 3.59)**	**0.004**	12/802	1.53 (0.80 - 2.91)	**0.048**
	Absent	59/6,876	0.80 (0.62 - 1.03)		35/6872	**0.72 (0.52 – 1.00)**	
Sleep duration							
Age[a]	≤67.5 y.	12/3,863	1.84 (0.97 - 3.50)	0.778	10/3859	1.68 (0.82 - 3.47)	0.406
	>67.5 y.	63/3,863	1.11 (0.86 - 1.44)		37/3859	1.14 (0.82 - 1.60)	
Sex	Male	42/3,330	1.02 (0.73 - 1.43)	0.218	24/3323	1.26 (0.79 - 2.01)	0.870
	Female	33/4,396	1.39 (0.98 - 1.97)		23/4395	1.21 (0.80 - 1.82)	
Park. signs	Present	16/807	1.00 (0.62 - 1.60)	0.270	12/805	1.09 (0.64 - 1.86)	0.460
	Absent	59/6,919	1.29 (0.98 - 1.71)		35/6913	1.31 (0.91 - 1.89)	

The associations of sleep quality and sleep duration with incident parkinsonism and Parkinson's disease are shown stratified for several potential effect-modifiers. Hazard ratios were obtained from Cox regression models, adjusted for (if applicable) age at baseline, sex, educational level, and smoking status, and are expressed per standard deviation increase of global Pittsburgh Sleep Quality Index score or sleep duration. Multiplicative interaction was tested in a model including the main effects of the stratified variable, and a untransformed and non-standardized variable of sleep quality or sleep duration. Split at median age in sample. **Bold** indicates statistical significance at P<0.05. Abbreviations: CI=Confidence interval; dis=disease; HR=Hazard ratio; N=sample size; Park=parkinsonian; P_{int}=P-value interaction term; SD=standard deviation; y=years.



Supplementary Figure 4. Associations of changes in sleep quality and duration between the baseline and follow-up visit with risk of parkinsonism and Parkinson's disease, per cumulatively increasing duration of follow-up

The associations of changes in (A) sleep quality ('worsening') and (B) sleep duration ('shortening') between the baseline and follow-up visit with incident parkinsonism and Parkinson's disease are shown for cumulatively increasing follow-up duration within the six-year study timeframe. Hazard ratio estimates were obtained from multivariate Firth's penalized Cox regression models by censoring all participants still at risk at year 2, 4, and after the total follow-up of 6 years. Hazard ratio estimates were adjusted for age at baseline, sex, educational level, smoking status, and time interval between measurements, are expressed per standard deviation increase of (A) worsening sleep quality, or (B) shorter sleep duration, and are plotted at a logarithmic (base 2) scale. Abbreviations: CI=Confidence Interval; PD=Parkinson's disease; PSQI=Pittsburgh Sleep Quality Index.

Supplementary Table 5. Characteristics of study population at follow-up visit

Characteristic (unit)	N = 5,450
Age at baseline (years)	68.4 ± 8.9
Female	3,127 (57%)
Educational level	
Primary education	398 (7%)
Lower/intermediate or lower vocational	2,161 (39%)
Higher or intermediate vocational	1,636 (30%)
Higher vocational or university	1,259 (23%)
Smoking status	
Never smoker	1,748 (32%)
Former smoker	3,068 (56%)
Current smoker	637 (12%)
Cognitive functioning (MMSE score)	29 (27-29)
Presence of any parkinsonian signs	806 (15%)
Time interval between baseline and follow-up visits (years)	6.0 ± 0.6
Missing	49 (1%)
Sleep quality (global PSQI score)	3 (1-6)
Missing	9 (0%)
Change in sleep quality compared to baseline (global PSQI score increase)	0.0 ± 3.1
Missing	211 (4%)
Sleep duration (hours)	6.9 ± 1.3
Change in sleep duration compared to baseline (hours decrease)	-0.1 ± 1.16
Missing	173 (3%)

Characteristics for eligible study population for analyses of sleep change at the follow-up visit. Values are expressed as frequency (%) for categorical variables and mean ± standard deviation or median (interquartile range) for continuous variables, unless specified otherwise. Includes imputed values for covariates. Abbreviations: MMSE=Mini-mental state examination; PSQI=Pittsburgh Sleep Quality Index.

....want tussen droom en daad,
staan wetten in de weg,
en praktische bezwaren,
en ook weemoedigheid,
die niemand kan verklaren,
en die des avonds komt,
wanneer men slapen gaat.

Willem Elsschot. Het Huwelijk. Rotterdam (1908).

4 NEUROBIOLOGICAL CORRELATES

4.1 PLASMA BIOMARKERS OF NEURODEGENERATIVE DISEASE

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Submitted

ABSTRACT

We aimed to investigate how disturbances in sleep and 24-hour activity rhythm are related to neurofilament light chain, an emerging plasma-based marker of neurodegenerative disease.

We included 4,712 persons from the Rotterdam Study who self-rated their sleep using the Pittsburgh Sleep Quality Index. A subset of 849 persons further underwent objective assessment of sleep and 24-hour activity rhythms using actigraphy. Simoa® assays were used to measure plasma levels of neurofilament light chain and additionally β-amyloid 40, β-amyloid 42, and total-tau. Cross-sectional associations of sleep and 24-hour activity rhythms with biomarkers were assessed with multivariable linear regression models, adjusting for relevant confounders.

Associations of self-rated sleep, actigraphy-estimated sleep and 24-hour activity rhythms with neurofilament light chain were not statistically significant after multivariable adjustment and correction for multiple testing, except for a non-linear association of self-rated time in bed with neurofilament light chain ($P=2.4*10^{-4}$). Similarly, we observed no significant associations with β-amyloid 40, β-amyloid 42, and total-tau after multiple testing correction.

Sleep and 24-hour activity rhythms are not associated with neuronal damage, as indicated by plasma neurofilament light chain, in the general middle-aged and elderly population. Previously reported associations of sleep and 24-hour activity rhythm disturbances with risk of neurodegenerative diseases such as all-cause dementia and Alzheimer's disease are likely mediated, or driven, by other factors.

INTRODUCTION

Sleep and 24-hour activity rhythm disturbances have been implicated in the etiology of neurodegenerative diseases such as dementia,[1-4] but it remains largely unclear what pathophysiological processes explain these findings.[4] Most studies have focused on beta-amyloid and tau pathology, both central hallmarks of Alzheimer's disease.[1,3,5] Yet, disturbed sleep and 24-hour activity rhythms may be linked to neurodegenerative disease risk through other pathophysiological processes as well.[6-12]

One key pathophysiological process in neurodegenerative diseases, including dementia, is neuronal damage.[12] Neuronal damage can be captured in vivo by cerebrospinal fluid (CSF) levels of the cytoskeletal protein neurofilament light chain (NfL).[13,14] Importantly, NfL cannot only be determined in CSF but also less invasively in blood.[15] This broad biomarker might therefore be well suited to capture any impact of sleep and 24-hour activity rhythms on neurodegenerative disease.

Studies that implemented blood-based NfL measurements have investigated the potential impact of sleep, but not 24-hour activity rhythm disturbance, on neuronal damage.[16-21] One study showed that chronic insomniacs have higher serum NfL than controls, which may decrease after treatment.[16] Others found no relation of disordered, subjectively impaired or experimentally deprived sleep with NfL in CSF or plasma.[17-21] To date, no large-scale population-based study investigated the relation of sleep and 24-hour activity rhythm disturbances with neuronal damage indicated by NfL.

We studied the associations of sleep and 24-hour activity rhythms with plasma NfL in individuals from the population-based Rotterdam Study cohort, hypothesizing that both poor sleep and disturbed 24-hour activity rhythms were associated with higher plasma NfL. For comparison, we also studied associations of sleep and 24-hour activity rhythms with other plasma biomarkers of neurodegenerative disease (β-amyloid 40 [$A\beta_{40}$], $A\beta_{42}$, and total tau [t-tau]).

METHODS

Study setting

This study is embedded in the population-based, prospective Rotterdam Study cohort, which includes individuals from a suburban district in Rotterdam, the Netherlands.[22] The cohort was initiated in 1990, including 7,983 participants aged ≥55 years, and was expanded first in 2000 with 3,011 participants aged ≥55 years, and again in 2006 with persons aged ≥45 years, totaling 14,926 participants. Examination rounds are repeated every 4 to 5 years.

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl) and into the WHO International Clinical Trials Registry Platform (ICTRP; www.who.int/ictrp/network/primary/en/) under shared catalogue number NTR6831. All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

Study population

Between 2002 and 2005, 6,044 participants from the initiation cohort and first expansion round underwent venipuncture at the dedicated research center. Of those, 5,069 had sufficient plasma stores available for analyzing biomarkers. We excluded 232 persons for whom valid data on plasma NfL could not be obtained, and 20 persons with all-cause dementia at baseline to focus on at-risk individuals only. From the remaining 4,817 participants, 4,712 provided valid data on one or more questionnaire-derived sleep parameters, of which 4,354 persons provided data on all parameters.

Also, of 4,817 participants, 1,346 individuals were invited to participate in an actigraphy study[23]; 970 agreed. Of these, 849 persons (88%) provided valid data for a minimum of 4 consecutive 24-hour periods.[23]

Self-rated sleep

Participants rated sleep using a Dutch version of the Pittsburgh Sleep Quality Index (PSQI[24]). The PSQI measures sleep over the past month and has a good test-retest reliability and validity in a non-clinical sample of older adults.[25] Items include bedtimes and total sleep time at night, from which we derived time in bed and sleep efficiency, and time to fall asleep (sleep latency). Additionally, all items were summed to obtain the global PSQI score, indicating subjective sleep quality. The PSQI score ranges from 0-21, and higher scores indicate a poorer subjective sleep quality.

We excluded persons missing ≥2 PSQI components (n=60), and calculated a weighted global PSQI score when only 1 component was missing (n=173) by multiplying the six-component sum score by 7/6. The PSQI was completed a median of 18 days (interquartile range [IQR] =17-19) before venipuncture.

Objectively estimated sleep and 24-hour activity rhythms

Participants wore an actigraph (Actiwatch model AW4, Cambridge Technology Ltd.) which measures acceleration summed as counts per 30-second epochs. We instructed participants to wear the actigraph for 7 days and nights around the non-dominant wrist, and to remove it only while bathing. Participants had to press a marker button on the

device when attempting to fall asleep (hereafter: 'lights out') and getting out of bed the next morning (hereafter: 'lights on'). They also kept a daily sleep diary.[23] Missing marker times were imputed from the diary, or estimated by inspecting recordings if diary times were missing. We removed 24-hour periods in which >3 continuous hours of no activity were recorded to minimize a 'time of day' effect. Actigraphy recordings averaged 137.9 ± 13.6 hours, and were initiated a median of 28 days (IQR=9-287) after venipuncture. Within the marker-defined time in bed, we estimated sleep (i.e. total sleep time) and wakefulness using a validated algorithm with a threshold of 20 counts.[23] We defined 'sleep start' as the midpoint of the first immobile ≥10 minute period after 'lights out' with ≤1 movement epoch.[23] Sleep onset latency was calculated as the time from 'lights out' to 'sleep start', and wake after sleep onset as wakefulness after 'sleep start'. We calculated sleep efficiency as total sleep time divided by time in bed * 100%.

We also used counts to calculate non-parametric indices of the 24-hour activity rhythm[26]: Intradaily variability (IV) which indicates the amount of alterations of activity-inactivity, interdaily stability (IS) which indicates how daily profiles in the recording resemble each other, and onset time of the least active 5 consecutive hours (L5 onset) which indicates the phase of lowest activity. A disturbed 24-hour activity rhythm is reflected by a high IV and a low IS.

Measurement of plasma concentrations of NfL, $A\beta_{40}$, $A\beta_{42}$, and t-tau

Participants came to the dedicated research center where a venipuncture was performed between 8:00-10:30 AM after an overnight fast. Blood was sampled in ethylenediamine tetra-acetic acid-treated containers and centrifuged. The plasma was aliquoted and frozen at -80^{0}C according to standard procedures. Measurements were performed in two batches. All measurements were performed at Quanterix (Lexington, MA, USA) on a single molecule array (Simoa®) HD-1 analyzer platform[27] and samples were tested in duplicate. Two quality control samples were run on each plate for each analyte. Neurofilament light chain was measured by using the NF-light advantage kit.[28] The Simoa Human Neurology 3-Plex A assay was used for measuring the concentration of $A\beta_{40}$, $A\beta_{42}$, and t-tau. Data was excluded if duplicates or single measurements were missing, if the concentration coefficient of variation exceeded 20%, or control samples were out of range.

Covariates

We considered age, sex, education (categorized as primary, secondary/lower vocational, intermediate vocational and higher vocational/university), batch number of biomarker analysis, time interval between measurements of sleep and biomarker, habitual alcohol consumption, presence of self-reported paid employment, smoking status (never, former, current), body mass index (BMI), presence of hypertension (resting blood pressure >140/90 mmHg, or use of blood pressure-lowering medication), presence of

diabetes mellitus (fasting serum glucose level ≥7.0 mmol/l, or use of glucose-lowering medication), total cholesterol level in serum in mmol/l, a positive history of heart disease (myocardial infarction, heart failure, or coronary revascularization procedure), and possible sleep apnea defined using PSQI items on loud snoring and respiratory pauses[29] as potential confounders, or as proxies for unmeasured confounders. Measurements were performed during the home interview or center visits, as detailed previously.[30]

Additionally, we assessed clinically relevant depressive symptoms defined as a score <16 on the validated Dutch version[31] of the Centre for Epidemiological Studies - Depression scale (CES-D), cognitive impairment defined by an Mini Mental State Examination (MMSE) score ≤25, and a history of stroke ascertained during examination rounds and by continuous monitoring as detailed previously.[30]

All sleep parameters were winsorized at 3 SD from the mean, and subsequently standardized. Biomarker values were log-transformed (base=2) to approach a normal distribution, winsorized to 3 SD and standardized to facilitate comparison across different biomarkers.

We used linear regressions to analyze the association of sleep and 24-hour activity rhythm parameters with plasma NfL. We investigated self-rated sleep (PSQI score, total sleep time, sleep onset latency, time in bed, and sleep efficiency), actigraphy-estimated sleep (total sleep time, sleep onset latency, wake after sleep onset, time in bed, sleep efficiency), 24-hour activity rhythms (intradaily variability, interdaily stability and L5 onset) and times of 'lights out' and 'lights on'. Analyses were adjusted for age, sex, educational level, batch, and time interval between measurements of sleep and biomarkers (model 1), and additionally for alcohol consumption, employment status, smoking status, BMI, hypertension, diabetes mellitus, total cholesterol, history of heart disease, and possible sleep apnea (model 2). Furthermore, as total sleep time and time in bed are known to show U-shaped relations with various poor health outcomes, we assessed non-linear associations of these parameters (self-rated and actigraphy-estimated) with NfL by adding quadratic terms of the determinant.

We additionally restricted analyses to persons without clinically relevant depressive symptoms, without cognitive impairment, and without prevalent stroke.

Besides NfL, other biomarkers may also be potentially important. Therefore, we also examined associations of sleep and 24-hour activity rhythms with other plasma biomarkers of neurodegenerative disease: $A\beta_{40}$, $A\beta_{42}$, and t-tau.

We performed statistical testing and considered associations below the threshold of $P<0.0046$ as statistically significant, which corrected for testing 15 self-rated and actigraphy-estimated parameters in this study. This threshold was defined by computing the number of effective tests ($M_{eff}=11.14$) based on correlations between all parameters, and applying a Sidak correction. We considered associations as nominally significant at $P<0.05$.

Table 1. Characteristics of study population

Characteristic (unit)	Total sample	Actigraphy subsample
	N=4,712	N=849
Age at sleep measurement (years)	71.1 (66.1 – 77.2)	66.7 (63.7 – 73.1)
Female	2,700 (57%)	433 (51%)
Medium or higher education	2,088 (45%)	428 (51%)
Alcohol consumption (gr/day)	7 (1-20)	9 (1-20)
Paid employment	303 (6%)	74 (9%)
Never smoker	1,480 (31%)	264 (31%)
Body mass index (kg/m^2)	27.6 ± 4.1	27.9 ± 4.0
Hypertension	2,569 (54%)	414 (49%)
Diabetes mellitus	472 (10%)	84 (10%)
Total cholesterol in serum (mmol/l)	5.6 ± 1.0	5.7 ± 1.0
History of heart disease	704 (15%)	89 (10%)
Possible sleep apnea	580 (12%)	113 (13%)
Self-rated sleep		
Global PSQI score	3 (2-6)	3 (1-6)
Duration (hours)	6.8 ± 1.3	6.9 ± 1.2
Latency (minutes)	10 (5 - 30)	10 (5 - 30)
Time in bed (hours)	7.7 ± 1.1	7.7 ± 1.0
Efficiency (%)	93 (83 – 99)	93 (86 – 100)
Actigraphic sleep and 24h activity rhythms		
Total sleep time (hours)	-	6.5 ± 0.8
Latency (minutes)	-	18 (12 – 26)
Wake after sleep onset (hours)	-	1.1 (0.9 – 1.4)
Time in bed (hours)	-	8.3 ± 0.8
Efficiency (%)	-	79 (74 – 83)
Intradaily variability (score)	-	0.41 (0.34 – 0.52)
Interdaily stability (score)	-	0.83 (0.76 – 0.88)
L5 onset (hh:mm)	-	01:53 ± 01:10
'Lights out' time (hh:mm)	-	23:51 ± 00:48
'Lights on' time (hh:mm)	-	08:10 ± 00:45
Neurofilament light chain (pg/ml)	13 (10-18)	11 (9-15)
Range	3 – 390	4 – 214

Values are expressed as frequency (%) for categorical variables and mean ± standard deviation or median (1st quartile – 3rd quartile) for continuous variables. Includes imputed values for covariates. Missing values for self-rated sleep parameters were 60 for PSQI score, 58 for sleep duration, 198 for sleep latency, 159 for time in bed, and 212 for sleep efficiency. Actigraphic time in bed was not automatically calculated but based on 'lights out' and 'lights on' times specified daily by participants using the actigraph marker buttons and a sleep diary. Abbreviations: L5=Least active 5 hours of the day; N=sample size; PSQI=Pittsburgh Sleep Quality Index.

Missing values on covariates were imputed using five multiple imputations with IBM SPSS Statistics version 24 (IBM Corp, Armonk, NY). Analyses were performed with R software.

RESULTS

For self-rated sleep parameters, we found no significant linear associations with plasma NfL in model 2 (Table 2). The association of self-rated longer time in bed with higher NfL in model 1 (beta per standard deviation [SD] increase of 0.038 SD increase in log(NfL), 95% confidence interval [CI] 0.015; 0.060, P=0.0013) was attenuated after additional multivariable adjustment (Table 2). The quadratic term of self-rated time in bed was significantly associated with NfL in model 2 ($P=2.4*10^{-4}$): Compared to a self-rated nor-

Table 2. Associations of self-rated and actigraphy-estimated sleep parameters with neurofilament light chain levels in plasma

Determinants	Model 1		Model 2	
	Mean diff. (95% CI)	P	Mean diff. (95% CI)	P
Self-rated				
PSQI score	0.023 (-0.001; 0.046)	0.06	0.014 (-0.009; 0.037)	0.23
Sleep duration	0.005 (-0.018; 0.027)	0.68	0.006 (-0.015; 0.028)	0.57
Sleep latency	0.017 (-0.010; 0.044)	0.23	0.006 (-0.021; 0.032)	0.66
Time in bed	0.038 (0.015; 0.060)	0.001	0.032 (0.009; 0.054)	0.01
Sleep	-0.032 (-0.056; -0.008)	0.01	-0.024 (-0.048; -0.001)	0.04
Actigraphy				
Total sleep time	-0.006 (-0.058; 0.047)	0.83	-0.030 (-0.082; 0.022)	0.26
Sleep latency	-0.008 (-0.062; 0.045)	0.76	-0.004 (-0.057; 0.048)	0.88
WASO	0.023 (-0.028; 0.073)	0.37	0.021 (-0.028; 0.071)	0.40
Time in bed[a]	0.001 (-0.051; 0.053)	0.97	-0.021 (-0.073; 0.030)	0.41
Sleep efficiency	-0.004 (-0.055; 0.047)	0.87	-0.016 (-0.066; 0.034)	0.52

Estimates indicate standard deviations change in NfL with a standard deviation increase in the determinant. Estimates were obtained with linear regression, adjusted for age and sex, educational level, batch, time interval between measurement of sleep and biomarkers (model 1), and additionally for alcohol consumption, employment status, smoking status, body mass index, presence of hypertension, presence of diabetes mellitus, total serum cholesterol level, history of cardiovascular disease, and possible sleep apnea (model 2). Analyses were performed in 4,652 persons for PSQI score, in n=4,654 for sleep duration, in n=4,514 for sleep latency, in n=4,553 for time in bed, and in n=4,500 for sleep efficiency. Actigraphy analyses were performed in 849 persons. Please note that actigraphy-derived time in bed was not automatically calculated but based on 'lights out' and 'lights on' times, specified daily by participants using actigraph marker buttons and a sleep diary. Abbreviations: CI=Confidence interval; diff.=difference; PSQI=Pittsburgh Sleep Quality Index; WASO=Wake after sleep onset.

mal time in bed (7-9 hours), spending a long time in bed (>9 hours) was significantly associated with higher NfL (0.174, 95% CI 0.087; 0.261, P=8.6*10^{-5}), but spending a short time in bed (<7 hours) was not (-0.007, 95% CI -0.056; 0.041, P=0.76).

Actigraphy-estimated sleep parameters were not related to NfL in plasma (Table 2). We found no non-linear associations for actigraphy-estimated total sleep time and time in bed.

We observed no significant associations of 24-hour activity rhythm parameters with NfL beyond the multiple testing corrected threshold (Table 3).

Restricting the abovementioned main analyses to individuals without clinically relevant depressive symptoms, without cognitive impairment or stroke did not substantially change effect sizes (Table 4).

For comparison, we also investigated associations of sleep and 24-hour activity rhythm parameters with other biomarkers of neurodegenerative disease. Median (IQR) plasma levels in pg/mL for 4,712 persons were 259.5 (230.3 – 294.0) for $A\beta_{40}$, 10.3 (8.8 – 11.9) for $A\beta_{42}$ and 2.4 (1.9 – 3.0) for t-tau. In comparison to associations with NfL, we observed slightly larger effect sizes and more associations exceeding P<0.05 including associations of poorer subjective sleep quality, longer self-rated time in bed and lower self-rated sleep efficiency with higher plasma concentrations of β-amyloid isoforms (Table 5). Yet, no association was statistically significant beyond the threshold corrected for multiple testing (Table 5).

Table 3. Associations of actigraphy-estimated 24-hour activity rhythm parameters and bedtimes with neurofilament light chain in plasma

Determinants	Model 1		Model 2	
	Mean difference (95% CI)	P	Mean difference (95% CI)	P
Intradaily variability	0.022 (-0.033; 0.078)	0.43	0.036 (-0.019; 0.092)	0.19
Interdaily stability	0.000 (-0.051; 0.052)	0.99	-0.017 (-0.068; 0.033)	0.50
L5 onset	-0.008 (-0.059; 0.043)	0.76	-0.005 (-0.054; 0.045)	0.85
'Lights out' time	-0.050 (-0.103; 0.004)	0.07	-0.033 (-0.086; 0.020)	0.22
'Lights on' time	-0.044 (-0.095; 0.008)	0.10	-0.049 (-0.100; 0.001)	0.06

Estimates indicate standard deviations change in NfL with a standard deviation increase in the determinant Outcome values of neurofilament light chain (NfL) in nmol/l were natural log-transformed and then expressed per standard deviation of ln(NfL). Estimates were obtained with linear regression, adjusted for age and sex, educational level, batch, time interval between measurement of sleep and biomarkers (model 1), and additionally for alcohol consumption, employment status, smoking status, body mass index, presence of hypertension, presence of diabetes mellitus, total serum cholesterol level, history of cardiovascular disease, and possible sleep apnea (model 2). Analyses were all performed in 849 persons. Please note that actigraphy-derived bedtimes were specified daily by participants using actigraph marker buttons and a sleep diary.Abbreviations: CI=Confidence interval; L5=average least active 5 hours of the day; SD=Standard deviation.

Table 4. Associations of sleep with neurofilament light chain in plasma in persons without depressive symptoms, cognitive impairment or stroke

Determinants	No depressive symptoms	No cognitive impairment	No stroke
	Mean diff. (95% CI)	Mean diff. (95% CI)	Mean diff. (95% CI)
Self-rated			
PSQI	-0.001 (-0.026; 0.025)	0.008 (-0.017; 0.032)	0.010 (-0.013; 0.033)
TST	0.015 (-0.009; 0.038)	0.001 (-0.023; 0.025)	0.006 (-0.016; 0.028)
SOL	0.000 (-0.029; 0.030)	-0.001 (-0.03; 0.028)	0.001 (-0.026; 0.028)
TIB	0.035 (0.012; 0.059)**	0.022 (-0.002; 0.046)	0.028 (0.006; 0.051)*
SE	-0.016 (-0.042; 0.009)	-0.022 (-0.047; 0.003)	-0.022 (-0.045; 0.001)
Actigraphy			
TST	-0.024 (-0.077; 0.030)	-0.027 (-0.085; 0.030)	-0.029 (-0.082; 0.025)
SOL	-0.013 (-0.069; 0.042)	-0.003 (-0.061; 0.054)	-0.003 (-0.055; 0.049)
WASO	0.011 (-0.040; 0.062)	0.016 (-0.037; 0.069)	0.021 (-0.029; 0.070)
TIB	-0.032 (-0.086; 0.021)	-0.029 (-0.085; 0.028)	-0.016 (-0.069; 0.036)
SE	-0.001 (-0.053; 0.051)	-0.010 (-0.064; 0.044)	-0.016 (-0.066; 0.034)
IV	0.018 (-0.039; 0.076)	0.046 (-0.015; 0.107)	0.042 (-0.012; 0.097)
IS	-0.006 (-0.058; 0.047)	-0.018 (-0.074; 0.038)	-0.010 (-0.060; 0.041)
L5 onset	-0.014 (-0.065; 0.037)	-0.023 (-0.076; 0.031)	0.003 (-0.047; 0.053)
Lights out	-0.035 (-0.089; 0.020)	-0.029 (-0.086; 0.027)	-0.039 (-0.091; 0.014)
Lights on	-0.063 (-0.115; -0.010)*	-0.055 (-0.111; 0.001)	-0.050 (-0.101; 0.001)

Absence of depressive symptoms was defined as CES-D score ≥16; absence of cognitive impairment as defined as MMSE score >25. Estimates were obtained with linear regression, indicate standard deviations change in NfL with a standard deviation increase in the determinant), and were adjusted for age and sex, educational level, batch, time interval between measurement of sleep and biomarkers, alcohol consumption, employment status, smoking status, body mass index, presence of hypertension, presence of diabetes mellitus, total serum cholesterol level, history of cardiovascular disease, and possible sleep apnea. For self-rated determinant analyses, cases per analysis ranged from 4,063-4,181 restricted to persons without depressive symptoms, from 3,909-4,042 in persons without cognitive impairment, and from 4,289-4,431 in persons without prevalent stroke. For actigraphy-derived determinants, cases in analyses were n=785 (depressive symptoms), n=756 (cognitive impairment) and n=817 (stroke). Please note that actigraphic time in bed was not automatically calculated but determined by 'lights out' and 'lights on' times specified through pressing actigraph marker buttons and the sleep diary. ** P=0.0035; *Nominal statistical significance at P<0.05. Abbreviations: CES-D= Center for Epidemiological Studies – Depression scale; CI=Confidence interval; diff.=difference; IS=Interdaily stability; IV=Intradaily variability; L5=average least active 5 hours of the day; MMSE=Mini-mental state examination; PSQI=Pittsburgh Sleep Quality Index; SD=Standard deviation; SE=Sleep efficiency; SOL=sleep onset latency; TIB=Time in bed; TST=Total sleep time; WASO=Wake after sleep onset

Table 5. Associations of sleep and 24-hour activity rhythms with biomarkers of neurodegenerative disease in plasma

Determinants	β-amyloid 40	β-amyloid 42	Total tau
	Mean diff. (95% CI)	Mean diff. (95% CI)	Mean diff. (95% CI)
Self-rated			
PSQI score	0.020 (-0.008; 0.047)	0.030 (0.002; 0.057)*	-0.016 (-0.045; 0.013)
TST	0.005 (-0.021; 0.032)	-0.007 (-0.034; 0.020)	0.018 (-0.009; 0.046)
SOL	0.008 (-0.025; 0.040)	0.009 (-0.023; 0.041)	-0.003 (-0.036; 0.031)
TIB	0.033 (0.006; 0.060)*	0.032 (0.005; 0.059)*	0.019 (-0.009; 0.047)
SE	-0.020 (-0.048; 0.008)	-0.038 (-0.066; -0.010)*	0.009 (-0.020; 0.038)
Actigraphy			
TST	-0.051 (-0.116; 0.013)	-0.025 (-0.086; 0.036)	0.034 (-0.032; 0.100)
SOL	-0.001 (-0.066; 0.064)	0.019 (-0.042; 0.080)	0.007 (-0.060; 0.074)
WASO	0.049 (-0.012; 0.110)	0.051 (-0.006; 0.109)	0.045 (-0.018; 0.108)
TIB[b]	-0.036 (-0.099; 0.028)	0.005 (-0.055; 0.065)	0.061 (-0.004; 0.127)
SE	-0.047 (-0.109; 0.015)	-0.050 (-0.108; 0.008)	-0.028 (-0.092; 0.036)
IV	0.066 (-0.002; 0.134)	-0.002 (-0.067; 0.062)	-0.007 (-0.077; 0.063)
IS	-0.022 (-0.085; 0.041)	0.018 (-0.041; 0.077)	-0.025 (-0.089; 0.040)
L5 onset	0.019 (-0.042; 0.080)	0.027 (-0.031; 0.085)	0.020 (-0.043; 0.083)
Lights out	0.007 (-0.058; 0.072)	-0.017 (-0.079; 0.044)	0.019 (-0.048; 0.085)
Lights on	-0.027 (-0.089; 0.036)	-0.017 (-0.076; 0.042)	0.074 (0.010; 0.139)*

Estimates were obtained with linear regression, indicate standard deviations change in biomarker with a standard deviation increase in the determinant, and are adjusted for age and sex, educational level, batch, time interval between measurement of sleep and biomarkers, alcohol consumption, employment status, smoking status, body mass index, presence of hypertension, presence of diabetes mellitus, total serum cholesterol level, history of cardiovascular disease, and possible sleep apnea. Numbers of cases per analysis differed as both determinants and outcomes had different numbers of missing values. For self-rated determinants, numbers varied from 4,486 (association total sleep time with total tau) to 4,146 (sleep efficiency with β-amyloid 42). For actigraphy-derived determinants (all n=849), numbers varied from 824 (total tau) to 806 (β-amyloid 42). Please note that actigraphic time in bed was not automatically calculated but based on 'lights out' and 'lights on' times specified by participants. *Nominal statistical significance at P<0.05. Abbreviations: CI=Confidence interval; diff.=difference; IS=Interdaily stability; IV=Intradaily variability; L5=average least active 5 hours of the day; PSQI=Pittsburgh Sleep Quality Index; SD=Standard deviation; SE=Sleep efficiency; SOL=sleep onset latency; TIB=Time in bed; TST=Total sleep time; WASO=Wake after sleep onset;

DISCUSSION

In this population-based study in middle-aged and elderly persons, sleep and 24-hour activity rhythms were not associated with plasma NfL, except for a non-linear association of self-rated time in bed with NfL.

We speculate that the association of self-rated long time in bed with higher plasma NfL might not reflect sleep per se, but instead overall poor health or underlying subclinical disease.[32,33] Sensitivity analyses suggested that cognitive impairment or stroke, but not depressive symptoms, could be examples of underlying impaired health explaining the association of self-rated longer time in bed and higher plasma NfL. Further research is needed to investigate to what extent self-rated time in bed is a more valid marker of overall health than sleep per se.

Recently, we demonstrated that actigraphy-estimated poor sleep was associated with the risk of all-cause dementia and Alzheimer's disease in the Rotterdam Study. (Lysen *et al*., *submitted*) Yet, sleep and 24-hour activity rhythm disturbances are not clearly associated with NfL in the current study which is embedded in the same cohort, suggesting that pathophysiological processes indicated by NfL do not play a role in the association of poor sleep with dementia. These findings could be explained in several ways.

First, the potentially harmful effects of poor habitual sleep or 24-hour activity rhythm disturbances on neuronal health may not lead to NfL release. At a cellular level, release of NfL, most abundantly present in the axon, occurs after apoptosis or axon-specific neuronal insults.[34,35] Sleep or 24-hour activity rhythm disturbances may involve neuronal insults that invoke various stress responses that potentially impair neuronal function, but not lead to apoptosis. Other pathophysiological processes are therefore likely to underlie the link between sleep, 24-hour activity rhythms and neurodegenerative disease.

Second, we measured sleep with questionnaires and actigraphy. These measures cannot diagnose sleep disorders such as insomnia or sleep-disordered breathing,[36] or important physiological aspects of sleep such as slow-wave activity. This could explain why a previous study showed higher serum NfL in chronic insomniacs versus controls, while we found no population-based association of subjective sleep quality, an insomnia-related construct, with NfL.[16]

Third, the sleep and 24-hour activity rhythm disturbances studied here may not have been severe enough to elevate NfL in plasma. Our hypothesis was based on mechanistic, animal-based studies[6-8,37-39] using mostly experimental sleep deprivation. However, we studied observational differences in habitual sleep, and these more chronic disturbances might pose less harm to neuronal health than experimentally induced reductions in sleep. Indeed, a previous study also did not find an association of observational differences in subjective sleep quality with NfL, using CSF measurements.[18] Additionally, experimental studies that used partially deprived sleep to only four hours for five nights

also found no effects on NfL in CSF,[17] or serum.[20] This suggest that the relation of sleep with NfL seems limited.

Compared to NfL, the associations of sleep and 24-hour activity rhythms with $A\beta_{40}$, $A\beta_{42}$ and t-tau in plasma were slightly more pronounced in effect size, yet no associations survived multiple testing correction. This is surprising as sleep is known to regulate brain β-amyloid levels,[6] and habitual sleep is related to CSF β-amyloid, and parenchymal β-amyloid deposition.[5] We measured $A\beta_{42}$ in plasma which may be subject to more disturbing factors and may differ from measurement in CSF.[40] This could have obscured detecting an association and should be studied further.

Several methodological considerations need to be mentioned. First, our largely negative findings could indicate invalidity of our measurement in plasma instead of CSF. Yet, high NfL and reduced $A\beta_{42}$ were associated with an increased risk of all-cause dementia and Alzheimer's disease in our cohort (De Wolf *et al*., *in press*). Second, correlations of NfL between CSF and plasma are lower in healthy versus diseased persons,[41] lowering our sensitivity to detect relevant plasma NfL increases, especially in the actigraphy subgroup. Third, associations with plasma NfL may not reflect increased damage but differential equilibration across fluid compartments, as poor sleep may disturb blood-brain barrier function.[42,43] Fourth, cross-sectional associations may not be detected as plasma NfL levels may lag behind neuronal injury by months.[44] Yet, our single sleep measures are relatively stable over time,[45] and we adjusted analyses for the time interval between sleep and NfL measurement. Fifth, actigraphy estimates may misclassify sleep and only indirectly reflect circadian functioning. Study strengths include using a large sample anchored in the general population, measuring sleep with two modalities, simultaneously investigating multiple relevant biomarkers, and correcting for various confounders.

In conclusion, sleep and 24-hour activity rhythm disturbances in the general middle-aged and elderly population are not consistently associated with plasma NfL, even though sleep disturbances and NfL have separately been associated with incident all-cause dementia and Alzheimer's disease. Therefore, associations linking sleep and 24-hour activity rhythms with these incident neurodegenerative disease are unlikely to be mediated, or driven, by neuronal damage as indicated by plasma NfL.

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4.2 ENLARGED PERIVASCULAR SPACES

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ABSTRACT

Sleep has been hypothesized to facilitate waste clearance from the brain. Reduced waste clearance may be indicated by an enlargement of perivascular spaces on brain magnetic resonance imaging (MRI). Therefore, we investigated the association of sleep with perivascular space counts.

In 561 participants (mean age 62 ± 6, 52% women) from the population-based Rotterdam Study cohort, we measured total sleep time, sleep onset latency, wake after sleep onset and sleep efficiency with actigraphy and polysomnography. The number of perivascular spaces was determined in four regions (centrum semiovale, basal ganglia, hippocampus and midbrain) via a machine learning algorithm using T2-contrast MR images. Associations were analysed with zero-inflated negative binomial regression models adjusted for potential confounders, taking into account multiple testing.

Higher actigraphy-estimated sleep efficiency was associated with a higher perivascular space count in the centrum semiovale (odds ratio 1.10, 95% confidence interval 1.04-1.16, P=0.0009). No polysomnographic sleep parameters were associated with perivascular space count in any region. Results were largely similar after additionally accounting for sleep-disordered breathing parameters, brain volumes, cerebral small vessel disease markers, or the time between measurements of sleep and MRI in our analyses.

The association of sleep with perivascular space counts in the middle-aged and elderly population remains limited to an association of higher actigraphy-estimated sleep efficiency with higher perivascular space load in the centrum semiovale. Further work is needed to determine the significance to glymphatic clearance, and sleep.

INTRODUCTION

Sleep has been hypothesized to be a key driver of waste clearance from the brain.[1] Brain waste clearance involves a glia-dependent, lymphatic-like system named the 'glymphatic' system.[2,3] Glymphatic clearance involves exchange of interstitial and cerebrospinal fluid across the perivascular space,[2] which surrounds small blood vessels throughout the brain.[2] Sleep has been shown to substantially enhance such clearance.[1] Although glymphatic clearance and its determinants have been primarily studied in animals,[4,5] emerging evidence also supports a role of sleep in waste clearance from the brain in humans.[6-8]

It has been suggested that glymphatic clearance in humans can be studied through visualizing perivascular spaces on brain magnetic resonance imaging (MRI).[9,10] Perivascular spaces can become visible on brain MRI when enlarged. This enlargement, although still a poorly understood phenomenon,[10,11] is deemed abnormal[5] as high perivascular space load on MRI is associated with vascular and neurodegenerative pathologies, and a related increased risk of stroke and dementia.[10,12-15] Impaired glymphatic clearance is also implicated in the pathophysiology of these diseases,[4,5,16-19] suggesting that perivascular space load on MRI could mark impaired glymphatic clearance.[9-11]

Several clinical and population-based studies determined the association of indicators of poor sleep with higher perivascular space load on MRI. Studies reported associations of lower sleep efficiency and shorter non-rapid eye movement (NREM) stage 3 sleep,[20] of obstructive sleep apnea,[21] of shorter total sleep time,[22] and of self-reported presence of interrupted sleep with higher perivascular space loads. Others reported no association.[23,24]One population-based study found a positive association of sleep efficiency with perivascular space load in the basal ganglia in a 97 participants, but not with sleep quality or apnea.[25] Considering these mixed results, it remains unknown to what extent sleep is important for brain waste clearance, as indicated by perivascular space load on brain MRI, in the general population. Determining this link may help support an etiological role of sleep disturbances, which are potentially amenable to treatment, in neurological diseases.

We explored the relation of sleep with perivascular space counts on MRI using data from middle-aged and elderly participants of the population-based Rotterdam Study cohort. We hypothesized that indicators of poor sleep were related to higher perivascular space counts.[20-23,25,26]

METHODS

Study setting and population

The Rotterdam Study cohort started in 1990 and aims to investigate common chronic diseases in the elderly.[27] The cohort has since been expanded twice and includes 14,926 participants aged 45 years and over. Examination rounds include a home interview and visits to the dedicated research center, and are repeated every 4-5 years.

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

Between 2011 and 2014, 2,135 persons were invited to participate in actigraphy substudy (1,773 agreed, 83%), and 1,750 persons were invited to participate in a polysomnography substudy (928 agreed, 53%). A total of 1,062 were invited to undergo both (656 agreed, 62%). No exclusion criteria were set except for being deemed able to understand instructions. In our main analysis, we included 561 individuals who had i) valid actigraphy for ≥4 days, ii) a valid 1-night polysomnography, and iii) a valid MRI-scan performed within a 2-year timeframe. Sensitivity analyses were performed in the full samples of 771 persons with valid polysomnography and MRI, and 1,228 persons with valid actigraphy and MRI.

Assessment of sleep

Participants wore an actigraph (Actiwatch, model AW4; Cambridge Technology, Cambridge, UK, or Geneactiv, Activinsights Ltd, Kimbolton, UK), measuring acceleration aggregated into activity counts per 30-second epochs.[28,29] We used a previously described method to ensure comparability of estimates obtained from these devices.[30,31] We instructed participants to wear the actigraph for 7 days and nights around the non-dominant wrist, to remove it only while bathing (only for Actiwatch), and to keep a daily sleep diary.[28]

Sleep data per night were considered invalid if no data was recorded, if the participant had discontinued wearing the actigraph, if it coincided with daylight savings or followed daylight savings occurring during the recording, or if sleep diary information on bedtime and get-up time from which time in bed was derived was invalid or missing.[31] Within the time in bed, the algorithm estimated the assumed sleep period based on sleep onset and sleep offset, as described previously.[28,32] It also estimated sleep versus wakefulness per 30-second epoch using a validated algorithm with a threshold of 20 activity counts.[32] The algorithm calculated total sleep time (the sum of all sleep epochs within the assumed sleep period), sleep onset latency (difference between diary-derived bedtime

and estimated sleep onset), wake after sleep onset (sum of all wake epochs within the assumed sleep period) and sleep efficiency (total sleep time / time in bed * 100%). Sleep parameters were averaged over all available nights.

Mean actigraphy recording duration was a median 144 hours (IQR=144-168).

For polysomnography at home, device and sensors were applied according to the American Association of Sleep Medicine (AASM) criteria by trained research assistants.[33] Sensors included six electroencephalography (EEG) channels (F3/A2, F4/A1, C3/A2, C4/A1, O1/A2, O2/A1), bilateral electro-oculography, chin electromyography, electrocardiography, respiratory belts on the chest and abdomen, oximetry, and a nasal pressure transducer and oronasal thermocouple. We instructed individuals to spend the night as normal as possible, without restrictions on bedtimes, activities or diet.

Participants signaled the times of 'lights out' and getting up on the device, from which time in bed was calculated. Missing times were extracted from the sleep diary. All recordings were scored by a Registered Polysomnographic Technologist to determine wake, non-rapid eye movement (NREM) sleep stage 1 (N1), N2, N3, and REM sleep.[33] We calculated total sleep time (sum of all sleep epochs regardless of stage), sleep onset latency (time from 'lights out' to the first epoch of sleep), wake after sleep onset (sum of wake epochs after sleep onset), and sleep efficiency (total sleep time / time in bed * 100%), and sleep stage durations (sum of epochs per stage).

Neuroimaging

Brain imaging was performed on a 1.5T MRI scanner (Signa Excite II, GE Healthcare, Milwaukee, WI, USA) providing T1-contrast (T1), T2 and T2*-weighted gradient-recalled-echo (T2*) sequences, as detailed previously.[34]

Perivascular spaces in the midbrain, hippocampi, basal ganglia and centrum semiovale were automatically classified, using a machine learning algorithm on T2-scans.[35] This algorithm was developed on visual ratings, performed with a standardized protocol,[10] defining perivascular spaces as linear, ovoid or round-shaped hyperintensities on T2 scans with a diameter of ≥1 mm, and <3 mm. Single slices were used to rate perivascular space counts in the centrum semiovale (1 cm above the uppermost part of the lateral ventricles) and the basal ganglia (slice involving the anterior commissure).[36] Counts in hippocampus and midbrain were evaluated in whole volumes.

Preprocessing and model development was detailed previously.[35] In short, preprocessing included extracting target brain regions on T1 with Freesurfer and masking surroundings. Images were then processed by a convolutional neural network.[37] This machine learning algorithm provides high reproducibility and low computation time, and is one of the most validated automated methods for quantifying enlarged perivascular spaces. Networks were trained per region, considering region-specific shapes and mimics of perivascular spaces. Models were trained (n=1,200) and validated (n=400)

on independent sets of scans,[35] and showed good performance based on specificity to perivascular spaces using attention maps, and agreement between automated and visual scores similar to human inter-observer agreement. Moreover, determinants of perivascular spaces were similar between using automatically calculated and visually rated counts.[35]

Covariates

We considered as potential confounders the following covariates: Age at sleep measurement, sex, education (categorized as primary, secondary/lower vocational, intermediate vocational and higher vocational/university), the time interval between measurements of sleep and MRI scanning, smoking status (never, former, current), habitual alcohol consumption (gr/day), body mass index (kg/m^2), presence of hypertension (resting blood pressure >140/90 mmHg, or use of blood pressure-lowering medication), presence of diabetes mellitus (serum glucose level ≥7.0 mmol/l [fasting] or ≥11.1 mmol/L [non-fasting], or use of glucose-lowering medication), a positive history of heart disease (myocardial infarction, heart failure, or coronary revascularization procedure), the systemic immune-inflammation index (blood-based biomarker calculated by multiplying counts, in 10^9/L, of platelets with granulocytes, divided by lymphocytes),[38] and napping (reported with the sleep diary during actigraphy recording as present versus absent per afternoon and evening, daily, i.e. ranging from 0 to 14). Measurements were performed during the home interview or center visits, unless stated otherwise.[39]

Additionally, we determined supratentorial intracranial volume, white matter hyperintensity volume, lacunar and cortical brain infarcts, and lobar cerebral microbleeds. Volumes were segmented automatically on T1-images and confirmed or corrected by trained raters.[40] Trained raters also rated cortical infarcts (lesions involving cortical gray matter with tissue loss), lacunar infarcts (subcortical lesions ≥3 mm and <15 mm), and lobar microbleeds as focal parenchymal areas of low signal on T2* images not involving deep gray and white matter structures.[40]

With regard to sleep we further determined the polysomnography-derived apnea-hypopnea index and desaturation rate (PRANA, PhiTools, Strasbourg, France), the apnea-hypopnea index was automatically calculated as the number of apneas and hypopneas, defined accordance to guidelines,[33] per hour of sleep. Similarly, desaturation rate was calculated as the number of desaturations of ≥3% from baseline, per hour of sleep.

Statistical analysis

We associated total sleep time, sleep onset latency, wake after sleep onset, and sleep efficiency, assessed with both actigraphy and polysomnography, with perivascular space counts in 4 regions. All sleep parameters were winsorized to 3 standard deviations from the mean, and subsequently standardized to facilitate comparison across characteristics.

We used zero-inflated negative binomial regression models to account for excess zeros in the perivascular space count data.[35] Analyses were adjusted for age, sex, education, and the time interval between measurements of sleep and MRI (model 1), and additionally for smoking status, habitual alcohol consumption, body mass index, hypertension, diabetes mellitus, a history of heart disease, the systemic immune-inflammation index, and napping (model 2). We additionally adjusted for white matter hyperintensity volume and intracranial volume in persons with valid segmentations (model 3a),[5,35] and separately also for apnea-hypopnea index and desaturation rate (model 3b).[21]

In addition, we investigated non-linearity in associations for total sleep time by modeling quadratic terms, as total sleep time may show a U-shaped relation to related poor health outcomes.[41] Second, we investigated as determinants separate polysomnography-derived sleep stages (N1, N2, N3, and REM), expressed proportional to total sleep time, which may relate differentially to perivascular spaces.[20] Third, we restricted our analysis to persons without cortical or lacunar infarcts on brain MRI (n=497) to determine the influence of comorbid cerebrovascular disease and reduce potential misclassification of infarcts as perivascular spaces.[35] Fourth, we restricted analyses to persons with a time interval between sleep and MRI measurement of ≤28 days to check the influence of the time interval in detecting cross-sectional associations. Lastly, we repeated analyses in the full samples of persons with valid data on either actigraphy and MRI, or polysomnography and MRI, to reduce selective inclusion and increase statistical power.

Posthoc, we examined if centrum semiovale-specific vascular pathology drove the association by additionally adjusting for lobar cerebral microbleeds, indicative of cerebral amyloid angiopathy.[42-45]

We considered associations at $P<0.00198$ as statistically significant correcting for multiple testing. This threshold was defined by first Bonferroni-correcting for testing 4 brain regions to $P<0.0125$, and then applying a Sidak correction using the number of effective tests[46] ($M_{eff}=6.43$) based on correlations amongst main analysis sleep parameters.

Missing values on covariates (median 0.4%, ranging from 0.2 to 9.4%, in n=561) were imputed using five multiple imputations with IBM SPSS Statistics version 24 (IBM Corp, Armonk, NY). All analyses were performed with R (package: *glmmADMB*).

RESULTS

We included 561 participants (mean age 62 ± 6, 52% female; Table 1). The absolute time interval between initiating actigraphy recording and MRI-scanning was a median 27 days (IQR=10-67); for polysomnography, this interval was a median 20 days (IQR=8-46). Correlations of perivascular space counts between brain regions were small ($r_{Spearman}=0.12$-0.27).

Table 1. Characteristics of study population

Characteristic (unit)	Study population (N = 561)	
	Actigraphy	Polysomnography
Age at sleep measurement (years)	62.3 ± 5.5	62.3 ± 5.5
Female	290 (52%)	-
Medium or higher education	338 (61%)	-
Absolute time interval sleep-MRI (days)	27 (10-67)	20 (8-46)
Never smoker	161 (29%)	-
Body mass index (kg/m2)	27.4 ± 4.1	-
History of diabetes mellitus	62 (11%)	-
History of hypertension	221 (39%)	-
History of heart disease	15 (3%)	-
Habitual alcohol consumption (grams/day)	8 (4-9)	-
Systemic immune-inflammation index	449 (345-601)	-
Naps during actigraphy recording	1 (0-2)	-
White matter hyperintensity volume (cm3)	2.3 (1.4 – 4.3)	-
Intracranial volume (cm3)	1,140 ± 115	-
Apnea-hypopnea index (events/hour)	11 (5-21)	-
Desaturation rate (events/hour)	19 (9-30)	-
No lacunar or cortical infarcts on brain MRI	497 (76%)	-
Presence of lobar cerebral microbleeds	53 (9%)	-
Total sleep time	6.2 ± 0.9	6.4 ± 1.0
N1 (%)	11 (9 – 17)	NA
N2 (%)	54 ± 9	NA
N3 (%)	11 (4 – 19)	NA
REM (%)	21 ± 5	NA
Sleep onset latency	13 (8-22)	14 (9-23)
Wake after sleep onset	0.9 ± 0.4	1.1 ± 0.7
Sleep efficiency	78 (72-83)	83 (78-89)
Perivascular space count		
Centrum semiovale	6.1 (3.9-9.9)	-
Basal ganglia	2.4 (1.8-3.3)	-
Hippocampus	2.3 (1.0-4.3)	-
Midbrain	1.3 (0.5-2.4)	-

Abbreviations: MRI=Magnetic resonance imaging; N=Sample size; NA=Not available.

Actigraphy-estimated higher sleep efficiency was associated with more perivascular spaces in the centrum semiovale in model 2 (Odds ratio [OR] per standard deviation increase 1.10, 95% confidence interval [CI] 1.04-1.16, P=0.0009; Table 2). For polysomnographic sleep parameters, we found no associations with perivascular space count in

Table 2. Associations of actigraphy-estimated sleep parameters with perivascular space counts

Determinant (N=561)	Rate ratio for association with perivascular space counts (OR [95% CI])			
	Centrum semiovale	Basal ganglia	Hippocampus	Midbrain
Total sleep time	1.05 (0.99-1.11)	0.98 (0.93-1.03)	0.99 (0.92-1.07)	1.02 (0.94-1.10)
Sleep onset latency	0.91 (0.86-0.97)	1.02 (0.96-1.08)	0.91 (0.84-1.00)	0.97 (0.89-1.06)
Wake after sleep onset	0.96 (0.90-1.02)	1.03 (0.97-1.08)	1.01 (0.94-1.10)	0.98 (0.91-1.07)
Sleep efficiency	**1.10 (1.04-1.16)**	0.99 (0.94-1.05)	1.00 (0.93-1.08)	1.05 (0.98-1.13)

Estimates are expressed as the relative increase in odds of the enlarged perivascular spaces count per standard deviation increase of the determinant. Estimates were obtained with zero-inflated negative binomial regression, adjusted for age, sex, education, time interval between measurements of sleep and MRI, smoking status, habitual alcohol consumption, body mass index, presence of hypertension, presence of diabetes mellitus, history of heart disease, systemic immune-inflammation index, and napping
Bold indicates statistical significance after correcting for multiple testing (P<0.00198).
Abbreviations: CI=Confidence Interval; N=Sample size; OR=Odds ratio.

Table 3. Associations of polysomnographic sleep parameters with perivascular space counts

Determinant (N=561)	Rate ratio for association with perivascular space counts (OR [95% CI])			
	Centrum semiovale	Basal ganglia	Hippocampus	Midbrain
Total sleep time	1.01 (0.96-1.08)	1.00 (0.95-1.06)	0.99 (0.92-1.07)	1.00 (0.92-1.08)
Sleep onset latency	0.92 (0.85-0.99)	0.96 (0.89-1.03)	0.96 (0.86-1.07)	0.96 (0.86-1.07)
Wake after sleep onset	0.94 (0.89-1.00)	1.01 (0.96-1.07)	1.00 (0.92-1.08)	1.03 (0.95-1.12)
Sleep efficiency	1.07 (1.01-1.13)	1.00 (0.94-1.06)	1.01 (0.93-1.09)	0.98 (0.91-1.07)

Estimates are expressed as the relative increase in odds of the enlarged perivascular spaces count per standard deviation increase of the determinant. Estimates were obtained with zero-inflated negative binomial regression, adjusted for age, sex, education, time interval between measurements of sleep and MRI, smoking status, habitual alcohol consumption, body mass index, presence of hypertension, presence of diabetes mellitus, history of heart disease, systemic immune-inflammation index, and napping
Abbreviations: CI=Confidence Interval; N=Sample size; OR=Odds ratio.

any region (Table 3). Model 1 estimates were similar (data not shown). The association of higher sleep efficiency with higher perivascular space count in the centrum semiovale remained after additional adjustment for white matter hyperintensity volume and intracranial volume (model 3a: OR 1.12, 95% CI 1.06-1.18, P=0.00004), and for sleep-disordered breathing parameters (model 3b: OR 1.09, 95% CI 1.04-1.16, P=0.001). Similar to model 2, we found no associations of other sleep parameters with perivascular space counts in model 3a and 3b.

Additional analyses demonstrated no non-linear associations of actigraphy-estimated or polysomnography-derived total sleep time by modeling quadratic terms (Supplementary Table 1). Polysomnography-derived separate sleep stages were also not associated with perivascular space counts (Supplementary Table 2). We observed the same associations after restriction to persons without cortical or lacunar infarcts (Supplementary Table 3).

After restricting to persons with a time interval between sleep and MRI assessment of ≤28 days, estimates for the association of higher sleep efficiency with higher perivascular space count in the centrum semiovale were similar, albeit non-significant (OR 1.12, 95% CI 1.04-1.20, P=0.0029). Also, non-significant estimates across brain regions seemed consistently slightly more pronounced (Supplementary Table 4).

Repeated analyses in full samples of participants with valid actigraphy and MRI (n=1,228, mean age 65.3 ± 7.3, 51% women), or valid polysomnography and MRI data (n=771; mean age 63.0 ± 6.6, 54% women), were similar to the main analysis (Supplementary Table 5). We observed a slightly attenuated, statistically significant association of higher actigraphy-estimated sleep efficiency with higher perivascular space count in the centrum semiovale (OR 1.07, 95% CI 1.03-1.11, P=0.0003).

Posthoc, we explored the association of sleep efficiency with higher perivascular space count in the centrum semiovale by additionally adjusting for presence of lobar cerebral microbleeds, which did not change estimates (OR 1.11, 95% CI 1.05-1.18, P=0.0001).

DISCUSSION

In this population-based study, we found that actigraphy-estimated sleep efficiency was associated with higher perivascular space count in the centrum semiovale.

We found no association of any other sleep parameters with higher perivascular space load in the basal ganglia, hippocampus or midbrain. Findings were not consistent with previous, mostly clinical studies who did suggest an association.[20-22,26] Another population-based cohort also mostly reported null findings,[24,25] except for the association of higher sleep efficiency with lower perivascular space load in the basal ganglia in a subgroup undergoing polysomnography.[25] Yet, as the authors noted, their findings may have had low generalizability.[25]

The direction of the association of higher sleep efficiency with perivascular space count was opposite to what we hypothesized based on aforementioned observations.[20-22,25,26] This could indicate that high sleep efficiency in our study did not represent good quality sleep but rather indicated short sleep opportunity accompanied by a high sleep pressure. Although we could not determine to what extent habitual sleep opportunity was too short, adjusting for napping did not influence the association, nor did we find an association for the proportion of N3 sleep. Together with no relation of short total sleep time with perivascular space load, findings suggest that the association of higher sleep efficiency with higher perivascular space count is not likely to be explained by a short sleep opportunity.

Equally, it could be hypothesized that enlarged perivascular spaces, at least in the centrum semiovale, may signify something else besides impaired clearance or accumulation

of pathology.[5] Several findings, including ours, seem to indeed support alternative interpretations. First, a higher hippocampal load of perivascular spaces was associated with better, not worse, memory performance in humans.[47] Second, age did not determine perivascular space count in the centrum semiovale in our cohort, nor did most vascular risk factors,[35] all of which are risk factors for brain pathology. Third, adjusting for lobar microbleeds, a marker of cerebral amyloid angiopathy associated with perivascular spaces in the centrum semiovale,[44,48] did not influence our estimates. Fourth, a previous study in our cohort found that higher actigraphy-estimated sleep efficiency related to better white matter microstructural integrity, in regions overlapping with the centrum semiovale.[29] Yet, we found the opposite relation to perivascular spaces, suggesting that perivascular space count, at least in relation to sleep, represents something else than vascular pathology. Together, these findings suggest that enlarged perivascular spaces in the centrum semiovale need not signal pathology per se.

Against the background of aforementioned considerations of how to interpret higher sleep efficiency and higher perivascular space count, different mechanisms may explain their association. One speculative explanation is that sleep state-related increases in fluid flow across the perivascular space[1] enlarge the compartment, e.g. through repetitively exerting mechanical force. Yet, total sleep time was not related to perivascular space count, nor was the proportion of deep sleep in which glymphatic flow may be strongest.[1,49] Vice versa, our cross-sectional association may also indicate that perivascular space caliber may help determine sleep. A higher caliber perivascular space may allow a higher rate of fluid exchange which, assuming that sleep functions to clear waste from the brain, offers a functional benefit and may increase the efficiency of waste clearance during sleep. In line with this interpretation, a recent study observed an association of functional genetic variation in aquaporin-4 (AQP4), an astrocytic water channel facilitating flow between perivascular space and interstitium,[50] with slow-wave power during the night in humans.[51] The functional importance of perivascular space caliber for waste clearance is also supported by another study in mice that showed that migraine-related closure of perivascular spaces could impair clearance.[52] Lastly, sleep efficiency and perivascular space enlargement may also share common biological causes, e.g. those related to astrocytic structure or function.[4,53]

Future studies may consider investigating the direction of our association by investigating determinants of perivascular space count in the centrum semiovale in humans, including glymphatic clearance, and investigate their relation with sleep, or investigate the temporality of our finding.

Several methodological considerations deserve mention. First, our algorithm was based on visually rated perivascular space counts as ground truth. Possibly, segmented volumes instead of counts may have better represented subtle caliber changes relevant for glymphatic functioning. Second, a 1.5-Tesla MRI scanner detects only the 'tip of the

iceberg' of perivascular space enlargement. Perivascular spaces detected with a 7-Tesla scanner may better represent physiological aspects,[54] potentially relevant to sleep. Third, we could not exclude that the visibility of perivascular spaces on MRI could have been influenced by sleeping during MRI-acquisition. As sleep strongly alters interstitial space volume and fluid exchange in mice,[1] perivascular space caliber may increase during sleep, which quickly and commonly occurs during scanning in the MRI.[55] Although speculative and untested in humans, such effects may have led to underestimation of our association. Study strengths include using two different modalities to objectively measure sleep, determining associations across several brain regions, and accounting for various potential confounding factors.

We conclude that the association of sleep with perivascular space counts in the middle-aged and elderly population remains limited to that of higher actigraphy-estimated sleep efficiency with higher perivascular space load in the centrum semiovale. Further work is needed to determine the significance of this association to glymphatic clearance, and sleep.

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SUPPLEMENTARY TABLES

Supplementary Table 1. Quadratic associations of total sleep time with perivascular space counts

Modeled total sleep time terms (N=561)	Centrum semiovale		Basal ganglia		Hippocampus		Midbrain	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Actigraphy-estimated total sleep time								
Main term	1.01 (1.00-1.02)	0.03	1.00 (0.99; 1.01)	0.88	1.00 (0.99-1.01)	0.91	1.00 (0.99-1.02)	0.67
Quadratic term	1.00 (1.00-1.00)	0.04	1.00 (1.00; 1.00)	0.80	1.00 (1.00-1.00)	0.93	1.00 (1.00-1.00)	0.71
Polysomnography-derived total sleep time								
Main term	1.00 (1.00-1.01)	0.35	1.00 (0.99-1.01)	0.97	0.99 (0.98-1.00)	0.04	1.00 (0.99-1.01)	0.76
Quadratic term	1.00 (1.00-1.00)	0.37	1.00 (1.00-1.00)	0.97	1.00 (1.00-1.00)	0.04	1.00 (1.00-1.00)	0.75

Estimates were obtained by modeling a main term of total sleep time that was not transformed nor standardized, and adding a quadratic term of total sleep time to the model. Estimates are expressed as the relative increase in odds of the enlarged perivascular spaces count per unit increase in total sleep time, i.e. hours. Estimates were obtained with zero-inflated negative binomial regression, adjusted for age, sex, education, time interval between measurements of sleep and MRI, smoking status, habitual alcohol consumption, body mass index, presence of hypertension, presence of diabetes mellitus, history of heart disease, systemic immune-inflammation index, and napping. Abbreviations: CI=Confidence Interval; N=sample size; OR=Odds ratio.

Supplementary Table 2. Association of separate sleep stages derived from polysomnography with perivascular space counts

Sleep stage duration expressed as	Rate ratio for association with perivascular space counts (OR [95% CI])			
% of total sleep time (N=561)	Centrum semiovale	Basal ganglia	Hippocampus	Midbrain
N1	0.97 (0.91; 1.03)	1.02 (0.96; 1.08)	0.97 (0.89; 1.06)	1.06 (0.98; 1.15)
N2	0.99 (0.94; 1.04)	1.00 (0.95; 1.05)	1.01 (0.94; 1.09)	0.97 (0.90; 1.04)
N3	1.02 (0.97; 1.08)	0.99 (0.93; 1.04)	1.01 (0.93; 1.09)	0.98 (0.91; 1.06)
REM	1.02 (0.97; 1.08)	1.00 (0.95; 1.05)	0.99 (0.92; 1.07)	1.03 (0.96; 1.10)

Estimates are expressed as the relative increase in odds of the enlarged perivascular spaces count per standard deviation increase of the determinant. We investigated relative sleep stage durations, i.e. as proportion of total sleep time. Estimates were obtained with zero-inflated negative binomial regression, adjusted for age, sex, education, time interval between measurements of sleep and MRI, smoking status, habitual alcohol consumption, body mass index, presence of hypertension, presence of diabetes mellitus, history of heart disease, systemic immune-inflammation index, and napping. Abbreviations: CI=Confidence Interval; N=Sample size; OR=Odds ratio.

Supplementary Table 3. Associations of actigraphy-estimated and polysomnographic sleep parameters with perivascular space counts, restricted to persons without lacunar of cortical brain infarcts on MRI

Determinant (N=497)	Rate ratio for association with perivascular space counts (OR [95% CI])			
	Centrum semiovale	Basal ganglia	Hippocampus	Midbrain
Actigraphy				
Total sleep time	1.07 (1.01-1.14)	0.98 (0.92-1.04)	1.02 (0.93-1.11)	1.01 (0.93-1.10)
Sleep onset latency	0.91 (0.85-0.97)	1.02 (0.95-1.09)	0.89 (0.81-0.98)	0.98 (0.89-1.08)
Wake after sleep onset	0.95 (0.89-1.01)	1.03 (0.96-1.09)	0.99 (0.91-1.09)	0.99 (0.90-1.08)
Sleep efficiency	**1.13 (1.06-1.19)**	0.99 (0.93-1.05)	1.04 (0.96-1.13)	1.05 (0.96-1.13)
Polysomnography				
Total sleep time	1.02 (0.96-1.08)	1.01 (0.95-1.07)	0.98 (0.90-1.06)	0.99 (0.91-1.08)
Sleep onset latency	0.93 (0.86-1.00)	0.96 (0.89-1.04)	0.96 (0.86-1.07)	0.96 (0.86-1.07)
Wake after sleep onset	0.93 (0.87-0.99)	1.01 (0.94-1.07)	1.00 (0.92-1.09)	1.05 (0.96-1.15)
Sleep efficiency	1.07 (1.01-1.14)	1.01 (0.95-1.08)	1.00 (0.91-1.09)	0.97 (0.89-1.06)

Estimates are expressed as the relative increase in odds of the enlarged perivascular spaces count per standard deviation increase of the determinant. Estimates were obtained with zero-inflated negative binomial regression, adjusted for age, sex, education, time interval between measurements of sleep and MRI, smoking status, habitual alcohol consumption, body mass index, presence of hypertension, presence of diabetes mellitus, history of heart disease, systemic immune-inflammation index, and napping. **Bold** indicates statistical significance after correcting for multiple testing (P<0.00198). Abbreviations: CI=Confidence Interval; MRI=Magnetic resonance imaging; N=Sample size; OR=Odds ratio

Supplementary Table 4. Associations of actigraphy-estimated and polysomnographic sleep parameters with perivascular space counts, restricted to persons with a time interval of ≤28 days between sleep and MRI measurements

Determinant (N [% of 561])	Rate ratio for association with perivascular space counts (OR [95% CI])			
	Centrum semiovale	Basal ganglia	Hippocampus	Midbrain
Actigraphy (N=350 [62%])				
Total sleep time	1.04 (0.96-1.12)	0.97 (0.90-1.04)	1.00 (0.90-1.11)	1.00 (0.90-1.11)
Sleep onset latency	0.90 (0.83-0.99)	1.02 (0.94-1.10)	0.95 (0.85-1.07)	0.94 (0.83-1.05)
Wake after sleep onset	0.91 (0.84-1.00)	0.98 (0.91-1.07)	1.03 (0.91-1.16)	0.94 (0.84-1.06)
Sleep efficiency	1.12 (1.04-1.20)	1.01 (0.94-1.08)	0.98 (0.89-1.09)	1.01 (0.91-1.12)
Polysomnography (N=288 [51%])				
Total sleep time	1.01 (0.94-1.08)	0.99 (0.93-1.06)	0.96 (0.87-1.06)	1.00 (0.91-1.10)
Sleep onset latency	0.87 (0.79-0.96)	0.93 (0.85-1.03)	0.92 (0.80-1.06)	0.89 (0.78-1.02)
Wake after sleep onset	0.95 (0.88-1.02)	1.02 (0.95-1.10)	1.03 (0.92-1.16)	1.03 (0.93-1.14)
Sleep efficiency	1.08 (1.00-1.16)	1.00 (0.93-1.08)	0.98 (0.88-1.10)	1.01 (0.91-1.12)

Estimates are expressed as the relative increase in odds of the enlarged perivascular spaces count per standard deviation increase of the determinant. Estimates were obtained with zero-inflated negative binomial regression, adjusted for age, sex, education, time interval between measurements of sleep and MRI, smoking status, habitual alcohol consumption, body mass index, presence of hypertension, presence of diabetes mellitus, history of heart disease, systemic immune-inflammation index, and napping. Please note that number of participants for analyses restricted on time intervals differed across modalities, as the start of actigraphy recording differed slightly from the date on which polysomnography was performed. Abbreviations: CI=Confidence Interval; N=Sample size; OR=Odds ratio

Supplementary Table 5. Associations of sleep parameters with enlarged perivascular space counts, separately for polysomnography and actigraphy in partly overlapping samples

Determinant	Rate ratio for association with perivascular space counts (OR [95% CI])			
	Centrum semiovale	Basal ganglia	Hippocampus	Midbrain
Actigraphy (n=1,228)				
Total sleep time	1.04 (1.00-1.08)	1.04 (1.00-1.08)	1.02 (0.97-1.08)	1.01 (0.96-1.06)
Sleep onset latency	0.96 (0.92-1.00)	1.01 (0.97-1.05)	0.94 (0.89-1.00)	1.00 (0.95-1.06)
Wake after sleep onset	0.95 (0.91-0.98)	1.00 (0.97-1.04)	0.98 (0.93-1.03)	0.97 (0.92-1.03)
Sleep efficiency	**1.07 (1.03-1.11)**	1.02 (0.98-1.05)	1.02 (0.97-1.08)	1.02 (0.97-1.07)
Polysomnography (n=771)				
Total sleep time	1.03 (0.98-1.07)	1.02 (0.98-1.06)	1.00 (0.94-1.06)	1.01 (0.95-1.08)
Sleep onset latency	0.96 (0.90-1.01)	0.96 (0.90-1.02)	0.99 (0.91-1.07)	1.01 (0.93-1.10)
Wake after sleep onset	0.95 (0.90-0.99)	1.00 (0.96-1.05)	1.00 (0.93-1.07)	0.97 (0.91-1.04)
Sleep efficiency	1.05 (1.01-1.10)	1.01 (0.97-1.06)	1.00 (0.94-1.07)	1.02 (0.95-1.09)

Estimates are expressed as the relative increase in odds of the enlarged perivascular spaces count per standard deviation increase of the determinant. Estimates were obtained with zero-inflated negative binomial regression, adjusted for age, sex, education, time interval between measurements of sleep and MRI, smoking status, habitual alcohol consumption, body mass index, presence of hypertension, presence of diabetes mellitus, history of heart disease, and systemic immune-inflammation index, and napping (polysomnography-derived sleep parameters were not adjusted for napping, which was assessed during actigraphy recordings).

Bold indicates statistical significance after correcting for multiple testing (P<0.00198). Abbreviations: CI=Confidence Interval; N=Sample size; OR=Odds ratio

4.3 RESTING-STATE FUNCTIONAL MAGNETIC RESONANCE IMAGING

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ABSTRACT

Sleep problems increase with aging. Increasing evidence suggest that sleep problems are not only a consequence of the aging process, but may independently contribute to developing vascular or neurodegenerative brain disease. Yet, it remains unclear what mechanisms underlie the impact sleep problems may have on brain health in the general middle-aged and elderly population.

Here, we studied sleep's relation to specifically brain functioning in 621 participants (median age 62 years, 55% women) from the population-based Rotterdam Study. We investigated cross-sectional associations of polysomnographic and subjectively measured aspects of sleep with intrinsic neural activity measured with resting-state functional magnetic resonance imaging on a different day. We investigated both functional connectivity between regions and brain activity (blood-oxygen-level-dependent signal amplitude) within regions, hierarchically towards smaller topographical levels.

We found that longer polysomnographic total sleep time is associated with lower blood-oxygen-level-dependent signal amplitude in (pre)frontal regions. No objective or subjective sleep parameters were associated with functional connectivity between or within resting-state networks.

Findings may indicate a pathway through which sleep, in a 'real-life' population setting, impacts brain activity or regional brain activity determines of total sleep time.

INTRODUCTION

Sleep is a homeostatic process serving vital functions for the brain to support performance the next day. As adults age, they increasingly experience sleep problems.[1] Sleep problems have been hypothesized to impair brain health, as they are associated with developing stroke[2] and dementia.[3] It is therefore important that we increase our understanding how sleep, beyond its homeostatic, night-to-day effect, may impact brain health in the general middle-aged and elderly population.

How sleep affects the brain can be investigated well by studying brain functional connectivity. Brain functional connectivity can be studied non-invasively with functional MRI (fMRI), which measures intrinsic neural activity indirectly through blood oxygenation. Applying fMRI when individuals are not engaged in a task ('resting-state' fMRI (rs-fMRI)) reveals how brain regions spontaneously communicate with each other in connected networks.[4] Intrinsic neural activity as measured with rs-fMRI can provide measures of activity between cortical regions, or within them. The organization of intrinsic neural activity in networks is remarkably robust and present across various conditions.[5]

That sleep is relevant for waking rs-fMRI neural activity has been shown using various approaches. Experimental sleep deprivation studies showed immediate widespread changes in functional connectivity during subsequent wakefulness[6,7] including an increase of global fMRI-signal variability,[8] also known as signal amplitude. Importantly, observational studies that associated habitual sleep quality or duration, or a sleep disorder such as insomnia with rs-fMRI measures suggest that sleep may impact intrinsic neural activity beyond the short term.[9-14] Yet, only few studies measured sleep objectively to minimize misclassification or used large samples to increase statistical power and decrease the chance that significant associations are overestimated. Findings from large-scale, population-based studies are more equivocal, reporting no associations of sleep quality with connectivity between networks[15] or of self-reported sleep duration with signal amplitude in the often-studied 'default mode' network.[12]

It is therefore unclear if variations in sleep, including total sleep time and duration of individual sleep stages, are related to intrinsic neural activity during daytime, measured as functional connectivity between or neural activity within different brain regions, in the general middle-aged and elderly population. We aimed to fill this knowledge gap using sleep parameters measured with polysomnography and the Pittsburgh Sleep Quality Index, and rs-fMRI measures from the population-based Rotterdam Study cohort. We explored associations between sleep and intrinsic neural activity using a hierarchical approach from global to more spatially-specific analyses, and subsequently examined associations of total sleep time more regionally based on initial findings.

METHODS

Study setting

The Rotterdam Study, starting in 1990, is a prospective population-based cohort of inhabitants of a suburban district in Rotterdam aged 45 years or over.[16] Participating inhabitants were interviewed at home and subsequently visited the research center. These examination rounds were repeated every 4-5 years. The cohort was expanded twice, in 2000 with persons aged ≤55, and in 2006 with persons aged ≤45. We studied individuals from all three inclusion rounds who participated in a polysomnography (PSG) study between January 2012 to September 2014, and also underwent a resting state fMRI (rs-fMRI) scan. Rs-fMRI was implemented routinely since 2012.[17]

The Rotterdam Study (RS) has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The RS has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl) and into the WHO International Clinical Trials Registry Platform (ICTRP; www.who.int/ictrp/network/primary/en/) under shared catalogue number NTR6831. The study was conducted in accordance with the guideline proposed in the World Medical Association Declaration of Helsinki. All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

Study sample

We invited 1,750 persons that visited the research center for in-home PSG; 928 consented. Invitees were deemed able to understand study purpose and instructions. Twenty-seven recordings failed or were of insufficient quality for sleep scoring. Of these, 724 persons without MRI contra-indications also underwent rs-fMRI. We excluded participants with poor quality[17] rs-fMRI data (n=49), cortical brain infarcts (n=20) or with prevalent dementia or missing dementia screening (n=2). From the remaining 653, we included in our main analyses 621 participants with a time interval between PSG and rs-fMRI of one year or less. From this population, we included 560 participants for analyses of PSG spectral power due to failure of critical EEG-leads in 61 individuals. Similarly, we included 603 participants for analyses on global PSQI score due to missing data of more than one component (see Sleep assessments below).

Sleep assessments

We recorded one night of PSG at home during weeknights. Polysomnography was applied by trained research assistants according to the American Association of Sleep Medicine (AASM) criteria,[18] including six electroencephalography (EEG) channels (F3/A2,

F4/A1, C3/A2, C4/A1, O1/A2, O2/A1), bilateral electro-oculography, chin electromyography, electrocardiography, respiratory belts on the chest and abdomen, oximetry, and a nasal pressure transducer and oronasal thermocouple. Participants were instructed to spend the night as normal as possible, without restrictions on bedtimes and use of alcohol, coffee or sleep medication. They pressed a button to signal when intending to go to sleep ("lights out") and getting out of bed ("lights on").

Sleep was scored[18] by a Registered Polysomnographic Technologist to determine total sleep time (TST), sleep onset latency (SOL), wake after sleep onset (WASO), sleep efficiency (SE), and the duration of the sleep stages non-rapid eye movement (NREM) 1 (N1), N2, N3 and REM.

We calculated spectral power and spindles in the C3/A2 derivation, using PRANA software (PhiTools, Strasbourg, France). For spectral power, band-pass filtering (0.125-128 Hz) and automated removal of artifacts were applied. Spectral analysis was performed using 4-second epochs with 50% overlap, averaged over 30-second epochs. We calculated the absolute spectral power in the delta (0.75-4.00 Hz), beta (15.50-22.50 Hz) and gamma (22.50-40.00 Hz) frequency bands.

Apneas were defined as an airflow reduction of ≥90% of baseline for ≥10 seconds, and a hypopnea was defined as an airflow reduction of ≥30% of baseline for ≥10 seconds and a desaturation of ≥3% from baseline or an arousal.[18] The apnea-hypopnea index (AHI) was automatically calculated as the number of apneas and hypopneas per hour of sleep.

Subjective sleep quality during the past 4 weeks was measured with the PSQI during the home interview. The PSQI has good test-retest reliability and validity in a non-clinical sample of older adults.[19] Items, including self-reported sleep duration, were scored to provide a global PSQI score ranging from 0-21. Higher scores indicate poorer sleep quality. We weighted the PSQI score for 36 out of 603 individuals with one component score missing, by multiplying scores by 7/6.

To validate our findings for polysomnography sleep measure and assess a possible first night effect we used actigraphy.[20] The night of polysomnography, participants also wore an actigraph (ActiWatch model AW4, Cambridge Technology Ltd), and were invited to wear it for 7 days and also keep a sleep diary. Of 621 participants, 428 completed at least 4 consecutive nights (recording duration: 153±16 hours [median=144]). We used diary-derived times of 'lights out' and getting up the next morning to estimate time in bed. Within the time in bed, total sleep time was estimated using a validated algorithm with a threshold of 20 activity counts, and was averaged over all available nights per participant to estimate habitual total sleep time.

Neuroimaging

Brain imaging was performed with a 1.5-tesla MRI scanner (Signa Excite II, GE Healthcare, Milwaukee, WI, USA) at the research center. Resting state fMRI acquisition time was 7m44s (TR= 2900 ms, TE= 60ms, Field of View= 21 cm^2, 31 axial slices, matrix size=64x64, slice thickness= 3.3 mm, 165 volumes). Details of rs-fMRI preprocessing and connectivity analyses are provided elsewhere.[17] In brief, participants were prompted before the start of the fMRI-sequence to lie still, keep their eyes open, and stay awake. Preprocessing of resting-state data was performed with the FMRIB Software Library FEAT package.[21] Subject-specific artifact removal was conducted using independent components which were automatically classified. We excluded scans that showing absolute head displacement >3 mm and/or mean relative frame-wise displacement >0.2 mm. Also, as mild ghosting artefacts were introduced during rs-fMRI acquisition, we did not include scans with a ghost-to-signal ratio>0.1 and added this ratio as a covariate in analyses.[17]

For functional connectivity analyses, we generated a study-specific functional parcellation using independent component analysis[17,22] resulting in 50 components of interest, or functional nodes (hereafter: nodes). A node thus is a region where voxels show the same temporal BOLD-signal pattern. This template was used to derive node-level time series and obtain values for the full temporal correlations per subject for all nodes. Using hierarchical clustering of the group-level node correlations,[22] we concatenated these nodes into 9 large-scale networks, labeled anterior default mode, posterior default mode, frontoparietal, dorsal attention, ventral attention, sensorimotor, visual, subcortical, and temporal network.[17] Networks thus contain multiple nodes showing similar temporal patterns. Defining small nodes and clustering them into networks allowed studying with more detail the functional specialization within networks, as well as large-scale networks as a whole.[23]

Using the functional parcellation of 50 nodes, we calculated functional connectivity between node regions, and brain activity within node regions. For functional connectivity, we calculated correlations between the BOLD-signal time-series of each of the 50 nodes with all others. At the network level, we obtained between-network functional connectivity by averaging correlation values between all nodes from one network with all nodes from the other network, for 9x9 networks. Within-network functional connectivity was thus defined by averaging correlations of node pairs within that network. We investigated brain activity within regions as the variability of that region's BOLD-signal, by calculating the standard deviation (SD) of each node's time series (hereafter: signal amplitude). Analogous to functional connectivity, network-level signal amplitude was obtained by averaging amplitudes across nodes within that network. Global signal amplitude was obtained by averaging over all 50 nodes.

Potential confounders

We adjusted for potential confounders selected based on relevant publications[17,24]: Age, sex, mean frame-wise head displacement, ghost-to-signal ratio, time interval between sleep and rs-fMRI measurement, habitual alcohol consumption, physical activity, systolic blood pressure, body mass index, history of diabetes mellitus, supratentorial gray matter volume and total intracranial volume.

The sensitivity analysis included additionally adjusting the main analyses for depressive symptoms and use of any antidepressant or hypnotic medication during PSG. Details of measurement are provided in the Supplementary Text.

Statistical analyses

Details are described in the Supplementary Text. We investigated cross-sectional associations of 12 sleep determinants (TST, WASO, SOL, SE, duration of stages N1, N2, N3, REM, spectral delta, beta and gamma power, and global PSQI score) with both functional connectivity between regions (and within where possible), and signal amplitude within regions. We used non-parametric permutation testing (n=5,000) implemented in FSL's 'randomise', with family-wise error (FWE) corrected P-values.

We hierarchically tested associations to examine regional heterogeneity if significant at a global level: We investigated associations with functional connectivity at the network level, and further analyzed node-level associations if nominally significant. Similarly, we first investigated associations with mean signal amplitude on a global level, and further analyzed the nominally significant associations on a network level. Furthermore, we investigated significant network-level associations on a node level.

As tests in 'randomise' are by default performed one-sided, we further Bonferroni-corrected the alpha level of 0.05 to $P_{FWE\text{-}corrected}<0.025$ (nominal significance level). As we tested multiple sleep determinants, we defined a more stringent threshold for significance at $P_{FWE\text{-}corrected}<0.00277$ (number of effective independent tests=9.23).

As sensitivity analysis, we repeated the analyses in persons with a shorter time interval between imaging and sleep measurements (<1 month for PSG parameters; <6 months for PSQI score). Also, we additionally adjusted analyses for i) depressive symptoms and use of any antidepressant or hypnotic medication during PSG; ii) AHI.

In post-hoc analyses based on initial findings for total sleep time, we i) explored associations of separate sleep stages with amplitude on a node level; ii) assessed possible non-linearity by analyzing 5 equal-sized categories (quintiles) of total sleep time and modeling a quadratic term; iii) repeated analyses with actigraphy-estimated total sleep time in n=428 with valid actigraphy data, and with self-reported sleep duration assessed in the PSQI.

RESULTS

We included 621 participants (median age=62 years [range 52-95 years], 55% women). The median absolute time interval between PSG and rs-fMRI was 17 days. Excluded participants did not differ by age, sex, head motion parameters or sleep stages duration from included participants. Correlations amongst sleep and fMRI parameters are provided in Supplementary Table 1.

We found no associations of objective or subjective sleep parameters with functional connectivity between or within resting state networks (all $P_{FWE\text{-}corrected}$ >0.025; Fig. 1).

We observed an association of longer total sleep time with lower mean global signal amplitude (beta per SD increase: -0.025 (95% CI -0.044; -0.006); P=5.0*e*-3; Table 2).

Investigating the regional heterogeneity of this association at a network level, we found it was present in the ventral attention, sensorimotor, subcortical, and temporal network (Table 3). In the ventral attention network, the association remained after correcting for testing multiple sleep parameters (-0.051 (95% CI -0.077; -0.024); $P_{FWE\text{-}corrected}$=1.2*e*-3; Supplementary Figure 1).

We further investigated associations of total sleep time with signal amplitude within aforementioned networks at the node level. We only observed associations of longer total sleep time with lower signal amplitude in nodes of the ventral attention network, distributed mainly in (pre)frontal regions (Fig. 2). The association in 'node 32' remained after correcting for multiple testing (-0.051 (95% CI -0.075;-0.027); $P_{FWE\text{-}corrected}$=1.6*e*-3). This node corresponds bilaterally to the anterior cingulate gyrus, and the juxtapositional lobule cortex (formerly: Supplementary motor cortex; Fig. 2).

Other sleep parameters were not associated with mean global signal amplitude, yet direction of effect sizes were mostly congruent with indicating 'poor' sleep (e.g. sleep onset latency, beta spectral power) versus 'good' sleep (e.g. sleep efficiency).

Restricting associations to persons with a shorter time interval between sleep and rs-fMRI measurement showed more pronounced effect sizes for the association of longer total sleep time with lower mean signal amplitude (n=450, Supplementary Table 2). Associations remained statistically significant in 'node 32' (-0.063 (95% CI -0.091; -0.034); $P_{FWE\text{-}corrected}$=1.0*e*-3), and 'node 23' (-0.080 (95% CI -0.120; -0.040); $P_{FWE\text{-}corrected}$=2.0*e*-3) corresponding mainly to the frontal pole and the anterior cingulate gyrus (Fig. 2). Longer stage N2 sleep related with lower global mean signal amplitude, driven mostly by the ventral attention and temporal networks (Supplementary Table 2), yet no node-level associations survived multiple testing correction.

In the total sample of n=621, additional adjustment for depressive symptoms and use of antidepressant and hypnotic medication during PSG did not change estimates on the global level (-0.025 (95% CI 0.044;-0.006); P=5.0*e*-3), or network level (ventral attention network: -0.051 (95% CI -0.078; -0.025); $P_{FWE\text{-}corrected}$=1.2*e*-3; other networks: all

Table 1. Characteristics of the study population

Characteristics (unit)	Value
Age (years)	62 (58; 66)
Female	340 (55%)
Time interval MRI-PSG (days)	6 (-12; 22)
No. of participants <1 month	450 (72%)
Time interval MRI-PSQI (days)	150 (104; 191)
No. of participants <6 months	438 (69%)
Habitual alcohol consumption (gr/day)	8 (4; 11)
Physical activity (MET-hours/week)	50 (24; 78)
Systolic blood pressure (mm Hg)	133 ± 18
Body mass index (kg/m^2)	27 ± 4
History of diabetes mellitus	73 (12%)
Supratentorial gray matter volume (cm^3)	538 ± 55
Intracranial volume (cm^3)	1,141 ± 115
Depressive symptoms (CES-D score)	12 (10; 15)
Use antidepressants/hypnotics during PSG	29 (5%)
Self-reported sleep duration (minutes)	408 ± 73
Apnea-hypopnea index (events/hour of sleep)	9 (5; 13)
Sleep parameters	
Total sleep time (minutes)	380 ± 65
Sleep onset latency (minutes)	14 (8; 23)
Wake after sleep onset (minutes)	71 ± 48
Sleep efficiency (%)	81% ± 11
Sleep stage duration (minutes)	
N1	49 ± 25
N2	203 ± 52
N3	48 ± 37
REM	79 ± 26
Absolute spectral power (μV^2/Hz)	
Delta (range: 0.75 - 4.50 Hz)	106 (72; 155)
Beta (range: 15.50 - 22.50 Hz)	2.5 (1.7; 3.7)
Gamma (range: 22.50 – 40.00 Hz)	1.9 (1.3; 2.9)
Missing	61 (10%)
Subjective sleep quality (PSQI score)	3 (1; 6)
Missing	18 (3%)

Values are frequency (%) for categorical variables, and mean ± standard deviation or median (1st quartile; 3rd quartile) for continuous variables, calculated over 621 participants unless specified otherwise. Values include imputed values for covariates.

Abbreviations: CES-D=Center for Epidemiological Studies – Depression Scale; MET=Metabolic equivalent of task; MRI=Magnetic resonance imaging; N=sample size; N[x]=non-REM stage x; PSG=polysomnography; PSQI=Pittsburgh Sleep Quality Index; REM=rapid-eye movement; TST=Total Sleep Time.

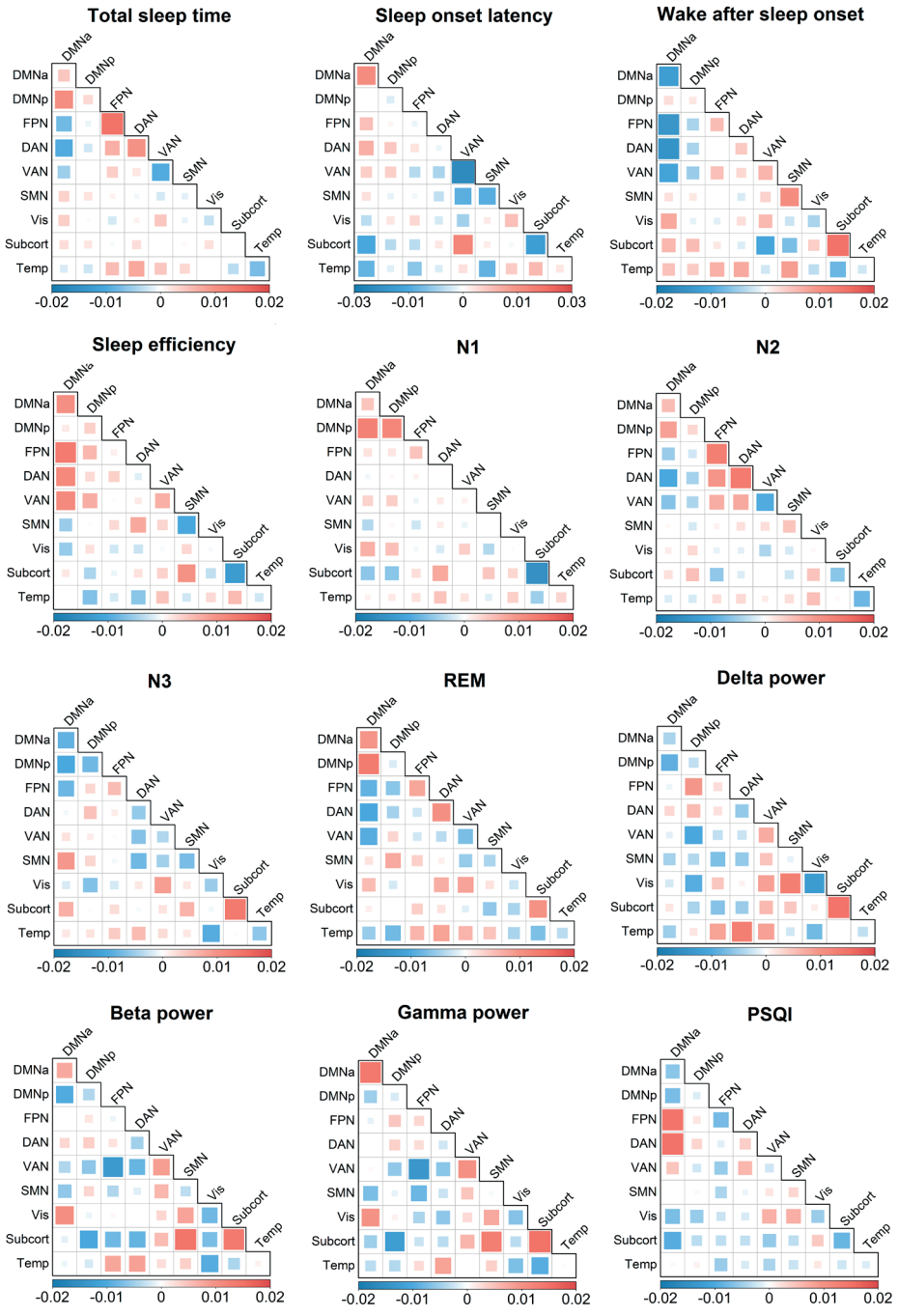

Figure 1. Associations of sleep parameters with functional connectivity between networks

Colors and sizes of blocks correspond to beta coefficients: Red indicates positive, and blue indicates negative associations. Values are obtained using linear regression, adjusted for age, sex, mean frame-wise head displacement, ghost-to-signal ratio, time interval between sleep and rs-fMRI measurement, habitual alcohol consumption, physical activity, systolic blood pressure, body mass index, history of diabetes mellitus, supratentorial gray matter volume and total intracranial volume. No associations were significant at the level of PFWE-corrected < 0.025. Abbreviations: DMNa=anterior default mode network; DMNp=posterior default mode network; DAN=dorsal attention network; FPN=frontoparietal network; N[x]=non-REM sleep stage x; PSQI=Pittsburgh Sleep Quality Index; REM=rapid eye movement; SMN=sensorimotor network; Subcort=subcortical network; Temp=temporal network, Vis=visual network; VAN=ventral attention network.

Table 2. Associations of sleep parameters with global mean signal amplitude

Sleep measures	Beta (95% CI)	P-value
Objective		
Sleep continuity measures		
Total sleep time	**-0.025 (-0.044; -0.006)**	**5.0e-3**
Sleep onset latency	0.015 (-0.020; 0.049)	0.19
Wake after sleep onset	-0.001 (-0.022; 0.019)	0.45
Sleep efficiency	-0.014 (-0.038; 0.010)	0.14
Sleep stage duration		
N1	-0.013 (-0.034; 0.007)	0.10
N2	-0.013 (-0.032; 0.005)	0.08
N3	-0.009 (-0.030; 0.013)	0.21
REM	-0.015 (-0.034; 0.004)	0.05
Spectral power		
Delta power	0.004 (-0.024; 0.032)	0.39
Beta power	0.013 (-0.013; 0.038)	0.16
Gamma power	0.003 (-0.023; 0.029)	0.41
Subjective		
Sleep complaints (global PSQI score)	0.009 (-0.010; 0.028)	0.18

Values represent difference (95% CI) in mean signal amplitude on a whole-brain level, per standard deviation increase in the determinant. Estimates are obtained using linear regression models adjusted for age, sex, mean frame-wise head displacement, ghost-to-signal ratio, time interval between sleep and rs-fMRI measurement, habitual alcohol consumption, physical activity, systolic blood pressure, body mass index, history of diabetes mellitus, supratentorial gray matter volume and total intracranial volume.
Bold values indicate statistical significance at P<0.025. Please note that P-values were uncorrected as only the 'global' region was tested. Abbreviations: PSQI=Pittsburgh Sleep Quality Index; Nx=non-REM sleep stage x; REM=Rapid eye-movement.

Table 3. Associations of total sleep time and mean signal amplitude in networks

Total sleep time	Beta (95% CI)	P-value	$P_{FWE\text{-}corrected}$
Networks			
1: Default Mode - anterior	**-0.046 (-0.083; -0.010)**	**5.8e-3**	0.04
2: Default Mode - posterior	-0.017 (-0.039; 0.006)	0.08	0.30
3: Fronto-parietal	-0.013 (-0.040; 0.013)	0.16	0.49
4: Dorsal Attention	-0.014 (-0.041; 0.013)	0.15	0.48
5: Ventral Attention	**-0.051 (-0.077; -0.024)**	**4.0e-4**	**1.2e-3**
6: Sensorimotor	**-0.030 (-0.049; -0.010)**	**1.6e-3**	**8.8e-3**
7: Visual	-0.013 (-0.033; 0.008)	0.12	0.39
8: Subcortical	**-0.021 (-0.036; -0.005)**	**4.2e-3**	**2.5e-2**
9: Temporal	**-0.032 (-0.053; -0.011)**	**1.2e-3**	**8.4e-3**

Values represent difference (95% CI) in mean signal amplitude on a network level, per standard deviation increase in total sleep time. Estimates are obtained using linear regression models and permutation tests, adjusted for age, sex, mean frame-wise head displacement, ghost-to-signal ratio, time interval between sleep and rs-fMRI measurement, habitual alcohol consumption, physical activity, systolic blood pressure, body mass index, history of diabetes mellitus, supratentorial gray matter volume and total intracranial volume. **Bold** indicates statistical significance at P<0.025.

$P_{FWE\text{-}corrected}$>8.6*e*-3). Additionally adjusting analyses for AHI did not influence global and network-level associations (Supplementary Table 3), and the association within 'node 32' remained highly similar (-0.051 (95% CI -0.075;-0.027); $P_{FWE\text{-}corrected}$=1.2*e*-3).

Posthoc, we explored the contribution of individual sleep stages to the association of total sleep time with mean signal amplitude found in four networks, at both the network and node level. As most of total sleep time was spent in stages REM and N2, these stages contributed most to the association (Fig. 3), yet no association survived multiple testing correction.

Analyzing categorized total sleep time did not suggest non-linearity in the relation with signal amplitude at a global or network level (Supplementary Table 4), supported by testing quadratic terms of total sleep time (global: P=0.27; networks: all $P_{FWE\text{-}corrected}$>0.025).

Actigraphy-estimated longer total sleep time was also associated with lower mean signal amplitude at a global level, driven by similar networks as when derived from PSG (Supplementary Table 5).

Self-reported sleep duration was not associated with mean signal amplitude on a global level (-0.011 (95% CI -0.028; 0.004); P=0.07), nor on a network level (all $P_{FWE\text{-}corrected}$>0.025).

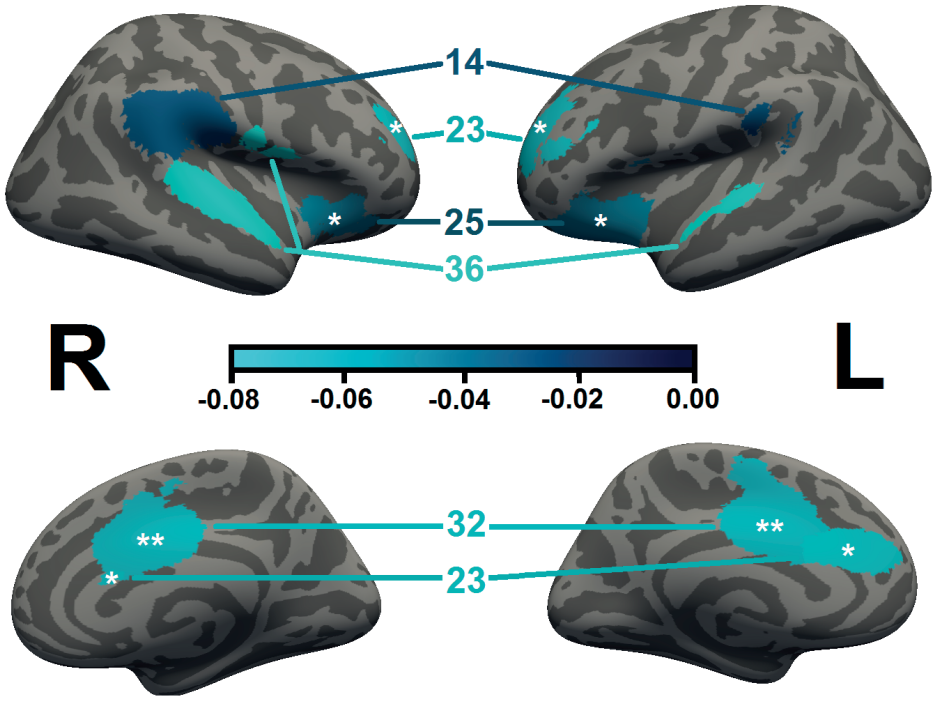

Figure 2. Topographical view of associations of total sleep time with signal amplitude within nodes of the ventral attention network

Negative associations of total sleep time with signal amplitude are shown for all 5 nodes of the ventral attention network on inflated right and left hemispheres, from a lateral (top row) and medial (bottom row) perspective. Lighter colors correspond to larger negative effect sizes (beta coefficients). Asterisks denote statistical significance as: *$P_{FWE\text{-}corrected}$<0.025; **$P_{FWE\text{-}corrected}$<0.00277. Please note that significance levels differ from effect sizes. Values represent difference in signal amplitude in that node per standard deviation increase in total sleep time, and are obtained through linear regression and permutation testing. Coefficients are adjusted for age, sex, mean frame-wise head displacement, ghost-to-signal ratio, time interval between sleep and rs-fMRI measurement, habitual alcohol consumption, physical activity, systolic blood pressure, body mass index, history of diabetes mellitus, supratentorial gray matter volume and total intracranial volume. Nodes correspond to the following regions (labeled using the probabilistic Harvard-Oxford cortical atlas found at https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases; top three overlapping regions): **Node 14**=Parietal operculum (16%), Posterior (16%) and Anterior (16%) Supramarginal cortex; **Node 36**= Superior Temporal cortex (21%), Temporal Pole (9%), Central Opercular cortex (9%); **Node 25**= Frontal Orbital cortex (28%), Insular cortex (17%), and Frontal Pole (8%); **Node 23**= Frontal pole (29%), Cingulate cortex - anterior division (9%), and Paracingulate cortex (6%); **Node 32**= Cingulate cortex – anterior division (24%), Juxtapositional Lobule (formerly: Supplementary Motor cortex) (13%), and Paracingulate cortex (5%). Threshold of node borders was set at z=5.0.

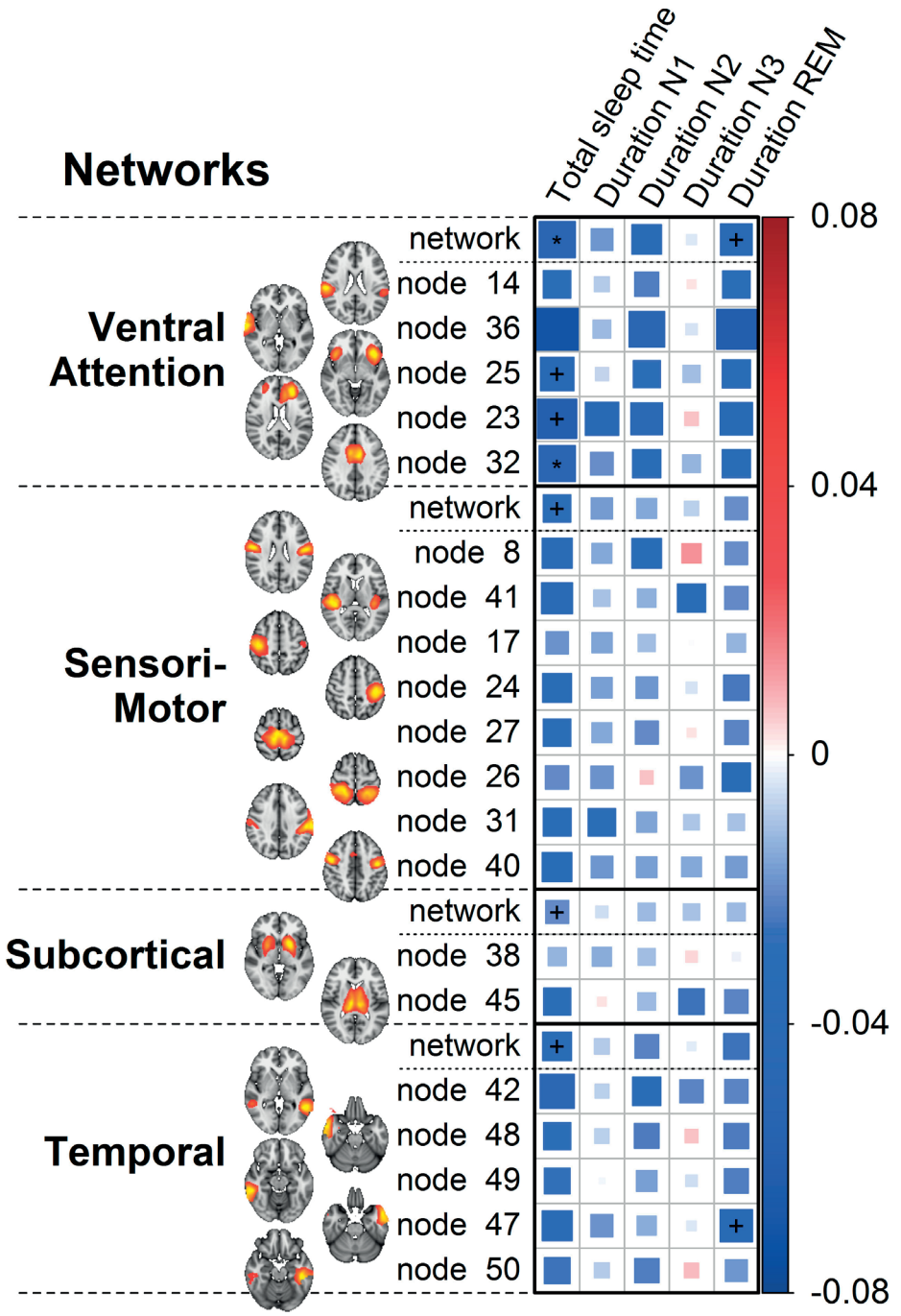

Figure 3. Associations of total sleep time, and duration of sleep stages, with signal amplitude within significant networks, and within their nodes

Associations of total sleep time and sleep stages with (mean) signal amplitude are shown for the four networks with a statistically significant relation. Corresponding nodes are depicted in the axial plane (right = anatomical left) at the level of highest node intensity. Colors and sizes of blocks correspond to effect sizes (beta coefficients): red indicates positive, and blue indicates negative associations.
Values are obtained through linear regression and permutation testing. Coefficients represent difference in signal amplitude in that network or node per standard deviation increase in the sleep parameter, adjusted for age, sex, mean frame-wise head displacement, ghost-to-signal ratio, time interval between sleep and rs-fMRI measurement, habitual alcohol consumption, physical activity, systolic blood pressure, body mass index, history of diabetes mellitus, supratentorial gray matter volume and total intracranial volume. FWE-corrected P-values for networks were corrected over all 9 networks, and for nodes were corrected for all 50 nodes. Symbols denote: + $P_{FWE\text{-}corrected}<0.025$; *$P_{FWE\text{-}corrected}<0.00277$. Please note that significance levels differ from effect sizes. Abbreviations: Nx= non-REM sleep stage x; REM=rapid eye movement.

DISCUSSION

In this population-based study, we found that PSG-determined longer total sleep time was associated with a lower mean BOLD-signal amplitude during daytime, primarily in the ventral attention network. In contrast, no objective or subjective sleep parameter was associated with functional connectivity between or within networks.

No study previously investigated the relation of objectively measured sleep with intrinsic neural activity measured at median 17 days apart, using a population-based design. In a large-scale study using UK biobank data, self-reported total sleep time was negatively correlated with signal amplitude in networks labeled as sensory/motor, not attentional networks.[12] We found no association for self-reported sleep duration assessed with the PSQI, but to the extent that PSG-derived total sleep time measured a similar construct, differences in study-specific parcellation, attributing the same functional node to different networks, may explain regional differences between studies.

We measured both sleep and rs-fMRI not within a 24-hour timeframe, which makes the association more robust to biases due to variable recording conditions of PSG and rs-fMRI. The association was more pronounced in persons who underwent measurements within a shorter, 1-month time interval, suggesting that effects were short-lived. Yet, both sleep[25,26] and resting state measures[27,28] exhibit 'trait'-like, time-stable properties, supporting that our association may extend beyond a night-to-day effect. Our findings were specific to BOLD-signal amplitude. Momentary increases in BOLD-signal may reflect local, task-triggered neural activity.[29] This amplitude does not refer to momentary increases but to increased *fluctuations over time*. Although its correlates have not been well characterized several observations suggest it is representative of a sleep-deprived state or lower vigilance.[7,30,31] After sleep deprivation, increased lapses in attentional maintenance can be observed[32] and such lapses may be accompanied by repeated intrusions of sleep.[30]

Alternatively, the amount of wakefulness could equally well underlie the association of total sleep time and signal amplitude as it was not driven by a specific sleep stage, and was also found when using actigraphy-estimated habitual total sleep time. Extended wakefulness increases synaptic potentiation,[33] and low-frequency EEG power[34] indicative of more synchronized activity. This power increase is most pronounced medio-frontally as was our association. Also, high amplitude activity on EEG observed in deep sleep indicates more synchronized fluctuations in membrane potential.[35] Against this background, we speculate that the association with BOLD-signal amplitude may also result from more synchronized, infra-slow neural activity during wakefulness.

Although we could not assess temporality in our cross-sectional study, these potential mechanisms favor a temporal association from sleep, or wakefulness, to brain intrinsic neural activity. Yet, the topographical overlap of our findings to the regions involved in the generation and propagation of sleep itself[36,37] may also suggest that signal amplitude determines total sleep time in a population-based, 'non-laboratory' setting. The temporality of the association of objectively estimated total sleep time and regional brain activity, or shared causes, should be studied further.

No sleep parameter was associated with network functional connectivity, in line with previous findings for the PSQI score.[15] Findings differ from experimental sleep deprivation studies that show a consistent impact on subsequent e.g. within-network connectivity of the default mode network.[6] Possibly, sleep deprivation effects may be too short-lived to be detected here. Furthermore, such effects inherently differ from our sleep measures which are more indicative of chronic, stable aspects of sleep. Importantly, methodological heterogeneity in e.g. study design, imaging processing, or modelling approaches may also explain finding null results in contrast to literature, as concluded recently for insomnia neuroimaging findings.[38] Also, bias by lack of adequate control for potential confounders or use of seed-based approaches[38] may have made previous studies more prone to finding false-positive results.

Several methodological considerations deserve mention. First, we did not monitor sleep during rs-fMRI acquisition and cannot rule out contamination of our measures by sleep.[39] Even light sleep stages[40] involve increases in global signal amplitude, consistent over networks. Individuals with a short total sleep time may have been at increased likelihood of falling asleep in the scanner, which may have biased our estimates. However, several observations suggest that contamination less likely explains our findings: i) We found no non-linearity in our associations for total sleep time, indicating that results were not driven by short sleepers only; ii) Total sleep time was not correlated with head motion, which may indicate sleepiness in the scanner[41]; iii) Even light stages of sleep involve substantially altered network connectivity.[39,42] This suggests that, if sleeping in the scanner drove our results for signal amplitude, one might expect to also find associations with functional connectivity between or within networks. Yet, we found none,

indicating that likely few participants slept during rs-fMRI acquisition. We ensured, by addressing participants, that they were awake at the start of rs-fMRI acquisition. Further vigilance monitoring with concomitant EEG was not deemed necessary nor feasible due to the population-based nature of our study. Second, we could not assess the influence of sleep the night preceding rs-fMRI acquisition. Third, performing fMRI at 1.5T instead of higher field strengths, and not controlling for variable conditions during rs-fMRI acquisition, may have reduced our sensitivity to detect associations. Similarly, retrospective assessment of sleep with the PSQI over the previous 4 weeks may have reduced chances to detect cross-sectional associations for PSQI-derived measures. Third, we could not assess how local differences in gray matter influenced our estimates beyond global volume. Study strengths include using PSG to study sleep over a broad and 'real-life' spectrum in a population-based study population, having substantial statistical power to detect small effect sizes, and adjusting for multiple potential confounders.

We conclude that, in the general middle-aged and elderly population, total sleep time affects the repertoire of (pre)frontal brain activity, or vice versa, beyond a night-to-day effect. At the same time, our results suggest there is no clear association of objective and subjective measures of sleep with functional connectivity between or within resting-state networks.

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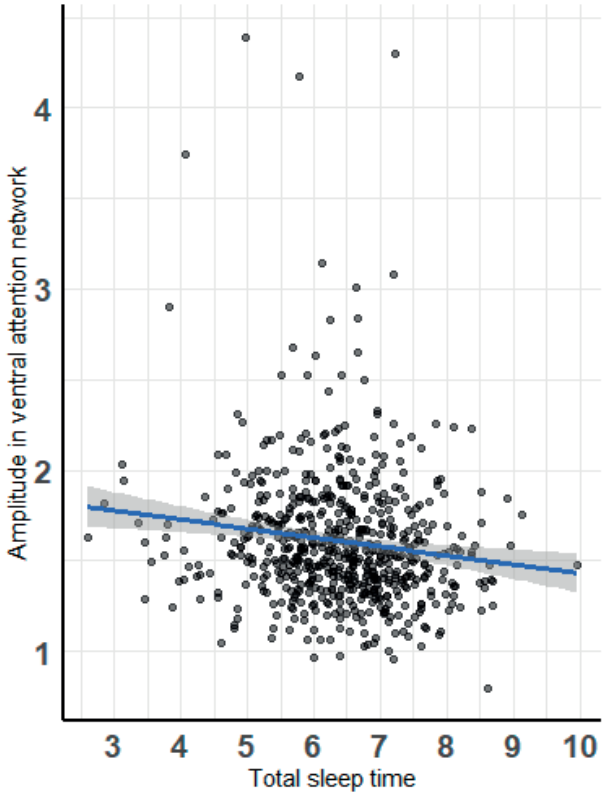
SUPPLEMENTARY MATERIAL

Supplementary Table 1. Correlations amongst sleep parameters and MRI quality parameters

TST	*TST*													
SOL	**-0.20**	*SOL*												
WASO	**-0.30**	**0.14**	*WASO*											
SE	**0.52**	**-0.48**	**-0.85**	*SE*										
N1	0.08	-0.03	**0.33**	**-0.20**	*N1*									
N2	**0.69**	-0.17	**-0.26**	**0.41**	0.02	*N2*								
N3	**0.18**	-0.01	**-0.19**	**0.19**	**-0.42**	**-0.33**	*N3*							
REM	**0.63**	**-0.14**	**-0.29**	**0.42**	-0.08	**0.30**	0.06	*REM*						
Delta	**0.15**	0.01	-0.10	0.11	**-0.44**	-0.07	**0.58**	0.03	*Delta*					
Beta	-0.04	0.09	**0.15**	**-0.13**	**-0.17**	**-0.14**	**0.26**	-0.04	**0.53**	*Beta*				
Gam	-0.06	0.11	**0.17**	**-0.15**	-0.11	**-0.17**	**0.22**	-0.06	**0.50**	**0.84**	*Gam*			
PSQI	-0.06	0.06	0.08	-0.11	-0.09	-0.02	0.06	-0.09	0.12	**0.17**	0.14	*PSQI*		
Gh	0.01	-0.06	0.02	0.03	0.11	0.07	**-0.17**	0.00	-0.12	-0.13	**-0.16**	**-0.14**	*Gh*	
FD	0.02	-0.03	0.00	0.01	0.08	0.01	-0.01	-0.02	-0.05	-0.12	-0.10	0.06	-0.04	*FD*

Spearman correlation values for polysomnography-derived parameters (sleep continuity measures, absolute sleep stage duration, spectral powers in three frequency bands), global PSQI score, and two MRI quality parameters. Values are obtained for complete case pairwise comparisons, using original variable distributions. Comparisons between spectral power frequency bands (n=560) and global PSQI score (n=603) were calculated in=543. **Bold** indicates statistical significance at P<0.001.

Abbreviations: Delta/Beta/Gam=Absolute spectral power in the delta/beta/gamma frequency band; FD=Frame-wise head displacement; Gh=ghost-to-signal ratio; Nx=non-REM sleep stage x sleep duration; PSQI=Global Pittsburgh Sleep Quality Index score; REM=Rapid eye movement sleep duration; SE=Sleep efficiency; SOL=Sleep onset latency; TST=Total sleep time; WASO=Wake after sleep onset



Supplementary Figure 1. Scatterplot of association of total sleep time with mean signal amplitude in the ventral attention network

The data points graphically depict the relation of longer total sleep time with mean signal amplitude in the ventral attention network. The regression line and corresponding shaded 95% confidence interval (CI) show the average relation obtained after linear regression. The multivariate adjusted linear association was statistically significant association after correcting for multiple testing only in this network (per standard deviation increase of total sleep time: -0.051 mean difference in signal amplitude (95% CI -0.077l -0.024); $P_{FWE\text{-}corrected}$=1.2e-3. Total sleep time is depicted in hours.

Supplementary Table 2. Associations of sleep parameters with mean signal amplitude at the global and network level, restricted to participants with a shorter time interval between sleep and rs-fMRI measurement

Level	Sleep parameters	Beta (95% CI)	P	$P_{FWE\text{-}corrected}$
Global	Total sleep time	**-0.033 (-0.055; -0.011)**	**2.4e-3**	-
	Sleep onset latency	-0.009 (-0.050; 0.032)	0.35	-
	Wake after sleep onset	-0.001 (-0.025; 0.023)	0.47	-
	Sleep efficiency	-0.005 (-0.033; 0.024)	0.38	-
	Stage N1 duration	-0.010 (-0.034; 0.014)	0.19	-
	Stage N2 duration	**-0.024 (-0.046; -0.003)**	**1.2e-2**	-
	Stage N3 duration	-0.007 (-0.031; 0.018)	0.30	-
	Stage REM duration	-0.016 (-0.038; 0.006)	0.08	-
	Spectral delta power	-0.003 (-0.031; 0.026)	0.44	-
	Spectral beta power	0.011 (-0.020; 0.041)	0.24	-
	Spectral gamma power	-0.009 (-0.041; 0.023)	0.31	-
	Global PSQI score	0.014 (-0.009; 0.037)	0.12	-
Network	Total sleep time			
	1: Default mode – anterior	**-0.058 (-0.100; -0.015)**	**5.2e-3**	**2.9e-2**
	2: Default mode – posterior	-0.025 (-0.051; 0.002)	0.04	0.17
	3: Frontoparietal	-0.024 (-0.054; 0.006)	0.06	0.25
	4: Dorsal attention	-0.023 (-0.055; 0.009)	0.07	0.29
	5: Ventral attention	**-0.062 (-0.094; -0.030)**	**6.0e-4**	**1.6e-3**
	6: Sensorimotor	**-0.039 (-0.061; -0.017)**	**4.0e-4**	**2.4e-3**
	7: Visual	-0.011 (-0.034; 0.012)	0.17	0.51
	8: Subcortical	**-0.029 (-0.047; -0.012)**	**6.0e-4**	**4.8e-3**
	9: Temporal	**-0.045 (-0.070; -0.020)**	**8.0e-4**	**2.2e-3**
	Stage N2 duration			
	1: Default Mode – anterior	-0.039 (-0.082; 0.003)	0.03	0.16
	2: Default Mode – posterior	-0.023 (-0.049; 0.004)	0.05	0.20
	3: Fronto-parietal	-0.025 (-0.055; 0.004)	0.04	0.20
	4: Dorsal Attention	-0.008 (-0.039; 0.023)	0.30	0.71
	5: Ventral Attention	**-0.049 (-0.081; -0.017)**	**1.2e-3**	**9.4e-3**
	6: Sensorimotor	**-0.025 (-0.047; -0.003)**	**1.3e-2**	0.07
	7: Visual	-0.003 (-0.026; 0.019)	0.39	0.79
	8: Subcortical	**-0.023 (-0.041; -0.006)**	**4.0e-3**	**2.6e-2**
	9: Temporal	**-0.038 (-0.063; -0.013)**	**1.6e-3**	**9.8e-3**

We analyzed associations at the global level, and further explored significant sleep parameters (total sleep time and stage N2 duration) at the network level. The absolute time interval between sleep and rs-fMRI measurement was ≤1 month for polysomnography (n=450 [72%], n=406 for spectral power variables [65%]), and ≤6 months for PSQI (n= 430 [69%]). Values represent difference (95% CI) in mean signal amplitude per standard deviation increase in the sleep parameter. Estimates are obtained using linear regression models and permutation tests, adjusted for age, sex, mean frame-wise head displacement, ghost-to-signal ratio, time interval between sleep and rs-fMRI measurement, habitual alcohol consumption, physical activity, systolic blood pressure, body mass index, history of diabetes mellitus, supratentorial gray matter volume and total intracranial volume. **Bold** indicates statistical significance at P<0.025. Please note that P-values at the global level were uncorrected as only the 'global' region was tested. Abbreviations: N=sample size; Nx=non-REM sleep stage x; PSQI=Pittsburgh Sleep Quality Index; REM=rapid eye movement

Supplementary Table 3. Associations of sleep parameters with mean signal amplitude at the global and network level, additionally adjusted for apnea-hypopnea index

Level	Sleep parameter	Beta (95% CI)	P	$P_{FWE\text{-}corrected}$
Global	Total sleep time	**-0.025 (-0.044; -0.006)**	**5.2e-3**	-
	Sleep onset latency	0.014 (-0.020; 0.049)	0.20	-
	Wake after sleep onset	-0.001 (-0.022; 0.019)	0.45	-
	Sleep efficiency	-0.014 (-0.038; 0.011)	0.14	-
	Stage N1 duration	-0.013 (-0.034; 0.007)	0.10	-
	Stage N2 duration	-0.013 (-0.032; 0.005)	0.08	-
	Stage N3 duration	-0.009 (-0.030; 0.012)	0.21	-
	Stage REM duration	-0.015 (-0.034; 0.004)	0.06	-
	Spectral delta power	0.004 (-0.024; 0.031)	0.40	-
	Spectral beta power	0.014 (-0.011; 0.039)	0.14	-
	Spectral gamma power	0.006 (-0.020; 0.032)	0.33	-
	Global PSQI score	0.009 (-0.010; 0.028)	0.18	-
Network	Total sleep time			
	1: Default mode – anterior	**-0.047 (-0.083; -0.01)**	**5.6e-3**	0.04
	2: Default mode – posterior	-0.016 (-0.039; 0.007)	0.09	0.32
	3: Frontoparietal	-0.013 (-0.040; 0.013)	0.16	0.50
	4: Dorsal attention	-0.014 (-0.042; 0.013)	0.15	0.47
	5: Ventral attention	**-0.051 (-0.078; -0.025)**	**4.0e-4**	**1.0e-3**
	6: Sensorimotor	**-0.030 (-0.049; -0.010)**	**1.6e-3**	**8.8e-3**
	7: Visual	-0.012 (-0.033; 0.008)	0.12	0.40
	8: Subcortical	**-0.020 (-0.036; -0.005)**	**5.0e-3**	0.03
	9: Temporal	**-0.032 (-0.052; -0.011)**	**1.6e-3**	**9.2e-3**

We analyzed associations at the global level, and further explored significant sleep parameters (total sleep time) at the network level. Values represent difference (95% CI) in mean signal amplitude per standard deviation increase in the sleep parameter. Estimates are obtained using linear regression models and permutation tests, adjusted for age, sex, mean frame-wise head displacement, ghost-to-signal ratio, time interval between sleep and rs-fMRI measurement, habitual alcohol consumption, physical activity, systolic blood pressure, body mass index, history of diabetes mellitus, supratentorial gray matter volume and total intracranial volume. **Bold** indicates statistical significance at P<0.025. Please note that P-values at the global level were uncorrected as only the 'global' region was tested.

Abbreviations: N=sample size; Nx=non-REM sleep stage x; PSQI=Pittsburgh Sleep Quality Index; REM=rapid eye movement

Supplementary Table 4. Effect sizes of associations of categorized total sleep time with mean signal amplitude, at both the global and network level

Level	Categories (quintiles) of total sleep time in hours				
	<5.6	5.6-6.2	6.2-6.6	6.6-7.2	>7.2
Global	0.035	0.006	0.000 (ref)	-0.046	-0.035
Network					
1: Default mode - anterior	0.020	-0.041	0.000 (ref)	-0.096	-0.116
2: Default mode - posterior	0.047	0.042	0.000 (ref)	-0.014	0.014
3: Frontoparietal	0.031	0.006	0.000 (ref)	-0.029	-0.017
4: Dorsal attention	0.002	-0.047	0.000 (ref)	-0.074	-0.060
5: Ventral attention	0.066	0.031	0.000 (ref)	-0.067	-0.055
6: Sensorimotor	0.052	0.016	0.000 (ref)	-0.031	-0.031
7: Visual	0.004	0.000	0.000 (ref)	-0.048	-0.035
8: Subcortical	0.031	-0.001	0.000 (ref)	-0.041	-0.025
9: Temporal	0.055	0.017	0.000 (ref)	-0.043	-0.025

Associations of categories (quintiles) of total sleep time with mean signal amplitude at the global level, and the network level. Values represent difference in mean signal amplitude for that quintile of total sleep time, referenced to the middle quintile. Values are obtained through linear regression adjusted for age, sex, mean frame-wise head displacement, ghost-to-signal ratio, time interval between sleep and rs-fMRI measurement, habitual alcohol consumption, physical activity, systolic blood pressure, body mass index, history of diabetes mellitus, supratentorial gray matter volume and total intracranial volume.

Supplementary Table 5. Associations of actigraphy-estimated sleep parameters with mean signal amplitude at the global and network level, in n=428 persons

Level	Sleep parameters	Beta (95% CI)	P	$P_{FWE\text{-}corrected}$
Global	Total sleep time	**-0.025 (-0.048; -0.001)**	**2.3e-2**	-
Network	Total sleep time			
	1: DMN – anterior	**-0.068 (-0.113; -0.023)**	**1.0e-3**	**8.6e-3**
	2: DMN – posterior	0.000 (-0.029; 0.029)	0.50	0.87
	3: Frontoparietal	-0.006 (-0.038; 0.027)	0.35	0.75
	4: Dorsal attention	-0.034 (-0.069; 0.000)	0.03	0.12
	5: Ventral attention	**-0.048 (-0.080; -0.016)**	**1.0e-3**	**9.4e-3**
	6: Sensorimotor	**-0.031 (-0.055; -0.007)**	**5.2e-3**	**3.6e-2**
	7: Visual	-0.005 (-0.032; 0.021)	0.33	0.73
	8: Subcortical	**-0.032 (-0.050; -0.014)**	**4.0e-4**	**2.2e-3**
	9: Temporal	-0.024 (-0.051; 0.003)	0.04	0.18

We analyzed associations at the global level, and further explored total sleep time at the network level. Values represent difference (95% CI) in mean signal amplitude per standard deviation increase in the sleep parameter. Estimates are obtained using linear regression models and permutation tests, adjusted for age, sex, mean frame-wise head displacement, ghost-to-signal ratio, time interval between sleep and rs-fMRI measurement, habitual alcohol consumption, physical activity, systolic blood pressure, body mass index, history of diabetes mellitus, supratentorial gray matter volume and total intracranial volume.
Bold indicates statistical significance at P<0.025. Please note that P-values at the global level were uncorrected as only the 'global' region was tested. Abbreviations: DMN=Default mode network; N=sample size; Nx=non-REM sleep stage x; PSQI=Pittsburgh Sleep Quality Index; REM=rapid eye movement

Supplementary Methods

Measurement of potential confounders and effect-modifiers

Potential confounders were selected based on impacting sleep derived from PSG, the neurovascular process underlying the fMRI BOLD-signal, or both.[1,2] Covariates, unless mentioned otherwise, were measured at the home interview or center visit, mostly before PSG. Age was determined at the polysomnography measurement. Values for ghost-to-signal ratio and mean frame-wise head displacement were obtained during fMRI preprocessing. Educational attainment was self-reported in four levels, expressed in corresponding average years (7, 9, 13, or 19). Habitual alcohol consumption was quantified with the Food Frequency Questionnaire[3] as grams/day intake. Physical activity was queried[4] and quantified in standardized measures of activity intensity (metabolic activity of task per week).[5] Systolic blood pressure in mm Hg was the average of two right-arm measurements when sitting up. Body mass index was calculated from measured weight and height (kg/m^2). Diabetes mellitus was defined as a fasting serum glucose level ≥7.0 mmol/L and/or self-reported use of anti-diabetic medication. Intracranial and supratentorial gray matter volume were obtained from structural MRI

(T1-weighted sequence) segmentations.[6] Depressive symptoms were assessed with the validated Dutch version[7] of the Centre for Epidemiological Studies Depression Scale.[8] Self-reported use of any antidepressant or hypnotic medication during the night of PSG was queried in an accessory sleep diary.

Statistical analysis

First, we calculated pair-wise correlations between all sleep determinants, and included frame-wise head displacement and ghost-to-signal ratio to examine how sleep related to MRI-parameters.[9] Main analyses were performed using general linear models with the intrinsic neural activity parameter as a dependent variable. We used group-level non-parametric permutation testing (n=5,000) implemented by FSL's randomise with family-wise error (FWE) corrected P-values to evaluate significance when testing associations in multiple regions within one topographical scale (i.e. at the network- or node-level). We chose sleep determinants informed by prior research.[10,11] Spectral power in the delta, beta and gamma bands were chosen based on the role of slow-wave activity on synaptic potentiation[12,13] and the role of high frequency bands as potential electrophysiological markers of hyperarousal in insomnia.[14-16] Lastly, we investigated global PSQI score as a measure of subjective sleep quality.

Thresholds for statistical significance and further exploring regional effects were a compromise between missing regional effects that may be 'averaged out' on a larger scale and type I error: Associations were regionally explored with $P_{FWE\text{-}corrected}<0.025$, halving the *alpha* of 0.05, as tests were performed one-tailed. A second, more stringent, significance threshold was defined to account for testing multiple sleep aspects in this study. For this, we computed the number of effective tests[17] (M_{eff}=9.23) based on Pearson correlations between the 12 sleep determinants, subsequently applied a Sidak correction,[18] and halved the new alpha level for two-tailed tests (P<0.00277).

In sensitivity analyses, we tested the robustness of findings by repeating the main analyses after including only participants with PSG and rs-fMRI measurements <1 month apart (n=450 for sleep stage scoring, n=425 for spectral analysis), or <6 months apart for PSQI (n=438). We additionally adjusted analyses for depressive symptoms and self-reported use of any versus no antidepressant or hypnotic medication at the night of PSG considering these factors may relate to both sleep and rs-fMRI parameters.[19,20] We also adjusted analyses for the apnea-hypopnea index, a prevalent indicator of potential obstructive sleep apnea in our study population.[21]

Posthoc, exploratory analyses included modeling a quadratic term of total sleep time (TST*TST), which was added besides the main effects term of TST, to statistically test potential non-linearity.

To minimize the effect of outliers, we winsorized outliers (changed to approach the mean) to 3 standard deviations from the mean. Sleep parameters were then standard-

ized (subtracting the sample mean and dividing by the standard deviation) to facilitate comparison of effect sizes. Missing data on covariates (mean=3%) were imputed using 5 multiple imputations, based on all analysis variables. Missing values imputation was performed with IBM SPSS Statistics version 22.0 (IBM Corp, Armonk, NY). Brain topographical depictions were created using Freesurfer Freeview. Figures including heat maps were created using R.

Supplementary references

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Ik dacht eens beter een nachtje niet
dan dat ik een nacht geniet
dus beter een dagje niet, dacht je niet?

De Jeugd van Tegenwoordig. Bertje vs. Yayo. De Machine (2008).

5 GENERAL DISCUSSION

This thesis discusses the role of sleep in neurodegenerative diseases and related brain outcomes, studied from a population perspective. Here I will discuss the main findings across studies, and highlight several methodological considerations relevant for interpreting our findings.

REVIEW OF FINDINGS

Sleep in the general population

We described self-reported sleep characteristics across all ages using population-based cohorts from the Netherlands, investigated their potential determinants and compared these sleep characteristics across countries and assessment methods (see Chapter 2.1). Using the National Sleep Foundation sleep duration recommendations[1] as a benchmark, we concluded that most people sleep for an acceptable duration. More importantly, sleep complaints or impaired sleep quality were more common than deviations of self-reported total sleep time from age-appropriate recommendations, across countries. Also, sleep characteristics assessed objectively through physiologic data systematically differed from subjective assessments.

Focusing on middle-aged and elderly individuals, an increased focus of research and public health professionals to increase sleep quality, not only duration,[2] seems relevant to improve sleep, and potentially related 'cognitive, physical and emotional health problems'[1] that may arise from poor sleep. However, we found that poor subjective sleep quality did not increase the risk of dementia in an elderly population. Recent studies showed that treating insomnia symptoms in adults through digital cognitive behavioral therapy for insomnia can reduce mental health problems,[3,4] of which application to older adults should be further studied. However, findings suggest that sleep problems when assessed subjectively are not necessarily related to an increased risk of all-cause dementia or Alzheimer's disease (see Chapter 3.1). This is not to say that ameliorating insomnia symptoms, or improving sleep quality, has no value.

Although most persons report a sleep duration deemed acceptable for their age, we could not adequately address questions regarding sleep deprivation, sleep debt or chronic sleep loss at a population level. These constructs are well not captured by one measurement of self-reported sleep characteristics. But more importantly, the discrepancy between self-reported and objective measurements[5] indicates that we are probably looking at more than sleep per se when judging self-reports on a population-based scale (also see Methodological considerations, paragraph on 'Subjective versus objective measurements'). Sleep characteristics that can be quantified using physiologic data, e.g. total sleep time, may be less adequately assessed using subjective, self-reported measurements.[5,6] It suggests we need to either recalibrate the whole debate to objec-

tive measures, or shift our focus more towards the constructs that are inherently validly measured through subjective appraisal.

Further research should investigate to what extent targeting individuals that report extreme durations or time in bed for their age and sex, or certain subgroups as those identified in this chapter, may yield improvement in sleep and well-being, and in health outcomes.

Sleep and dementia

We found no association of subjective sleep quality, measured with the Pittsburgh Sleep Quality Index (PSQI), with the risk of all-cause or Alzheimer's disease dementia over 13 years of follow-up (see Chapter 3.1). Moreover, PSQI components, including the often-investigated parameters of self-reported sleep duration and efficiency, were also not related to dementia risk. We discussed that potential biases do not seem to explain our negative findings, which are, however, largely inconsistent with meta-analyzed results.[7-9] On the one hand this suggests that chance may have played a role, on the other hand meta-analysis authors have cautioned for potential publication bias.[7] Interestingly, the most recent meta-analysis showed that there was no association of poor sleep with risk of cognitive disorders including dementia when the analysis was restricted to longer follow-up studies (>10 years). This suggests reverse causation, or the effect of preclinical or prodromal dementia on sleep at baseline, may have driven the largely positive results. Repeating survival analysis across studies, using our approach of time-stratified analyses on individual-level data may be an important next step to assess such effects (see Methodological considerations, paragraph 'Reverse causation').

While self-reported sleep quality was not associated with dementia risk, we found a relation of having objectively disturbed sleep with increased dementia risk (see Chapter 3.2). Evidently, subjective and objective measurements of sleep differ. Finding only an association using actigraphy-estimated sleep suggests that some disturbances are not recognized or not experienced as problematic by participants. Presence of prodromal subtle cognitive problems may hinder recognizing poor or short sleep, or contribute to downplaying issues with sleep, possibly to avoid further enquiry.[10] Yet, we cannot exclude that participants or their spouses may be aware of sleep problems relevant to dementia risk. Beyond awareness of sleep problems, we could also not determine to what extent prioritizing sleep, or negligence of sleep, contributed to increased dementia risk.

Besides sleep, we also investigated actigraphy-estimated 24-hour activity rhythms in relation to risk of dementia, identified as a knowledge gap in Chapter 2.2.[11-13] We found no relation of fragmented or unstable 24-hour activity rhythms with incident dementia or Alzheimer's disease. Yet, we found associations of a phase advance of sleep with incident dementia only in the next 2 years of follow-up, and of a stronger association of an

earlier 'lights out' time with incident dementia in short versus longer follow-up durations. These findings suggest that underlying neurodegeneration, or concomitant behavioral or neuropsychiatric disease features disturb the 24-hour activity rhythm closely before the diagnosis, not vice versa. This is not in line with prevailing interpretations of mostly cross-sectional data in previous reviews.[11-13] We feel repeated studies similar to ours are necessary. Also, future studies may consider investigating determinants of 'lights out' time as a novel indicator of dementia risk, with the objective to determine whether this symptom is indicative of behavior choices or of underlying circadian disruption.[14]

We discussed which neurobiological correlates may potentially confound the associations of actigraphy-estimated nighttime wakefulness and higher risk of dementia, especially Alzheimer's disease (see Chapter 3.2). Here, I provide a brief background for one potentially important factor hypothesized to have a bidirectional relation with sleep disturbances: Disease-related neurodegenerative pathology.

Alzheimer's disease is characterized pathologically by plaques of beta-amyloid and tau neurofibrillary tangles. When Alzheimer's disease is clinically diagnosed, patients (or relatives) often also report sleep or circadian disturbances. Such disturbances are not only a consequence of the disease but have been hypothesized to independently contribute to development of progression or the disease.[11-13,15-17] Studies have especially focused on the role of sleep, and extended wakefulness, in beta-amyloid metabolism. Animal studies show that beta-amyloid concentrations in interstitial fluid fluctuate with sleep and wake.[18] Sleep has been hypothesized to drive beta-amyloid clearance,[19] while wakefulness drives beta-amyloid production through neuronal activity.[20,21] Human observational and experimental studies mostly confirm this regulatory role of sleep on beta-amyloid concentrations,[22-28] and also sleep's role in regulating concentrations of pathological tau proteins relevant to Alzheimer's disease pathogenesis.[29-33] The relation of sleep disturbances with Alzheimer's disease pathology is likely bidirectional.[15]

Against this background, determining an association of sleep with incident neurodegenerative disease requires accounting for neurodegenerative pathology at baseline to obtain unbiased results. We discussed the possibility of such confounding (see Chapter 3.2), and addressed it by investigating the cross-sectional relation of sleep with biomarkers of neurodegenerative disease (see Chapter 4.1). Interestingly, we found that sleep and biomarkers were unrelated. This seemed unattributable to poor validity of our biomarkers measurements, as another recent study in our cohort found that higher NfL and lower Ab-42 in plasma were associated with an increased risk of all-cause dementia and Alzheimer's disease in non-demented individuals.[34] We do not know to what extent our plasma-based measurements may have not picked up small, strategic neurodegenerative changes in sleep-wake regulating regions in brainstem and prefrontal regions.[16,35,36] Further research may focus on such local lesions using neuroimaging methods. Nevertheless, findings suggests that neurodegenerative pathologies including those related

to Alzheimer's disease were not likely a confounder, or a mediator, of the association of actigraphy-estimated sleep disturbances with dementia risk. Thus, at the same time, to explain the link between sleep disturbances and dementia risk we feel it is warranted to look beyond Alzheimer's disease pathology.[16] After all, beta-amyloid and tau pathology are not sufficient[37] causes for developing clinical Alzheimer's disease dementia.[38,39] Also, other pathophysiological processes in the brain play a role in dementia,[32,40] that may also disturb sleep. Interestingly, some of these processes have also been described to occur as a consequence of disturbed sleep, e.g. excitotoxic activity or hyperexcitability, neuroinflammation, DNA damage, oxidative stress, or impaired glucose metabolism.[41-46] This overlap argues that we further investigating these factors as potential confounders, or mediators, of the link of sleep disturbances with risk of dementia in the general population.

This thesis further studied two such neurobiological correlates. We first determined sleep's relation with glymphatic functioning as indicated by the structural appearance of the perivascular space on magnetic resonance imaging (MRI; see Chapter 4.2). Similar to findings for plasma biomarkers, we found no consistent associations of poor sleep with higher perivascular space burden on MRI. Contrary to findings from the small number of previous studies on this topic, we found an association of higher sleep efficiency with higher perivascular space count in the centrum semiovale, i.e. an association in the opposite direction. Results could be explained by, among others, perivascular space count indicating brain physiological aspects beneficial to sleep, and we suggest further study of this surprising finding.

We also determined the association of sleep with brain functioning measured with resting-state fMRI (see Chapter 4.3). This method probes the functional organization of the brain and may represent subtle global or regional brain changes possibly relevant to neurodegeneration.[47-49] We found that longer total sleep time, measured with polysomnography and also actigraphy, was associated with a lower BOLD-signal amplitude, driven by prefrontal brain regions. The significance of this finding to risk of neurodegenerative disease remains unclear, although it seems limited as the absolute amount of actigraphy-estimated total sleep time was not associated with dementia risk (see Chapter 3.2).

Together, the neurobiological correlates investigated in chapter 4 could not explain the relation of actigraphy-estimated poor sleep with increased dementia risk (see Chapter 3.2). Further study of neurobiological correlates potentially confounding or mediating the sleep-dementia link is needed to learn what sleep characteristics, if any, contribute to risk of dementia in middle-aged and elderly persons.

Sleep and Parkinson's disease

We found that poor sleep quality and short sleep duration increase the risk of Parkinson's disease only in the first 2 years of follow-up, but not thereafter (see Chapter 3.3). Analyses over repeated measurements of sleep showed that a deterioration of sleep, i.e. a shortening of duration and a decrease in quality, was related to developing Parkinson's disease. These observations are congruent with sleep being a prodromal feature of Parkinson's disease. This interpretation also fits with neuropathological findings in the model proposed by Braak and colleagues, stating the involvement of sleep-wake regulating brain regions before onset of motor symptoms.[50-53]

We could not determine if specific sleep disorders drove our findings. Rapid eye movement sleep behavior disorder (RBD) may be considered a likely candidate as it occurs in around 30% of patients around diagnosis[54,55] and is highly specific to developing Parkinson's disease or related synucleinopathies.[56] Yet, current limited evidence suggests persons with RBD in the general population do not report their sleep as shorter or poorer than otherwise healthy individuals,[57] and may even report longer sleep durations. If, however, RBD precedes Parkinson's disease by over a decade, it could be involved in higher baseline levels of self-reported sleep duration and quality that make for steeper declines in these constructs when prodromal disease sets in. Alternatively, obstructive sleep apnea (OSA) has also been reported to precede Parkinson's disease in registry-based studies.[58] We feel further study of the involvement of OSA in the etiology of Parkinson's disease, and as driver of our findings is warranted based on several observations. First, the etiology of obstructive respiratory events strongly involves factors related to the airways, and not only central nervous system integrity. It may therefore be less susceptible to potential reverse causation effects than other sleep disorders in its relation to risk of neurodegenerative disease. Second, sequelae of OSA may potentially impact Parkinson's disease and its pathological features.[59,60] Third, a meta-analysis showed that OSA may be less prevalent in early Parkinson's disease cases versus controls,[61] which seems incongruent with Parkinson's disease as the primary cause of OSA. This has been attributed to increased rigidity in the upper airway reduces sleep-related collapse and obstructive events around the time of diagnosis.[58] However, most studies reporting a link of OSA and incident Parkinson's disease are registry-based studies which may be prone to diagnostic bias,[58] supporting the need for population-based prospective cohort studies implementing multimodal ascertainment of Parkinson's disease.

Further research into the role of sleep disturbances as marker of prodromal Parkinson's disease, or as potential risk factors to disease development seems warranted. To this end, population-based, prospective cohort studies such as the Rotterdam Study, that implement aforementioned ascertainment for incident disease as well as measurement of endophenotypes such as gait or symptoms of brady- or hypokinesia or rigidity, may well complement findings from cohort with individuals with RBD. Future studies may

also want to determine to what extent our results are generalizable to patients with early-onset Parkinson's disease.

METHODOLOGICAL CONSIDERATIONS

Sleep seems to be a highly variable phenomenon between persons and over time (see Chapter 2.1).[1,62] As hinted on in the 'General introduction' of this thesis, how normal we think sleep is contrasts sharply with how poorly we understand sleep in terms of its causes and consequences. This lack of knowledge is what makes sleep an interesting topic to study, especially in a population-based setting. At the same time, this inherently involves making several assumptions, some of which are not explicitly mentioned in the discussion sections of each chapter. The focus of this thesis was mainly on determining sleep's consequences. Here I further discuss sleep's neurobiological underpinnings and measurements, and how these are relevant to interpret the link with risk of neurodegenerative disease.

Multidimensionality of sleep

Sleep is a complex process or state, involving the orchestrated activity of diverse neuronal populations across the entire brain.[50,63,64] The dominant model for understanding how sleep and wake fluctuate at a systems level is the two-process model: Sleep depends on an interaction between a sleep homeostatic process and a circadian timing process.[65] Sleep homeostasis compensates sleep loss with extra sleep, operates throughout the brain and is indicated by slow-wave activity on the sleep electroencephalogram. Circadian timing is characterized at a cellular level by expression of proteins that inhibit their own production, fluctuating with a period of about 24-hours.[11] The master clock in the hypothalamic suprachiasmatic nucleus integrates circadian rhythms throughout the body.[66] Various brain nuclei and projections throughout the brainstem, frontal lobe and limbic system, using different neurotransmitter and hormonal systems, effectuate aforementioned processes.[50]

The approach to study this intangible process is 'multidimensional', reflected by the variety of levels, neurobiological to psychological, or characteristics on which sleep is measured.[63] Sleep can be appreciated through e.g. subjective appraisal, lack of movement, or slow-wave activity on electro-encephalography, all of which can estimate the same quantifiable characteristics such as total sleep time.[63] In line with this multidimensionality, we measured sleep using self-report, actigraphy in combination with diaries, or polysomnography, or a combination of these where deemed possible or appropriate.

Please note that our population-based measures were largely not designed to diagnose participants with sleep disorders, or in a larger sense, to identify persons with disordered

or deficient sleep versus 'normal' sleep (the 'tip of the iceberg' of sleep disturbances). Analogously, we studied sleep characteristics on a continuous scale, assuming that this conveyed information on subclinical but relevant abnormal sleep. Also, we assumed that our single measurements were to some extent stable over time and thus indicative of chronic exposure to a certain level of normal/abnormal, or good/poor, sleep.

Subjective versus objective measurements

Subjective measurements of sleep have been preferred in large-scale studies for their ease of administration and low costs. In general, subjective appraisal is inherently valuable as it expresses well-being. In sleep research, such measures are also relevant as they may drive seeking healthcare, and signal sleep problems that matter to individuals. Objectively measured sleep can only explain a part of the subjective appraisal of sleep's quality.[67,68] The role of subjective evaluation in sleep medicine, for example in insomnia diagnosis and treatment,[69] is, and remains, important regardless of increasing technological advances.

Yet, subjective quantification of sleep characteristics such as total sleep time may substantially differ from those obtained by methods taking physiological measurements, e.g. actigraphy.[6,70] This disagreement itself could of course be of interest, e.g. to assess insomnia severity.[67] Nevertheless, disagreement between methods is not random and may introduce bias.[6,71] If we are primarily interested in studying e.g. total sleep time, a characteristic best quantified physiologically, use of self-reported total sleep time means it will be misclassified and as such may introduce bias and hamper etiological inference. This issue is eloquently voiced by Bianchi and colleagues, who also highlight that using self-reported total sleep time increases the potential for confounding by unknown factors leading to systematically over- or underestimated total sleep time.[5] Especially cognition should be considered here. The importance of cognitive processes for reporting sleep is well illustrated by a study showing consistent differences for different constructs according to using a direct or indirect method of querying sleep.[72] Cognitive impairment may further reduce the validity of self-reporting sleep (see discussion of Chapter 3.1), possibly so that persons with lower cognitive functioning overstate their actigraphy-estimated sleep duration,[6] and patients with Alzheimer's disease underreport problematic sleeping in the face of evidently poor sleep estimated with actigraphy.[10] Besides cognition, affective factors are also relevant, as self-reported total sleep time is inextricably linked to mood.[6,73,74] Health-related factors as discussed by researchers from the Sleep Heart Health Study are also important to consider.[75]

We encountered inaccuracy in self-reporting time-related sleep characteristics in our meta-analysis (see Chapter 2.1), where up to 10% of individuals in some cohorts reported longer total sleep time than their time spent in bed. Moreover, this disagree-

ment in methods determined the difference in findings with regard to dementia risk in this thesis (see Chapters 3.1 and 3.2).

Some researchers respond to these inherent limitations of self-reported sleep data by carefully discussing these challenges, while others advocate we radically stop querying self-reported total sleep time.[5] We discussed possible biases and, where possible, used more objective methods to quantify sleep. Also, not knowing what determines these self-reported measures precludes actionable, preventive interventions to benefit public health when studying these measures (see paragraph "From sleep epidemiology to prevention").[76]

Reverse causation

Alzheimer's disease, the most common form of dementia, and Parkinson's disease are degenerative diseases hypothesized to be present long before diagnosis can be made.[52,53,77] Prospective cohort studies using structured repeated assessments show that subtle cognitive or motor deficits are already appreciable for up to a decade before the diagnosis in patients versus controls.[78-80] Besides typical disease-related characteristics, more non-specific neuropsychiatric symptoms may also be present in this prediagnostic phase, such as depressive symptoms,[78,81,82] or physical inactivity for Alzheimer's disease.[83,84] When such factors are investigated as potential risk factors for incident dementia in non-demented individuals followed up over time, they may temporally precede a dementia diagnosis and be labeled a risk factor when truly there is no causal relation. Instead, the temporal relation is causal but reversed, which can also be thought of as confounding by the underlying pathological processes. Sleep is also subject to this phenomenon. Neurodegenerative pathology may directly influence brain regions that generate or propagate sleep,[36,85,86] or may affect sleep and 24-hour activity rhythms through other prodromal or non-specific symptoms or signs, e.g. physical inactivity, apathy, decreased light exposure.

We examined potential reverse causation by stratifying analyses on follow-up time, simulating premature study endings. We restricted follow-up to the first e.g. 2 years after baseline, censoring all at-risk participants, and then incrementally increased follow-up from 2 years towards the duration of the overall follow-up, simulating shorter-duration studies within our own study. We did not exclude the first years of follow-up, selecting persons on not getting the outcome for the first e.g. 2 years, which has been described to potentially lead to selection bias.[87] We assumed that a decrease in strength of effect sizes with increasing follow-up time indicates preclinical or prodromal disease disturbing sleep at baseline (see Chapter 3.3).

This analytical approach to reverse causation seems worthwhile to pursue in an individual-participant data framework on sleep and incident dementia, as done by others,[83] to tease out to what extent studies suffer from reverse causation.[7-9] Importantly,

stratifying existing studies on median total follow-up time as previously performed[7,9] is a less sensible approach to examine potential reverse causation, as single risk estimates averaged over long study follow-up may still be driven only by a strong relation in the first few years of follow-up.

Please note that aforementioned approach to detect reverse causation only shows a temporal relation, and cannot prove reverse causation. This means that a typical pattern indicating reverse causation does not prove the absence of any causal effect of the exposure on the outcome. The exposure may also be a step in a multistage process that harms only in a certain opportune window. Evidence for such a multistage process, requiring accumulation of several sequential pathological 'hits', may be found in incidence data in prospective cohorts for dementia.[88] Also, a temporal relation indicative of reverse causation does not exclude the possibility of confounding of the relation of the exposure and risk of the outcome by genuine, unknown risk factors.

From sleep epidemiology towards prevention or treatment

Epidemiological studies not only aim to provide quantifiable insight into the etiology of disease but also to contribute information to prevent disease. This second step should be highlighted to show that identifying risk factors does not necessarily allow taking preventive action.[89] A difference between the two can be identified within the potential outcomes framework, or counterfactual framework, of causal inference.[90] The difference is that the sleep exposures studied by epidemiologists may differ from what is reasonably intervened upon to change that exposure.

Take the following example: Reducing high BMI may seem like a reasonable objective in public health. Yet, different interventions to reduce BMI tackle different underlying biological processes. Examples include giving lifestyle advice, prescribing diets, performing bariatric surgery, but also amputating a limb.[91,92] Amputation seems effective to reduce BMI, yet everybody would agree it would not reduce risk of cardiovascular outcomes or mortality. Why not? Clearly, the underlying biological substrates of increased BMI, its directly identifiable upstream causes, increase the risk, not necessarily BMI itself. Considering BMI as risk factor for mortality still lacks the actionable information needed to inform public health policies.

As discussed earlier, sleep is a process involving various neurobiological and neurotransmitter systems, and is pragmatically measured across multiple dimensions. Analogous to the BMI example, this suggest a potential for a disconnect between observational exposures and potential interventions. Let's pretend that we performed the perfect observational study on the relation of actigraphy-estimated sleep with dementia risk, and found an association of short total sleep time with increased dementia risk. How do we then increase total sleep time, and will this reduce dementia incidence? Pharmacological interventions, typically sedative hypnotics, may not necessarily mimic

naturalistic sleep.[93] Interventions such as cognitive behavioral therapies are designed to address dysfunctional thoughts or behaviors regarding sleep. This may renormalize a short total sleep time, yet problematic cognition or behavior may have not necessarily been the problem underlying short total sleep time. Moreover, behavioral changes to increase sleep, i.e. deciding to get more sleep, is only indirectly achieved by extending sleep opportunity in the hopes of getting more sleep.

Even if we studied an exposure that was more clearly defined in terms of the underlying biology, e.g. slow-wave sleep, there may still be a disconnect between observational exposure and potential interventions. Pharmacological interventions that enhance slow-wave activity and therefore slow-wave sleep, may differ in other effects that differentially relate to the outcome under study.[94] Specifically enhancing slow-wave activity during sleep may also be achieved through waking activities (meditation, cognitive activity, physical activity), sensory stimulation during sleep (acoustic, olfactory, vestibular stimuli), or non-invasive transcranial electromagnetic stimulation.[95] Interestingly, these different interventions are also associated with a better performance on cognitive tasks,[95] even in older adults,[96] supporting a key role for slow-wave activity or sleep in cognition and providing a basis for targeted treatment or prevention of cognitive impairments.

A more thorough understanding of the neurobiological determinants of sleep may help to design interventions towards preventative action. This does however not preclude that appropriate interventions may have a different effect than what was derived from observational studies.[97]

Identifying a risk factor in observational studies is a process of reasonably excluding biases and chance and then accepting that whatever remains is the causal relation of that exposure with your outcome. Aforementioned example suggests that not only is short total sleep time not defined well enough in terms of its corresponding intervention, but that this lack of specificity in its definition is hampering our ability to know to what extent our association is unconfounded.[92] This principle seems to apply not only to total sleep time but to a number of sleep exposures in epidemiological studies, including ours. It is therefore important to stress that current sleep epidemiological findings should be considered more an important first step than research efforts lacking actionable information. Epidemiologists advocating the use of well-defined interventions in the potential outcomes framework are in my opinion advocating pragmatism, and as such may understand that current population-based studies pragmatically investigate sleep through feasible measures first. If no relation exists, valuable resources are better invested elsewhere.

Threats to validity of sleep findings

Several threats, or biases, may have affected the validity of the findings in this thesis. These concern confounding, selection bias, and information bias as threats to interval

validity, and limited generalizability. We tried to account for these potential biases in the analysis phase, or discussed them, per chapter. Here, I want to briefly highlight the issues of confounding, and generalizability.

Confounding indicates that a third factor, a cause of both the exposure and outcome, distorts their relation.[98] Confounding in observational research is ubiquitous. Recognizing the potential for confounding, trying to reduce confounding or at least discuss the potential for confounding based on someone's expert knowledge is a prerequisite in any attempt to produce methodologically sound results.[99] Selection of potential confounders was informed on literature where possible.[98] Further studies on what determines our population-based measures of sleep, especially brain determinants, seems important to improve adequate control for confounding in future.

The importance of recognizing potential confounding in observational sleep research is illustrated by an example focused on the rare, neurodegenerative disease Fatal Familial Insomnia (FFI). This disease involves abnormal folding of the brain's own prion proteins, related to a specific genetic polymorphism in the gene encoding prion protein. It is characterized by a progressive, severe lack of sleep, and patients often die within a year of diagnosis. One thus observes a lack of sleep linked to a high mortality rate. While sleep disturbance can certainly impact health and well-being, and may contribute to an increased risk of dying, the apparent association of sleep disturbance in FFI with high mortality is likely confounded by the underlying neurodegenerative process. Sleep disturbances in FFI are therefore not proof that a lack of sleep is life-threatening in humans.

As a rule of thumb, one should be very critical in interpreting observational associations as causal. This is especially important as aforementioned example may lead families of patients with FFI to believe that treating sleep disturbances may have prolonged the life of their loved one, for which currently no evidence exists. I find this an interesting example as it featured in the popular book on sleep "Why We Sleep" by Matthew Walker published in 2017,[100] which evoked criticisms in the form of blogs on social media,[101,102] and a sportsman-like response by the author.[103] It is also of personal interest as I have been in personal contact with patients with prion disease and their families, during my work as physician for the Dutch National Prion Registry. Having witnessed how especially family members deal with scarce information available on these severe disorders, I find it all the more important that the information on possible treatments is accurate.

Regarding generalizability, studies embedded in the Rotterdam Study were based on predominantly individuals from European descent, with a middle-to-high income.[104] We found that subjective appraisal of sleep was poorer in the US compared to the Netherlands and the UK (see Chapter 2.1). Interestingly, meta-analyses on the relation of mostly self-reported sleep characteristics with dementia and Alzheimer's disease risk showed that results obtained from European studies were similar to those from North-American studies.[7,9] Nevertheless, cross-cultural heterogeneity in the social timing of sleep and

its role in daily life,[105] especially in aged individuals studied in this thesis, may limit the generalizability of our findings.

IMPLICATIONS

Clinical

We aimed to study sleep's role in the etiology of neurodegenerative disease, in a population-based setting, and studied sleep mostly in otherwise healthy individuals. Therefore, findings have limited implications for patients and healthcare professionals. Nevertheless, several points may be of clinical interest.

First, descriptive sleep data from our meta-analysis provides a data-driven view on extremes in sleep, e.g. through percentile curves, which may be used as an evidence-based starting point to actively screen for underlying sleep disorders. Cut-offs are applicable to the general population, and further evaluation of their accuracy and overall usefulness in more selected populations, e.g. patients visiting a general practitioner with a sleep complaint or something related, or visiting a sleep clinic, should be performed.

Our data show that sleep complaints are common, especially with increasing age in older adults, providing a potential target for sleep improvement at the population level. At the same time, the same data can be interpreted as sleep problems being something 'normal'. If indeed sleep complaints, after evaluation by a healthcare professional, are not in need of further diagnostic tests or therapeutic interventions, our data could be used to reassure individuals with sleep complaints that their problems are common.

Second, dementia patients and their families can be informed that poor sleep in late life is associated with an increased risk of dementia, or vice versa, that poor sleep may precede a diagnosis of dementia by years. This may not necessarily be reflected in subjective appraisal of sleep, although we did not investigate whether in retrospect sleep problems may be recognized. Also, our results show that typical changes in 24-hour activity rhythms that may constitute prodromal dementia features are an advance in sleep phase and earlier bedtime. Explaining these disease-related changes to patients and loved ones may help them gain a sense of understanding of, and therefore perhaps control over, the very serious problems they are faced with.

Third, patients with Parkinson's disease with questions regarding sleep could be informed that having more sleep complaints and reporting a shorter sleep duration are prodromal features of the disease that may occur on average at least two years before a diagnosis.

Although we could not reasonably show relations of sleep and incident outcomes that indicate a causal effect, optimizing sleep and circadian rhythms seem reasonably inexpensive secondary treatment goals, that matter to patients or caregivers. Enquiring

about perceived sleep problems seems warranted, for which Dutch healthcare professionals may find the structured *NHG-standaard* approach useful.[106]

Public health

Our meta-analysis results show what is 'normal' for different sleep characteristics, different from the expert recommendations of the US National Sleep Foundation about what constitutes 'good' sleep. This provides an alternative, more pragmatic benchmark for future sleep studies. Our findings show that sleep complaints are common and not necessarily explained by aberrant sleep times. An association of more insomnia symptoms with above-normal time in bed suggests a place for interventions to reduce the time in bed as used in insomnia disorder treatment, i.e. non-pharmacological, cognitive-behavioral interventions. Education is a key part of such interventions, so large-scale education of the public seems at first glance a potentially efficacious route to try and shift the population distribution of insomnia symptoms. Debunking false myths about sleep that have public health sleep significance[107] may be part of that approach. Possibly, as mentioned above, providing state-of-the-art cognitive behavioral therapy for insomnia via digital channels may provide a scalable alternative to effectively reduce insomnia complaints and related mental health problems.[3,4]

Important caveats that should be kept in mind is that digital health interventions may not reach elderly persons, especially the more vulnerable, cognitively impaired persons who are expected to have substantial benefit.[108,109] Nevertheless, use of smartphone in elderly persons seems to be on the rise, at least in the Netherlands,[110] and with it may come increased openness to engage with digital health solutions. Also, an important caveat in any attempt to communicate the importance of sleep to the general public is that attention to sleep equals worry about sleep, which is bad for sleep.

Future research

Designing future sleep research focused on etiology of neurodegenerative diseases may be well informed by thinking about the most optimal observational study, with infinite resources at our disposal, that may be performed to support causal claims.

Ideally, we would need a large-scale (10,000+ participants) cohort study, that from midlife onwards[111] repeatedly measures sleep with polysomnography and actigraphy, measure state-of-the-art, disease-related brain markers (CSF, blood, non-invasive neuroimaging), combined with continuous follow-up to diagnose neurodegenerative disease. Imaging approaches may focus on specific sleep-regulatory nuclei such as the locus coeruleus,[112-114] which shows Alzheimer's disease-related tau pathology early in life,[35,115] and may play a role in RBD, a sleep disorder specific to development of alpha-synucleinopathies.[116] Functional imaging approaches would need to ensure simultaneous vigilance/sleep measurement, e.g. by combining fMRI/EEG to properly assess

sleep's role in functional changes of the brain across time.[117] Measuring from mid-life onwards may help establish a potential window of opportunity for preventive action.[118] Polysomnography must include a screening approach to further evaluate persons with possible RBD. Data analysis may include implementing causal inference methods, e.g. g-methods to deal with unmeasured confounding and time-varying confounding,[119] or a 4-way decomposition analysis to deconstruct the potential interaction and mediation of sleep with Alzheimer's disease pathology on risk of dementia.[120,121]

Unfortunately, this sleep study will remain a dream. Until then, we need to investigate both determinants as well as consequences of sleep in the general middle-aged and elderly population. Important avenues to pursue are linking brain structure and function to objective sleep and 24-hour activity rhythm characteristics, and leverage genetic data to establish the biological basis of our sleep measures, for which there is increasing attention.[122] Both approaches are probably best executed in collaboration, such as the ENIGMA consortium for sleep neuroimaging studies, or setting up new collaborations to achieve large sample sizes for much-anticipated genome-wide associations studies on objective sleep and 24-hour activity rhythm phenotypes. Understanding the link of sleep and dementia may also be better achieved by using Mendelian randomization,[123] or leveraging genetic risk scores, to assess the associations of genetic correlates of certain sleep characteristics with dementia risk and vice versa. Using repeated measures of sleep to investigate what determines trajectories of sleep in aging will help elucidate relevant underlying factors in the context of slowly progressive neurodegenerative diseases. Yet, most importantly, one of the key first steps towards better understanding the potential causal role of sleep disturbances in dementia is to account for disease-related neuropathological factors. Our approach using plasma-based biomarkers is an example of a feasible design to study this in large, population-based samples.

Besides pragmatic studies on the risk of neurodegenerative disease and related neurobiological correlates, several assumptions regarding the relation of sleep and Alzheimer's disease pathology should also be addressed. These mostly concern the translation of laboratory findings to a 'real world' setting. For example, it is unclear how effects of acute sleep deprivation on pathology relate to the often less severe but chronic disturbances observed in real-life. For example, a 5-day chronic sleep restriction regime differed from acute sleep deprivation in microglia activation in mice,[44] and the history of sleep may be carried forward and help determine behavioral performance days later.[124] It is therefore unclear if a) chronic disturbances equate repeated acute disturbances, i.e. repeatedly elevate beta-amyloid levels, and if b) this leads to higher rates of plaque deposition, and if c) this leads to accelerated cognitive and functional deterioration. Acute excesses of beta-amyloid may also be adequately removed from interstitial or cerebrospinal fluid compartments,[125] and partial sleep deprivation in humans to 5 nights of 4 hours did not

elevate beta-amyloid isoforms or other biomarkers of detrimental processes in cerebrospinal fluid or plasma.[126,127]

Population-based studies may also provide insights into how sleep determines Alzheimer's disease pathology over time. Yet, so far only one study determined longitudinal changes in amyloid deposition.[128] Authors reported that excessive daytime sleepiness in non-demented individuals increased amyloid deposition over 2.2 years on average. Potential confounding was discussed but not yet taken into account in the analyses.

CONCLUSION

We conclude that sleep complaints are common in elderly persons, more so than an inadequate sleep duration. Poor sleep was associated with incident neurodegenerative disease. In the case of self-reported sleep quality and duration in relation with Parkinson's disease, patterns of associations suggest that poor sleep is a prodromal feature of the disease, whereas in the case of actigraphy-estimated nighttime wakefulness preceding all-cause dementia and Alzheimer's disease, the link seemed not explained by known potential confounders.

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"The bed is open; the toothbrush hangs on the wall,
Put your shoes at the door, sleep, prepare for life."

The last twist of the knife.

T.S. Eliot. Rhapsody on a Windy Night (1911).

6 SUMMARY

6.1 ENGLISH SUMMARY

Sleep is a normal phenomenon, yet poorly understood. Emerging neurobiological insight into sleep suggests it serves several vital functions for the brain. These imply that disturbed sleep may contribute to brain disease. Specifically a link of sleep disturbances with age-related neurodegenerative diseases seems interesting as sleep's role in brain health overlaps with pathological processes of these diseases. In this thesis, I examined how sleep is related to risk of dementia, including Alzheimer's disease, and Parkinson's disease, and related neurobiological correlates.

First, we aimed to describe sleep in the general population (Chapter 2.1). We collected individual-level data from 36 Dutch sleep cohorts, as well as objective and subjective sleep data from different countries, and investigated potential determinants. Summaries of basic sleep characteristics such as sleep duration per night were given across the lifespan in percentile curves. We found that, of the 200,358 individuals from Dutch population-based studies, most persons had a sleep duration within the "acceptable" range according to US guidelines. Clearly delineable groups with poorer sleep were teenagers, and women. Adults that reported spending 7-8 hours in bed report the least sleep problems, and problems seemed to differ whether time in bed is higher or lower than that. While self-reported sleep duration in adult populations from the Netherlands, UK and US is similar, insomnia symptoms are more prevalent in the US than in the Netherlands.

Objective sleep estimates showed that while women report sleep as shorter or poorer, actigraphy estimates suggest that they actually sleep longer and more efficient than men. When using the self-report recommendations, actigraphy-estimated sleep duration is below the recommended level for over 80% of adults aged 40 years and over. Recommendations for actigraphy-estimated sleep are currently lacking. Consistent across studies and countries, poor sleep quality is a greater perceived problem than short sleep, a finding that calls for targeting sleep quality improvement

We also reviewed the relation of 24-hour activity rhythms, as a measure of circadian rhythm functioning, with various disease outcomes, including neurodegenerative disease (Chapter 2.2). An increasing number of studies uses actigraphy to study the circadian rhythm, yet most studies are cross-sectional or are not performed in the setting of a prospective cohort, making it hard to draw temporal inference. Especially neurodegenerative diseases are commonly investigated, where the few longitudinal studies that were performed suggest that 24-hour activity rhythm disturbances precede dementia, which should be studied further (see chapter 3.2).

Based on previous studies suggesting a relation of disturbed sleep with incident dementia, we further investigated the relation of sleep, assessed both with questionnaire (chapter 3.1) and with actigraphy (chapter 3.2), with risk of all-cause dementia

and Alzheimer's disease. First, in chapter 3.1, we specifically focused on subjective sleep quality assessed with the Pittsburgh Sleep Quality Index (PSQI). We found no relation of the PSQI score, nor its components, with incident dementia or Alzheimer's disease. In contrast, actigraphy-estimated poorer sleep was associated with a higher risk of dementia, including Alzheimer's disease.

In line with the knowledge gap identified in chapter 2.2, we also investigated the association of actigraphy-estimated 24-hour activity rhythm characteristics with dementia. Disturbed activity rhythms, based on its variability and stability, were not related to dementia risk. We found an association of an earlier 'lights out' with increased dementia risk, which was especially strong in the first 2 year of follow-up. Also, an earlier onset of the least 5 active hours of the day were associated with a higher risk of dementia in the first 2 years of follow-up, which may be congruent with an advance in activity rhythms, as marker of circadian disturbance, as a late prodromal feature of dementia.

Besides dementia, we also investigated Parkinson's disease (Chapter 3.3). We determined the relation of self-reported sleep duration and sleep quality, as well as the changes in these sleep characteristics over two measurements, with incident parkinsonism, including Parkinson's disease. Poorer sleep quality and shorter sleep duration were associated with an increased risk of Parkinson's disease in the first 2 years of follow-up, disappearing with increasing follow-up. Also, a shortening of sleep duration and a worsening of sleep quality over repeated measurements were associated with an increased risk of Parkinson's disease. Both observations were congruent with a progressive deterioration of sleep towards the diagnosis of Parkinson's disease, i.e. of a deterioration of sleep as a prodromal feature of Parkinson's disease.

In chapter 4, we further investigated potential neurobiological correlates of sleep. We investigated the relations of questionnaire-assessed and actigraphy-estimated sleep with neurofilament light chain (NfL), and also beta-amyloid isoforms and total tau, assessed in plasma. We found no association of sleep characteristics with these plasma biomarkers of neurodegenerative disease, except for an association of a longer self-rated time in bed with a higher NfL concentration. The lack of associations of sleep characteristics with biomarkers of neurodegenerative disease suggest that these processes do not confound, or mediate, the association of poor sleep with incident dementia.

Sleep has been hypothesized to clear waste from the brain, enhancing fluid exchange across the 'glymphatic' system. We investigated the relation of sleep with enlarged perivascular spaces on brain MRI as a potential marker of impaired waste clearance through the glymphatic system. We found an association of higher actigraphy-estimated sleep efficiency with higher perivascular space count in the centrum semiovale. The direction of this cross-sectional association contrast with previous clinical and population-based studies in humans. We discuss the various explanations of this finding, including

a speculation that perivascular spaces may signal a physiological aspect of the brain beneficial to sleep.

In chapter 4.3, we investigated the brain's functional connectivity through resting-state functional MRI which may sensitively detect any relevant consequences poor sleep may have on the brain in middle-aged to elderly persons. We found that a longer polysomnography-derived total sleep time was associated with a lower signal amplitude in especially (pre)frontal regions. This cross-sectional association may indicate that total sleep time affects the repertoire of brain activity in those regions, that regional brain activity determines total sleep time in the general population, or that both have common causes.

6.2 NEDERLANDSE SAMENVATTING

Slaap is, voor een alledaags fenomeen, slecht begrepen. Nieuwe inzichten vanuit de neurobiologie geven aan dat slaap het brein op verschillende belangrijke manieren ondersteunt in zijn functioneren. Er wordt hierbij gesuggereerd dat verstoorde slaap bij kan dragen aan het ontstaan van hersenziekten. Specifiek is de invloed van slaap op veelvoorkomende neurodegeneratieve ziekte interessant, aangezien de biologische effecten van slaap op het brein volgens dezelfde processen zouden verlopen als de pathologische processen in deze ziekten. In dit proefschrift onderzoek ik de relatie tussen slaap en het risico op dementie, waaronder het Alzheimer subtype, de ziekte van Parkinson, en andere aspecten van het brein die gerelateerd zijn aan deze ziekten.

Eerst beschreven we slaap op populatieniveau (hoofdstuk 2.1). We verzamelden slaapdata van personen uit 36 onderzoekscohorten in Nederland, en subjectieve en objectieve slaapdata uit andere landen ter vergelijking. We beschreven verschillen naar leeftijd en geslacht, en onderzochten de relatie van diverse andere factoren met slaap variabelen. We vatten de data van slaapduur samen met percentiel curves over leeftijden van 1 tot 100 jaar, van 200,358 Nederlanders. De meeste mensen rapporteerden een slaapduur die paste bij de aanbevolen slaapduur voor hun leeftijd. Adolescenten en ook vrouwen waren subgroepen die relatief vaak slechte slaap rapporteerden of een inadequate slaapduur aangaven. Volwassenen die een tijd in bed rapporteerden tussen de 7 en 8 uur klaagden het minst over hun slaap. Het type slaapproblemen leek te verschillen tussen mensen die te kort versus te lang in bed lagen. We vonden dat zelfgerapporteerde slaapduur vergelijkbaar is tussen Nederland, het Verenigd Koninkrijk en de Verenigde Staten (VS), maar dat klachten over de slaap vaker voorkomen in de VS dan in Nederland. Objectieve schattingen van slaap middels beweging gemeten met actigrafie lieten zien dat vrouwen juist iets beter slapen dan mannen, tegenovergesteld aan wat zij rapporteerden. Aanbevelingen voor slaap gemeten met actigrafie zijn er momenteel niet, maar is nodig aangezien de huidige, op vragenlijsten gebaseerde aanbevelingen, stellen dat de meeste volwassenen te weinig zouden slapen gebaseerd op actigrafie (80% van de populatie heeft dan een slaapduur korter dan aanbevolen voor de leeftijd). De belangrijkste bevinding van dit onderzoek lijkt dat klachten over de slaap meer voorkomen dan een afwijkende slaapduur, wat suggereert dat verbeteringen van de slaap op populatieniveau niet zozeer te bereiken zijn door mensen te adviseren over de juiste slaapduur.

Naast deze grote beschrijvende studie maakten we ook een niet-systematisch overzicht van studies naar de relatie van 24-uurs activiteitsritmes, als maat van het circadiane ritme, met neurodegeneratieve ziekten en andere veelvoorkomende aandoeningen op oudere leeftijd. Het aantal studies naar 24-uurs activiteitsritmes neemt de afgelopen jaren duidelijk toe, maar voornamelijk nog met een dwarsdoorsnede studie opzet.

Longitudinale studies zijn nodig om een temporeel verband te vinden tussen verstoringen van activiteitsritmes met dementie en andere neurodegeneratieve ziekten. Daar maakten we werk van in hoofdstuk 3.2.

Op basis van eerdere studies, die een verband tussen slechte slaap en het risico op dementie lieten zien, onderzochten we enkele specifieke aspecten van slechte slaap verder, zoals slaapkwaliteit. We vonden dat slaapkwaliteit, ingeschat met de Pittsburgh Sleep Quality Index (PSQI), niet was gerelateerd aan het risico op dementie, noch het Alzheimer subtype. Ook de componenten van deze (zelf-gerapporteerde) slaapkwaliteit, die door anderen als risico-verhogend waren aangewezen, waren in onze studie niet geassocieerd met een hoger risico op dementie. In tegenstelling tot deze bevindingen voor slaapkwaliteit vonden we wel een relatie tussen slechter slapen en hoger dementie risico als slaap was bepaald met actigrafie. Met alle andere studies op dit gebied in overweging genomen, is het niet onwaarschijnlijk dat slecht slapen inderdaad geassocieerd is met een hoger risico op het krijgen van dementie. Of dat verband causaal blijft echter de vraag.

In navolging van de in hoofdstuk 2.2 geïdentificeerde behoefte aan longitudinale studies onderzochten we ook de relatie van actigrafie-bepaalde 24-uurs activiteitsritmes met dementierisico. Verstoorde ritmes, dat wil zeggen variabele en onstabiele ritmes, waren niet gerelateerd aan dementierisico. Vroeger het licht uitdoen om te gaan slapen was wel geassocieerd met een hoger dementierisico. Ook als de meest inactieve 5 uur van de dag eerder viel, was er een associatie met dementie in de daaropvolgende 2 jaar, maar niet daarna. Beide bevindingen zijn congruent met prodromale dementie die het activiteitsritme verstoren, en het totaal aan bevindingen is niet ondersteunend voor een temporeel, mogelijk causaal, verband van verstoorde 24-uurs activiteitsritmes op dementierisico.

Ook onderzochten we de relatie van zelf-gerapporteerde kwaliteit en duur van slaap met het krijgen van een diagnose van een hypo-kinetisch rigide syndroom, inclusief de ziekte van Parkinson. Slechtere kwaliteit en kortere duur van slaap waren geassocieerd met een verhoogd risico op de ziekte van Parkinson, maar alleen in de eerste 2 jaar van de studie dan wanneer we langere periodes van *follow-up* analyseerden. Daarnaast vonden we ook dat een achteruitgang in slaap over twee rapportages gemiddeld enkele jaren uiteen, i.e. een verslechtering van de slaapkwaliteit en een verkorting van de slaapduur, geassocieerd was met een hoger Parkinson-risico nadien. De slaap lijkt dus progressief te verslechteren naarmate de diagnose dichterbij komt, wat we interpreteerden als slaapverslechtering als vroegtijdig signaal van de ziekte van Parkinson.

In hoofdstuk 4 onderzochten we middel dwarsdoorsnede onderzoeken de relatie van slaap met breinmaten die gerelateerd zijn aan neurodegeneratieve ziekten. We bepaalden de relatie van slaap, gemeten met de PSQI en actigrafie, met eiwitten indicatief voor neurodegeneratieve ziekten gemeten in bloedplasma. Deze zogenoemde 'biomarkers'

waren het 'lichte-keten' neurofilament (NfL), twee vormen van beta-amyloid, en de totaal hoeveelheid tau. We vonden geen relaties tussen verschillende slaapaspecten en deze biomarkers, behoudens een associatie van een langere zelf-gerapporteerde tijd in bed met een hogere concentratie NfL. Die laatste associatie wijst waarschijnlijk op onderliggende aandoeningen. Het gebrek aan associaties in deze studie lijkt belangrijk, want deze suggereert dat de neurodegeneratieve processen gemeten met de biomarkers niet een gemeenschappelijke oorzaak zijn, noch een mediërende factor, van de eerdergenoemde relatie tussen slecht slapen en het risico op dementie.

Een hypothese over de functie van slaap is dat deze een nachtelijke 'hersenspoeling' faciliteert waarin toxische afbraakproducten verwijderd worden. Die verwijdering van brein-'afval' zou plaatsvinden over het zogenaamde 'glymfatische' systeem, een systeem analoog aan het lymfatisch stelsel in de rest van het lichaam. Wij onderzochten de relatie tussen slaap en verwijde perivasculaire ruimten op een MRI van de hersenen, die dysfunctie van voorgenoemd glymfatisch systeem zouden indiceren. We vonden een associatie van hogere, actigrafie-bepaalde slaap efficiëntie met meer perivasculaire ruimte in één hersengebied, het centrum semiovale. De richting van de associatie was tegengesteld aan wat we hadden verwacht op basis van eerdere studies in de mens. In het hoofdstuk bespreken we de diverse interpretaties van deze bevindingen. Eén speculatieve verklaring is dat verwijding van de perivasculaire ruimten iets zegt over de fysiologie van het brein dat van voordeel is voor de slaap.

In hoofdstuk 4.3 onderzochten we de relatie van slaap met het functioneren van de hersenen, gemeten met functionele MRI opgenomen tijdens een zogenaamde 'ruststaat'. Zo'n functionele MRI kan erg gevoelig zijn voor subtiele hersenveranderingen die mogelijk optreden als het gevolg van slecht slapen. We vonden dat een langere slaapduur, gemeten met polysomnografie, geassocieerd was met een lagere amplitude van het fMRI-signaal, in met name (pre)frontale hersengebieden. Deze associatie kan erop wijzen dat de slaapduur het repertoire van hersenactiviteit bepaalt in specifieke gebieden, of, vice versa, dat activiteit in specifieke hersengebieden bepalend is voor hoe lang je slaapt. Een derde verklaring is dat de slaapduur en de lokale hersenactiviteit een gemeenschappelijke oorzaak hebben.

Als je het niet ziet zitten, ga liggen.

Based on: Jiggy Djé featuring Pete Philly. Ik Heb Je. De Ark De Triomf (2009).

APPENDICES

I. PHD PORTFOLIO
II. LIST OF PUBLICATIONS
III. ACKNOWLEDGEMENTS
IV. ABOUT THE AUTHOR

I. PORTFOLIO

Name PhD student	Thom Sebastiaan Lysen
Erasmus MC Department	Epidemiology
Research School	NIHES
Period	May 2016 – December 2019

Education

- MSc in Clinical Epidemiology at the Netherlands Institute for Health Sciences (2016-2018; *cum laude*)
- R software for beginners 1-wk course, MolMed graduate school, Erasmus MC (2016)
- Integrity Scientific Research, Erasmus MC 1-day course (2017)
- Sleep and Circadian Neuroscience 1-wk Summer School, Oxford, UK (2017)

Symposia, seminars & workshops

- Annual CoSTREAM consortium meetings (2016-2019)
- Epidemiology
  - o Weekly seminars, bi-weekly '2020' PhD-focused meetings, journal club
- Neurology (Erasmus MC)
  - o Weekly department meetings (seminar; case discussion)
- Big Brother meets Science (Oct 2016)
- Rot.Jong Meets … Toekomst van de zorg (Nov 2016)
- Medical Business Masterclass (Apr 2017)
- Young@Heart event 'Mobility' – Dutch Heart Foundation (Oct 2017)
- 'Lof der Geneeskunst' – Erasmus MC (Oct 2017)
- Yearly event 'Deltaplan Dementie' (Nov 2017)
- Innovation for Health (Feb 2018)
- Medical Business Masterclass (Apr 2018)
- SteLA Europe & NIHES Leadership/teamwork (Apr 2018)
- Dutch Society Sleep-wake research (NSWO) spring lecture (Apr 2018)
- Mix & Match Memorabel 2018 (Jun 2018)
- Valedictory symposium prof. dr. Albert Hofman, Erasmus University (Jun 2018)
- Dutch Society Sleep-wake research (NSWO) fall symposium (Nov 2018)
- University of The Netherlands – A Night about Sleep (Nov 2018)
- Dutch Society for Neurology symposium 'Neurology and Sleep' (Nov 2018)
- Dutch Society Sleep-wake research (NSWO) *Open House@SEIN* (Mar 2018)

Conferences

- SLAAP – Conference of the NSWO and the Dutch Society of Sleep Medicine (SVNL; Ermelo, NL; 2016)
- SLAAP2017 (Ermelo, NL; 2017)
  - o Oral: *Preclinical work on sleep and cognition in the Rotterdam Study – current projects on dementia and resting state functional connectivity of the brain*
  - o Poster: *Self-reported sleep quality and the risk of dementia: Rotterdam Study*
- 'Update@Kempenhaeghe' International symposium-sleep (Heeze, NL; 2018)
- European Sleep Research Society (ESRS) Conference (Basel, CH; 2018)
  - o Oral: *A polysomnographic sleep and resting state fMRI study in the general population*
- 'Update@Kempenhaeghe' International symposium-Sleep (Heeze, NL; 2019)
  - o Oral: *Actigraphic sleep and 24-h rhythms in relation to incident dementia: The Rotterdam study*
- SLAAP2019 (Ermelo, NL; 2019)
  - o Oral: *Neurobiological correlates of sleep in the general population*
  - o Poster: *Sleep, 24-hour activity rhythms, and plasma biomarkers of neurodegeneration (awarded 'Piet Visser* poster prize' [0.5k])

Research activities

Within the Rotterdam Study framework

- Physician at ERGO centre performing exit interview
  - o Discuss test results
  - o Structured screening for lifetime depression and for TIA/stroke
- Contactperson/scheduler for physicians performing exit interviews at the ERGO centre
- Dementia screening with structured interview (CAMCOG/CAMDEX)
- Training physicians to screen for lifetime depression using the LIDAS questionnaire
- Rating incidental findings on routinely performed brain MRI-scans
- Inspection and correction of automated segmentations of brain MRI-scans

Other

- Physician at the Dutch National Surveillance Center for Prion Disease, Erasmus MC
- Member of the Young Scientists committee (Dutch Society for Sleep Research)
- Peer-review (BJPsych; Sleep Med)

II. MANUSCRIPTS AND PUBLICATIONS

Featured in this thesis

Chapter 2.1: Kocevska D, **Lysen TS** et al. Sleep characteristics across the lifespan in 1.1 million persons from the general population of the Netherlands, UK and USA. *Under revision*

Chapter 2.2: De Feijter M, **Lysen TS,** Luik AI. 24-hour activity rhythms and health in older adult. *Current Sleep Medicine Reports* 6, 76–83 (2020)

Chapter 3.1: **Lysen TS**, Wolters FJ, Ikram MK, Luik AI, Tiemeier H, Ikram MA. Subjective sleep quality is not associated with incident dementia: The Rotterdam Study. *Journal of Alzheimer's disease* 2018;64(1):239-247.

Chapter 3.2: **Lysen TS**, Luik AI, Ikram MK, Tiemeier H, Ikram MA. Actigraphy-estimated sleep and 24-hour activity rhythms and the risk of dementia. *Alzheimer's & Dementia, 2020*

Chapter 3.3: **Lysen TS**, Darweesh SKL, Ikram MK, Luik AI, Ikram MA. Sleep and risk of parkinsonism and Parkinson's disease. *Brain* 2019 Jul 1;142(7):2013-2022.

Chapter 4.1: **Lysen TS**, Ikram MA, Ghanbari M, Luik AI. Sleep, 24-hour activity rhythms and plasma biomarkers of neurodegenerative disease. *Submitted*.

Chapter 4.2: **Lysen TS**, Yilmaz P, Dubost F, Ikram MA, De Bruijne M, Vernooij MW, Luik AI. Sleep and enlarged perivascular spaces in the middle-aged and elderly population. *In preparation*.

Chapter 4.3: **Lysen TS**, Zonneveld HI, Muetzel RL, Ikram MA, Luik AI, Vernooij MW, Tiemeier H. Sleep and resting-state functional MRI connectivity in middle-aged adults and elderly: A population-based study. *Journal of Sleep Research* 2020 Feb 26:e12999

Other

Kocevska D, Cremers LGM, **Lysen TS**, Luik AI, Ikram MA, Vernooij MW, Tiemeier H. Sleep complaints and cerebral white matter: A prospective bidirectional study. *J Psychiatr Res.* 2019 May;112:77-82.

Kocevska D, Tiemeier H, **Lysen TS**, de Groot M, Muetzel RL, Van Someren EJW, Ikram MA, Vernooij MW, Luik AI. The prospective association of objectively measured sleep and cerebral white matter microstructure in middle-aged and older persons. *Sleep*. 2019 Oct 9;42(10)

Karamujić-Čomić H, Ahmad S, **Lysen TS**, Heshmatollah A, Roshchupkin GV, Vernooij MW, Rozemuller AJM, Ikram MA, Amin N, Van Duijn CM. Prion protein codon 129 in mild cognitive impairment and dementia: a population-based cohort study. *Brain Communications, fcaa30, 2020 [ePub]*

Freak-Poli R, Wang R, Wagemaker N, **Lysen TS**, Ikram MA, Vernooij MW, Dintica CS, Vernooij-Dassen M, Melis RJM, Laukka EJ, Fratiglioni L, Xu W, Tiemeier H. Loneliness and the risk of cognitive decline and dementia across two countries. *In preparation*

III. ACKNOWLEDGEMENTS

"*De beperkingen zijn voorbij. De vertraging neemt af*" - Dutch Railways

This thesis was made possible by the support, direct or indirect, of many persons. Here, I will highlight a few.

My promotors, prof. dr. M.A. Ikram and prof. dr. H. Tiemeier. I am grateful to have been able to study under direct supervision of such skilled and enthusiastic epidemiologists. Aside from being frustrated in the earlier meetings because of not being able to understand and converse at your level, working together has been a joy.

Dear Arfan, I appreciate your ideas on 'intrinsic motivation' as a guiding principle in a PhD track, and am inspired by the great clarity in your teaching, and how you keep smiling through the busy, or very busy, times. I will not forget the flashy watch too big for your wrist, using an antique pen, the hand gesturing, and ending sentences with a double 'etcetera' (surely the inspiration for my tautology-like writing). I felt lucky to be able to return the favor and teach you a trick or two on the tennis court. Hope we get to play once more, or work together in future.

Dear Henning, too bad you had to leave halfway through my PhD to work somewhere else. Luckily for you, I heard, that that 'Harvard' place might not be all that bad. That you, upon leaving, expressed your confidence in me being able to complete my PhD without your direct supervision was a great compliment. I am grateful for your efforts to measure sleep in the Rotterdam Study already years ago. I really enjoyed your very personal style of supervising, including your keen eye for sharing compliments for even the smallest of achievements. I will not forget the psych epi group meetings that never ended, giving personally baked 'Torte' for first accepted papers, your energetic lecture style (no slides or microphone required) that almost feels like watching a little play, and you organizing dinner parties at your home.

I was most lucky to have the gap left by Henning filled by dr. A.I. Luik, who agreed to supervise me as co-promotor. Dear Annemarie, having a sleep expert and former PhD-student of our department scrutinize my work undoubtedly elevated this thesis to a higher level. I am grateful for all the tips you shared the past 2 years, and for helping me connect with other sleep researchers. I am proud to have been present at the start of your continuously growing research group of Psychiatric epidemiology, and hope we might get to work together once more in future.

Pivotal for my appointment at the Epidemiology department at the Erasmus MC, and concomitant career opportunities, was the help of the highly learned Koudstaal, professor in neurology. Dear professor Koudstaal, I will not forget Minah's call, inviting me to

come see you and chat about research. Thank you for giving me an opportunity to show my worth.

Members of the reading and defense committee: Professor M.W. Vernooij, dear Meike, thank you for being a driving force of the open atmosphere at the department. I admire you for being a role model for medical doctors in research, and by combining it with being an involved supervisor and an overall enjoyable person. Professor M.K. Ikram, dear Kamran, thank you for your open-door policy and sincerity in our discussions on various PhD and residency issues. Your attention to emotional reflection and personal growth in your students is an inspiration. I look forward to working with you in the clinic. Dear prof. dr. Sebastiaan Overeem (Eindhoven University of Technology), dr. Jurgen A.H.R. Claassen (Radboud University), dr. Sebastian Köhler (Maastricht University), dr. Mirjam I. Geerlings (Universitair Medisch Centrum Utrecht), and dr. Gert-Jan Lammers (Leiden University), your contributions to approving this thesis or discussing its contents during my defense are gratefully acknowledged.

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With the large data volume came data analysis, and days spent 'computering', mainly learning R. That involved at times being totally tuned-out or frustrated from spending too much time at some annoying code. Inspired by Erasmus's idea of 'space separates our bodies, not our minds', I would conclude that at times the Erasmus MC, Dept of Epi, was a place where 'minds separate from bodies'. I am therefore thankful that victim support in the form of many great colleagues was closeby. Thank you to the many colleagues that helped build and maintain an atmosphere of openness to different ideas. It was a joy spending time with intelligent individuals discussing many things epi, research, or other. Especially thanks to Hazel (Amsterdam!), Lotte (Aagje Beton), Desi (D! Dobre dobre dobre?), and neuro-buddies Noor and Alis (AIOSsen!) for the socials, talks and laughs. Frank (scholar, writer) and Silvan (when others go to sea he does not come mea, mega-perseverance and totally 'knettah'), thanks for sharing knowledge, coffees, beers, laughs and supporting the sleep cause as paranymphs. Cheers to still being 'at risk'!

I got by with a little help from my friends and family. Please note that this modest acknowledgement in no way reflects your importance to me on a personal level. Special thanks to contributions by Ruben who jokingly personified innumeracy under medical doctors (I tell patients that chances of having the disease are '50-50': you either have it or

you don't): It's funny because it's true. Also, thanks to Jorien for suggesting that the act of me explaining my research shows promise as new sleep therapy.

Lastly, thanks to Christine, the love of my life, for her continued love and support. Christine, you help me keep my feet on the ground and be half as busy, and twice as happy.

IV. ABOUT THE AUTHOR

Thom Sebastiaan Lysen was born on October 9th, 1987 in Amersfoort, the Netherlands. After graduating from high school in 2006, he studied Movement Sciences at the University of Groningen. In 2007 he switched to Medicine at that same university. He obtained his medical degree in 2014, moved to Amsterdam with his girlfriend, and started working as a resident (not in specialist training) at the Neurology department at the Kennemer Gasthuis (currently Spaarne Gasthuis) in Haarlem (supervision: dr. M. Weisfelt). In 2016 he started the work described in this thesis at the department of Epidemiology at the Erasmus MC, University Medical Center Rotterdam, supervised by prof. dr. M. Arfan Ikram, prof. dr. Henning Tiemeier, and dr. Annemarie I. Luik. In 2018 he obtained a Master of Science degree in Clinical Epidemiology (*cum laude*) at the Netherlands Institute for Health Sciences in Rotterdam. Upon finishing the work described in this thesis he started working at the department of Neurology at the Albert Schweitzer hospital in Dordrecht (supervision: dr. H. Kerkhoff), after which he will start working at the department of Neurology at the Erasmus MC (chair: prof. dr. P.A.E. Sillevis Smitt; supervision: dr. J.E.C. Bromberg).