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# PLASMA BIOMARKERS OF NEURODEGENERATIVE DISEASE

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## 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 (1$^{st}$ quartile – 3$^{rd}$ 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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