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