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ORIGINAL ARTICLE

Linking sleep microstructure, blood markers of inflammation and metabolism, and cognition: mediation analysis in the osteoporotic fractures in men study

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Abstract The sleep electroencephalographic (EEG) microstructure is explicitly related to brain functions, such as sleep spindle and memory consolidation. On the other hand, given the crosstalk between the central nervous system and the body, including inflammation regulation and metabolic systems, there is a gap in understanding the bidirectional relationship between sleep and these systems that contribute to cognitive health. We used data from the Osteoporotic Fractures in Men (MrOS) Study, a six-site cohort study of community-dwelling men 65 years or older. We analyzed 1898 participants who participated in the Sleep Visit and Visit 2. We analyzed 41 sleep EEG microstructures and 9 blood markers. The outcome was the Modified Mini-Mental State Examination (3MS) in Visit 2. We used partial Spearman's correlation to investigate the pairwise associations and performed 3MS prediction. We then performed mediation analyses using each blood marker as the exposure and each sleep EEG microstructure as the mediator, and then the other way around. Sleep EEG microstructures were more strongly correlated with 3MS than blood markers in general. The best Pearson's correlation

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between the actual and predicted 3MS scores was 0.45 (95% confidence interval 0.38–0.47) using sleep EEG microstructures and covariates, which provided little improvement compared to using covariates alone (0.43, 0.39–0.48). Leptin and 3MS score were associated (Spearman's $\rho = 0.053$, $p = 0.02$) while adjusting for covariates. The association between leptin and 3MS score was mediated by fast spindle density (indirect effect = 0.030, $p = 0.02$) and spindle-slow oscillation (SO) overlap (indirect effect = 0.029, $p = 0.02$). No blood marker mediated the association between sleep EEG microstructure and 3MS. Instead, the following sleep EEG microstructures had significant direct associations with 3MS independent of the blood markers: theta power during N1 and REM (direct effect = −0.57 and −0.46, $p = 0.0002$ and 0.007, respectively), spindle density (direct effect = 0.39, $p = 0.006$), and spindle-SO coupling overlap (direct effect = 0.29, $p = 0.01$). The blood markers of inflammation and metabolism were less predictive of and indirectly associated with global cognition compared to the sleep EEG microstructures. Future work is needed to confirm these results in an experimental setting.

Keywords Cognition · Sleep · Electroencephalography · Blood markers · Metabolism · Leptin · Inflammation

Introduction

Good sleep is essential for brain health, including cognitive health. Experimental acute sleep deprivation leads to impaired next-day cognition [1–3] and higher systemic inflammation. In observational studies, sleep disturbances, such as long wake after sleep onset and sleep-disordered breathing, are independently associated with cognitive impairment [4] and an increased risk of Alzheimer's disease and Alzheimer's disease–related dementias (AD/ADRD) [5]. However, the pathway from sleep disturbances to cognitive impairment and AD/ADRD is complex and multifactorial, involving a combination of genetic, environmental, and biological factors that are not yet fully understood [6, 7]. While sleep disturbances result in impaired sleep macrostructure, the sleep electroencephalographic (EEG) microstructure is also affected [8] as measured by the electrical activity in the brain. Sleep EEG microstructure is explicitly related to brain cognitive functions, pathology, and structures. For example, sleep spindle-slow oscillation coupling is associated with memory consolidation [9]; sleep spindle density is associated with total tau in cerebrospinal fluid [10] and thalamic volume [11]; reduced slow-wave activity (SWA) is associated with high cerebrospinal fluid Aβ42 [12], and frontal lobe and thalamic volumes [11].

Sleep also involves a network of organ systems in the body other than the brain, including inflammation regulation [13] and metabolic systems [14], which also contribute to cognitive health. It is known that acute peripheral inflammation impairs memory [15] and reaction time [16] via randomized controlled trials (RCTs) of endotoxins that induce acute pro-inflammatory markers, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). On the other hand, chronic peripheral inflammation is usually found in aging, increasing the vulnerability to and risk of subsequent pathogenesis [17]. The peripheral inflammation can lead to neuroinflammation [18], resulting in neuronal apoptosis, synaptic dysfunction, and ultimately dementia and neurodegeneration [19]. Metabolic dysfunction is also closely linked to elevated pro-inflammatory blood markers such as IL-6, TNF-α, and C-reactive protein (CRP) [20]. However, it is unknown how much additional information can inflammation and metabolic dysfunction provide in terms of predicting cognitive impairment in addition to sleep disturbances.

At the same time, there is a bidirectional relationship between sleep and the systems of inflammation [13] and metabolism [21]. Sleep deprivation leads to an acute increase in pro-inflammatory markers in the blood, possibly via disrupted hypothalamic–pituitary–adrenal axis and non-dipping of the sympathetic nervous system [22]. Sleep deprivation also leads to metabolic dysfunction via disrupted hormone regulation involved in appetite regulation [14] and glucose metabolism [23]. While sleep deprivation is an extreme case, it is unknown if disturbances in sleep EEG microstructures are also associated with these dysfunctions. On the other hand, inflammation regulates sleep via direct neural innervation, hormone mediators, and blood–brain barrier changes [22]. Metabolic dysfunction also impairs sleep. For example, people with type 2 diabetes have an increased risk of nocturnal hypoglycemia, restless leg syndrome, and sleep-disordered breathing [24]. However,

there is a gap in understanding how the bidirectional relationships together contribute to cognitive health.

Although experiments or RCTs are needed to clarify the underlying mechanism, here, we aim to provide statistical evidence using a large observational cohort. First, we studied the pairwise associations among global cognition, sleep EEG microstructures, and blood markers of inflammation and metabolism. We then predicted the global cognitive scores as the outcome. We conducted two sets of mediation analyses, including one sleep EEG microstructure as the mediator and one blood marker as the exposure, and the other way around. The results generate hypotheses to elucidate the possible pathways among sleep EEG microstructure, inflammation and metabolism, and cognition.

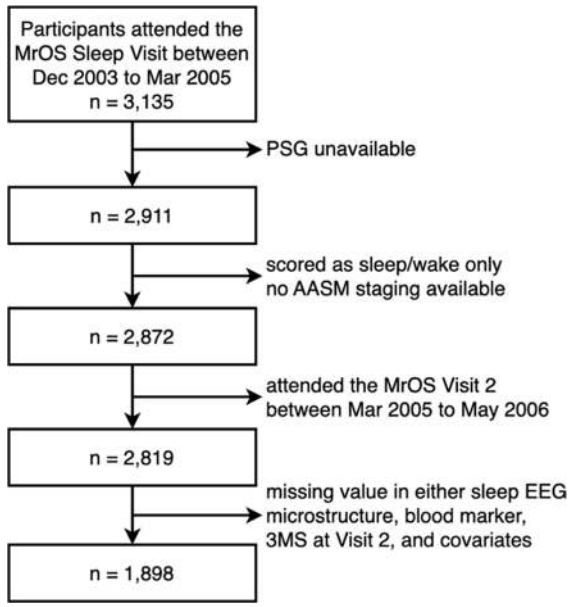

Fig. 1 Cohort flowchart from the initial cohort to the final analysis

Methods

Study design

We used the Osteoporotic Fractures in Men (MrOS) Study [25, 26], a six-site cohort study of 5994 community-dwelling men 65 years or older, including Birmingham, AL; Minneapolis, MN; Palo Alto, CA; Monongahela Valley near Pittsburgh, PA; Portland, OR; and San Diego, CA. The cohort flowchart is shown in Fig. 1. Between December 2003 and March 2005, 3135 participants from the initial cohort were recruited to undergo full unattended polysomnography (Sleep Visit), where the participants could not use any sleep apnea treatment (CPAP, BiPAP, mouthpiece, oxygen therapy, or tracheotomy) within 3 months before the Sleep Visit. Between March 2005 and May 2006, 5229 participants from the initial cohort attended the second visit of the MrOS parent study (visit 2). Visit 2 was 1.2 years (standard deviation 0.3 years) after the Sleep Visit. We used the cognitive scores from visit 2. In our study, the inclusion criterion was attending the Sleep Visit. The exclusion criteria were (1) polysomnography (PSG) unavailable; (2) the PSG was only scored as sleep or wake; and (3) missing value in either sleep EEG microstructure, blood marker measurement (not measured or below detectable limit), cognitive outcomes at visit 2, and covariates. The MrOS committee approved the study. We have followed the AGReMA: A Guideline for Reporting Mediation Analyses [27], as included in the Appendix.

Blood markers

Fasting blood serum was sampled during the Sleep Visit and then stored at − 80 °C. The blood markers of inflammation included C-reactive protein (CRP), interleukin-6 (IL-6), interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and tumor necrosis factor-α soluble receptor 2 (TNF-αSRII). The metabolic blood markers included leptin, insulin, adiponectin, and glucose. All concentrations of blood markers were log-transformed. The details for measuring these blood markers are in the Appendix.

Sleep EEG microstructure

One night of at-home unattended PSG was performed at the Sleep Visit. Staff returned the next morning to collect the equipment and download the data, which was transferred to the Central Sleep Reading Center for centralized scoring by a trained technician. Details have been previously published [28].

We used two EEG channels: C3-M2 and C4-M1. The sampling rate was 256 Hz. They were

notch-filtered at 60 Hz and then bandpass-filtered at 0.3–35 Hz. The EEG signals were then segmented into 30-s epochs. To remove artifacts, 30-s epochs with either of the following conditions were removed: (1) absolute amplitude higher than 500uV at any point; (2) more than 10% flat signal; or (3) Hjorth's parameters (activity, mobility, complexity) more than three times standard deviation higher or lower than mean. ECG artifacts were removed by subtracting the template EEG time-aligned at the ECG R peak. After artifact removal, the sleep EEG microstructures were extracted, including spectral band powers, spindle metrics, slow oscillation (SO) metrics, and spindle-SO coupling metrics. A list of all sleep EEG microstructures is available in Table S1 in the Appendix.

To estimate the band powers, we used multitaper spectral estimation [29, 30] with a bandwidth of 0.5 Hz. The band powers included delta (1–4 Hz) and alpha (8–12 Hz) in N3, delta in N2, theta (4–8 Hz) in N1, delta and theta in REM, and also the slope of delta power across the whole night in N2 or N3 stages. All band powers were averaged between C3-M2 and C4-M1. All band powers had two versions: absolute power measured in decibels and relative power normalized by the total power (1–30 Hz).

The spindle, SO, and their coupling metrics were derived from N2 or N3 using Luna [31]. The spindles were detected using wavelet transformation with a cycle number of 7 and multiplicative threshold of 4.5. To account for the diverse spindle frequency both across and within individuals, we considered all possible frequencies in the broad sigma range (10.5 to 15.5 Hz, step size 0.5 Hz), and then combined the detection results based on temporal overlap within each channel. For quality control, we first ran the detection with a relaxed quality threshold ($q = 0$ in Luna), then randomly selected 900 spindles and manually labeled them as spindle or not spindle. We obtained the best quality threshold $q = 0.47$ based on Youden's operating point on the receiver-operator curve (ROC), where the area under ROC was 0.73, the area under the precision-recall curve (PRC) was 0.43, sensitivity was 0.73, specificity was 0.61, F1 score was 0.43, and Cohen's kappa was 0.22 (Fig. S1 in the Appendix). We finally re-ran the detection with the optimal quality threshold. The spindle metrics included frequency, density, density of the SO-coupled spindles, chirp (frequency change rate within spindle), and integrated activity (duration and amplitude, normalized by the individual's baseline). We further split the spindles into fast (≥13Hz) or slow (< 13 Hz), and calculated these metrics for fast and slow separately.

The SOs were detected by first band-pass filtering the signal between 0.5 and 4 Hz, finding all zero-crossings, retaining intervals between 0.8 and 2.0 s and negative-peak and peak-to-peak amplitudes, both higher than three times the median. The SO metrics included peak-to-peak amplitude, duration, rate, slope, negative slope, and positive slope. The spindle-SO coupling was measured by the proportion of spindles that overlap with SO in the same channel. The coupling metrics were calculated separately for fast and slow spindles.

Cognitive assessments

The outcome was the Modified Mini-Mental State Examination (3MS) [32] at Visit 2, with a full range from 0 to 100. A higher 3MS score indicates better global cognition. Not all participants in the Sleep Visit attended Visit 2, due to reasons including lack of interest, health issues, relocation, excessive study contact, caregiver responsibilities, and death.

Covariates

The covariates included age at the time of Sleep Visit, self-reported race (Asian, Black, Hispanic, White, Other), body mass index (BMI), education level, medication use at the time of Sleep Visit including benzodiazepine, antidepressant, opioid, and anti-inflammation medication (Table 1), self-report of a diagnosis of hypertension, diabetes, and stroke, APOE e4 carrier, and also the apnea–hypopnea index (AHI). AHI was defined as the number of apneas regardless of desaturations or arousals, or hypopneas with > 30% flow reduction and ≥ 4% oxygen desaturation or with arousal, divided by total sleep time in hours. The details for APOE genotype ascertainment are in the Appendix.

Pairwise associations among global cognition, sleep EEG microstructures, and blood markers

We calculated partial Spearman's correlations of the 3MS score vs. each sleep EEG microstructure, the 3MS score vs. each blood marker, and each sleep

Table 1 Cohort characteristics

Variable	Value
Age at the Sleep Visit, mean (SD), year	76.2 (5.4)
Race and ethnicity, *n* (%)	
Asian	55 (2.9%)
Black	37 (1.9%)
Hispanic	38 (2.0%)
White	1745 (91.9%)
Other	23 (1.2%)
Education, *n* (%)	
Lower than college	829 (43.7%)
Higher or equal to college	1,069 (56.3%)
APOE e4 carrier, *n* (%)	455 (24.0%)
Body mass index at the Sleep Visit, median (IQR), kg/m^2	26.7 (24.5–29.2)
Apnea–hypopnea index at the Sleep Visit, median (IQR),/hour	9.1 (3.3–18.6)
Medical conditions at the Sleep Visit, *n* (%)	
Hypertension	925 (48.7%)
Type 2 diabetes	215 (11.3%)
Stroke	73 (3.8%)
Any cardiovascular disease*	487 (25.7%)
Medication use at the Sleep Visit, *n* (%)	
Benzodiazepine	110 (5.8%)
Antidepressant	143 (7.5%)
Opioid	77 (4.1%)
Anti-inflammation medication^	505 (26.6%)
Modified Mini-Mental State (3MS) test score at Visit 2, median (IQR)	94 (91–97)

*Atrial fibrillation, congestive heart failure, or myocardial infarction

^Corticosteroids (oral or inhaled), or nonsteroidal anti-inflammatory drugs (NSAIDs)

EEG microstructure vs. each blood marker, while adjusting for covariates as listed above. The reason for using Spearman's correlation was to account for monotonic relationships since the variables are not necessarily linear and normal distributed.

Cognitive score predictions

We used the sleep EEG microstructures, the blood markers of inflammation and metabolism, and the covariates at the Sleep Visit to predict the 3MS score at Visit 2. We tested various settings with increasing levels of adjustment. Before model training, we transformed 3MS scores using logit transformation: log(p/(1-*p*)), where $p =$ 3MS score/101, to make it closer to the normal distribution. Note that the choice of 101 was to make sure $p < 1$ since the maximum 3MS score is 100. After model training, we inverse-transformed the predicted 3MS back to its original scale. We performed tenfold cross-validation to estimate the out-of-sample prediction performance, as measured by Pearson's correlation, to test their linear correlation. During model training in each fold, due to the large number of predictors, we used linear regression with Bayesian sparsity constraints on its coefficients to select features [33]. We applied the trained model to the testing fold to get predicted 3MS scores. The Pearson's correlation was estimated using the actual 3MS scores vs. the predicted 3MS scores pooled from all testing folds.

Mediation analysis

The sleep recording and the blood draw were performed concurrently with a negligible time interval (about 0–7 days). The mediation analysis was done in two ways to include the bidirectional relationship between sleep vs. inflammation and metabolism. First, each blood marker was used as the exposure, and each sleep EEG microstructure was used as the mediator. Second, each sleep EEG microstructure was used as the exposure, and each blood

marker was used as the mediator. In both cases, the 3MS score at Visit 2 was the outcome. In each case, exposure-mediator-outcome was the indirect pathway, and exposure-outcome was the direct pathway. The Average Causal Mediation Effect (ACME) is the expected difference in outcomes when the exposure is held constant and the mediator changes from a value when the exposure is held at a low value to a value when the exposure is held at a high value. The Average Direct Effect (ADE) is the expected difference in outcomes when the exposure is varied from low to high, while the mediator is held constant. The sum of ACME and ADE is the total effect (TE). The estimation of ACME and ADE requires a mediator model, using exposure and covariates to predict the mediator, and an outcome model, using exposure, mediator, and covariates to predict the outcome. We used linear models for both mediator and outcome models.

Importantly, mediation analysis is traditionally done by comparing two levels of exposure. However, both sleep microstructure and blood markers were continuous numbers. Here, we defined the two levels of exposure by 25% and 75% percentiles within the dataset. The ACME and ADE were both defined as the contrast between these two levels. One could alternatively define other percentiles to have larger or smaller contrast and hence larger or smaller effect sizes, but would not alter the statistical significance under linear models.

Mediation analysis in observational data is based on the sequential no unmeasured confounding assumption: (1) the exposure is independent of outcome and mediator when conditional on observed confounders; (2) the mediator is independent of outcome when conditioned on exposure and observed confounders [34]. Therefore, we performed sensitivity analyses for unmeasured confounding and covariate strata. First, we examined how ACME and ADE change as a function of unmeasured confounding strength, defined as the correlation (ρ) between the residuals of the mediator and outcome models. We varied ρ and assessed if the confidence intervals of ACME and ADE include 0, i.e., null result. The range of ρ corresponding to null results is called the sensitive region. The sensitive region should be interpreted in the context of literature to determine the feasibility of such unmeasured confounding. Second, we examined the sensitivity to covariate strata by comparing the ACME and ADE across different values of covariates. Specifically, we tested the age of 70 years vs. 80 years, and BMI of 25 kg/m^2 vs. 30 kg/m^2. We obtained the *p*-value of the difference in ACME or ADE under these two covariate values. The mediation analysis and sensitivity analysis were conducted using R 4.3.2 and the "mediation" package version 4.5.0.

Statistical analysis

Bonferroni correction was applied for multiple comparisons. Due to the correlations among many sleep EEG microstructure and blood markers, principal component analysis (PCA) was used to reduce the number of effective comparisons [35] by identifying the dimensions that explain 99% of the variance. For band powers, the effective number of comparisons was 6. For spindle and SOs, the effective number of comparisons was 2. For blood markers, the effective number of comparisons was 8. Non-parametric bootstrapping 1000 times was used to estimate the confidence intervals for both prediction and mediation analysis.

Results

Cohort characteristics

The final cohort included 1898 men. The average age was 76.2 years at the Sleep Visit. The majority were of White race (91.9%). The median BMI was 26.7 kg/m^2, which was in the overweight category. The median AHI was 9.1/h, consistent with mild sleep apnea.

Pairwise associations

As shown in Fig. 2, the overall magnitude of correlation was small, between -0.1 and $+0.1$, as shown in the color bar. Among the blood markers, only leptin was associated with 3MS (top row), but the significance did not survive after Bonferroni correction (no middle dot). Among the sleep EEG microstructures, multiple spindles and SO metrics were associated with 3MS (right column) after the Bonferroni correction (with middle dot). Between blood markers and sleep EEG microstructures, six pairs were significant after Bonferroni correction, i.e., delta band power at N3 at the central channel vs. leptin, insulin, and

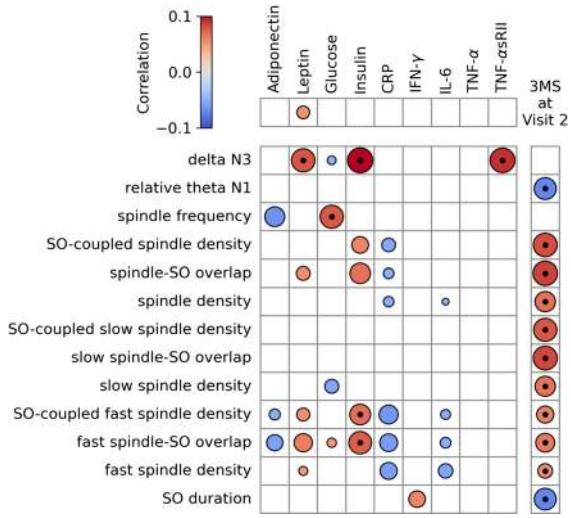

Fig. 2 The pairwise associations among 3MS at Visit 2, sleep EEG microstructures at the Sleep Visit, and blood markers at the Sleep Visit. The associations were obtained by partial Spearman's correlation after adjusting covariates. Each circle represents an association with $p < 0.05$. The middle dot represents statistical significance after the Bonferroni correction. The size of the circle is proportional to 1-$\sqrt{p}$. The color of the circle is proportional to the correlation, where red means positive value and blue means negative value

TNF-αsRII, spindle frequency and glucose, and insulin vs. fast spindle-SO coupling.

Cognitive score prediction from sleep EEG and blood markers

In Table 2, we show the prediction performance, measured by Pearson's R, using seven different predictor settings. Using sleep EEG alone had a higher point estimate of Pearson's R (Setting 3, $R = 0.22$) than using blood markers alone (Setting 2, $R = 0.10$), although their confidence intervals overlap. The combination of sleep EEG and blood markers (Setting 4, $R = 0.22$) did not improve the performance compared to sleep EEG alone. When comparing Setting 5 ($R = 0.40$) to Setting 1 ($R = 0.43$), the use of blood markers in addition to covariates (see Methods) did not improve the performance. When comparing Setting 6 (R = 0.45) to Setting 1 (R = 0.43), the use of sleep EEG in addition to covariates had a small improvement in terms of the point estimate. When comparing Setting 7 (R = 0.44) to Setting 1 (R = 0.43), the joint use of both sleep EEG and blood markers, in addition to covariates, had a negligible difference in terms of the point estimate. In Fig. 3, we showed the 3MS vs. the predicted 3MS using Setting 7: sleep EEG, blood markers, and covariates.

Mediation with blood marker as the exposure and sleep EEG microstructure as the mediator

There were two pathways with a significant mediation effect as shown in Fig. 4. The pathway in Fig. 4a shows a positive indirect effect of 0.030 and a *p*-value of 0.02 for leptin on the 3MS score mediated through fast spindle density. The pathway in Fig. 4b shows a positive indirect effect of 0.029 and a *p*-value of 0.02 for leptin on the 3MS score mediated through spindle-SO overlap. The reason why the indirect mediation effect sizes were small is that the ACME reflects the multiplicative effect that involves two steps, i.e., from the exposure to the mediator and from the mediator to the outcome. There was no pathway with a significant direct effect. In other words, all significant

Table 2 Pearson's R between the actual and the predicted future 3MS at Visit 2 using different predictor settings. The time interval from the Sleep Visit to the Visit 2 is about one year. "Y" means the predictor was used in the prediction. The Pearson's correlations were obtained using tenfold cross-validation, hence measuring the out-of-sample performance

Setting	Covariates	Blood markers	Sleep EEG	Pearson's *R* (95% confidence interval)
1	Y			0.43 (0.39–0.48)
2		Y		0.10 (0.10–0.22)
3			Y	0.22 (0.15–0.24)
4		Y	Y	0.22 (0.18–0.27)
5	Y	Y		0.40 (0.37–0.47)
6	Y		Y	0.45 (0.38–0.47)
7	Y	Y	Y	0.44 (0.39–0.47)

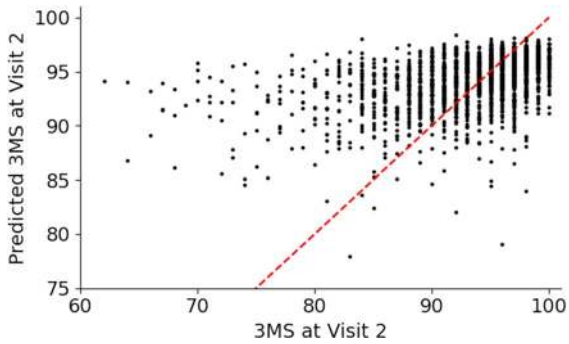

Fig. 3 The scatter plot of the actual 3MS score at Visit 2 (*x*-axis) vs. the predicted 3MS score (*y*-axis), using sleep EEG, blood markers, and covariates at the Sleep Visit. The Pearsons R is 0.44 (95% confidence interval 0.39–0.47). Each point represents a participant. The red dashed line represents the diagonal line where the predicted 3MS equaled the actual 3MS. 3MS: Modified Mini-Mental State examination

associations between blood markers and 3MS score (in this case, leptin and 3MS) could be explained by changes in sleep EEG. Indeed, the covariate-adjusted partial Spearman's ρ between exposure (leptin) and outcome (3MS) was significant (ρ = 0.053, p = 0.02, Fig. 2), and became non-significant after further adjusting for fast spindle density or spindle-SO overlap.

We performed sensitivity analyses to assess unmeasured confounding. For the pathway in Fig. 4a, the sensitivity analysis indicated that the ACME became null when the unmeasured confounding strength, i.e., the correlation between the residuals of the mediator and outcome, was between −0.02 and 0.13. For the pathway in Fig. 4b, the sensitivity analysis indicated that the ACME became null when the unmeasured confounding strength was between −0.02 and 0.12. We also performed sensitivity analyses comparing the ACME between age 70 years vs. 80 years, and between BMI 25 kg/m^2 vs. 30 kg/m^2, for both pathways in Fig. 4. The ACME remained the same across both age and BMI categories.

Mediation with sleep EEG microstructure as the exposure and blood markers as the mediator

There was no pathway with a significant mediation effect. There were 11 sleep EEG microstructures with a significant direct effect on the 3MS score, independent of the inflammation and metabolic blood markers studied here. We categorized them into four categories as shown in Fig. 5: (a) absolute and relative theta power during N1, which had negative direct effects on the 3MS score; (b) absolute theta power during REM, which had a negative direct effect on the 3MS score; (c) spindle density for all, fast, and slow types, as well as SO-coupled spindle density for all and fast types, which had positive direct effects on the 3MS score; and (d) spindle-SO coupling overlap for all and slow types, which had positive direct effects on the 3MS score.

We performed sensitivity analyses to assess unmeasured confounding. For the pathway in Fig. 5a, the sensitivity analysis indicated that the ADE became null when the unmeasured confounding strength was lower than −0.97. For Fig. 5b, the ADE became null when the unmeasured confounding strength was greater than 0.84 or lower than −0.63. For Fig. 5c, the ADE became null when the unmeasured confounding strength was lower than −0.22. For Fig. 5d, the ADE became null when the unmeasured confounding strength was greater than 0.41 or lower than −0.72. We also performed sensitivity analyses comparing the ADE between age 70 years vs. 80 years, and between BMI 25 kg/m^2 vs. 30 kg/m^2, for all pathways in Fig. 5. The ADE remained the same across both age and BMI categories.

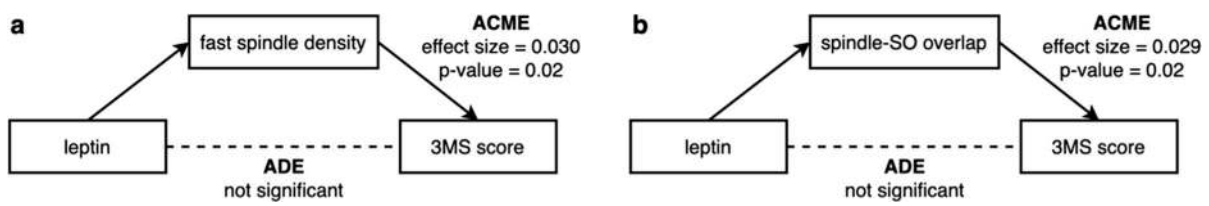

Fig. 4 Two pathways with significant indirect effects. **a** Leptin was significantly associated with 3MS score, requiring fast spindle density. **b** Leptin was significantly associated with 3MS score requiring spindle-SO overlap. 3MS: Modified Mini-Mental State examination. ACME: average causal mediation effect. ADE: average direct effect. SO: slow oscillation

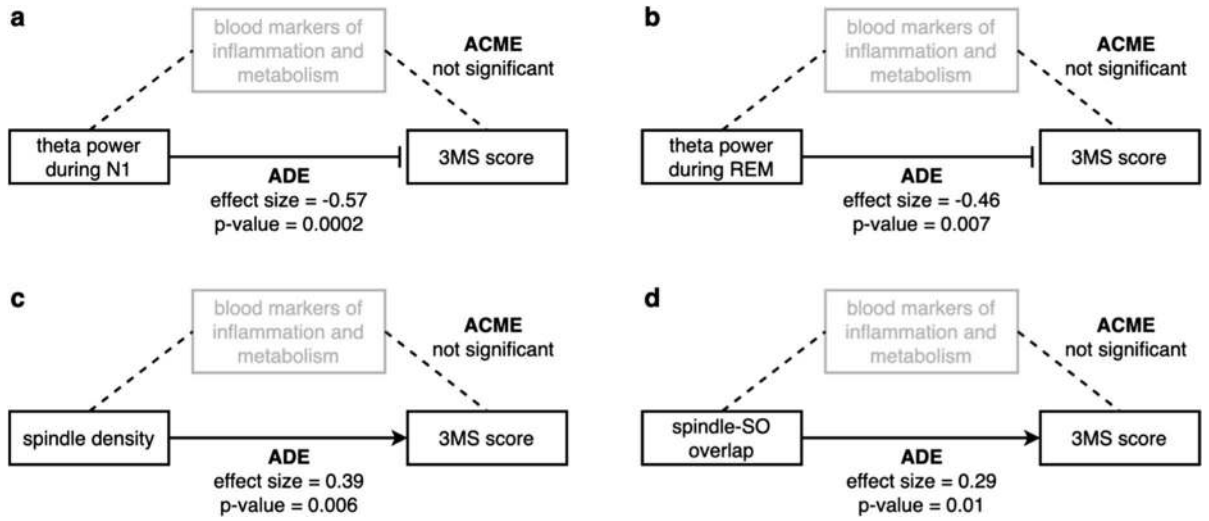

Fig. 5 Four pathways with significant direct effects. **a** Theta power during N1 was negatively associated with 3MS score, independent of the blood markers we examined. **b** Theta power during REM was negatively associated with 3MS score, independent of the blood markers we examined. **c** Spindle density was positively associated with 3MS score, independent of the blood markers we examined. **d** Spindle-SO overlap was positively associated with 3MS score, independent of the blood markers we examined. The blood marker boxes were grayed to indicate the non-significant mediating pathway. 3MS, Modified Mini-Mental State examination. ACME, average causal mediation effect. ADE, average direct effect. SO, slow oscillation

Discussion

Sleep EEG and blood markers of inflammation and metabolism provided little predictive value for future global cognitive scores in addition to covariates. The mediation analyses suggested a role of leptin on global cognition mediated by sleep spindle and spindle-SO coupling (Fig. 4). On the other hand, theta power in N1 and REM, spindle density, and spindle-SO coupling were directly associated with cognition, not mediated by the blood markers studied here (Fig. 5).

Results leading to new hypotheses

Our results suggested a potential role of leptin, fast spindle density, and spindle-SO coupling (measured at the central channel) in cognitive health (Fig. 4). Leptin is known as "obesity protein" which is a protein hormone mostly made by adipocytes. Its primary function is assumed to regulate energy balance [36]. Leptin is associated with improved sleep macrostructure parameters and circadian rhythms [37]. Leptin is also associated with lower 4-year cognitive decline measured by 3MS in a prospective cohort study [38], as well as the risk of dementia or mild cognitive impairment in the Study of Osteoporotic Fractures in old women with normal BMI [39]. However, the association of leptin with sleep EEG microstructure has not been explored. Our results led to a new hypothesis that the brain's ability to generate spindles (especially fast spindles), and the brain's ability to couple spindles with slow oscillation through corticothalamic interaction, is related to different levels of energy balance marked by different levels of leptin in the blood, and hence connect to cognitive health. This new hypothesis is rooted in the general context of the close relationship between sleep and metabolic dysfunction. For instance, our previous study found that sleep EEG predicts the risk of developing type 2 diabetes over 10 years, with participants in higher-risk quartiles having a 2.6-fold increased risk of diabetes than the middle quartile [40]. The predictive EEG features for diabetes include lower spindle-SO overlap and longer SO duration. Another study shows that SO-spindle coupling plays a key role in glucose homeostasis and insulin sensitivity, which is more predictive than sleep architecture at the macrostructure level, such as total sleep time or sleep stage distribution [41]. Our results, together with these

findings, contribute to the growing body of evidence linking sleep disturbances, such as sleep apnea and reduced sleep duration, with insulin resistance and metabolic syndrome.

Another (partially) new finding was the direct negative association of theta power during N1 on global cognition, independent of the inflammation and metabolic blood markers studied here (Fig. 5a). N1 resembles drowsiness and transitional sleep. The proportion spent in N1 over the night increases with age. On EEG, N1 is characterized by theta oscillation at 4–8 Hz, as a result of a continuous slowing down of the alpha oscillation at 8–12 Hz at wake before sleep onset. On the other hand, the alpha peak frequency at wake before sleep onset decreases with age, and can enter the theta frequency range [42]. Our previous work has shown that theta power in N1 is an important driving factor in sleep EEG-based brain age prediction. In the literature, higher theta power in NREM sleep, which includes N1, is negatively associated with Mini-Mental Status Exam scores [43]; higher theta power in resting state is negatively associated with executive functioning and memory performance, and is more often observed in AD patients compared to the control group [44]. However, overall, theta power during N1 is less studied and requires additional research to investigate its implications on cognitive functions.

The next (partially) new finding was the direct negative association of theta power during REM on global cognition (Fig. 5b). Although many studies have discovered the association between time spent in REM, the EEG microstructure during REM is less studied. The EEG during REM typically has mixed frequency and low amplitude, resembling open-eye wake (hence also called paradoxical sleep). Past studies have found that REM EEG is slower in the parietal, frontal, and temporal regions of AD samples compared to controls [45, 46]. A recent study used a REM EEG slowing ratio, defined as the band power ratio (delta + theta)/(alpha + sigma + beta), and found a moderate association between REM EEG slowing and poorer visuospatial performance in mild cognitive impairment but without association with other cognitive performance measures [47]. The slowing of EEG during REM could be due to the degeneration of cholinergic neurons, leading to memory loss and attention deficits [48].

Our study did not find any blood markers of inflammation and metabolism in the mediation pathway (Fig. 5). We also performed the same analysis using blood markers assessed in Visit 1, which was about 3 years before the Sleep Visit. The sample size became 337 since fewer participants with blood samples were collected at Visit 1. With the smaller sample size, we could not find any significant mediation pathway. IL-6 at Visit 1 showed a trend level ($p < 0.1$) mediation effect with the 3MS score at Visit 2 mediated through theta power at N1 and REM. But theoretically, it is reasonable to hypothesize the involvement of inflammatory markers such as cytokines. As a supporting point from the literature, studies show that cytokines can activate the JAK/STAT pathway involved in synaptic plasticity [49], which supports synaptic homeostasis during sleep [50] in the long term, ensuring overall cognitive health. Another possible mechanism is based on inflammatory molecules affecting the integrity of the blood–brain barrier and the oxidation status of brain cells in the long term, triggering glial activation, neuroinflammation, and ultimately ADRD [51].

Results supported by the literature

Predicting cognition from sleep EEG has been studied. Ujma et al. used 2,720 participants from the same MrOS cohort, predicting 3MS and other cognitive scores at the Sleep Visit (not at the future Visit 2, as in our study), and reported a Pearson correlation of 0.28 [52]. In comparison, our analysis had a Pearson correlation of 0.22 (0.15–0.24) (Table 2), which was lower, but we predicted cognitive scores at Visit 2, 1 year after the Sleep Visit. Similarly, Adra et al. used 150 participants from a clinical population, predicting total, fluid, and crystallized cognition using the NIH Toolbox Cognitive Battery with Pearson correlations of 0.37, 0.56, and −0.07 (not significant), respectively [53, 54]. However, their cognitive scores were collected within 40 days of the sleep recordings, which did not predict future cognition as in our case.

Predicting cognition from blood markers of inflammation has also been studied, but the overall effect sizes were small, consistent with our results (Table 2). Garés-Caballer et al. performed a prospective cohort study with 165 participants, and measured peripheral blood inflammatory and oxidative stress markers [55]. At 1 year follow-up, the blood markers had

a small-to-moderate effect size of association with cognition functions. A similarly small effect size was found in another related study [56]. Chi et al. studied 1159 community-dwelling adults aged 75 or older who were free of dementia at baseline and followed for up to 7 years [57]. Their combined inflammation score predicted psychomotor speed and memory at follow-up, also with a small effect size: every twofold higher inflammation was associated with a 0.0044 SD decline in psychomotor speed per 6 months and a − 0.0029 SD decline in memory.

There are also many studies about the associations between sleep and cognition, but not in terms of prediction (Table 2) or mediation analysis (Fig. 5c, d). For example, many studies [9] have reported positive associations between spindle density vs. global cognition, and spindle-SO coupling vs. global cognition. This is consistent with the role of spindles and their coupling with SO in memory consolidation, i.e., transferring short-term memory in the hippocampus into long-term memory in the cortex [58]. There have been studies about the beneficial effects of memory processing, declarative learning [59], and problem-solving skills [60].

Limitations

First, the cohort consisted of primarily White men 65 years and older; hence, the generalizability to younger, men of different race/ethnicities and female populations was unknown. Second, the study was retrospective and observational, so there were biases, including selection bias and unmeasured confounding bias (unsolvable but partially addressed by sensitivity analysis). Another limitation was that the outcome was measured about 1 year after the sleep study and blood test, which was a short-term outcome compared to long-term outcomes such as dementia.

Conclusion

The blood markers of inflammation and metabolism were less predictive of and indirectly associated with global cognition, compared to the sleep EEG microstructures. Future experimental studies and external validations are needed to confirm these results.

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Data and code availability Data from MrOS are available at https://mrosonline.ucsf.edu. The analysis dataset for this specific manuscript is also available from the corresponding author upon request. The code is available at https://github.com/Hockey86/sleep-bloodmarker-cognition.

Declarations

Ethnical approval and consent to participate They were approved and obtained by the MrOS committee.

Consent for publication All authors consent for publication. The MrOS committee approved the publication.

Conflict of interest Dr. Westover is the co-founder of Beacon Biosignals, which is not involved in any part of this article. Dr. Thomas discloses general sleep medicine consulting: GLG Councils, Guidepoint Global. Dr. Stone reports grant funding from Eli Lilly (unrelated to this work). Dr. Redline is an unpaid member of the ApniMed Scientific Advisory Board and an unpaid Board member of the National Sleep Foundation and Alliance of Sleep Apnea Partners.

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