SLEEP DISORDERS EDITOR'S PICK 2021

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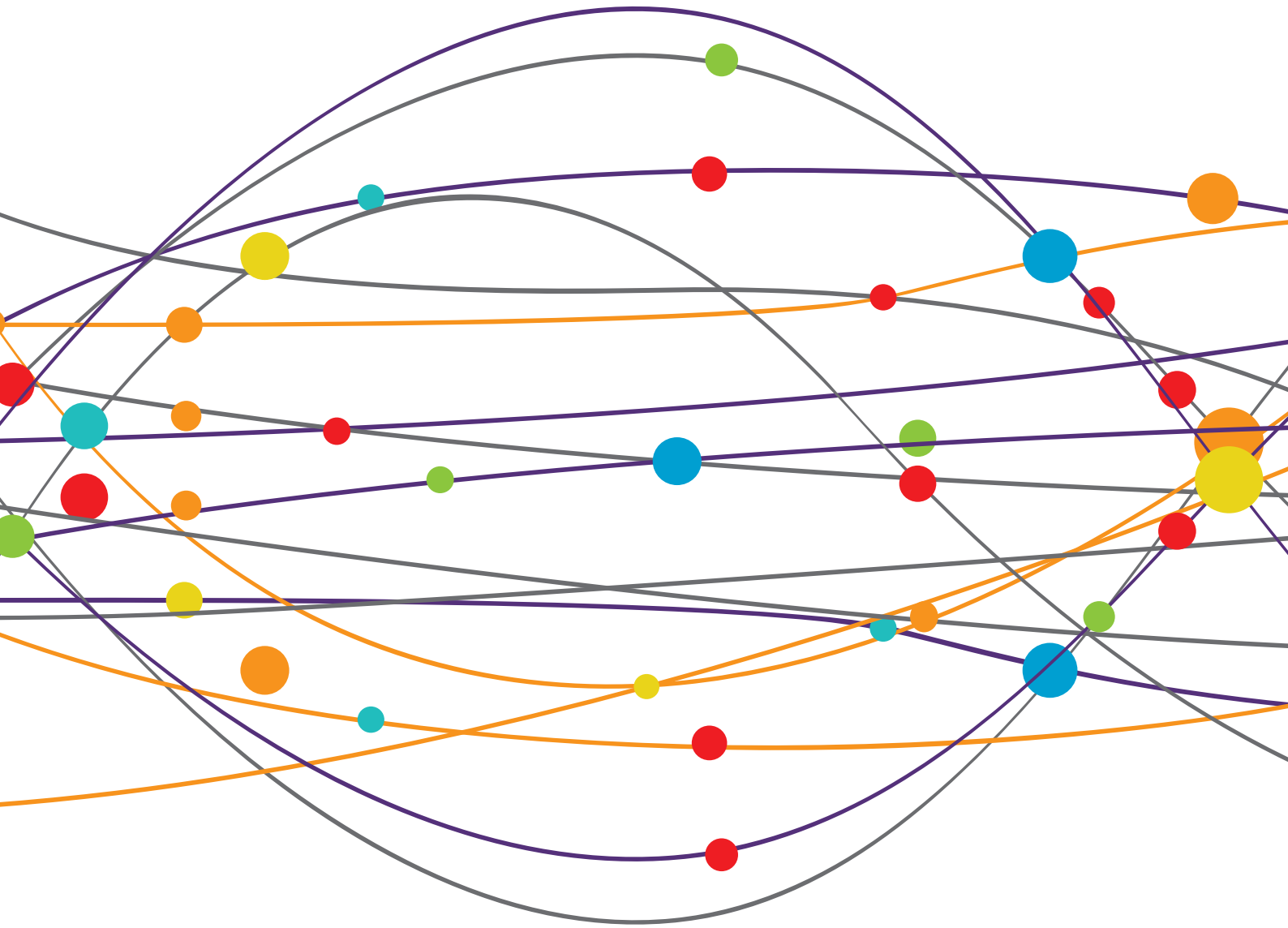

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SLEEP DISORDERS EDITOR'S PICK 2021

Topic Editor:
Yves A. Dauvilliers, Hôpital Gui De Chauliac, France

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CLINICAL TRIAL
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Non-REM Sleep Characteristics Predict Early Cognitive Impairment in an Aging Population

Jacques Taillard[1,2*], *Patricia Sagaspe*[1,2,3], *Christian Berthomier*[4], *Marie Brandewinder*[4], *Hélène Amieva*[5,6], *Jean-François Dartigues*[5,6], *Muriel Rainfray*[5], *Sandrine Harston*[5], *Jean-Arthur Micoulaud-Franchi*[1,2,3] *and Pierre Philip*[1,2,3]

[1] USR CNRS 3413 SANPSY Sommeil, Addiction et NeuroPSYchiatrie, Bordeaux, France, [2] SANPSY, USR 3413, Université Bordeaux, Bordeaux, France, [3] CHU de Bordeaux, Pôle Neurosciences Cliniques, Bordeaux, France, [4] PHYSIP, Paris, France, [5] CMRR, CHU Bordeaux, Bordeaux, France, [6] Bordeaux Population Health Center, INSERM U1219, Université de Bordeaux, Bordeaux, France

Objective: Recent research suggests that sleep disorders or changes in sleep stages or EEG waveform precede over time the onset of the clinical signs of pathological cognitive impairment (e.g., Alzheimer's disease). The aim of this study was to identify biomarkers based on EEG power values and spindle characteristics during sleep that occur in the early stages of mild cognitive impairment (MCI) in older adults.

Methods: This study was a case-control cross-sectional study with 1-year follow-up of cases. Patients with isolated subjective cognitive complaints (SCC) or MCI were recruited in the Bordeaux Memory Clinic (MEMENTO cohort). Cognitively normal controls were recruited. All participants were recorded with two successive polysomnography 1 year apart. Delta, theta, and sigma absolute spectral power and spindle characteristics (frequency, density, and amplitude) were analyzed from purified EEG during NREM and REM sleep periods during the entire second night.

Results: Twenty-nine patients (8 males, age = 71 ± 7 years) and 29 controls were recruited at T0. Logistic regression analyses demonstrated that age-related cognitive impairment were associated with a reduced delta power (odds ratio (OR) 0.072, $P < 0.05$), theta power (OR 0.018, $P < 0.01$), sigma power (OR 0.033, $P < 0.05$), and spindle maximal amplitude (OR 0.002, $P < 0.05$) during NREM sleep. Variables were adjusted on age, gender, body mass index, educational level, and medication use. Seventeen patients were evaluated at 1-year follow-up. Correlations showed that changes in self-reported sleep complaints, sleep consolidation, and spindle characteristics (spectral power, maximal amplitude, duration, and frequency) were associated with cognitive impairment ($P < 0.05$).

Conclusion: A reduction in slow-wave, theta and sigma activities, and a modification in spindle characteristics during NREM sleep are associated very early with a greater risk of the occurrence of cognitive impairment. Poor sleep consolidation, lower amplitude, and faster frequency of spindles may be early sleep biomarkers of worsening cognitive decline in older adults.

Keywords: sleep, EEG, slow-wave sleep, spindle, cognition, cognitive decline, aging, mild cognitive impairment

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Mehmet Y. Agargün, Yuzuncu Yil University, Turkey

Reviewed by:
Axel Steiger, Max-Planck-Institut für Psychiatrie, Germany
Luigi De Gennaro, Sapienza University of Rome, Italy

***Correspondence:**
Jacques Taillard
jacques.taillard@u-bordeaux.fr

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INTRODUCTION

Western societies are marked by aging of the general population which favors the increasing prevalence of neurological and sleep disorders. These disorders contribute to the morbidity and the mortality of the general population, in particular through daily life activities.

Recent evidence suggests that sleep disorders or modifications in sleep stage or electroencephalogram (EEG) waveform precede over time the onset of the clinical signs of mild cognitive impairment (MCI). This may be viewed as a transitional stage from normal cognition to dementia and Alzheimer's disease (AD), a neurodegenerative disorder characterized by progressive decline in memory and other cognitive domains.

A study (1) suggested that older adults suffering from sleep-disordered breathing, characterized by repeated episodes of hypoxemia and brief arousal, had a higher risk of developing long-term cognitive decline. An underlying mechanism of this relationship would seem to be hypoxemia rather than sleep fragmentation or sleep duration. However, a longitudinal study (2) showed that subsequent cognitive decline was associated with reduced sleep efficiency, greater wake after sleep onset (WASO), greater number of long wake episodes, and poor self-reported sleep quality. A recent study (3) showed that long sleep latency could also serve as an early marker of cognitive decline in MCI.

In addition to the sleep architecture or presence of sleep disorders, a new line of research is moving toward electroencephalogram oscillations as being involved in age-related cognitive decline.

The "Active System Consolidation theory of memory" posits that sleep, especially NREM sleep, promotes long-term consolidation of memories involving a dialog between the hippocampus and neocortex. (4–7) The key factor in hippocampal to neocortical transmission is the triple-phase that locks slow oscillations (cortex)-spindles (thalamus)-sharp wave-ripples (hippocampus) (7–9). Depolarizing slow oscillation up-states are involved in the generation of fast spindle (13–15 Hz) and sharp wave ripples, thus leading to the constitution of "spindle-ripple events" (9–11). Slow spindles (11–13 Hz) coincide with the slow oscillation up to down state transition but their role in memory consolidation is less well-documented. On the other hand, slow spindles are accompanied by an increase in lower frequencies, especially in the 5–8 Hz theta range (11). Theta oscillations during NREM might also be involved in strengthening memories (12). A study (13) demonstrated that reactivation of memory not only occurred in synchrony with spindles but was modulated by spindle amplitude: the higher amplitude, the stronger was the activity in the hippocampus.

There is growing evidence that sleep spindles, especially spindle characteristics (frequency, density, and/or amplitude), participate in memory formation, learning and synaptic plasticity (9, 14, 15).

The synaptic homeostasis hypothesis postulates that NREM sleep, especially slow wave sleep, restores synaptic, and cellular homeostasis that has been potentiated toward saturation during wakefulness (16). This theory predicts that the process of synaptic renormalization during sleep increases the capacity to acquire information during the following day (16, 17).

Healthy aging is accompanied by changes in sleep quantity, especially a decreased total sleep time, increased WASO, and decreased sleep efficiency (18, 19).

A highly important change in sleep with aging is a reduction in slow-wave sleep and in EEG slow-wave activity (18, 20–23). Sigma activity during NREM is decreased and the number, density and amplitude of sleep spindles are also reduced (23, 24).

A meta-analysis on sleep in patients with MCI showed less total sleep time and sleep efficiency and longer sleep latency (25). Another study (26) in patients with amnestic MCI demonstrated that slow wave sleep, delta, and theta power during NREM sleep were dramatically reduced. REM sleep, REM latency and sleep efficiency were also reduced. Spindle density, especially among fast spindles, was reduced. A recent study confirmed that parietal fast spindle density is decreased in MCI patients (27). As MCI progresses to Alzheimer's disease, sleep disturbances worsen. The amount of SWS is greatly reduced and spindles diminish in frequency (28–31) especially fast spindles (27, 29). The development of dementia in Parkinson patients was linked with sleep spindle density in posterior regions and sleep spindle amplitude in parietal regions. Lower sleep spindle amplitude in posterior regions was associated with poorer visuo-spatial abilities in patients who developed dementia at follow-up (32). The K-complex (KC) density during stage N2 decreases in AD patients but not in MCI patients (33, 34). KC density is positively correlated with cognitive deterioration. KC density cannot be considered as an early biomarker of AD but as a measure of cognitive decline (33). The reduction in KC density could reflect a dysfunction in synaptic plasticity linked with a deterioration in memory consolidation (34).

Slow oscillatory transcranial direct current stimulation in MCI patients led to enhanced endogenous slow wave-spindle coupling in the following way: spindle amplitude was significantly amplified during the depolarizing slow oscillation up-phases and synchronization between slow oscillation and fast spindle amplitude, involving an enhancement of visual declarative memory (35).

Moreover, alteration in sleep quantity, and quality facilitates the accumulation of amyloid-β, potentially initiating earlier cognitive decline and conversion to Alzheimer's disease. The disturbance of NREM sleep may represent a novel pathway though which cortical amyloid-β impairs hippocampus-dependent memory consolidation (31).

The aim of the SCOAL study is to determine polysomnographic biomarkers that occur at a very early stage of MCI. The objectives are the following:

- To compare the sleep architecture and/or presence of sleep disorders and neuropsychological performance in patients with isolated subjective cognitive complaints (SCC) or MCI, vs. cognitively normal controls.
- To study the prospective association of sleep and cognitive decline in patients with isolated SCC or MCI over a 1-year follow-up period.

METHODS

Study Population

Patients

The patients in the SCOAL study were recruited from the MEMENTO cohort at the University Hospital of Bordeaux from October 2011 to October 2015. MEMENTO (deterMinants and Evolution of AlzheiMer's disEase aNd relaTed disOrders) is a 5-year prospective large cohort of patients with either isolated SCC or recently diagnosed MCI while not demented attending an outpatient memory clinics (CMRR—Center Mémoire de Ressource et de Recherche) of public hospitals in France (36). The medical staff from the Bordeaux Memory Clinic invited eligible patients to participate in the SCOAL protocol (see **Figure 1**), which involved a 2-day stay in hospital and neuropsychological testing on attentional and executive functions (not detailed here).

MEMENTO inclusion criteria were as follows:

- Having at least MCI defined by a performance of more than 1.5 standard deviation from the mean (defined according to age, gender and level of education) in one or more cognitive domains (assessed from a battery of neuropsychological tests exploring memory, language, praxis, vision, executive functions), the deficit being identified for the first time by tests performed <6 months before the inclusion date;
- Or presenting an isolated cognitive complaint if the participant was over 60 years old;
- Having a clinical dementia rating [CDR] (37) Scale score $\leq$0.5 and being non-demented (DSM-IV).

As part of the MEMENTO study, participants completed a battery of neuropsychological tests administered by a trained neuropsychologist. In particular, the Mini-Mental State Examination (MMSE), which tests global cognition (38), the Free and Cued Selective Reminding Test (FCSRT) (39), the Trail-Making Test (TMT) (40) and the CDR (37) were administered. To assess the presence and severity of neuropsychiatric symptoms, the Neuropsychiatric Inventory (NPI) was used.

MCI was diagnosed using the Petersen Criteria (41).

The specific inclusion criteria for patients in the SCOAL study were as follows:

- Being at least 18 years old
- Having stable health (i.e., no medical condition involving imminent care or hospitalization)
- Being treated for obstructive sleep apnea syndrome (OSAS), if diagnosed.

Controls

Healthy controls considered cognitively normal at baseline and matched on age, gender, and level of education with patients underwent clinical and cognitive assessment. To verify the absence of objective cognitive deficit, participants were administered the MMSE (38), FCSRT (39), TMT (40), and CDR (37) by a trained neuropsychologist.

Design

The 1-year follow-up SCOAL study was a case-control cross-sectional study with follow-up at 1 year.

Patients with isolated SCC or MCI from the MEMENTO cohort in Bordeaux and cognitively normal controls were recruited. The controls with patients were matched on age, gender, and level of education.

Participants were recorded with 2-night polysomnography (PSG) monitoring and were tested on a battery of neuropsychological tests assessing attention and executive functions at T0 and T+1 year.

To determine usual sleep patterns, volunteers' sleep was recorded via an actimeter over 3 days in a natural environment.

To assess sleep disorders, participants underwent a first night of polysomnographic sleep recording (PSG) at the hospital. Questionnaires were used to capture sleep complaints and excessive daytime sleepiness.

The participants also completed a battery of neuropsychological tests.

The second night of PSG recording was used for sleep and EEG analysis.

Information on current pathologies and medical treatment was also collected.

All participants gave written informed consent. The study was approved by the local ethical committee (consultative committee for the protection of persons participating in biomedical research, CPP Sud-Ouest et Outre Mer III). The study was registered with ClinicalTrials.gov, identifier: NCT01650454.

Neuropsychological Evaluation

Patients completed their neuropsychological evaluation as part of their cognitive follow-up at the Memory Clinic, at T0 and then after 1 year of follow-up. Controls completed a neuropsychological evaluation at the time of the inclusion visit.

Subjective cognitive complaints (SCC) were assessed by visual analog scales ranging from 0 to 10 on several domains (e.g., memory, attention, language).

The Mini-Mental State Examination (MMSE) (42) is one of the most widely used psychometric tests for quantifying global cognitive functioning and cognitive change in population-based longitudinal studies (total score, range 0–30).

The free and cued selective reminding test (FCSRT) (39) evaluates the ability to learn a list of 16 written words that are presented with a semantic cue to control for memory encoding. It distinguishes between simple difficulties in retrieval of stored information (facilitated by cuing) and genuine storage deficits characterizing typical Alzheimer's disease (not facilitated by cuing). The learning phase is followed by three trials of recall, each consisting in retrieving the words first spontaneously ("free recall") and then with the help of a semantic cue ("cued recall") for those items not retrieved by free recall. The three measures evaluated were free recall (the cumulative sum of free recall from the three trials; range 0–48), total recall (the cumulative sum of free recall + cued recall from the three trials, range 0–48), and delayed recall (sum of delayed free recall + delayed cued recall, range 0–16).

The Trail-Making Test (TMT) (43) is a two-part paper and pencil neuropsychological test which assesses executive function. Participants are required to connect numbered circles in a sequential order in the TMT-A, whereas they have to

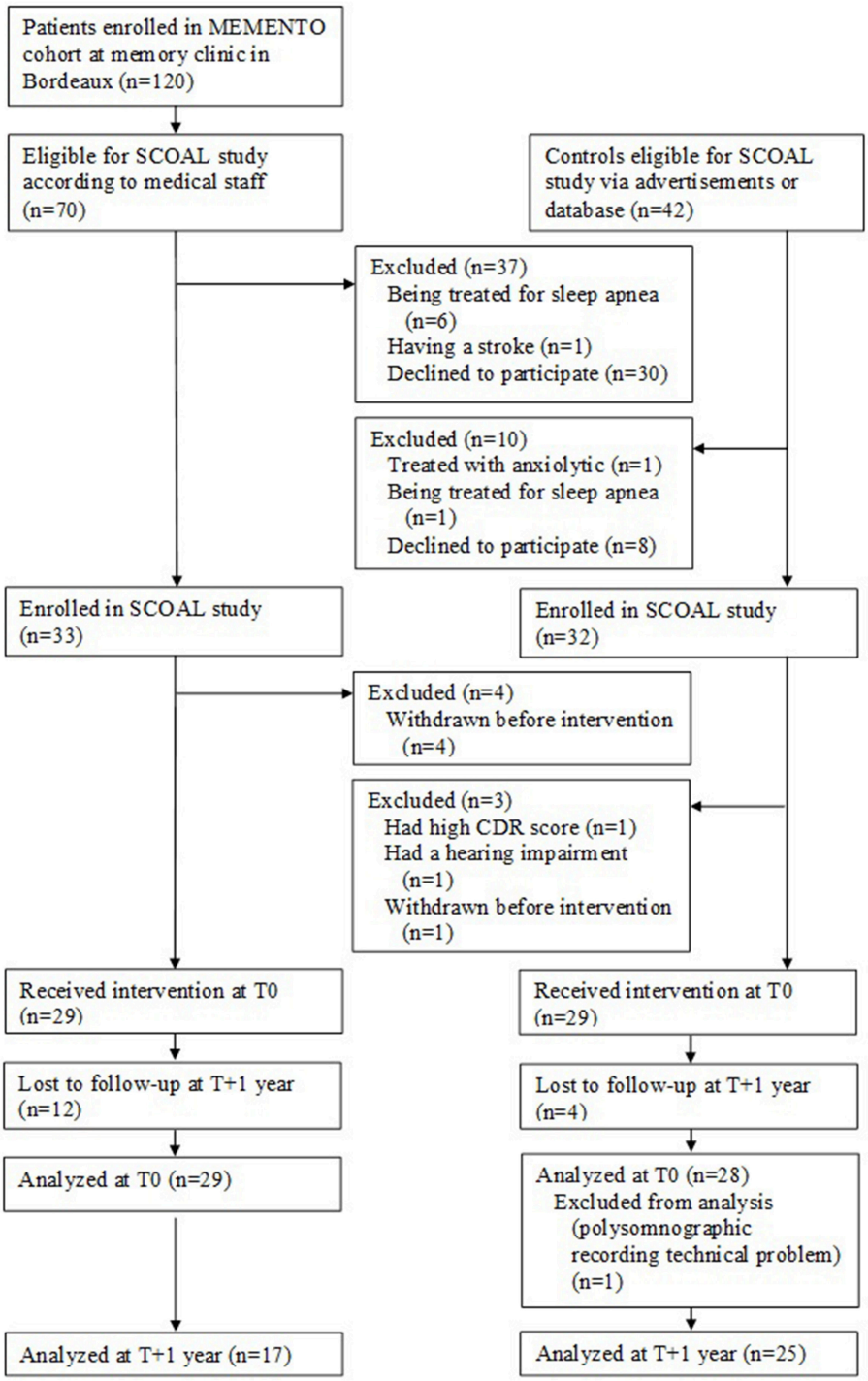

FIGURE 1 | Flow charts SCOAL study.

connect numbered and lettered circles in alternating sequential-alphabetical order in the TMT-B (i.e., 1-A-2-B, etc.). TMT-A time is taken as a measure of processing speed, while TMT-B time is considered an index of flexibility. The dependent variables are the number of seconds needed to complete the sequence and the number of correct responses for Part B and an interference index (Part A/Part B).

The Clinical Dementia Rating (CDR) (37) is a global scale developed to clinically denote the presence of dementia of the Alzheimer type and stage its severity. The clinical protocol

incorporates semi-structured interviews with the patient and informant to obtain information necessary to rate the SCC in six domains (memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care). The score is the CDR Scale Sum of Boxes (CDR-SOB).

Self-Reported Sleep Measures

Participants completed questionnaires during the follow-up visits.

The Epworth Sleepiness Scale (ESS) (44), a self-administered questionnaire, is used to measure subjective daytime sleepiness. Scores range from 0 to 24, a score of >10 indicating excessive daytime sleepiness.

The Pittsburgh Sleep Quality Index (PSQI) (45) is a 19-item questionnaire assessing subjective sleep quality and disturbances over the past month. Scores range from 0 to 21, a score of >5 indicating poor sleep quality.

The Insomnia Severity Index (ISI) (46) is a seven-item questionnaire assessing the nature, severity and impact of insomnia symptoms over the past month. The total score ranges from 0 to 28.

Objective Sleep Measures

Actigraphy

Participants had 3 days of monitoring with actimeters (Actiwatch, Cambridge Neurotechnology, Cambridge, United Kingdom). To study disrupted daily activity/rest cycles (47), the criteria assessed were as follows:

- Inter-daily stability (IS): measure of stability across days.
- Intra-daily variability (IV): relative consolidation within days.
- Rhythm amplitude (RA): difference in activity level between the 10 most active and five least active hours in the day.

Polysomnography

Participants slept two successive nights at the sleep unit. Polysomnographic signals were recorded with a Braintronics Brainbox EEG-1042 digital sleep recorder (Almere, The Netherlands, resolution 16-bit, stop band frequency 100 Hz, passband ripple 0.027 dB, stopband ripple −40 dB) at a sampled rate of 256 Hz. Nine Ag-AgCl electrodes were placed according to the international 10–20 System (F4, C4, O2, F3, C3, O1, Fz, Cz, Pz, Oz, M1, and M2) and referenced to linked-mastoids. Additionally, an electro-occulogram (EOG), an electromyogram (EMG, chin), and an electrocardiogram (ECG) were recorded as recommended by AASM (48) for the routine scoring of polysomnography. During the first night, nasal pressure, rib cage and abdominal movements, snoring sounds, transcutaneous finger pulse oximeter, and leg movements were also recorded. The first night was for adaptation and to identify the presence of any organic sleep disorders, while the second night was used for sleep and EEG analysis. Following the application of a notch filter (50 Hz) and a band pass filtered at 0.53–35 Hz, sleep stages and associated events were visually scored according to standard procedures (48) by the same experienced technician. The analyses of sleep EEG recordings were performed after an automatic artifact rejection using the ASEEGA software (version 3.30.14, Physip, France) (49–52). Artifacts are detected automatically based on both temporal and frequential criteria, where non-physiological abrupt variations are discarded. EEG spectral power per 30 s artifact-free epoch (Cz-Oz) was calculated using the fast Fourier transform with the Hanning window after an automatic artifact rejection. The spectral power was computed in the frequency bands delta (0.1–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), sigma (12–16 Hz), and beta (16–50 Hz), for all the automatically scored R and NREM epochs. After their automatic detection, the spindles were characterized by their duration (s), power (squared microvolts), maximum amplitude (microvolts), and frequency (Hz) [for more details on spindle detection method see (53)]. The density of spindles was computed as the average number of detected spindles per 30 s epoch for each subject. The spindle maximal amplitude is measured on the EEG filtered in the sigma frequency band and corresponds to the maximum of the filtered signal envelope. Spindles falling within the 11–13 Hz frequency range were considered as "slow spindles," and those falling in the 13–15 Hz range as "fast spindles" (27, 54). In addition, delta, theta, alpha, and beta spectral power and variations in spindle activity were computed for each successive NREM sleep cycles as defined by Feinberg and Floyd criteria (55).

STATISTICAL ANALYSES

Quantitative variables were expressed as mean ± standard deviation (*SD*), and qualitative variables were expressed as relative frequency.

Cross-Sectional Analyses at T0

First, univariate analyses with *t*-test comparisons for continuous variables or chi-square tests (χ^2) for categorical variables were used to compare demographic, clinical, neuropsychological, polysomnographic and log-transformed EEG characteristics in subjects with isolated SCC or MCI, with those characterized as cognitively normal.

Variables were expressed as continuous variables or as categorical variables as follows:

Age; Gender; Body mass index (BMI); Educational level; Self-reported pathologies: diabetes or hypertension; Self-reported current medication use: antidepressants, benzodiazepines or non-benzodiazepine anxiolytics; Neuropsychological performance: MMSE score, number of words at Free recall, Total recall and Delayed recall, TMT-B scores, TMT interference index; CDR score; ESS score; PSQI score; ISI score; Actimetric parameters: inter-daily stability (IS), intra-daily variability (IV), rhythm amplitude (RA); Polysomnographic characteristics: Time in bed (TIB); Total sleep time (TST); Sleep structure: Sleep latency, Stages N1, N2, N3, REM, sleep efficiency; Wake after sleep onset (WASO-PTS): >58, index of micro-arousals: >20 events/h; Apnea/Hypopnea Index (AHI): >10 events/h, >30 events/h; Apnea/Hypopnea central index; Periodic limb movements (PLM) index: >15 events/h; Respiratory effort-related arousals index (RERAs); Respiratory disorder index (RDI); Snoring; Mean SaO2; Minimum SaO2; SaO2 <90%; Oxygen desaturation index.

Then, multivariate analyses with logistic regression models were conducted to control for potential confounding factors

on the association between sleep parameters and cognitive impairment. Multivariate models were adjusted on age, gender, BMI, education level, use of antidepressant, benzodiazepine and non-benzodiazepine anxiolytics.

Results are presented as odds ratios (ORs) with 95% confidence intervals (CIs).

The alpha risk threshold was set at $P = 0.05$.

Longitudinal Analyses

Yearly changes in self-reported and objective sleep variables and cognitive variables were computed as the subtraction of the T0 score from the T+1-year score for individual performance.

Then, non-parametric correlations (Spearman) were computed for patients between sleep and cognitive variables scores.

All statistical analyses were performed using the SPSS statistical software package (PASWR Statistics 18).

RESULTS

Characteristics of Patients vs. Controls at T0

Population

Twenty-nine patients with isolated SCC or MCI enrolled from the MEMENTO cohort received the intervention at T0 (see **Figure 1**).

Concerning cognitive status, 21% had amnestic MCI (17% single-domain amnestic MCI), 48% non-amnestic MCI (79% single-domain non-amnestic MCI) and 31% had isolated SCC.

Twenty-nine matched controls characterized as cognitively normal were recruited after the clinical and neuropsychological examination at baseline (see **Figure 1**).

Demographic Characteristics

Concerning patients, most were female ($n = 21$; 72.4%), they had a mean age of 71 ($SD = 7$) years (range: 58–85 years), a mean BMI of 24.1 ($SD = 3.9$), and an education level of 12.4 ($SD = 3.8$) years. 69% had graduated from high school and attended university.

The latter were matched on age, gender, and educational level with cognitively normal controls. Most were female ($n = 21$; 72.4%), had a mean age of 68.1 ± 4.4 years (range: 58–77 years) and an education level of 11.8 ± 4.2 years. 48.3% had graduated from high school and attended university.

Participants with isolated SCC or MCI did not differ from cognitively normal controls on demographic parameters (see **Table 1**).

Clinical Characteristics

Regarding cardiovascular risk factors, there was no difference in the proportion of individuals suffering from diabetes or hypertension between those with isolated SCC or MCI and the controls.

Regarding medication use, a significantly higher proportion of patients with isolated SCC or MCI used antidepressants (12.1%) compared to controls (0%) ($P < 0.01$). There was no difference in the proportion of patients with isolated SCC or MCI and controls

TABLE 1 | Demographic, clinical, neuropsychological and polysomnographic characteristics (Mean ± SD) in patients with isolated subjective cognitive complaints or mild cognitive impairment, and in cognitively normal controls, at first intervention (T0). Statistical significance (*P* values) for independent groups with *T*-tests for continuous variables or Chi-square test (χ^2) for categorical variables.

	Patients with isolated cognitive complaints or mild cognitive impairment T0 ($n = 29$)	Controls T0 ($n = 29$)	*P* value
DEMOGRAPHIC AND CLINICAL CHARACTERISTICS			
Age (years)	71.0 ± 7.0	68.1 ± 4.4	ns
Gender (% females)	72.4	72.4	ns
Body Mass Index (BMI) (kg/m^2)	24.1 ± 3.9	23.9 ± 4.3	ns
Educational level (years)	12.4 ± 3.8	11.8 ± 4.2	ns
PATHOLOGIES (%)			
Diabetes	0.0	3.5	ns
Hypertension	15.8	8.8	ns
CURRENT MEDICATION USE (%)			
Antidepressants	12.3	0.0	<0.01
Benzodiazepines	3.5	1.8	ns
Non-benzodiazepine anxiolytics	1.8	0.0	ns
MINI-MENTAL STATE EXAMINATION (MMSE)			
Score	28.1 ± 1.5	28.2 ± 1.6	ns
≥24	100%	100%	ns
FREE AND CUED SELECTIVE REMINDING TEST			
Free recall	29.1 ± 6.8	31.5 ± 6.9	ns
Total recall	45.3 ± 5.4	46.1 ± 2.5	ns
Delayed recall	15.6 ± 1.2	12.3 ± 2.5	<0.001
TRAIL-MAKING TEST (TMT)			
TMT-B (RTs)	96.9 ± 40.8	107.1 ± 79.0	ns
TMT-B (correct)	22.5 ± 4.3	22.4 ± 2.4	ns
TMT interference index (RTs)	0.5 ± 0.1	0.4 ± 0.2	ns
CLINICAL DEMENTIA RATING (CDR)			
	0.40 ± 0.21	0.01 ± 0.09	<0.001
SELF-REPORTED QUESTIONNAIRES			
Epworth Sleepiness Scale (ESS) score	8.6 ± 4.7	6.8 ± 3.9	ns
>10	34%	17%	
Pittsburgh Sleep Quality Index (PSQI)	7.4 ± 3.8	6.8 ± 3.6	ns
>5	66%	55%	
Insomnia Severity Index (ISI)	8.7 ± 5.1	8.9 ± 6.5	ns
≥15	17%	21%	
ACTIMETRIC PARAMETERS			
Inter-daily stability (IS)	0.73 ± 1.09	0.70 ± 0.10	ns
Intra-daily variability (IV)	0.77 ± 0.23	0.82 ± 0.20	ns

(Continued)

TABLE 1 | Continued

	Patients with isolated cognitive complaints or mild cognitive impairment T0 ($n = 29$)	Controls T0 ($n = 29$)	*P* value
Rhythm amplitude (RA)	0.92 ± 0.05	0.93 ± 0.03	ns
POLYSOMNOGRAPHIC CHARACTERISTICS			
Time in bed (min)	478 ± 19.8	472 ± 23.7	ns
Total sleep time (min)	382 ± 62.7	391 ± 52.4	ns
Sleep latency (min)	9.0 ± 9.0	10.6 ± 10.4	ns
Stage N1 (%)	7.1 ± 3.3	7.1 ± 4.4	ns
Stage N2 (%)	50.2 ± 11.2	48.8 ± 8.4	ns
Stage N3 (%)	20.5 ± 8.1	20.8 ± 10.0	ns
Stage REM (%)	22.1 ± 6.8	23.3 ± 5.2	ns
Sleep efficiency (%)	80.0 ± 13.0	82.7 ± 9.2	ns
Wake After Sleep Onset—PTS (WASO)	74.6 ± 57.7	51.8 ± 29.3	ns
>58 (min) (median)	58.6%	39.3%	ns
Index of micro-arousals	25.7 ± 13.2	25.6 ± 14.3	ns
>20 events/h (*n*)	65.5%	55.2%	ns
Apnea/Hypopnea Index (AHI)	19.1 ± 14.1	19.4 ± 13.6	ns
>10 events/h (*n*)	72.4%	65.5%	ns
>30 events/h (*n*)	13.8%	20.7%	ns
Apnea/Hypopnea Central Index	0.24 ± 1.1	1.25 ± 6.4	ns
Periodic Limb Movements (PLM) Index	16.6 ± 20.3	9.2 ± 15.9	ns
>15 events/h (n)	37.9%	17.2%	ns
Respiratory Effort Related Arousals Index (RERAs)	1.25 ± 1.8	0.81 ± 1.3	ns
Respiratory Disorder Index (RDI)	20.3 ± 13.9	20.2 ± 19.6	ns
Snoring	5.5 ± 10.2	2.2 ± 4.8	ns
Mean SaO2 (%)	92.9 ± 2.2	93.0 ± 1.4	ns
Minimum SaO2 (%)	83.7 ± 7.2	85.9 ± 5.4	ns
SaO2 <90% (*n*)	47.1 ± 88.5	30.1 ± 41.4	ns
Oxygen desaturation index, events/h	19.2 ± 16.9	17.1 ± 14.0	ns

SD, Standard Deviation; n, Number; SaO2, Oxygen saturation.

regarding benzodiazepine or non-benzodiazepine anxiolytic use (see **Table 1**).

Regarding the baseline neuropsychological evaluation, the CDR score was higher in patients with isolated SCC or MCI than in controls (see **Table 1**).

TABLE 2 | Log-transformed EEG characteristics (Mean ± SD), in patients with isolated subjective cognitive complaints or mild cognitive impairment, and in cognitively normal controls, at first intervention (T0).

	Patients with isolated cognitive complaints or mild cognitive impairment T0 ($n = 29$)	Controls T0 ($n = 29$)	*P* value
EEG CHARACTERISTICS			
Spindle spectral power			
During total stage 2	1.247 ± 0.281	1.420 ± 0.291	<0.05
In stage 2 cycle 1	1.309 ± 0.285	1.466 ± 0.309	=0.051
In stage 2 cycle 2	1.240 ± 0.281	1.392 ± 0.290	=0.053
In stage 2 cycle 3	1.228 ± 0.279	1.389 ± 0.299	=0.052
In stage 2 cycle 4	1.143 ± 0.342	1.374 ± 0.292	ns
During total stage 3	1.112 ± 0.270	1.257 ± 0.287	=0.055
In stage 3 cycle 1	1.197 ± 0.224	1.322 ± 0.293	ns
In stage 3 cycle 2	1.097 ± 0.302	1.241 ± 0.282	ns
In stage 3 cycle 3	1.046 ± 0.281	1.235 ± 0.284	<0.05
In stage 3 cycle 4	1.005 ± 0.384	1.182 ± 0.304	ns
Spindle maximal amplitude			
During total stage 2	0.915 ± 0.137	1.003 ± 0.142	<0.05
In stage 2 cycle 1	0.947 ± 0.138	1.027 ± 0.152	<0.05
In stage 2 cycle 2	0.912 ± 0.135	0.993 ± 0.140	<0.05
In stage 2 cycle 3	0.907 ± 0.137	0.987 ± 0.148	<0.05
In stage 2 cycle 4	0.866 ± 0.167	0.983 ± 0.141	=0.052
During total stage 3	0.847 ± 0.133	0.923 ± 0.142	<0.05
In stage 3 cycle 1	0.883 ± 0.104	0.957 ± 0.144	<0.05
In stage 3 cycle 2	0.844 ± 0.141	0.916 ± 0.138	ns
In stage 3 cycle 3	0.810 ± 0.133	0.912 ± 0.140	<0.05
In stage 3 cycle 4	0.782 ± 0.177	0.901 ± 0.145	ns
Spindle duration			
During total stage 2	−0.078 ± 0.031	−0.073 ± 0.027	ns
In stage 2 cycle 1	−0.082 ± 0.039	−0.081 ± 0.042	ns
In stage 2 cycle 2	−0.078 ± 0.028	−0.077 ± 0.029	ns
In stage 2 cycle 3	−0.080 ± 0.040	−0.071 ± 0.031	ns
In stage 2 cycle 4	−0.077 ± 0.032	−0.072 ± 0.035	ns
During total stage 3	−0.098 ± −0.042	−0.099 ± 0.031	ns
In stage 3 cycle 1	−1.107 ± 0.040	−1.008 ± 0.041	ns
In stage 3 cycle 2	−0.101 ± 0.059	−0.100 ± 0.053	ns
In stage 3 cycle 3	−0.101 ± 0.055	−0.082 ± 0.078	ns
In stage 3 cycle 4	−0.093 ± 0.054	−0.066 ± 0.061	ns
Spindle frequency			
During total stage 2	1.136 ± 0.023	1.130 ± 0.018	ns
In stage 2 cycle 1	1.135 ± 0.023	1.129 ± 0.018	ns
In stage 2 cycle 2	1.136 ± 0.023	1.130 ± 0.017	ns
In stage 2 cycle 3	1.133 ± 0.023	1.130 ± 0.017	ns
In stage 2 cycle 4	1.136 ± 0.022	1.126 ± 0.018	ns
During total stage 3	1.132 ± 0.023	1.126 ± 0.017	ns
In stage 3 cycle 1	1.131 ± 0.025	1.123 ± 0.016	ns
In stage 3 cycle 2	1.131 ± 0.023	1.127 ± 0.016	ns
In stage 3 cycle 3	1.130 ± 0.024	1.125 ± 0.023	ns
In stage 3 cycle 4	1.134 ± 0.026	1.124 ± 0.023	ns
Spindle counts			
Slow	0.468 ± 0.759	0.499 ± 0.665	ns

(Continued)

TABLE 2 | Continued

	Patients with isolated cognitive complaints or mild cognitive impairment T0 ($n = 29$)	Controls T0 ($n = 29$)	*P* value
Fast	1.793 ± 1.077	1.583 ± 0.959	ns
NREM delta spectral power			
During total NREM	2.365 ± 0.310	2.558 ± 0.321	<0.05
In NREM cycle 1	2.434 ± 0.347	2.488 ± 0.338	ns
In NREM cycle 2	2.272 ± 0.337	2.601 ± 0.371	<0.05
In NREM cycle 3	2.234 ± 0.299	2.491 ± 0.368	<0.05
In NREM cycle 4	2.091 ± 0.443	2.371 ± 0.370	ns
NREM theta spectral power			
During total NREM	1.290 ± 0.280	1.497 ± 0.219	<0.05
In NREM cycle 1	1.327 ± 0.290	1.521 ± 0.262	<0.05
In NREM cycle 2	1.270 ± 0.290	1.496 ± 0.216	<0.05
In NREM cycle 3	1.294 ± 0.308	1.458 ± 0.227	<0.05
In NREM cycle 4	1.253 ± 0.355	1.509 ± 0.201	<0.05
NREM alpha spectral power			
During total NREM	0.936 ± 0.285	1.094 ± 0.235	<0.05
In NREM cycle 1	0.982 ± 0.289	1.119 ± 0.267	ns
In NREM cycle 2	0.938 ± 0.292	1.087 ± 0.243	<0.05
In NREM cycle 3	0.923 ± 0.286	1.094 ± 0.249	<0.05
In NREM cycle 4	0.864 ± 0.350	1.073 ± 0.207	ns
NREM sigma spectral power			
During total NREM	0.518 ± 0.264	0.672 ± 0.266	<0.05
In NREM cycle 1	0.560 ± 0.255	0.687 ± 0.285	ns
In NREM cycle 2	0.523 ± 0.277	0.643 ± 0.272	ns
In NREM cycle 3	0.474 ± 0.268	0.677 ± 0.289	<0.05
In NREM cycle 4	0.460 ± 0.315	0.652 ± 0.258	ns
NREM beta spectral power			
During total NREM	0.437 ± 0.280	0.524 ± 0.227	ns
In NREM cycle 1	0.431 ± 0.262	0.519 ± 0.240	ns
In NREM cycle 2	0.387 ± 0.282	0.484 ± 0.236	ns
In NREM cycle 3	0.341 ± 0.290	0.537 ± 0.249	<0.05
In NREM cycle 4	0.317 ± 0.315	0.491 ± 0.233	ns
REM theta spectral power			
During total REM	1.150 ± 0.323	1.321 ± 0.245	<0.05
In REM cycle 1	1.154 ± 0.318	1.330 ± 0.251	<0.05
In REM cycle 2	1.176 ± 0.336	1.340 ± 0.254	<0.05
In REM cycle 3	1.159 ± 0.331	1.307 ± 0.266	ns
In REM cycle 4	1.107 ± 0.424	1.344 ± 0.229	ns
REM alpha spectral power			
During total REM	0.824 ± 0.327	1.007 ± 0.258	<0.05
In REM cycle 1	0.811 ± 0.318	1.016 ± 0.269	ns
In REM cycle 2	0.850 ± 0.355	1.047 ± 0.267	<0.05
In REM cycle 3	0.854 ± 0.324	1.000 ± 0.276	ns
In REM cycle 4	0.828 ± 0.381	0.971 ± 0.229	ns
REM beta spectral power			
During total REM	0.591 ± 0.319	0.731 ± 0.302	ns
In REM cycle 1	0.629 ± 0.340	0.770 ± 0.301	ns
In REM cycle 2	0.606 ± 0.316	0.728 ± 0.311	ns
In REM cycle 3	0.551 ± 0.320	0.729 ± 0.318	=0.052
In REM cycle 4	0.483 ± 0.285	0.635 ± 0.247	ns

Statistical significance (P values) for independent groups with T-tests for continuous variables.
SD, Standard Deviation.

Patients with isolated SCC or MCI do not report more excessive daytime sleepiness, or insomnia complaints than controls.

Actimetric Parameters

There was no difference in actimetric data between patients with isolated SCC or MCI and controls (see **Table 1**).

EEG Characteristics (PSG)

The PSG was not correctly recorded in one patient.

Out of 28 patients, 11 presented at least 4 NREM sleep periods (vs. 18 controls) and 24 presented at least 3 NREM sleep periods (vs. 26 controls) during their PSG recording.

Macro-architecture of sleep

Two-thirds to three-quarters of the participants (65.5% of controls and 72.4% of patients, respectively) met the criteria for sleep-disordered breathing with an AHI of 10 or more events per hour.

37.9% met the criteria for PLM disorder with an index of 15 or more events per hour vs. 17.2% in controls ($P = 0.08$).

There was no difference in sleep structure (% stage 1, 2, 3, and REM), sleep duration (TST), sleep propensity (sleep onset latency) between patients with isolated SCC or MCI and controls (see **Table 1**).

Regarding sleep consolidation parameters, the mean WASO was of greater duration in patients with isolated SCC or MCI (74.6 ± 57.7 min) than in cognitively intact controls (51.8 ± 29.3 min, $P = 0.07$, tendency). There was no difference in sleep efficiency between patients with isolated SCC or MCI and controls.

Micro-architecture of sleep

Delta power, theta power and sigma power during NREM sleep periods were lower in patients with isolated SCC or MCI than in cognitively normal controls (see **Table 2**, **Figure 2**).

Theta power and alpha power during REM sleep periods were lower in patients with isolated SCC or MCI than in cognitively normal controls (see **Table 2**, **Figure 2**).

Regarding spindle parameters across NREM, spindle maximal amplitude was lower in patients with isolated SCC or MCI in the four NREM sleep periods than in controls. There was no difference in spindle density, duration and frequency between the groups (see **Table 2**).

Association Between Cognitive Impairment and Sleep Parameters

Logistic regression analyses showed that a reduced spindle maximal amplitude (OR = 0.002, $p < 0.05$), delta power (OR = 0.072, $p < 0.05$), theta power (OR = 0.018, $p < 0.01$), and sigma power (OR = 0.033, $p < 0.05$) during NREM sleep periods were risk factors associated with isolated SCC or MCI in aging. Variables were adjusted on age, gender, BMI, educational level, and medication use (see **Table 3**).

One-Year Follow-Up Analyses

Seventeen patients were evaluated in follow-up.

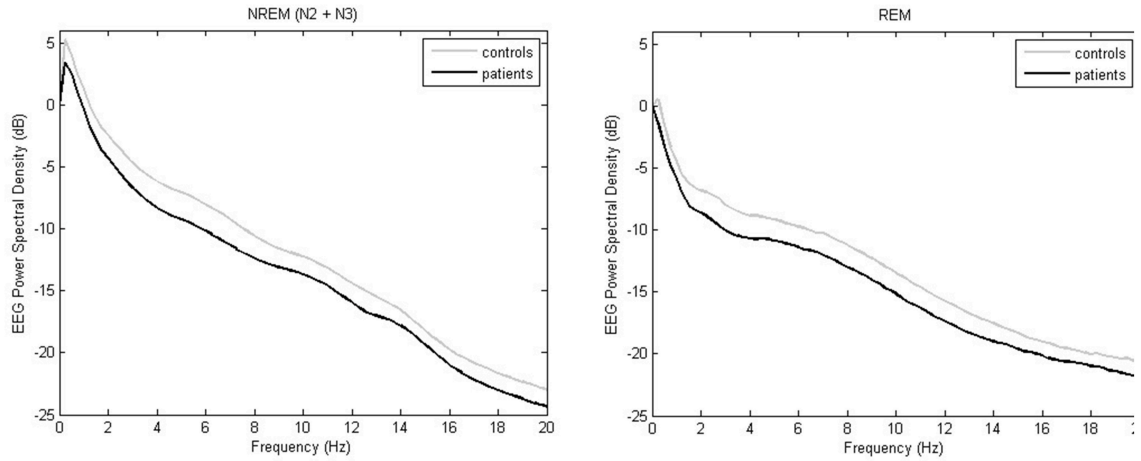

FIGURE 2 | Whole-night EEG power spectral densities during NREM sleep and REM sleep in controls (gray) or SSC and MCI patients (black). Mean absolute values (expressed in logarithmic scale) are plotted in the frequency range from 0 to 20 Hz for 0.25 Hz bins.

TABLE 3 | Multivariate logistic regression results for predicting isolated subjective cognitive complaints or mild cognitive impairment in older volunteers at first intervention (T0). Figures are adjusted odds ratios and 95% confidence intervals (CI) for multivariate model.

	Odds ratio (95 %CI)	*P*-value
SPINDLE MAXIMAL AMPLITUDE		
During total stage 2	**0.002** (0.000–0.354)	<0.05
NREM DELTA SPECTRAL POWER		
During total NREM	**0.072** (0.008–0.621)	<0.05
NREM THETA SPECTRAL POWER		
During total NREM	**0.018** (0.001–0.321)	<0.01
NREM SIGMA SPECTRAL POWER		
During total NREM	**0.033** (0.002–0.527)	<0.05

Multivariate models were adjusted on age, gender, BMI, education level, use of antidepressant, benzodiazepine, and non-benzodiazepine anxiolytics.
CI, Confidence Intervals; BMI, Body Mass Index; NREM, Non-Rapid Eye Movement.
Bold values correspond to Odds ratio (no unit)

Concerning their cognitive status, 35% had amnestic MCI (33% single-domain aMCI), 35% non-amnestic MCI (67% single-domain naMCI), and 30% had isolated SCC.

Considering decline at group level in patients with isolated SCC or MCI, there was no difference in neuropsychological performance during the 1-year follow-up.

Considering decline at an individual level, there were significant correlations between changes on self-reported or objective sleep parameters and cognitive impairment in patients with isolated SCC or MCI during the 1-year follow-up (see **Table 4**).

MMSE score was positively correlated with EEG spindle amplitude during NREM, with higher cognitive degradation for 1 year associated with smaller spindle amplitude.

FCSRT (total recall) was correlated with awakenings during the night measured by sleep efficiency with higher memory degradation associated with weaker sleep consolidation.

TMT (TMT-B RTs) was correlated with EEG spindle frequency activity with higher impairment in executive function associated with faster spindle frequency or with a lower number of slow spindles or with a higher number of fast spindles.

DISCUSSION

To our knowledge, this study is the first to examine prospectively whether electroencephalogram (EEG) power values and sleep architecture measured by highly controlled in lab polysomnography are directly or indirectly informative of progressive cognitive decline in patients with isolated SCC or MCI.

The cross-sectional investigation (i.e., patients with isolated SCC or MCI/cognitively normal controls) demonstrates that during NREM sleep, a reduction in delta (slow wave), theta and sigma activities and in spindle maximal amplitude are associated very early with an increased risk of the occurrence of isolated SCC or MCI (25–32).

The present findings confirm that changes in NREM sleep patterns seem to be predisposing factors for the early onset of MCI. No difference was observed in sleep architecture, sleep apnea, or periodic limb movement indices between the groups.

Our results showed a marked decline in NREM and REM sleep EEG power in SSC and MCI patients. As reported previously (21, 22, 56), the decrease in power spectral density associated with normal aging was not limited to slow wave activity but also affected theta and sigma activity in NREM and in REM. These EEG modifications, especially low-frequency delta activity during both NREM and REM sleep, were associated with thinning of the frontal and prefrontal gray matter (56, 57). In contrast to our results and the change in EEG with age, Latreille et al. (58) observed that Parkinson's patients who developed dementia had

TABLE 4 | Spearman correlations between yearly changes (subtraction of T0 score from T+1-year score for individual performance) on neuropsychological and polysomnographic and log-transformed EEG characteristics in patients with isolated subjective cognitive complaints or mild cognitive impairment.

Yearly Changes	PSQI	ISI	Sleep efficiency	WASO	Fast spindle (counts)	Slow spindle (counts)	Spindle spectral power (during total stage 3)	Spindle maximal amplitude (during total stage 2)	Spindle maximal amplitude (during total stage 3)	Spindle duration (during total stage 2)	Spindle frequency (during total stage 2)	Spindle frequency (during total stage 3)
MINI-MENTAL STATE EXAMINATION (MMSE)												
Score							0.56*	0.50*	0.62*			
FREE AND CUED SELECTIVE REMINDING TEST												
Free recall												
Total recall	−0.67**	−0.50*	0.53*	−0.51*						0.51*		
Delayed recall												
TRAIL MAKING TEST (TMT)												
TMT-B (RTs)					0.63**	−0.67**					0.66**	0.56*
TMT-B (correct)						0.48*						
TMT interference												
Index (RTs)												

RTs, reaction times; PSQI, Pittsburgh Sleep Quality Index; ISI, Insomnia Severity Index; WASO, Wake After Sleep Onset—PTS.

*$P < 0.05$, **$P < 0.01$.

a slowing of EEG in NREM sleep (higher power in delta and theta bands).

The 1-year follow-up demonstrates that the worsening cognitive decline in SCC/MCI patients is associated with changes in spindle characteristics (spectral power, maximal amplitude, and frequency) and with the impairment of sleep consolidation. In particular, a decrease in spindle maximal amplitude was associated with overall cognitive decline (i.e., MMSE), while an increase in spindle frequency was associated with a decline in executive functions. Moreover, changes in sleep consolidation parameters (i.e., sleep efficiency and WASO) together with changes in subjective sleep complaints (i.e., sleep quality and insomnia) were associated with episodic memory decline. Therefore, sleep consolidation and/or spindle characteristics (amplitude and frequency) could be early biomarkers that determine which SCC/MCI patients are at the greatest risk of suffering impaired cognitive or memory functions.

Sleep, especially slow-wave sleep plays an important role in the consolidation of long-term memory (5). Slow-wave activity reflects neural synchrony mainly within the prefrontal cortex, which may increase cortical connections that are important for cognition (57). A study has shown that sleep increases beta amyloid peptide (Aβ) clearance in interstitial fluid, promoting the removal of Aβ from the brain (59). Therefore, sleep disturbances, or modifications may be related to impairment of Aβ clearance and Aβ accumulation in the central nervous system, which leads to amyloid plaques, a characteristic of AD. Amyloid deposition in the preclinical stage of AD appears to be associated with worse sleep quality, especially sleep consolidation (60). Moreover, reduced SWS is associated with high cerebrospinal fluid Aβ in cognitively normal elderly (61). Finally, a recent study linked Aβ pathology with a reduction of SWS and the associated sleep-dependent memory consolidation, further supporting the existence of links between Aβ pathology, cognitive decline and sleep disturbances (62). Increased WASO and decreased SWS lead to relative increases in synaptic and metabolic neuronal activity, increased soluble CSF Aβ levels during the sleep period, increased Aβ aggregation, and sequestration into plaques, and attenuation of the Aβ diurnal pattern (63). Mander et al. (62) propose that sleep fragmentation and sleep efficiency quantified by actigraphy recorded during more than 10 days may be an early independent or complementary biomarker of AD risk. Although our results do not demonstrate that sleep fragmentation and efficiency differs between patients and controls, 1-year follow-up shows that more than a reduction in delta activity, it is above all the increase in wake time during sleep which is responsible for the worsening of memory in SCC/MCI patients.

In a recent study that examined the association between quantitative sleep EEG changes measured at home and clinical manifestations of MCI and/or incident dementia, [64] baseline EEG power values were higher in the group that developed dementia/MCI. Values were higher in the alpha and theta bands in NREM sleep, and in the alpha and sigma bands in REM sleep.

Like Djonlagic et al. (64), we did not find relationship between worsening of memory and modification in the delta bands during NREM. Either the association between EEG delta activity and cognition across the lifespan is more complex, reflecting an

age-related dissociation of the functional relationship between delta activity and cognition, or it is the alteration of delta activity which precedes the clinical onset of cognitive decline.

Our results confirm that sigma activity and spindle characteristics are the marker of cognitive functioning in older adults. Like Djonlagic et al. (64), we found that the worsening of memory in MCI patients is not associated with a modification of sigma activity during NREM. On the other hand, we show that the worsening of memory in MCI patients is associated with a modification of spindle characteristics (amplitude and frequency). A decrease in spindle maximal amplitude is associated with overall cognitive deterioration (i.e., MMSE) and we confirm that spindle amplitude is positively correlated with cognitive ability (65).

We found that a higher density of fast spindles or an increase in spindle frequency during NREM were associated with poorer performance on executive function (i.e., mental flexibility). This result is in disagreement with one of the few studies to focus on changes in the density of spindle subtypes in association with overall cognitive decline (27). However, studies in children and adolescents have shown an association between slow spindle activity, learning efficiency, and general cognitive abilities (66) or a negative association between spindle activity and cognitive performance (67).

These results are seemingly at odds with findings in younger adults. However, Bang et al. (68) demonstrated that slow sigma activity corresponding to slow spindles was involved in the consolidation of a texture discrimination task. Further studies should investigate the relationships between sleep spindles, episodic memory, and overall cognitive abilities in aging and/or during life time. Moreover, the local specificity of alterations in slow and fast spindle activity and its relation to the severity of specific cognitive decline remain unclear.

An important question is whether slow wave activity or sleep spindle activity could be stimulated to prevent, or at least slow down, cognitive decline in the elderly. Slow oscillatory transcranial direct current stimulation in MCI (35) would be a good approach to improve the physiology of disordered sleep and memory deficits.

Sleep-disordered breathing is frequent among elderlies and is known to alter cognition in aging (69). We observed a high apnea/hypopnea index and high measures of sleep-related hypoxemia in both our groups. Therefore, the impact of sleep-disordered breathing on cognitive impairment could not be investigated here.

The strengths of this study are the examination of self-reported and objectively measured sleep parameters in the laboratory on several nights in the least severely affected patients of a cohort with isolated SCC or MCI. Moreover, we adjusted on potentially important confounding factors such as age, gender, BMI, education level, use of antidepressant, benzodiazepine, and non-benzodiazepine anxiolytics.

To overcome the limitations of our study, additional studies with larger sample sizes and a longer follow-up period involving clear clinical deterioration are required. Patients with SCC or MCI and controls suffered from psychiatric, cardiovascular, metabolic, and sleep (especially Obstructive Sleep Apnea Hypopnea) disorders and were receiving treatment (especially antidepressants). These disorders can modify sleep patterns and cognitive measures. We believe that the sleep EEG modifications or cognitive impairments in patients with SCC or MCI might not have been caused by these disorders, since there was no difference in the proportion of individuals suffering from these disorders between those with isolated SCC or MCI and the controls. Antidepressants can modify REM sleep parameters, but they did not differ between MCI patients and controls in this study. To account for these factors (pathologies and treatments), we adjusted the regression analyses. EEG was analyzed only at the CzOz localization, yet power activity and spindle characteristics were localized according to age. A study demonstrated that changes in sleep spindles related to age follow topographical patterns that are specific to each spindle characteristic, and that age-related changes in spindle density and frequency differ between men and women homogeneously across brain regions (70). Moreover, it would have been interesting to evaluate which of these patients will develop Alzheimer's disease in the future to determine whether sleep disturbances are key to an early diagnosis.

To conclude, our results demonstrate the following: (1) cognitive decline in SCC/MCI patients is associated with a reduction in slow-wave delta, theta and sigma (spindle) activities, and in spindle maximal amplitude during NREM sleep; (2) spindle characteristics (amplitude and frequency) and sleep consolidation parameters (sleep efficiency or WASO) could potentially serve as early sleep biomarkers for worsening cognitive decline with aging. Memory decline is associated with an increase in wake time during sleep. Overall cognitive decline is associated with a decrease in spindle amplitude, and the impairment of executive functions is associated with an increase in spindle frequency.

The findings of this study support the use of quantitative sleep EEG analysis as a promising biomarker for older people at risk of cognitive decline. An algorithm for the automatic detection of MCI and dementia markers in sleep EEG would be a welcome development.

Further research is necessary to unravel more precisely the associations between specific sleep modifications, sleep disorders and pre-dementia and their impact on the progression of cognitive and behavioral impairment.

AUTHOR CONTRIBUTIONS

JT participated in the study concept and design, data acquisition, data analysis and interpretation, interpretation of results, and writing of the manuscript. PS participated in study concept and design, data analysis and interpretation, interpretation of results, writing of the manuscript, and study supervision. CB and MB participated in study concept and design, data acquisition, data analysis and interpretation, interpretation of results, and manuscript revision. HA and J-FD participated in study concept and design and manuscript revision. MR and SH participated in data acquisition and manuscript revision. J-AM-F participated in manuscript revision. PP participated in study concept and

design, interpretation of results, writing of the manuscript, and study supervision.

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Clinical Features and Pathophysiology of Disorders of Arousal in Adults: A Window Into the Sleeping Brain

Tommaso Baldini[1], *Giuseppe Loddo*[1], *Elisa Sessagesimi*[2], *Francesco Mignani*[3], *Fabio Cirignotta*[4], *Susanna Mondini*[4], *Laura Licchetta*[1,3], *Francesca Bisulli*[1,3], *Paolo Tinuper*[1,3] *and Federica Provini*[1,3]*

[1] Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy, [2] Radiology Unit, Experimental, Diagnostic and Specialty Medicine, University Hospital S. Orsola-Malpighi, Bologna, Italy, [3] IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy, [4] Unit of Neurology, S.Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy

Introduction: Disorders of Arousal (DoA) are NREM parasomnias that have been typically regarded as self-limited childhood manifestations. It is now clear that DoA can persist in adults, often presenting with distinctive characteristics. So far, few studies have described the clinical course and characteristics of DoA in adulthood, therefore a large part of their semiology is ignored. The aim of this study is to describe the clinical manifestations of DoA in an adult population and to provide a pathophysiological interpretation of their features.

Methods: We screened our database for all 1,600 adult (≥15 years) patients with sleep-related motor behaviors between 1995 and 2016. We identified 45 patients with typical DoA episodes, of whom a complete history, neurological examination and diagnostic video-polysomnography (VPSG) were available. All patients provided a detailed description of their episodes (with particular regards to semiology, frequency, and association with stressful life events) in different life periods. VPSG recordings were reviewed and DoA episodes were identified and assigned to three different categories according to their complexity.

Results: Our population was composed of 45 adult patients ranging between 15 and 76 years. Sleepwalking was reported by 86% of patients, possibly associated with complex interactions with the environment and violent behaviors in 53% of cases; distressing mental contents were reported by 64%. Recall of the episodes was reported in 77% of patients. Non-restorative sleep was reported in 46% of patients. Stress was a potential episode trigger in 80% of patients. VPSG recordings documented 334 DoA episodes. According to our classification of motor patterns, 282 episodes (84%) were Simple Arousal Movements (SAMs), 34 (10%) Rapid Arousal Movements (RAMs) and 18 (5%) Complex Arousal Movements (CAMs).

Discussion: Our study confirms that DoA in adulthood present with distinctive characteristics, such as non-restorative sleep, violence and complex, or bizarre behaviors. Alternative classifications of DoA based on motor patterns could be useful

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***Correspondence:**
Federica Provini
federica.provini@unibo.it

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to characterize DoA episodes in adults, as different motor patterns often coexist in the same individual and minor episodes are more common but generally underreported by patients. Prospective studies are needed for a definitive characterization of DoA in adulthood throughout the life course.

Keywords: disorder of arousal (DoA), NREM sleep, parasomnia, pathophysiology, sleep-related behaviors, adults, video-polysomnography (VPSG)

INTRODUCTION

Disorders of Arousal (DoA) are NREM parasomnias characterized by involuntary movements or behaviors of different complexity that occur as incomplete arousals from deep sleep (1). These events are accompanied by variable degrees of vegetative activation, automatic behaviors, misperception and reduced responsiveness to external stimuli, mental confusion and frequent retrograde amnesia (2). According to the International Classification of Sleep Disorders (ICSD-3), DoA include Confusional Arousals (CA), Sleep Terrors (ST) and Sleepwalking (SW) (1). Confusional arousals consist of confusion and disorientation without major accompanying behaviors or autonomic responses. Sleep terrors are characterized by a sudden arousal usually accompanied by a sharp scream, intense agitation and fear, confusion, and heightened autonomic discharge. Sleepwalking includes any form of complex behavior, ranging from walking to performing semi-purposeful activities. Although classified as distinct entities, DoA actually represent a spectrum of manifestations of increasing complexity (3). Indeed, these disorders share similar genetic and familial patterns, similar pathophysiology and similar priming by sleep deprivation and bio-psychosocial stressors (4).

DoA have been typically labeled as self-limited childhood manifestations that tend to disappear during adolescence (5, 6); in the last decades, however, it has progressively been understood that DoA can persist into adulthood or appear *de novo* in adults (7). A recent meta-analysis reports that the significant difference in current sleepwalking rates between children and adults is an artifact of not being observed, rather than a true effect (8). Remarkably, disorders of arousal in adults exhibit different characteristics from childhood DoA (9). They are more often associated with excessive daily sleepiness (with an impact on daytime activities) and violent or potentially harmful behaviors, which are rare in childhood DoA (9–13). These can include running into walls and furniture, trying to escape imaginary threats, leaving one's house, destruction of property, driving motor vehicles, suspected suicide, and even homicide or attempted homicide (14–16).

Additionally, the semiological manifestations of adult DoA include a spectrum of motor patterns of different complexity and duration that are not contemplated in the ICSD-3 classification, so that their video-polysomnographic (VPSG) recognition and interpretation can sometimes be problematical. Indeed, while in typical cases the diagnosis of DoA can be done with clinical history alone, in adult DoA diagnostic uncertainty often exists and VPSG is needed (7, 17). This is particularly true in the differential diagnosis with Sleep-related Hypermotor Epilepsy (SHE), a form of focal epilepsy in which motor seizures appear predominantly during sleep (7, 18–21). Therefore, knowledge of the precise characteristics of DoA in adults is essential for a correct diagnosis.

So far, few studies have described the clinical course of DoA in adulthood and have been usually performed after medical or psychological therapy (10, 22, 23). Moreover, few studies have reported specific description of DoA episodes in adulthood, therefore a big part of their semiology remains neglected, with negative repercussions to the diagnosis of these conditions. (2, 10, 13, 20, 24) The aim of this study is to describe the clinical features of DoA in an adult population—with particular attention to their trend during lifetime, the semiology of the episodes and the presence of predisposing and precipitating factors. Additionally, we provide a pathophysiological interpretation of these clinical characteristics on the basis of the most recent evidence from the literature.

MATERIALS AND METHODS

Patient Selection and Interview

We screened our electronic database for all 1,600 patients ≥15 years of age who underwent video-polysomnography (VPSG) at our sleep laboratory for complex motor behaviors during sleep in the time period from 1995 to 2016. We identified all patients presenting with at least one typical DoA episode according to the ICSD-3 criteria. We excluded those patients for whom a complete personal, family and medical history and a neurological examination were not available. All patients underwent a telephone interview and subsequently a semi-structured face-to-face interview (along with any potential witness of the events), paying particular attention to the description of DoA episodes. Patients were also asked to report the frequency of DoA episodes in different periods of their life (5–15 years; 15–25 years; 25–35 years; 35–50 years). For each life period, patients were subdivided into two subgroups: a high-frequency group (more than one DoA episode per week) and a low-frequency group (<1 DoA episode per week). For each of these periods we also explored the presence of stressful life events.

VPSG Analysis and Classification of Motor Patterns

We reviewed the VPSG recordings of all patients and identified any DoA episodes. We classified the episodes on the basis of their main motor pattern according to Loddo et al. dividing them into three groups with increasing intensity and complexity: pattern I or simple arousal movements (SAMs), pattern II or rising

arousal movements (RAMs) and pattern III or complex arousal with ambulatory movements (CAMs), i.e., sleepwalking. SAMs are further subclassified in A: head flexion/extension; B: head flexion/extension and limb movement; C: head flexion/extension and partial trunk flexion/extension (25). Sleep stages were scored according to the standard American Academy of Sleep Medicine (AASM) criteria, and the percentages of NREM and REM sleep stages and sleep efficiency (the percentage of total sleep time/time in bed) were evaluated.

Statistical Analysis

Continuous variables were presented as mean ± standard deviation (SD) and categorical variables as absolute frequency and relative frequency (%).

RESULTS

Clinical Data

Our population included 45 DoA patients ≥ 15 years (25 males and 20 females). At the time of VPSG, patients' ages ranged from 15 to 76 years (mean ± SD: 33±17 years); only one patient was younger than 18. DoA onset was at 13 ± 11 years (range 5–58). We didn't find any differences between males and females in our cohort of patients.

On the base of the age of onset, it was possible to distinguish between two set of patients: relapsing and adult-onset DoA. Forty patients (89%) have had a disease onset before 18 years of age but persisting over time (i.e., relapsing DoA), with a mean duration of disease of 21 ± 14 years (range: 1–64). At the time of our observation, those patients had a mean age of 31 ± 16 (range 13–74). The remaining five patients (11%) have had a disease onset after 18 years of age (i.e., adult-onset DoA), mean 36 ± 15; range: 19–58; no one after 65. At the time of our observation, those patients had a mean age of 40 ± 14 (range: 24–76), with a mean duration of disease of 10 ± 11 years (range: 2–20). There were no differences between the two groups regarding the number of episodes per night or the VPSG data. Unfortunately, due to the small number of the cohort, it wasn't possible to statistically compare the two groups.

Family and Personal History of the Patients

Twenty patients (44%) reported one or more first-, second- or third-degree relatives positive for DoA. DoA in relatives were usually described as sporadic, occurring mostly in childhood and persisting into adulthood only in two cases. Six patients reported a family history for sleep terrors (13%), ten for sleepwalking (22%) and four for confusional arousal (8%). One patient had a positive family history for epilepsy. All patients reported normal birth and psychomotor development. Two patients reported infantile febrile convulsions; one patient underwent surgical removal of a pilocytic astrocytoma of the IV ventricle and posterior brainstem 4 years after DoA onset. Eight patients (16%) reported headache. Nineteen (42%) patients reported concomitant medical diseases, 8 (16%) reported headache and twelve (26%) had been suffering from psychiatric disorders. Seven patients (15%) reported other concomitant sleep disorders (**Table 1**).

TABLE 1 | Clinical features of the studied cohort.

Concomitant medical disease	Number of patients (%)
Allergic diseases	6 (13%)
Thyroid diseases	5 (11%)
Gastritis	3 (6%)
Hypertension	3 (6%)
Previous cancer	2 (4%)
CONCOMITANT NEUROLOGIC DISEASE	
Migraine	4 (8%)
Tension-type headache	4 (8%)
CONCOMITANT PSYCHIATRIC DISEASES	
Depression	5 (11%)
Anxiety	5 (11%)
Post-traumatic stress disorder	2 (4%)
CONCOMITANT SLEEP DISORDERS	
Insomnia	3 (6%)
Bruxism	2 (4%)
Obstructive sleep apnea (AHI 5-10)	2 (4%)

AHI, apnea-hypopnea index.

TABLE 2 | Clinical characteristics of DoA episodes reported by patients.

Motor activity	Number of patients (%)
Sleepwalking	39 (86%)
Leaving the room	15 (33%)
Leaving the house	6 (13%)
Sitting in the bed	39 (86%)
BEHAVIORS AND DEGREE OF INTERACTION	
Sleep talking	44 (97%)
Interaction with the environment	31 (68%)
Simple actions mimicking daily activities	20 (44%)
Bizarre behaviors	23 (51%)
Violent behaviors	24 (53%)
Interaction with other people	29 (64%)
Verbal interaction	17 (33%)
Physical interaction	12 (26%)
MENTAL ACTIVITY	
Frightening or distressing content	29 (64%)
Quite mental activity	6 (13%)
Recall for the episode	35 (77%)

Clinical Description of DoA Episodes as Reported by the Patients

The type of motor activation and of interaction with the environment and the presence of mental activity during the episode in our cohort are summarized in **Table 2**.

Motor Activity

Thirty-nine patients (86%) reported they could get out of bed and wander around during a typical DoA episode. Fifteen patients (33%) could leave their room and six of them left their house at least once. Thirty-nine (86%) reported also episodes during

which they could stay in bed, sit up and often speak or shout, look around, turn on the light or handle objects. For twenty patients (44%) the bed partner described a frightened facial expression during DoA episodes.

Behaviors and Interaction With the Environment

Thirty-one patients (68%) described some kind of interaction with the environment, with variable degrees of complexity. Twenty patients (44%) exhibited simple actions or behaviors mimicking daily activities such as: turning on the light, looking at or using the mobile phone, opening a door or looking out of the window, raising the blinds, checking if the door was locked, ringing the bell or knocking on the door of a neighbor, getting dressed, preparing breakfast, laying the table, taking actions related to working activity, washing clothes, preparing luggage, doing their makeup, going up or down the stairs, climbing on the bike in the garage. Twenty-three patients (51%) reported also more bizarre behaviors: hiding objects (e.g., a watch, a remote control), tinkering with components of the furniture in the room (e.g., handling, unplugging or lifting and then letting a lamp fall to the floor, moving a wardrobe or the bed, pulling down a shelf, lifting a heavy compact disc holder off the ground, overturning a bedside table, trying to take the door off its hinges, looking for something—a fox—inside the wardrobe, punching the door or a shutter), cutting something with a knife or scissors (an electric cable, the sheet), removing the sheets from the bed and piling them on the ground, wrapping themselves in the sheets, throwing a pillow, climbing a spiral staircase, climbing over a window, waving their arms and knocking them on the bed or on the ground, pushing hands and feet against the wall, getting on a couch, acting as if throwing something (such as some water) toward a lamp.

Twenty-four patients (53%) reported violent and/or injuring behaviors, including simple dream enactment and/or complex behaviors potentially or actually dangerous for the patient and/or bed partner. Examples include hitting or tripping over objects in their room (the door, a glass, the dog, the dresser) and in some cases falling on the ground, falling out of bed, or trying to climb over a window. Injuries included cracked ribs in three cases, knee dislocation in one patient, a cut to the chin requiring stitches in one patient, and more frequently other minor lesions (excoriation, soreness in the bruised area for a few days after the trauma).

All patients except one (97%) reported they could utter vocal sounds, including moaning, mumbling, shouting, saying a few words (more or less intelligible), articulating complex sentences or even singing. Twenty-nine patients (64%) reported that during DoA episodes they could interact with their bed partner or other family members, being verbal in 33% of subjects; this included speaking with the bystanders (often excitedly) either using a few words or more complex and articulated sentences. Physical interaction was reported by 26% of subjects and mostly described as aggressive or even violent.

End of the Episodes

Awakening from episodes was possible (although not constant) in twenty-three patients (51%) and could be caused both by internal or external stimuli, such as shouting or feeling pain after a fall. Once awake the majority of patients reported they felt confused and disorientated.

Twenty-one patients (46%) complained of non-restorative sleep and twenty-two (48%) reported that they often felt tired during the day.

TABLE 3 | Patient distribution according to the different DoA episodes frequency in the different life periods.

Life period	Number of patients (%) with a high frequency	M	F	Number of patients (%) with a low frequency	M	F
5–15 years	10 (30%)	6	4	23 (70%)	13	10
15–25 years	17 (51%)	8	9	16 (49%)	6	10
25–35 years	13 (50%)	7	6	13 (50%)	7	6
35–50 years	2 (20%)	1	1	8 (80%)	5	3

M, males; F, females.

High frequency is defined as ≥ 1 episode/week, while low frequency < 1 episode/week. The total number of patients in the different life periods can be <45 due to the different age of onset and the different degree of episodes' recall.

Mental Activity Associated With DoA Episodes

Thirty-five patients (77%) reported that, even if not constantly, they could recall some kind of mental activity at the end of the episode. Of them, twenty-nine (64%) reported frightening and distressing contents, variably alternating with neutral contents in four of them. Fearful contents included someone chasing or trying to kill the patient, the ceiling falling on the patient, a truck running over the patient, mice infesting the house, being inside a box from which it was impossible to escape, a fire, walls crashing during an earthquake, thieves entering the house or a fox in the room. Because of these mentations, some patients could rise abruptly, jump out of bed, run and/or carry out protective behaviors like kicking or moving the arms. Finally, six patients (13%) reported a quiet mental activity (such as reliving the experiences of the same day or being late for work with colleagues waiting outside in the street) in association with DoA episodes.

DoA Frequency and Lifetime Occurrence

Almost all patients reported that DoA episodes usually occurred in the first or in the central part of the night. All patients reported that DoA frequency and intensity varied during the course of the disease, alternating periods of DoA high frequency (nightly or weekly episodes) with free periods lasting from few weeks to years. Four patients reported a free period more than 5 years long.

At the time of our VPSG, 18 patients (40%) reported nightly episodes; 17 patients (38%) had more than one episode per week; seven patients (15%) <1 episode per week but more than one per month; 3 patients (7%) reported <1episode per month.

The detailed patient distribution according to DoA episode frequency in the different life periods is reported in **Table 3**.

TABLE 4 | Sleep parameters in our DoA patients cohort.

Sleep parameters	Values
Total sleep time, minutes	411 ± 103
Sleep efficiency, % (NV > 85)	86 ± 11
REM latency, min (NV 60–120)	102 ± 56
Sleep stage 1, % (NV 2- 5)	8 ± 6
Sleep stage 2, % (NV 45–55)	45 ± 10
Sleep stage 3, % (NV 15–25)	25 ± 10
Sleep stage REM, % (NV 20–25)	22 ± 7
PLMI	2 ± 6

NV, normal value; REM, rapid eye movement; PLMI, periodic limb movement index.

Triggering Events and Predisposing Conditions

Two patients (4%) reported a head trauma before the onset of DoA. Thirty-six patients (80%) reported that both stressful and positive emotional events or situations could increase the frequency of DoA episodes, whereas sleeping in familiar places or with a light on could reduce it.

VPSG Analysis: Sleep Parameters and Motor Patterns

Our cohort of 45 patients underwent a total of 103 VPSG showing 334 DoA episodes. Of these, 72% appeared in the first third of the night and 80% emerged from slow-wave sleep. In all episodes, we didn't identify any features suggestive of epilepsy, such as asymmetric o dystonic posturing, kicking, cycling and rocking body movements, which are required to make a diagnosis of SHE (19).

Sleep efficiency, light and deep non-rapid eye movement (NREM) and rapid eye movement (REM) sleep stages, and REM latency were normal in all patients (**Table 4**). In the two patients with OSAS the episodes of DoA didn't occur after an obstructive apnea. According to Loddo's classification of motor patterns, 282 episodes (84%) were SAMs (of which 60 SAM-A, 128 SAM-B, and 94 SAM-C), 34 (10%) RAMs and 18 (5%) CAMs (**Figure 1**) (25).

DISCUSSION

Clinical Characteristics of DoA in Adults

In our cohort of patients DoA began at an average of 13 years and persisted at the time of our VPSG (to a maximum of 76 years), supporting the hypothesis that DoA could represent a lifelong sleep disorder, arising in childhood and/or adolescence and persisting in adulthood at a variable level of intensity. The frequency and intensity of the episodes varied greatly during lifetime and among different patients. Quite often DoA showed a fluctuating course, alternating periods of high frequency and intensity of the episodes followed by intervals of months or even years free from episodes. Such fluctuations may partially explain the long delay between episodes' onset and the diagnosis.

The accurate description of DoA episodes provided by the patients or by their witness revealed a considerable degree of variability and complexity that are not usually observed during laboratory VPSGs (26–28). This supports the need for an extensive history taking, both from the patients and from any bed partner, in order to make a correct diagnosis and also to identify and possibly prevent any dangerous behaviors during an episode of DoA.

The ICSD-3 categorizes DoA as confusional arousals, sleep terrors and sleepwalking (1). However, as underlined by our study, the semiological manifestations of DoA in adults comprehend a spectrum of motor patterns of different complexity that often coexist in the same individual. Therefore, it can be difficult to make a definite diagnosis of a DoA subtype in adults. Instead, semiological-based classifications could be more precise in the description of the episodes. Loddo et al. recently proposed a classification of the motor patterns of DoA in adult patients, identifying three categories with increasing complexity: pattern I or SAMs, pattern II or RAMs and pattern III or CAMs (25, 29). The majority of our patients reported to suffer mainly from complex behaviors (sleepwalking and structured and bizarre actions), however during VPSGs the number of minor episodes (SAMs) largely exceeds that of the major episodes (RAMs and CAMs). This can be explained with the following considerations. First, patients probably do not recall the majority of the minor episodes, as they are too short and devoid of mental activity to be remembered after awakening; therefore, SAMs are typically underreported by patients. Secondly, patients usually exhibit more than one motor pattern, either in different periods of the night or in different life periods. It is therefore expected that the majority of VPSG recordings of adults complaining of sleepwalking will document only minor episodes. Also, it is possible that in a consistent number of adults who have a positive past medical history for DoA in childhood, such "minor" episodes are not recognized but actually persist at a subclinical level. This is reasonable as the mechanisms responsible for such conditions and therefore the predisposition to suffer from DoA are life-lasting (see below). Hence, we believe that the proposal of a classification based on motor patterns could be useful in the diagnosis of DoA in adults. For example, in the setting of a patient whose history is suggestive of sleepwalking but without documentation of major episodes, recording of SAMs could corroborate the diagnosis of a DoA. Still, further studies are necessary to validate the usefulness of this model in clinical practice.

Violent behaviors, posing threats to the patient itself or to others, were reported in more than half of our cases. These data confirm that DoA in adults can be a major cause of injuries during sleep, complicating the differential diagnosis with other motor behavioral manifestations during sleep (9). However, the high variability of the episodes' semiology, intensity and frequency over time are typical of DoA and therefore represent a critical diagnostic feature to differentiate DoA from epilepsy. On the other hand, a progressive increase of the episodes' frequency and the presence of motor stereotypy typically suggest Sleep-related Hypermotor Epilepsy (SHE) (20, 21, 30–33).

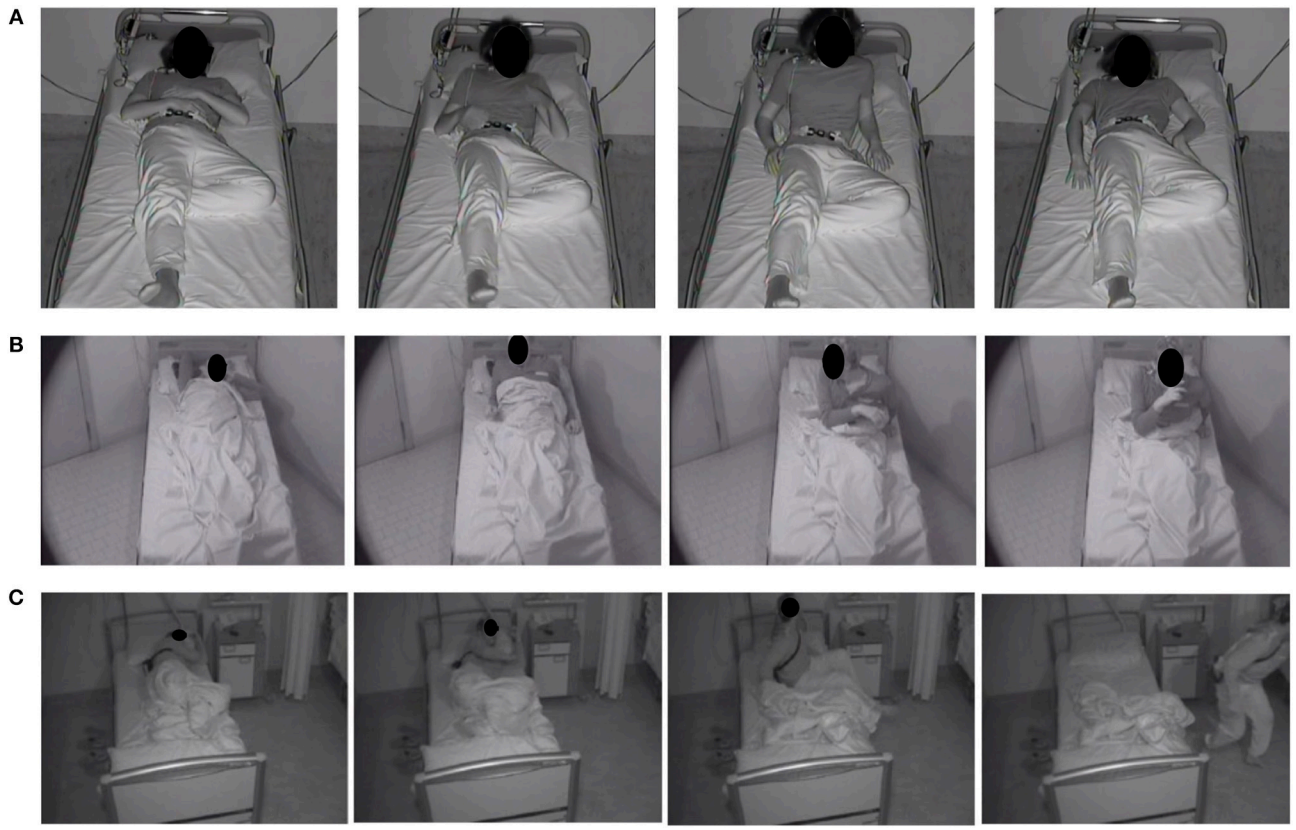

FIGURE 1 | Photographic sequences of the three different motor patterns of DoA in adults. **(A)** Simple Arousal Movement (SAM): this pattern was characterized by head flexion and/or head extension and/or limb movement; **(B)** Rising Arousal Movement (RAM): the core feature of this pattern was a trunk flexion followed by sitting with feet in or out of the bed; **(C)** Complex Arousal with ambulatory Movements (CAM): this pattern was characterized by sitting up, getting out of bed, and walking.

Once awake, the majority of patients reported that they felt confused and disorientated, and more than half of patients could not recollect the episode. Approximately one-third had complete amnesia. This raises fundamental questions about the medico-legal and forensic implications of DoA, given the neurophysiologic and cognitive states that characterize patients during such episodes (34).

When recollected, however, more than half of the patients reported distressing mental contents during the episodes. This is consistent with the view that in adults at least some, if not all, the episodes of DoA may originate from cortical activity and be associated with NREM sleep mentation (see below).

Finally, almost half of the patients complained of non-restorative sleep and daytime tiredness, opening the question if non-refreshing sleep is the only reason explaining the presence of daytime tiredness or if it is an intrinsic characteristic of the disorder itself (35).

Pathophysiology of DoA in Adults

Disorders of arousal result from a NREM sleep-wake state dissociation (36). In the last decades it has progressively become clear that wake and sleep are not mutually exclusive, and admixture of features of the different states can occur (37, 38). During transitions between NREM sleep and wakefulness (as it occurs during arousals) a temporary, pathological dissociation of states can occur across different brain structures, resulting in a state of altered consciousness manifesting as DoA (39).

As depicted by the description of the episodes and specifically the most bizarre ones, patients appear to be simultaneously awake (with retention of their motor and behavioral functions) and asleep (with impairment of cognition, judgment and memory for the events) (40). Indeed, a SPECT study performed during sleepwalking showed a decrease in regional blood flow in the fronto-parietal associative cortices, and an increase in blood flow in the posterior cingulate cortex and in the anterior cerebellum (41). Also, stereo-EEG studies during DoA identified local fast wake-like EEG activity on the motor, cingulate, insular, temporopolar and amygdalar cortices and sleep-like EEG with increased delta activity on the fronto-parietal associative cortices and in the hippocampus (**Figure 2**) (42, 43).

Patients with DoA seem to be predisposed to state dissociation, probably due to abnormal neuronal excitability in different cortical areas (44, 45). A high-density EEG study and a SPECT study showed persistent, localized changes in neuronal excitability of DoA patients: specifically, an increased arousability of motor and limbic areas, in contrast with a reduced arousability of associative cortices (especially frontal) (40, 45–47).

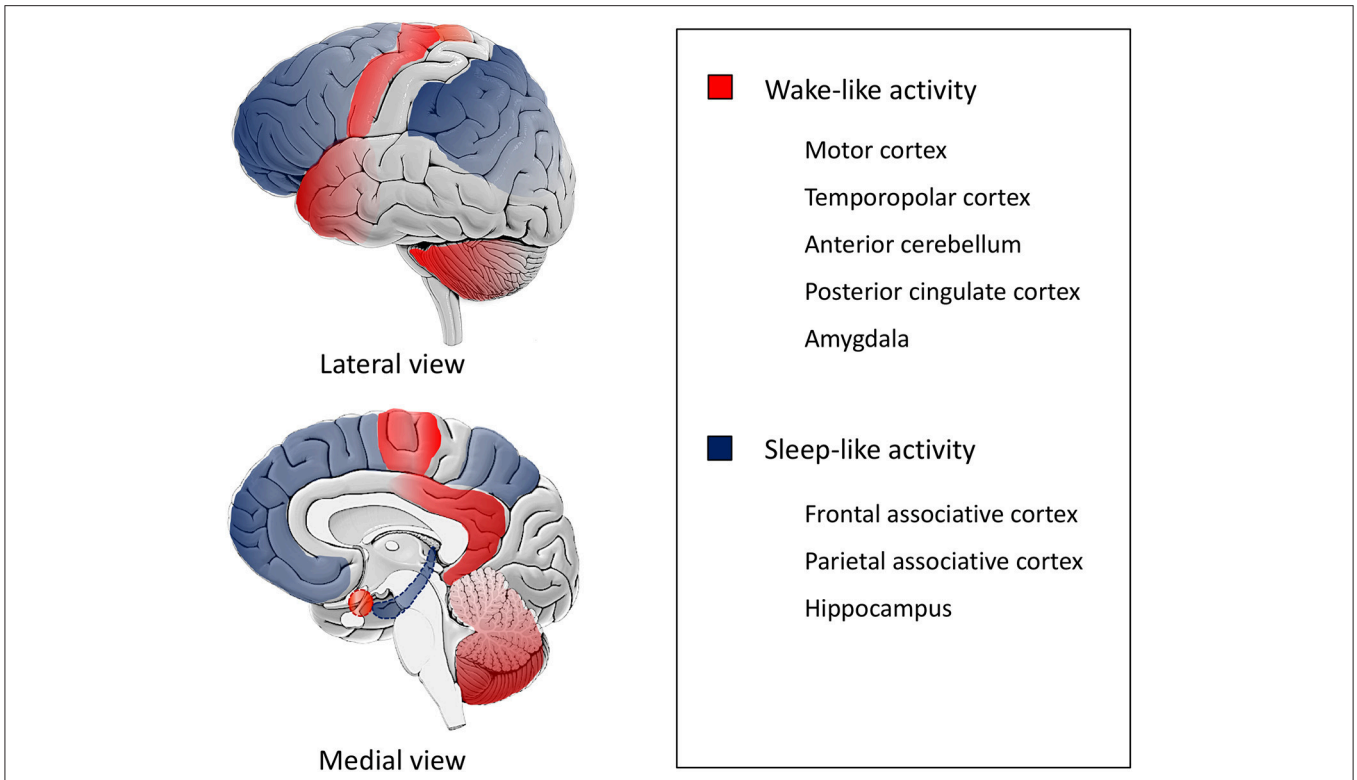

FIGURE 2 | Schematic representation of state dissociation during DoA. The figure is a schematic representation based on data from SPECT and stereo-EEG studies, (41–43) illustrating state dissociation in disorders of arousal (DoA), i.e., co-occurrence of different local activity patterns in the human brain. Motor, temporopolar, anterior cerebellar, posterior cingulate cortices and the amygdala exhibit a wake-like activity (red) while fronto-parietal associative and hippocampal cortices show a sleep-like activity (blue).

The impaired inhibitory control of motor systems and increased motor impulsivity could partly explain the violent behaviors observed in DoA patients (47, 48).

Hence, during arousals from SWS, motor and limbic areas are more easily aroused while associative areas more difficulty transition into wakefulness, especially in conditions of increased SWS pressure.

Therefore, two types of conditions are thought to increase the likelihood of DoA episodes in predisposed individuals. First, all conditions increasing the amount of SWS, such as sleep deprivation, physical or emotional stress, fever and medications affecting sleep increase the likelihood of DoA episodes occurring. Indeed, most of our patients reported that the episodes occurred mainly in the first half of the night, which is consistent with a physiological higher proportion of slow wave sleep (SWS) during this period. Secondly, arousal by whatever mechanism (internal or external) can precipitate a DoA episode (1). The list includes comorbid sleep disorders (particularly Obstructive Sleep Apnea Syndrome, OSAS, and Periodic Limb Movement Disorder, PLMD), bladder distention, physiological ending of a sleep cycle, mental activity and noises (49). In our study, we found a significant relation between stress and DoA episode frequency, as one would expect from the effects of stress on sleep, i.e., SWS reduction (and therefore increase in SWS pressure) and an increase in the number of arousals (50).

Other physiologic phenomena have been proposed to contribute to disorders of arousal (1). Indeed, some behaviors observed in DoA resemble stereotyped, archaic behaviors (such as defensive postures, violent gestures and feeding) that result from the activation of neural circuits (mainly subcortical), namely central pattern generators (CPGs). As these structures in humans are largely under neocortical control, it has been suggested that some manifestations of DoA could result from CPG disinhibition, as a result of prefrontal cortex dysfunction (51, 52). In many cases of adult DoA, however, more complex and learned behaviors, of likely cortical origin, are performed. Interestingly, these can be accompanied by a congruent dream-like mentation (34). Our study confirmed these data, as the majority of patients exhibited quite intricate behaviors (e.g., getting dressed, preparing breakfast, doing their makeup) and were able to recall mental contents (often distressing) related to the episodes. These data suggest a role of NREM sleep dreaming in the genesis of some episodes of DoA, which could then represent "dream-enactment behaviors" (34, 53–55).

In conclusion, DoA occurs when internal or external precipitating factors trigger the episodes in predisposed individuals. The precise cause of the "pathological" state dissociation and cortical abnormal excitability, however, is unknown, but probably results from an interplay of genetic and environmental factors (56). A familial predisposition have been

reported suggesting a genetic basis for DoA (23, 57, 58) and family studies have recognized several potential candidate genes, including the adenosine deaminase gene (59).

CONCLUSION

In this study we described the clinical and pathophysiological characteristics of DoA in adults, underlying their unique characteristics such as violent behaviors and non-restorative sleep. Our results seem to suggest that DoA could be lifelong disorders, whose frequency oscillates over time, often in association with stress.

Our study was performed in the absence of any treatment and included data of patients during different periods of life and reported specific description of DoA episodes. However, there are some limitations to the present study: particularly, the small sample of the cohort and the retrospective self-evaluation of frequency and severity of the episodes, possibly not reflecting the real spectrum of DoA manifestations. The episodes are followed by variable degrees of amnesia, therefore the information may not be entirely accurate. We tried to minimize this risk by requiring a face-to-face interview with a witness as an inclusion criterion. Also, patients attending a sleep center are often not really representative of the general population because they could have more frequent and/or severe episodes. Therefore, prospective and longer follow-up studies are needed for a definitive characterization of clinical course of DoA in adulthood.

We encourage the implementation of the classification of motor patterns of DoA as they could help in the diagnosis of these conditions, since different patterns often coexist in the same patients and episodes of minor intensity are generally underreported by patients.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the International Good Clinical Practice guidelines, ethical committee Area Vasta Emilia Centro (CE-AVEC), with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the CE-AVEC (protocol number 17176, 14/12/2017).

AUTHOR CONTRIBUTIONS

TB: data collection, literature revision and critical revision of the manuscript. GL and ES: data collection, writing of the manuscript. FM: VPSG analysis. FC, SM, LL, FB, and PT: data collection and interpretation of results. FP: data collection, literature revision, and revision of the manuscript.

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The Effect of Anxiety and Depression on Sleep Quality of Individuals With High Risk for Insomnia: A Population-Based Study

Chang-Myung Oh[1], *Ha Yan Kim*[2], *Han Kyu Na*[3], *Kyoo Ho Cho*[3]* *and Min Kyung Chu*[3]

[1] Division of Endocrinology and Metabolism, CHA Bundang Medical Center, School of Medicine CHA University, Seongnam-si, South Korea, [2] Biostatistics Collaboration Unit, Yonsei University College of Medicine, Seoul, South Korea, [3] Department of Neurology, Yonsei University College of Medicine, Seoul, South Korea

Introduction: One of the most common sleep disorders, insomnia is a significant public health concern. Several psychiatric disorders, such as anxiety disorders and depression, have shown strong relationships with insomnia. However, the clinical impact of the combination of these two conditions on insomnia severity and sleep quality remains unknown. We investigated the relationship between sleep disturbance and psychiatric comorbidities in subjects with high risk for insomnia.

Methods: We analyzed data from a nation-wide cross-sectional survey of Korean adults aged 19 ∼ 69 years conducted from November 2011 to January 2012. The survey was performed via face-to-face interviews using a structured questionnaire. We used the insomnia severity index (ISI) to evaluate insomnia and defined respondents with ISI scores of ≥10 were considered to be at high risk for insomnia. To diagnose anxiety and depression, we used the Goldberg anxiety scale (GAS) and Patient Health Questionnaire-9 (PHQ-9), respectively.

Results: Of the 2,762 respondents, 290 (10.5%) were classified as subjects with high risk for insomnia; anxiety [odds ratio (OR), 9.8; 95% confidence interval (CI), 7.3–13.1] and depression (OR, 19.7; 95% CI, 13.1–29.6) were more common in this population than in participants without insomnia. Of the participants with insomnia, 152 (52.4%) had neither anxiety nor depression, 63 (21.7%) only had anxiety, 21 (7.2%) only had depression, and 54 (18.6%) had both anxiety and depression. The group with both anxiety and depression was associated with worse scores on sleep-related scales than the other groups [high ISI, Pittsburgh Sleep Quality Index (PSQI), and Epworth Sleepiness Scale]. The relationship between outcome measures (ISI and PSQI) and psychiatric problems was significant only when anxiety and depression were present. The PSQI has a significant mediation effect on the relationship between psychiatric comorbidities and insomnia severity.

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Maurice M. Ohayon, Stanford University, United States

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Thomas Penzel, Charité Medical University of Berlin, Germany
Sunao Uchida, Sunao Clinic, Japan

***Correspondence:**
Kyoo Ho Cho
zhau@yuhs.ac

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Conclusion: Among the respondents with insomnia, psychiatric comorbidities may have a negative impact on daytime alertness, general sleep quality, and insomnia severity, especially when the two conditions are present at the same time. Clinicians should, therefore, consider psychiatric comorbidities when treating insomnia.

Keywords: insomnia, mood disorder, depression, anxiety, sleep quality

INTRODUCTION

As one of the most common sleep disorders, insomnia has become a significant public health problem. While the prevalence of insomnia varies considerably across countries, its global prevalence of insomnia as defined by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria is estimated to be ∼6–10% (1–3).

Insomnia reportedly increases the rate of car accidents (4), decreases job performance, results in self-medication with alcohol as well as socio-economic problems (5–7), and has been associated with the onset of cardiovascular diseases (8).

Although a previous (second) edition of the International Classification of Sleep Disorders (ICSD-2) distinguished psychophysiological insomnia from mental-illness induced insomnia (9), there are substantial overlap features between the two: e.g., conditioned arousal, poor sleep hygiene, and excessive worry about sleep (10). Also, many subjects with insomnia have psychiatric comorbidities, rendering the discrimination of insomnia subtypes difficult. Because of these issues (overlapping primary and secondary insomnia), in the next version, ICSD-3, insomnia was recategorized according to time course (10). Nevertheless, it is still of great value to recognize the most common psychiatric comorbidities such as anxiety disorders and depression in patients with insomnia (11). Among patients with insomnia, the prevalence of anxiety disorder, including generalized anxiety disorder, panic disorder, post-traumatic stress disorder, and phobia, is 24–36% (11, 12), while that of major depression is 14–31% (11, 12). Conversely, about 90% of patients with depression complain of sleep disturbance (13, 14). Similarly, sleep problems are much more common among individuals with anxiety disorders (15, 16).

Multiple studies have investigated the relationship between sleep disorders and psychiatric comorbidity. A study that used data collected by the national survey in the United States revealed that individuals with any comorbid sleep problem (especially non-restorative sleep) are more prone to impairments of daytime activities (17). Using data obtained by the same survey, another investigation reported that the rate of insomnia complaints was highest among individuals with anxiety and mood disorders (42–63%) (18). However, to date, it remains unclear whether the combination of anxiety and mood (depression) problems aggravate the severity of insomnia itself and the sleep quality of people at high risk for insomnia.

This study explored the relationships among insomnia severity, sleep quality and daytime sleepiness, and comorbidities with anxiety and depression among individuals at high risk for insomnia. In addition, we used mediation modeling to evaluate whether insomnia severity is mediated by changes in general sleep quality.

METHODS

Survey Procedure

We used data from a nationwide, cross-sectional survey of headache and anxiety in the general Korean population conducted from November 2011 to January 2012 (19). Trained interviewers performed structured, face-to-face interviews that included questionnaires regarding sleep and headache disorders and mood problems (anxiety and depression). Adults between the ages of 19 and 69 years were included. Besides, we collected the respondents' demographic and geographic information. The target area, sampling method, and detailed survey procedures were same as the previously documented process (19). The distribution in this study and the total population were not significantly different in sex, age groups, size of a residential area, and educational level.

Diagnosis of Insomnia, Anxiety, and Depression

We used the insomnia severity index (ISI) to define high risk for insomnia. Subjects who received an ISI score of $\geq$10 were classified as high risk for insomnia according to a previous community-based study (20). Using an ISI score of 10 as the cutoff, insomnia was detected with 86.1% sensitivity and 87.7% specificity (20).

For the diagnosis of anxiety and depression, we used the Goldberg anxiety scale (GAS) and Patient Health Questionnaire-9 (PHQ-9), respectively. The GAS consists of four screening and five supplementary questions. The validated Korean version of the GAS features a sensitivity of 82.0% and a specificity of 94.4% (21). At least two positive answers to screening and five or more positive answers to complementary questions in GAS questions indicated anxiety. The PHQ-9 was used to diagnose depression (22). The Korean version of the PHQ-9 features 81% sensitivity and 89.9% specificity (23). Participants with a PHQ-9 score of $\geq$10 were considered to have depression. We classified the respondents into four groups: individuals (1) without anxiety or depression, (2) with anxiety but without depression, (3) without anxiety but with depression, and (4) with both anxiety and depression. The demographic data of the participants are shown in **Table 1**.

Measures Related to Sleep Quality

Each individual was asked to complete questionnaires, including the Pittsburgh Sleep Quality Index (PSQI), which accesses

TABLE 1 | Sociodemographic distribution of all survey participants, the total Korean population, and of cases identified as insomnia, anxiety, and depression.

	Insomnia ($n = 290$)	*P*	Anxiety ($n = 268$)	*P*	Depression, ($n = 116$)	*P*
Gender		<0.01		<0.01		<0.01
Male (1,345)	117 (8.7%)		109 (8.1%)		43 (3.2%)	
Female (1,350)	173 (12.8%)		159 (11.8%)		73 (5.4%)	
Age		0.53		0.71		0.75
19–29 (542)	59 (10.9%)		53 (9.8%)		23 (4.2%)	
30–39 (604)	53 (8.8%)		51 (8.4%)		32 (5.3%)	
40–49 (611)	66 (10.8%)		67 (11.0%)		24 (3.9%)	
50–59 (529)	63 (11.9%)		53 (10.0%)		22 (4.2%)	
60–69 (409)	49 (12.0%)		44 (10.8%)		15 (3.7%)	
Size of residential area		0.95		0.71		0.76
Large city (1,248)	136 (10.9%)		130 (10.4%)		57 (4.6%)	
Medium-to-small city (1,186)	125 (10.5%)		112 (9.4%)		47 (4.0%)	
Rural are (261)	29 (11.1%)		26 (10.0%)		12 (4.6%)	
Educational level*		<0.01		0.03		0.70
Middle school or less (393)	62 (15.8%)		55 (14.0%)		20 (5.1%)	
High school (1,208)	116 (9.6%)		111 (9.2%)		49 (4.1%)	
College or more (1,068)	109 (10.2%)		100 (9.4%)		47 (4.4%)	
Type of job**		0.58		0.57		0.32
Shift work (145)	18 (12.4%)		17 (11.7%)		9 (6.2%)	
Regular work (2,157)	224 (10.4%)		209 (9.7%)		87 (4.0%)	

*Total subjects: 2,695; P, p-value; *non-responder: 26; **non-responder: 393.*

the multifactorial construct of sleep dysfunction with strong reliability and validity (24), and the Epworth Sleepiness Scale (ESS). which assesses each participant's daytime sleepiness (25). The subjective and objective sleep qualities measured with ISI feature a high concordance with daytime disability (26). Even young adults with insomnia frequently complain of daytime sleepiness (27). Additionally, each subject's ISI score was categorized into one of three groups (mild insomnia, $10 \leq \text{ISI} < 15$; moderate, $15 \leq \text{ISI} < 20$; severe, $\text{ISI} > 20$).

Ethics

This study was approved by the institutional review board/ethics committee of the Severance Hospital, and written informed consent was obtained from each participant.

Statistical Analysis

We used the chi-square test to examine whether the number of diagnoses differed according to sex, age, size of a residential area, educational level, and types of work shift. We compared group differences in sleep time and ESS and PSQI scores using the Kruskal-Wallis test followed by Bonferroni's multiple comparison correction. Group differences in ISI severity and insomnia characteristics were compared with Jonckheere's trend test. Univariate and multivariate logistic regression analysis was performed to evaluate the odds ratio (OR) of anxiety and depression in individuals with high risk for insomnia compared to those without high risk for insomnia. The same procedure was used to compare the PSQI and ISI scores stratified by the presence of depression or anxiety between groups; sex, age group, size of residential area, and education level were included in the multivariate analysis. To examine whether the association between anxiety and depression comorbidities and insomnia risk is mediated by sleep quality, we performed a mediation analysis based on a previously developed method (28). In all the statistical analyses, two-tailed $p < 0.05$ were considered statistically significant.

RESULTS

Demographic Data of the Subjects

Of the total 7,430 interviewees, 3,114 completed the survey (acceptance rate of 41.9%), and 352 subjects suspended the interview. The final sample that completed the survey and the interview included 2,762 individuals (19). The enrollment flow chart of this study is presented in **Figure 1**. The prevalence of insomnia, anxiety, and depression according to sex, age distribution, size of the residential area, and education level is depicted in **Table 1**. Insomnia, depression, and anxiety were significantly more prevalent among women. Participants with the lowest educational levels were associated with a higher prevalence of insomnia and anxiety than were those who had completed high school, college, or graduate school.

Prevalence of Insomnia, Anxiety, and Depression and Mean Scores on Sleep-Related Scales

Of the 2,762 participants, 290 (10.5%) were classified as having a high risk for insomnia. The frequencies of insomnia, anxiety, and depression are presented in **Table 1** according to sex, age group, size of the residential area, and education level. The mean ISI, ESS, and PSQI scores of individuals with high risk for insomnia were 14.37 ± 4.39, 7.73 ± 4.87, and 7.42 ± 2.59, respectively. Of the 290 subjects with high risk for insomnia, 152 (52.4%) had neither anxiety nor depression (Group 1), 63 (21.7 %) had anxiety only (Group 2), 21 (7.2%) had depression only (Group 3), and 54 (18.6%) had both anxiety and depression (Group 4). Anxiety was more common among individuals who had a high risk for insomnia than among those who did not [40.3% vs. 6.1%; OR, 10.1; 95% confidence interval (CI), 7.6–13.4]; this finding remained consistent after adjustment for demographic variables (OR, 9.8; 95% CI, 7.3–13.1). Depression was also more common among individuals who had a high risk for insomnia than those who did not (25.9% vs. 1.7%; OR, 20.1; 95% CI, 13.4–30.2); this finding also remained consistent after adjustment for demographic variables (OR, 19.7l 95% CI, 13.1–29.6) (**Table 2**).

Difference in Sleep-Related Scales Among the Four Groups

Participants at high risk for insomnia with both anxiety and depression were associated with significantly higher ESS and PSQI scores relative to the anxiety-only group (**Figure 2**).

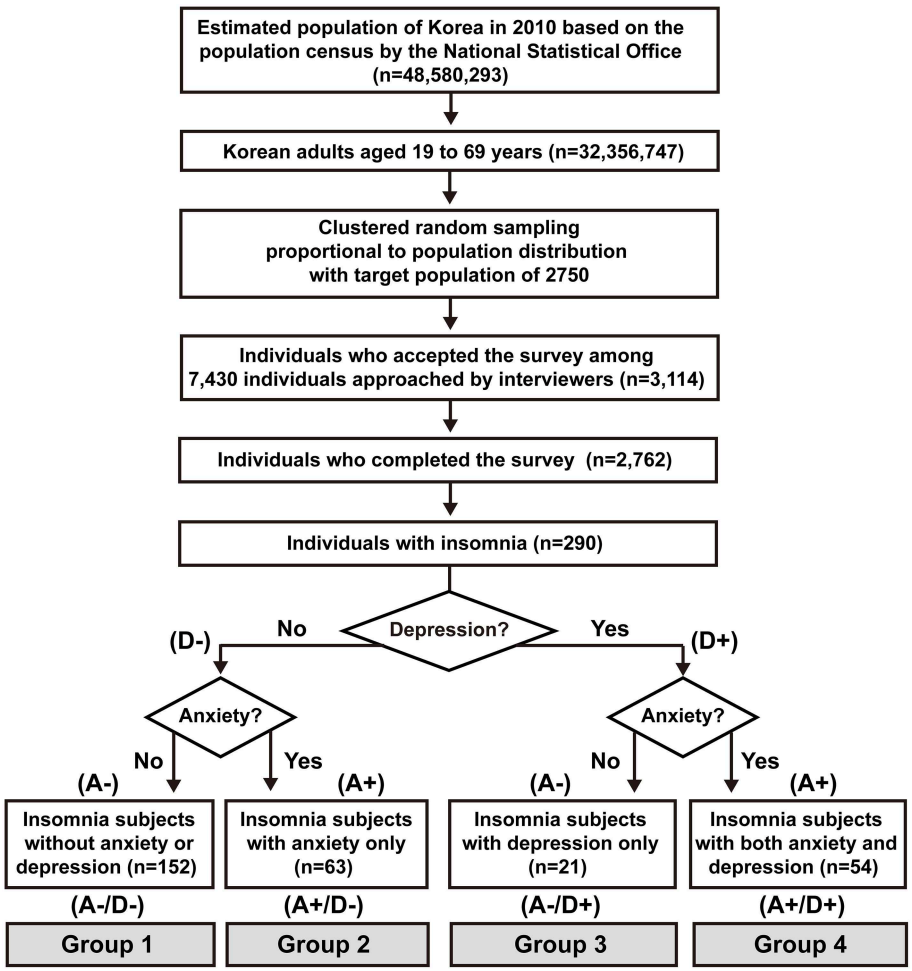

FIGURE 1 | Flow chart of participants in the Korean Headache-Sleep study. Whether individuals had anxiety (A) or depression (D) are denoted by positive (+) and negative (–). A–/D–, without anxiety or depression; A+/D–, with anxiety only; A–/D+, with depression only; A+/D+, with both anxiety and depression.

Meanwhile, individuals with no psychiatric comorbidities were associated with lower PSQI scores relative to the three other groups. ISI scores were significantly higher among participants with both anxiety and depression than those with any psychiatric comorbidities. When stratified by the presence of anxiety and depression, severe ISI tended to increase from Groups 1–4 (**Figure 3**).

Insomnia Characteristics According to the Presence of Anxiety or Depression

No statistical differences were observed among the 4 groups in average sleep time during workdays, weekends, and overall sleep. The three insomnia-related symptom scales of each group are presented in **Figure 3**. Regarding the difficulty in initiating sleep and feelings of impaired quality of life, only Groups 1 and 4 featured a statistically significant difference. Group 4 was more likely to have difficulties in maintaining sleep than were Groups 1 and 2. The groups did not differ in their scores on the frequent awakening scale.

Mediation Model Analysis

When insomnia was comorbid with both anxiety and depression, PSQI and ISI were significantly correlated with each other (**Tables 3**, **4**). The relationship between anxiety and ISI (beta = 4.21) was attenuated when PSQI was used as a mediator (**Figure 4**). The Sobel test revealed the significance of the indirect effect of PSQI in mediating both anxiety and depression and ISI (**Table 5**).

DISCUSSION

We found the prevalence of high risk for insomnia in the Korean adult population to be 10.5%, which is significantly different from previously reported rates in the Korean population (about 20%). Differences between the methodology of our study and those of previous investigations, which used telephone-based interviews without validated questionnaires, may account for the divergent findings (29, 30). Measuring residential noise levels in addition to employing a structured questionnaire, a population-based study

TABLE 2 | Logistic regression analysis.

	Insomnia, $n = 290$	Non-insomnia, $n = 2,472$	Univariate analysis		Multivariate analysis[¶]	
			OR	CI	OR	CI
Anxiety	117 (40.3%)	151 (6.1%)	10.1	7.6–13.4	9.8	7.3–13.1
Depression	75 (25.9%)	41 (1.7%)	20.1	13.4–30.2	19.71	13.1–29.6

Insomnia vs. non-insomnia population. OR, odds ratio; CI, 95% confidence interval; [¶]adjusted for age, gender, education level, and size of residential area.

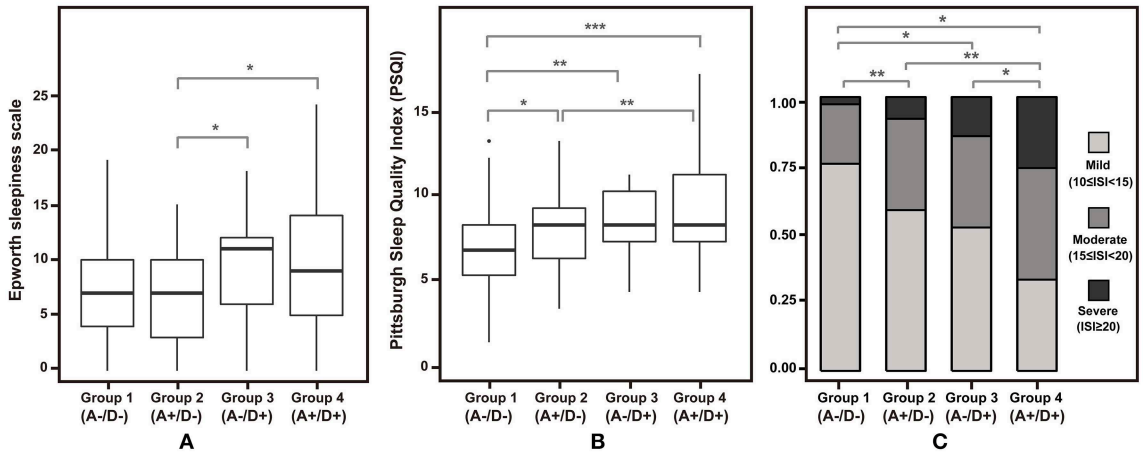

FIGURE 2 | Differences in scores on sleep-related scale across four groups. **(A)** Epworth sleepiness scale and **(B)** Pittsburg sleep quality index is depicted in box plots. **(C)** Proportion of each insomnia severity index category is shown in a stacked bar plot. A–/D–, without anxiety or depression; A+/D–, with anxiety only; A–/D+, with depression only; A+/D+, with both anxiety and depression; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

conducted in Japan, reported a crude prevalence of insomnia of 8.8% (31), which better accords with our results. The prevalence of overall anxiety and depression observed in our study was also similar to or slightly higher than the rates reported by prior Korean epidemiologic studies (32, 33). Similar to a previous study (34), we found that the prevalence of insomnia to be higher among women and individuals with low levels of education. We also found no association between age and the prevalence of insomnia. This discrepancy may be due to the changes in the lifestyles of young Korean adults, which might cause higher rates of insomnia among adults below the age of 30 (35).

We observed that 47.6% of individuals with high risks for insomnia had comorbidities of anxiety or depression. This finding in the Korean population was similar to a previously reported rate: 40% of individuals with insomnia had comorbid psychiatric disorders (11), among which depression and anxiety disorders were reportedly the most common.

As expected, Group 1 had lower PSQI and ISI scores than those with anxiety-only, depression-only, or both (Groups 2, 3, and 4). The ESS scores of subjects with anxiety-only or depression-only (Groups 2 and 3), but not both (Group 4), did not differ significantly from those of individuals without any anxiety or depression (Group 1) as depicted in **Figure 1**. This might be due to individuals with insomnia having excessive hyperarousal continuing throughout the day time rather than daytime sleepiness. Group 4 was consistently associated with poorer sleep-related indices than was Group 2. While Groups 3 and 4 differed significantly in the proportion of participants who had higher ISI scores (**Figure 3C**), they did not differ significantly in ESS and PSQI scores. This result might be due to a lack of statistical power associated with sample sizes (Group 3 was relatively small).

Individuals with high risk for insomnia are associated with an increased incidence of anxiety and depression relative to those without insomnia. After controlling for possible confounding variables, subjects with insomnia were found to be 9.8 times more likely to have anxiety than subjects without insomnia and 19.7 times more likely to have depression. In addition, we found Groups 2, 3, and 4, in order, had significantly higher rates of insomnia symptoms.

Numerous studies have reported an association between insomnia and depression and anxiety (36–40). Indeed, patients with persistent insomnia are reportedly predisposed to developing psychiatric illness and are more prone to the recurrence of depression (41, 42). Among patients with partially treated depression, residual symptoms such as anxiety and insomnia are among the most powerful predictors for relapse (41). Additionally, insomnia and anxiety share a pathogenetic mechanism: hyperarousal caused by dysregulation of neurotransmitter systems including cholinergic and GABA (gamma-aminobutyric acid) eric mechanisms (43). Hyperarousal and insufficient sleep disrupt the function of corticolimbic circuitry, which leads to impaired affective reactivity and regulation (44). Genetic studies also showed a strong overlap

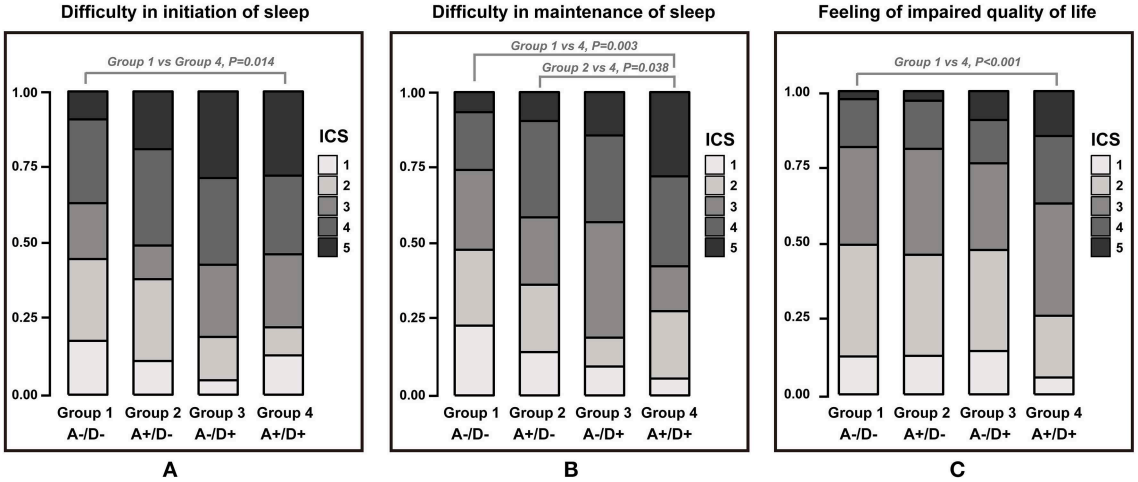

FIGURE 3 | Stacked bar plot showing the proportion of each insomnia characteristic scale 1–5 across groups. A–/D–, without anxiety or depression; A+/D–, with anxiety only; A–/D+, with depression only; A+/D+, with both anxiety and depression. Insomnia characteristic scale (ICS) was graded on a scale of 1–5.

TABLE 3 | Mediation analysis.

Variables		Beta	*SE*	*P*
Outcome	**Predictors**			
ISI	PSQI	0.50	0.08	<0.0001
ISI	Both anxiety and depression	0.21	0.56	<0.0001
PSQI	Both anxiety and depression	0.33	0.37	<0.0001
Indirect effect	0.17	0.36	<0.0001	
Total effect	0.37	0.61	<0.0001	

ISI, insomnia severity index; PSQI, Pittsburgh Sleep Quality Index; SE, standard error; P, p-value.

TABLE 4 | Linear regression analysis, endpoint: ISI.

Univariate analysis	**Beta**	***SE***	***P***	**Multivariate analysis¶**	**Beta**	***SE***	***P***
Anxiety only	0.2	0.6	0.70	Anxiety only	0.2	0.6	0.75
Depression only	0.8	1.0	0.40	Depression only	1.0	1.0	0.30
only one of anxiety or depression	0.5	0.6	0.41	Only one of anxiety or depression	0.5	0.6	0.38
Anxiety + Depression	4.2	0.6	<0.0001	Anxiety + Depression	4.1	0.6	<0.0001

ISI, insomnia severity index; ¶adjusted for age, gender, education level, and size of residential area; SE, standard error; P, p-value.

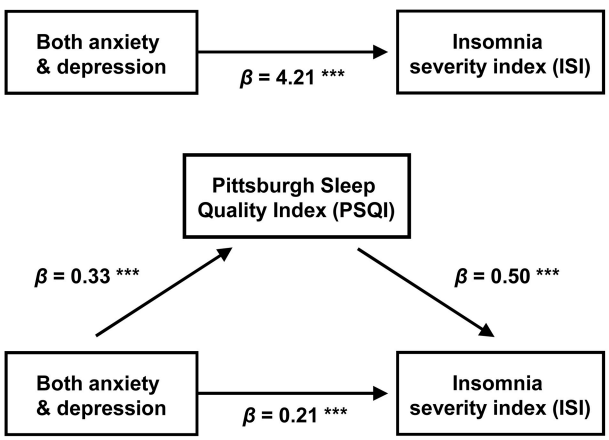

FIGURE 4 | Mediation model: the indirect effect of combined anxiety and depression on ISI scores through PSQI. Unstandardized regression coefficients are presented for each path ***$p < 0.001$.

between genetic influences on insomnia, depression, and anxiety (44). This is reflected in high comorbidity of the two conditions (70–90% of patients with anxiety report insomnia) (45).

The mediational model revealed that the effect of combined anxiety and depression on insomnia severity is mediated by poor sleep behaviors. Considering that the essential feature of insomnia is conditioned arousal affected by dysfunctional efforts to sleep and negative expectations, patients with insomnia become anxious and frustrated as insomnia symptoms persist. The mediator PSQI covers general sleep problems including sleep latency; sleep duration and efficiency; and sleep-disturbing factors, such as nocturia, nocturnal breathing problems, pain, feeling too hot or cold, use of sleeping pills, and daytime dysfunction. Even in the absence of direct insomnia-related factors, nearly all of these components are associated with somatic symptoms of depression (46), and more than half of patients with anxiety disorders report those somatic complaints (47). These observations are in agreement with the findings of our mediation analysis: depression and anxiety have indirect relationships with the degree of insomnia severity and poor sleep-related somatic symptoms, which in turn aggravates insomnia severity.

There was a systemic review on the relationships among anxiety, depression, and sleep disturbance. They showed

TABLE 5 | Linear regression analysis, endpoint: PSQI.

Univariate analysis	Beta	*SE*	*P*	Multivariate analysis¶	Beta	*SE*	*P*
Anxiety only	0.1	0.4	0.85	Anxiety only	0.1	0.4	0.86
Depression only	0.9	0.6	0.11	Depression only	1.0	0.6	0.10
Only one of anxiety or depression	0.4	0.3	0.28	Only one of anxiety or depression	0.4	0.3	0.27
Anxiety + Depression	4.1	0.3	<0.0001	Anxiety + Depression	2.2	0.4	<0.0001

PSQI, Pittsburgh Sleep Quality Index; ¶adjusted for age, gender, education level, and size of residential area; SE, standard error, P, p-value.

that insomnia and sleep quality features have "bidirectional" relationships with anxiety and depression, respectively (48). Moreover, regarding treatment, cognitive behavioral therapy that focuses on attenuating anxiety and depression reduces insomnia severity and the symptoms of the two psychiatric conditions (49).

Our study is subject to several limitations. First, we did not consider comorbidities of anxiety and depression with other sleep disorders, including obstructive sleep apnea, narcolepsy, and restless legs syndrome. The presence of any pain or other significant medical illnesses was also not investigated. Second, even though we performed a population-based study with a low sampling error, the statistical power for the examination of subgroups might have been diminished due to the small samples (especially Group 3). Third, in the mediation analysis, we did not conduct a detailed investigation to elucidate the component of PSQI that features a close relationship with insomnia severity. Third, we did not analyze longitudinal data which causes a problem to establish causality, despite a quite large sample. Last, we didn't consider the impact of medication use when we analyzed our data. The medication status is an important factor that can change the subjects' mental status. Unfortunately, our research target was the general population. So, it was not easy to obtain optimal medical information from the survey method.

This report is, to the best of our knowledge, the first to evaluate how anxiety and depression affect sleep quality and the severity of insomnia. The prevalence of high risk for insomnia and the comorbidities with anxiety and depression is comparable to the findings of previous reports. Daytime sleepiness, general sleep quality, and insomnia severity were consistently poorer in subjects with both depression and anxiety. Also, we found that the effect of the combination of both psychiatric conditions was mediated by general sleep quality indices, which encompass insomnia and related somatic symptoms. We surmise that anxiety and depression affect insomnia in a supra-additive manner. When treating insomnia patients, clinicians should look for underlying comorbid psychiatric conditions to determine the appropriate therapy and enhance the therapeutic effect.

DATA AVAILABILITY

The datasets analyzed in this manuscript are not publicly available. Requests to access the datasets should be directed to chumk@yuhs.ac.

AUTHOR CONTRIBUTIONS

C-MO, KC, and MC: study concept and design. C-MO, HK, KC, and MC: acquisition, analysis, and interpretation of data. C-MO and KC: drafting of the manuscript. HK, KC, and HN: statistical analysis. C-MO: obtained funding. KC and MC: study supervision. All authors read and approved the manuscript.

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Respiratory Mandibular Movement Signals Reliably Identify Obstructive Hypopnea Events During Sleep

Jean-Benoit Martinot[1,2], Nhat-Nam Le-Dong[3], Valerie Cuthbert[3], Stephane Denison[3], Jean C. Borel[4], David Gozal[5] and Jean L. Pépin[4]*

[1] Sleep Laboratory, CHU UCL Namur Site Sainte-Elisabeth, Namur, Belgium, [2] Institute of Experimental and Clinical Research, UCL Bruxelles Woluwe, Brussels, Belgium, [3] Research and Development Center, Respisom, Erpent, Belgium, [4] HP2 INSERM U1042, Université Grenoble Alpes, Grenoble, France, [5] Department of Child Health and Child Health Research Institute, University of Missouri, Columbia, MO, United States

Context: Accurate discrimination between obstructive and central hypopneas requires quantitative assessments of respiratory effort by esophageal pressure (OeP) measurements, which preclude widespread implementation in sleep medicine practice. Mandibular Movement (MM) signals are closely associated with diaphragmatic effort during sleep.

Objective: We aimed at reliably detecting obstructive off central hypopneas events using MM statistical characteristics.

Methods: A bio-signal learning approach was implemented whereby raw MM fragments corresponding to normal breathing (NPB; $n = 501$), central ($n = 263$), and obstructive hypopneas ($n = 1861$) were collected from 28 consecutive patients (mean age = 54 years, mean AHI = 34.7 n/h) undergoing in-lab polysomnography (PSG) coupled with a MM magnetometer, and OeP recordings. Twenty three input features were extracted from raw data fragments to explore distinctive changes in MM signals. A Random Forest model was built upon those input features to classify the central and obstructive hypopnea events. External validation and interpretive analysis were performed to evaluate the model's performance and the contribution of each feature to the model's output.

Results: Obstructive hypopneas were characterized by a longer duration (21.9 vs. 17.8 s, $p < 10^{-6}$), more extreme low values ($p < 10^{-6}$), a more negative trend reflecting mouth opening amplitude, wider variation, and the asymmetrical distribution of MM amplitude. External validation showed a reliable performance of the MM features-based classification rule (Kappa coefficient = 0.879 and a balanced accuracy of 0.872). The interpretive analysis revealed that event duration, lower percentiles, central tendency, and the trend of MM amplitude were the most important determinants of events.

Conclusions: MM signals can be used as surrogate markers of OeP to differentiate obstructive from central hypopneas during sleep.

Keywords: sleep apnea syndrome, hypopnea, respiratory effort, mandibular movements, obstructive hypopnea, central hypopnea

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****Correspondence:***
Jean-Benoit Martinot
martinot.j@respisom.be

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INTRODUCTION

Hypopnea is the most frequent respiratory event reported during sleep (1). AASM rules recommend, as an option, to sub-divide hypopnea as either obstructive or central, depending on the underlying respiratory effort (RE) (2), reflecting increases, or decreases in the central respiratory command, respectively. The gold standard marker of RE during sleep is the amplitude of the esophageal pressure (OeP) curve, a surrogate of the diaphragmatic muscular contraction in the presence of increased flow resistance within the airways (3). Alternative non-invasive technique for assessing RE such as thoraco-abdominal inductance plethysmography, that detects phase angle differences or inspiratory flow limitation assessed by nasal pressure recordings or snoring loudness remain to be validated against OeP for routine hypopnea characterization (2, 3). Correct characterization of the hypopnea sub-type provides information about its origin and contributes to the therapeutic personalized decision-making process (4).

We have recently shown that analysis of respiratory mandibular movements (MM) during sleep reproducibly and reliably identifies RE in patients being evaluated for suspected obstructive Sleep Apnea Syndrome (OSAS). The amplitude of MM mid-sagittal and vertical displacements at the breathing frequency change across different types of scored events similarly to the amplitudes of the EMG activity of the crural diaphragm (5). These findings strongly suggest that MM amplitudes reflect the intensity of RE (6), and also that hypopneas characterized by different levels of RE reflecting more or less recruitment of the central ventilatory command can be potentially identified and, as such, serve as a reliable marker of OeP, a technique that is seldom, if ever, implemented in clinical sleep studies (5).

In this study, we aimed to identify in patients being evaluated for suspected OSAS, whether hypopneas scored as either obstructive or central, based on the OeP measurement and strictly following 2012 AASM rules, would be predictably identified by MM analyses. To this effect, MMs were recorded as time series data and, in the context of the large amount of raw data acquired, we searched for surrogate features of specific patterns to make quantitative comparisons between the scored hypopneas.

MATERIALS AND METHODS

Study Subjects

Thirty-six consecutive adult patients referred for suspected OSAS in a single sleep center (CHU UCL Namur, Saint Elisabeth site, Namur, Belgium) were invited to participate. All participants had symptoms suggestive of underlying OSAS. The study was approved by the local human ethics committee (IRB 00004890-number B707201523388), and all participants provided a written informed consent.

Abbreviations: AASM, American Academy of Sleep Medicine; EMG, Electromyography; MM, Mandibular movement; NPB, Normal Period of Breathing; OeP, Esophageal Pressure; OSAS, Obstructive Sleep Apnea Syndrome; PSG, Polysomnography; RE, Sleep Respiratory Effort; SpO2, Pulsed O2 Saturation.

Study Design

This was a prospective cross-sectional study performed during a single night PSG.

Measurements and Data Acquisition

Polysomnography

A commercial digital acquisition system (Somnoscreen Plus, Somnomedics, Randersacken, Germany) was used for recording in laboratory PSG. The parameters monitored included EEG (Fz-A+, Cz-A+, Pz-A+), right and left electro-occulogram, submental EMG, tibial EMG, chest and abdominal wall motion by respiratory inductance plethysmography (SleepSense S.L.P.Inc, St. Charles, IL, USA), nasal and oral flows, respectively with a pressure transducer and a thermistor, and O2 saturation by digital oximeter displaying pulse wave form SpO2 (Nonin, Nonin Medical, Plymouth, MN, USA). Following instillation of local anesthetic, a 2.5 mm external diameter soft silicone covered catheter (Gaeltec Ltd, Dunvegan, Isle of Skye, Scotland, UK) was inserted through the nares into the esophagus.

The catheter is mounted with 1 pressure transducer, which is a thin film resistive strain gauge sensors. Proper positioning of the catheter was verified by visual inspection of the signal itself. The catheter was secured with tape to the patient's nose, lip, and cheek. The transducer was calibrated relative to atmospheric pressure (zero) before each recording.

The catheter was connected to a miniature computer recorder (digitraper) that is placed on the bedside locker overnight (7).

Mandibular Movements (MM)

MM were assessed with a midsagittal mandibular movement magnetic sensor (Brizzy® Nomics, Liege, Belgium) which measures the distance between two parallel, coupled, resonant circuits placed on the forehead, and on the chin. It was used to record mandibular movements (8). The transmitter generates a pulsed magnetic wave of low energy. The change in the magnetic field recorded at the receiver is inversely related to the cube of the distance between the chin and forehead probes. The distance between the two probes is measured in mm with a resolution of 0.1 mm. Basically, this signal provides the instantaneous position of the mandible (**e-Figure 1**).

Polysomnography Scoring

PSG scoring (sleep stages and respiratory events) was performed by two trained technicians who were blinded to the study aims and an in strict accordance with the American Academy of Sleep Medicine rules (2).

Analysis was restricted to 28 of the 36 originally recruited patients who spent a minimum of 4 h sleeping along with good quality signals on all recorded channels including the OeP. Normal breathing periods (NPB), central, and obstructive hypopnea events were scored.

Scoring of the conventional polysomnography supplemented with Esophageal pressure measurement showed a strong agreement between the two readers: ICC (2.1) = 0.927 (95%CI: 0.901–0.962; $p < 0.001$).

A hypopnea was defined as a reduction in nasal pressure signal (flow) of $>$ 10 s, ended by an arousal or a decrease in SpO2 of

at least 3% relative to baseline. Hypopneas were characterized in obstructive vs. central events depending on the presence or absence of RE during at least one respiratory cycle. This was assessed by changes in OeP swings accompanied by at least one other PSG signal reporting RE (namely, flow pressure limitation, respiratory belt asynchrony, or snoring). OeP swings consisted in progressively more negative amplitudes terminated by a sudden increase to a less negative level.

A central hypopnea was identified if there was a clear reduction in OeP swings from baseline along all the episode time (3). The hypopneas combining periods of no RE or decreasing RE and then at least one respiratory cycle with marked increasing RE (mixed hypopneas) were scored as obstructive (2). An example of fragment is shown in **e-Figure 1** after unblinding.

Nevertheless, to optimize the validity of labeling, only the labels which represent a perfect agreement between 2 scorers have been included for main analysis. Ambiguous fragments were excluded from training data.

Data Processing Analysis

The analysis plan is summarized in the **Figure 1**. Feature extraction, data processing and descriptive were done in R statistical programming language (8), while Machine learning experiments were conducted using sci-kit learn and SHAP packages in Python language.

(1) After PSG scoring, individual raw data were acquired from 28 patients. Each dataset contained OeP and MM signals (synchronized at 10 Hz frequency and processed with noise reduction). From this database, 2625 fragments including raw MM signal during normal breathing periods (NPB; $n = 501$), obstructive hypopneas ($n = 1{,}861$), or central hypopneas ($n = 263$) were analyzed.
(2) A customized algorithm (**e-Table 1**) was applied to extract 23 features from MM raw signal of each event (**Figure 2**) or each 10 s of NPB (9). Those features included: the central tendency (mean, median and mode) of MM amplitudes; MM distribution (raw or enveloped signals): skewness, Kurtosis, IQR, 25th, 75th, and 90th centiles; extreme values: Min, Max, 5th and 95th centiles of MM amplitudes; the tendency of variation: linear trend and coefficients of Tensor product-based spline factors (S1, 2, 3, 4) from a generalized additive model to evaluate MM in function of Time; and the duration of each event.
(3) The extracted features and corresponding target labels were integrated to a tabular dataset.
(4) Exploratory data visualization, one-way ANOVA and pairwise student-t tests with Bonferroni correction were performed to compare 23 MM features among 3 groups: normal breathing, obstructive and central hypopneas. Significance level was set at highly stringent criteria ($p = 0.001$) (10) for null-hypothesis testing.
(5) Model development: The data were randomly split into 2 subsets: a larger set (70%) for model development and a smaller set (30%) for model validation. Because the original training set was unbalanced between central (minority class) and obstructive hypopneas (majority class), a synthetic minority over-sampling technique (SMOTE) on the trainset before model development was applied (11).

A multiclass classification rule was built to classify the 3 groups using 23 input features. This consisted of a Random Forest algorithm that combined 500 distinct decision trees (each one was constructed on a random subset of 5 features).
(6) Model interpretation: The content of the Random Forest model was analyzed in order to evaluate the importance of each feature and the possible coalition that contributed to the classification (potential combinations among them to differentiate obstructive from central hypopnea). To evaluate the contribution of each features to the prediction, the Lundberg's Shapley additive explanation (SHAP) method was adopted (12). The theory of this method is explained in the Online Supplement (13, 14).

RESULTS

Characteristics of the Studied Population and Scoring Performance

Thirty-six OSA patients were recruited and 28 had at least 4 h of tracings without artifacts. The characteristics of the group are presented in **Table 1**.

Scoring of the conventional polysomnography including esophageal pressure measurement showed a strong agreement between the two readers: ICC (2.1) = 0.927 (95%CI: 0.901–0.962; $p < 0.001$).

Exploratory Analysis of MM Signal Features

MM signal characteristics were evaluated during obstructive hypopneas ($n = 1{,}861$), in comparison with NPB ($n = 501$) and central hypopneas ($n = 263$). The first features group measured the lower and upper extremities of MM amplitudes. As presented in the **Figure 3A**, there was a clear contrast between the two types of hypopnea in terms of extremity levels of MM amplitude. All features, including minimum, maximum and 4 centiles (5th, 25th, 75th, and 95th) were 2 to 4 times larger during obstructive hypopnea compared to central hypopnea events (all differences were significant at p value thresholds below 10^{-6}).

The second feature set describes the central tendency (mean, median, mode), the dispersion (interquartile range) and the distribution shape (skewness and kurtosis) of MM signals (**Figure 3B**).

NPB were characterized by a steady and symmetrical MM pattern with the amplitude centralized at zero. By contrast, there was a high variability in distribution of MM values during hypopnea events, that could be symmetrical, right or left skewed, leptokurtic, or platykurtic. The differences were consistent for all 3 centrality parameters, suggesting that obstructive hypopneas had a significantly higher MM amplitude compared with central hypopnea events ($p < 10^{-6}$). No significant difference was found in terms of skewness and kurtosis.

The third feature set allows to capture the duration (in s), the linear and the curvilinear trends of MM in function of time (**Figure 3C**). Compared to the central events, the obstructive events had a significantly longer duration (21.9 vs. 17.8 s,

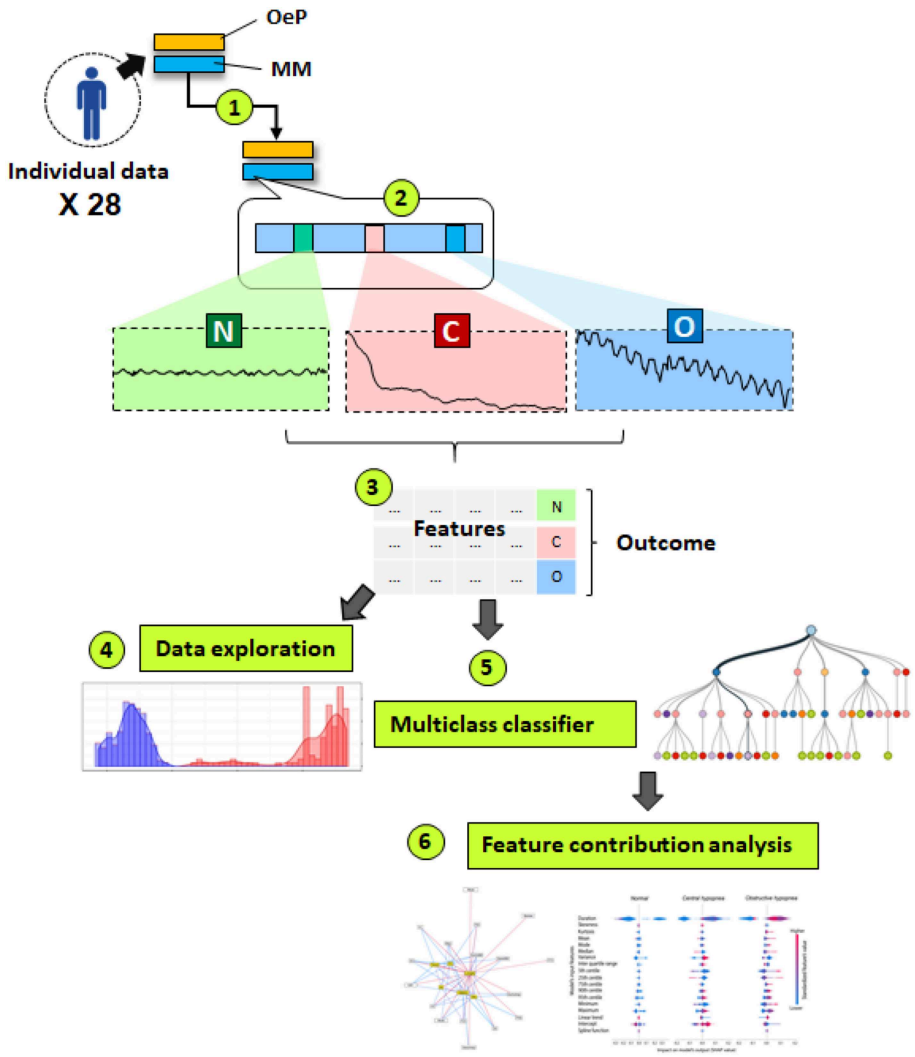

FIGURE 1 | This schematic diagram summarizes a 6 steps data analysis plan. (1) Acquisition and preprocessing of individual data in 28 patients. OeP, Esophageal pressure signal; MM, Mandibular movement signal; (2) Manual label scoring based on OeP signal: N, Normal breathing ($n = 501$), O= Obstructive hypopneas ($n = 21861$) and C, Central hypopneas ($n = 263$); (3) Feature extraction and data compilation; (4) Exploratory data analysis; (5) Developing a classification rule based on Random Forest algorithm; (6) Model explanation, to determine the role of each contributor and their interactions to identify the 3 target events.

$p < 10^{-6}$). During NPB, the MM trend was null, confirming that MM signal was in a steady state. The linear and curvilinear trend of MM time series became negative during central and obstructive hypopneas, though no significant differences emerged between these two groups.

MM Based Classification Rule to Differentiate Central and Obstructive Hypopneas

Model's Performance by External Validation

The optimized Random Forest model implied randomly 5 features for each one in 500 different decision trees. When validated on unseen data ($n = 788$ events), the model showed a good performance to classify the 3 classes (normal breathing, central and obstructive hypopneas), with a balanced accuracy of 0.876 and a high agreement with the manual blind scoring based on esophageal signal (Cohen's Kappa coefficient = 0.879). A confusion matrix of the model validation is provided in the Online Supplement (**e-Figure 2**).

Model Interpretation

The interpretation consisted in two steps: (1) Understanding the model structure and (2) Evaluating the contribution of the features to the model's output (**e-Figure 3**).

The model's complex structure is described through a network by examining all possible interconnection among 23 features across all decision trees (**Figure 4**). The network showed that all 23 features contributed to the model's structure, although some features may be more important than others. The event duration played a central role, it was present in all decision rules and collaborated with every other feature. The 5th centile, linear trend and minimum values also had an important role, as these features

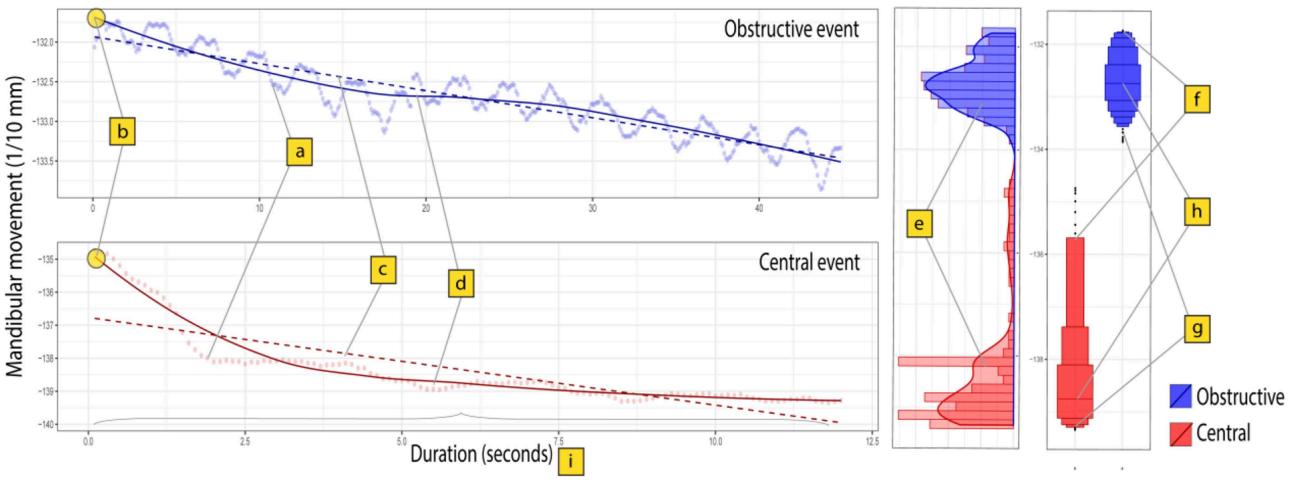

FIGURE 2 | Surrogate features to describe the characteristics of MM signal during central and obstructive hypopnea events. Mandibular movement signal was recorded as a time series (sampling rate = 10 Hz). The figure presents two distinct series: a 12.5 s central hypopnea event (125 points, in red) and an obstructive hypopnea event (45 s or 450 points, in blue). Each point (a) indicates a single value in the series. The slope of a linear model (c) allows to describe the linear trend of MM signal. A generalized additive model (b,d) with polynomial smoothing spline function was fitted for estimating the time dependent variability of MM signal in 2 series, then the intercept (b) and 4 coefficients of spline function (denoted as S1, S2, S3, and S4) were extracted from the model as surrogate features to characterize the complex trajectories of MM in time. The distribution shape parameters (e) (skewness, Kurtosis, Variance) were estimated to describe the shape of MM signal distribution in each series. Other parameters aimed to describe the centrality (h), including mean, mode and median), lower extremities (g, including the minimum, 5th and 25th centiles) and upper extremities (f, including maximum, 95th and 75th centiles) of MM amplitude. Finally, the event duration (i, measured in second) was also included as characteristic feature.

TABLE 1 | Characteristics of the OSA patients ($n = 28$).

Parameter	Median	IQR	95%CI
Age (year)	54.0	19.5	29.5 – 77.0
BMI (kg/m^2)	29.6	11.3	22.2 – 51.8
TST (min)	381.0	93.1	242.8 – 522.5
AHI (n/h)	34.7	32.9	2.8 – 95.8
Arl (n/h)	30.9	23.1	13.9 – 86.1

BMI, Body mass index; TST, Total sleep time; AHI, Apnea/Hypopnea index; Arl, Cortical Arousal index.

were linked to many other features in more than 250 rules. The variance of MM amplitude and intercept was also highlighted as these emerged as important elements in the network. The connection among those 6 relevant features formed the core of the ensemble model.

According to the SHAP value analysis (**Figure 5**, **e-Figure 4**), the contribution of the input features could be interpreted as follows:

The most relevant features allowing to distinguish central from obstructive hypopnea included: event duration, variance in signal amplitude, lower extremities (minimum, 5th and 25th centiles), while the central tendency (mean, median and intercept), and the linear trend of the signal showed only moderate contribution.

More specifically, a longer event duration, higher value of centrality (mean, median), and more important linear trend supported the prediction of obstructive hypopnea; in contrast, a shorter event duration, higher values of minimum, 5th and 25th centiles, lower values of mean, or median allowed to rule-out the obstructive hypopnea. On the other hand, a coalition of moderate event duration, lower central tendency (mean, mode or median), lower values of minimum, 5th and 25th centiles, and less important linear trend predicted central hypopnea.

Although the upper extremities (Max, 90th, or 95th centiles), as well as the intercept were found relevant for the prediction, their contributions were equally distributed in both hypopnea types, and thus remained impervious for either central hypopnea or obstructive hypopnea. Other features such as skewness, interquartile range, 75th centile and spline functions were least important as their contribution rarely impacted the model's output (SHAP values were close to zero).

DISCUSSION

This study expands on our previous findings (5) indicating the clinical utility of MM signals as a surrogate marker of RE during sleep, by extracting more features from the raw MM data and focusing on the differentiation between obstructive and central hypopnea events, an issue that provides more informative content than the general classification of sleep breathing disorders. The Random Forest algorithm was adopted as a statistical inference tool because it offers the capacity to handle multiclass problems as well as delineate complex interactions among input features. The findings not only confirmed the ability of MM to differentiate obstructive from central hypopneas and periods of NPB, but also provided enhanced understanding about the changes in MM signal patterns during these episodes. In general, hypopneas can

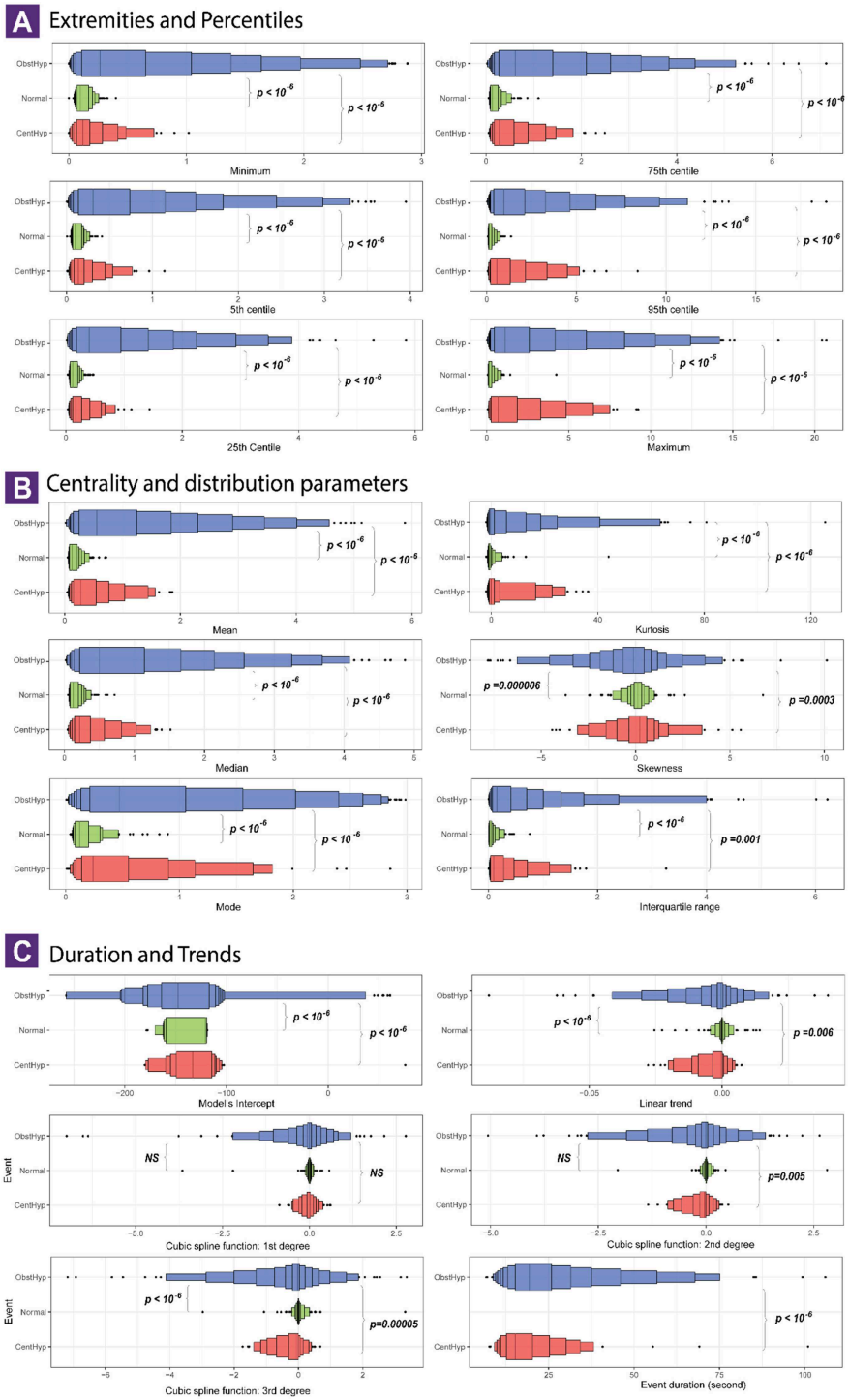

FIGURE 3 | Characteristics of mandibular movement signal during obstructive and central hypopnea events. Each panel visualizes the distribution of a surrogate feature of MM signal in 3 event types: normal breathing (501 series, green color), central hypopneas ($n = 263$ series, red color) and obstructive hypopnea ($n = 1,861$ series, blue color). The surrogate features are presented in 3 groups: **(A)** The extreme levels of signal amplitude, with lower extremities on left side and upper extremities on the right side; **(B)** The centrality or location parameter of the signal (left) and distribution shape parameters (right); **(C)** The event duration, linear trend and coefficients of the smoothing spline time series model (S1–S3). The letter-value boxplot was used to ensure a better description for large data (10). Multiple boxes were drawn, each one represents a pair of lower and upper letter values. The procedure starts with the median, followed with quartiles, and so on. The innermost box is equivalent the conventional boxplot. As moving toward the tails, the boxes became incrementally narrower until we reached the extremes values (outliers, minimum, and maximum). The p_values correspond to a pairwise comparison using *t*-test with Bonferroni correction. A difference is considered significant if p_value is lower than 10^{-6}.

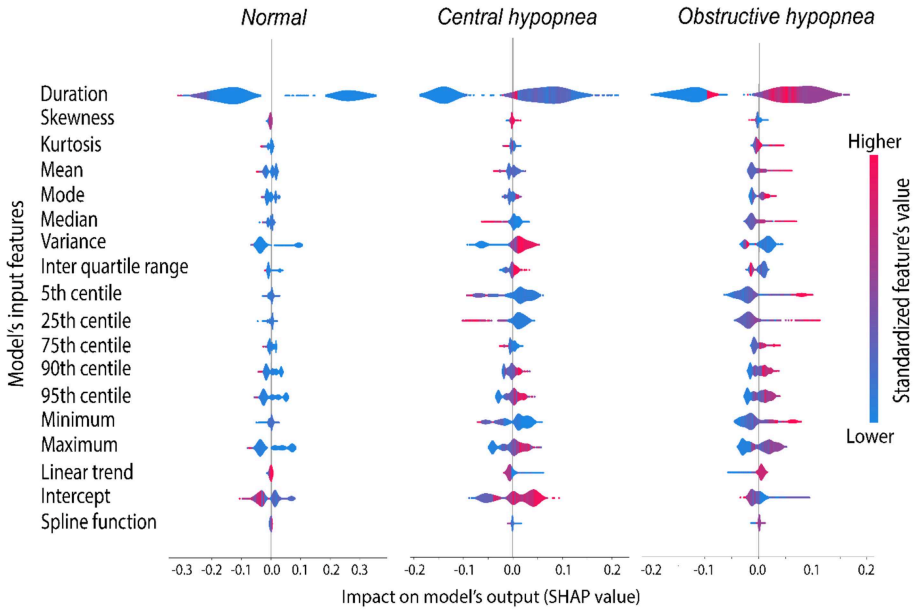

FIGURE 4 | Average marginal contribution of the 18 most relevant features to the model's prediction. Each line in this graph corresponds to a single feature and consists of 3 violin plots showing the contribution of that feature to the prediction of 3 target groups. Each column corresponds to a target group (normal, central and obstructive hypopneas). The violin plots and X scale indicate the distribution of the SHAP value, a score assigned to each feature to measure the average marginal contribution of that feature across all possible coalitions with other features to make a certain prediction. The shape and location of the violin plots indicate the impact of each feature on the model's output, or how much a feature may contribute to a certain prediction. A negative SHAP value indicates that the feature participates to rule out that group (by decreasing the predicted probability), whilst a feature with positive SHAP value would increase the probability of that group, thus supporting its identification. Larger absolute SHAP scores (the density plot extends further to the left or right side) indicate more important role of that feature. A zero SHAP value (concentrated density plot) indicates that the feature does not contribute at all to the prediction of that group. The feature values were normalized and mapped to a blue/red color scale (blue = lower values, red = higher values), allowing to estimate the tendency of prediction as the feature value increases or decreases.

be reproducibly and correctly characterized as either central (without signs of increasing RE) or obstructive (presence of marked or increasing RE) when considering an ensemble of statistical features in sleep MM signals.

We have previously shown that MM signals provide accurate estimates on the degree of RE in patients with OSAS (15). In a group of consecutive patients clinically referred for evaluation in the sleep laboratory for suspected OSA, MM analyses allowed for successful detection of RE as defined by the conventional scoring rules. Moreover, as shown herein MM can readily differentiate between obstructive and central hypopnea events.

Since many patients exhibit a preponderance of events during sleep that are scored as hypopnea rather than apnea, and since obstructive hypopnea likely share the same pathophysiological determinants and obstructive apnea (4), the AHI does not provide accurate clinical risk stratification since it includes both central and obstructive events, and therefore other PSG-derived features are needed to better define sleep-disordered breathing (16). In the context of the last iteration of AASM scoring rules in 2012, it did not seem that differences with previous scoring guidelines would enhance the prediction of the risk of cardio-vascular morbidities (17). However, the importance of identifying the sub-type of hypopneas to better stratify morbidity risks and overall outcomes cannot be over emphasized.

The current study pointed to both predictive and interpretative goals. However, there is always a trade-off between the predictive power and the interpretability when adopting a statistical learning algorithm. Due to the high dimensionality of the extracted features data and multiclass classification problem, we had to adopt the Random Forest classifier.

We found, however, that both the extremities (min, max) and the lower centiles of the MM amplitudes, as well as the central tendency parameters (mean, median or intercept of a linear regression) provided informative content about the position of the mandible during sleep events, and that obstructive hypopneas lasted longer as compared to central hypopneas. The parameters of the values distribution shape showed no significant differences. In contrast, a smaller variance in the mandibular position and in peak amplitudes was more typically observed during central hypopneas.

Similarly, the linear and the curvilinear trend analyses along the hypopnea spectrum highlighted that the obstructive events generated a more negative signal (mouth more open). The coefficients of the spline function in the non-linear trend analysis were similar between both hypopnea sub-types; it is likely because the signal curve shape of the temporal series was dependent on the individual characteristics. Notably, hypopnea duration contributed importantly to the distinction between central from obstructive hypopnea, the

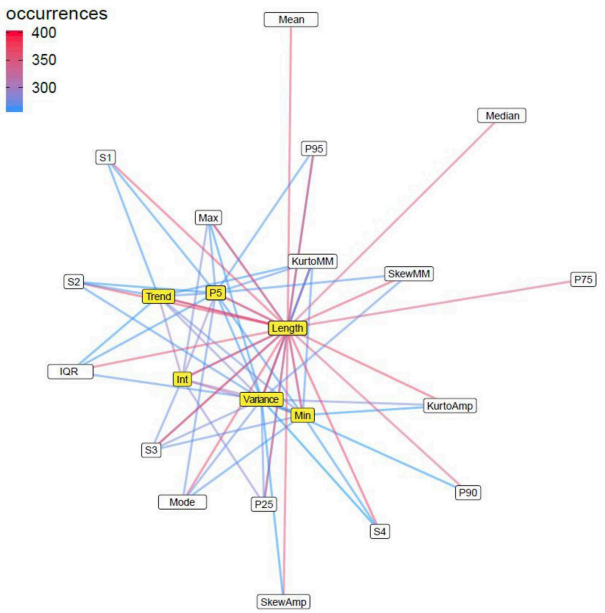

FIGURE 5 | Cooperation network among MM signal features in Random Forest classifier. The figure presents a network of all possible combinations among the 23 potential surrogate features of MM signals that occurred in 500 classification rules in the Random Forest model. Pi, ith percentile; IQR, interquartile range; Amp, amplitude of MM signal; Int, intercept of the linear model estimating MM signal amplitude in function of time; S1–S4, spline functions determining the curvilinear trend of MM signal amplitude in function of Time; Skew, skewness; Kurto, Kurtosis; Trend, linear trend of the MM signal. Each node in the network indicates a feature and the link connecting between two nodes indicates that these two features did co-exist in at least 250 decision trees. The color intensity of connection links is proportionate with the frequency of that combination. The nodes are positioned in function of their ability to inter-connect with other nodes, thus the more centralized nodes (colored in yellow; more links), such as event duration (length), 5th centile, linear trend, intercept, variance, and Min were the most important features, because those features did participate in almost every classification rule.

latter being more likely to be of longer duration. Those findings were consistent in both traditional statistical inference (ANOVA) and model interpretative analysis using the SHAP method.

The importance of identifying the sub-type of hypopneas to better stratify morbidity risks and overall outcomes of sleep apnoea patients is now widely recognized. Identification of central breathing events without RE or with decreasing RE is paramount to formulate tailored treatment decisions (18). Indeed, emergence of central respiratory disturbances provides clinically important information on underlying pathological conditions: frequent sleep/wake transitions, excessive loop gain with or without excessive arousability and sleep fragmentation, prolonged circulatory time as in congestive heart failure with increased risk for Cheyne-Stokes breathing, or central alveolar hypoventilation of various causes (19–22). However, all these conditions associated with central hypopnea tend in general to result in shorter events. Episodes, including those with longer decreases in RE are in fact of mixed nature, and were regrouped in this study with the obstructive hypopneas. Previous algorithmic analyses had to consider multiple PSG parameters in combination (flattening of nasal flow-pressure signal, paradoxical breathing, arousal, event termination profile, and sleep stage) to characterize the hypopnea with a satisfactory overall accuracy. In the search for a useful single signal source that manifest overnight stability in the absence of magnetic interferences, MM emerges as a good candidate that can be used in machine-based classifiers aiming at separating central from obstructive hypopneas.

LIMITATIONS

Similar to any study, our study has a few important limitations that deserve comment. Even though a classification rule was built in our analyses, this approach was simply aimed as an experimental demonstration of feasibility and proof-of-concept, rather than reflecting a final and authoritative diagnostic rule. Extracted feature-based algorithms may not be the best solution for clinical practice, due to their higher computation cost and other biases. Therefore, more advanced methods such as recurrent or convolutional neural networks are more efficient for bio-signal learning, pattern recognition, as these algorithms can innately handle both feature extraction and model optimisation, independently from human knowledge-based interventions. As such, implementation of the approaches proposed herein should incorporate a multicentre large cohort from which derivation of more robust and validated rules could be then implemented and disseminated.

We should also point out the imbalance among the 3 event groups, as central hypopneas were less frequent compared to the larger number of obstructive events. However, this problem was handled by conducting an over-sampling process on the training set before building the model and by setting a high threshold of statistical significance for null hypothesis testing ($p < 10^{-6}$).

CONCLUSIONS

The signal characteristics of MM can be used as surrogate markers of OeP to correctly classify obstructive and central hypopneas as well as periods of NPB in patients being evaluated for suspected OSAS. MM signal opens doors to automate the more complex part of respiratory events scoring. It also opens the possibility of scoring by less trained physicians and reduces medical errors.

DATA AVAILABILITY

The datasets analyzed in this manuscript are not publicly available. Requests to access the datasets should be directed to martinot.j@respisom.be.

AUTHOR CONTRIBUTIONS

J-BM, JB, DG, and JP designed research. J-BM, VC, SD, and JB performed data acquisition and interpretation. N-NL-D

analyzed data. VC and J-BM performed *post-hoc* validation and interpretation of the findings. N-NL-D, DG, J-BM, and JP wrote the paper. JB, DG, and JP revised the manuscript. J-BM is the guarantor of the paper. The final manuscript has been reviewed and approved by all authors.

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SUPPLEMENTARY MATERIAL

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Absence of Relationship Between Self-Reported Sleep Measures and Amyloid Load in Elderly Subjects

*Audrey Gabelle[1,2,3], Laure-Anne Gutierrez[2,3], Isabelle Jaussent[2,3], Fayçal Ben Bouallegue[3,4,5], Delphine De Verbizier[4], Sophie Navucet[1,2], Caroline Grasselli[1,2], Karim Bennys[1], Cécilia Marelli[1], Renaud David[6], Denis Mariano-Goulart[3,4,5], Sandrine Andrieu[7], Bruno Vellas[7], Pierre Payoux[8], Claudine Berr[2,3] and Yves Dauvilliers[2,3,9]**

[1] Department of Neurology, Memory Research and Resources Center, CHU Montpellier, Montpellier, France, [2] INSERM, U1061, Neuropsychiatrie, Recherche Clinique et Epidémiologique, Montpellier, France, [3] Université de Montpellier, MUSE, Montpellier, France, [4] Nuclear Medicine Department, CHU Montpellier, Montpellier, France, [5] PhyMedExp, INSERM-CNRS, Montpellier University, Montpellier, France, [6] Department of Psychiatry, Memory Research and Resources Center, CHU Nice, Nice, France, [7] Gérontopôle de Toulouse, Inserm UMR1027, Toulouse Université III, Toulouse, France, [8] Nuclear Medicine Department, CHU Toulouse, Toulouse, France, [9] Department of Neurology, Narcolepsy National Reference Center, Sleep Center, CHU Montpellier, Montpellier, France

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Maurice M. Ohayon, Stanford University, United States

Reviewed by:
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***Correspondence:**
Yves Dauvilliers
y-dauvilliers@chu-montpellier.fr

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Objective: To determine the relationships between self-reported sleep profile and cortical amyloid load in elderly subjects without dementia.

Methods: This cross-sectional study included 143 community-dwelling participants aged ≥70 years (median: 73 years [70–85]; 87 females) with spontaneous memory complaints but dementia-free. Sociodemographic characteristics, health status, neuropsychological tests, sleep, and ^{18}F-florbetapir (amyloid) PET data were collected. The clinical sleep interview evaluated nighttime sleep duration, but also daytime sleep duration, presence of naps, and restless leg syndrome (RLS) at time of study. Validated questionnaires assessed daytime sleepiness, insomnia, and risk of sleep apnea. The cortical standardized uptake value ratio (SUVr) was computed across six cortical regions. The relationship between sleep parameters and SUVr (cut-off ratio>1.17 and tertiles) was analyzed using logistic regression models.

Results: Amyloid-PET was positive in 40.6% of participants. Almost 40% were at risk for apnea, 13.5% had RLS, 35.5% insomnia symptoms, 22.1% daytime sleepiness, and 18.8% took sleep drugs. No significant relationship was found between positive amyloid PET and nighttime sleep duration (as a continuous variable, or categorized into <6; 6–7; ≥7 h per night). Logistic regression models did not show any association between SUVr and daytime sleep duration, 24-h sleep duration, naps, RLS, daytime sleepiness, insomnia symptoms, and sleep apnea risk (before and after adjustment for APOEε4 and depressive symptoms).

Conclusion: Our study did not confirm the association between amyloid-PET burden, poor sleep quantity/quality in elderly population, suggesting that the interplay between sleep, and amyloid is more complex than described.

Keywords: amyloid, amyloidosis, sleep, elderly, PET—positron emission tomography, NAPS, dementia, cognition

INTRODUCTION

Experimental and human studies suggest that sleep-wake alterations contribute to brain amyloid-beta (Aβ) dysregulation and showed that Aβ load in interstitial fluid proportionally increases with time awake (1). Short sleep duration, sleep fragmentation, and reduced slow-wave sleep may thus affect Aβ brain deposition, one of the key pathophysiological mechanisms of Alzheimer's disease (AD) (1–3). Sleep-wake disturbances (i.e., sleep fragmentation, frequent and long awakenings, and excessive daytime sleepiness), and sleep disorders (i.e., insomnia and sleep apnea syndrome) are frequently found in patients with AD at early and also late stages of the disease (4–10).

One reference study on 62 older adults found that people with self-reported short sleep had higher Aβ burden than long sleep/night (5). Other studies on cognitively healthy individuals at risk of developing AD reported associations between amyloid load and other sleep parameters (i.e., sleep quality, sleep latency, sleep efficiency, wake after sleep onset, excessive daytime sleepiness, and napping) (5–8, 11–14). Similarly, in a 1-year prospective study, we found that the risk of cognitive decline is higher in frail elderly subjects with excessive daytime sleepiness and longer nighttime in bed (15). However, all these results need to be replicated due to the frequent sleep misperception in older people, and the differences in sleep assessment methodology, populations and sample size, treatment intake, confounding factors and study design (5–14). Moreover, the relationships between sleep profile, cognitive status, and amyloid load measured by positron imaging tomography (PET) remain unknown in elderly population with memory complaints.

The main aim of this study was to determine the relationship between self-reported nighttime sleep duration and cortical amyloid load, measured by PET with the ^{18}F-florbetapir amyloid ligand (^{18}F-florbetapir PET), in elderly subjects with memory complaints. We also assessed using a detailed, and comprehensive clinical interview the relationships between other sleep characteristics and brain amyloid load.

METHODS

Participants

The MAPT-AV45 sleep ancillary study (www.clinicaltrials.gov NCT00672685) included 143 community-dwelling participants aged ≥70 years (median age: 73 years-old; range [70 to 85]; 87 females) with spontaneous memory complaints, but free of dementia (16–19). Sociodemographic characteristics, health status, and neuropsychological test results were available for all patients who also underwent sleep assessment and ^{18}F-florbetapir-PET brain imaging.

The MAPT-AV45 study and the sleep ancillary study were approved by the Toulouse ethics committee (Comité de Protection des Personnes- Sud-Ouest et Outre-Mer I et II). The methods were carried out in accordance with the approved guidelines. Each participant signed legal consent forms. Informed consent was obtained from all subjects.

^{18}F-florbetapir-PET Brain Imaging

Brain imaging was performed using five different hybrid PET-CT scanners. Image acquisition started 50 min after injection of 4 MBq/kg of ^{18}F-florbetapir. PET sinograms were reconstructed with an iterative algorithm, with corrections for randomness, scatter, photon attenuation, and decay, producing images with an isotropic voxel of $2 \times 2 \times 2$ mm^3, and a spatial resolution of approximately 5-mm full width at half maximum at the field of view center. ^{18}F-florbetapir images were co-registered to a template provided by Avid Radiopharmaceuticals (Philadelphia, PA) (20) using SPM for normalization to the Montreal Neurological Institute (MNI) space. Tracer retention in the cerebral cortex was quantified using the standardized uptake value ratio (SUVr) relative to the whole cerebellum. The SUV of the cortical retention index were computed in six cortical regions of interest (i.e., frontal, parietal, temporal, precuneus, anterior, and posterior cingulate cortices). Cortical SUV ratios were obtained by normalizing the cortical SUV with the mean uptake relative to the whole cerebellum that defines the amyloid load. According to a previously established cut-off, the amyloid PET scan was considered positive when the global cortical SUVr value was higher than 1.17[3]. As threshold may depend of the targeted population, we further grouped participants according to the tertiles of their global cortical SUVr, the highest tertile group compared with the other two.

Self-Reported Sleep Parameters

All participants had a face-to-face interview to assess last month the duration of nighttime sleep (in hour and also categorized as <6; 6–7; ≥7 h per night according to a reference study [10]), daytime sleep (in minutes), presence and duration of naps (recorded as: no naps, naps <30 min, naps ≥30 min per day), total sleep time (daytime and nighttime sleep), and sleep efficiency (i.e., total sleep time divided by time spent in bed reported by subjects, expressed as a percentage). The presence of restless legs syndrome [RLS; on the basis of the five minimal diagnostic criteria (21)], and of rapid eye movement sleep behavioral disorder (RBD; violent nocturnal agitation accompanied by shouting often at the end of the night and associated with dreamlike memories) were also investigated by a semi-structured clinical interview. Participants completed, with the help of medical staff when required, the following sleep questionnaires: the Epworth Sleepiness Scale (ESS) to evaluate excessive daytime sleepiness (EDS; cut-off score ≥11) (22); the 7-item self-report Insomnia Severity Index (ISI) to evaluate insomnia symptoms [score <8: low insomnia; score (3–9): sub-threshold insomnia; and score ≥15: moderate-severe insomnia] (23); and the Berlin questionnaire to assess the risk of sleep apnea (24).

Other Biological and Clinical Characteristics

A standardized interview with questions on sociodemographic characteristics, health status and use of medications was performed at baseline and each year during the 5-year-follow-up. Drugs were coded according to the World Health

Organization's Anatomical Therapeutic Chemical Classification. Hypnotics were classified as benzodiazepine, benzodiazepine-like compounds (zolpidem, zopiclone), sedative antidepressants, and miscellaneous medications (including barbiturates, antihistamines, and other pharmacological categories, such as neuroleptics). Drugs administered during the year of the ^{18}F-florbetapir-PET were taken into account for the analysis. Height and weight were measured and used to calculate the body mass index. Cerebro-cardiovascular and metabolic diseases were defined as self-report history of stroke and cardiovascular events, diabetes, or hypertension (defined by measured systolic blood pressure $\geq$140 mmHg or diastolic blood pressure $\geq$95 mmHg, or current antihypertensive treatment). Depressive symptoms were evaluated using the Beck Depression Inventory II (BDI-II) scale (25) (no depressive symptoms: 0–11 score; moderate to severe depressive symptoms, when score $\geq$12).

Based on the Clinical Dementia Rating (CDR) scale and the validated Petersen criteria (26), participants with a CDR score = 0.5 were classified as having Mild Cognitive Impairment (MCI). Participants with dementia at baseline (CDR score $\geq$ 1) were not included. The Mini Mental State Examination (MMSE) test was performed in all participants.

APOE was genotyped using a classical PCR digestion method (primers: 5′-GGGCACGGCTGTCCAAGGAGCTG-3′ and 5′-TCGCGGGCCCCGGCCTGGTA CACT-3′; restriction enzyme: *Hha*1).

Statistical Analysis

The sample was described using percentages for categorical variables, and medians [range] for quantitative variables, because the Shapiro-Wilk test indicated the data distribution was mostly skewed. The negative and positive amyloid PET groups were compared using univariate logistic regression models. Sociodemographic and clinical variables associated with brain amyloid load at $p < 0.10$ in the univariate analysis were included in logistic models to estimate the adjusted odds-ratios (OR) and their 95% confidence intervals (CI) for sleep parameters. For all analyses, the significance level was set at $p < 0.01$. Analyses were performed using the SAS statistical software (version 9.4; SAS Inc, Cary, North Carolina).

TABLE 1 | Socio-demographic and clinical characteristics of the participants divided according the pathological cut-off value for the global cortical SUVr calculated using ^{18}F-florbetapir-PET data.

Variable	Global cortical SUVr				*p*
	≤1.17 *N* = 85		>1.17 *N* = 58		
	N	%	*n*	%	
Sex					
Men	55	64.71	32	55.17	0.25
Women	30	35.29	26	44.83	
Age, in years[(a)]	74 [70–85]		73 [70–85]		0.86
Educational level					
Low	22	25.88	8	13.79	0.20
Intermediate	35	41.18	30	51.72	
High	28	32.94	20	34.48	
MMSE score[(a)]	29 [24–30]		29 [20–30]		0.45
MCI					
No	65	76.47	42	72.41	0.58
Yes	20	23.53	16	27.59	
APOEε4					
Not carrier	62	81.58	31	57.41	**0.003**
Carrier	14	18.42	23	42.59	
BMI, kg/m2					
<25	33	38.82	26	44.83	0.75
[25–30]	36	42.35	23	39.66	
≥30	16	18.82	9	15.52	
BDI-II score					
<12	67	78.82	35	61.40	**0.03**
≥12	18	21.18	22	38.60	
Cardiovascular events					
No	29	34.12	20	34.48	0.96
Yes	56	65.88	38	65.52	
Hypnotic intake					
No	70	82.35	46	79.31	0.65
Yes	15	17.65	12	20.69	

[(a)]*Continuous variables were expressed as median [minimal value–maximal value]. SUVr, standardized uptake value ratio; MMSE, Mini Mental Score Examination; MCI, Mild Cognitive Impairment; BMI, Body Mass Index; BDI-II, Beck Depression Inventory II. Bold indicates signficant p values.*

RESULTS

Clinical Characteristics, Sleep Profile, and Amyloid PET

For the whole population (n = 143), the education level was intermediate in 45.5% and high in 33.6% of participants, and the median MMSE score was 29 [20–30]. Moreover, 25% of participants had MCI, and 28.5% were APOEε4 allele carriers. Regarding the vascular and metabolic profile, 41.3% were overweight, 17.5% obese, and 65.7% reported history of cardiovascular and/or metabolic diseases. Moderate to severe depressive symptoms were reported by 30.0% of participants.

The median sleep duration was 7 h [3.25–9.5] for nighttime and 20 min [0–120] for daytime. About 56% of participants were nappers, and 29.5% had naps longer than 30 min. The median sleep efficiency was 87.5% [44.4–100]. Almost 40% of participants were at risk of sleep apnea, 13.5% had RLS (mild in 52.6% of them), 22.1% had EDS, 35.5% presented insomnia symptoms (moderate to severe for 8.5% of them), 8.2% had clinically-defined RBD, and 18.8% took often hypnotics.

Amyloid PET was positive (global cortical SUVr >1.17) in 40.6% of participants (**Table 1**). Participants with positive amyloid PET were more frequently APOEε4 carriers ($p = 0.003$) and had more often depressive symptoms (BDI-II score $\geq$12) ($p = 0.03$) (**Table 1**).

Association Between Sleep Parameters and Cortical Amyloid Load

^{18}F-florbetapir-PET was performed within a median interval of 134 [10–360] days from the sleep evaluation. No significant

TABLE 2 | Sleep characteristics of participants divided according to the pathological cut-off value and to tertiles for the global cortical SUVr calculated using the ^{18}F-florbetapir-PET data.

	Global cortical SUVr															
	≤1.17 *N* = 85		>1.17 *N* = 58		Model 0		Model 1		≤1.22 *N* = 94		>1.22 *N* = 49		Model 0		Model 1	
Variable	***n***	**%**	***N***	**%**	**OR [95% CI]**	***P***	**OR [95% CI]**	***P***	***n***	**%**	***n***	**%**	**OR [95% CI]**	***P***	**OR [95% CI]**	***P***
Nighttime sleep duration, hours																
<6	13	15.29	11	18.97	1	0.63	1	0.89	17	18.09	7	14.29	1	0.38	1	0.39
6–7	44	51.76	32	55.17	0.86 [0.342; 0.16]		1.01 [0.362; 0.87]		46	48.94	30	61.22	1.58 [0.594; 0.28]		2.15 [0.686; 0.87]	
≥7	28	32.94	15	25.86	0.63 [0.231; 0.75]		0.82 [0.262; 0.61]		31	32.98	12	24.49	0.94 [0.312; 0.84]		1.53 [0.425; 0.55]	
Nighttime sleep duration, hours	7 [4–10]		7 [4–9]		0.87 [0.661; 0.15]	0.33	0.89 [0.641; 0.22]	0.47	7 [4–10]		7 [4.5–9]		0.92 [0.691; 0.24]	0.60	0.98 [0.701; 0.37]	0.90
Daytime sleep duration, minutes																
Daytime sleep duration, *min* (1)	15 [0–120]		20 [0–90]		1.00 [0.991; 0.02]	0.71	1.01 [0.991; 0.02]	0.49	15 [0–120]		20 [0–90]		1.00 [0.991; 0.02]	0.97	1.01 [0.991; 0.02]	0.55
Naps																
No	35	45.45	23	44.23	1	0.77	1	0.48	37	44.05	21	46.67	1	0.81	1	0.59
Yes <30 min	21	27.27	12	23.08	0.87 [0.362; 0.10]		1.81 [0.664; 0.97]		23	27.38	10	22.22	0.77 [0.311; 0.91]		1.71 [0.594; 0.95]	
Yes ≥30 min	21	27.27	17	32.69	1.23 [0.542; 0.82]		1.47 [0.573; 0.84]		24	28.57	14	31.11	1.03 [0.442; 0.40]		1.36 [0.513; 0.67]	
Sleep efficiency (%)																
<82.35	28	32.94	19	32.76	1	0.90	1	0.53	29	30.85	18	36.73	1	0.77	1	0.61
[82.35–93.75]	29	34.12	18	31.03	0.91 [0.402; 0.09]		0.85 [0.332; 0.15]		32	34.04	15	30.61	0.76 [0.321; 0.77]		0.67 [0.261; 0.77]	
≥93.75	28	32.94	21	36.21	1.11 [0.492; 0.49]		1.41 [0.563; 0.59]		33	35.11	16	32.65	0.78 [0.341; 0.81]		1.05 [0.402; 0.75]	
Sleep efficiency (%)(a)	87.5 [44.4–100]		88.9 [50-100]		1.01 [0.991; 0.04]	0.39	1.02 [0.991; 0.06]	0.12	87.5 [44.4–100]		87.5 [62.5–100]		1.01 [0.981; 0.03]	0.64	1.02 [0.991; 0.05]	0.18
ESS score																
<11	66	78.57	40	76.92	1	0.82	1	0.68	71	77.17	35	79.55	1	0.76	1	0.98
≥11	18	21.43	12	23.08	1.10 [0.48; 2.52]		1.22 [0.483; 0.09]		21	22.83	9	20.45	0.87 [0.362; 0.10]		1.01 [0.372; 0.77]	
ESS score(a)	6 [0–21]		6 [1–15]		1.01 [0.931; 0.10]	0.86	1.01 [0.911; 0.11]	0.89	6 [0–21]		6 [1–14]		0.99 [0.901; 0.08]	0.82	0.99 [0.891; 0.10]	0.84
Insomnia severity scale																
<8	55	65.48	36	63.16	1	0.75	1	0.44	60	65.22	31	63.27	1	0.29	1	0.12
8–14	21	25.00	17	29.82	1.24 [0.582; 0.66]		1.08 [0.422; 0.78]		22	23.91	16	32.65	1.41 [0.653; 0.06]		1.24 [0.473; 0.28]	
≥15	8	9.52	4	7.02	0.76 [0.212; 0.72]		0.38 [0.071; 0.93]		10	10.87	2	4.08	0.39 [0.081; 0.88]		0.11 [0.011; 0.11]	
Insomnia severity scale(a)	5 [0-23]		4 [0-23]		0.99 [0.931; 0.06]	0.81	0.95 [0.871; 0.04]	0.25	5 [0-23]		4 [0-15]		0.96 [0.901; 0.03]	0.31	0.91 [0.821; 0.00]	0.04

(Continued)

TABLE 2 | Continued

	Global cortical SUVr											
	≤1.17 N = 85		>1.17 N = 58		Model 0		Model 1		≤1.22 N = 94		>1.22 N = 49		Model 0		Model 1	
Variable	***n***	**%**	***N***	**%**	**OR [95% CI]**	***P***	**OR [95% CI]**	***P***	***n***	**%**	***n***	**%**	**OR [95% CI]**	***P***	**OR [95% CI]**	***P***
Restless legs syndrome																
No	71	84.52	51	89.47	1	0.40	1	0.41	77	82.80	45	93.75	1	0.08	1	0.07
Yes	13	15.48	6	10.53	0.64 [0.231; 0.80]		0.63 [0.211; 0.91]		16	17.20	3	6.25	0.32 [0.091; 0.16]		0.28 [0.071; 0.10]	
Risk of sleep apnea																
Low	39	60.00	30	61.22	1	0.89	1	0.57	44	60.27	25	60.98	1	0.94	1	0.51
High	26	40.00	19	38.78	0.95 [0.442; 0.03]		1.28 [0.543; 0.01]		29	39.73	16	39.02	0.97 [0.442; 0.13]		1.35 [0.553; 0.29]	
Clinically-defined RBD																
No	64	94.12	36	87.80	1	0.26	1	0.38	68	93.15	32	88.89	1	0.45	1	0.70
Yes	4	5.88	5	12.20	2.22 [0.568; 0.80]		1.98 [0.439; 0.09]		5	6.85	4	11.11	1.70 [0.436; 0.76]		1.37 [0.276; 0.95]	

[a]*Continuous variables were expressed as median [minimal value-maximal value].*

ESS, Epworth Severity Scale; RBD, REM sleep behavioral disorder.

Model 0: Crude association.

Model 1: Adjustment for APOEε4 and Beck Depression Inventory-II score in two classes (score 0–11: no depressive symptoms, and score ≥12: moderate to severe depressive symptoms).

relationship was found between positive amyloid PET and nighttime sleep duration [taken as a continuous variable and categorized into <6; 6–7; ≥7 h per night as previously reported (5)] before and after adjustment for APOEε4 and depressive symptoms (BDI-II score ≥12) (**Table 2** and **Figure 1**). Similarly, no association was found between amyloid PET status and other sleep characteristics: daytime sleep duration, 24-h sleep duration, napping, sleep efficiency, EDS, insomnia symptoms, risk of sleep apnea, RLS, and clinically-defined RBD (**Table 2**). These results remained unchanged after excluding subjects with MCI ($n = 36$).

Comparison of participants subdivided in two groups according to their global cortical SUVr values in tertiles (highest tertile, SUVr >1.22, vs. the other two) showed almost similar results, with no between-group differences (**Table 2**).

DISCUSSION

Our study provides an extensive assessment of the sleep-wake profile and brain amyloid load measured with ^{18}F-florbetapir-PET in a cohort of elderly subjects without dementia. No association was found between the global cortical (using the pathological cut-off of 1.17 and tertiles of the population) and nighttime sleep duration, but also with daytime sleep duration, naps, daytime sleepiness, insomnia, risk of apnea, clinically-defined RBD, and sleep efficiency in unadjusted and adjusted models for APOEε4 and depressive symptoms.

A similar key study on 62 community-dwelling older adults found that people with self-reported sleep ≤ 6 h/night had higher Aβ burden measured by ^{11}C-Pittsburgh compound B (PiB) PET in cortical and precuneus areas than those with >7 h of sleep/night. Amyloid burden was intermediate in subjects with 6–7 h of sleep per night (5). These results remained significant after adjustment for potential confounders (APOEε4 carrier status, comorbidities and sleep-related treatments) (5). Despite similar self-reported assessment of sleep duration among both studies, our study did not confirm such association in unadjusted and adjusted models for APOEε4 status and depressive symptoms. Conversely, the number of participants (143 in our study vs. 62), amyloid tracer (^{18}F-florbetapir vs. ^{11}C-PiB), tracer uptake quantification (SUVr vs. distribution volume ratio), segmentation method (PET vs. MRI template), and cognitive profile (25.2% of subjects with MCI in our population vs. cognitively normal participants; but results from our study remained unchanged after excluding subjects with MCI) were different (5). Except for the low sample size at risk of obtaining a Type I error, other differences are less likely to explain such result discrepancies. We also reanalyzed our data using linear instead of logistic regression models, like in the previous study (5), but results remain unchanged. Also, we found no association between amyloid load in the precuneus specifically and nighttime sleep duration (data not shown).

Although the potential relationship between sleep duration and amyloid load (1, 16) is attractive in the context of amyloid clearance, sleep-wake patterns (27), orexin involvement (28), and glymphatic alterations in AD (29–31), the links between sleep and AD pathology are complex, often bidirectional and

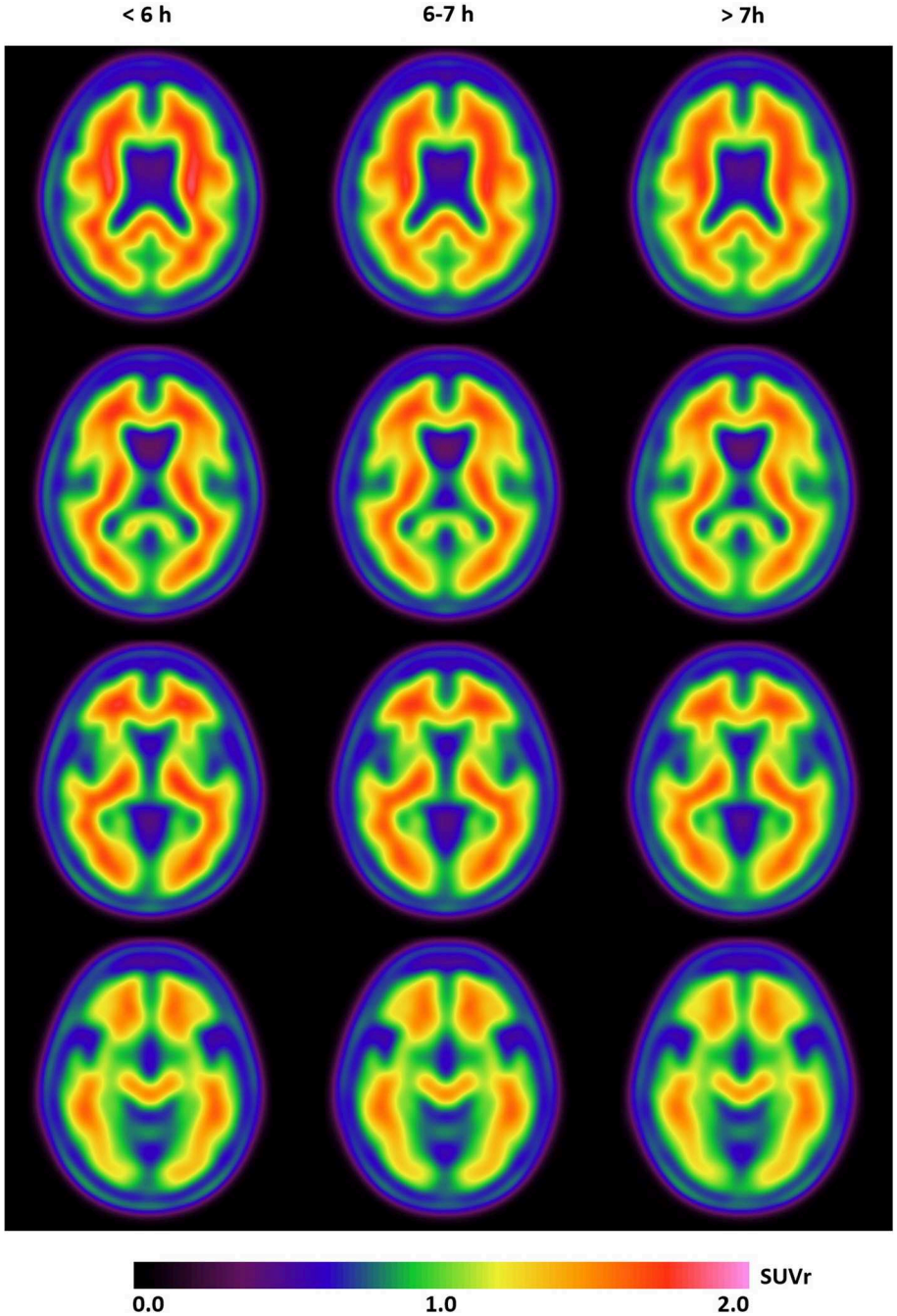

FIGURE 1 | The mean SUVr (^{18}F-florbetapir images) from four axial slices in participants divided according to their sleep duration (≤6, 6–7, and >7 h) show no difference in Aβ burden in these three groups.

variable during disease progression (32). Extracellular amyloid accumulation could be reduced during sleep and increased during wakefulness (33). Recent studies performed in young healthy adults showed that slow-wave sleep, rather that the entire sleep period, plays a key role in regulating the cerebrospinal fluid (CSF) levels of Aβ (34). As slow waves change in amplitude, frequency and shape with aging (35), we could hypothesize that the links between sleep and production/clearance of soluble amyloid proteins are different in older individuals compared with young people. Nevertheless, our current findings do not confirm that insufficient sleep duration is a clinically significant risk factor for brain amyloid deposition in elderly subjects.

Our study also did not find any association between amyloid PET positivity and napping, EDS, and sleep efficiency.

Conversely, a study with a different design (actigraphy and CSF Aβ quantification in 142 healthy volunteers) showed an association between low CSF $A\beta_{42}$ levels and frequent napping, sleep efficiency, but again not with nighttime sleep duration (12). A cross-sectional study on 184 cognitively normal participants older than 60 years found that longer sleep latency was associated with higher levels of amyloid burden (by PET), independently of the APOEε4 status (14). Similarly, another study found that cognitively healthy adults with less adequate sleep, more sleep problems, and greater somnolence (assessed using the Sleep Scale from the Medical Outcomes Study) had higher amyloid load in angular gyrus, frontal medial orbital cortex, cingulate gyrus, and precuneus (6). However, amyloid burden was not associated with the daytime sleepiness assessed by ESS (6). On the other hand, a recent study on 283 ≥70-year-old participants without dementia found that baseline excessive daytime sleepiness (ESS score ≥10) was significantly associated with longitudinal Aβ accumulation in the anterior and posterior cingulate, precuneus and parietal regions within a mean interval between two ^{11}C-PiB-PET scans of 2.2 years (36). Unfortunately, the absence of reported cross-sectional results on amyloid burden, sleep profile, particularly nighttime sleep duration and excessive daytime sleepiness, does not allow comparing our findings. Using the Berlin questionnaire, we did not find any association between amyloid PET status and the risk of sleep apnea. In contrast, several recent studies underlined the impact of self-reported clinical diagnosis of obstructive sleep apnea on the longitudinal increases in florbetapir PET uptake in normal and MCI subjects (37, 38). The mechanistic processes that link sleep apnea to accumulation of amyloid plaques and dementia need to be better assessed before proposing novel targets for intervention (37, 38).

The strengths of our study are the well-characterized population with spontaneous memory complaints but free of dementia, the standardized ^{18}F-florbetapir-PET imaging with cortical SUVr measurements, the clinical face-to-face sleep interview associated with validated questionnaires, and the analysis of a large number of potential confounding factors. The absence of relationship between positive amyloid PET and nighttime sleep duration persists despite adjustment for APOEε4 and depressive symptoms. To fill the gap between cognitively normal participants and prodromal AD, subjects with memory complaints constitute a well-targeted population.

The present study also presents some limitations. The sleep profile assessment was self-reported only, based on a clinical interview and questionnaires completed by the patients with the help of a caregiver/clinical team member if required, to detail sleep characteristics and complaints at time of study instead of objective measurements, such as actigraphy or polysomnography. The self-reporting nature of the evaluation could have led to recall biases and lack of accuracy in the responses, with potential unstable sleep phenotype/complaints and sleep misperception. However, the use of self-reported questionnaires to detect individuals with sleep disturbances is particularly relevant in the clinical practice and was easier and less expensive than polysomnography in a sleep laboratory but preclude to detect sleep apneas. Finally, we are aware that small sample size is often a limitation especially when reporting negative results. However, the number of participants was more than twice that of the previous study (5). We have computed a *post-hoc* statistical power calculation between the groups in our population. With the SUVR threshold of 1.17 and the means of sleep duration of 6.83 h in subjects below 1.17 and 6.63 h in those above, common standard deviation of 1.2 and alpha risk of 0.05, 567 subjects per group (1,134 in total) would have been necessary to show significant between-group differences with a power of 0.80. To date, no study arranges data with both PET-amyloid and sleep assessments on more than 1,000 participants at-risk to develop AD.

CONCLUSIONS

Our study failed to confirm previous findings on the association between poor sleep quantity/quality and amyloid load, despite the fairly large population, the detailed, and comprehensive sleep profile, the large number of potential confounding factors, and the cortical amyloid load measurements by ^{18}F-florbetapir-PET. Conversely, our results show that the interplay between sleep and amyloid is more complex than previously described. Before developing tailored therapeutic approaches, the sleep profile of subjects with amyloid pathology at different disease stages need to be better understood.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the supplementary files.

ETHICS STATEMENT

The MAPT-AV45 study and the sleep ancillary study were approved by the Toulouse ethics committee (Comitéde Protection des Personnes- Sud-Ouest et Outre-Mer I et II). The methods were carried out in accordance with the approved guidelines. Each participant signed legal consent forms. Informed consent was obtained from all subjects.

AUTHOR CONTRIBUTIONS

YD and AG: drafting, revising the manuscript for content, including medical writing for content, study concept or design, interpretation of data analysis, study supervision, and coordination. L-AG, CB, and IJ: revising the manuscript for content, interpretation of data analysis, study concept or design, and statistical analysis. FB and DM-G: revising the manuscript for content, interpretation of data analysis, and acquisition of data. SN, DD, CG, KB, CM, RD, SA, BV, and PP: revising the manuscript for content and acquisition of data.

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MAPT Study Group:

In Toulouse: *Investigators* Sophie Guyonnet; Project leader: Isabelle Carrié; *CRA*: Lauréane Brigitte; Catherine Faisant, Françoise Lala, Julien Delrieu, Hélène Villars; *Psychologists*: Emeline Combrouze, Carole Badufle, Audrey Zueras; *Methodology, statistical analysis and data management*: SA, Christelle Cantet, Christophe Morin; *Multi-domain group*: Gabor Abellan Van Kan, Charlotte Dupuy, Yves Rolland (physical and nutritional components), Céline Caillaud, Pierre-Jean Ousset (cognitive component), Françoise Lala (preventive consultation), Bertrand Fougère (Toulouse).

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MRI group: Carole Dufouil (Bordeaux), Stéphane Lehéricy, Marie Chupin, Jean-François Mangin, Ali Bouhayia (Paris); Michèle Allard (Bordeaux); Frédéric Ricolfi (Dijon); Dominique Dubois (Foix); Marie Paule Bonceour Martel (Limoges); François Cotton (Lyon); Alain Bonafé (Montpellier); Stéphane Chanalet (Nice); Françoise Hugon (Tarbes); Fabrice Bonneville, Christophe Cognard, François Chollet (Toulouse).

PET scan group: Thierry Voisin, Julien Delrieu, Sophie Peiffer, Anne Hitzel (Toulouse); Michèle Allard (Bordeaux); Michel Zanca (Montpellier); Jacques Monteil (Limoges); Jacques Darcourt (Nice).

Medico-economics group: Laurent Molinier, Hélène Derumeaux, Nadège Costa (Toulouse).

Biological sample collection: Christian Vincent, Bertrand Perret, Claire Vinel (Toulouse).

Safety management: Pascale Olivier-Abbal

DSA Group: Stéphanie Savy, Christelle Cantet, Nicola Coley.

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French Language Online Cognitive Behavioral Therapy for Insomnia Disorder: A Randomized Controlled Trial

Régis Lopez[1,2]*, *Elisa Evangelista*[1,2,3], *Lucie Barateau*[1,2,3], *Sofiene Chenini*[1], *Adriana Bosco*[1], *Michel Billiard*[4], *Anne-Dominique Bonte*[5], *Séverine Béziat*[2], *Isabelle Jaussent*[2] *and Yves Dauvilliers*[1,2,3]*

[1] Centre National de Référence Narcolepsie Hypersomnies, Unité des Troubles du Sommeil, Département de Neurologie, Hôpital Gui-de-Chauliac, Montpellier, France, [2] Inserm U1061, Montpellier, France, [3] Université de Montpellier, Montpellier, France, [4] Département de Neurologie, Hôpital Gui-de-Chauliac, Montpellier, France, [5] Meta-coaching, Paris, France

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***Correspondence:**
Régis Lopez
r-lopez@chu-montpellier.fr
Yves Dauvilliers
y-dauvilliers@chu-montpellier.fr

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Background: Despite cognitive-behavioral therapy for insomnia (CBT-I) being the recommended treatment for insomnia disorder, its access remains very limited. Automated Internet-delivered CBT-I (eCBT-I) is an emerging cost-effective strategy for adults with insomnia, however no such program is currently available in French Language. We evaluated a French-speaking, eCBT-I intervention to improve insomnia disorder in comparison to minimal psychoeducation therapy (mPT).

Methods: Forty-six adults with insomnia disorder were randomly allocated to eCBT-I or mPT. The eCBT-I program consisted of seven sessions that delivered the typical components of CBT-I during 12 weeks. The mPT provided structured and non-tailored information about sleep and insomnia during a 1 h session. Insomnia severity Index (ISI, primary outcome), measures of fatigue, sleepiness, anxiety, depressive symptoms and quality of life were collected at baseline and endpoint. Electronic sleep diaries were completed over 2 week periods pre- and post-intervention.

Results: Compared to mPT, eCBT-I resulted in greater decrease in ISI scores between baseline and endpoint. Sleep diaries parameters improved in both groups, with a greater improvement in the eCBT-I group. Patients allocated to eCBT-I group also improved depressive, fatigue, anxiety symptoms, and quality of life. Among patients with CNS-active drug at baseline, 91.7% reduced or stopped their hypnotic medication, and 16.7% in the mPT group.

Conclusions: The present eCBT-I program seems feasible, acceptable and effective in reducing insomnia severity and insomnia-related functional outcomes in this small clinically-derived population. Given the high prevalence of insomnia, our data are supportive of the use of such program as an effective alternative to treat insomnia in daily clinical practice in French speaking countries.

Keywords: insomnia, cognitive-behavioral therapy, internet-based intervention, adults, randomized controlled trial

INTRODUCTION

Insomnia disorder is very prevalent and affects 6–15% of the general population according to various definitions, severity and phenotypes (1). Insomnia is characterized by dissatisfaction with sleep quality or quantity with difficulty initiating or maintaining sleep, associated with daytime dysfunction and deterioration of the quality of life (2, 3). Insomnia disorder is often chronic, and its persistence may be associated with psychiatric and cardiovascular diseases (4), and even mortality (5).

According to international recommendations, cognitive behavioral therapy for insomnia (CBT-I) should be the first-line treatment of persistent insomnia disorder, instead of hypnotic benzodiazepines or Z-drugs or sedative antidepressants with mixed long-term results, frequent side effects, dependence, and tolerance over time (6). Several meta-analyses have highlighted the efficacy of CBT-I improving both sleep and functional outcomes, with effects being maintained for years (7, 8). Traditionally, CBT-I is delivered face-to-face, individually or in groups, but with limited access due to the insufficient availability of trained therapists, and its high cost. Insomnia remains an unrecognized and undertreated condition despite its important societal cost, high level of use of health care, and impaired work productivity and absenteeism (9, 10).

To overcome the limitation of CBT-I accessibility to the general population, promising methods were proposed to deliver automated CBT-I program with self-help materials (11), and more recently over the Internet with several existing commercial CBT platforms (i.e., Sleepio, SHUTi, Restore…). Since the first randomized controlled trial (RCT) evaluating online CBT-I (eCBT-I) (12), several programs have been evaluated showing improvements in several sleep outcomes, including self-assessed insomnia severity, total sleep time (TST), sleep efficiency (SE), sleep latency (SL), wake after sleep onset (WASO) and the number of nighttime awakenings (NWAK). Two recent meta-analyses of RCT of eCBT-I have been recently conducted and found improvement in both severity of insomnia and sleep efficiency (13, 14). These changes were comparable to improvement reported after either face-to-face delivered CBT-I or pharmacotherapy for insomnia (13). Some studies also highlighted the benefit of behavioral interventions to attenuate withdrawal symptoms and prevent insomnia relapse (8, 15–17), however contrasting results were obtained using eCBT-I in achieving hypnotic tapering (12, 18–25).

Nowadays, eCBT-I is accepted as an effective treatment for adults with chronic insomnia and as a cost-effective strategy in health care (26, 27). Although the French language is the sixth most spoken in the world, with 220 million speakers, including France, but also other predominantly French-speaking areas such as Belgium, Switzerland, Quebec and some African countries, to our knowledge, no eCBT-I program was currently available.

The aim of this study was to compare the efficacy of a French-speaking automated eCBT-I program in a small population of clinically-derived population of patients with persistent insomnia disorder in comparison to an active control intervention.

METHODS

Study Design

The study was a parallel-group, RCT with two treatment arms: (1) eCBT-I program and (2) minimal psychoeducation therapy (mPT: placebo). The randomization sequence was computer-generated using random blocks in an order unknown by the investigators.

Participants

Participants were recruited from the outpatient clinic for sleep disorders of the Gui-de-Chauliac Hospital, Montpellier, France, from July 2015 to February 2016. All patients underwent a structured interview with experimented clinicians to assess the presence of insomnia disorder, its characteristics (insomnia subtype, duration, frequency, and functional impairment) and severity using the insomnia severity scale (ISI), and potential comorbidities. They also completed an electronic sleep diary for 2 weeks before the randomization. Eligible participants were men and women, aged 18–64 years, who met the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria for insomnia disorder (2). Participants were also required to have a sleep efficiency $\leq$79% based on two-week sleep diary and an ISI score $>$14. Patients were excluded in presence of comorbid sleep disorders (sleep apnea syndrome clinically defined, restless legs syndrome at least twice a week), clinically significant psychiatric (mood, anxiety, psychotic, substance use or post-traumatic stress disorder) or medical disorders (chronic pain, respiratory, cardiovascular disease).

Central nervous system (CNS)-active drugs prescribed for insomnia (i.e., hypnotic, anxiolytic or low doses antidepressants) were accepted, however patients with more than two different CNS-active medications intake and those taking antipsychotic, opioid, antiepileptic or antiparkinsonian agents were not included.

All patients gave written consent to take part in the study. The study was approved by the local ethics committee (CPP Sud Méditerranée I, France, Number 2014-A01796-41; ClinicalTrials.gov; identifier NCT02539862).

Procedures

Patients assigned to eCBT-I group received a 12 week automated program divided in seven sessions. The eCBT-I program was conceived by MB and ADB, and reviewed by RL. Patients ran the first session with the following components: self-assessment questionnaires about their sleep habit and complaints, followed by quiz testing their knowledge about sleep and insomnia with real time feedback. During the second session, the sleep restriction protocol was started. During the third session, behavioral therapy and stimulus control techniques were implemented. During the fourth session, three relaxation techniques (progressive muscle relaxation, imagery rehearsal and mindfulness-based approaches) were proposed and patients were invited to choose one and practice. During the fifth and sixth session, the cognitive component of the therapy was introduced with patients being invited to answer questions related to insomnia beliefs, with an automated personalized

feedback to restructure dysfunctional thinking and to reduce evening ruminations. The last session consisted in revising the entire program and gave information and advices to prevent the relapse.

Among all sessions, structured information related to normal sleep, normal daytime functioning, insomnia mechanisms, hypnotic treatments, sleep hygiene rules were progressively delivered. On a daily basis, patients completed online a sleep diary in the morning to complete information on the past night, and in the evening to provide information on the daytime functioning. Based on the data collected, an automatic algorithm gave appropriate feedback to help the patients analyzing their progression and to adequately adapt the behavioral instructions. Another challenge was to ensure adherence to behavioral instructions especially when targeted to sleep restriction. Motivational advices with reward/incentive tools have been implemented all along the program. They included challenges to be accomplished within 5 to 14 days, classified into four different categories (behavioral, emotional, mindfulness, health) with habit tracking systems. Successful challenges allowed participants to earn points that unlock rewards.

Patient randomized to the control arm had a face-to-face 1 h single session of insomnia psychoeducation therapy that included structured information on normal sleep, sleep hygiene, insomnia mechanisms, and pharmacological treatment, without any behavioral instruction given to the patient.

Outcomes

The primary efficacy outcome measure was the change in ISI from baseline to endpoint (2 weeks after the end of intervention) between the two groups. ISI, a 7-item self-report questionnaire assessing insomnia symptoms including the degree of resultant distress, was completed by all participants at baseline and endpoint. A cut-off score of 14 indicates clinically relevant insomnia while 21 indicates severe insomnia.

Secondary nighttime efficacy outcome measures were changes from baseline to endpoint between the two groups on TST, SE, SOL, WASO, NWAK assessed from sleep diaries. Patients completed an electronic sleep diary for 2 weeks before the randomization and at the end of the intervention. The electronic sleep diaries completed by participants assigned to the eCBT-I arm within the program were not used for this study. Sleep efficiency was calculated as the ratio of TST to time in bed (TIB), multiplied by 100 to yield a percentage. Information about SOL, WASO and NWAK was also retrieved from sleep diaries. Daytime efficacy outcomes included fatigue, anxiety, depressive and daytime sleepiness symptoms and quality of life assessed on validated questionnaires from baseline to endpoints. Patients completed the questionnaires twice, at baseline and endpoint to measure symptoms of sleepiness (Epworth sleepiness scale—ESS), fatigue (14-items Chalder Fatigue Scale—CFS), depression (21-items Beck depressive inventory—BDI), anxiety (State Trait Anxiety Inventory Form Y—STAI). The EuroQol group questionnaire EQ-5D is a simple instrument that provides a generic measure of health outcomes for a wide range of health conditions. It consists of two sections, the EQ-5D descriptive section and the visual analogic scale (VAS). The descriptive section assesses five dimensions of health-related quality of life (Mobility, Self-Care, Usual Activities, Pain/Discomfort, and Anxiety/Depression), each described by three response levels (no, some or extreme problems). An utility score can be calculated from individual descriptive responses. The EQ-5D VAS scores are anchored on 100 as the best imaginable health and 0 as the worst (28–32). CNS-active drugs, number, classes and doses were recorded in the two groups at both evaluations.

Power Calculation

Statistical power was based on a sample of patients with chronic insomnia (33) with a total mean score of ISI equaled to 16.4 (SD = 3.8). Assuming that this mean would be unchanged at the end of trial for the mPT group, 18 patients per group were required to show a significant mean difference between the two groups equal to 4.23 for ISI total score with a 90% statistic power, a 2-sided 2-sample *t*-test with a significance level of 0.05.

Statistical Analyses

Efficacy analyses were based on the modified intention-to-treat population (mITT). The primary and secondary outcomes were analyzed using the modified Full Analysis Set (mFAS), including all subjects who had one baseline and one post-baseline evaluation. The characteristics of the study population were described using the median [interquartile range (IQR)] for quantitative variables, and number and percentage for categorical variables. Chi-square or Fisher's exact tests were used to compare categorical variables between the two groups, and Mann-Whitney U test for continuous variables. Changes in each outcome between baseline and endpoint were compared between the groups using Mann-Whitney U test. For the between-group differences, 95% Confidence Intervals (CI) were constructed. To compare differences between baseline and endpoints within group, Wilcoxon signed-rank tests were used. The agreement between the two periods was evaluated with the Cohen's Kappa coefficient for dichotomous variables. The statistical analysis was done by an independent external statistician. Statistical significance was set at $p < 0.05$. Statistical analyses were performed with SAS version 9.4.

RESULTS

Fifty-nine patients were screened with insomnia disorder, of whom 13 were excluded due to not fully meeting eligibility criteria (**Figure 1**). The remaining 46 patients [34 females, median age 45.49 years (11.00)] were randomized to receive the study treatment: 23 were assigned to the eCBT-I and 23 to the mPT. Baseline characteristics, severity of insomnia, and rates of patients under CNS-active drug were not different between the two randomized arms (**Table 1**). At baseline, median BMI was 21.74 kg/m^2 (4.92) with 11 patients being overweight. The median duration of insomnia complaints was 10.99 years (14.99). Nineteen patients had difficulties in initiating sleep, 32 in maintaining sleep and 37 reported early-morning awakenings with 33 patients having at least two symptoms. Sleep difficulties occurred with a median of 5.5 times per week (3.0). The median ISI score was 19.00 (4), with 11 patients having severe insomnia

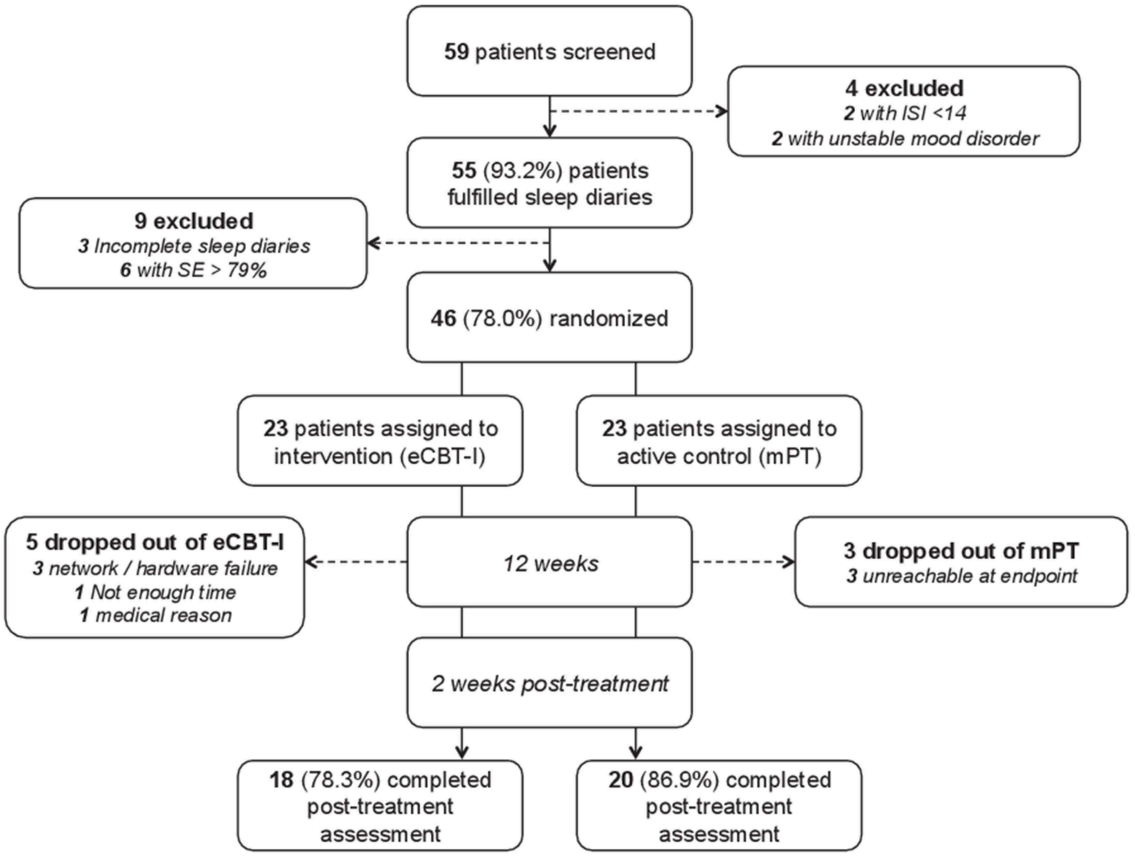

FIGURE 1 | Study flowchart. SE, Sleep Efficiency; ISI, Insomnia Severity Index; eCBT-I, Online Cognitive Behavioral Therapy for Insomnia; mPT, Minimal Psychoeducation.

complaints. The sleep diaries of the first 2 weeks showed a median sleep efficiency of 66.67% (23.49). Thirty patients (65.2%) were taking at least one CNS-active drug, all on a daily basis.

Eight patients prematurely discontinued the trial (5 in eCBT-I group and 3 in mPT group) (see details, **Figure 1**). The mITT population consisted of 38 patients: 18 patients [15 females, median age 46.50 years (11.00)] in the eCBT-I group and 20 patients [14 females, median age 45.99 years (9.50)].

Concerning the primary outcome, a significant between-group difference was found for changes in ISI between baseline and endpoint in the mITT population [eCBT-I group: −9 (7.00)] vs. mPT group −3.00 (7.00), $p = 0.0004$) (**Table 2**). Results remained significant in the ITT population (eCBT-I group: −8 (10.00) vs. mPT group: −3.00 (8.00), $p = 0.004$). ISI improved in both groups, with a median relative diminution of −43.91% (35.59) and −19.38% (39.08) in the eCBT-I and the mPT groups from the mITT population. Additional analyses were performed by subgroups of patients, according to their age, gender, and baseline severity (**Table 3**). We found significant between-group differences for ISI score with improvement in patients aged <45.5 years old, female, and in patients with baseline ISI<19 and BDI ≥14 in the CBT-I group compared to mPT group.

Significant changes between groups were also found for secondary nighttime outcomes, SE, TIB, SOL, NWAK between baseline and endpoint, with greater improvement in the eCBT-I group (**Table 2**). All parameters (i.e., TIB, TST, SE, SOL, WASO, and NWAK) improved in the eCBT-I group while only TST improved in the mPT group with similar tendency for SE.

Between-group differences were also significant for anxiety symptoms and fatigue, while depressive symptomatology improved in both groups, and perceived quality of life in the eCBT-I group only (**Table 2**). Daytime sleepiness levels remained stable along the study in both groups; however the frequency of EDS (ESS > 10) increased from 31.6 to 47.4% in the mPT group ($p = 0.18$), and decreased from 44.4 to 22.2% in the eCBT-I group ($p = 0.05$).

Most patients with CNS-active drug at baseline in the eCBT-I group decreased their hypnotic consumption (11/12, 91.7%) that includes eight patients who completely stopped the drugs during the study. In contrast, two out of 12 patients (16.7%) either reduced (one patient) or stopped (one patient) their medication in the mPT group.

Randomized participants from both groups did not report any treatment-emergent adverse effect.

TABLE 1 | Demographic and clinical characteristics of participants randomized to Minimal psychoeducation therapy (mPT) vs. Online cognitive and behavioral therapy (eCBT-I).

	mPT group $N = 23$		eCBT-I group $N = 23$		
	n	**%**	***n***	**%**	***p*-value**
Baseline demographic characteristics					
Gender (female)	15	65.22	19	82.61	0.18
Age (years) [(1)]	45.00 (13.00)		46.00 (11.00)		0.93
BMI (kg/m^2) [(1)]	21.87 (4.90)		21.48 (6.35)		0.99
BMI > 25 kg/m^2	5	22.73	6	26.09	0.79
Insomnia and related-characteristics during the randomization phase					
Difficulty initiating sleep (yes)	11	47.83	8	34.78	0.37
Difficulty maintaining sleep (yes)	17	73.91	15	65.22	0.52
Early morning awakening (yes)	20	86.96	17	73.91	0.26
Insomnia disorder duration (years) [(1)]	11.00 (14.00)		11.00 (15.00)		0.71
Frequency of insomnia (day per week) [(1)]	5.00 (4.00)		7.00 (2.00)		0.12
At least one CNS-active medication (yes)	13	56.52	17	73.91	0.22
ISI score [(1)]	18.00 (6.00)		20.00 (3.00)		0.11
ISI score >21	6	26.09	5	21.74	0.73
TIB (min) [(1)]	487.14 (82.50)		513.93 (53.57)		0.28
TST (min) [(1)]	278.21 (117.83)		296.07 (113.21)		0.48
SE [(1)]	0.60 (0.22)		0.58 (0.22)		0.74
Sleep onset latency (min) [(1)]	33.93 (33.57)		52.86 (63.93)		0.08
Number of awakenings [(1)]	1.00 (0.81)		1.14 (1.07)		0.63
Wake after sleep onset (min) [(1)]	36.43 (36.90)		36.43 (46.79)		0.87
ESS score [(1)]	9.00 (6.00)		10.00 (7.00)		0.52
ESS > 10	7	31.82	10	43.48	0.42
BDI score [(1)]	13.00 (11.00)		16.00 (15.00)		0.25
BDI score ≥14	11	47.83	14	60.87	0.37
EQ-5D utility score [(1)]	0.78 (0.31)		0.69 (0.12)		0.03
EQ-5D VAS [(1)]	60.00 (22.50)		60.00 (25.00)		0.56
STAI-E [(1)]	34.00 (15.00)		40.00 (16.00)		0.29
CFS physical score [(1)]	4.00 (3.00)		5.00 (2.00)		0.11

[(1)]*Continuous variables were expressed as median (interquartile range).*

DISCUSSION

In a small clinical population of adult patients with insomnia disorder, our randomized controlled study showed the efficacy of online automated CBT-I interventions. Even preliminary, this program seems effective in reducing insomnia severity and improving sleep efficiency, sleep duration, sleep onset latency, number of nighttime awakenings but also fatigue, depressive and anxiety-related symptoms as well as perceived quality of life. Our results also support the abilities of this Internet-delivered program to help CNS-active medication discontinuation in patients with insomnia disorder. Altogether, this eCBT-I confirms the extensive evidence-based results associated with several existing commercial CBT platforms such as Sleepio, SHUTi, and Restore. However, to our knowledge this is the first online CBT-I program in French-speaking language.

The reduction of insomnia severity assessed on ISI in the eCBT-I group was impressive, 43.9%, and 19.4% in the mPT group in the mITT population, a result in line with other online CBT-I interventions (13, 14). Data retrieved from the sleep diaries completed along the program also revealed a strong improvement in SE, SOL, and NWAK for patients following the eCBT-I program compared to patients assigned to the mPT group. These results were similar to those found in meta-analyses of automated CBT-I, and to those reported after either face-to-face or group delivered conventional CBT-I (13, 14, 34, 35).

We also reported that compared to the control group, fatigue, and anxiety symptoms improved in the eCBT-I group while a better quality of life assessed on a widely used visual analogic scale of the EQ5D was perceived in the eCBT-I group only. In contrast, depressive symptoms improved in both groups, a result in line with previous studies using eCBT-I programs showing reduction in affective symptoms even in the absence of specific interventions targeting depressive or anxiety symptoms (36). In contrast, no change in EDS complaint was found that may relate to a floor effect (rare patients with insomnia with high level of EDS) and sleep restriction instructions (37, 38). In contrast to most of sleep diary data, most of self-report functional parameters did not reveal between group differences from baseline to endpoint. These findings may relate to the

TABLE 2 | Effects of online cognitive and behavioral therapy (eCBT-I) compared to Minimal psychoeducation therapy (mPT) on sleep diaries parameters and self-report functional outcomes in the mITT population.

	mPT Group ($N = 20$)				eCBT-I Group ($n = 18$)					
	Baseline	Post-treatment	Within-Group Difference[(1)]		Baseline	Post-treatment	Within-Group Difference[(1)]		Between-Group Difference [(2)]	
Variable	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	***p***	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	***p***	**Median [95% IC]**	***p***
Primary outcome: ISI score										
ISI score	17.00 (5.00)	16.00 (6.50)	3.00 (7.00)	0.009	19.50 (3.00)	12.00 (9.00)	9.00 (7.00)	<0.0001	−7.00 [−10.00;−3.00]	0.0004
Secondary outcomes: Sleep diaries parameters										
TIB (min)	489.46 (59.64)	512.32 (53.60)	−7.50 (37.71)	0.44	508.57 (47.86)	421.79 (89.40)	68.75 (80.96)	<0.0001	−67.20 [−95.66; −41.07]	<0.0001
TST (min)	289.29 (114.63)	319.64 (112.65)	−26.07 (89.29)	0.04	303.57 (113.21)	335.18 (61.07)	−53.04 (79.29)	0.002	+24.64 [−17.14; +66.79]	0.26
SE	0.61 (0.22)	0.68 (0.19)	−0.04 (0.18)	0.06	0.61 (0.17)	0.82 (0.17)	−0.18 (0.19)	<0.0001	+ 0.15 [+0.09; +0.23]	<0.0001
Sleep onset latency (min)	33.21 (31.79)	32.50 (22.14)	1.96 (25.12)	0.60	41.96 (55.25)	15.89 (26.07)	23.80 (33.57)	0.0008	−23.93 [−42.50; −9.29]	0.004
Number of awakenings	1.04 (0.80)	0.64 (0.86)	0.11 (0.79)	0.09	1.14 (1.00)	0.32 (0.64)	0.79 (1.14)	0.0003	−0.50 [−0.93; −0.07]	0.03
Wake after sleep onset (min)	36.61 (28.57)	32.86 (39.64)	8.04 (22.68)	0.10	35.36 (25.00)	9.64 (33.93)	22.68 (33.57)	0.02	−11.79 [−28.21; +6.43]	0.16
Other outcomes: Self-report functional outcomes										
ESS score	9.00 (6.00)	10.00 (8.00)	1.50 (5.00)	0.29	10.00 (6.00)	8.00 (4.00)	0.50 (4.00)	0.13	0.00 [−2.00; +2.00]	0.93
BDI score	14.50 (10.00)	8.00 (8.00)	5.00 (10.00)	0.02	14.50 (12.00)	5.00 (4.00)	6.50 (10.00)	<0.0001	−3.00 [−8.00; +2.00]	0.21
EQ-5D utility score	0.78 (0.12)	0.78 (0.09)	0.00 (0.05)	0.92	0.76 (0.09)	0.78 (0.09)	0.00 (0.09)	0.16	0.02 [0.00; +0.09]	0.21
EQ-5D VAS	60.00 (20.00)	61.00 (20.00)	−3.50 (22.00)	0.35	67.50 (20.00)	75.00 (10.00)	−15.00 (25.00)	0.004	+10.00 [−5.00; +25.00]	0.17
STAI-E	35.00 (14.00)	37.00 (16.00)	−0.50 (7.00)	0.79	35.00 (14.00)	32.00 (12.00)	7.50 (12.00)	0.02	−6.00 [−11.00;0.00]	0.03
CFS physical score	4.00 (3.00)	4.00 (4.00)	0.00 (2.00)	0.63	5.00 (2.00)	3.00 (4.00)	1.00 (2.00)	0.003	−1.50 [−3.00;0.00]	0.01

[(1)] Indicates median change between baseline and post-treatment evaluation.

[(2)] Indicates median change difference between the mPT group and the eCBT-I group.

TABLE 3 | Change in Insomnia Severity Index from baseline in response to either online cognitive and behavioral therapy (eCBT-I) or minimal psychoeducation therapy (mPT) by participant characteristics in the mITT population.

Variable	mPT group		eCBT-I group			*p*-value
	No. of patients	Within-group ISI change from baseline[2]	No. of patients	Within-group ISI change from baseline[2]	Between-group difference [3]	
		Median (IQR)		Median (IQR)	Median [95% CI]	
Age, years[1]						
<45.5 years	10	1.00 (7.00)	8	10.50 (6.00)	−8.00 [−14.00; −2.00]	0.005
≥45.5 years	10	4.00 (5.00)	10	8.00 (11.00)	−5.00 [−10.00; 0.00]	0.07
Gender						
Male	6	4.00 (3.00)	3	13.00 (10.00)	−7.00 [−17.00; 7.00]	0.21
Female	14	2.00 (7.00)	15	8.00 (8.00)	−7.00 [−10.00; −3.00]	0.001
ISI score[1]						
<19	13	1.00 (7.00)	6	7.50 (8.00)	−6.00 [−12.00; 0.00]	0.02
≥19	7	5.00 (6.00)	12	9.50 (6.50)	−4.00 [−10.00; 1.00]	0.09
BDI score[1]						
<14	9	5.00 (2.00)	8	8.00 (7.50)	−3.00 [−10.00; 2.00]	0.12
≥14	11	1.00 (8.00)	10	11.00 (7.00)	−8.00 [−13.00; −4.00]	0.003

[1] The cut-off values were based on the median values of the whole sample.
[2] Indicates median change between baseline and post-treatment evaluation.
[3] Indicates median change difference between the mPT group and the eCBT-I group.

nature of the control condition. Our control group was in fact an active group, as it consisted on a 1 h face-to-face psychoeducation that offers high-quality information about sleep physiology and insomnia. Some of these patients benefited to this educational program (i.e., −3.0 points on ISI score) that may be a confounding factor thus minimizing the observed global differences between eCBT-I and control groups. In contrast, most of RCT on eCBT-I typically used a passive waiting list as the control group with thus higher between-group differences expected (13, 14, 38).

Overall, the online CBT-I intervention was well-received by participants, without adverse effects. Dropout attrition (subjects not completing final evaluation measures) was similar to previous RCT on eCBT-I, with 21.74% of lost.

Our eCBT-I program might also help in tapering off hypnotic medication use (with more than 90% of patients allocated to eCBT-I reducing or stopping sleep aids), even in the absence of specific given instructions. Several studies have highlighted the benefit of behavioral interventions (stimulus control, sleep restriction, relaxation) within the context of gradual tapering of benzodiazepines among patients with chronic insomnia. Such interventions may attenuate withdrawal symptoms and prevent relapse up to 12-month follow-up (15, 16, 39). This unexpected outcome is of major interest for general and socio-economic public health perspectives since 30% of the general population in France occasionally use anxiolytics or hypnotics (with 5–7% chronic users), making French users two to three time superior to most industrialized countries (40, 41). Dissemination of online mental health services has increased in the recent years and may thus change the management of high prevalence disorders. Our results showed comparable efficacy on insomnia measures than face-to-face CBT-I delivered by psychologists. Altogether, these findings have important policy implications, and eCBT-I should be part of initiative to educate the public and to manage some patients with insomnia after a comprehensive diagnosis made by physicians (42).

Several limitations should be taken into consideration when interpreting our results. The sample size is really small; however it is a clinically-derived population with strict eligibility criteria (i.e., sleep efficiency $\leq$ 79% based on two-week sleep diary, an ISI score $>$ 14). The exclusion of participants with clinically significant comorbid medical disorders or multiple psychiatric comorbidities may limit the generalizability of the results (43). Although the participants were recruited from a tertiary outpatient sleep center, the population included was well-educated and comfortable using the Internet. The post-treatment evaluation was conducted 2 weeks after the last intervention that preclude to assess on the long-term maintenance of efficacy of the eCBT-I. No long-term benefit-risk assessment was planned for this study. No objective measures of sleep such as polysomnography or actigraphy were used. However, the severity of insomnia complaints was assessed using the Insomnia Severity Index and sleep diaries, which are reliable and valid insomnia measures.

In conclusion, we developed the first eCBT-I program in French language and reported that this program delivered using online support seems feasible, acceptable and effective in improving sleep and related functional impairments in a small clinical population of adults with insomnia disorder but also in reducing CNS-active drug consumption. Our preliminary results should be replicated in larger, potentially less severe populations of patients with insomnia, and with multiple comorbid conditions to clarify if our findings are generalizable and maintained (44). Given the high prevalence of insomnia, the

large use of Internet worldwide and the robust effects reported, our data are supportive of the use of eCBT-I as an effective alternative to treat insomnia in daily clinical practice in French speaking countries.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by CPP Sud Méditerranée I, France, Number 2014-A01796-41. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YD and RL: drafting/revising the manuscript for content, including medical writing for content, study concept or design, interpretation of data analysis, and study supervision and coordination. MB and A-DB: authors of the French version of eCBT-I. SB and IJ: revising the manuscript for content, interpretation of data analysis, study concept or design, and statistical analysis. EE, LB, SC, and AB: revising the manuscript for content, interpretation of data analysis, and acquisition of data.

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Evaluation of Chronotype Among Children and Associations With BMI, Sleep, Anxiety, and Depression

Bassam Eid[1,2†], *Mary Bou Saleh*[3†], *Imad Melki*[1,2], *Paul-Henry Torbey*[1,2], *Joelle Najem*[3], *Maroun Saber*[3], *Nada El Osta*[4,5] *and Lydia Rabbaa Khabbaz*[3*]

[1] *Faculty of Medicine, Saint-Joseph University of Beirut, Beirut, Lebanon,* [2] *Department of Pediatrics, Hôtel-Dieu de France Hospital, Saint-Joseph University of Beirut, Beirut, Lebanon,* [3] *Laboratoire de Pharmacologie, Pharmacie clinique et Contrôle de qualite des medicaments, Faculty of Pharmacy, Saint-Joseph University of Beirut, Beirut, Lebanon,* [4] *Craniofacial Research Laboratory, Oral Health Unit, Faculty of Dental Medicine, Saint-Joseph University, Beirut, Lebanon,* [5] *Department of Prosthodontics, Faculty of Dental Medicine, Saint-Joseph University, Beirut, Lebanon*

Objectives: To evaluate possible associations between chronotype, weight, sleep problems, anxiety, and depression among children from 6 to 12 years of age.

Method: One-hundred children aged between 6 and 12 years were randomly recruited in five pediatrician clinics in the capital city of Beirut, Lebanon. The protocol was approved by the ethics committee of Saint-Joseph University and Hotel-Dieu Hospital and an informed written formal consent was obtained from one of the parents. The Sleep Disturbance Scale for Children (CCTQ), the Revised Child Anxiety and Depression Scale (RCADS)-Parent version, and the Children's Chronotype Questionnaire (CCTQ) were used.

Results: The majority of the sample (47%) presented an intermediate chronotype. There was a shift toward evening chronotype with increased age and a significant association between electronic devices use and an evening chronotype. Higher sleep disturbances were also observed among children with an evening chronotype. In particular, disorders of initiating and maintaining sleep, non-restorative sleep, excessive somnolence, and total SDSC were significantly higher among evening type children in our study. Finally, major depression domain scores were significantly higher among children with an evening chronotype.

Conclusions: Several findings of this study are important and explain factors associated to chronotype in children. Two important future perspectives can be highlighted: limiting electronic devices use among children in an effort to reduce circadian rhythm disturbances and identifying and treating sleep problems associated with eveningness, taking into account the possible presence of major depression among this population.

Keywords: chronotype, weight, sleep, anxiety, depression, children

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Maurice M. Ohayon,
Stanford University, United States

Reviewed by:
Lourdes DelRosso,
University of Washington,
United States
Kristen Knutson,
Northwestern Medicine, United States

***Correspondence:**
Lydia Rabbaa Khabbaz
lydia.khabbaz@usj.edu.lb

[†] *These authors have contributed equally to this work*

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INTRODUCTION

Humans show cyclic rhythmicity in a wide range of psychological, cognitive, and physiological behaviors as well as in hormonal variations. This natural rhythmicity is called the circadian rhythm and it affects several processes such as sleep-wake cycles, mood, hormone levels, cognition, and temperature. The trait determining individual circadian preference in rhythm is known as

chronotype, which is relative to cycles of external light and dark (1–5). Chronotypes are divided into three main categories: morning, intermediate, and evening.

Young children (2–6 years of age) seem to show a relatively strong preference for morningness (6–8) but transition toward eveningness begins in early childhood (9) and this shift is significantly more marked in adolescence (10) when a delay in the timing of sleep tends to be seen (11). At the end of the adolescence, a change toward morningness occurs (12). Studies among adolescents and adults have shown that eveningness was significantly associated with obesity when it is compared to morningness (13, 14) and evening chronotype was associated with changes in eating behavior (14–16) such as poor dietary control, high total calories and cholesterol intakes, consumption of a larger portion, late-night food intake, and a tendency to omit breakfast among adults (17, 18). Studies of the links between chronotype and weight among children are very scarce. Only one study was conducted among children with ADHD and revealed that evening preference plays a role in a mechanism linking ADHD to obesity (19). In addition to its possible association with weight, circadian preference was strongly linked with sleep quality in adults (20, 21) and eveningness was related to more sleeping difficulties and in particular to insomnia (22). In pre-school children (aged 4.5 years old), those with an evening chronotype seem to present more sleep difficulties than morning types as reported by parents, and consequently, more negative social consequences (23). Sleep problems were also found to be concurrent with anxiety, depression, conduct problems, and hyperactivity in both children (24, 25) and adolescents (26, 27).

Existing evidence suggests that sleep dysfunction during childhood could be an early manifestation of future adjustment problems (28, 29). Thus, sleep may be used as an early marker of psychopathology developing later and it may enable specialists to identify individuals at risk before the development of more serious symptoms (30). Indeed, among preschool children, sleep problems predicted depression and anxiety at 9–13 years (29). Furthermore, eveningness and sleep difficulties during childhood have been linked to worse academic performance later on, both at school and in university (31) and to adjustment difficulties, defined as internalizing and externalizing problems (32).

Even though several studies examined sleep among children, few of them focused on chronotype, and those were mainly conducted among adolescents. Eveningness has been previously linked to anxiety, depression, and general affective problems among adolescents (33–35) but no such data are available for children.

The period just before adolescence can be critical in determining future sleep pattern and psychological problems and very little is known on the associations between sleep, circadian preference, anxiety, and depression in children during this period. Thus, the primary objective of this study was to investigate the links between the chronotype, weight, sleep problems, anxiety, and depression among children aged between 6 and 12 years.

MATERIALS AND METHODS

Study Design and Population

The study was conducted between March and December 2018. First, five pediatrician clinics were randomly selected from a list of clinics based in Beirut. and randomly recruited in five pediatrician clinics in the capital city of Beirut, Lebanon. Inclusion was done during vaccination visit (before the administration of the vaccine) to make sure that the child is not sick at the time of the inclusion. Briefly, a list of appointments was provided on Monday morning (and for the coming week) by the staff, highlighting vaccination visits. From this list, selection was randomly performed. The exclusion criteria were: children taking any medication, children with any chronic diseases. A trained research assistant interviewed the parents in the waiting room. One-hundred and fifty parent's children were approached, of whom 28 refused to participate in the study, and 22 children presented at least one exclusion criteria. The final study sample consisted of 100 children aged between 6 and 12 years. In addition to filling the questionnaires listed below, socio-demographic information was recorded. Physical activity and electronic devices use were also collected. For physical activity, since the number of participants with no activity ($n = 3$) was small, the two categories of physical activity (no activity and 1 h/week) were combined. The categories were 0–1 h/week, 2 h/week, and $\geq$3 h/week. For electronic devices use, four categories were used: 0, 1, 2, and $\geq$3 h/week.

Sleep Disturbances

The Sleep Disturbance Scale for Children (SDSC) consists of 26 questions evaluating sleep problems during the 6 previous months. SDSC uses a five-point scale and a total sleep time of 1 (9–11 h) to 5 (<5 h) and for sleep latency of 1 (<15 min) to 5 (>60 min). The frequency of the symptoms of sleep disorder is measured on a likers scale of five-point: never (1); occasionally, meaning once or twice per month (2); sometimes, meaning once, or twice per week (3); often, meaning 3–5 times per week (4) and always, meaning six or seven times per week (5). Furthermore, in children, SDSC identifies six types of symptoms of sleep disorders: early and late sleep disorders (sleep latency, sleep duration, nocturnal awakenings, and sleep anxiety), sleep breathing disorders (snoring, breathing) disturbances of arousal (such as sleepwalking, nightmares, and night terrors), disorders of sleep-wake transition (rhythmic movements, hypnotic saccades, and bruxism), disorders of excessive sleepiness (difficulty to wake up, tiredness in the morning, and inappropriate nap) and sleep hyperhidrosis (or night sweating). Calculation of a total score and a score for each symptom is performed for each child. A higher score indicates higher risk of developing sleep disorders (36, 37). The Chronbach alpha of the questionnaire was 0.661.

Chronotype

The Children's Chronotype Questionnaire (CCTQ) (38) was developed based upon the previous work of Roenneberg et al. (39–41) and Carskadon et al. (42, 43).

The CCTQ is a 27-item, mixed format. Parents can respond to several open-ended questions concerning sleep/wake parameters for scheduled days (non-holidays) and holidays (free days). It has 27 items and a five-point chronotype (CT) score (44). It contains: 16 questions about sleep and wake parameters (e.g., lights-off time, bedtime, sleep latency, rising time, wake-up time, fully alert time, and regular naps) for scheduled and free days; In addition, there is a 10-item morningness/eveningness scale (range of 10–48); and one item number 27 named "chronotype (CT)" (range of 1–5). This is a single-item measure. Parents read a short description of different chronotypes and selected one of five categories that best represents their child's circadian phase preference (i.e., definitely a morning type, rather a morning type than an evening type, neither/nor type, rather an evening type than a morning type, or definitely an evening type).

The total score from the MES [morningness/eveningness (M/E) scale] is a sum of scores of items 17–26 only, and ranges from 10 to 48 (38). This score is used to classify individuals as:

TABLE 1 | Socio-demographic characteristics, eating patterns, physical activity, and electronic devices use of the participants and results obtained for the questionnaires of SDSC, CCTQ, and RCADS-P.

	*N**	Minimum	Maximum	Average	SD**
Age	100	6	12	9.10	2.250
Gender					
Males	38				
Females	62				
BMI (Kg/m^2)	100	11.0	30.9	19.004	4.118
Underweight	4				
Normal	57				
Obese	19				
Overweight	20				
Number of siblings	100	0	7	1.73	1.145
Crowding index	100	0.4	1.8	1.085	0.3153
At least one of the parents smoke			Premature birth		
No	62		No	94	
Yes	38		Yes	6	
Electronic devices use			Physical activity		
No	45				
1 h/day	20		0–1 h/week.....35		
2 h/day	12		2 h/week.....61		
≥3 h/day	23		≥3 h/week.....4		
Total CCTQ score	100	18	43	29.57	5.487
Chronotype categories					
Evening type	37				
Intermediate	47				
Morning type	16				
Total SDSC score	100	26	60	41.27	8.875
Parasomnias	100	2	23	11.73	4.417
Disorders of initiating and maintaining sleep	100	6	19	10.91	3.232
Sleep breathing disorders	100	3	7	3.66	1.335
Disorders of excessive somnolence	100	2	10	3.11	1.729
Sleep hyperhydrosis	100	3	11	5.85	2.418
Non-restorative sleep	100	4	16	6.01	2.699
Total RCADS-P score	100	12	102	40.32	15.592
Social phobia	100	1	26	12.45	5.709
Panic disorder	100	0	19	3.52	3.789
Separation anxiety	100	0	11	4.34	2.811
Generalized anxiety	100	0	17	7.91	3.861
Obsessive-compulsive disorder	100	0	39	6.51	5.743
Major depression	100	0	14	5.59	3.444
Total anxiety score	100	8	88	34.73	14.424

**N = % (total N = 100); **SD, standard deviation.*

morning type, intermediate type, and evening type (scores of ≤23, 24–32, and ≥33, respectively) (2). The questionnaire yielded a Cronbach alpha of 0.619.

RCADS-P

The Revised Child Anxiety and Depression Scale (RCADS)-Parent version is a self-assessment scale developed to identify and screen for the clinical symptoms of anxiety and depression in children or adolescents and is filled by the parents. This questionnaire includes 47 questions grouped into six subscales: Separation Anxiety Disorder, Anxiety Disorder (General Anxiety Disorder), Social Phobia, Panic Disorder, Major Depressive Disorder, and Obsessive Compulsive Disorder. RCADS provides a score for each subscale as well as a total score for anxiety, which is the sum of all anxiety subscales except MDD, a total score for all the scales (sum of six subscales). The higher the score, the more the child presents the clinical symptomatology of anxiety and depression (45, 46). The questionnaire had a Cronbach alpha of 0.759.

Ethical Considerations

The study protocol obtained the approval of Saint-Joseph University ethics committee of and Hotel-Dieu Hospital ethic committee (USJ, HDF, number CEHDF 1102). Prior to participating, an informed written formal consent was given by one of the parents. The study was conducted between March and December 2018. The self-administered anonymous questionnaires were filled by the children's parent(s).

Statistical Analysis

The statistical analyses were performed with SPSS software for Windows (version 24.0, Chicago, IL, USA). The level of significance was set at 0.05. The mean and standard deviation were calculated for continuous variables and percentage was calculated for categorical variables. The normality of the distribution of continuous variables was assessed by Kolmogorov-Smirnov tests. In the first step, univariate analyses using the Student's *t*-test or non-parametric Mann-Whitney test and ANOVA (analysis of variance) or its equivalent non-parametric Kruskal-Wallis test were performed. ANOVA (analysis of variance) followed by Tukey *post-hoc* tests or its equivalent non-parametric Kruskal-Wallis test were performed to evaluate the association between continuous variables.

To evaluate the association between continuous variables, Pearson and Spearman correlation coefficients were calculated, and to assess the relationship between categorical variables, Fisher Exact tests, and Chi-square independence tests were performed. In the second step, multiple regression analyses were performed according to the Enter Method; all independent variables with a -*p*- < 0.200 were entered into the equation simultaneously. Collinearity among independent variables was also examined. Finally, two regression models were executed; the first model included age, electronic device, social phobia, major depression, separation anxiety, and SDSC. Since, the variables disorders of initiating and maintaining sleep, non-restorative sleep, and sleep breathing disorders are domains that belong to SDSC, they were not included in the same multivariate model. Hence, the second model includes: age,

TABLE 2 | Association between chronotype categories and participants' characteristics.

		Chronotype			*p*-value***
		Evening type (*N** = 37)	Intermediate type (*N* = 47)	Morning type (*N* = 16)	
Age (years)	Mean ± SD**	9.97 ± 2.279[b]	8.79 ± 2.176[a,b]	8.00 ± 1.713[a]	**0.005**
BMI (Kg/m^2)	Mean ± SD	19.77 ± 3.976	18.55 ± 4.313	18.55 ± 3.828	0.632
Number of siblings	Mean ± SD	1.65 ± 0.789	1.74 ± 1.343	1.88 ± 1.258	0.801
Gender	Males	17 (44.7%)	18 (47.4%)	3 (7.9%)	0.173
	Females	20 (32.3%)	29 (46.8%)	13 (21.0%)	
Premature birth	No	34 (36.2%)	45 (47.9%)	15 (16.0%)	0.761
	Yes	3 (50.0%)	2 (33.3%)	1 (16.7%)	
Electronic devices use	No	9 (20.0%)	25 (55.6%)	11 (24.4%)	**0.009**
	1 h/week	7 (35.0%)	10 (50.0%)	3 (15.0%)	
	2 h/week	9 (75.0%)	2 (16.7%)	1 (8.3%)	
	≥3 h/week	12 (52.2%)	10 (43.5%)	1 (4.3%)	
Physical activity	≤1 h/week	15 (40.5%)	18 (38.3%)	2 (12.5%)	**0.217**
	2 h/week	22 (36.1%)	27 (44.3%)	12 (19.7%)	
	3–4 h/week	0 (0%)	2 (50.0%)	2 (50.0%)	
At least one parent smokes	No	22 (35.5%)	27 (43.5%)	13 (21.0%)	0.220
	Yes	15 (39.5%)	20 (52.6%)	3 (7.9%)	

**N = % (total N = 100); **SD, standard deviation; ***p-values in bold are significant.*

Chi-Square tests and Fisher Exact tests for the comparisons of categorical variables.

ANOVA followed by Tukey post-hoc tests and Kruskal-Wallis tests for the comparison of continuous variables.

[a,b]Different letters indicate the presence of a significant difference according to Tukey post-hoc tests. Bold values are significant.

electronic device, social phobia, major depression, separation anxiety, and disorders of maintaining sleep, non-restorative sleep and sleep breathing disorders.

The sample size was calculated according to the formula of Tabachnick and Fidell (47) taking into consideration the number of independent variables to include in the model: the formula used was $N = 50 + 8m$ (m being the number of independent variables for the primary outcome); Given that $m = 6$, we had to include at least 98 subjects in the study.

RESULTS

Sociodemographic Characteristics of the Participants

The total number of children included in this study was 100 children. The mean age of the population is 9.10 (±2.25) years old. Twenty percent of the children were overweight and 19% were obese (**Table 1**).

Physical activity and electronic devices use are also reported.

Scores of the CCTQ, SDSC, and RCADS-P Questionnaires

Approximately, half of the sample (47%) were classified as having an intermediate chronotype. The total SDSC and CCTQ scores were respectively 41.27 ± 8.875 and 29.57 ± 5.487. Detailed results are presented in **Table 1**.

Association Between Chronotype, BMI, Sleep, and RCADS-P Domains: Results of the Univariate Analysis

Evening type children were significantly older, but no associations were observed between chronotype and BMI (**Table 2**). Furthermore, there was a significant association between chronotype and electronic devices use: among evening type children, 52% used electronic devices ≥ 3 h/week vs. 4.3% only among morning type children (**Table 2**). **Table 3** shows significant associations between chronotype categories and SDSC or RCADS-P domains and **Table 4** presents correlations between chronotype scores and SDSC or RCADS-P domains.

Association Between Chronotype, BMI, Sleep, and RCADS-P Domains: Results of the Multivariate Analysis

Later chronotype (higher CCTQ score) was associated with higher SDSC score, indicating greater sleep disturbance; It was also associated with older age, electronic devices use and with higher score on the major depression domain of the RCADS-P (**Table 5**).

TABLE 3 | Associations between chronotype categories, SDSC, and RCADS-P domains ($N = 100$).

	CCTQ	N*	Average	SD**	p-value
Disorders of initiating and maintaining sleep	Evening type	37	11.46[b]	2.950	0.005
	Intermediate type	47	11.28[b]	3.450	
	Morning type	16	8.56[a]	2.128	
Disorders of excessive somnolence	Evening type	37	3.70[b]	2.259	0.020
	Intermediate type	47	2.87[a]	1.296	
	Morning type	16	2.44[a]	0.892	
Non-restorative sleep	Evening type	37	6.97[b]	3.655	0.019
	Intermediate type	47	5.55[a]	1.767	
	Morning type	16	5.12[a]	1.628	
Total SDSC score	Evening type	37	44.14[b]	8.331	<0.000
	Intermediate type	47	41.47[b]	8.637	
	Morning type	16	34.06[a]	6.981	
Social phobia	Evening type	37	10.35[a]	4.996	0.005
	Intermediate type	47	14.32[b]	5.809	
	Morning type	16	11.81[a,b]	5.431	
Major depression	Evening type	37	6.89[b]	3.332	0.002
	Intermediate type	47	5.28[a,b]	3.437	
	Morning type	16	3.50[a]	2.503	
Separation anxiety	Evening type	37	3.24[a]	2.510	0.009
	Intermediate type	47	4.89[a,b]	2.846	
	Morning type	16	5.25[b]	2.720	

Only significant associations are shown in the table (-p-value < 0.05).

**N = % (total N = 100); **SD, standard deviation.*

ANOVA followed by Tukey post-hoc tests and Kruskal-Wallis tests for the comparison of continuous variables.

[a,b]Different letters indicate the presence of a significant difference according to Tukey post-hoc tests.

TABLE 4 | Correlations between SDSC, CCTQ, and RCADS-P scores ($N = 100$).

		TAS	TS	SP	PD	MD	SA	GA	OCD	PS	DIMS	SBD	DES	SH	NRS	SDSC
Total anxiety score (TAS)	CC	1														
	-p-value															
Total RCADS-P score (TS)	CC	**0.977**	1													
	-p-value	**<0.000**														
SP	CC	**0.559**	**0.496**	1												
	-p-value	**<0.000**	**<0.000**													
PD	CC	**0.711**	**0.747**	0.068	1											
	-p-value	**<0.000**	**<0.000**	0.501												
MD	CC	**0.234**	**0.437**	−0.095	**0.407**	1										
	-p-value	**0.019**	**<0.000**	0.345	**<0.000**											
SA	CC	**0.476**	**0.431**	**0.321**	0.102	−0.039	1									
	-p-value	**<0.000**	**<0.000**	**0.001**	0.314	0.703										
GA	CC	**0.684**	**0.674**	0.169	**0.476**	0.187	0.128	1								
	-p-value	**<0.000**	**<0.000**	0.094	**<0.000**	0.062	0.203									
OCD	CC	**0.795**	**0.803**	0.093	**0.688**	**0.306**	**0.232**	**0.501**	1							
	-p-value	**<0.000**	**<0.000**	0.357	**<0.000**	**0.002**	**0.020**	**<0.000**								
Parasomnias (PS)	CC	**0.332**	**0.393**	0.139	**0.258**	**0.390**	0.026	0.202	**0.377**	1						
	-p-value	**0.001**	**<0.000**	0.169	**0.010**	**<0.000**	0.796	0.044	**<0.000**							
Disorder initiating maintaining sleep (DIMS)	CC	−0.025	0.029	0.056	−0.108	**0.235**	0.175	−0.039	−0.107	0.151	1					
	-p-value	0.803	0.777	0.581	0.283	**018**	0.082	0.702	0.290	0.133						
Sleep breathing disorders (SBD)	CC	**0.335**	**0.363**	0.039	**0.261**	**0.242**	−0.042	**0.349**	**0.415**	0.137	−0.138	1				
	-p-value	**0.001**	**<0.000**	0.701	**0.009**	**0.015**	0.681	**<0.000**	**<0.000**	0.175	0.170					
Disorder excessive somnolence (DES)	CC	−0.180	−0.141	**−0.206**	−0.152	0.113	−0.047	−0.162	−0.015	**0.321**	0.179	**0.199**	1			
	-p-value	0.073	0.160	**0.040**	0.130	0.264	0.640	0.107	0.883	**0.001**	0.075	**0.047**				
Sleep hyperhydrosis (SH)	CC	0.154	**0.207**	**0.141**	0.046	**0.221**	0.014	0.124	0.126	**0.434**	−0.002	**0.325**	**0.305**	1		
	-p-value	0.126	**0.039**	0.162	0.649	**0.027**	0.894	0.219	0.210	**<0.000**	0.986	**0.001**	**0.002**			
Non-restorative sleep (NRS)	CC	**−0.286**	**−0.240**	**−0.262**	−0.128	0.113	−0.190	**−0.246**	−0.116	**0.234**	0.048	0.040	**0.422**	**0.264**	1	
	-p-value	**0.004**	**0.016**	**0.008**	0.205	0.261	0.059	**0.014**	0.249	**0.019**	0.638	0.691	**<0.000**	**0.008**		
Total SDSC	CC	0.126	**0.212**	0.014	0.072	**0.433**	0.007	0.066	**0.208**	**0.746**	**0.467**	**0.293**	**0.551**	**0.605**	**0.468**	1
	-p-value	0.211	**0.034**	0.891	0.476	**<0.000**	0.943	0.513	**0.038**	**<0.000**	**<0.000**	**0.003**	**<0.000**	**<0.000**	**<0.000**	
CCTQ score	CC	−0.114	−0.030	**−0.201**	0.035	**0.343**	**−0.248**	0.105	−0.059	0.067	**0.326**	**0.200**	0.186	0.035	**0.241**	**0.301**
	-p-value	0.257	0.766	**0.044**	0.732	**<0.000**	**0.013**	0.299	0.558	0.509	**0.001**	**0.046**	0.064	0.726	**0.016**	**0.002**

Pearson and Spearman correlation coefficients were calculated to assess the relationship between categorical variables. The CC is used for Spearman and Pearson Correlation Coefficient. SDSC, Sleep Disturbance Scale for Children; RCADS-P, Revised Child Anxiety and Depression Scale parent version; SP, Social phobia; PD, panic disorder; SA, separation anxiety; GD, generalized anxiety; OCD, obsessive-compulsive disorder; MD, major depression; TAS, total anxiety score; CC, correlation coefficient. Values in bold are significant. Pearson and Spearman correlation coefficients.

TABLE 5 | Multivariate analysis according to Enter Method: chronotype score taken as the dependent variable.

	Unstandardized coefficients		Standardized coefficients	*t*	Sig.	95.0% Confidence Interval for *B*	
	B	Standard error	Beta			Lower bound	Upper bound
Age	0.497	0.236	0.204	2.104	0.038	0.028	0.966
Electronic devices use	1.034	0.473	0.230	2.185	0.031	0.095	1.973
Social phobia	−0.107	0.092	−0.112	−1.160	0.249	−0.291	0.076
Major depression	0.449	0.149	0.282	3.017	0.003	0.154	0.744
Separation anxiety	−0.281	0.194	−0.144	−1.446	0.151	−0.667	0.105
SDSC total score	0.169	0.055	0.273	3.052	0.003	0.059	0.279
Age	0.513	0.239	0.210	2.141	0.035	0.037	0.988
Electronic devices use	1.016	0.463	0.226	2.192	0.031	0.095	1.936
Social phobia	−0.111	0.088	−0.116	−1.269	0.208	−0.286	0.063
Major depression	0.207	0.149	0.130	1.384	0.170	−0.090	0.503
Separation anxiety	−0.330	0.187	−0.169	−1.766	0.081	−0.702	0.041
Disorders of initiating and maintaining sleep	0.530	0.156	0.312	3.406	0.001	0.221	0.839
Non-restorative sleep	0.226	0.179	0.111	1.265	0.209	−0.129	0.582
Sleep breathing disorders	1.072	0.385	0.261	2.784	0.007	0.307	1.837

SDSC: Sleep Disturbance Scale for Children.

DISCUSSION

Our results showed that the majority of the sample (47%) presented an intermediate chronotype which is consistent with several previously conducted studies (42, 48); However, 37% of the sample presented an evening chronotype, which was higher than other studies (48). This could be explained by the fact that different instruments are used, such as the youth self-report with the Morningness/Eveningness Scale in Children (MESC) in the study of Carskadon et al. (42). Furthermore, differences in age and gender distribution exist between the different study's samples. Among our sample, the average age was around 9 years old, which is closer to puberty onset, especially in females (consisting 62% of our sample). Indeed, it was previously reported that the onset of puberty triggers an evening preference among approximately 40% of youth, which is compounded by social changes (e.g., less parental control, technology) (49, 50). In addition, the chronotype depends on genetic and environmental factors (51) and these factors are specific to each population and each culture.

There was a shift toward evening chronotype with increased age: evening type children were significantly older than morning type (9.97 ± 2.279 vs. 8.00 ± 1.713 years of age). It was previously reported that a shift toward later sleep rhythm occurs from early to late adolescence (48, 52) and eveningness was associated with older participants in another recent study conducted among participants aged from 11 to 19 years (48).

Excessive use of electronic media (such as computers, tablets, smartphones, gaming consoles, etc.) among adolescents was known to be associated with a disruption in the circadian clock, irregular, shortened, and later sleep onset (53). Little is known about this topic among children, but one could predict that the same consequences would be seen. Indeed, our results showed a significant association between electronic devices use and an evening chronotype.

Higher sleep disturbances were also observed among children with an evening chronotype, similarily to previous report about eveningness among youth resulting in sleep deprivation (49, 50). Several authors previously noted that an evening preference was associated with an irregular sleep-wake schedule (54). In particular, disorders of initiating and maintaining sleep, non-restorative sleep, excessive somnolence, and total SDSC were significantly higher among evening type children in our study. In fact, the most common symptom of sleep disorders is non-restorative sleep, which results in daytime sleepiness (55). Several studies demonstrated that non-restorative sleep is associated with other various health problems such as heart disease, respiratory diseases, obesity, depressive symptoms, and suicide among adults (56). Non-restorative sleep and a short sleep duration were significantly linked to suicidal ideation in adolescents (57). Very few data exist about the consequences of non-restorative sleep among children but they are probably problematic. Thus, interventions aiming at screening sleep problems, especially non-restorative sleep associated with eveningness among children seem important because they might prevent further sleep complications later on in life.

Finally, anxiety domains of the RCADS-P were not associated to chronotype after performing the multivariate analysis but major depression scores were significantly higher among children with an evening chronotype (38–44). Exhibiting preference for eveningness has already been associated with several negative outcomes, in particular, depression (58–64), poor academic performance (34, 61), physical inactivity, higher rates of alcohol use, of smoking, and obesity (65, 66) among adolescents and adults. A recent report among children and adolescents between 11 and 19 years old (42) also showed that eveningness was associated with higher levels of depression.

The reciprocal effects between chronotype and depression are poorly understood during the critical developmental period extending from childhood to adolescence and the nature of our study (cross-sectional) does not allow to draw hypothesis on the cause-effect relationship between chronotype and depression. In addition, the nature of the questionnaire used (RCADS-P), which is a screening and not a diagnostic tool, does not allow to assume a diagnosis of major depression, only a high suspicion of it.

Our findings should take into account several limitations. The results were obtained through questionnaires filled out by the parent of the child. Although, parents are aware of when they put their children to bed and when they wake up, the ability of parents to report on their child's preference for evening vs. morning is more limited. No information about Tanner Stage was available even though it could affect chronotype. The cross-sectional design of this study limits the authors' ability to determine whether later chronotype preceded or followed the existence of high depression scores on the RCADS-P; thus, the methodology is not adapted to establish a causal relationship between chronotype and depression suspicion. Finally, we excluded any chronic disease and of chronic medication intake, which prevented us from studying the impact of comorbidities on chronotype.

Despite these limitations, several findings observed in this study explain factors associated to circadian rhythm disturbances in children; the aspects explored are important and warrant further investigations.

CONCLUSION

Significant associations were observed in this study between chronotype and sleep as well as between chronotype and major depression domain of the RCADS-P among children. Based on our results, two important future perspectives can be highlighted: (i) limiting electronic devices use among children in an effort to reduce circadian rhythm disturbances and (ii) identifying and treating sleep problems associated with eveningness, taking into account the possible presence of major depression among this population.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by ethics committee of Saint-Joseph University and Hotel-Dieu Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

BE and MB: data collection and analysis. P-HT and IM: data collection and protocol design. NE: statistical analysis and protocol design. JN and MS: data collection and writing manuscript draft. LR: protocol design, interpretation of data, writing the draft, and final manuscript.

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Efficacy of Tasimelteon (HETLIOZ®) in the Treatment of Jet Lag Disorder Evaluated in an 8-h Phase Advance Model; a Multicenter, Randomized, Double-Blind, Placebo-Controlled Trial

Christos M. Polymeropoulos, Michael A. Mohrman, Madison S. Keefe, Jennifer L. Brzezynski, Jingyuan Wang, Lydia S. Prokosch, Vasilios M. Polymeropoulos, Changfu Xiao, Gunther Birznieks and Mihael H. Polymeropoulos*

Vanda Pharmaceuticals Inc., Washington, CA, United States

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Ahmed S. BaHammam, King Saud University, Saudi Arabia

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Yoshiaki Yamaguchi, Kyoto University, Japan
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***Correspondence:**
Christos M. Polymeropoulos christos.polymeropoulos@vandapharma.com

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Background: Most travelers experience Jet Lag Disorder (JLD) symptoms due to misalignment of their circadian rhythms with respect to the new time zone. We assessed the efficacy and safety of tasimelteon (HETLIOZ®) in healthy participants using a laboratory model of JLD induced by an 8-h phase advance of the sleep-wake cycle (JET8 Study). We hypothesized that tasimelteon treatment in participants experiencing JLD would cause increased sleep time, increased next-day alertness, and reduced next-day sleepiness.

Methods: We undertook a randomized, double-blind, placebo-controlled trial in 12 US clinical research sleep centers. We screened healthy adults ages 18–73 years, who were eligible for the randomization phase of JET8 if they typically went to bed between 21:00 and 01:00, slept between 7 and 9 h each night, and slept at a consistent bedtime. We used block randomization stratified by site to assign participants (1:1) to receive a single oral dose of tasimelteon (20 mg) or placebo 30 min before their 8-h phase-advanced bedtime. The primary endpoint was Total Sleep Time in the first 2/3 of the night ($TST_{2/3}$), which was measured by polysomnography during the 8-h sleep episode, and assessed in the intent-to-treat population. The trial is completed and registered with ClinicalTrials.gov, NCT03373201.

Results: Between October 16, 2017 and January 17, 2018, we screened 607 healthy participants for JET8, of whom 320 (53%) were assigned to receive tasimelteon ($n = 160$) or placebo ($n = 160$). Tasimelteon treatment increased $TST_{2/3}$ (primary endpoint) by 60.3 min (95%CI 44.0 to 76.7, $P < 0.0001$) and whole night TST by 85.5 min (95% CI 64.3 to 106.6, $P < 0.0001$), improved next day alertness, next day sleepiness, and shortened latency to persistent sleep by −15.1 min (95% CI −26.2 to −4.0, $P = 0.0081$).

Conclusion: A single dose of tasimelteon improves the primary symptoms of JLD, including nighttime insomnia and next day functioning among participants in a laboratory model of JLD simulating eastward trans-meridian travel by inducing an 8-h phase advance of the sleep-wake cycle.

Keywords: jet lag disorder, circadian rhythm, tasimelteon, sleep, circadian, Hetlioz, jet lag

INTRODUCTION

Jet Lag Disorder (JLD), also known as Jet Lag and Circadian Rhythm Sleep-Wake Disorder—Jet Lag Type, is a Circadian Rhythm Sleep-Wake Disorder (CRSWD) characterized by a mismatch between the timing of an individual's endogenous circadian cycle and the sleep and wake patterns required following a rapid change in time zone. After traveling across two or more time zones, a traveler's endogenous circadian clock becomes misaligned with the destination's local time zone (1, 2). The mismatch between a traveler's endogenous circadian cycle and their extrinsic 24-h light-dark cycle may cause them to experience symptoms of JLD due to a misaligned circadian rhythm with respect to the new time zone. The essential features of JLD, according to the International Classification of Sleep Disorders Third Edition, are night-time insomnia and daytime sleepiness when the sleep-wake schedule is shifted in the new time zone (1). Other symptoms that may be associated with JLD include impairment of daytime functioning and malaise (3). Excessive daytime sleepiness and circadian misalignment can lead to impaired concentration, attention, performance, and alertness (4). JLD can also be associated with gastrointestinal symptoms, headache, fatigue, general malaise, decreased appetite, indigestion, and menstrual symptoms in women, though these are not the cardinal features of the disorder (5).

The endogenous circadian clock drives an intrinsic 24-h rhythm in humans that regulates hormone levels, body temperature, metabolism, and the sleep-wake cycle. The circadian timing system (CTS) is composed of a hypothalamic circadian pacemaker located in the suprachiasmatic nuclei (SCN), an array of SCN outputs, and a system of molecular clocks in peripheral tissues (6). The light-dark cycle is the major environmental time cue and most powerful synchronizer ("zeitgeber") of the CTS to the Earth's 24-h day. Light sensed by intrinsically photosensitive Retinal Ganglion Cells (ipRGCs) provides information on the daily light-dark cycle to the SCN via the retinohypothalamic tract (RHT). Through this input, the SCN is synchronized (entrained) to a 24-h light-dark cycle. The SCN also functions as the master body clock by entraining the body's peripheral clocks to a 24-h rhythm through endocrine, neuronal, and physiological signals that regulate cyclic levels of melatonin, cortisol, and core body temperature (7, 8). Through the synchronization of peripheral clocks, the SCN governs the optimal timing of key physiological processes, including those of the cardiovascular system, metabolism, immune regulation, and the rest-activity and sleep-wake cycles (9–11).

Sleep disruption is the primary symptom of JLD, and can be especially severe when traveling in an eastward direction, which requires a phase advance of both sleep-wake timing and circadian rhythmicity (5, 12). Following eastward travel, the first two thirds of the night is usually very disrupted, as that portion of the night overlaps with the traveler's wake-maintenance zone (13). Further, a phase advance of longer than 7 h can induce an "antidromic" response, wherein the individual will start delaying to the new phase instead of advancing (for instance, delaying 16-h instead of advancing by 8-h) (12). Due to the phase advance associated with eastward flights across 3–8 time zones, travelers have difficulty going to sleep at the new bedtime, whereas after westward flights across 3–8 time zones, travelers have difficulty remaining awake in the evening and have early morning awakenings in the new time zone (13, 14). Additionally, depending on the number of hours of the phase advance and speed to physiologically adjust to the new time zone, the main effect can be on sleep latency and may only affect the first night or two of the trip (15). For instance, west coast to east coast US travel results in a 3-h phase advance, which is equivalent to being required to sleep 3 h before an individual's usual bedtime. This would result in difficulty falling asleep, but after the first 3 h, the individual would be attempting to sleep during their typical sleep cycle. Additionally, for this phase advance, the effect may only be experienced on the first night, with adaptation to the new time zone on subsequent nights, as demonstrated in a previous study (15).

The hormone melatonin has a robust endogenous circadian secretory profile that peaks during night hours. Misaligned melatonin rhythms are associated with CRSWD including Non-24-H Sleep-Wake Disorder (Non-24), Delayed Sleep-Wake Phase Disorder, and JLD. Two types of G-protein-coupled receptors for melatonin, MT_1 and MT_2, are located in the SCN. Melatonin exerts sleep-promoting effects, although the exact mechanism is not clear. It has been demonstrated that activation of the MT_1 receptor by melatonin suppresses firing of SCN neurons (16), which may interfere with the wake-promoting signal. It has also been suggested that the sleep-promoting effects may result from melatonin's ability to induce hypothermia (17).

Tasimelteon is a Dual Melatonin Receptor Agonist (DMRA) that has been shown to regulate the timing of melatonin secretion when administered to participants in advance of the usual dim light onset of melatonin (18). We have previously shown that tasimelteon can entrain the circadian clock of individuals who are not entrained to the 24-h day and have Non-24 by administering tasimelteon every night 30 min before the target bedtime (18). We have further shown that administration of tasimelteon advances the circadian clock during a 5-h phase advance (15). It has also been reported that exogenous melatonin administration can entrain and shift the circadian clock in humans (19–21). Tasimelteon has not previously been tested to phase advance

individuals more than 5 h, and there are no phase response curve data available for tasimelteon. However, phase response curve data for melatonin suggests that the maximal resetting effect of melatonin for inducing a phase advance occurs at about 3-h before dim light melatonin onset (DLMO) timing (about 5-h before the start of the scheduled sleep episode) and can advance the endogenous circadian phase by about 1-h after one night (21). Little is known about the impact of administering a melatonin-receptor agonist following a phase advance in sleep timing of more than 5-h.

Here we present the results of the JET8 study, a phase III clinical trial to determine the efficacy and safety of a single oral dose of tasimelteon in healthy participants in a laboratory simulation of JLD associated with an 8-h phase advance of sleep-wake timing.

METHODS

Study Design

The study used a randomized, double-blind, placebo-controlled, parallel design and was approved by institutional review boards, either Chesapeake IRB or BioMed IRB, at all sleep centers. Screening consisted of a clinical site visit to evaluate eligibility followed by a screening interval lasting from 1 to 4 weeks. Evaluation, which consisted of a clinical site overnight stay, followed successful completion of the screening procedure (**Figure 1**). Additionally, the end of Daylight Saving Time (DST) occurred during the study. Study sites were instructed not to schedule any participants for evaluation for the day before, or the week following, November 4, 2017. This was done to allow participants to naturally adjust to the 1-h phase delay induced by DST. Twelve US study sites with single-bed suites were utilized for the evaluation visit. Suites were free of time cues such as light and sound.

Participants

Participants recruited were men and women aged 18 to 73 years, in good health (determined by medical and psychiatric history, physical examination, electrocardiography, serum chemistry, hematology, urinalysis, and urine toxicology), who were not at high risk for common sleep disorders including obstructive sleep apnea and chronic insomnia, as assessed using validated questionnaires. Study participants provided written informed consent before any screening procedures began.

At the inpatient screening visit, participants self-reported their habitual bedtime and self-determined target bedtime. Participants were excluded at screening if their habitual and target bedtimes were not between 21:00 and 01:00, or if their average habitual total reported sleep durations were less than 7-h, or more than 9-h. After completion of the screening visit, participants were required to maintain a consistent sleep-wake schedule for at least 1 week prior to the start of the study. Participants were instructed to adhere to their self-selected target bedtime for the duration of screening. Participants were eligible for randomization if they met their target bedtime, ± 30 min, for the three nights prior to the inpatient evaluation visit, and at least five of the seven nights before the evaluation visit. Exclusions also occurred if participants did not have an average sleep episode duration of at least 6.7-h and no more than 9-h for the seven nights before evaluation. For the last three nights prior to the inpatient evaluation visit, the participant must have had at least 7-h and no more than 10-h, of self-reported total sleep time for each night.

Randomization and Masking

Randomization was performed through an interactive web response system (IWRS). When each participant arrived at the evaluation visit, the investigator or designee utilized the IWRS system to distribute study medication from the capsule-containing bottle. Participants were randomly assigned to either tasimelteon (20 mg) or placebo, in a 1:1 ratio. All capsules were size 1, opaque, hard gelatin, and the color was dark blue with two white bars printed on both the cap and body of the capsule. Placebo was provided in size and appearance identical to those containing tasimelteon. An unblinded, third-party statistician prepared the randomization scheme. Randomization was stratified by study site.

Procedures

During screening, assessments taken included the Karolinska Sleepiness Scale (KSS), a Visual Analog Scale (VAS), the Morningness-Eveningness Questionnaire (MEQ), and a post-sleep questionnaire (PSQ). The KSS and VAS were subjective measures of next day functioning and alertness. The KSS queried participants as to how sleepy they felt on a 9-point scale with 1 being extremely awake and 9 being extremely sleepy/fighting to stay awake. The VAS was a self-rated scale to assess sleepiness. Participants marked along a 100 mm line to represent their current state of sleepiness, 0 being very sleepy and 100 being very alert. The PSQ was used as a subjective assessment of wakefulness after sleep onset (WASO), sleep latency, total sleep time (TST), number of nocturnal awakenings, and overall sleep quality. Overall sleep quality was reported on a scale from 1 to 5, corresponding to "poor," "fair," "average," "good," or "excellent."

Daily electronic diaries and wrist actigraphy were recorded to ensure compliance with eligibility parameters during outpatient screening. These eligibility parameters were used to ensure that the participants were not acutely or chronically sleep deprived in the days preceding the inpatient overnight phase advance of sleep-wake timing.

For the 8-h sleep-wake phase advance, each participants' bedtime was advanced by 8-h compared to the target bedtime established during screening (**Figure 2**). A single oral dose administration of medication, tasimelteon or placebo, occurred 30 min (± 5 min) prior to lights off. Tasimelteon and placebo were manufactured by Patheon Pharmaceuticals Inc. in Cincinnati, Ohio, US. Participants' sleep duration, timing, and architecture were monitored objectively by polysomnography (PSG), and centrally scored in 30-s epochs. Polysomnographic data were analyzed for the single inpatient 8-h sleep episode during the evaluation visit.

During evaluation, assessments taken included the KSS, VAS, and PSQ. The KSS and VAS were each administered four times following the dose administration after awakening. The first KSS

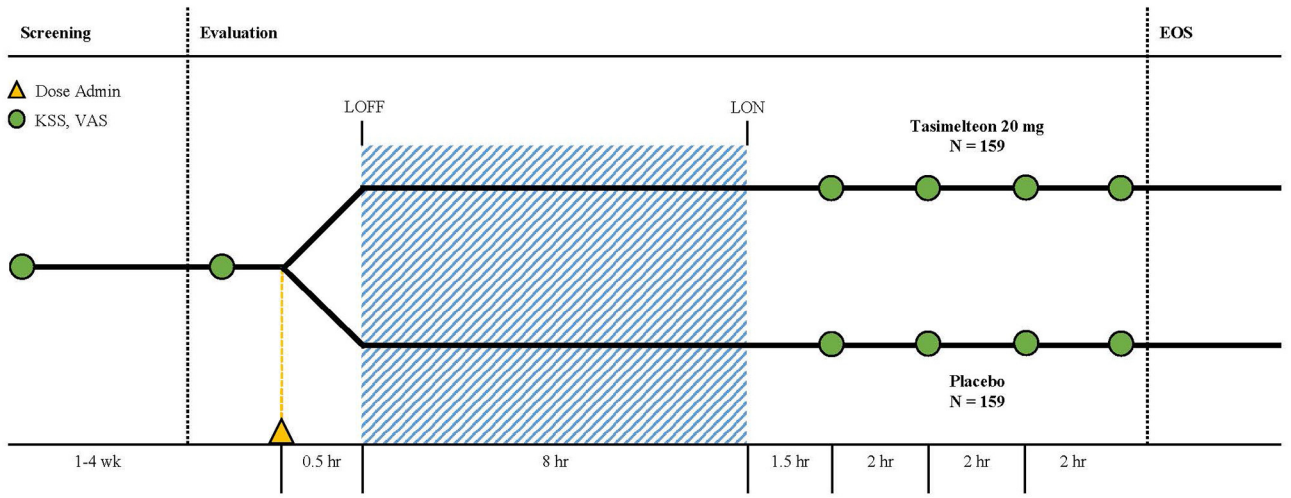

FIGURE 1 | The study consisted of a 1–4 week screening period followed by an evaluation visit where patients were dosed with tasimelteon 20 mg or placebo 30-min prior to their pre-determined 8-h phase advance bedtime. Polysomnography was performed during the 8-h sleeping period. Karolinska Sleepiness Scale (KSS) and Visual Analog Scale (VAS) assessments were completed once during screening, once before the phase advance, and four times after waking.

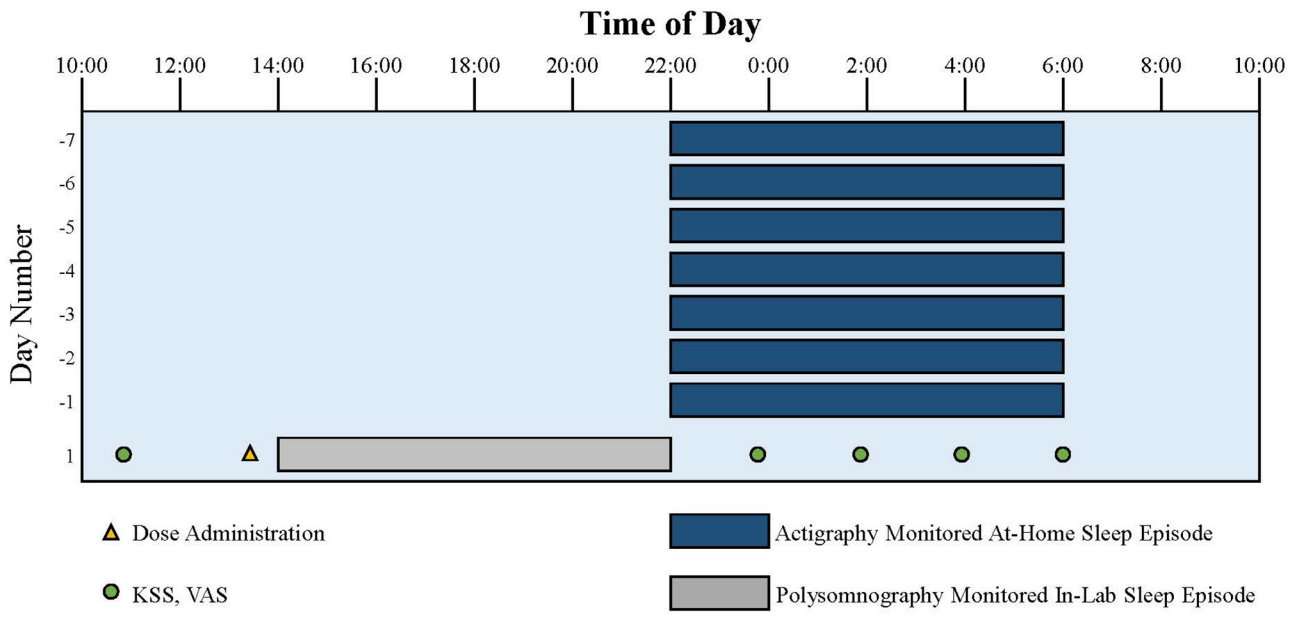

FIGURE 2 | Representation of the study protocol for an individual with a usual target bedtime of 22:00. Dark blue bars indicate at-home sleep monitored with actigraphy and photometry. Gray bar indicates in-lab sleep monitored with polysomnography following the 8-hour phase advance imposed to model the change of sleep timing required after a trip from Los Angeles to London. Yellow triangle indicates dose administration. Green circles indicate KSS and VAS assessments.

and VAS assessments were conducted 90 min (± 15 min) after wake time, and the following three KSS and VAS assessments were conducted once every 2-h, over the following 6-h. The PSQ was administered once, approximately 30 min (± 15 min) after the end of the bedrest episode, following the 8-h phase advance.

Outcomes

The primary outcome measure was total sleep time in the first two-thirds of the night ($TST_{2/3}$) following an 8-h phase advance of the sleep episode. $TST_{2/3}$ was selected as the primary endpoint for multiple reasons. The first two-thirds of the night are maximally overlapped with the previous circadian day, including the circadian wake maintenance zone (13), when the output of the central circadian pacemaker is most likely to interfere with sleep. Further, many individuals habitually sleep less than 8 h per night and therefore wakefulness in the final third of an 8-h interval may represent the normal morning after a sufficient night of sleep.

Secondary objective outcomes measured by PSG included TST, WASO, and latency to persistent sleep (LPS). WASO was defined as the time spent awake in the interval between onset of persistent sleep and the end of the bedrest episode. LPS was defined as the length of time elapsed between the start of the bedrest episode and onset of persistent sleep. Subjective secondary outcome measures included the next day residual effects of tasimelteon, as measured by individual and average KSS scores, individual and average VAS scores, and the PSQ. Average Night 1 KSS and Average Night 1 VAS combined all four KSS and VAS assessments collected after dose administration. These primary and secondary outcomes were centrally assessed across all study centers.

Safety assessments included regular monitoring and recording of adverse events; monitoring of hematology, serum chemistry, and urinalysis values; monitoring of vital signs; performance of physical examinations; and performance of electrocardiograms. Safety assessments were performed at the inpatient screening and evaluation visits, and any supplementary unscheduled visits.

Statistical Analysis

For hypothesis testing, a sample size of 150 individuals per treatment group was estimated to provide ∼3% power to detect a 30-min difference in $TST_{2/3}$ following an 8-h phase advance bedtime between the treatment group and placebo based on a two-tailed *t*-test with $\alpha = 0.05$ (15) and a standard deviation (SD) of 75 min, which was estimated from a previous study comparing doses of tasimelteon and placebo in healthy volunteers in a 5-h phase advance model (15). Additionally, this sample size provided at least 85% power to detect a difference of 28 min (SD 80) in TST between the treatment group and placebo.

The intent-to-treat (ITT) population was defined as all individuals randomized into the study who received a dose of study drug and had complete PSG data. The difference between treatment groups was summarized by the difference between the least squared means (LS) and the 95% confidence interval (95% CI). Hypotheses tested were declared statistically significant if the calculated *P*-values were ≤ 0.05. All primary and secondary objective outcome measures between treatment groups were analyzed by analysis of variance (ANOVA). The Wilcoxon Rank-Sum test of $TST_{2/3}$ was performed as a sensitivity analysis. All primary and secondary endpoints were pre-specified in the statistical analysis plan. All analyses and tabulations were performed using SAS® version 9.3 or higher. This study is registered with clinicaltrials.gov under NCT03373201.

RESULTS

Between October 16, 2017 and January 17, 2018 a total of 607 participants were assessed for eligibility in the JET8 study. 320 (52.7%) participants met the inclusion criteria and were enrolled. Following randomization, one participant (0.3%) in the placebo group withdrew consent and one participant (0.3%) in the tasimelteon group discontinued due to a serious adverse event determined to be unrelated to therapy. 318 individuals completed

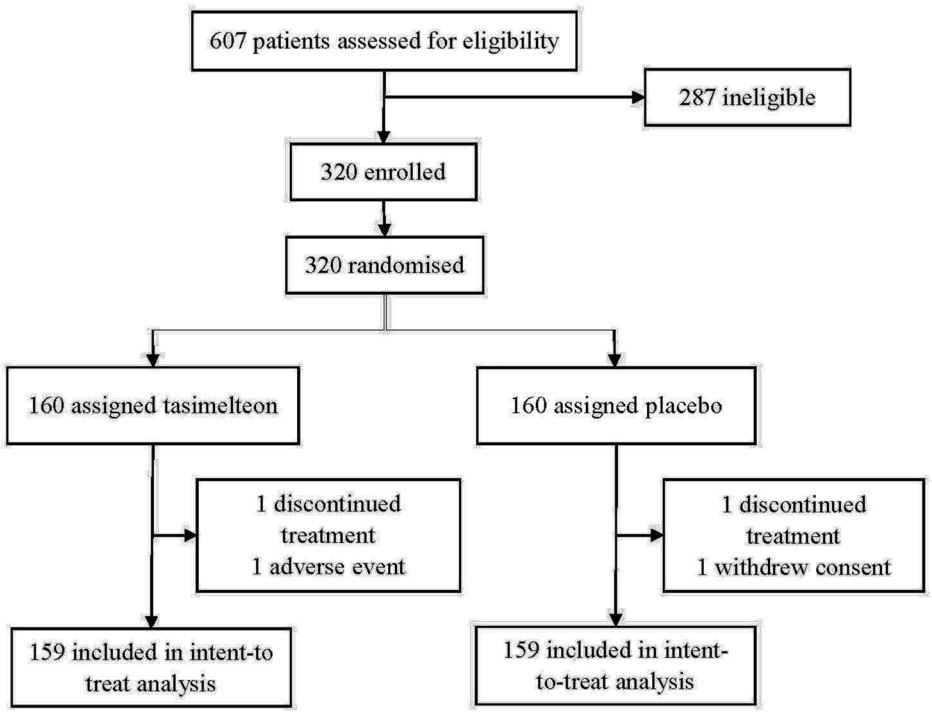

FIGURE 3 | Number of patients screened, enrolled, randomized, and discontinued for the JET8 study.

TABLE 1 | Baseline characteristics for the ITT population.

	Tasimelteon 20 mg ($N = 159$)	Placebo ($N = 159$)	Total ($N = 318$)
Age (year)	35.8 (11.20)	35.9 (12.63)	35.8 (11.92)
Sex			
Male	73 (45.9%)	78 (49.1%)	151 (47.5%)
Female	86 (54.1%)	81 (50.9%)	167 (52.5%)
Baseline BMI (kg/m^2)	25.1 (3.04)	24.8 (3.00)	25.0 (3.02)
Baseline MEQ	59.0 (7.78)	58.4 (8.52)	58.7 (8.16)
Race			
White	94 (59.1%)	97 (61.0%)	191 (60.1%)
Black or African American	44 (27.7%)	47 (29.6%)	91 (28.6%)
Asian	15 (9.4%)	13 (8.2%)	28 (8.8%)
Native Hawaiian or Other Pacific Islander	0 (0.0%)	0 (0.0%)	0 (0.0%)
American Indian or Alaska Native	1 (0.6%)	0 (0.0%)	1 (0.3%)
Other	5 (3.1%)	2 (1.3%)	7 (2.2%)

Data are n (%), or mean (SD). Baseline is defined as the last non-missing measurement prior to start of study drug.
BMI, Body Mass Index; MEQ, Morningness-Eveningness Questionnaire.

the study and were included in the ITT population analysis (**Figure 3**).

Baseline demographics were similar between tasimelteon and placebo groups. Of the 318 ITT participants, 151 (47.5%) were male. The average age was 35.8 (SD 11.92), and average BMI was 25.0 (SD 3.02). There was no significant difference in the baseline MEQ scores between the groups (**Table 1**). The racial breakdown was 191 (60.1%) White, 91 (28.6%) Black or African American, 28 (8.8%) Asian, 0 (0.0%) Native Hawaiian or Other Pacific Islander, 1 (0.3%) American Indian or Alaska Native, and 7 (2.2%) Other (**Table 1**).

Tasimelteon treatment resulted in increased $TST_{2/3}$ by 60.3 min (95% CI 44.0 to 76.7, $P < 0.0001$) in the first two-thirds of the night (**Table 2**).

Over the full 8-h sleep episode, TST increased by 85.5 min (95% CI 64.3 to 106.6, $P < 0.0001$) for the tasimelteon group compared to the placebo group. WASO was reduced by 74.6 min (95% CI −94.8 to −54.3, $P < 0.0001$) for the tasimelteon group compared to the placebo group. LPS was decreased by 15.1 min (95% CI −26.2 to −4.0, $P = 0.0081$) for the tasimelteon group compared to the placebo group (**Table 2**). Additionally, for each individual third of the night, average TST was significantly increased in the tasimelteon group compared to the placebo group. Average TST was increased for the tasimelteon group compared to the placebo group in the first third of the night (from minute 0 to 160) by 22.2 min (95% CI 14.1 to 30.3, $P < 0.0001$), in the second third of the night (from minute 161 to 320) by 38.1 min (95% CI 26.7 to 49.5, $P < 0.0001$), and in the final third of the night (from minute 321 to 480) by 25.1 min (95% CI 13.8 to 36.5, $P < 0.0001$) (**Table 2**). For each hour of the night, average TST was also significantly increased for the tasimelteon group compared to the placebo group (**Figure 4**).

Secondary outcome measures of subjective sleep efficacy were assessed by subject reported measures including the KSS, VAS, and PSQ. Average Night 1 KSS was decreased for the tasimelteon group by −0.5 pts (95% CI −0.9 to −0.1, $P = 0.0083$) compared to the placebo group (**Table 2**). Average Night 1 VAS was increased for the tasimelteon group by 6.6 mm (95% CI 1.6 to 11.6, $P = 0.0099$) compared to the placebo group (**Table 2**). KSS and VAS administered at each time point are shown in **Figure 5**. Subjective measures assessed by the PSQ including subjective WASO, subjective TST, and overall sleep quality were significantly improved for the tasimelteon group. Subjective WASO was reduced by −38.2 min (95% CI −64.2 to −12.2, $P = 0.0041$) for the tasimelteon group compared to the placebo group. Subjective TST was increased by 61.7 min (95% CI 34.5 to 88.9, $P < 0.0001$) for the tasimelteon group compared to the placebo group. Overall sleep quality increased by 0.6 pts (95% CI 0.3 to 0.9, $P = 0.0001$) in the tasimelteon group compared to the placebo group. Trends in subjective sleep latency and the subjective number of nocturnal awakenings were also observed (**Table 2**).

A pre-specified responder analysis of participants on tasimelteon and placebo showed that 11.3% of participants taking placebo achieved a sleep efficiency of $\geq$ 85% during the phase advance night vs. 32.7% of participants taking tasimelteon achieving this sleep efficiency ($P < 0.0001$).

The safety analysis included data from 320 participants (tasimelteon $n = 160$, placebo $n = 160$) who received one treatment dose after randomization. Discontinuation due to treatment emergent adverse events was insignificant between treatment groups. The most common treatment emergent adverse event reported in the JET8 study was headache (8[5%] tasimelteon vs. 4[2.5%] placebo). One serious adverse event was reported but was determined by the Principal Investigator to be unrelated to study drug.

DISCUSSION

Treatment with 20 mg tasimelteon achieved clinically meaningful improvements in objective and subjective measures of sleep duration and sleep quality for participants exposed to an 8-h phase advance, in a model of the phase shift induced when travelers fly eastward across eight time zones. The effects of tasimelteon were observed on the first night of treatment. Participants treated with tasimelteon not only fell asleep an average of 15.1 min faster, but also slept 85.5 min longer than placebo during the 8-h sleep episode scheduled at a time equivalent to the circadian challenge induced by a flight from Los Angeles to London. Participants taking tasimelteon reported lower sleepiness scores and higher alertness scores the day after the phase advance, compared to placebo. Participants taking tasimelteon, self-aware of the improvement, also reported that they fell asleep faster, spent less time awake during the night, slept longer, and rated their overall sleep quality higher as compared to individuals taking placebo.

Tasimelteon treatment also resulted in increased alertness and decreased sleepiness the day following the phase advance, as measured by the VAS and the KSS, which has been validated against objective EEG measures of sleepiness and

TABLE 2 | Summary of endpoints for the ITT population.

	Tasimelteon 20 mg (*N* = 159)	Placebo (*N* = 159)	Difference (95% CI)	*P*-value
Primary Endpoint				
$TST_{2/3}$ (min)	216.4	156.1	60.3 (44.0 to 76.7)	$P < 0.0001$
Objective Secondary Endpoints				
WASO (min)	144.6	219.1	−74.6 (−94.8 to −54.3)	$P < 0.0001$
LPS (min)	21.8	36.8	−15.1 (−26.2 to −4.0)	$P = 0.0081$
TST_{full} (min)	315.8	230.3	85.5 (64.3 to 106.6)	$P < 0.0001$
$TST_{firstthird}$ (min)	124.6	102.4	22.2 (14.1 to 30.3)	$P < 0.0001$
$TST_{secondthird}$ (min)	91.8	53.7	38.1 (26.7 to 49.5)	$P < 0.0001$
$TST_{finalthird}$ (min)	99.4	74.2	25.1 (13.8 to 36.5)	$P < 0.0001$
Subjective Secondary Endpoints				
Average Night 1 KSS (pt)	4.0	4.5	−0.5 (−0.9 to −0.1)	$P = 0.0083$
Average Night 1 VAS (mm)	60.8	54.2	6.6 (1.6 to 11.6)	$P = 0.0099$
Subjective WASO (min)	75.3	113.5	−38.2 (−64.2 to −12.2)	$P = 0.0041$
Subjective Sleep Latency (min)	27.0	40.9	−13.9 (−29.8 to 2.0)	$P = 0.0857$
Subjective TST (min)	393.6	331.9	61.7 (34.5 to 88.9)	$P < 0.0001$
No. Nocturnal Awakenings	2.6	2.8	−0.3 (−0.7 to 0.2)	$P = 0.2389$
Subjective Sleep Quality (pt)	3.3	2.7	0.6 (0.3 to 0.9)	$P = 0.0001$

Data are average measurements recorded by polysomnography for the primary and objective endpoints and recorded by questionnaire for the subjective endpoints. Tasimelteon treatment resulted in significant improvements in objective and subjective sleep time, wake after sleep onset, latency to persistent sleep, and next day alertness as measured by KSS and VAS.

$TST_{2/3}$, Total Sleep Time in the first 2/3 of the night; TST, Total Sleep Time; WASO, Wake After Sleep Onset; LPS, Latency to Persistent Sleep; KSS, Karolinska Sleepiness Scale; VAS, Visual Analog Scale; No., Number.

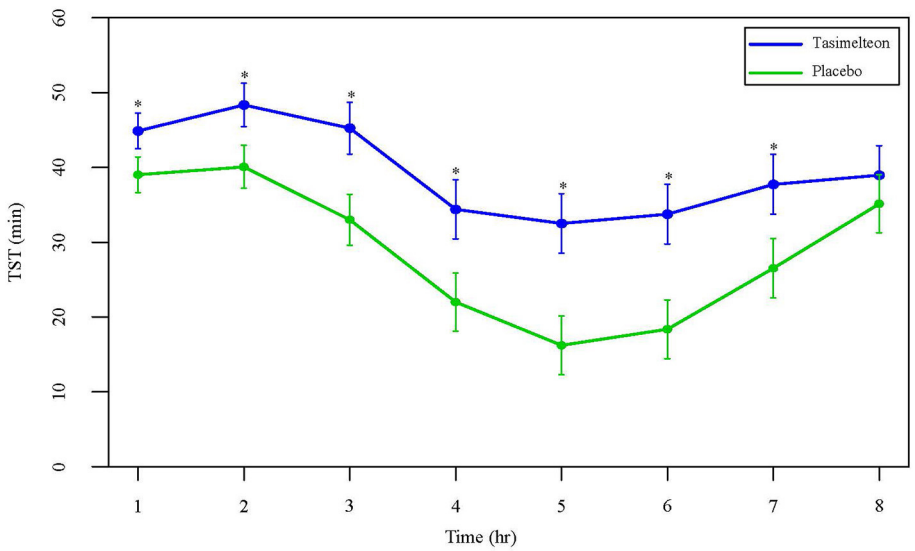

FIGURE 4 | Data are LS Mean (95% CI) for average TST by hour between tasimelteon and placebo. TST during the 8-h phase advance demonstrated a statistically significant increase in the tasimelteon group compared to placebo for hours 1–7. *$P < 0.001$.

objective measures of neurobehavioral performance (22). These improvements in next-day functioning are clinically important given the safety consequences of reduced alertness and sleepiness among individuals experiencing JLD resulting in occupational hazards and motor vehicle accidents. The symptoms of JLD can be severe in up to a quarter of patients. Further, the fatigue and cognitive effects can result in significant errors when performance is critical, such as when operating machinery or being a member of the flight crew. Although it is well-known that sleep deprivation causes significant morbidity and mortality annually as a result of performance impairment, fatigue during times of eastward JLD itself has been implicated as the causative agent of several airplane accidents (14, 23, 24).

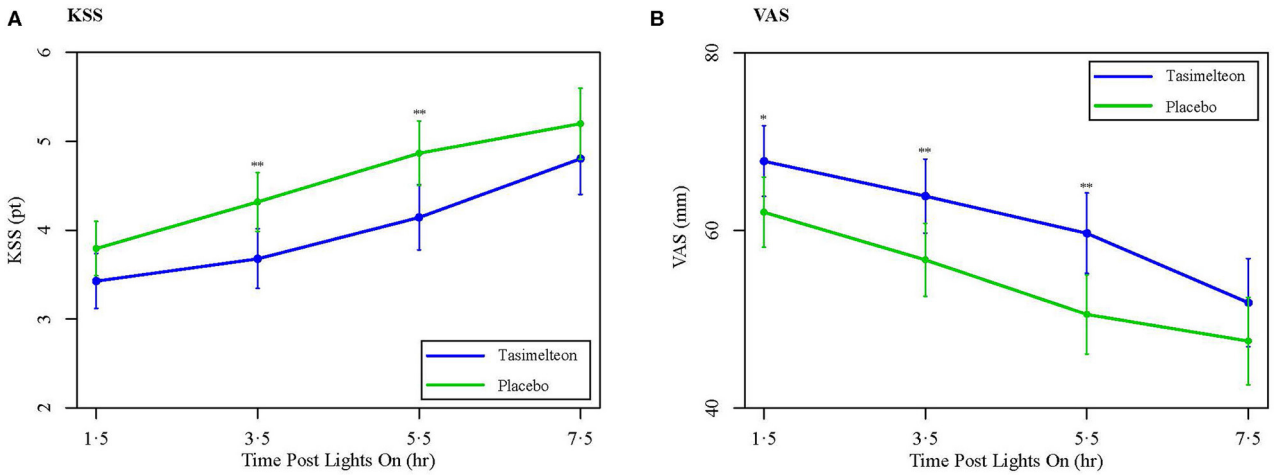

FIGURE 5 | (A) Data are LS Mean (95% CI) for average Night 1 KSS values between tasimelteon and placebo. KSS values, 1 pt being extremely awake and 9 pts being extremely sleepy/fighting to stay awake, demonstrated a statistically significant decrease following the 8-h phase advance in the tasimelteon group compared to the placebo group for 3.5 and 5.5 h post-lights on. $^{**}P < 0.01$. **(B)** Data are LS Mean (95% CI) for average Night 1 VAS values between tasimelteon and placebo. VAS values, 0 mm being very sleepy and 100 mm being very alert, demonstrated a statistically significant increase following the 8-h phase advance in the tasimelteon group compared to the placebo group for 1.5, 3.5, and 5.5 h post-lights on. $^{**}P < 0.01$, $^{*}P < 0.05$.

There is currently no treatment for JLD approved by the US Food and Drug Administration (FDA) or the European Medicines Agency (EMA). The American Academy of Sleep Medicine (AASM) recommends the use of light therapy, slowly changing sleep-wake schedule timing in advance of a trip, and the nutritional supplement melatonin, although an appropriate dosage form has not been determined (25). Caffeine may counteract jet lag-induced sleepiness but may also disrupt nighttime sleep (25). The AASM acknowledges the use of hypnotic sleeping pills as a rational treatment for JLD; however, they mention hypnotics are not necessary, have risks of adverse effects like global amnesia, and should be used on a short-term basis (25). Travelers who follow even the above recommendations continue to report symptoms of JLD.

It is estimated that about two-thirds of people who complete eastward, transmeridian travel across three or more time zones experience at least moderate JLD, and almost one-third experience severe symptoms of JLD (24, 26). In 2018, 93 million US citizens traveled internationally. Outbound overseas travel from the US totaled 41.8 million travelers (27). Of the millions of travelers who cross time zones each year, 80% report disrupted sleep during the scheduled sleep episode in the new time zone (28).

Although previous studies demonstrated that treatment with oral melatonin after a phase advance may reduce sleep onset latency and improve sleep quality (19, 20), increasingly studies are focusing on synthetic Dual Melatonin Receptor Agonists, as they may have a more stable pharmacokinetic profile, may be more potent, and may produce a greater effect than melatonin (5, 29). Prior studies with tasimelteon, which has selective agonist activity at the MT_1 and MT_2 receptors (15, 30), have demonstrated that when taken on a fixed time schedule, can entrain the circadian clock of blind individuals with Non-24 to the 24-h day (18). The central circadian pacemaker regulates the circadian rhythms of hormones, many aspects of physiology, metabolism, and behavior, including melatonin and cortisol, and synchronizes them with the 24-h day (6, 7). For this study, we hypothesized that tasimelteon would provide an alternative 24-h time cue necessary to resynchronize the CTS to the newly imposed 24-h day.

Tasimelteon has been shown to have different affinities for the MT_1 and MT_2 receptors, with a 2 to 4-fold higher affinity for the MT_2 receptor than the MT_1 receptor, and a 60% higher affinity for the MT_2 receptor than melatonin itself, but about equal affinity to melatonin to the MT_1 receptor (16). This is notable since the MT_2 receptor is responsible for mediating circadian phase shifting (16). The differential affinity between tasimelteon and melatonin at the MT_2 receptor may in part account for both the failure of melatonin to show benefit in a large JLD study and the success of tasimelteon in this model for JLD (26).

The robust results observed in this study confirm that participants who took tasimelteon had longer and better quality sleep. Lastly, we found that the morning following tasimelteon dosing, participants experienced fewer symptoms of JLD, as they reported feeling both more alert and less sleepy.

We believe that the underlying mechanism involves tasimelteon replacing the light-dark cycle as the time cue that synchronizes the CTS to the 24-h day. If the CTS of an individual with JLD can be re-synchronized to the new 24-h light-dark cycle, we predict that the master body clock will be able to regulate the secretion of hormones like melatonin and cortisol in phase with the new time zone, thus allowing an individual to feel alert during the day and sleepy at night.

While there are inter-individual differences in susceptibility to JLD, responder analysis demonstrates that only 11% of participants achieved a high (≥85%) sleep efficiency when treated with placebo during an 8-h sleep phase advance, whereas a third (32.7%) of participants reach that high sleep efficiency when

treated with tasimelteon following the 8-h sleep phase advance ($P < 0.0001$). Moreover, inter-individual differences in chronotype cannot account for this robust effect of tasimelteon, as the average MEQ score of the participants in the group treated with placebo was comparable to that of participants in the group treated with tasimelteon (**Table 1**).

In this study, tasimelteon was safe and well-tolerated. This clinical trial demonstrates that tasimelteon is effective for treating the symptoms of JLD following a shift in the timing of the sleep-wake cycle, comparable to that required following eastward travel across eight time zones.

Current therapies available for the treatment of JLD have not been validated and do not fundamentally address the underlying circadian dysfunction. These include sedative hypnotics such as zolpidem and eszopiclone, and wake promoting agents such as modafinil. In the largest ($N = 257$) double-blind trial to evaluate the efficacy of melatonin for the treatment of JLD, 0.5 and 5 mg of melatonin were reported to be ineffective (26). Similarly, treatment of healthy participants with 1, 4, or 8 mg of ramelteon failed to improve sleep (31). Moreover, 26% of over-the-counter formulations of melatonin, which is weakly regulated by the FDA as a food supplement and not as a drug, have been reported to be contaminated with serotonin, putting users at risk for serotonin syndrome (32).

Potential limitations of the study include that Jet Lag was induced by an immediate phase advance of the sleep-wake cycle in a sleep clinic, rather than jet travel in the eastward direction. However, the model in the JET8 study helps eliminate confounders unrelated to JLD, which is fundamentally a circadian dysfunction unrelated to the actual travel in a jet plane. Further, this potential limitation was tested in another study with tasimelteon, the JET study, wherein participants flew from the US to Europe. The JET study met its primary endpoint (source: in publication), demonstrating that tasimelteon is effective to treat JLD in a model that uses transatlantic flights. Additionally, JET8 participants were studied and dosed for one night, as opposed to multiple night dosing. However, JLD may affect individuals for only one night, depending on the degree of phase advance, the length of the trip, and the speed at which the individual adapts to the new time zone. Finally, the JET8 study protocol results in a "first night effect," in which a new sleeping environment induces insomnia. One potential limitation therefore is that a sleep study in a clinic may be at least partially causing first night effect-induced insomnia in addition to phase advance insomnia. However, given that following transmeridian travel many individuals will be sleeping in an unfamiliar environment such as a hotel, the present study also successfully simulates that aspect as well. Further, comparison of the differences in results between this study, which employed an 8-h phase advance, and the JET5 study, which employed a 5-h phase advance, demonstrates that a difference in the number of time zones advanced results in significant differences in JLD symptoms with and without treatment (15).

In summary, the results of the JET8 study demonstrate effectiveness of tasimelteon in treating the symptoms of JLD as shown in this model for the disorder. The magnitude of the total benefit over placebo is significant and clinically meaningful. The results of the study strongly suggest that tasimelteon may be an effective therapeutic tool in the treatment of individuals with JLD.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Boards of both Chesapeake and BioMed. The patients/participants provided their written informed consent to participate in this study. This clinical trial is registered at: HTTP://clinicaltrials.gov/, under TRN: NCT0337320.

AUTHOR CONTRIBUTIONS

CP, GB, CX, and MP contributed to the study concept and design. JW, CX, CP, and MP developed the statistical analysis plan. CP wrote the report in collaboration with MK, JB, VP, LP, and MM. CP revised the report with participation from all authors. All authors reviewed and approved the report before submission.

FUNDING

The funder of the study designed the study, performed data analysis, data interpretation, and writing of the report. The corresponding authors had full access to all of the data in the study and had final responsibility for the decision to submit for publication.

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Conflict of Interest: CP, MK, JB, MM, VP, LP, GB, JW, CX, and MP are employees of Vanda Pharmaceuticals. The clinical trial was sponsored by Vanda Pharmaceuticals.

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Sleep Inconsistency and Markers of Inflammation

Joseph M. Dzierzewski[1], Emily K. Donovan[1], Daniel B. Kay[2], Timothy S. Sannes[3,4] and Keighly E. Bradbrook[5]*

[1] Department of Psychology, Virginia Commonwealth University, Richmond, VA, United States, [2] Department of Psychology, Brigham Young University, Provo, UT, United States, [3] Division of Psychosocial Oncology and Palliative Care, Dana-Farber Cancer Institute, Boston, MA, United States, [4] Harvard Medical School, Boston, MA, United States, [5] Department of Biostatistics, Virginia Commonwealth University, Richmond, VA, United States

Objective: Poor sleep is associated with higher levels of inflammatory biomarkers. Conventionally, higher average time awake, lower average time asleep, and lower sleep efficiency define poor sleep. Recent research suggests that, in addition to average sleep, sleep inconsistency is an important indicator of sleep dysfunction. The current study sought to extend our knowledge of the relationship between sleep and inflammation through an examination of sleep inconsistency and inflammatory biomarkers.

Methods: Secondary analyses of the Survey of Midlife in the United States (MIDUS) sleep study were conducted. Five hundred thirty-three individuals completed nightly sleep diaries, actigraphy, and underwent a blood draw for the inflammatory biomarkers C-reactive protein, interleukin-6, and fibrinogen. Sleep inconsistency was derived from 7 consecutive nights of assessment and was operationalized as nightly fluctuations in the following variables: terminal wakefulness, number of awakenings, time in bed, sleep onset latency, and wake after sleep onset. Structural equation modeling was used to examine the influence of a latent average sleep and a latent sleep inconsistency variable on a latent inflammation variable. Models were subsequently adjusted for age, sex, BMI, health, and medication. Stratified models by sex were also analyzed.

Results: The average sleep model would not converge. The sleep inconsistency model fit the data well. A significant positive association between the latent factors sleep inconsistency and inflammation was observed ($\beta = 10.18$, $SE = 4.40$, $p = 0.021$), suggesting inconsistent sleep is associated with higher levels of inflammatory biomarkers. When stratified by sex, the association between the latent sleep inconsistency factor and inflammation was significant for women ($\beta = 1.93$, $SE = 0.82$, $p = 0.018$), but not men ($\beta = 0.20$, $SE = 0.35$, $p = 0.566$). The association between sleep inconsistency and inflammation weakened following multivariate adjustment ($\beta = 6.23$, $SE = 3.71$, $p = 0.093$).

Conclusions: Inconsistent sleep may be an associated feature of inflammatory dysfunction, especially in women. Future studies should build upon this preliminary work and examine these associations longitudinally and through treatment trials.

Keywords: sleep, actigraphy, biomarkers, cytokines, multivariate analyses, psychoneuroimmunology

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David Gozal,
University of Missouri, United States

Reviewed by:
Tianyi Huang,
Brigham and Women's Hospital and Harvard Medical School, United States
Masaya Takahashi,
National Institute of Occupational Safety and Health, Japan

****Correspondence:***
Joseph M. Dzierzewski
dzierzewski@vcu.edu

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INTRODUCTION

Sleep inconsistency, also called intraindividual variability and night-to-night variability in sleep patterns, has emerged as an important approach to quantifying sleep with implications for physical and mental functioning (1–3). Studies linking poor sleep to clinical health outcomes have traditionally focused on aggregate markers of sleep disturbance, such as average sleep duration and poor subjective sleep quality (2). For example, in one study all participants with self-reported short sleep (<5 h) and participants with self-reported long sleep (≥8.5 h) who also reported poor sleep quality had elevated allostatic load, measured by a multisystem biological risk index comprised of various biomarkers representing seven different physiological systems (4). Evidence suggests sleep inconsistency may provide unique information beyond that of average sleep (5). The importance of sleep inconsistency highlighted by previous studies mirrors a broader trend in medical research recognizing that the presence of individual-level variation in behavior and cognitive functioning may reflect impaired physiologic systems and brain processes (6–9). Likewise, sleep inconsistency may represent an underlying collection of dysfunction in biological systems involved in sleep-wake regulation, dysfunction that may be missed by only examining average sleep.

Biological mechanisms through which poor sleep adversely impacts health outcomes continue to be identified and include proinflammatory responses (10, 11), the sympathetic nervous system (12), the renin-angiotensin-aldosterone system (13), and endothelial renal functioning (14). Systemic inflammation has been one of the most intensely studied of these potential mechanisms (10). Markers of systemic inflammation, such as C-reactive protein (CRP), interleukin 6 (IL-6), and fibrinogen have been linked to poor sleep, with higher levels of markers associated with poorer sleep (15, 16). The association between sleep and inflammation may be most robust in women (17, 18). Systemic markers of inflammation have been also observed to be altered in several clinical samples with sleep disturbance including rheumatoid arthritis and end-stage renal disease (15, 19). While the relationship between sleep and inflammation is likely bidirectional, systemic inflammation may also mediate the association between sleep dysfunction and adverse clinical outcomes (20–22). However, several studies have failed to find an association between sleep characteristics and markers of inflammation (10, 23). One reason for these mixed findings may be the countervailing association between inflammation and sleep, as sleep dysfunction can alter inflammation and inflammation can also lead to sleep recuperation (15).

Due to the nuanced suspected associations between sleep and inflammation, aggregate measures of sleep, such as sleep duration and quality may not adequately capture the complex interrelationships that exist between sleep and inflammation. Sleep inconsistency, conversely, may serve as a more sensitive marker of these dynamic processes and as a more robust measure for studying the associations between sleep and inflammation (5). There are several reasons why sleep inconsistency and acute disruptions in an individual's circadian rhythm may drive inflammation. For instance, awakening someone from sleep typically results in acute inflammation (24); however, over a more prolonged period of awakening, there is a release of both proinflammatory and anti-inflammatory cytokines (25). Thus, while poor sleep quality overall is associated with a general inflammatory state (26), anti-inflammatory mechanisms following prolonged awakenings may represent a compensatory mechanism to disrupted circadian rhythms (25) and this balance may lead to greater inflammation overall. Additionally, the circadian clock is present in the majority of the body's cells and corresponds to a 24-h cycle (27), and inconsistent sleep may promote greater inflammation overall by continual disruption and resetting of these underlying cellular mechanisms. In fact, one study in older adults found that greater sleep inconsistency, defined as the within-person standard deviation of bedtime, waketime, and time in bed, was associated with greater circulating inflammatory markers IL-6 and Tumor Necrosis Factor-alpha (28). The current study aims to extend these previous findings by investigating the association between sleep inconsistency and systemic inflammation in middle-aged and older adults. We hypothesized that inconsistency in sleep would be associated with greater systemic inflammation.

MATERIALS AND METHODS

Procedures

Data from the National Survey of Midlife Development in the United States (MIDUS) 2, the second wave of a longitudinal study of health and well-being, were used for all analyses. The MIDUS study involved numerous ancillary studies, including investigations focused on sleep and inflammation (29). Of the 1255 participants in the MIDUS 2 biomarker project, data from the 533 participants who had actigraphy data collected at the University of Wisconsin-Madison were included in analyses. Participants in the MIDUS study provided written informed consent, and study procedures were approved by the Education and Social/Behavioral Sciences and the Health Sciences Institutional Review Boards at the University of Wisconsin-Madison. In order to participate in the MIDUS 2 biomarker project, individuals had to have met eligibility criteria for and participated in MIDUS 1 (i.e., aged 25–75, English speaking, non-institutionalized, and living in the coterminous United States) and have completed the MIDUS 2 phone survey and self-administered questionnaire (30).

Measures

Sleep

Sleep data were collected on participants for 7 consecutive days and nights. Participants were asked to wear a Mini-Mitter Actiwatch®-64 (Koninklijke Philips N.V., Amsterdam, Netherlands) on their non-dominant wrist, record bedtime and rise time using the event marker function of the watch, and complete a sleep diary daily. Both sleep diaries and event markers were used to determine bedtimes and rise times for each participant. Additionally, using Actiware 5 software and a medium threshold for sleep/wake detection, sleep onset latency (SOL: amount of time to fall asleep), number of awakenings (NWAK: count of nocturnal awakenings), wake after sleep onset

(WASO: amount of time awake during the night), and terminal wakefulness (TWAK: amount of time awake in the morning prior to rising from bed) variables were generated (5). To assess average sleep and sleep inconsistency, the individual means and standard deviations of SOL, NWAK, WASO, and TWAK were calculated using actigraphy data. Additionally, the individual means and standard deviations of time in bed (TIB: total amount of time spent in bed at night) using data available from the sleep diaries were calculated. Participants had exceptional compliance to the sleep assessments, with a mean number of missing actigraph data points of 0.18 (SD = 0.65). The median number of missing sleep measurements was 0 (range 0–4).

Inflammation

Methods for specimen collection and processing are described elsewhere (31), but briefly, venous blood for measurement of CRP, IL-6, and fibrinogen was collected between 06:30 and 07:00 from the non-dominant arm, when possible, into vacutainer tubes. For assays requiring serum (i.e., IL-6, high-sensitivity CRP) red/black serum separator tube(s) were used, and blue citrated tubes were used for assays requiring plasma (i.e., CRP and fibrinogen). Concentrations of IL-6 in serum samples were determined by using high-sensitivity enzyme-linked immunosorbent assays (ELISA; Quantikine® High-sensitivity ELISA kit #HS600B, R & D Systems, Minneapolis, MN) with a standard curve, for an assay range of 0.156–10 pg/mL. Citrated plasma levels of CRP were determined by a particle enhanced immunonepholometric assay and BNII nephelometer (Dade Behring, Deerfield, IL) for an assay range of 0.175–1,100 ug/mL. CRP samples falling below the assay range were re-analyzed in sera utilizing immunoelectrochemiluminescence and a high-sensitivity assay kit (kit #K151STG, Meso Scale Diagnostics, Rockville, MD) for an assay range of 0.014–216 ug/mL. Fibrinogen was measured in citrated plasma samples using the BNII nephelometer (N Antiserum to Human Fibrinogen; Dade Behring, Deerfield, IL) for an assay range of 60–1,200 mg/dL.

These three cytokines were selected for investigation for several reasons. CRP is an acute-phase protein that increases in the body's response to inflammation. Higher levels of CRP are seen across a number of disease states including diabetes and heart disease (32). IL-6 is a critical pro-inflammatory cytokines and is involved in the up or down-regulation of the inflammatory cascade, guiding the body's immune response (33). Both IL-6 and CRP are more strongly related to psychosocial factors when compared to other cytokines (34). Finally, fibrinogen is a protein that aids in tissue repair and increases with greater inflammation, (35) and has been previously linked to poor sleep patterns (36).

Covariates

Potential demographic and clinical covariates included age (years since birth), sex (male/female), BMI (self-reported weight in kilograms/self-reported height in m^2), health (0–10 self-rating of health from "worst possible health" to "best possible health," respectively), and medication (sum of three yes/no variables: antihypertensive, antidepressant, and cholesterol-lowering medication).

Data Analyses

Continuous covariates were summarized using means and standard deviations and categorical covariates using frequencies and percentages. Indicators of average sleep and sleep inconsistency were computed as the 7-days within-person mean sleep and standard deviation, respectively. Within-person variables were summarized using means and standard deviations. All analyses were performed using the lavaan package in R (37, 38), using a full-information maximum likelihood (FIML) technique which handles missing data within the analysis thereby using all available data (39). An alpha level of 0.05 was used for all tests, where appropriate.

Structural Equation Modeling

In order to examine the relationship between the latent factors, sleep and inflammation, structural equation modeling was used. A model with average sleep and a model with sleep inconsistency were built. Both models employed a two-step modeling approach to assess model fit (40). First, confirmatory factor analysis was used to validate and check the model fit of the measurement model, which included two-factors, either average sleep or sleep inconsistency and inflammation. The latent sleep factors were defined with either mean-values or within-person standard deviation values of TIB, NWAK, WASO, TWAK, and SOL; while a latent inflammation factor was defined with CRP, IL-6, and fibrinogen. SOL, TWAK and WASO inconsistency had variances several times larger than the other variables in the model so they were rescaled by a factor of 1/100 in the fitted model to place them on a similar scale to the other variables (rescaling has no impact on assessment of model fit). Once a good fit of the measurement model was established, the full theorized, unadjusted model was fit and a subsequent multivariate adjusted model (with predetermined covariates; **Figure 1** for unadjusted and multivariate adjusted models) (40). Models were also examined stratified by sex to explore possible heterogeneity in the sleep inconsistency to inflammation association.

Fit Indices

Model fit indices were evaluated for the measurement model, unadjusted model, and multivariate adjusted model, and the normalized residual covariance was examined to evaluate local fit of the model in any cases where the global fit indices indicated poor fit. All models described were assessed for global and local model fit issues. The Comparative Fit Index (CFI), Root Mean Square Error of Approximation (RMSEA), Standardized Root Mean Square Residual (SRMR), and the Chi-Squared Test of Model Fit were used to assess global fit. A CFI >0.90 or >0.95, RMSEA or SRMR <0.05, or non-significant Chi-Square value were all indications of good model fit. In order to assess local fit, the standardized residual covariance matrix was evaluated, and residuals >2 were considered an indication of an area in which the model was not adequately fitting the data.

RESULTS

Descriptive statistics for the covariates and observed sleep inconsistency and inflammatory variables are provided in

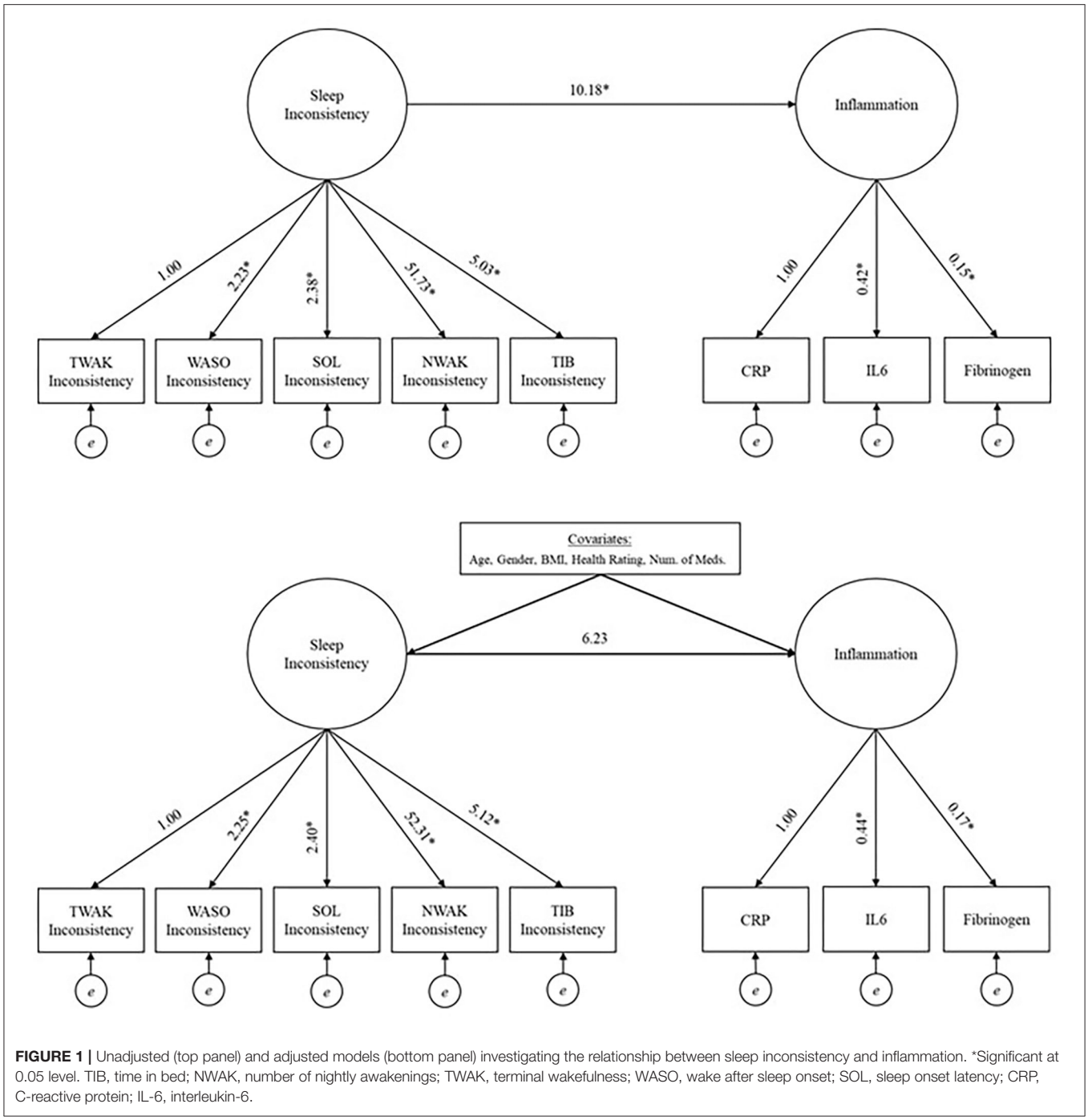

FIGURE 1 | Unadjusted (top panel) and adjusted models (bottom panel) investigating the relationship between sleep inconsistency and inflammation. *Significant at 0.05 level. TIB, time in bed; NWAK, number of nightly awakenings; TWAK, terminal wakefulness; WASO, wake after sleep onset; SOL, sleep onset latency; CRP, C-reactive protein; IL-6, interleukin-6.

Table 1. The coefficients of variation for the inflammatory biomarkers are as follows: 1.61, 0.93, 0.25 for CRP, IL-6, and fibrinogen, respectively. The measurement model for average sleep failed to converge, and subsequent analyses were not performed. The fit indices for the second measurement model (i.e., model building latent sleep inconsistency and inflammation variables from observed sleep and inflammation indicators), including the CFI (0.978), RMSEA (0.040), and SRMR (0.044), suggest good model fit (**Table 2**). The chi-squared test is significant ($p = 0.015$), but this result is expected given its sensitivity to large sample sizes. Overall, the measurement model fit well and was used to examine the full structural model, both unadjusted and multivariate adjusted models.

The model fit indices for the unadjusted model (i.e., the structural model, without covariates but including a path from the latent sleep inconsistency variable to the latent inflammation variable) are presented in **Table 2**, and the model results are presented in **Table 3** and **Figure 1** (top panel). The model

exhibited good fit as specified by a CFI (0.978), RMSEA (0.028), and SRMR (0.038). The chi-squared test is significant ($p = 0.015$), but, as above, this result is expected in large sample sizes. The manifest sleep inconsistency variables all significantly loaded on the latent sleep inconsistency factor (all p's < 0.003), and all manifest inflammation variables significantly loaded on the latent inflammation factor (all p's < 0.001). In the unadjusted model, there is a significant positive relationship between the latent sleep inconsistency factor and the latent inflammation factor ($\beta = 10.18$, $SE = 4.4$, $p = 0.021$) indicating that those with greater sleep inconsistency had higher levels of inflammation.

The model fit indices for the unadjusted model stratified by sex are presented in **Table 2**, and the model results are presented in **Table 4**. The models exhibited good fit as specified by a CFI (0.999, 0.940), RMSEA (0.000, 0.071), and SRMR (0.052, 0.073), respectively for the male and female models. The chi-squared tests were significant (p's = <0.0001); however, as above, this result is expected in large sample sizes. In the male model, the manifest sleep inconsistency variables did not significantly load onto the latent sleep inconsistency factor (all p's > 0.05); however, the manifest inflammation variables did significantly load on the latent inflammation factor (all p's < 0.001). In the male model, there was not a significant association between the latent sleep inconsistency factor and the latent inflammation factor ($\beta = 0.20$, $SE = 0.35$, $p = 0.566$). In the female model, the manifest sleep inconsistency variables did significantly load on the latent sleep inconsistency factor (all p's < 0.002), and the manifest inflammation variables did significantly load onto the latent inflammation factor (all p's < 0.001). In the female model, there was a significant association between the latent sleep inconsistency factor and the latent inflammation factor ($\beta = 1.93$, $SE = 0.82$, $p = 0.018$), suggesting that women with higher levels of inconsistent sleep also exhibit higher levels of inflammation.

The model fit indices for the multivariate adjusted model (i.e., full model including covariates) are presented in **Table 2** and the results are presented in **Table 3** and **Figure 1** (bottom panel). The model fit for the multivariate adjusted model is ambiguous, but several fit indices indicate that it may be less than adequate with both RMSEA and SRMR >0.05 and a significant chi-squared test ($p < 0.001$). However, the CFI (0.901) meets the minimal standard for good model fit. The manifest sleep inconsistency variables all significantly loaded on the latent sleep inconsistency factor (all p's < 0.003), and all manifest inflammation variables significantly loaded on the latent inflammation factor (all p's < 0.001). Age, sex, BMI, and health status were significant covariates, suggesting that these demographic characteristics help explain some of the variation in inflammation and should therefore be adjusted for in future studies. The relationship between the latent factors of sleep inconsistency and inflammation is weakened when controlling for covariates ($\beta = 6.23$, $SE = 3.71$, $p = 0.093$). The multivariate adjusted model would not converge when stratified by sex.

TABLE 1 | Descriptive statistics for covariates, sleep characteristics, and inflammatory markers.

	Mean (SD) or Frequency (%)
Gender	
Male	213 (39.96%)
Female	320 (60.04%)
Age	56.12 (11.56)
Race—White	314 (94.6%)
BMI	30.76 (7.23)
Self-rated health (0: Worst–10: Best)	7.60 (1.40)
Number of medications	2.23 (0.86)
Average sleep	
TIB	8.22 (2.55)
NWAK	32.47 (15.05)
TWAK	16.74 (15.05)
WASO	49.00 (36.40)
SOL	31.27 (49.22)
Sleep inconsistency	
TIB	1.22 (1.34)
NWAK	9.29 (5.31)
TWAK	22.18 (36.13)
WASO	20.32 (18.70)
SOL	26.60 (29.47)
Inflammatory markers	
CRP (ug/mL)	3.56 (5.74)
IL-6 (pg/mL)	3.44 (3.21)
Fibrinogen (mg/dL)	356.24 (89.09)

TIB, time in bed measured in hours; NWAK, number of nightly awakenings; TWAK, terminal wakefulness measured in minutes; WASO, wake after sleep onset measured in minutes; SOL, sleep onset latency measured in minutes; CRP, C-reactive protein; IL-6, interleukin-6. Average sleep was calculated as the 7-days average of each sleep characteristic. Sleep inconsistency was calculated as the 7-days individual standard deviation for each participant.

TABLE 2 | Fit indices from structural equation modeling predicting inflammation from sleep inconsistency.

		Unadjusted models			
Model fit indices	**Measurement model**	**Full model**	**Male model**	**Female model**	**Adjusted model**
Comparative fit index (CFI)	0.978	0.978	0.999	0.940	0.901
Root mean square error of approximation (RMSEA)	0.040	0.028	0.000	0.071	0.055
Standardized root mean square residual (SRMR)	0.044	0.038	0.052	0.073	0.073
Chi-Squared test p-value	0.015	0.015	<0.001	<0.001	<0.001

The following values indicate good fitting models: CFI > 0.90 or > 0.95; RSMEA < 0.05; SRMR < 0.08; Chi-Squared Test p-value > 0.05.

TABLE 3 | Results from structural equation modeling predicting inflammation from sleep inconsistency.

Measurement model	Unadjusted model			Adjusted model		
	Factor loadings	**Standard error**	***P*-value**	**Factor loadings**	**Standard error**	***P*-value**
SLEEP INCONSISTENCY						
TWAK	1.00	–	–	1.00	–	–
WASO	2.23	0.58	<0.001*	2.25	0.59	<0.001*
SOL	2.38	0.64	<0.001*	2.40	0.65	<0.001*
NWAK	51.73	13.77	<0.001*	52.31	14.06	<0.001*
TIB	5.03	1.71	0.003*	5.12	1.74	0.003*
INFLAMMATION						
CRP	1.00	–	–	1.00	–	–
IL-6	0.42	0.05	<0.001*	0.44	0.05	<0.001*
Fibrinogen	0.15	0.02	<0.001*	0.17	0.02	<0.001*
Regressions	**Structural coefficients**	**Standard error**	***P*-value**	**Structural coefficients**	**Standard error**	***P*-value**
INFLAMMATION						
Sleep inconsistency	10.18	4.40	0.021*	6.23	3.71	0.093
Age	–	–	–	0.05	0.02	0.008*
Sex	–	–	–	1.31	0.38	0.001*
Medication	–	–	–	−0.37	0.24	0.132
BMI	–	–	–	0.18	0.03	<0.001*
Health	–	–	–	−0.60	0.19	0.002*

**Significant at the 0.05 level. TIB, time in bed; NWAK, number of nightly awakenings; TWAK, terminal wakefulness; WASO, wake after sleep onset; SOL, sleep onset latency; CRP, C-Reactive Protein; IL-6, interleukin-6. Sleep inconsistency was calculated as the 7-days individual standard deviation for each participant. Unstandardized coefficients, as presented in this table, reflect the expected linear change in y (inflammation) for each unit increase in x (i.e., manifest variable or covariate).*

TABLE 4 | Results from unadjusted structural equation modeling predicting inflammation from sleep inconsistency stratified by sex.

Measurement model	Male model			Female model		
	Factor loadings	**Standard error**	***P*-value**	**Factor loadings**	**Standard error**	***P*-value**
SLEEP INCONSISTENCY						
TWAK	1.00	–	–	1.00	–	–
WASO	0.10	0.12	0.84	0.35	0.10	<0.001*
SOL	0.11	0.13	0.41	0.36	0.10	0.001*
NWAK	2.66	3.21	0.41	7.45	2.18	0.001*
TIB	0.19	0.25	0.77	1.08	0.34	0.002*
INFLAMMATION						
CRP	1.00	–	–	1.00	–	–
IL-6	0.45	0.08	<0.001*	0.43	0.06	<0.001*
Fibrinogen	0.13	0.03	<0.001*	0.16	0.02	<0.001*
Regressions	**Structural coefficients**	**Standard error**	***P*-value**	**Structural coefficients**	**Standard error**	***P*-value**
INFLAMMATION						
Sleep inconsistency	0.20	0.35	0.57	1.93	0.82	0.018*

**Significant at the 0.05 level. TIB, time in bed; NWAK, number of nightly awakenings; TWAK, terminal wakefulness; WASO, wake after sleep onset; SOL, sleep onset latency; CRP, C-Reactive Protein; IL-6, interleukin-6. Sleep inconsistency was calculated as the 7-days individual standard deviation for each participant. Unstandardized coefficients, as presented in this table, reflect the expected linear change in y (inflammation) for each unit increase in x (i.e., manifest variable or covariate).*

DISCUSSION

The present study sought to investigate possible associations between an emerging, novel quantification of sleep patterns, sleep inconsistency, and markers of systemic inflammation. Commensurate with our hypothesis, we found that greater sleep inconsistency was associated with greater inflammation. This association was most robust in women. Importantly, the observed sleep and inflammation variables all loaded onto common factors suggesting that both sleep inconsistency and

inflammation were highly correlated across various measures. These common factors were not observed in aggregate (i.e., mean) levels of sleep quality, the more commonly used markers of sleep dysfunction (i.e., a model aimed at building a latent mean-level sleep variable would not converge). As such, our analytic approach may signify underlying shared physiologic patterns of sleep inconsistency and inflammation. Past research on associations between sleep and inflammation has resulted in mixed findings, possibly due to evaluating aggregate measures of sleep rather than examining sleep inconsistency. However, preliminary research on sleep inconsistency has begun to identify patterns of associations between sleep inconsistency and a variety of inflammatory outcomes. As mentioned previously, one study of older adults found that inconsistency in sleep was associated with elevated peripheral inflammatory cytokines (28). Another study demonstrated that even one night of sleep disruption, as occurs frequently with on-call physicians, can impact an individual's immune modulation (41). Furthermore, in adolescents, sleep inconsistency measured across 1 week via actigraphy, was associated with higher levels of CRP (42). Nevertheless, the literature on sleep inconsistency and inflammation in adults remains limited, and the present study provides further preliminary insight into this association.

Finally, we found that, after controlling for covariates (i.e., age, sex, BMI, health rating, and medication), the association between the sleep inconsistency and inflammation latent factors was no longer significant. Since the adjusted model was no longer significant after the addition of covariates, it can be assumed that part of the relationship between sleep inconsistency and inflammation is due to demographic and clinical factors that account for differences in sleep inconsistency and inflammation. Previous research has shown that age (43), sex (44), BMI (45), self-rated health (46), and medication use [e.g., antihypertensives (47)], are associated with changes in sleep. These clinical and demographic factors have also been independently associated with inflammation [i.e., age (48), sex (49), BMI (50), self-rated health (51)], and medication use [e.g., antidepressants (52)]. Therefore, the findings of the present study are consistent with prior literature evaluating demographic and clinical predictors of sleep disturbance and inflammation.

There are a number of mechanistic pathways that may explain the relationship between sleep inconsistency and inflammation. For instance, when individuals are woken from sleep on just one occasion, an inflammatory cascade unfolds that can be measured in altered gene expression (53) and multiple awakenings and inconsistent sleep from night-to-night may further exacerbate the inflammatory response. Inflammation increases during stages 1 and 2 of sleep, as well as rapid eye movement (REM) sleep, while levels of inflammation during slow wave sleep are comparable to levels of awakening hours (54). Thus, returning to a level of homeostasis during slow wave sleep (lower levels of inflammation) may represent an integral physiological function of the various stages of sleep. However, if an individual wakes up at different times each night—or goes to bed at highly inconsistent times each evening—an inflammatory profile may be promoted, eventually making its way "downstream" to the physiologic markers, such as CRP and fibrinogen, measured in this study. Such inflammatory profiles may be particularly pronounced in populations known to have increased proportions of REM sleep to slow wave sleep, such as older adults or those suffering from depression (55–57).

Other factors could contribute to our findings. First, the overall levels of inflammation in this sample deserve further comment. For example, CRP is considered clinically relevant above the cutoff of 3 mg/L (58). One-third of the population has minor elevation in CRP, defined as levels >3 mg/L but <10 mg/L (58). This minor elevation has been associated with a variety of health outcomes, including those associated with tissue damage or environmental irritants (58). In our sample, mean CRP was 3.56 mg/L, indicating that our sample fell within this mildly elevated range. This could indicate that the association between sleep inconsistency and inflammation could be unique to individuals with at least mild systemic inflammation. Second, the current findings are noteworthy in light of other studies of sleep inconsistency. For instance, while our goal was to take the initial step toward identifying a common sleep inconsistency factor and examine associations with inflammation, previous work has identified that sleep inconsistency may be moderated by sex (28). In the current analyses, females indeed showed greater levels of inflammation. These sex differences and minor elevation in inflammation specific to our sample could be responsible for at least some of the associations of sleep inconsistency with inflammation.

The results of this study may help guide research on the mechanisms linking altered sleep processes and circadian misalignment to adverse health outcomes. Sleep and circadian processes are involved in the regulation of inflammatory cytokines, and experimental manipulation of both has been shown to increase blood concentrations of inflammatory cytokines (59–61). Likewise, inconsistent sleep may reflect a pattern of altered sleep and circadian processes that have implications on inflammatory regulation and health. For example, recent evidence suggests that sleep inconsistency confers risk for cardiometabolic disease (62–65). Given that inflammation plays a critical role in the pathogenesis of metabolic abnormalities (66), research is needed to explore whether inflammation is a mechanism through which sleep inconsistency and metabolic health are linked. Because sleep inconsistency is readily modifiable, this line of research may lead to sleep interventions aimed at preventing diseases that have been linked to altered sleep, including cardiovascular disease. Further, our finding of an association between sleep inconsistency and inflammation in women but not men (when stratified by sex) may be the result of gender differences in biological and socioeconomic factors. Future research is warranted.

There are a number of limitations warranting further comment in this study. First, peripheral markers of inflammation were only available at one time point. Such cross-sectional data limits our ability to quantify inconsistency in inflammatory processes over time, which has been suggested as a meaningful approach to linking inflammation to other health outcomes (6). Despite the current study being limited in only capturing sleep inconsistency, future research should target comparison of inconsistency across physiologic systems when possible (e.g.,

endocrine output coupled with behavioral activity throughout the day). Second, we were limited by the health behavior and comorbidity variables available in MIDUS. In order to extend the current findings, future research should implement collection of more robust time series of data within individuals. Examining longer epochs of data collection would allow researchers to identify antecedents of changes in sleep patterns or potential response to intervention, such as cognitive behavioral therapy for insomnia. Third, sources of sleep inconsistency (i.e., social jetlag, sleep disorders) were not readily discernable in the dataset and should be examined in future studies to identify potential differential effects on inflammation. Fourth, longitudinal investigations are needed to investigate whether the cross-sectional results hold over time. Lastly, the racial composition of the present sample prohibited important analyses based on race.

This study identified a significant relationship between sleep inconsistency and inflammation. Specifically, a latent factor of sleep inconsistency was found to be related to an overall inflammatory factor; however, this association was no longer significant after controlling for covariates. These novel findings extend prior work linking poor sleep patterns to physiologic dysfunction, further underscoring sleep inconsistency as a meaningful approach to quantifying sleep. Future research should extend measures of inconsistency to other physiologic systems as well as measuring how sleep inconsistency changes over time in conjunction with markers of inflammation.

DATA AVAILABILITY STATEMENT

The datasets analyzed for this study can be found in the Midlife in the United States study website: http://www.midus.wisc.edu.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Education, Social/Behavioral Sciences, and Health Institutional Review Boards at the University of Wisconsin-Madison. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JD, DK, and TS contributed to the conception and design of the study. KB organized the database and performed statistical analyses. JD wrote the first draft of the manuscript. JD, ED, DK, TS, and KB wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Resting Heart Rate Variability Is Associated With Subsequent Orthostatic Hypotension: Comparison Between Healthy Older People and Patients With Rapid Eye Movement Sleep Behavior Disorder

Yukiyoshi Sumi[1], Chikao Nakayama[2], Hiroshi Kadotani[3], Masahiro Matsuo[1], Yuji Ozeki[1], Takafumi Kinoshita[2], Yuki Goto[2], Manabu Kano[2], Toshitaka Yamakawa[4], Masako Hasegawa-Ohira[5], Keiko Ogawa[6] and Koichi Fujiwara[2,7]*

[1] Department of Psychiatry, Shiga University of Medical Science, Otsu, Japan, [2] Department of Systems Science, Kyoto University, Kyoto, Japan, [3] Department of Sleep and Behavioral Sciences, Shiga University of Medical Science, Otsu, Japan, [4] Department of Priority Organization for Innovation and Excellence, Kumamoto University, Kumamoto, Japan, [5] Faculty of Education, Shiga University, Otsu, Japan, [6] Graduate School of Humanities and Social Sciences, Hiroshima University, Higashihiroshima, Japan, [7] Department of Materials Process Engineering, Nagoya University, Nagoya, Japan

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André Diedrich,
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Almazov National Medical Research Centre, Russia

***Correspondence:**
Hiroshi Kadotani
kadotanisleep@gmail.com

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Background: Orthostatic hypotension (OH) caused by autonomic dysfunction is a common symptom in older people and patients with idiopathic rapid eye movement sleep behavior disorder (iRBD). The orthostatic challenge test is a standard autonomic function test that measures a decrease of blood pressure during a postural change from supine to standing positions. Although previous studies have reported that changes in heart rate variability (HRV) are associated with autonomic dysfunction, no study has investigated the relationship between HRV before standing and the occurrence of OH in an orthostatic challenge test. This study aims to examine the connection between HRV in the supine position and the occurrence of OH in an orthostatic challenge test.

Methods: We measured the electrocardiograms of patients with iRBD and healthy older people during an orthostatic challenge test, in which the supine and standing positions were held for 15 min, respectively. The subjects were divided into three groups: healthy controls (HC), OH-negative iRBD [OH (–) iRBD], and OH-positive iRBD [OH (+) iRBD]. HRV measured in the supine position during the test were calculated by time-domain analysis and Poincaré plots to evaluate the autonomic dysfunction.

Results: Forty-two HC, 12 OH (–) iRBD, and nine OH (+) iRBD subjects were included. HRV indices in the OH (–) and the OH (+) iRBD groups were significantly smaller than those in the HC group. The multivariate logistic regression analysis for OH identification for the iRBD groups showed the model whose inputs were the HRV indices, i.e., standard deviation 2 (SD2) and the percentage of adjacent intervals that varied by more than 50 ms (pNN50), had a receiver operating characteristic curve with area under the curve of 0.840, the sensitivity to OH (+) of 1.000, and the specificity to OH (–) of 0.583 ($p = 0.023$).

Conclusions: This study showed the possibility that short-term HRV indices in the supine position would predict subsequent OH in iRBD patients. Our results are of clinical importance in terms of showing the possibility that OH can be predicted using only HRV in the supine position without an orthostatic challenge test, which would improve the efficiency and safety of testing.

Keywords: orthostatic hypotension, OH, rapid eye movement sleep behavior disorder, RBD, heart rate variability, HRV, Poincaré plot, autonomic dysfunction

INTRODUCTION

Orthostatic hypotension (OH) is a major health concern in older people, affecting approximately one-third to two-thirds of them (1–3). A recent systematic review and meta-analysis found that OH is associated with falls in older people (4). OH symptoms, which include dizziness, titubation, blurry vision, syncope, nausea, and falls, occur due to temporary cerebral hypoperfusion and sympathetic hyperactivity (5). In particular, it is important to prevent falls associated with OH because injuries caused by falls may significantly impair quality of life.

OH is defined as a persistent decrease of systolic blood pressure (BP) $\geq$20 mm Hg, a persistent decrease of diastolic BP $\geq$10 mm Hg, or a decrease in systolic BP to $<$90 mm Hg within 3 min of standing or head-up tilt, or the manifestation of any OH clinical symptoms (2, 6, 7). It has been reported that a drop in BP at 1 min after standing from supine is important because it is associated with future adverse outcomes, including falls, fractures, and syncope (8). The American Society of Hypertension recommends measuring BP at 1 and 3 min after standing to detect BP decrease within 3 min (9). While aging, drug side effects, and dehydration can cause OH, autonomic dysfunction is one of the most important factors of OH, but it is often difficult to be aware of it (5, 10).

Rapid eye movement (REM) sleep behavior disorder (RBD) is REM parasomnia characterized by dream-enacting behaviors (11). Although RBD is widespread among patients with neurodegenerative disorders, such as Parkinson's disease (PD), dementia with Lewy bodies (DLB), or multiple system atrophy (MSA) (12–17), RBD is often a precursor of the clinical appearance of PD, DLB, or MSA. The idiopathic form without any neurological disorder is called idiopathic RBD (iRBD) (18). Although some iRBD patients have stayed disease-free for more than 10 years after being diagnosed with RBD (19), most iRBD patients phenoconvert to synucleinopathy over time (20), and it has been reported that severe cardiovascular autonomic dysfunction in iRBD is associated with phenoconversion to DLB (21).

In order to prevent falls of iRBD patients caused by OH, the severity of OH needs to be evaluated. One standard test for evaluating the severity of OH is the orthostatic challenge test, which usually takes place in clinics or hospitals (3). In the test, a subject is asked to change his/her position from supine to standing, and his/her BP is measured at fixed intervals during both the supine and the standing positions. Since the orthostatic challenge test requires medical staff to ensure subject safety, it is difficult to perform it in daily life. Thus, we should develop a simple alternative method for evaluating OH severity that can be easily performed even in daily life.

Autonomic dysfunction and OH are common among older people, particularly patients with α-synucleinopathies, such as PD, DLB, and MSA (10, 22). It has been reported that the more severe motor symptoms become, the more severe the autonomic dysfunction becomes in patients with PD (23).

The pathology of RBD involves brain regions that are responsible for the autonomic nervous system as well as the brainstem which regulates REM sleep (24, 25). Various clinical studies have reported that autonomic dysfunction is associated with iRBD. According to a multicentral study involving 24 institutes, 156 out of 531 patients with iRBD (29.4%) had orthostatic symptoms (26). In addition, the uptake of ^{123}I-metaiodobenzylguanidine (^{123}I-MIBG) in the iRBD patients tends to decrease, which indicates cardiac sympathetic denervation (27, 28). Thus, patients with iRBD potentially have autonomic dysfunction, and the severity of autonomic dysfunction may be utilized as a proxy variable for assessing OH severity.

Although various methods for evaluating the severity of autonomic dysfunction have been proposed, they have some problems. Interviews or questionnaires such as the Scales for Outcomes in Parkinson's Disease-Autonomic questionnaire (SCOPA-AUT) (23) may overlook OH symptoms of which patients are not aware. ^{123}I-MIBG is a quantitative test of autonomic dysfunction; however, it uses a radioactive substance and is expensive.

In this work, we focus on heart rate variability (HRV) which is spontaneous beat-to-beat oscillation in the R-R interval (RRI) of an electrocardiogram (ECG) and reflects the autonomic nervous function (29). According to research using RRI data collected during sleep by means of polysomnography (PSG), HRV indicators of patients with PD and iRBD are different from healthy controls (30–32), which indicates that patients with PD and iRBD have autonomic dysfunction. However, studies on HRV during an OH test of iRBD patients have not been performed.

We assumed that signs of autonomic dysfunction associated with OH appear even when in the supine position before standing in the orthostatic challenge test. Therefore, we conducted the orthostatic challenge test with ECG measurement for patients with iRBD and healthy older people in order to investigate the relationship between HRV in the supine position and the occurrence of OH. In addition, we discuss the possibility of the

use of HRV analysis as an alternative method to the orthostatic challenge test for evaluating OH severity.

METHODS

Subjects

Participants in this study consisted of healthy subjects and patients with iRBD. The details of this study were explained to them and written informed consent was obtained from each subject. In addition, the protocol was approved by the ethical committee at the Shiga University of Medical Science (R2017-199). Healthy subjects and patients with iRBD were recruited between December 2017 and August 2019.

We recruited healthy subjects over 60 years of age and without history of autonomic dysfunction.

In this study, patients who satisfied diagnostic criteria of RBD according to the 3rd edition of the International Classification of Sleep Disorders (ICSD-3) were recruited from among patients who visited the Shiga University of Medical Science. PSG was performed on all of the patients; who were then diagnosed with RBD. In order to focus on iRBD (18), patients with PD (33), DLB (34), or MSA (35) were excluded. Patients on antidepressants or with severe sleep apnea were also excluded because they might have symptoms mimicking RBD, such as medication-related dream enactment behaviors and apnea-related behaviors (36, 37). Patients with a history of cerebral infarction were also excluded.

A total of 60 healthy subjects and 29 iRBD patients were enrolled. One healthy subject was excluded because of a history of autonomic failure. Since a comorbidity of arrhythmia could affect the results of HRV analysis, 9 healthy participants and 3 iRBD patients with the comorbidity of arrhythmia were excluded (**Figure 1**).

The orthostatic challenge test (the details are described in the "Experimental Protocol" section) was conducted on the remaining 50 healthy subjects and 26 iRBD patients. One healthy subject who did not maintain the standing position during the standing phase was excluded, and 3 healthy subjects who satisfied the OH criteria were also excluded (the details of OH criteria are described in "Orthostatic Hypotension Criteria and Grouping" in the "Orthostatic Challenge Test" section). One iRBD patient to whom the examiner failed to attach devices was also excluded. The adaptation of the Hampel and the quotient filters (38, 39) resulted in the exclusion of 4 healthy subjects and 4 patients with iRBD.

Finally, 42 healthy controls (HC) and 21 patients with iRBD [12 OH (–) iRBD and 9 OH (+) iRBD] were included in this study. The definition of the subject grouping are described in "Orthostatic Hypotension Criteria and Grouping" in the "Orthostatic Challenge Test" section.

Experimental Protocol

The study consisted of a clinical interview and an orthostatic challenge test. The participants were instructed not to ingest

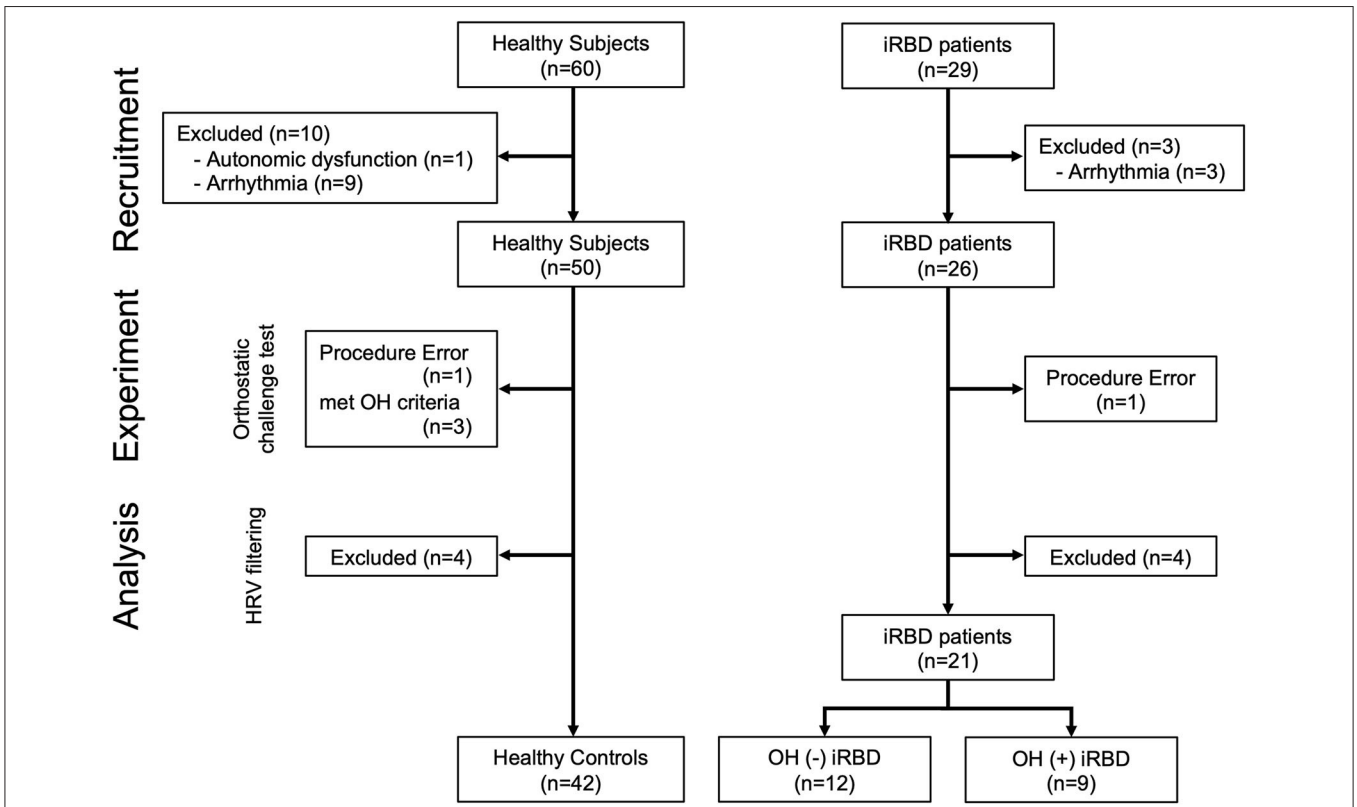

FIGURE 1 | Flowchart of subject enrollment. iRBD, idiopathic rapid eye movement sleep behavior disorder; OH, orthostatic hypotension; HRV, heart rate variability.

alcohol, caffeine, or to smoke the night before the experiment because these substances could affect the autonomic nervous system (40). The experiment was conducted between 2 p.m. and 4 p.m. to minimize the effect of the circadian rhythm on HRV (41). Subjects were instructed to finish meals 2 h before the start of the experiment (42).

Clinical Interview

The subjects were interviewed regarding their past histories including arterial hypertension, coronary artery disease, myocardial infarction, and diabetes mellitus, as well as their smoking habits, which could affect BP dynamics and autonomic nervous functions (43). Interviews about medication which could affect BP or HRV, including beta-blockers, calcium channel blockers, diuretics, angiotensin-converting-enzyme (ACE) and angiotensin II type 1 (AT II) inhibitors, organic nitrates, arrhythmic medications, atropine, scopolamine, atenolol, metoprolol, loop diuretics, monoamine oxidase inhibitors, antipsychotics, antidepressant agents, phenothiazine, phosphodiesterase type 5 inhibitors, parkinsonism agents, barbiturates, anesthetics, opioids, muscle relaxants, vincristine, or doxorubicin were also conducted (7, 43–49). The cognitive functions of the subjects were evaluated using the Mini-Mental State Examination (MMSE), which is a screening tool for dementia with a cut-off score of 23/24 (maximum score of 30) (50). The participants were asked to answer the REM sleep behavior disorder screening questionnaire (RBDSQ), which consists of 13 questions (maximum possible score = 13), to assess RBD symptoms such as nocturnal movements, injuries, and motor behavior during the night (51, 52). In this study, the Japanese version of the RBDSQ (RBDSQ-J) was used (53).

In addition, patients with iRBD were asked about the duration of their RBD disease, the onset of which was confirmed by family members.

Orthostatic Challenge Test

In order to evaluate behavior in daily life, an active standing test called the orthostatic challenge test was performed instead of passive standing tests such as the tilt test (3). The orthostatic challenge test was performed in our laboratories, in which the temperature and humidity were set to 23.0 ± 2.0°C [mean ± standard deviation (SD)] and 35.2 ± 12.7 % (mean ± SD), respectively.

Measurement Devices

A wearable RRI sensor (T. Yamakawa Lab, wireless R-R monitor Bluetooth LE model ver 0.7) was used for RRI measurement (**Figure 2**) (54). This sensor is able to record ECG signals at a 1,000 Hz sampling rate and to measure RRI automatically without requiring any special skills. The measured RRI data were sent to a Nexus5X smartphone [Google, LG. Operating system Android 6.0 (Marshmallow)] via Bluetooth Low Energy (BLE). The smartphone was placed in a running pouch attached to the back of the waist and the received RRI data were stored by a custom-made smartphone app (**Figure 2**) (55). The RRI error handling procedure in this study is described in the "Heart Rate Variability Analysis" section.

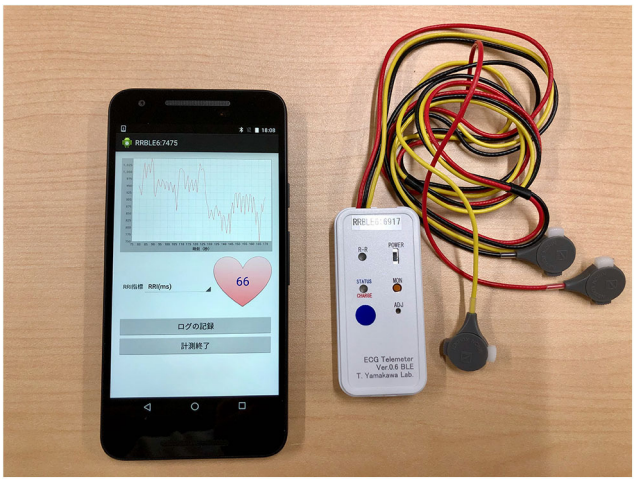

FIGURE 2 | Smartphone Nexus 5X and wearable RR intervals sensor. The RRI data acquired by the wearable RRI sensor was sent to the smartphone application in real time. This figure shows the heart rate 66 calculated from the RRI at that time. In this experiment, the electrodes of the wearable RRI sensor were attached to the three poles of the trunk of the subject, just as in a normal electrocardiogram. The smartphone was in a running pouch attached to the back of the waist of the subject. RRI, RR intervals.

In addition, BP and pulse rate (PR) were measured with a digital brachial BP monitor HEM-7500 (OMRON HEALTHCARE Co., Ltd., Japan). The BP monitor was attached to the left upper arm of the participant so that the cuff of the sphygmomanometer was at heart level. When the examiner pressed a button on the sphygmomanometer, the cuff was automatically pressurized and depressurized to record systolic BP (sBP), diastolic BP (dBP), and PR.

Orthostatic Challenge Protocol

The orthostatic challenge test was composed of two phases: supine- and standing-position phases (**Figure 3**). Before the test, the participants were equipped with the wearable RRI sensor, the smartphone, and the sphygmomanometer, and instructed to lie in bed at about 40 cm from the floor. In addition, they were instructed to avoid talking and not to fall asleep during the test.

After checking the device operation in the supine position (>2 min), the measurements of BP and PR began, they were performed four times with 3-min intervals. After being in the supine position for 15 min, the participants were instructed to quickly stand up and to keep their standing position for 15 min. After standing for 1 min, BP and PR measurement was performed once per min for 15 min. The examiner carefully monitored the safety of the participant during the test. After the orthostatic challenge test, the examiner, by asking the participants, confirmed the presence/absence of the following symptoms: dizziness, titubation, blurry vision, syncope, or nausea.

Orthostatic Hypotension Criteria and Grouping

The average of five BP and PR measurements of each participant in the supine position was defined as the baseline. The orthostatic

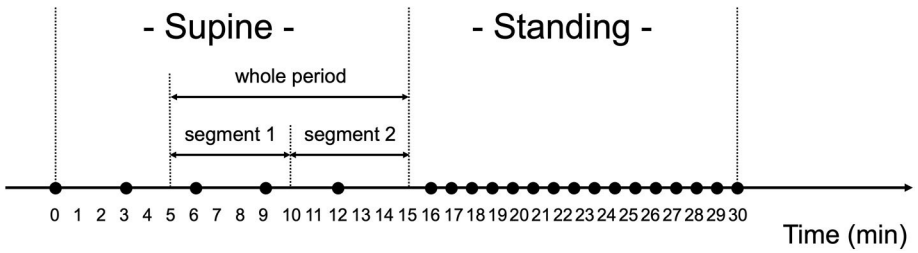

FIGURE 3 | Orthostatic challenge protocol. Black circles (•) show when blood pressure and pulse rate were measured. The measurements were done every 3 min when in the supine position, and every 1 min when in the standing position. Ten min worth of RRI data in the supine position were analyzed for heart rate variability, which were divided into two segments (segment 1: 6–10 min and segment 2: 11–15 min).

challenge test was determined to be positive when the participant satisfied one of the following conditions: (1) decrease of sBP ≥20 mm Hg, or decrease of dBP ≥10 mm Hg in comparison with the baseline BP at 1 or 3 min after standing, (2) a decrease in systolic BP to <90 mm Hg, or (3) manifestation of any clinical symptom including falling, dizziness, blurry vision, syncope, and nausea (2, 6, 7, 9). However, for the patients with baseline sBP ≥160 mm Hg, the criterion of a decrease of sBP ≥30 mm Hg was applied thereto, based on criteria proposed by the American Society of Hypertension (3, 9). In all other cases, the orthostatic challenge test was determined to be negative.

In patients with iRBD, those who met the orthostatic challenge test criteria were defined as "OH (+) iRBD," and those who did not meet the criteria were defined as "OH (–) iRBD." Among healthy subjects, those who did not meet the OH criteria were defined as "HC (healthy control);" on the other hand, those who met the OH criteria were excluded because they might have potential autonomic nervous dysfunction (**Figure 1**).

Heart Rate Variability Analysis

The HRV data extracted from the RRI data measured when in the supine position were analyzed (**Figure 3**). Data from the first 5 min in the supine position were excluded from the HRV analysis because RRI for the first 5 min may have been impacted by the posture change from the sitting to the supine position (56). Thus, data from the remaining 10 min were analyzed, which were named "whole period" and divided into two segments (segment 1: 6–10 min and segment 2: 11–15 min). In the manuscript, the results for segment 2 were mainly described because the subjects had been in the supine position for a long time; the results for segment 1 and the whole period are shown in the **Supplementary Table 1**.

Incidental arrhythmias such as premature atrial contractions (PAC) or premature ventricular contractions (PVC) are often observed in continuous ECG measurements of elderly people, although arrhythmias are not identified in short-term ECG examination. A high proportion of PAC and PVC in the elderly has been reported (57, 58); thus, it is necessary to filter ectopic RRI caused by PAC or PVC to suppress the influence of such ectopic RRIs on the HRV analysis (59). Therefore, this study adopted two HRV filtering methods: the Hampel filter and the quotient filter (38, 39). RRIs that deviate from the 3-σ range, which refers to data within three standard deviations from a mean, are detected by means of the Hampel filtering. In addition, quotient filtering detects significant RRI fluctuations: RRI that has a 10% or more change than the mean of before and after the RRI. Additionally, all data recorded from the participant were removed from the analysis when the sum of the RRIs detected with these filters became >150 s within the 5-min section (or 300 s within the 10-min section), because reliable analysis of such ectopic data was difficult.

Because frequency-domain analysis is significantly affected by incidental arrhythmias, the Poincaré plot and time-domain analysis were employed in this study (60). The Poincaré plot is based on a simple scatter of plots where the x value is the nth RRI and its corresponding y value is the $(n + 1)$th RRI. A typical Poincaré plot forms an ellipse with about a 45 tilt, and the diffusion ranges around the major axis and the minor axis of the ellipse are defined as the standard deviation 2 and 1 (SD2 and SD1), respectively. In addition, their ratio is defined as SD1/SD2 (**Figure 4**).

In addition to the Poincaré plot, time-domain analysis, which includes the standard deviation of all R-R intervals (SDNN), the root mean square of successive differences (RMSSD), and the percentage of adjacent intervals that varied by more than 50 ms (pNN50) was performed.

Various indices of HRV have been reported to be associated with the sympathetic and the parasympathetic nervous system (61). For the time-domain analysis, SDNN is influenced by the sympathetic and the parasympathetic nervous system; pNN50 and RMSSD mainly correlate to the parasympathetic nervous system. In particular, pNN50 <3% and RMSSD <25 ms are regarded as low parasympathetic activity (62, 63). For the Poincaré plots, SD1 represents the parasympathetic nervous function, SD2 is related more strongly to the sympathetic than to the parasympathetic tone, and SD1/SD2 represents the balance between the sympathetic-parasympathetic arms (60, 64–66).

Outcome Measurement

The demographic data of the HC, OH (–) iRBD, and OH (+) iRBD groups were examined. In addition, the experimental data of these three groups—BP and PR of the baseline and the delta values at 1 and 3 min after standing, and the ratio of clinical symptoms manifested after standing—were compared. The HRV

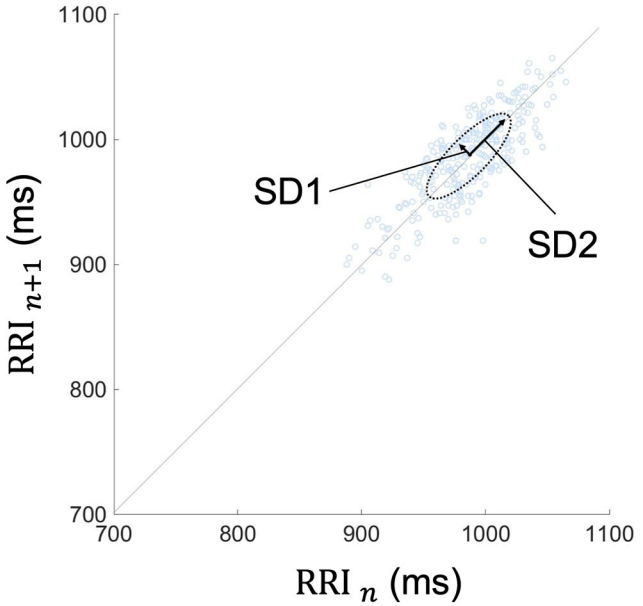

FIGURE 4 | Typical Poincaré plot of RRI. The Poincaré plot was based on a simple scatter of plots where the x value is *n*th RRI and its corresponding y value is the ($n + 1$)th RRI. Each plot is described in the (RRI n, RRI $n + 1$) space. This figure represents the Poincaré plot of a 5-min RRI. The Poincaré plot typically forms an ellipse with about a 45-degree tilt, and the diffusion range around the major axis and the minor axis of the ellipse are defined as SD2 and SD1, respectively. Their ratio is defined as SD1/SD2. RRI, RR intervals; SD1, standard deviation 1; SD2, standard deviation 2.

features when in the supine position were compared among the three groups.

Statistical Analysis

For comparison with the three groups, nominal data were compared using the χ^2-test. The normality of the distribution of the variable was checked by the Shapiro-Wilk test ($p < 0.05$ was considered statistically significant). Parametric variables were presented as mean ± SD; non-parametric variables were presented as the median and interquartile range (IQR). For the three groups, parametric variables were compared using one-way analyses of variance (ANOVAs) with Tukey's *post-hoc* analysis followed by Bonferroni correction; non-parametric variables were compared using the Kruskal-Wallis test and *post-hoc* Mann-Whitney test followed by Bonferroni correction. For comparison with the two groups [OH (–) iRBD and OH (+) iRBD], parametric variables were compared using Student's *t*-test.

Age and BMI were compared using ANOVAs. Years of education, a score of MMSE, and a score of RBDSQ-J were compared using the Kruskal-Wallis test. Gender differences, rate of comorbidities, and rate of medication usage were compared using the χ^2-test. The disease duration of RBD was analyzed using the Student's *t*-test. In the orthostatic challenge test, BP and PR of the baseline and the delta values at 1 and 3 min after standing were compared using ANOVAs at each time point. For the correction for the multiple comparisons, Tukey's *post-hoc* analysis was used for the comparison of multiple groups, and Bonferroni adjustment was performed for multiple time points ($p < 0.05/3 \approx 0.017$). The ratio of the clinical symptoms that appeared after standing was assessed using the χ^2-test. In addition, HRV indices when in the supine position were compared using the Kruskal-Wallis test and *post-hoc* Mann-Whitney test followed by Bonferroni correction.

A value of $p < 0.05$ was considered significant in this study. For parametric variables, the Cohen's *d* effect size index was used to calculate the pairwise differences. Cohen classified the effect sizes into small ($d = 0.2$), medium ($d = 0.5$), and large ($d = 0.8$) (67). For non-parametric variables, the effect size *r* was used to calculate the pairwise differences. The effect sizes of *r* were classified into small ($r = 0.1$), medium ($r = 0.3$), and large ($r = 0.5$) (68).

Next, among the OH (–) iRBD and OH (+) iRBD patients, a multivariate logistic regression analysis was conducted to focus on the relationship between the OH (+/–) and HRV indices, with OH (–) or OH (+) as the dependent variable and HRV indices (SDNN, RMSSD, pNN50, SD1, SD2, and SD1/SD2) as the independent variables. The Bayesian information criterion (BIC) was used to select the appropriate independent variables. In a model that minimized the BIC, we calculated the area under the curve (AUC) of the receiver operating characteristic (ROC) curve, the sensitivity of OH (+), and the specificity of OH (–). Besides, the value of HRV indices that maximizes Youden's index was calculated to determine the optimal cutoff values (69).

These statistical tests were performed using IBM SPSS Statistics for Macintosh, Version 22.0 (IBM Corp. Armonk, NY). HRV were evaluated with an HRV Analysis software (Kubios HRV v.1.1 for Windows, Biomedical Signal Analysis Standard).

RESULTS

Demographics

The demographic data of the included participants are shown in **Table 1**. HC, OH (–) iRBD, and OH (+) iRBD subjects were not significantly different in age, BMI, years of education, or the scores of MMSE. Since the patients were predominantly male, reflecting the male-dominant nature of this disease, the sex proportions were significantly different among the three groups. The RBDSQ-J scores were significantly different among the three groups. The RBD disease durations were not significantly different between OH (–) and OH (+) iRBD groups.

Of the comorbidities that could affect BP or HRV, there were no significant differences in the promotions of arterial hypertension, coronary artery disease, and diabetes mellitus. However, the proportion of myocardial infarction was significantly higher in the OH (+) iRBD group.

For medications that could affect BP or HRV, calcium channel blockers, ACE and AT II inhibitors, and organic nitrates were used by the participants. There was a significant difference in the proportion of ACE and AT II inhibitor use. Of the calcium channel blockers, diltiazem may decrease the low-frequency component of HRV and nifedipine may

TABLE 1 | Demographic data of healthy controls, OH (–) iRBD, and OH (+) iRBD groups.

Demographics	HC ($n = 42$)	OH (–) iRBD ($n = 12$)	OH (+) iRBD ($n = 9$)	p
Age (years)[a]	70.2 ±6.6	74.8 ±6.3	73.2 ±5.3	0.068
Gender (male: female)[b]	16:26	9:3	8:1	**0.005**
BMI (kg/m^2)[a]	22.4 ±3.0	21.6 ±2.6	23.2 ±2.9	0.468
Years of education[c]	13.0 (4.0)	16.0 (4.0)	12.0 (4.0)	0.347
MMSE[c]	29.0 (3.0)	29.0 (4.0)	29.0 (5.0)	0.958
RBDSQ-J[c]	1.0 (2.0)	3.0 (2.5)	3.0 (3.0)	**0.002**
Duration of RBD (years)[d]	–	10.2 ±6.8	9.2 ±6.2	0.748
Comorbidities[b]				
Arterial hypertension (*n*)	9 (21.4%)	4 (33.3%)	3 (33.3%)	0.592
Coronary artery disease (*n*)	1 (2.4%)	2 (16.7%)	1 (11.1%)	0.165
Myocardial infarction (*n*)	0 (0.0%)	0 (0.0%)	2 (22.2%)	**0.002**
Diabetes mellitus (*n*)	3 (7.1%)	3 (25.0%)	1 (11.1%)	0.222
Medication[b]				
Calcium channel blockers (*n*)	6 (14.3%)	3 (25.0%)	3 (33.3%)	0.353
ACE and AT II inhibitors (*n*)	4 (9.5%)	1 (8.3%)	2 (22.2%)	**<0.001**
Organic nitrates (*n*)	1 (2.4%)	1 (8.3%)	0 (0.0%)	0.492

ACE, angiotensin-converting-enzyme; AT II, angiotensin II type 1; BMI, body mass index; HC, healthy control; OH, orthostatic hypotension; iRBD, idiopathic rapid eye movement sleep behavior disorder; MMSE, Mini-Mental State Examination; RBDSQ-J, rapid eye movement sleep behavior disorder screening questionnaire Japanese version.

Results are presented with mean ± standard deviation for parametric variables, median (interquartile range), or percentage in parentheses.

$p < 0.05$ was considered significant. Significant values are given in bold.

[a] *One-way ANOVA.*

[b] *Chi-square test.*

[c] *Kruskal-Wallis test.*

[d] *Student t-test.*

not (45). ACE and AT II inhibitors may increase the low-frequency component of HRV (44). Nobody used any of the other medications listed below, which might influence BP or HRV; arrhythmic medications, atropine, scopolamine, atenolol, metoprolol, beta-blockers, loop diuretics, monoamine oxidase inhibitors, antipsychotics, antidepressant agents, phenothiazine, phosphodiesterase type 5 inhibitors, parkinsonism agents, barbiturates, anesthetics, opioids, muscle relaxants, vincristine, or doxorubicin.

As to smoking habit and the use of dopaminergic agonists, there were few participants (zero or one) in each group of HC, OH (–), and OH (+) iRBD.

Orthostatic Challenge

The results of the orthostatic challenge test are shown in **Figure 5** and **Table 2**. sBP tended to decrease after standing, in the order of OH (+) iRBD, OH (–) iRBD, and HC (**Figures 5A1,A2**). In the OH (+) iRBD group, sBP decreased remarkably by 28.6 mm Hg on average at 1 min after standing, and the trend of decreasing sBP continued thereafter. dBP increased after standing in HC; however, it decreased in OH (+) iRBD (**Figures 5B1,B2**). In addition, dBP decreased by 11.4 mm Hg on average at 1 min after standing and the trend of decreasing dBP continued thereafter as with sBP.

PR increased after standing in all three groups (**Figures 5C1,C2**). Although the increase in PR at 1 min after standing was 11.1 beats/min on average in HC, the increase of PR in OH (+) iRBD was 4.9 beats/min and smaller than that of HC ($p = 0.003$). PR in the HC group increased greatly after standing and then decreased rapidly. Similar changes were not observed in the OH (+) or OH (–) iRBD groups.

Delta values of BP and PR (written as ΔsBP, ΔdBP, and ΔPR) and the occurrence of subjective symptoms related to the OH +/– criteria were compared among the three groups (**Table 2**). sBP, dBP, and PR at the baseline were not significantly different among the three groups. There were statistically significant differences in ΔsBP at 1 min ($p < 0.001$) and at 3 min after standing ($p < 0.001$), and ΔdBP at 1 min ($p < 0.001$) and at 3 min after standing ($p < 0.001$). Subsequent *post-hoc* analyses revealed that ΔsBP at 1 min after standing in the OH (+) iRBD group was significantly lower than that in the HC group and OH (–) iRBD group with large effect sizes (Cohen's $d = 2.585, 2.037$, respectively). Similarly, ΔsBP at 3 min after standing in the OH (+) iRBD group was significantly lower than the HC group with a large effect size (Cohen's $d = 1.727$). ΔdBP at 1 min after standing in the OH (–) iRBD and OH (+) iRBD groups were significantly lower than that in the HC group with large effect sizes (Cohen's $d = 1.057, 2.182$, respectively).

Only dizziness was reported in this test as a subjective symptom in the OH (+) iRBD group ($n = 2$; 22.2%), and the incidence of dizziness was significantly different in the three groups ($p = 0.002$).

Heart Rate Variability Analysis

The results of the time-domain analysis and the Poincaré plots for HRV during segment 2 are shown in **Table 3**, and those during segment 1 and whole period are shown in **Supplementary Table 1**. Examples of Poincaré plots during segment 2 in the supine position are shown for a single HC subject, a single OH (–) iRBD subject, and a single OH (+) iRBD subject in **Figure 6**. HRV indices were significantly different among the three groups in time-domain analysis and the Poincaré plots.

First, as for the time-domain analysis during segment 2, significant differences in SDNN, RMSSD, and pNN50 were confirmed among the three groups ($p < 0.001$, $p = 0.003$, 0, 010, *respectively*). Subsequent *post-hoc* analyses revealed that SDNN in the OH (–) iRBD and the OH (+) iRBD groups were significantly smaller than that in the HC group with medium and large effect sizes ($r = 0.477, 0.597$, *respectively*); RMSSD in the OH (–) iRBD and the OH (+) iRBD groups were significantly smaller than that in the HC group with medium effect sizes ($r = 0.364, 0.358$, *respectively*); and pNN50 in the OH (–) iRBD group was significantly smaller than that in the HC group with a medium effect size ($r = 0.370$).

Second, in terms of the Poincaré plots, there were no significant differences among the three groups in SD1/SD2; however, significant differences were confirmed in SD1 and

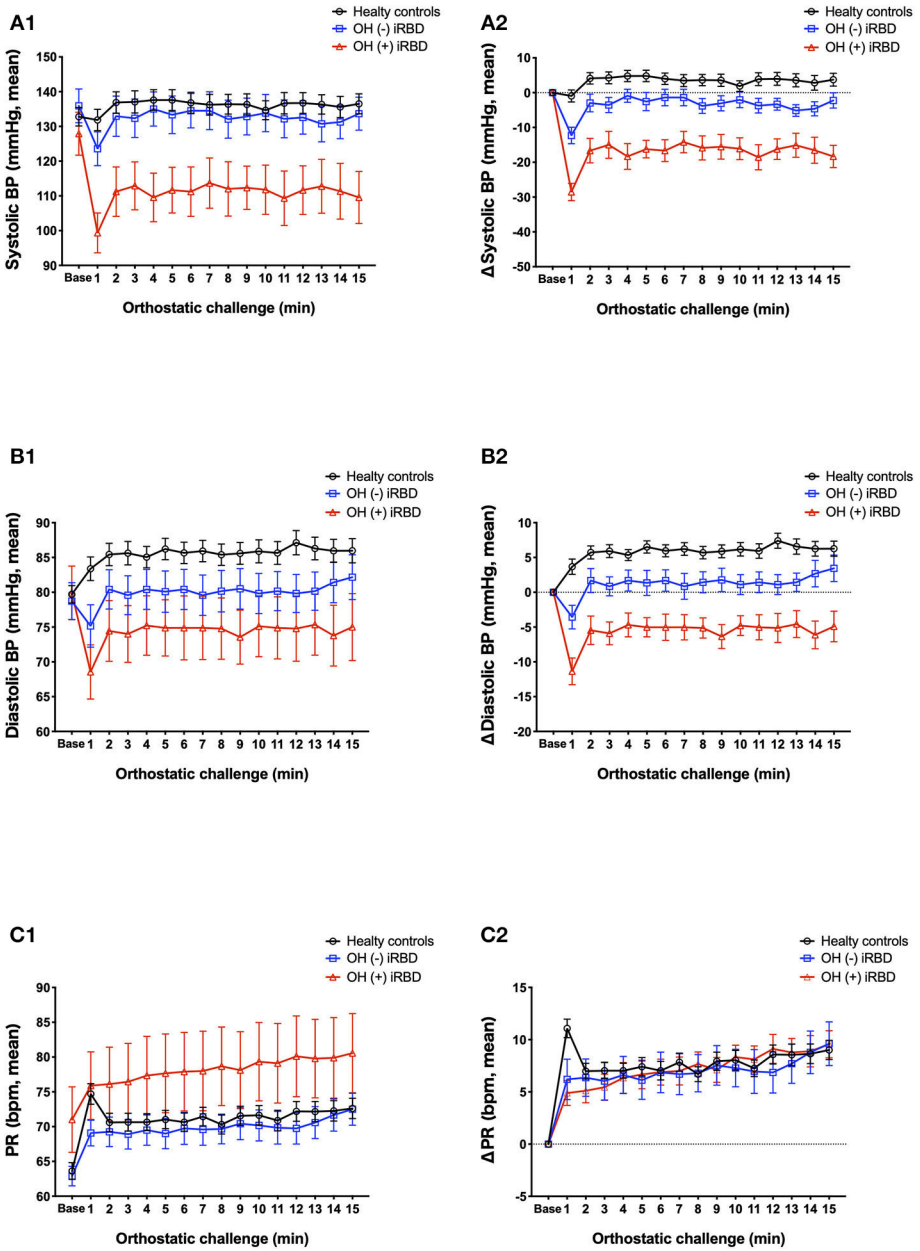

FIGURE 5 | Comparison of orthostatic challenge test among healthy controls, OH (–) iRBD, and OH (+) iRBD groups. **(A1–C1)**, absolute changes (mean ± standard error of the mean); **(A2–C2)**, delta values compared to baseline supine position (mean ± standard error of the mean). BP, blood pressure; HR, heart rate; iRBD, idiopathic rapid eye movement sleep behavior disorder; OH, orthostatic hypotension. x-axis: base, mean baseline values in supine position; 1–15 min, after standing.

SD2 ($p = 0.003$, $p < 0.001$, *respectively*). Subsequent *post-hoc* analyses revealed that SD1 in the OH (–) iRBD and the OH (+) iRBD groups were significantly smaller than that in the HC group with medium effect sizes ($r = 0.367, 0.541$, *respectively*); SD2 in the OH (–) iRBD and the OH (+) iRBD groups were significantly smaller than that in the HC group with medium and large effect sizes ($r = 0.471, 0.602$, *respectively*).

RRI usually fluctuates and the magnitude of the RRI fluctuations is represented by SDNN and RMSSD, or visualized by the Poincaré plot (**Figure 3**). The results in **Table 3** are visualized in the examples in **Figure 6**. In the HC group, RRI fluctuated within a certain range, and the Poincaré plot showed an oval-like shape (**Figure 6A**). However, in the OH (–) iRBD group, RRI fluctuated within a small range, and the Poincaré plot showed a narrow distribution (**Figure 6B**) with a smaller median

TABLE 2 | Results of orthostatic challenge test.

	HC ($n = 42$)	OH (–) iRBD ($n = 12$)	OH (+) iRBD ($n = 9$)	p	Pairwise differences	Effect sizes (Cohen's d) HC– OH (–) iRBD	HC– OH (+) iRBD	OH (–) iRBD– OH (+) iRBD
Systolic BP (mm Hg)[a]								
Baseline	132.8 ±17.3	135.9 ±16.8	127.9 ±18.5	0.580	N/A	−0.181	0.280	0.456
ΔsBP at 1 min after standing	−1.0 ±11.2	−12.3 ±8.3	−28.6 ±7.5	**<0.001**	HC > OH (–) iRBD > OH (+) iRBD	**1.068**	**2.585**	**2.037**
ΔsBP at 3 min after standing	4.3 ±11.1	−3.6 ±7.5	−15.0 ±11.6	**<0.001**	HC > OH (+) iRBD	0.752	**1.727**	1.215
Diastolic BP (mm Hg)[a]								
Baseline	79.7 ±8.0	78.7 ±9.2	79.9 ±11.6	0.936	N/A	0.118	−0.023	−0.115
ΔdBP at 1 min after standing	3.7 ±7.1	−3.6 ±5.9	−11.4 ±5.8	**<0.001**	HC > OH (–) iRBD = OH (+) iRBD	**1.057**	**2.182**	1.334
ΔdBP at 3 min after standing	5.9 ±6.0	0.9 ±4.7	−5.9 ±4.9	**<0.001**	HC > OH (+) iRBD	0.879	**2.022**	1.411
Pulse rate (beats/min)[a]								
Baseline	63.6 ±7.8	62.9 ±7.8	71.0 ±14.1	0.055	N/A	0.100	−0.805	−0.819
ΔPR at 1 min after standing	11.1 ±5.7	6.2 ±6.7	4.9 ±3.6	**0.003**	HC > OH (+) iRBD	0.818	**1.135**	0.234
ΔPR at 3 min after standing	7.0 ±5.1	6.0 ±6.3	5.4 ±3.8	0.646	N/A	0.186	0.324	0.110
Subjective symptoms[b]								
Dizziness	0 (0.0%)	0 (0.0%)	2 (22.2%)	**0.002**				

OH, orthostatic hypotension; iRBD, idiopathic rapid eye movement sleep behavior disorder; BP, blood pressure; PR, pulse rate.
Results are presented with mean ± standard deviation or percentage in parentheses.
One-way analysis of variance (ANOVA) for systolic BP, diastolic BP and PR, or Kruskal-Wallis for nominal variables are conducted.
$p < 0.05$ was considered significant. Significant values are given in bold.
Effect sizes (Cohen's d) are shown for pairwise difference.
[a]*One-way ANOVA and Tukey's post-hoc analysis with Bonferroni correction for multiple comparison of three time points.*
[b]*Chi-square test.*

TABLE 3 | Results of heart rate variability analysis during segment 2 and comparison among healthy controls, OH (–) iRBD, and OH (+) iRBD groups.

	HC ($n = 42$)	OH (–) iRBD ($n = 12$)	OH (+) iRBD ($n = 9$)	p	Pairwise differences	Effect sizes (r) HC– OH (–) iRBD	HC– OH (+) iRBD	OH (–) iRBD– OH (+) iRBD
Time-domain analysis								
SDNN (ms)	34.9 (20.5)	21.0 (11.8)	13.8 (10.7)	**<0.001**	HC > OH (–) iRBD = OH (+) iRBD	**0.477**	**0.597**	0.380
RMSSD (ms)	19.9 (12.7)	13.9 (3.7)	9.5 (11.8)	**0.003**	HC > OH (–) iRBD = OH (+) iRBD	**0.364**	**0.358**	0.132
pNN50 (%)	1.14 (3.78)	0.00 (0.97)	0.60 (0.71)	**0.010**	HC > OH (–) iRBD	**0.370**	0.257	0.065
Poincaré plots								
SD1 (ms)	13.58 (6.44)	9.53 (3.53)	6.76 (8.32)	**0.003**	HC > OH (–) iRBD = OH (+) iRBD	**0.367**	**0.351**	0.588
SD2 (ms)	46.9 (30.4)	27.4 (16.6)	17.6 (12.8)	**<0.001**	HC > OH (–) iRBD = OH (+) iRBD	**0.471**	**0.602**	0.349
SD1/SD2	0.287 (0.112)	0.300 (0.254)	0.463 (0.172)	0.071	N/A	0.132	0.318	0.178

HC, healthy control; iRBD, idiopathic rapid eye movement sleep behavior disorder; OH, orthostatic hypotension; SDNN, standard deviation of all N–N intervals; RMSSD, root mean square of successive differences; pNN50, percentage of successive RR intervals that differ by more than 50 ms; SD1, standard deviation 1; SD2, standard deviation 2.
Results are presented with median (interquartile range).
Kruskal-Wallis test was conducted and post-hoc Mann-Whitney test followed by Bonferroni correction were used.
$p < 0.05$ was considered significant. Significant values are given in bold.
Effect sizes (r) are shown for pairwise difference.

of SD1 and SD2 than those of the HC group (**Table 3**). In the OH (+) iRBD group, the Poincaré plot showed a narrow distribution as well as the OH (–) iRBD group (**Figure 6C**); in addition, the median of SD1 and SD2 were much smaller than those of the HC group (**Table 3**).

These results indicated that changes in HRV due to autonomic dysfunction may appear even in the supine position before standing, and iRBD patients, especially OH (+) iRBD patients, had smaller values of HRV indices than the HC group.

Relationship Between OH (+/–) and HRV Indices

The multiple logistic regression analysis for OH (+/–) was performed using HRV indices during segment 2. We identified SD2 and pNN50 associated with OH (+/–) with the minimal

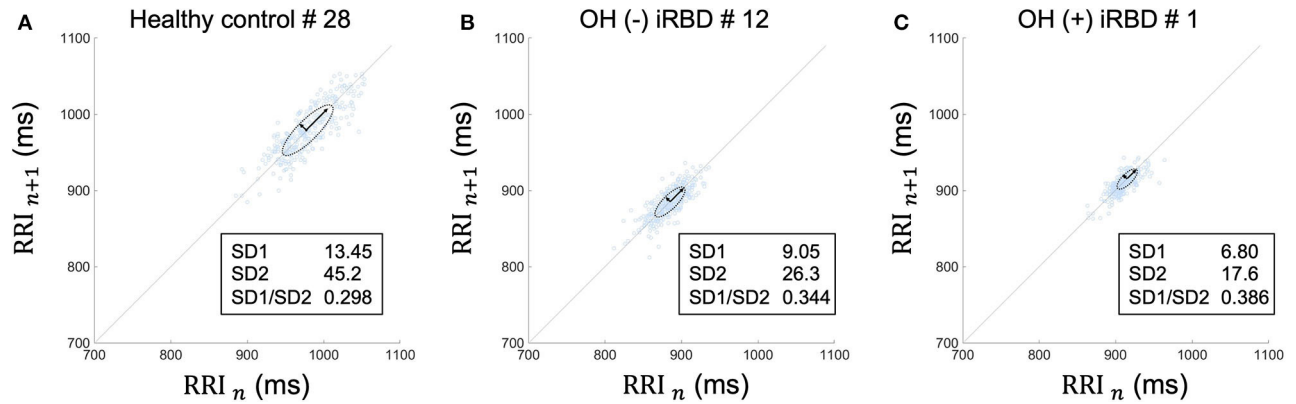

FIGURE 6 | Poincaré plots during segment 2 in supine position of **(A)** a healthy control subject, **(B)** an OH (–) iRBD subject, and **(C)** an OH (+) iRBD subject. The Poincaré plot was based on a simple scatter of plots where the x value is the *n*th RRI and its corresponding y value is ($n + 1$)th RRI. **(A)** RRI plots were sparsely distributed between 900 and 1,050 ms. The distribution approximates a 45-degree tilted oval. **(B)** RRI plots were densely distributed between 840 and 920 ms. Both SD1 and SD2 were smaller than **(A)**. **(C)** RRI plots were closely distributed between 890 and 940 ms. SD1 and SD2 were smaller than **(A)**, in particular, SD2 was only ∼40% of **(A)**. OH, orthostatic hypotension; iRBD, idiopathic rapid eye movement sleep behavior disorder; RRI, R–R intervals; HR, heart rate; SD1, standard deviation 1; SD2, standard deviation 2.

value of BIC ($p = 0.012$). In this model, the AUC of the ROC curve was 0.840, and the sensitivity of OH (+) was 1.000, and the specificity of OH (–) was 0.583 (**Figure 7**). The cutoff values of SD2 and pNN50 were 17.6 and 0.69, respectively.

The same procedures were conducted for segment 1 and the whole period. SD2 and pNN50 were also associated with OH (+/–) with the minimal values of BIC in segment 1 and the whole period ($p = 0.008, 0.005,$ *respectively*). In the segment 1 model (**Supplementary Figure 1**), the AUC of the ROC curve was 0.870, the sensitivity of OH (+) was 0.778, and the specificity of OH (–) was 0.917. The cutoff values of SD2 and pNN50 were 12.9 and 0.00, respectively. In the whole period model (**Supplementary Figure 2**), the AUC of the ROC curve was 0.870, the sensitivity of OH (+) was 0.889, and the specificity of OH (–) was 0.750. The cutoff values of SD2 and pNN50 were 21.0 and 0.12, respectively.

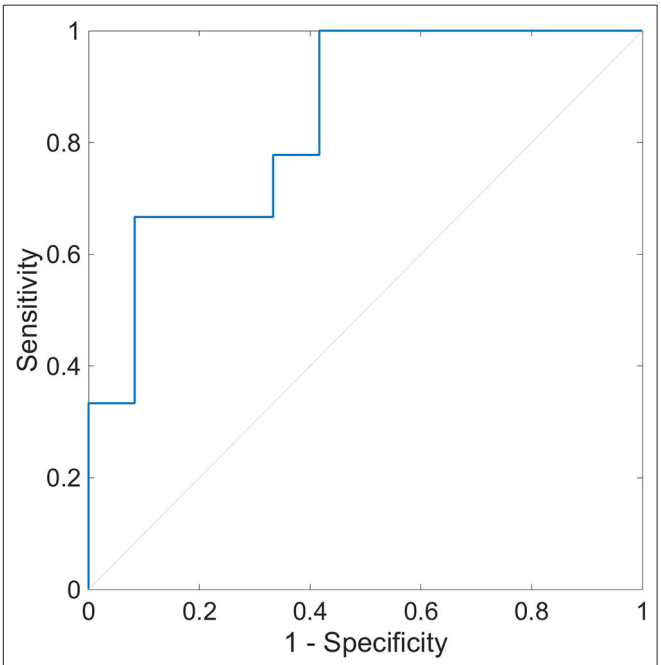

FIGURE 7 | Receiver operating characteristic curve of heart rate variability during segment 2 for the detection of orthostatic hypotension. The ROC curve with the AUC of 0.840 is shown ($p = 0.012$). The sensitivity to OH (+) is 1.000, and the specificity to OH (–) is 0.583. The optimal cutoff point is pNN50 of 0.69 and SD2 of 17.6. ROC, receiver operating characteristic; AUC, area under the curve; OH (+), orthostatic hypotension-positive; OH (–), orthostatic hypotension-negative; pNN50, percentage of successive RR intervals that differ by more than 50 ms; SD2, standard deviation 2.

DISCUSSION

Orthostatic hypotension is a prominent symptom not only in neurodegenerative diseases such as PD, DLB, and MSA, but also in iRBD. It is important to find indicators of autonomic nerve activities associated with OH in order to prevent unintended falls. Dahms et al. conducted the orthostatic challenge test on patients with iRBD and healthy subjects (43), and they reported that 20 patients with iRBD showed significantly greater decrease in both sBP and dBP after standing than healthy controls; however, the decrease rate thereof was not clear. Miyamoto et al. reported that cardiac ^{123}I-MIBG uptake decreased in patients with iRBD, which indicates cardiac sympathetic denervation (27, 28). In addition, the HRV data of iRBD patients were different from those of HC (30, 31). Thus, we aimed to investigate connections between rapid changes in HRV in the supine position and the OH occurrence.

Conventional time-domain and frequency-domain analyses have been used for HRV analysis in previous studies. Frequency-domain analysis, in particular, has been adopted for diagnosing the severity of autonomic dysfunction; however, the use of

frequency-domain analysis is not appropriate for older people because frequency-domain analysis is significantly affected by arrhythmia and most older people have PVC and PAC (57, 58). In addition, it is difficult for conventional frequency-domain analysis to capture rapid changes in the autonomous nerve activities since it usually requires 2–5 min of RRI data. In order to deal with these problems, we adopted a robust HRV analysis method: a time-domain analysis and Poincaré plots for short-term HRV analysis after adopting the Hampel filter and the quotient filter for modifying arrhythmia appropriately.

Previous studies about the relationship between iRBD and the autonomic dysfunction mainly focused on nocturnal HRV data collected from PSG; however, there are some reports about HRV analysis using the RRI data collected during daytime. Rocchi et al. and Dahms found autonomic dysfunction in iRBD patients by analyzing HRV data collected during postural changes (43, 70). We also performed the orthostatic challenge test between 2 p.m. and 4 p.m. to investigate daytime changes in autonomic nerve activities.

It has been reported that HRV during rest is related to the severity of autonomic dysfunction. Diabetic autonomic dysfunction is related to increases in heart rate and RRI variations in the rest condition (71, 72). These indicate that HRV during rest, as well as during postural changes, may be useful for evaluating autonomic dysfunction. In addition, Sannino et al. tried to predict decreases in BP of young, healthy people after standing (56).

Our experiments revealed two important findings regarding HRV. The first was that short term HRV before standing was found to represent autonomic dysfunction in patients with iRBD. SDNN, RMSSD, pNN50, SD1, and SD2 differed in comparison in the three groups (**Table 3**). It is noteworthy that the HRV indices of iRBD patients in the supine position as well as in the standing position were different from those of the HC group, which is a new finding of this study. These results show that physiological RRI fluctuations are reduced in both of OH (–) and OH (+) iRBD patients (**Figure 6**), indicating sympathetic and the parasympathetic nervous dysfunction. In particular, SDNN and SD2 significantly differed from the OH (+) iRBD and HC groups with large effect sizes, thus OH (+) iRBD patients were indicated to have severe autonomic dysfunction compared to HC. Decreases in HRV indices in OH (–) and OH (+) iRBD are in good agreement with previous studies (30, 31).

The second finding is that the combination of HRV indices showed the possibility of predicting OH. Multivariate logistic regression analysis for OH (–) and OH (+) iRBD patients using HRV indices presented a good model and ROC curve (**Figure 7**). HRV indices may reflect the severity of autonomic dysfunction in iRBD patients, which may have led to the relationship between HRV indices and OH. These findings were obtained by the analysis of HRV during segment 2; similar results were obtained by the analysis of HRV during segment 1 and the whole period (**Supplementary Table 1**, **Supplementary Figures 1, 2**).

Our results are of clinical importance in terms of showing the possibility that OH of older people can be predicted using only HRV data in the supine position without an orthostatic challenge test, which could improve the efficiency and safety of testing. However, several steps are required for the clinical application of OH detection by HRV indices. Since the number of OH patients were limited in this study, we performed a multivariate logistic regression analysis for only the construction of models. In the future, it is needed to validate the current model. Besides there are issues to be addressed to meet the clinical needs, such as whether using only the HRV indices is enough for OH detection or whether a combination of the OH questionnaire and the HRV indices is useful. A new device that can predict the occurrence of a fall can thereby be realized, since a wearable device for measuring RRI and calculating HRV in real-time has already been developed, which would contribute to preventing the falls of older people.

There were two benefits of recruiting iRBD patients in this study. The first was that the evaluation of autonomic dysfunction in the whole population of iRBD patients [OH (–) and OH (+) iRBD] was carried out, and the second was that we were able to perform an analysis to detect severe cases of autonomic dysfunction [OH (+) iRBD group]. iRBD patients have a high rate of autonomic dysfunction, and the degree of autonomic dysfunction was considered to be graded from mild to severe. Since PD, DLB, and MSA are associated with severe autonomic dysfunction, and most of the iRBD patients phenoconvert to PD, DLB, and MSA over a long period of time (14, 15, 20), patients with iRBD were thought to have various degrees of autonomic dysfunction. In fact, in this study, iRBD patients were split approximately halfway between OH (–) and OH (+), and there were significant decreases in BP after standing and decreases in HRV indices during the supine position in the OH (–) and OH (+) iRBD groups compared to HC (**Tables 2**, **3**). Therefore, the recruitment of iRBD patients, who were diagnosed with autonomic dysfunction and had had its severity graded, was consistent with the purpose of this study.

Demographics of the patients with iRBD included in our experiments were not much different from previous studies (73), although previous reports on OH in iRBD patients vary widely in their testing methods and results. Lee reported that 59% (10 of 17) of patients with iRBD displayed OH during the tilt test, and it was confirmed that 94% of patients had some autonomic dysfunction through a composite autonomic severity score which comprehensively evaluates autonomic nervous dysfunction (74). In Fraucher's study, only 13% (2 of 15) of patients with iRBD had OH; however, the composite autonomic scoring scale was significantly higher in patients with iRBD than healthy controls (75). A recent multicenter study found that 29% (156 of 531) of patients with iRBD had orthostatic symptoms (26). Although these studies have reported that most patients with iRBD have autonomic dysfunction, the proportion of OH in iRBD patients in these studies was different from each other. This variation might be explained by the age of the participants, duration of iRBD, the prevalence of cardiovascular disease, and experimental techniques. On the other hand, 43% (9 of 21) of patients with iRBD displayed OH (**Tables 1**, **2**) in this study, which is a modest prevalence in comparison with these previous studies. Patients with iRBD is prevalent in older males (76), and the population of this study matched the epidemiological characteristics of iRBD. In addition, patients with dementia were excluded, and we

confirmed that their MMSE scores exceeded the cutoff. It is also consistent with previous reports, in which only iRBD patients with no apparent cognitive impairment were included (73). Thus, it is concluded that our study has external validity.

On the other hand, the RBDSQ-J score of our population was lower than in the previous report (53), and the average disease duration was rather long: ∼10 years after symptom emergence. The RBDSQ-J score may have been low due to the long disease duration of iRBD (77). According to a longitudinal observational study of patients with a long disease duration of iRBD, almost all subjects showed multiple features of neurodegeneration including autonomic dysfunction after a follow-up of at least 10 years (19). Therefore, the participants in the present study with a long disease duration are an appropriate population for evaluating autonomic dysfunction.

The time of the experiment, the last meal, and the temperature and humidity in the laboratory were controlled to reduce their effects on the autonomic activities. The participants were instructed to stop consuming alcohol and tobacco the night before. However, autonomic activities in daily life are affected by various factors such as circadian rhythms and diet as well as alcohol, tobacco, and temperature (40, 42, 78–80). Niu et al. demonstrated significant changes in HRV after hemodialysis in elderly patients with diabetes (81). Thus, changes in the autonomic nervous activities, including HRV and BP fluctuations in daily life, may be greater than the results of this study. In order to accurately identify the indicators of OH occurrence, it is necessary to record the amount of daily alcohol and water consumption, the time of meal, temperature, humidity, and other factors as well as the RRI data. Since our device can measure RRI continuously for 24 h, we will perform additional experiments in a daily-life setting.

Because Xie et al. suggested that cognitive impairment-related gait disorders occur while walking in daily life among patients with amnestic mild cognitive impairment (82), comprehensive assessments of falls including the autonomic nervous functions and cognitive impairment-related gait disorders are also needed. In addition, from the viewpoint of fall prevention, we should account for orthostatic cerebral hypoperfusion syndromes, which are possible underlying pathophysiologies of orthostatic dizziness without orthostatic hypotension (83).

The limitations of this research include medication and comorbidities; although older people regularly use blood pressure-related medications due to hypertension or complications of angina, our study did not control for these medications and comorbidities. Some patients took calcium channel blockers, ACE and AT II inhibitors, and organic nitrates. There were significant differences in the proportion of myocardial infarction comorbidity and the use of ACE and AT II inhibitors (**Table 1**). To address this problem, we performed an analysis related to HRV after excluding patients with the comorbidity of myocardial infarction or the use of ACE and AT II inhibitors. After excluding one OH (–) iRBD patient and two OH (+) iRBD patients, HRV indices were calculated and the multivariate logistic regression analysis was performed (**Supplementary Table 2**, **Supplementary Figure 3**). The results of the analysis showed similar results in **Table 3** and **Figure 7**. Although the patients with other comorbidities or medication use should have been excluded from the analysis, we could not perform the analysis due to the reduced sample size. The undeniable influence of some comorbidities and medication is a limitation of this study. In order to assess the autonomic dysfunction without drug-related effects, it was necessary to instruct the subjects to discontinue these medications. Dahms performed the orthostatic challenge test after having the patients stop taking their regular medications (43); however, it would not have been appropriate for the patients to stop taking their regular medication for our experiments, because our purpose was to identify indicators of risk of falls in daily life.

Another limitation was the ratio of male to female in the HC group. In this study, there were more females than males in HC (female: 62%), although the number of males was more significant than that of females in the patient group reflecting the characteristics of RBD prevalence. There was the possibility that this gender difference might affect HRV and decreases in BP. To address this issue, we prepared randomized HC samples with the number of HC adjusted for the ratio of males to females in the iRBD groups (female: 19%) because the sample size of iRBD was smaller than that of HC. Namely, of the 26 HC females, we randomly selected 4 females 10 times, then created a sample of HC (16 males and 4 females) 10 times. The mean or median of variables of the 10 randomized samples of the 20 HC, whose male-to-female ratio was matched to the iRBD, are shown in **Supplementary Table 3**. Although the comorbidities with arterial hypertension and diabetes mellitus and the rate of medication use of calcium channel blockers and ACE and AT II inhibitors were higher than the original HC group (**Table 1**), the values of BP and PR and the HRV indices were not markedly different from those of the original HC group (**Tables 2**, **3**). Therefore, we believe that the gender ratio in HC does not largely affect the results of this study.

CONCLUSION

In this study, iRBD patients showed significant decreases in BP after standing and significant decreases in HRV indices during a supine position compared to HC. The combination of HRV indices would predict subsequent OH in iRBD patients. Our results are clinically useful in terms of showing the possibility that OH can be predicted without an orthostatic challenge test using only HRV data in the supine position, which could improve the efficiency and the safety of testing. Future studies are required to develop a system that can predict OH before standing up by analyzing HRV in real-time.

DATA AVAILABILITY STATEMENT

The datasets analyzed in the current study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethical committee at Shiga University of Medical Science. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YS, MM, HK, KF, and CN conceived the idea. YS, CN, and TK performed the experiments and analyzed the data. KF and TY contributed to the reagents, materials, and analysis tools. YS, KF, and HK wrote the draft. MM, YO, HK, KF, CN, YG, MK, TY, MH-O, and KO interpreted the data and revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.567984/full#supplementary-material

Supplementary Figure 1 | Receiver operating characteristic curve of heart rate variability during segment 1 for the detection of orthostatic hypotension. The ROC curve with the AUC of 0.870 is shown ($p = 0.008$). The sensitivity to OH (+) is 0.778, and the specificity to OH (–) is 0.917. The optimal cutoff point is pNN50 of 0.00 and SD2 of 12.9. ROC, receiver operating characteristic; AUC, area under the curve; OH (+), orthostatic hypotension-positive; OH (–), orthostatic hypotension-negative; pNN50, percentage of successive RR intervals that differ by more than 50 ms; SD2, standard deviation 2.

Supplementary Figure 2 | Receiver operating characteristic curve of heart rate variability during the whole period for the detection of orthostatic hypotension. The ROC curve with the AUC of 0.870 is shown ($p = 0.005$). The sensitivity to OH (+) is 0.889, and the specificity to OH (–) is 0.750. The optimal cutoff point is pNN50 of 0.12 and SD2 of 21.0. ROC, receiver operating characteristic; AUC, area under the curve; OH (+), orthostatic hypotension-positive; OH (–), orthostatic hypotension-negative; pNN50, percentage of successive RR intervals that differ by more than 50 ms; SD2, standard deviation 2.

Supplementary Figure 3 | Receiver operating characteristic curve of heart rate variability during segment 2 for the detection of orthostatic hypotension, excluding the patients with a history of myocardial infarction or use of angiotensin-converting-enzyme or angiotensin II type 1 inhibitors. The ROC curve with the AUC of 0.818 is shown ($p = 0.023$). The sensitivity to OH (+) is 1.000, and the specificity to OH (–) is 0.636. The optimal cutoff point is pNN50 of 0.71 and SD2 of 23.9. ROC, receiver operating characteristic; AUC, area under the curve; OH (+), orthostatic hypotension-positive; OH (–), orthostatic hypotension-negative; pNN50, percentage of successive RR intervals that differ by more than 50 ms; SD2, standard deviation 2.

Supplementary Table 1 | Results of heart rate variability analysis during segment 1 and the whole period and comparison among healthy controls, OH (–) iRBD, and OH (+) iRBD groups.

Supplementary Table 2 | Results of heart rate variability analysis during segment 2 of OH (–) iRBD and OH (+) iRBD groups without history of myocardial infarction or use of angiotensin-converting-enzyme or angiotensin II type 1 inhibitors.

Supplementary Table 3 | Demographic data and results of the study of randomly sampled, sex ratio-adjusted healthy control.

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