











ORIGINAL RESEARCH

# Association Between Sleep Microstructure and Incident Hypertension in a Population-Based Sample: The HypnoLaus Study

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**BACKGROUND:** Poor sleep quality is associated with increased incident hypertension. However, few studies have investigated the impact of objective sleep structure parameters on hypertension. This study investigated the association between sleep macrostructural and microstructural parameters and incident hypertension in a middle- to older-aged sample.

**METHODS AND RESULTS:** Participants from the HypnoLaus population-based cohort without hypertension at baseline were included. Participants had at-home polysomnography at baseline, allowing assessment of sleep macrostructure (nonrapid eye movement sleep stages 1, 2, and 3; rapid eye movement sleep stages; and total sleep time) and microstructure including power spectral density of electroencephalogram in nonrapid eye movement sleep and spindles characteristics (density, duration, frequency, amplitude) in nonrapid eye movement sleep stage 2. Associations between sleep macrostructure and microstructure parameters at baseline and incident clinical hypertension over a mean follow-up of 5.2 years were assessed with multiple-adjusted logistic regression. A total of 1172 participants (42% men; age 55±10 years) were included. Of these, 198 (17%) developed hypertension. After adjustment for confounders, no sleep macrostructure features were associated with incident hypertension. However, low absolute delta and sigma power were significantly associated with incident hypertension where participants in the lowest quartile of delta and sigma had a 1.69-fold (95% CI, 1.00–2.89) and 1.72-fold (95% CI, 1.05–2.82) increased risk of incident hypertension, respectively, versus those in the highest quartile. Lower spindle density (odds ratio, 0.87; 95% CI, 0.76–0.99) and amplitude (odds ratio, 0.98; 95% CI, 0.95–1.00) were also associated with higher incident hypertension.

**CONCLUSIONS:** Sleep microstructure is associated with incident hypertension. Slow-wave activity and sleep spindles, 2 hallmarks of objective sleep continuity and quality, were inversely and consistently associated with incident hypertension. This supports the protective role of sleep continuity in the development of hypertension.

**Key Words:** delta power ■ hypertension ■ power spectral density ■ sleep architecture ■ sleep structure ■ slow wave sleep ■ spindle

**H**igh blood pressure (BP) is a leading risk factor for cardiovascular disease–related mortality.<sup>1</sup> Sympathetic nervous system activity and BP tend to decrease with the progressive deepening

of nonrapid eye movement (NREM) sleep<sup>2</sup> and return to levels similar to wakefulness during rapid eye movement sleep.<sup>3</sup> BP also demonstrates transient changes over the course of sleep, such as a

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## CLINICAL PERSPECTIVE

### What Is New?

- In contrast to previous data on the relationship between sleep structure and hypertension, this study performed a more in-depth analysis, including comprehensive objective sleep microstructural and more conventional sleep macrostructural parameters.
- We found that slow wave activity and sleep spindles, 2 hallmarks of sleep continuity and objective sleep quality, were inversely associated with incident hypertension and were robust to multiple adjustments for potential confounders, while sleep macrostructure was not associated with the development of hypertension.

### What Are the Clinical Implications?

- These findings reinforce the concept of a protective role of sleep continuity in the development of hypertension, implying that general practitioners and cardiologists should consider sleep quality when conducting clinical hypertension risk assessment.

## Nonstandard Abbreviations and Acronyms

<b>AASM</b>	American Academy of Sleep Medicine
<b>N1</b>	nonrapid eye movement sleep stage 1
<b>N2</b>	nonrapideye movement sleep stage 2
<b>N3</b>	nonrapid eye movement sleep stage 3
<b>NREM</b>	nonrapid eye movement
<b>PSD</b>	power spectral density
<b>SWAN</b>	Study of Women's Health Across the Nation
<b>SWS</b>	slow wave sleep

gradual rise during airway obstruction events and surges following arousal and awakening events.<sup>4</sup> Several epidemiological studies have demonstrated that reduced BP dipping or “nondipping” during sleep increases cardiovascular risk.<sup>5–7</sup> In addition, growing evidence suggests that short sleep duration and subjective poor sleep quality are associated with cardiovascular comorbidities.<sup>8–12</sup> Experimental studies suggest that the magnitude of nocturnal BP dipping is significantly altered by deep slow wave sleep (SWS; or NREM sleep stage 3 [N3]) deprivation in young healthy individuals with normotension.<sup>13</sup> The importance of SWS in the modulation of BP was further supported in 2 community-based cohorts

showing that the percentage of SWS was inversely associated with incident hypertension.<sup>14,15</sup>

However, conventional “macroscopic” sleep stage scoring represents a superficial simplification of electroencephalogram (EEG) temporospatial and frequency domains, and may vary between scorers because of visual-based rules that remain subjective.<sup>16</sup> Thus, quantification of sleep-related brain oscillations via power spectral density (PSD) analysis of EEG signals have been developed, allowing for more objective and finer-grained analysis of sleep microstructure. This method allows the decomposition of EEG brain waves across a range of power frequency bands from slow wave activity (delta EEG power, 1–4 Hz) to fast frequency activity (beta EEG power, 18–30 Hz) using fast Fourier transform algorithms. SWS is characterized at the microscopic level by the presence of high delta power, which reflects the presence of high-amplitude slow waves typical of deep sleep. Several studies suggest that quantitative EEG may provide more sensitive markers for identifying patient phenotypes at risk for adverse health outcomes compared with conventional sleep scoring.<sup>17–20</sup> However, few studies have investigated the association between sleep microstructure and future development of hypertension. One study performed in a small sample of middle-aged women showed that those with low delta power during NREM sleep may be at increased risk of developing hypertension.<sup>21</sup> However, obstructive sleep apnea was not controlled for, which clearly impacts quantitative EEG,<sup>19</sup> and thus may have confounded the association with hypertension.

Furthermore, novel computed algorithms allow more in-depth analysis of EEG graphical elements, such as spindle characteristics during NREM sleep stage 2 (N2). Although previous studies have shown that lower spindle density could determine neural dysfunction and cognitive decline,<sup>22,23</sup> none have investigated the impact of spindle characteristics in the development of hypertension.

Therefore, the aim of this study was to investigate the potential association between both sleep macrostructure and microstructure and incident hypertension in middle- to older-aged adults of the general population.

## METHODS

### Data Availability

Because of the sensitivity of the data and the lack of consent for online posting, individual data cannot be made accessible. Only metadata will be made available in digital repositories on reasonable request. Metadata requests can also be made via the study website: <https://www.colaus-psycolous.ch>.

## Study Design and Population

The sample was derived from the prospective CoLaus|PsyCoLaus population-based cohort<sup>24,25</sup> and HypnoLaus, a nested study, designed to assess the prevalence and determinants of sleep disorders in participants randomly selected from the city of Lausanne, Switzerland. The design, sampling, and procedures of the study have been described elsewhere.<sup>26</sup> Briefly, 2162 individuals from CoLaus|PsyCoLaus underwent a home-based polysomnography and multiple clinical assessments, including demographic, medical history, anthropometric, and office BP measurements, between 2009 and 2012 (considered as baseline hereafter). Between 2014 and 2017, participants underwent a second examination in which clinical parameters were reassessed. Inclusion criteria were: (1) absence of hypertension at baseline, (2) complete data for hypertension diagnosis at baseline and follow-up, and (3) full polysomnography performed at baseline. HypnoLaus and CoLaus|PsyCoLaus were approved by the ethics committee of the Vaud Canton (CER-VD n° PB\_2018-00038 (239/09)) and all participants provided written informed consent.

## BP Measurements

BP was assessed in triplicate on the left arm at 5-minute intervals with the participant seated and resting for at least 10 minutes using calibrated automated oscillometric sphygmomanometers (Omron HEM-907). The mean of the second and third measurements was used for analysis, as recommended.<sup>27</sup> Hypertension was defined as systolic BP  $\geq 140$  mm Hg and/or diastolic BP  $\geq 90$  mm Hg and/or antihypertensive medication use.

## Outcome

Incident hypertension was defined as the absence of hypertension at the time of polysomnography (baseline) and the development of hypertension assessed at the clinical follow-up of the CoLaus|PsyCoLaus cohort in 2014–2017.

## Clinical Assessment and Covariates

Information on sociodemographic characteristics and medical and treatment history was obtained by trained interviewers using standardized questionnaires at baseline. Alcohol consumption was defined using weekly mean consumption of standard drinks containing 10 g of alcohol. Food sodium content was assessed using a validated food frequency questionnaire querying the consumption of 97 different food items including portion size over the previous 4 weeks. Medication was coded according to World Health Organization Anatomical, Therapeutic,

Chemical classification. Psychotropic drugs included antidepressants, anxiolytics, antipsychotics, benzodiazepines, and “Z-drugs.” Body weight and height were measured with participants standing without shoes in light clothes to calculate body mass index. Diabetes was defined as fasting blood glucose  $\geq 7$  mmol/L and/or use of antidiabetic drugs.<sup>28</sup> Rheumatoid arthritis was self-reported. Restless leg syndrome was investigated using the International Restless Legs Syndrome Study Group criteria.<sup>29</sup>

Self-reported sleep quality was assessed with the Pittsburgh Sleep Quality Index,<sup>30</sup> where a score  $>5$  indicates poor sleep quality. Sleepiness was evaluated with the Epworth Sleepiness Scale,<sup>31</sup> where a score  $>10$  indicates excessive daytime sleepiness.

## Polysomnography

Home-based polysomnography (Titanium) was conducted with multiple EEG leads (C3, C4, F3, F4, O1, and O2; 256 Hz sampling rate). Polysomnography setup specifications followed the 2007 American Academy Sleep Medicine (AASM) recommendations.<sup>32</sup> Polysomnography data included EEG (central, occipital, frontal), electrooculogram (right and left eyes), chin and anterior tibialis electromyogram, ECG, nasal pressure, thoracic and abdominal effort bands, oximetry, snoring, and body position signals. Manual scoring of sleep stage was performed using the 2007 AASM criteria,<sup>32</sup> while respiratory events were scored according to the 2012 AASM criteria.<sup>33</sup> Obstructive sleep apnea was defined by an apnea-hypopnea index  $\geq 15$  events per hour.

## EEG PSD Analysis

Detailed descriptions of EEG PSD data preprocessing including artifact detection and management are described elsewhere.<sup>34</sup> Synchronized European data format and sleep stage files were generated using Embla RemLogic Polysomnography Software (Natus Medical, Incorporated). PSD values were calculated in C3-M2 using fast Fourier transformation and the Welch algorithm on artifact-free consecutive, nonoverlapping 6-second epochs (Hamming windows, 8 segments, 50% overlap) and used to compute absolute signal power ( $\mu\text{V}^2$ ) in typical frequency bands including delta (1–4 Hz), theta (5–8 Hz), alpha (8–12 Hz), sigma (12–16 Hz), and beta (18–30 Hz).

## Spindle Characteristics

Spindles were separately identified in the 10-Hz to 16-Hz range in central derivations (C3 and C4) during N2 based on an automatic algorithm, as previously described.<sup>35</sup> The following spindle characteristics were computed for each detected spindle: spindle density

defined as total number of spindles in N2 divided by the N2 time in minutes; spindle frequency, defined as the frequency with the highest power in the 10-Hz to 16-Hz range; spindle amplitude, defined as the highest power within the spindle (expressed in microvolts squared;  $\mu V^2$ ); spindle duration, defined as the average spindle length (second) of each spindle event; and percentage of fast spindles (12- to 16-Hz range) of all identified spindles (Figure 1). Spindle characteristics were then averaged across both C3-M2 and C4-M1 when artifact-free.

## Statistical Analysis

Descriptive and inferential statistics were performed using IBM SPSS Statistics version 26. Data are presented as number (percentage) of patients, median (interquartile range [IQR]), or mean $\pm$ SD, unless otherwise stated. Data distribution was graphically assessed through normal Q-Q plot. Pearson chi-square test, independent samples *t* test or Mann-Whitney pairwise comparisons were used as appropriate to compare differences in baseline characteristics based on the presence or absence of hypertension. Associations between sleep macrostructure and microstructure and the incidence of hypertension were evaluated using unadjusted (model 0) and multivariable-adjusted logistic regression (model 1 and 2).

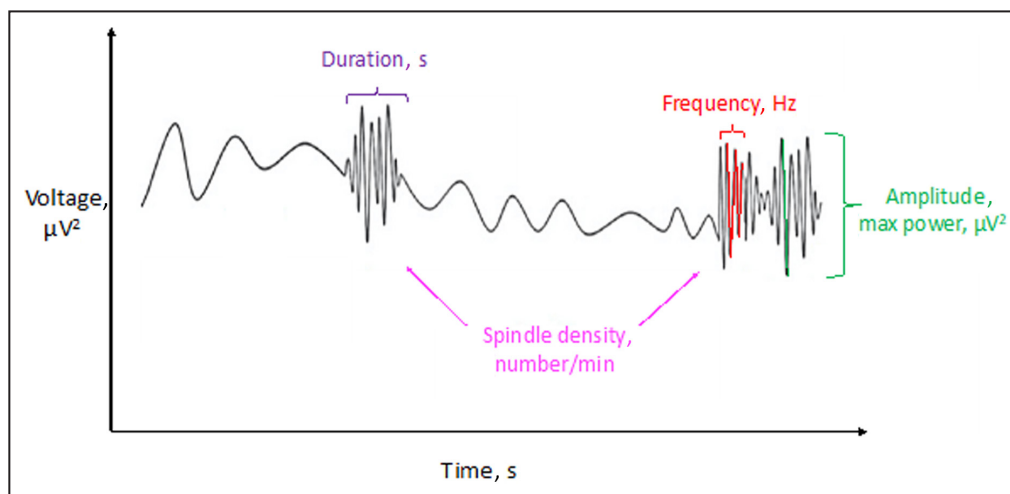
Locally weighted scatterplot smoothing was drawn to check the assumption of linearity for the logit of each continuous independent variable. In the case of nonlinearity, continuous variables were transformed into categorical variables based on clinically relevant cutoffs or median. Model 1 was adjusted for age (continuous), sex (categorical), and body mass index (continuous), while model 2 was additionally adjusted for alcohol consumption (continuous), baseline systolic BP (continuous), diabetes (categorical), dyslipidemia

(categorical), obstructive sleep apnea (categorical, ie, apnea-hypopnea index >15 events per hour), sleep efficiency (continuous), and duration of follow-up (continuous). These confounding factors were included in the multivariate models either because of significant difference in bivariate analysis or because of their recognized clinical associations with hypertension (sex, alcohol). Since patients who developed hypertension had higher BP at baseline, baseline systolic BP was included as a covariate in model 2. Results are presented as odds ratio (ORs) and 95% CIs. When a sleep macrostructure or microstructure continuous parameter was significantly associated with incident hypertension in the fully adjusted model, it was further categorized into quartiles to account for potential nonlinear associations.

To facilitate interpretation, significant continuous scale results in fully adjusted models were further categorized into quartiles. As the association between spindle density and incident hypertension appeared nonlinear in quartile analysis, a complementary restricted cubic spline regression was performed using the rms package in R. In secondary analysis, given the a priori hypothesis that sleep quality may vary according to sex,<sup>36</sup> we tested the interaction between sex and SWS, sleep microstructure, and spindles characteristics. Statistical significance was defined as a 2-sided *P* value of <0.05.

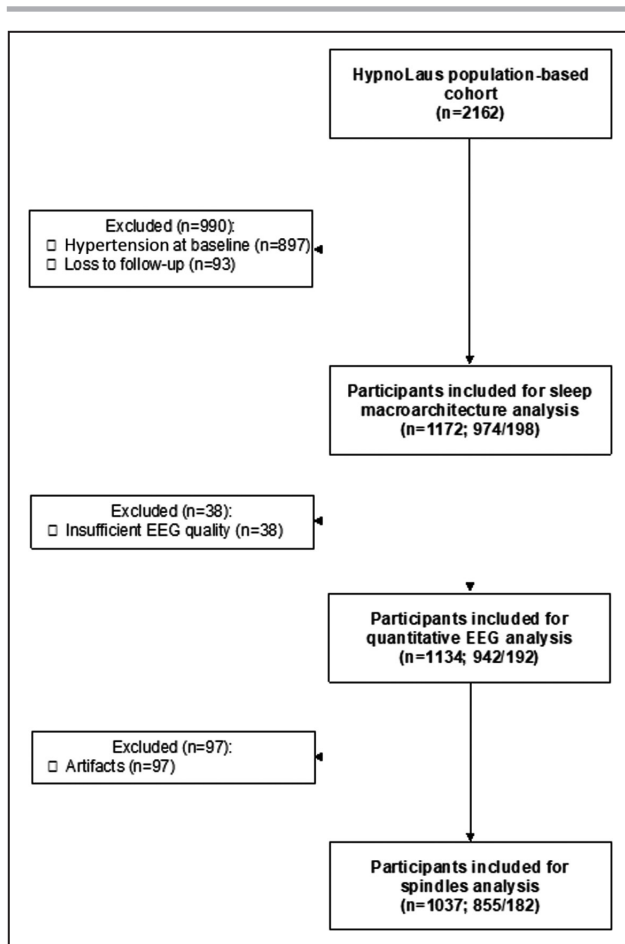
## RESULTS

Of the 2162 participants included in HypnoLaus, 1172 who had hypertension at baseline with complete polysomnography and known hypertension status at follow-up were included in this analysis (Figure 2). Approximately 17% (n=198) of the sample developed hypertension during a mean $\pm$ SD



**Figure 1.** Sleep spindle characteristics.





**Figure 2. Study flow chart.**

n/n indicates number of participants without hypertension at follow-up/number of participants who developed hypertension during follow-up. EEG indicates electroencephalogram.

follow-up of  $5.2 \pm 0.6$  years (minimum: 2.2 years; maximum: 7.8 years). Participants who developed hypertension were older (median age, 56 years [IQR, 49–66 years] versus 52 years [47–62 years]), with higher body mass index (median body mass index,  $26.2 \text{ kg}\cdot\text{m}^{-2}$  [IQR,  $23.6\text{--}28.8 \text{ kg}\cdot\text{m}^{-2}$ ] versus  $24.5 \text{ kg}\cdot\text{m}^{-2}$  [IQR,  $22.2\text{--}26.9 \text{ kg}\cdot\text{m}^{-2}$ ]) and more comorbidities than those who did not (Table 1). Regarding objective sleep, although participants who developed hypertension during follow-up had similar TST to those without hypertension, there was a clinically nonrelevant but statistically significant difference in sleep efficiency (median sleep efficiency, 89.0% [IQR, 80.1%–93.0%] versus 89.9% [IQR, 84.2%–93.5%];  $P=0.024$ ). Moreover, patients who developed hypertension had higher arousal index, apnea-hypopnea index, and hypoxemic parameters compared with those without hypertension (Table 1). Last, patients who developed hypertension had higher BP at baseline compared with those without hypertension (mean $\pm$ SD systolic BP,  $126 \pm 9$  mm Hg versus  $116 \pm 11$  mm Hg;  $P<0.001$ ).

## Association of Sleep Macrostructure With Incident Hypertension

A higher percentage of NREM sleep stage 1 (N1) sleep (mean difference, +1.4; 95% CI, 0.4–2.3 [ $P=0.002$ ]) and a lower percentage of N3 sleep (mean difference,  $-1.8$ ; 95% CI,  $-3.0\text{--}0.5$  [ $P=0.008$ ]) in participants who developed hypertension was identified in bivariate analysis (Table 2). After multiple adjustments, no significant associations were found between sleep macrostructure and incident hypertension (Table 3).

## Associations of EEG PSD With Incident Hypertension

Bivariate analysis showed that participants who developed hypertension had lower EEG power in almost all power frequency bands during NREM sleep, except in the beta band (Table 2). After adjustment for age, sex, and body mass index (model 1), only the absolute delta power (OR, 0.96; 95% CI, 0.93–1.00) and absolute sigma power (OR, 0.94; 95% CI, 0.89–0.99) remained associated with incident hypertension (Table 3). These associations persisted in the fully adjusted model (model 2; Table 3). When delta and sigma were categorized in quartiles, participants with delta and sigma values in the lowest quartile (quartile 1) had an  $\approx 1.7$ -fold increased risk of developing hypertension during follow-up compared with those who had delta and sigma values in the highest quartile (quartile 4) (OR, 1.69 [95% CI, 1.00–2.89] for quartile 1 delta and 1.72 [95% CI, 1.05–2.82] for quartile 1 sigma compared with quartile 4 delta and sigma, respectively) (Figure 3).

## Associations of Spindle Characteristics and Incident Hypertension

Participants who developed hypertension had lower spindle density (mean difference,  $-0.37$ ; 95% CI,  $-0.61$  to  $-0.14 \text{ min}^{-1}$  [ $P=0.002$ ]) and lower spindle amplitude (mean difference,  $-1.92$ ; 95% CI,  $-3.22$  to  $-0.61 \mu\text{V}^2$ ) in bivariate analysis compared with those who did not develop hypertension (Table 2). After multiple adjustments (models 1 and 2), both spindle density (OR, 0.87; 95% CI, 0.76–0.99) and spindle amplitude (OR, 0.98; 95% CI, 0.95–1.00) remained significantly associated with incident hypertension (Table 3). Quartile analysis suggested a possible U-shaped relationship between spindle density and incident hypertension ( $P$  for quadratic trend=0.039). Participants with spindle density in quartile 1 and quartile 2 had a 2.13-fold (95% CI, 1.25–3.63) and 2.24-fold (95% CI, 1.32–3.80) increased risk of developing hypertension, respectively, compared with quartile 3, while the fourth quartile did not differ significantly (OR, 1.62; 95% CI, 0.94–2.80) (Figure 3). However, the U-shaped association did not remain when spindle density was modeled as a continuous variable using restricted

**Table 1. Baseline Demographic and Clinical Characteristics in Patients With and Without Incident Hypertension**

	No hypertension	Incident hypertension	P value	No.
	(n=974)	(n=198)		
Age, y	52 (47–62)	56 (49–66)	0.001*	1172
Women, %	549 (58.5%)	105 (54.7%)	0.334	1172
BMI, kg/m <sup>2</sup>	24.5 (22.2–26.9)	26.2 (23.6–28.8)	<0.001*	1166
SBP, mm Hg	116±11	126±9	<0.001*	1172
DBP, mm Hg	73±8	79±7	<0.001*	1172
Alcohol consumption <sup>†</sup>	4 (1–8)	4 (1–10)	0.323	1172
Food sodium content			0.587	1111
Medication use, %				
Antidiabetic	8 (0.9%)	7 (3.6%)	0.002*	1172
Lipid-lowering	57 (6.1%)	31 (16.1%)	<0.001*	1172
Psychotropic <sup>‡</sup>	13.1 (128)	13.1 (26)	0.997	1172
Comorbidities, %				
Diabetes	29 (3.1%)	18 (9.4%)	<0.001*	1171
Dyslipidemia	191 (20.4%)	61 (31.8%)	0.001*	1171
OSA <sup>§</sup>	230 (24.5%)	70 (36.5%)	0.001*	1172
RLS	148 (15.2%)	26 (13.1%)	0.457	1172
Arthritis	21 (2.2%)	3 (1.5%)	0.784	1172
Smoking, %				
Current smoker	190 (20.5%)	39 (20.6%)	0.863	
Ex-smoker	332 (35.7%)	71 (37.6%)		
Excessive daytime sleepiness <sup>  </sup>	124 (13.7%)	24 (13.0%)	0.780	1087
Subjective sleep				
PSQI score	5.0±3.2	4.9±3.2	0.809	1018
Poor sleep quality <sup>¶</sup>	299 (34.9%)	57 (35.2%)	0.950	1018
Objective sleep				
TST, min	408±70	408±73	0.970	1172
Sleep efficiency, %	89.9 (84.2–93.5)	89.0 (80.1–93.0)	0.024*	1172
Arousal index, events per h	17.3 (12.7–23.7)	19.7 (14.3–26.9)	0.002*	1172
AHI, events per h	7.1 (2.8–14.8)	9.9 (3.8–21.0)	<0.001*	1172
ODI, events per h	6.7 (3.0–14.0)	10.3 (4.1–19.2)	<0.001*	1172
T90, %	0 (0–0.5)	0.1 (0–1.4)	<0.001*	1163
Mean SpO <sub>2</sub> , %	94.8 (93.7–95.7)	94.4 (93.3–95.4)	0.003*	1171
PLMSI, events per h	1.0 (0–11.6)	1.5 (0–15.7)	0.320	1172

Data are presented as number (percentage), median (interquartile range), or mean±SD, unless otherwise stated. AHI indicates apnea-hypopnea index; BMI, body mass index; DBP, diastolic blood pressure; ODI, oxygen desaturation index; PLMSI, periodic leg movements during sleep; RLS, restless leg syndrome; SBP, systolic blood pressure; SpO<sub>2</sub>, oxygen saturation; TST, total sleep time; and T90, percentage of total sleep time with oxygen saturation <90%.

\*Indicates significant P-values.

<sup>†</sup>Alcohol consumption was defined as weekly mean consumption of standard drinks containing 10 g of alcohol.

<sup>‡</sup>Psychotropic drugs included antidepressants, hypnotics, anxiolytics, antipsychotics, benzodiazepines, and “Z-drugs.”

<sup>§</sup>Obstructive sleep apnea (OSA) was defined by an apnea-hypopnea index ≥15 per hour.

<sup>||</sup>Excessive daytime sleepiness was defined by an Epworth Sleepiness Scale score ≥11.

<sup>¶</sup>Poor sleep quality was defined by a Pittsburgh Sleep Quality Index (PSQI) score ≥6.

cubic spline ( $P=0.085$ , Figure S1). In addition, categorization of spindle amplitude in quartiles produced a linear relationship, with participants in the first quartile having a significant increased risk of incident hypertension compared with participants in the fourth quartile (OR, 1.91; 95% CI, 1.14–3.22 [ $P$  for trend=0.058]) (Figure 3).

## Secondary Analysis

No interaction of either sex, age, OSA, or excessive daytime sleepiness was found in the relationship between incident hypertension and SWS, delta in NREM, sigma in NREM, spindle density, and spindle amplitude (Table 4).

**Table 2. EEG Power Spectral Density During NREM Sleep (N1, N2, N3) and N2 Spindle Characteristics in Patients With and Without Incident Hypertension**

	No hypertension	Incident hypertension	P value	No.
	(n=939)	(n=192)		
Sleep macrostructure				
N1 sleep, %	9.2 (7.0–13.0)	11.0 (7.2–15.6)	0.002*	1172
N2 sleep, %	45.8±9.8	45.9±9.4	0.870	1172
N3 sleep, %	21.0±8.2	19.3±7.4	0.008*	1172
REM sleep, %	22.6±5.7	22.8±5.4	0.583	1172
Sleep microstructure				
Delta, $\mu\text{V}^2$	92.3 (66.3–123.8)	80.7 (60.5–107.9)	0.002*	1134
Theta, $\mu\text{V}^2$	12.5 (9.1–17.5)	11.5 (8.2–16.0)	0.043*	1134
Alpha, $\mu\text{V}^2$	10.5 (7.2–15.3)	9.0 (6.4–14.1)	0.003*	1134
Sigma, $\mu\text{V}^2$	5.4 (3.8–7.7)	4.8 (3.1–7.1)	0.001*	1134
Beta, $\mu\text{V}^2$	1.9 (1.4–2.6)	1.7 (1.3–2.4)	0.083	1134
EEG activation index (log (beta/delta))	-1.66±0.27	-1.64±0.23	0.279	1134
N2 spindle characteristics <sup>†</sup>				
Density, $\text{min}^{-1}$	2.8±1.4	2.4±1.5	0.002*	1037
Amplitude, $\mu\text{V}^2$	28.1±8.1	26.2±8.2	0.004*	1037
Frequency, Hz	12.4±0.5	12.4±0.6	0.211	1037
Duration, s	1.33±0.06	1.33±0.07	0.642	1037
Fast spindles, %	63.7±18.3	61.0±20.2	0.095	1037

EEG indicates electroencephalogram; N1, nonrapid eye movement sleep stage 1; N2, nonrapid eye movement sleep stage 2; N3, nonrapid eye movement sleep stage 3; NREM, nonrapid eye movement; and REM, rapid eye movement.

\*Indicates significant *P*-values.

<sup>†</sup>A total of 1037 of the 1131 participants had complete data for spindle analysis.

## DISCUSSION

This study investigated the associations between sleep macrostructure and microstructure and incident hypertension in a middle- to older population-based sample over a 5.2-year follow-up. Our results showed that sleep microstructural but not sleep macrostructural parameters were associated with incident hypertension. In particular, we found significant and robust associations between incident hypertension and low delta and low sigma power after multiple adjustments. Participants with values in the lowest quartile of delta and sigma had an  $\approx 1.7$ -fold increased incidence of hypertension compared with those with values in the highest quartile. Furthermore, we found that spindle density and amplitude were inversely associated with incident hypertension.

### Comparison With Previous Studies

Contrary to 2 previous community-based studies that focused on sleep macrostructure,<sup>14,15</sup> we did not find an association between SWS and incident hypertension. The reason for this discrepancy remains uncertain but may be attributable to the lack of consistency of the classification method for human sleep stage scoring.

Even if standard AASM rules are strictly followed,<sup>32</sup> conventional sleep stage scoring is based on visual assessment of EEG, electrooculogram, and electromyogram data, based on arbitrary 30-second epochs of sleep, which show considerable intrascorer and interscorer variability, particularly for N1 and SWS.<sup>37–39</sup> Moreover, sleep staging is generally less reliable if sleep is fragmented, which commonly occurs in patients with sleep-disordered breathing.<sup>40</sup> Furthermore, conventional sleep stage scoring does not allow analysis of more subtle and potentially more clinically useful EEG features, such as the density, duration, and amplitude of spindles in NREM sleep.

Conversely, quantitative EEG provides a more objective and reliable alternative that is not prone to human error/variation to evaluate sleep structure using a continuous scale of sleep depth by classifying EEG according to its frequency content using automated and validated fast Fourier transformation-based algorithms. Interestingly, we found that higher sigma and delta power, which characterize important features of deep NREM sleep, were associated with reduced incidence of hypertension, independently of classical clinical covariates and obstructive sleep apnea, sleep efficiency, and systolic BP at baseline.

**Table 3. Incident Hypertension According to Subjective and Objective Sleep Parameters**

	Crude		Model 1		Model 2	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Subjective sleep quality (each 1-PSQI point increase)	0.99 (0.94–1.05)	0.809	0.99 (0.94–1.05)	0.840	1.00 (0.95–1.06)	0.914
Sleep macrostructure						
N1 (each 1% increase)	1.03 (1.01–1.06)	0.005*	1.02 (0.99–1.04)	0.174	1.01 (0.98–1.04)	0.678
N2 (each 1% increase)	1.00 (0.99–1.02)	0.738	0.99 (0.98–1.01)	0.496	1.00 (0.98–1.01)	0.716
N3 (each 1% increase)	0.97 (0.95–0.99)	0.007*	0.99 (0.97–1.01)	0.180	0.99 (0.97–1.01)	0.262
REM (each 1% increase)	1.00 (0.98–1.03)	0.904	1.02 (0.99–1.05)	0.130	1.03 (1.00–1.06)	0.083
Sleep microstructure (absolute PSD)						
Delta power (each 10- $\mu\text{V}^2$ increase)	0.95 (0.91–0.98)	0.003*	0.96 (0.93–1.00)	0.043*	0.96 (0.92–1.00)	0.040*
Theta power (each 1- $\mu\text{V}^2$ increase)	0.99 (0.97–1.01)	0.166	0.99 (0.97–1.01)	0.232	0.99 (0.97–1.01)	0.325
Alpha power (each 1- $\mu\text{V}^2$ increase)	0.97 (0.95–0.99)	0.026*	0.98 (0.96–1.00)	0.055	0.98 (0.96–1.01)	0.113
Sigma power (each 1- $\mu\text{V}^2$ increase)	0.93 (0.88–0.98)	0.005*	0.94 (0.89–0.99)	0.022*	0.94 (0.89–0.99)	0.018*
Beta power (each 1- $\mu\text{V}^2$ increase)	0.96 (0.89–1.04)	0.298	0.96 (0.88–1.04)	0.309	0.95 (0.87–1.04)	0.256
EEG activation index	1.37 (0.77–2.44)	0.279	0.87 (0.52–1.82)	0.932	0.98 (0.50–1.96)	0.975
N2 spindle characteristics						
Density (each 1-spindle.min <sup>-1</sup> increase)	0.83 (0.74–0.94)	0.002*	0.87 (0.77–0.99)	0.027*	0.87 (0.76–0.99)	0.032*
Amplitude (each 1- $\mu\text{V}^2$ increase)	0.97 (0.95–0.99)	0.004*	0.98 (0.96–1.00)	0.041*	0.98 (0.95–1.00)	0.049*
Frequency (each 1-Hz increase)	0.83 (0.62–1.11)	0.211	0.95 (0.70–1.29)	0.734	0.88 (0.64–1.21)	0.427
Duration (each 1-s increase)	1.97 (0.16–23.9)	0.596	1.62 (0.13–19.8)	0.705	2.98 (0.23–38.6)	0.403
Fast spindles (each 1% increase), %	0.99 (0.98–1.00)	0.075	1.00 (0.99–1.01)	0.371	1.00 (0.99–1.01)	0.275

Data were analyzed using multivariable-adjusted logistic regression. EEG indicates electroencephalogram; NREM, nonrapid eye movement; N1, nonrapid eye movement sleep stage 1; N2, nonrapid eye movement sleep stage 2; N3, nonrapid eye movement sleep stage 3; OR, odds ratio; PSD, power spectral density; PSQI, Pittsburgh Sleep Quality Index; and REM, rapid eye movement.

Model 1 was adjusted for age, sex, and body mass index; and model 2 was additionally adjusted for alcohol consumption, systolic blood pressure, diabetes, dyslipidemia, obstructive sleep apnea, sleep efficiency, and duration of follow-up.

\*Indicates significant P-values.

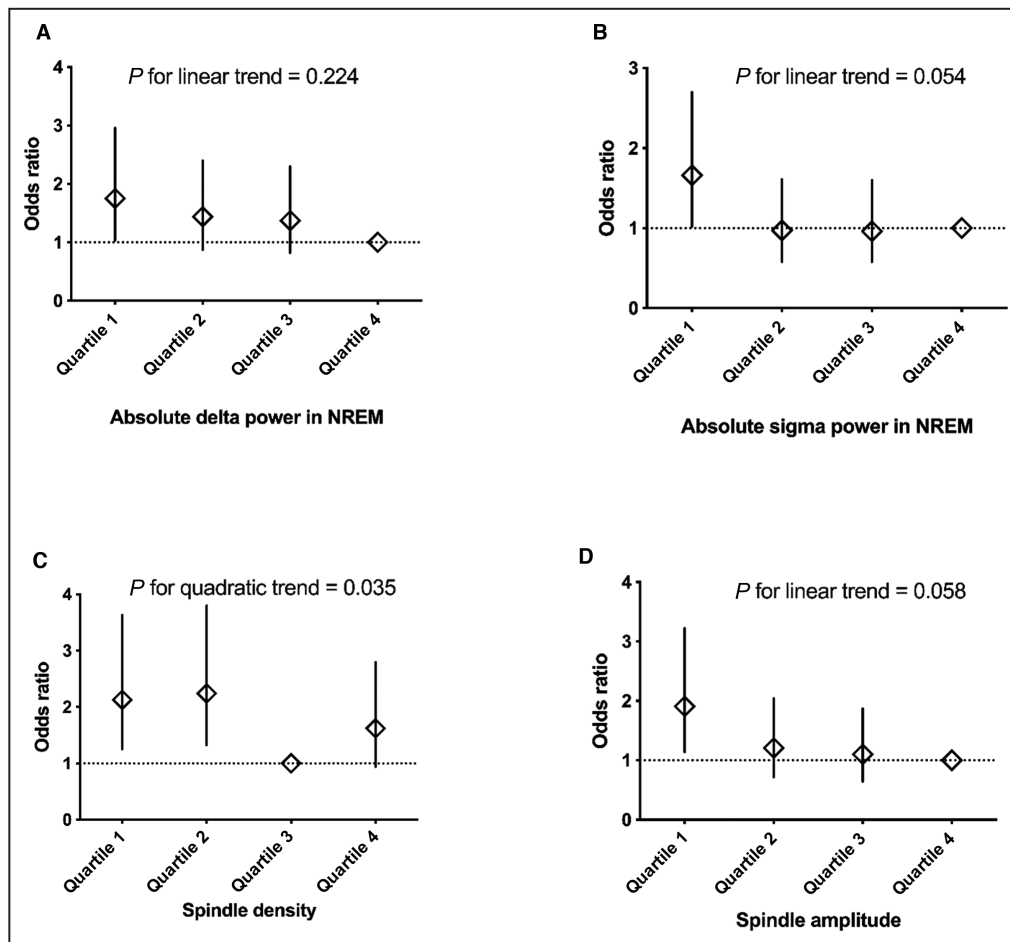
Our results confirm those of SWAN (Study of Women’s Health Across the Nation), a sleep study which showed that women with lower NREM delta power had a greater increase in diastolic BP during follow-up and tended to be at increased risk for incident hypertension, independent of obstructive sleep apnea.<sup>21</sup> However, to our knowledge, no previous studies have investigated other EEG power frequency bands. Thus, the present study is the first to demonstrate that not only slow wave activity (ie, power in the delta frequency band) but also faster brain activity (ie, power in the sigma frequency band reflecting spindles activity) in NREM sleep may protect against the development of hypertension. Furthermore, our findings extend the current literature by showing that some specific spindle characteristics, namely spindle density and amplitude, were also associated with a lower risk of incident hypertension after adjustment for multiple confounding factors.

### Potential Pathophysiological Mechanisms

The observed relationship between reduced slow wave activity (delta waves) and increased hypertension could reflect an underlying causal relationship with altered autonomic nervous system activity. Indeed, delta waves predominate during SWS, and studies investigating cardiac hemodynamics during sleep in healthy participants have shown decreased sympathetic activity and a concomitant increase in parasympathetic activity and baroreflex sensitivity with deeper sleep, which decreases BP and heart rate.<sup>41</sup> As a result, non-dipping BP associated with altered SWS could contribute to persistent elevation of daytime BP and further hypertension over the long term through mechanisms such as silent vascular damage, oxidative stress, and inflammation.<sup>11,42</sup>

Sleep spindles are an EEG hallmark of N2 and are reflected in the sigma power frequency band.<sup>43</sup> Although their functions are not clearly understood,





**Figure 3.** Association between incident hypertension at 5 years of follow-up and: (A) quartiles of delta power in nonrapid eye movement sleep (NREM); (B) quartiles of sigma power in NREM; (C) quartiles of spindle density in NREM sleep stage 2 (N2); and (D) quartiles of spindle amplitude in N2.

Results were analyzed using multivariable logistic regression with adjustment for baseline age, body mass index, sex, alcohol consumption, systolic blood pressure, diabetes, dyslipidemia, obstructive sleep apnea, sleep efficiency, and duration of follow-up (model 2).

observational and experimental evidence emphasize an important role of sleep spindles in memory consolidation and learning.<sup>44,45</sup> Higher sleep spindle density correlates with longer N2 sleep duration and greater resilience to sleep disruption from external perturbations.<sup>43,45</sup> At ages >40 years, studies even suggest that sleep spindles may become more relevant determinants of sleep quality than slow wave activity, which gradually decrease with age.<sup>46</sup> Of interest, animal studies have shown that mice tend to wake up from sleep during spindle-depleted periods, which may indicate a sleep “fragility period” where awakening is more likely.<sup>47</sup> Conversely, spindle-enriched periods, also called continuity periods, appear to be protective against arousals.<sup>47</sup> Furthermore, autonomic control correlates with these fragility and continuity periods, and continuity periods with high spindle density are accompanied by lower heart rate and higher parasympathetic activity.<sup>47,48</sup> These observations may

help to explain the observed protective effect of high sigma activity and spindle density in the development of hypertension. Last, although no longer consistent after multiple adjustments, the higher arousal index and proportion of N1 in bivariate analysis suggest that patients who developed hypertension may have more fragmented sleep.

### Strengths and Limitations

The main strengths of this study are the large number of participants included and the unselected population-based sample including both men and women, unlike SWAN.<sup>21</sup> Compared with earlier studies that investigated the relationship between sleep structure and hypertension, we performed a more in-depth analysis, assessing not only sleep macrostructure but also more comprehensive and objective sleep microstructural parameters using PSD EEG and novel spindle metrics.

**Table 4.** Interaction Analysis Between Sex, Age, OSA, Excessive Daytime Sleepiness, and Sleep Parameters for Incident Hypertension

	Sex interaction		Age interaction		OSA interaction		EDS interaction	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
SWS	1.01 (0.97–1.06)	0.642	1.03 (0.98–1.08)	0.208	1.04 (0.99–1.08)	0.133	1.02 (0.95–1.09)	0.649
Delta in NREM	1.00 (0.99–1.01)	0.538	1.00 (0.99–1.01)	0.152	1.01 (1.00–1.01)	0.166	1.00 (0.99–1.01)	0.956
Sigma in NREM	0.97 (0.86–1.09)	0.599	1.01 (0.90–1.12)	0.891	1.00 (0.89–1.12)	0.997	0.99 (0.83–1.20)	0.944
Spindle density	0.86 (0.65–1.12)	0.248	0.94 (0.73–1.22)	0.661	0.80 (0.59–1.08)	0.142	1.06 (0.72–1.56)	0.762
Spindle amplitude	0.97 (0.92–1.02)	0.225	1.01 (0.96–1.06)	0.702	1.01 (0.95–1.06)	0.860	0.99 (0.92–1.06)	0.747

Data are presented as odds ratios (ORs) and 95% CIs. Each factor (sex [men versus women]; age [ $<55$  years vs  $\geq 55$  years]; obstructive sleep apnea [OSA]; apnea-hypopnea index [ $<15$  events per hour versus  $\geq 15$  events per hour]; excessive daytime sleepiness [EDS;  $<11$  versus  $\geq 11$ ]), and each continuous objective sleep parameter (slow wave sleep [SWS]; delta in nonrapid eye movement [NREM]; sigma in NREM; spindle density; and spindle amplitude) were successively included together with an interaction term into the statistical model 2 adjusted for age, sex, body mass index, alcohol consumption, systolic blood pressure, diabetes, dyslipidemia, obstructive sleep apnea, sleep efficiency and duration of follow-up.

Moreover, comprehensive adjustment for covariates were assessed within the CoLaus study allowing for identification of robust independent associations between sleep metrics and incident hypertension.

Nevertheless, this study has some limitations. First, 24-hour ambulatory BP monitoring data and nocturnal BP measurements would have been desirable. However, these measures are challenging in population cohorts and contribute to arousals and awakenings that disturb sleep quality likely to confound EEG metrics. Second, a familiarization night was not performed and the polysomnography equipment may have altered participants' sleep quality even though polysomnography remains the gold standard to assess sleep. However, these effects would be expected to influence all participants relatively similarly. Furthermore, in-home polysomnography, which allows participants to sleep in their usual environment, is likely to be less disturbing than sleep laboratory polysomnography. Third, one intrinsic limitation of the use of cortical EEG is related to the limited spatial resolution of this technique, which allows the measurements of signals close to the cortical surface. Although the central EEG derivations are the most common area analyzed, further studies are needed to determine whether our results are consistent across other cortical areas.<sup>49</sup> Fourth, our study included almost exclusively White patients, which prevents the generalizability of our results to other ethnicities. Fifth, we acknowledge that baseline BP was higher in patients who developed hypertension during the follow-up period, but this difference was addressed in the statistical analysis by adding baseline systolic BP as a covariate. Last, given the large sample size, it is likely that some differences, albeit statistically significant, are not clinically relevant. Nevertheless, the quartile analysis showed both statistically and clinically significant increased risk (from +69% to +124%) among participants with low delta power, sigma power, spindle density, and amplitude. As those sleep microstructure parameters have seldom been studied, it would

be important that our results are replicated in other cohorts.

## Perspectives

Our findings indicate that sleep microstructural features are associated with incident hypertension. Slow wave activity and sleep spindles, 2 hallmarks of sleep continuity and objective sleep quality, were inversely associated with incident hypertension and were robust to multiple adjustments for potential confounders. These findings support the concept of a protective role of sleep continuity in the development of hypertension. However, additional studies are needed to confirm these results in other samples and to investigate potential underlying physiological mechanisms.

## ARTICLE INFORMATION

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## Disclosures

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## Supplemental Material

Figure S1

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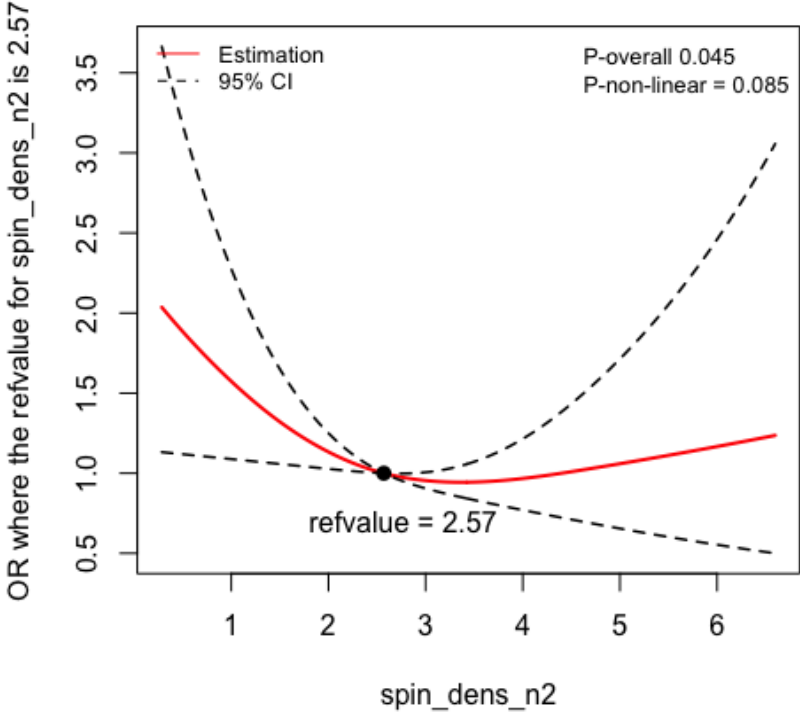
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# SUPPLEMENTAL MATERIAL



**Figure S1. Restricted cubic spline regression for the association between spindle density and incident hypertension.**



The red solid line represents the OR and the black dashed lines represent the 95% confidence intervals. Knots were placed at the 05<sup>th</sup>, 50<sup>th</sup> and 95<sup>th</sup> percentiles of spindle density. The reference value was 2.57. The model was adjusted for baseline age, body mass index, sex, alcohol consumption, systolic blood pressure, diabetes, dyslipidemia, obstructive sleep apnea, and sleep efficiency (Model 2).