Aus dem Interdisziplinärem Schlafmedizinischen Zentrum
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Altered Autonomic Function in Patients with Obstructive Sleep Apnea during Wakefulness

Veränderte autonome Funktion bei Patienten mit obstruktiver Schlafapnoe im Wachzustand

zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

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von

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To the Past, Present, and Future Me

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Abbreviations

OSA: Obstructive sleep apnea

HRV: Heart rate variability

BMI: Body mass index

AHI: Apnea-hypopnea index

ECG: Electrocardiography

PSG: Polysomnography

CPAP: Continuous positive airway pressure

meanNN: mean value of normal to normal (NN) interval time series

CVNN: ratio of SDNN divided by meanNN

SDNN: Standard deviation of NN intervals

SDANN1: Standard deviation of the average of 1-minute NN intervals over five minutes

RMSSD: Square root of the mean squared differences of consecutive NN intervals

pNN50: the percentage of NN>50ms counts divided by the total number of all NN intervals

pNNl20: NN intervals differences<20ms in percentage

5-min TP: Total power over five minutes

LF Power: Power in low frequency range

HF Power: Power in high frequency range

LF/HF: Ratio between low frequency and high frequency

LF nu: Low frequency power in normalized units

HF nu: High frequency power in normalized units

LF/p: Ratio of low frequency power and total power

HF/p: Ratio of high frequency power and total power

ShanEn: Shannon entropy

Renyi4: Renyi entropy with a weighting coefficient of α=4

Fwshannon: Frequency of Shannon entropy of word distribution

Fwrenyiα: Frequency of Renyi entropy of word distribution using a weighting coefficient of α (α=025,4)

Forbword: Forbidden Word (number of seldom ($p< 0.001$) or never occurring word types)

Wpsum02: relative portion of words consisting only of the symbols ‘0’ and ‘2’

Wpsum13: relative portion of words consisting only of the symbols ‘1’ and ‘3’

Wsdvar: Standard deviation of the word sequence

Plvar20: Portion of low-variability patterns in the NN interval time series (< 20 ms)

RRI: R peak to R peak intervals

SAGIC: Sleep Apnea Global Interdisciplinary Consortium

Abstract in English

Background: Patients with obstructive sleep apnea (OSA) show impaired cardiac autonomic function with heterogeneous profiles of heart rate variability (HRV) during sleep-wake states. However, the effect of OSA and its severity on autonomic modulation assessed by HRV during the wake period in a large multicenter clinical cohort is unclear.

Methods: A total of 1247 participants (426 non-OSA controls and 821 OSA patients, according to apnea-hypopnea index≥5) were globally recruited from the Sleep Apnea Global Interdisciplinary Consortium. HRV measures were computed from 5-minute ECG data in wake status with relaxed tidal breathing prior to the sleep onset, using time-domain, frequency-domain and non-linear approaches. Differences in HRV measurements were estimated among groups using analysis of covariance, adjusted for age, gender, body mass index, race/ethnicity, comorbidities and sites.

Results: OSA patients exhibited significantly reduced time-domain variations (SDNN, SDANN1, RMSSD, pNN50) and less complexity of cardiac rhythms (Shannon entropy, Fwshannon, Forbword) compared to non-OSA subjects. Those with severe OSA had considerably lower HRV compared to in comparison to other groups, both in time-domain and non-linear measurements. Compared to patients with OSA, those with severe OSA had reduced HRV based on SDNN (adjusted mean [95% CI]: 37.40 [34.55, 40.25] vs.46.19 [43.77, 48.60] ms; p<0.0001), SDANN1 (17.98.0 [15.87, 20.10] vs. 22.77 [20.97, 24.56] ms; p<0.0001), RMSSD (21.51 [19.59, 23.42] vs.27.98 [26.35, 29.60] ms; p<0.0001), pNN50 (5.1% [3.7%, 6.5%] vs. 9.2% [8.0%, 10.4%]; p=0.0001), Shannon entropy (1.8 [1.8, 1.9] vs. 2.0 [2.0, 2.1]; p<0.0001), Fwshannon 2.7 [2.6, 2.7] and Forbword (37 [35, 38] vs. 33[32, 34]; p=0.0001). There were no significant differences in overall frequency-domain metrics. Among obese patients, there is an increase in sympathetic tone in patients with severe OSA with higher LF/HF ratio compared to those without OSA (4.2 vs. 2.7; p = 0.009).

Conclusions: HRV is significant correlative with OSA severity. OSA patients show reduced HRV patterns during pre-sleep wakefulness compared to individuals without OSA, especially patients with severe OSA having significantly decreased time-domain HRV metrics and less complex non-linear HRV dynamics. Only obese OSA patients show enhanced sympathetic activity with increased LF and LF/HF. Thus, HRV could be a promising tool to investigate autonomic modulation and cardiovascular pathophysiology in patients with OSA.

Abstract in German

Hintergrund: Patienten mit obstruktiver Schlafapnoe (OSA) zeigen eine eingeschränkte kardiale autonome Funktion mit heterogenen Profilen der Herzratenvariabilität (HRV) im Wachzustand und im Schlaf. Die Wirkung von OSA und ihrer Schwere auf die autonome Modulation, die durch HRV während der Wachphase in einer großen multizentrischen klinischen Kohorte bewertet wurde, sind jedoch unklar.

Methoden: Insgesamt 1247 Probanden (426 ohne OSA und 821 Patienten mit OSA, basierend auf dem Apnoe-Hypopnoe-Index ≥ 5) wurden weltweit aus einem globalen interdisziplinären Konsortium für Schlafapnoe rekrutiert (the Sleep Apnea Global Interdisciplinary Consortium, SAGIC). Die HRV-Parameter wurden während einer Periode von 5-minütigen Wachheit mit Spontanatmung vor der eigentlicher Schlafstudie unter Verwendung von Zeitbasis-, Frequenzbasis- und nichtlinearen Methoden berechnet. Unterschiede in den HRV-Parametern wurden zwischen den Gruppen unter Verwendung von Kovarianzanalysen bewertet, wobei Alter, Geschlecht, Body-Mass-Index, Ethnizität, Komorbiditäten und Standort des Schlaflabors kontrolliert wurden.

Ergebnisse: Patienten mit OSA zeigten im Vergleich zu Personen ohne eine OSA signifikant geringere Variationen der Parameter in der Zeitbasis (SDNN, SDANN1, RMSSD, pNN50) und eine geringere Komplexität der Herzschläge (Shannon-Entropie, Fwshannon, Forbword). Diejenigen mit schwerer OSA hatten im Vergleich zu allen anderen Gruppen eine auffallend reduzierte HRV, sowohl in den Zeitbasis als auch in nichtlinearen Parametern. Im Vergleich zu Patienten ohne OSA hatten Patienten mit schwerer OSA eine niedrigere HRV basierend auf SDNN (adjustierter Mittelwert [95% CI]: 37.40 [34.55, 40.25] vs.46.19 [43.77, 48.60] ms; p<0.0001), SDANN1 (17.98.0 [15.87, 20.10] vs. 22.77 [20.97, 24.56] ms; p<0.0001), RMSSD (21.51 [19.59, 23.42] vs.27.98 [26.35, 29.60] ms; p<0.0001), pNN50 (5.1% [3.7%, 6.5%] vs. 9.2% [8.0%, 10.4%]; p=0.0001), Shannon entropy (1.8 [1.8, 1.9] vs. 2.0 [2.0, 2.1]; p<0.0001), Fwshannon 2.7 [2.6, 2.7] and Forbword (37 [35, 38] vs. 33[32, 34]; p=0.0001). Bei Ergebnissen der Methoden im Frequenzbereich wurden insgesamt keine signifikanten Unterschiede gefunden. Bei adipösen Patienten gibt es einen Anstieg des sympathischen Tonus bei schwerer OSA mit einem höheren LF/HF-Verhältnis im Vergleich zu adipösen nicht-OSA-Patienten (4,2 vs. 2,7; p = 0,009).

Schlussfolgerungen: Die HRV korreliert signifikant mit dem Schweregrad der OSA. OSA-Patienten zeigen im Wachzustand reduzierte HRV-Messwerte im Vergleich zu Personen ohne OSA, insbesondere Patienten mit schwerer OSA zeigen deutlich verringerten HRV-Messwerten in der

Zeitbasis und weniger komplexitatin parametern der nichtlinearen HRV-Dynamik. Nur adipöse OSA-Patienten zeigen eine sympathische Hyperaktivität mit erhöhtem LF und LF/HF. Somit könnte HRV zusätzliche Informationen über die autonome Modulation und die kardiovaskuläre Physiologie bei OSA-Patienten liefern.

1. Introduction

1.1 Obstructive sleep apnea (OSA) and autonomic function

Obstructive sleep apnea (OSA) is a heterogeneous sleep disordered breathing with high prevalence, which affects approximately 37% males and 17% females in the general population.[1] OSA is independently associated with cardiovascular diseases such as hypertension, arrhythmias, stroke and sudden death.[2-4] Furthermore, OSA is the leading cause of secondary hypertension.[5] OSA is characterized by complete and partial airway collapsibility, arousal and hypoxia.[6] Accordingly, these OSA-related physiologic changes may lead to activation of the autonomic nervous system. The autonomic nervous system mediates circulatory responses to internal and external stimulus via the sympathetic and parasympathetic nerve branches. It plays a key role in the control of heartbeat. Heart rate variability (HRV) measures variations between consecutive normal sinus beats (NN intervals) obtained from electrocardiography (ECG) data and is commonly used to quantify cardiac autonomic nervous activity.[7]

A number of previous studies provide primary evidence of negative associations between autonomic function and OSA under different conditions.[8] These findings suggested that autonomic dysregulation, particularly sympathetic hyperactivity and autonomic imbalance, could contribute to the pathogenesis and the development of cardiovascular diseases in patients with OSA. OSA can carry residual effects without any clinical symptoms even during daytime.[9, 10] To date, the clinical implication of altered autonomic function in OSA and the underlying mechanisms of high prevalence and risk of unfavorable cardiovascular outcomes in OSA are still unknown.

1.2 Heart rate variability (HRV)

OSA affects cardiac autonomic modulation.[8] Currently, OSA severity is defined by the apnea–hypopnea index (AHI, the number of apnea and hypopnea events per hour of sleep), which only captures the respiratory aspect of disease heterogeneity among patients.[11] Additional measurements combined with AHI, such as HRV, may help to improve the present characterization of OSA severity and cardiovascular implications. OSA patients appear to have a markedly elevated incidence of autonomic dysfunction, showing a diversity of HRV patterns during wake and sleep.[8] HRV could provide important encoded information in these fluctuations of RR sequence with regard to cardiovascular pathophysiology associated with OSA, without additional measuring

beyond polysomnography (PSG).[12]

Three main analytical approaches have been established to quantify linear and non-linear HRV: (1) time-domain analysis for quantifying the degree of variation of heartbeats (2) frequency-domain analysis for identifying the sympathetic and parasympathetic activity (3) non-linear analysis for capturing the randomness and complexity of heartbeats.[7] Given the non-linear and stochastic phenomenon of autonomic regulation for the cardiovascular system, any of these variables convey an individual biomedical message about HRV both in static and dynamic perspectives.

However, many confounding factors during sleep (e.g. sleep stages and sleep apneas) on HRV have been reported.[13, 14] In particular, temporal fluctuation in cardiac rhythms during sleep is associated with the time course of apneic events, exhibiting cyclic alternations of bradycardia and tachycardia during episodes of sleep apnea and respiratory restoration.[15] 24-hour HRV is also influenced by circadian rhythms, physical activity and emotional changes.[7] In addition, there are no standardized methods on sleep HRV and 24-hour HRV analysis. As a result, it is challenging to compare sleep HRV data and to interpret their results among different studies due to the nature of HRV measures and various methodologies. Unlike nocturnal and 24-hour ECG recordings, short-term records are easy to control and perform. Nevertheless, sparse data is reported in OSA patients regarding addressing abnormal changes of cardiac autonomic regulation and impaired cardiovascular variability during pre-sleep wakefulness.[9, 16, 17]

1.3 Study objectives

Thus, differences in multiple HRV parameters during pre-sleep wakefulness among OSA severity groups are unknown. With regards to this, this study takes advantage of a large-scale multicenter OSA clinical cohort recruited from the Sleep Apnea Global Interdisciplinary Consortium (SAGIC) to show the cardiovascular autonomic characteristics in OSA patients at normal breathing during wakefulness prior to sleep onset. It aims to study whether HRV measures could serve as a potent and reliable indicator to assess OSA severity and to evaluate autonomic nervous system regulation

2. Methods

2.1 Study population

426 controls without OSA and 821 patients with OSA, on the basis of AHI>5 events/hour were enrolled as part of the SAGIC study. All subjects included in this study untaken nocturnal sleep study at one of seven centers (Charité – Universitätsmedizin Berlin, Germany; Royal North Shore Hospital, Australia; Sir Charles Gairdner Hospital, Australia; University of Pennsylvania, the United States; The Ohio State University, the United States; Médicado Instituto do Sono, Brazil; and Chang Gung Memorial Hospital, Taiwan). Demographic and anthropometric characteristics were assessed before overnight in-lab polysomnography. Clinical profiles were obtained from medical history-taking and questionnaires. Demographic, anthropometric and clinical data included age, sex, body mass index (BMI), previous medical history, current medication usage and clinical symptoms. 1622 subjects in total with HRV and completed information on AHI, age, gender, BMI, and race/ethnicity were available in our SAGIC study cohort database. Of these, 321 participants with wake ECG recording after sleep study from the Icelandic site, 4 subjects with ECG sampling rate<128Hz, and 50 subjects with no normal-to-normal time>30000s (10% ectopic time in 5-minute HRV measurement) were excluded from the analysis sample. A final sample of 1247 subjects were eligible to test hypotheses in the present study (**Figure 1**). Every participant offered the informed consent as according to the Helsinki protocol. The experimental protocol as part of the SAGIC projects were individually approved by the ethical committee of every institution and hospital involved.

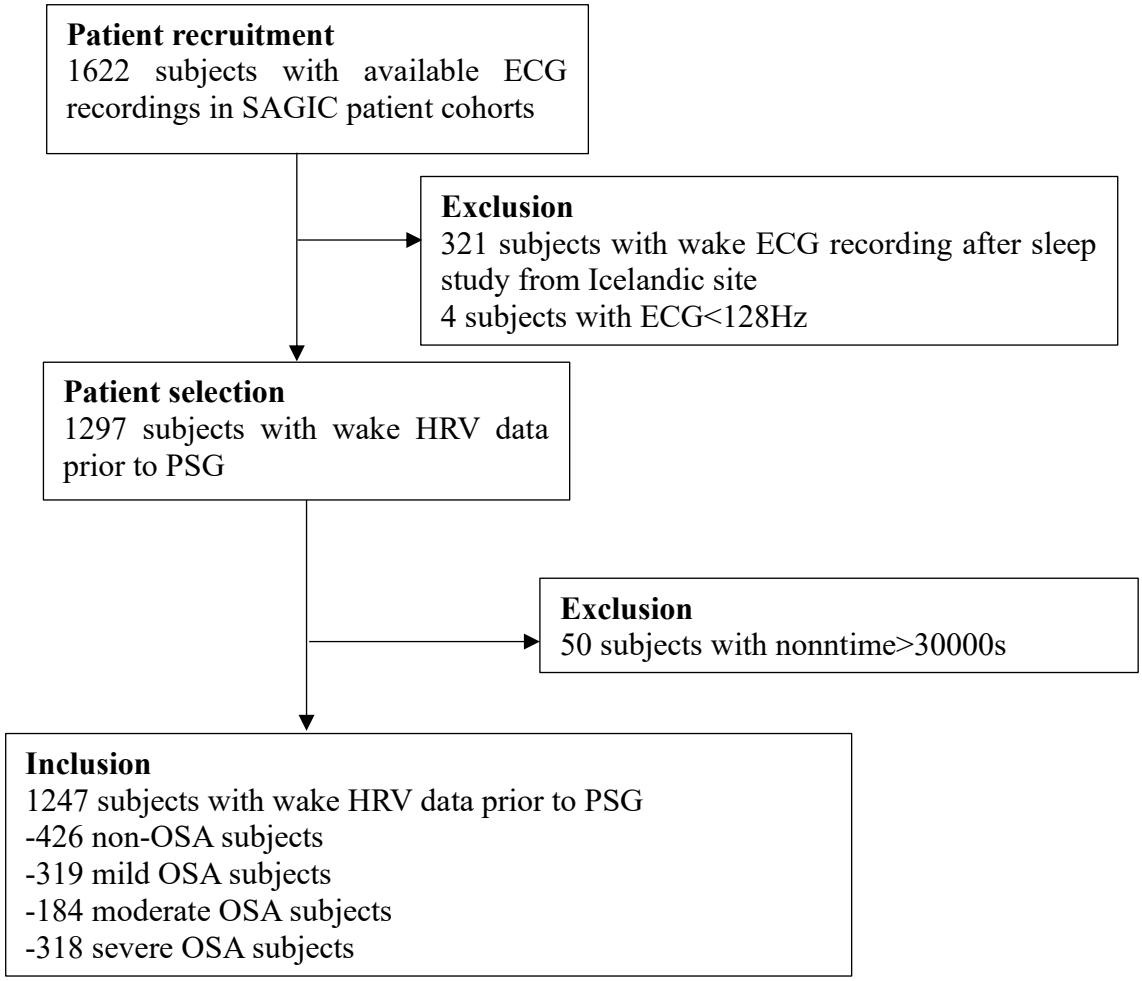

Figure 1 Flowchart of patient selection.

2.2 ECG acquisition and HRV analysis

2.2.1 ECG recording and signal processing

A standardized operating procedure for recording ECG was introduced across all centers to obtain comparable data (**Figure 2**). Each sleep lab implemented standard clinical protocols before completing overnight PSG for traditional and methodological control; all sites except Sydney and Perth site instruct patients that they should avoid alcohol and caffeine-containing beverages (e.g. tea and coffee) during the day of study. ECG data was collected from a modified single lead II channel (**Figure 2**).

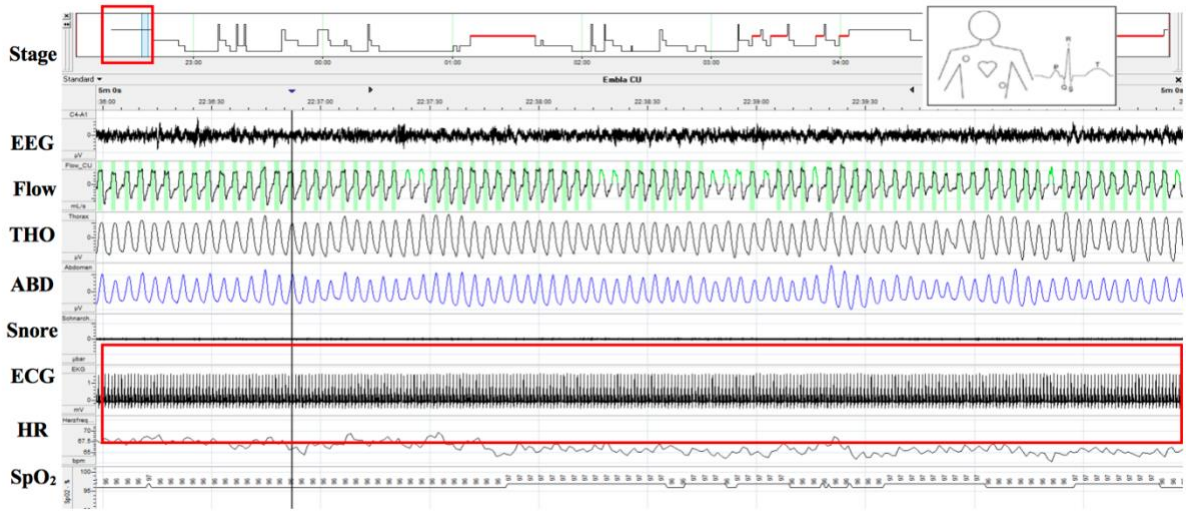

Figure 2 Five-minute polymographic tracings and the ECG Lead II placement in a patient during wakefulness prior to sleep onset.

Each channel from top to bottom is hypnogram of sleep stage, electroencephalography (EEG), flow, thoracic effort (THO), abdominal effort (ABD), snore, electrocardiogram (ECG), heart rate (HR), pulse oxygen saturation (SpO2). The panel at top right corner illustrated the placement of ECG electrodes with a single modified lead II channel (left) and ECG with large R-wave and baseline at the lower level (right).

The sampling frequency applied to ECG acquisition in each sleep laboratory ranged from 128 to 512 Hz. All subjects were instructed to maintain resting spontaneous breathing for 15 minutes in waking condition prior to the initiation of overnight sleep study. Electroencephalogram (EEG) was employed to assess whether subjects were awake during ECG data collection. All participants were awake without any abnormal breathing patterns (e.g. obstructive sleep apnea and central sleep apnea) during HRV measurements. The Pan Tompkins algorithm was utilized to detect R peaks in ECG data[18]. The beat-to-beat interval series was derived from a series of time differences between successive R peaks. Then, as previously described, an adaptive filtering algorithm was used to automatically detect and remove artifacts and ectopic beats from the interbeat intervals. Finally, HRV measures was calculated from a selected 5-minute ECG segment preceding sleep onset for each participant, as 5 minutes is thought to be a preferred time window for short-term linear and non-linear dynamic HRV analysis (**Figure 3**).

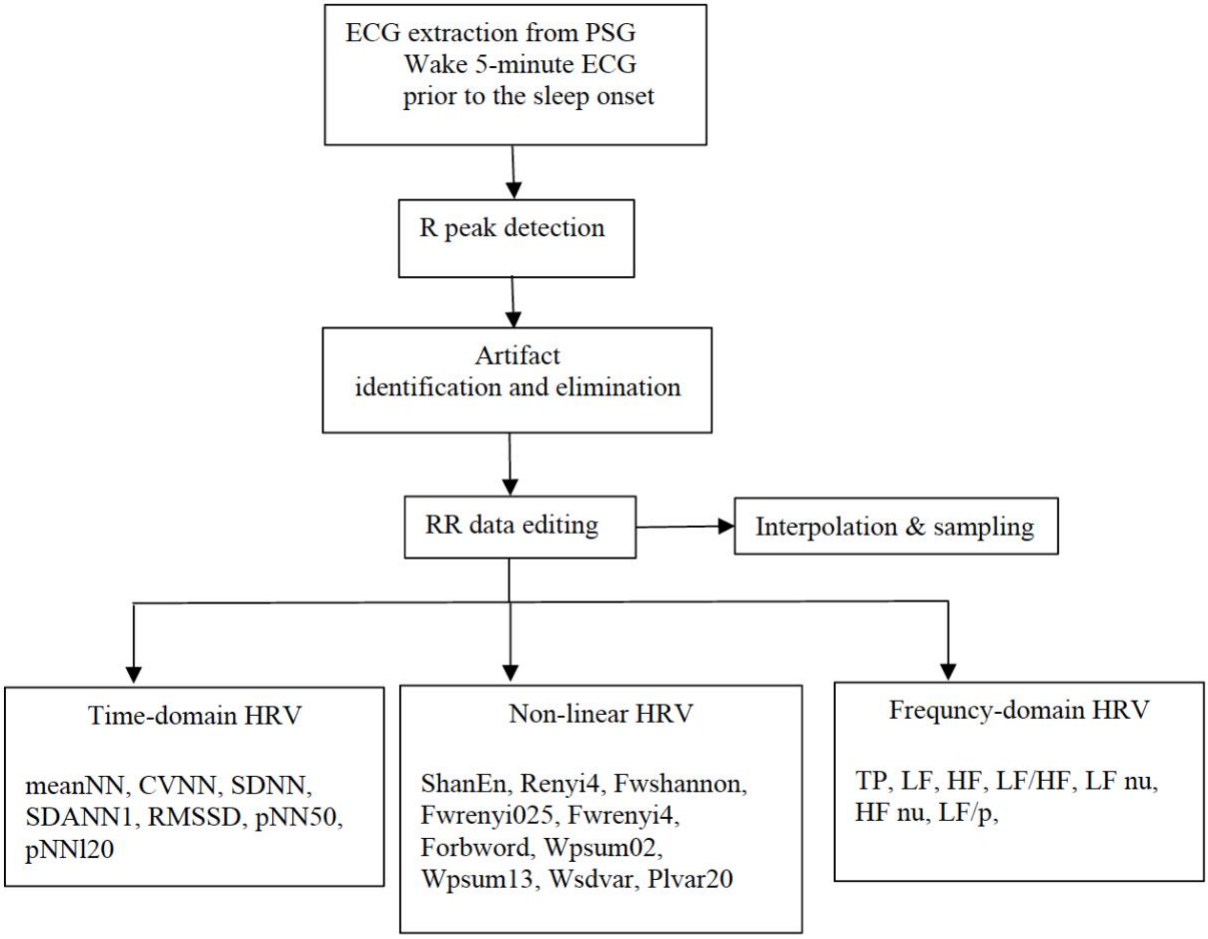

Figure 3 Heart rate variability.

ECG: electrocardiography; PSG: polysomnography; HRV: heart rate variability; meanNN: the mean value of normal to normal (NN) interval time series; CVNN: the ratio of SDNN divided by meanNN; SDNN: the standard deviation of NN intervals; SDANN1: the standard deviation of the average of 1-minute NN intervals over five minutes; RMSSD: square root of the mean squared differences of successive NN intervals; pNN50: the percentage of NN>50ms counts divided by the total number of all NN intervals ; pNNl20: Percentage of NN intervals differences<20ms; 5-min TP: total power over five minutes; LF: low frequency, HF: high frequency range; LF/HF: the ratio between low frequency and high frequency; LF nu: low frequency power in normalized units; HF nu: high Frequency power in normalized units; LF/p: ratio of low frequency power and total power; HF/p: ratio of high frequency power and total power; ShanEn: Shannon entropy; Renyi4: Renyi entropy with a weighting coefficient of α=4; Fwshannon: frequency of Shannon entropy of word distribution; Fwrenyi4: frequency of Renyi entropy of word distribution using a weighting coefficient of α (α=025,4); Forbword=forbidden word (number of seldom ($p< 0.001$) or never occurring word types); Wpsum02: relative portion of words consisting only of the symbols ‘0’ and ‘2’; Wpsum13: relative portion of words consisting only of the symbols ‘1’ and ‘3’; Wsdvar: standard deviation of the word sequence; Plvar20: portion of low-variability patterns in the NN interval time series (< 20 ms).

2.2.2 HRV measurements

The time-domain measures were calculated based on RR intervals (RRI) over time series to quantify the amount of variation between heartbeats.[7] Seven common time-domain variables were reported in our study including standard deviation of the normal-to-normal interval (SDNN) aggregates RRI over time into an ensemble measure of HRV and is the most common index; standard deviation of the average of 1-minute NN intervals over five minutes (SDANN1), square root of the mean squared differences of consecutive NN intervals (RMSSD), and the percentage of NN>50ms counts divided by the total number of all NN intervals (pNN50), mean value of NN interval time series(meanNN), ratio of sdNN divided by meanNN (CVNN), and NN intervals differences<20ms in percentage (pNNl20). RMSSD and pNN50 are closely correlatives with parasympathetic nervous activity.

The spectral analysis identifies components of ECG data to quantify the cardiac autonomic modulation.[7] Eight frequency-domain measures were demonstrated in this study including 5-minute total power (5-min TP, 0.0001-0.4 Hz), very low frequency (VLF, 0.003–0.04 Hz), low frequency (LF, 0.04-0.15 Hz) and high frequency (HF, 0.15-0.4 Hz), low frequency presented in normalized units (LF nu), high frequency presented in normalized units (HF nu), and low-frequency to high-frequency power ratio (LF/HF).[7] Normalized LF (LF nu)and normalized HF(HF nu) are defined as relative proportions, equal to LF/(TP-VLF)x100 and HF/(TP-VLF)x100, respectively. LF/P and HF/P are ratios of LF and total power and ratio of HF and total power, respectively. The HF component is believed to reflect parasympathetic activity and the LF component possibly represents both sympathetic and vagal control, which is mainly mediated by baroreflexes.[19] LF/HF reflects the autonomic balance.[7]

The non-linear analysis explore self-similarity and randomness of cardiac rhythm as heartbeat is a complex non-linear physiological signal.[7] In this study, Shannon entropy and symbolic dynamics parameters were used to measure the complex dynamics of heartbeats.[20] A higher Shannon entropy indicated more irregularity in HRV. Symbolic dynamics transform RR intervals into a symbol series in order to dynamically investigate the behavior of heart rate oscillations.[20] First, the RR intervals are transformed into symbolic dynamics sequences employing 3-symbol words (w = 3) and an alphabet consisted of four symbols.[20] Then, the complexity of the symbols dynamics sequences is evaluated by eight metrics – the frequency of word distribution of Shannon entropy (Fwshannon), forbidden words (Forbword), relative portion of words including only of the

symbols “0” and “2” (wpsum02), relative portion of words consisting only of the symbols “1” and “3” (wpsum13), standard deviation of the word sequence (wsdvar), portion of low-variability patterns in the NN interval time series (plvar20), Renyi entropy of the word distribution with weight coefficent α=X (X=025,4; fwrenyiX, bit), portion of low-variability patterns in the NN interval time series<20 ms (plvar20). Accordingly, the Fwshannon is introduced to quantively assess the randomness and regularity of the interbeat tachograms. Larger values of Fwshannon indicate higher complexity of heart rate. Forbword is the number of words of length 3 that never or rarely appear. Lower values of Forbword suggest a higher irregularity, as it is found that the number of Forbword will be larger when the time series is highly regular. Wpsum02 is measured by quantifying low variation in mean of heartbeat intervals for decreased HRV, while wpsum13 is for increased HRV by quantifying high variation in mean of heartbeat intervals. Fwrenyi025 weight smaller probabilities and Fwrenyi4 weight larger probabilities. Plvar20 measures probability for low variation of NN<20ms. Renyi entropy with a weighting coefficient of α=4 (renyi4). Renyi4 tends to estimate larger probabilities more than lower coefficients of variation.

Shannon entropy of the HRV histogram is applied as an entropy measure to quantify the distribution of NN intervals:

$$\text{Shannon entropy} = -\sum_{j\in\Omega} p_j \ \log(p_j)$$

where p_j is the histogram of the time series.

The Shannon entropy of the probabilities of occurrence of the words of the symbol sequence (Fwshannon):

$$\text{Fwshannon} = -\sum_{k=1}^{4^w} p_k \ \log(p_k)$$

where p_k are the probabilities of each word of w symbols of the sequence. The larger Fwshannon, the higher the complexity of the corresponding tachograms.

Renyi entropy (α=4) of the HRV tachogram is calculated to measure the weighted probability distributions (Renyi4):

$$\text{Renyi entropy} = -\frac{1}{3}\log_2 \sum_{i=1}^{n} P_i^4$$

where P_i is the class probabilities (i=1,2,3..,n) using a weighting coefficient of α=4. It weights the larger probability more than the lower coefficient.

Symbolic dynamics transformation:

$$\mathbf{S_i}(X_i) = \begin{cases} 0: & \mu & < X_i \leq & (1+\alpha)\mu \\ 1: & (1+\alpha)\mu & < X_i < & \infty \\ 2: & (1-\alpha)\mu & < X_i \leq & \mu \\ 3: & 0 & < X_i \leq & (1-\alpha)\mu \end{cases}$$

where $i = 1, 2, 3$ ...the time series $\mathbf{X_1}$, $\mathbf{X_2}$, $\mathbf{X_3}$,…$\mathbf{X_N}$. $\mathbf{X_i}$is transformed into the symbol sequence $\mathbf{S_1}$, $\mathbf{S_2}$, $\mathbf{S_3}$,…$\mathbf{S_N}$. $\mathbf{S_i}$∈A based on the alphabet A = (0, 1, 2, 3). The transformation into symbols refers to three given levels where μ represents the mean RR interval and α is a variable that is chosen to be 0.05.

2.3 Sleep studies

A standard nocturnal polysomnography was conducted for assessment of OSA. The following polysomnographic signals were recorded via electroencephalography (EEG), electrooculogram (EOG), ECG as described above, chin electromyogram (EMGchin), thoracoabdominal movements bands to measure respiratory effort, tracheal sound by microphone, body position sensor, pulse oximetry measure oxygen saturation, and airflow (thermistor and nasal pressure) monitor breathing after skin preparation with gel and alcohol. All participants were encouraged to sleep supinely. According to AASM criteria.[11] Apnea episodes were scored as a decrease ≥90% of pre-event baseline airflow amplitude for ≥10 seconds and hypopnea episodes were defined as a dorp ≥30% of pre-event baseline airflow with ≥4% desaturation or an arousal for ≥10 seconds, respectively. OSA categories were stratified by apnea-hypopnea index (AHI, numbers of apneaic and hyponeaic events per hour over sleep period). Patients were divided into four groups based on AHI severity: non-OSA (AHI<5 events per hour), mild OSA (5≤AHI<15 events per hour), moderate OSA (15<AHI≤30 events per hour), and severe OSA (AHI≥30 events per hour).

2.4 Statistical analyses

Numerical data were reported as mean ± standard deviation (SD) or median (range). Categorical data were present as numbers (percentages). Comparison of quantitative data among groups using analysis of variance (ANOVA) and categorical data compared among groups using chi-squared tests. To assess differences in HRV parameters among different AHI severity groups, unadjusted ANOVA or adjusted analysis of covariance (ANCOVA) controlling for important clinical covariates, including age, sex, BMI, race/ethnicity, presence of comorbidities (hypertension,

hyperlipidemia, coronary artery disease, heart failure, stroke and diabetes), SAGIC site, and ECG sampling rate and time from the end of ECG to sleep onset, was utilized in this study. To better investigate further modifying effects of obesity on observed relationships among group, exploratory analyses were conducted in the full sample. The obesity stratification was based on BMI ≥ 27 kg/m^2 among subjects of self-reported Asian ethnicity and BMI ≥ 30 kg/m^2 among others ethnicities.[21] Statistical interaction tests assessing the significance of a product term [obesity (1 = obese, 0 = non-obese)] by [AHI severity group] in models including the main effects were used to evaluated for discrepancies among OSA severity groups stratified by obesity status.

Additionally, Pearson's linear and partial correlations (adjusted for the same covariates) to study the associations between continuous AHI or Epworth Sleepiness Scale (ESS) and HRV metrics were employed among groups. In accordance with observed non-normal distributions of variables, meanNN, CVNN, SDNN, SDANN1, RMSSD, 5-min TP, LF/HF and HF/P were in natural log-transformations in all analyses. Similarly, the natural log of AHI + 1 was applied to examine continuous relationships with HRV variables.

Statistical significance was according to the results of a domain-specific Bonferroni correction (e.g. $p<0.0125$ for time-domain parameters, $p<0.0083$ for frequency-domain parameters and $p<0.0167$ for non-linear domain parameters). If significant or nominal ($p<0.05$) differences among groups were found in overall ANOVA/ANCOVA analyses, post-hoc comparisons were performed, using a Bonferroni corrected threshold of $p<0.0083$ for statistical significance. In all analyses, $p<0.05$ was considered nominal evidence of an association. We used SAS version 9.4 (SAS Institute, Cary, NC) and Stata/SE 14.2 (StataCorp LLC, College Station, TX) to perform statistical analyses.

3. Results

3.1 Patient characteristics

The comparison of baseline characteristics among subjects with and without OSA according to AHI classification is summarized in **Table 1**.[22] There are significant differences among groups for each demographic, anthropometric and ECG sampling variable including age, gender, BMI, AHI, ethnicity, site and sampling rate (all $p<0.0001$). Only a small number of participants in the analysis (n=4 [0.3%]) had a sampling frequency <128 Hz; these individuals were excluded. Among those

included in the analysis, 62.7% of all recordings had a sampling frequency <256 Hz, including 128Hz (11.3%), 200Hz (51.2%), 250Hz (0.2%), 256Hz (28.0%) and 512Hz (9.3%). While there were some differences in sampling frequencies across sites, we do not expect this to influence the distributions of OSA severity presented in **Table 1**; we also included site as a covariate in our adjusted analyses to control for possible confounding due to site-specific factors. Similarly, differences were observed in all reported comorbidities, with higher prevalence of cardiovascular diseases and diabetes among patients with OSA. No significant difference was found in time from end of 5-minute ECG recordings to sleep onset (P=0.554). Overall, the mean (standard deviation) time between end of the ECG and sleep initiation was 11.8 (18.2) minutes. There was indeed a large range from 0 to 244 minutes; however, 91.8% of the sample had <30 minutes between the end of ECG and the start of sleep. There was only a small negative correlation between this duration and Epworth scores (rho=-0.07, p=0.032), and no significant correlation between this duration and log-transformed AHI (rho = -0.05, p=0.102). All of these variables, except AHI, were included as covariates in adjusted comparisons of HRV parameters from non-OSA to severe OSA.

Table 1 Summary of characteristics of participants with and without obstructive sleep apnea at baseline[22]

Variable	No OSA AHI<5	Mild OSA 5≤AHI<15	Moderate OSA 15≤AHI<30	Severe OSA AHI≥30	p-value
N	426	319	184	318	
Age, years	46.2 ± 14.5	53.1 ± 13.5	55.1 ± 13.9	52.5 ± 14.0	<0.0001
Male, N (%)	170 (39.9)	168 (52.7)	99 (53.8)	221 (69.5)	<0.0001
BMI, kg/m^2	27.4 ± 5.6	30.8 ± 7.4	32.6 ± 7.2	33.3 ± 8.8	<0.0001
AHI, events/h	1.7 ± 1.5	9.5 ± 2.9	21.0 ± 4.3	59.0 ± 24.1	<0.0001
Ethnicity, N (%)					
Caucasian	158 (37.1)	124 (38.9)	74 (40.2)	118 (37.1)	<0.0001
Asian	11 (2.6)	18 (5.6)	15 (8.2)	96 (30.2)	
C/S American	205 (48.1)	129 (40.4)	61 (33.2)	72 (22.6)	
Afr./Afr. American	11 (2.6)	18 (5.6)	13 (7.1)	12 (3.7)	
Others	41 (9.6)	30 (9.4)	21 (11.4)	20 (6.3)	
Site, N (%)					
Berlin	22 (5.2)	25 (7.8)	12 (6.5)	18 (5.7)	<0.0001
Brazil	241 (56.6)	156 (48.9)	75 (40.8)	92 (28.9)	
OSU	25 (5.9)	28 (8.8)	18 (9.8)	41 (12.9)	
Penn	13 (3.1)	19 (6.0)	16 (8.7)	13 (4.1)	
Perth	40 (9.4)	20 (6.3)	23 (12.5)	17 (5.3)	
Sydney	79 (18.5)	67 (21.0)	27 (14.7)	44 (13.8)	
Taiwan	6 (1.4)	4 (1.3)	13 (7.1)	93 (29.2)	
Comorbidity, N (%)					
Hypertension	84 (19.7)	100 (31.3)	75 (40.8)	127 (39.9)	<0.0001
CAD	10 (2.3)	21 (6.6)	16 (8.7)	28 (8.8)	0.0007
Heart Failure	6 (1.4)	8 (2.5)	8 (4.3)	18 (5.7)	0.008
Stroke	7 (1.6)	9 (2.8)	4 (2.2)	16 (5.0)	0.048
High Cholesterol	88 (20.7)	100 (31.3)	79 (42.9)	126 (39.6)	<0.0001
Diabetes	18 (4.2)	42 (13.2)	17 (9.2)	35 (11.0)	0.0002
ECG Latency*, mins	12.4 ± 20.3	12.3 ± 19.8	11.2 ± 14.0	10.7 ± 15.3	0.554
Sampling Rate, N (%)					
128 Hz	46 (10.8)	39 (12.2)	28 (15.2)	28 (8.8)	<0.0001
200 Hz	262 (61.5)	181 (56.7)	87 (47.3)	108 (34.0)	
250 Hz	0 (0.0)	0 (0.0)	1 (0.5)	2 (0.6)	
256 Hz	112 (26.3)	95 (29.8)	55 (29.9)	87 (27.4)	
512 Hz	6 (1.4)	4 (1.3)	13 (7.1)	93 (29.2)	

Continuous data presented as mean ± standard deviation (SD) and categorical data as frequency and percentage; Abbreviations: BMI = body mass index, AHI = apnea-hypopnea index, OSU = The Ohio State University, Penn = University of Pennsylvania, C/S = Central/South, Afr. = African, CAD = coronary artery disease; *Time (minutes) from end of 5-minute ECG recording to sleep onset.

Note: this table is adopted from my doctoral work (Ref 22) as there are the same samples included in this study, which means the values of the clinical and technical features are same.

3.2 Linear and non-linear HRV measures among OSA severity groups

The comparisons of HRV measurements among groups are shown in unadjusted analysis (**Table 2**) and adjusted analysis with controls for confounding factors (**Table 3**).

Table 2 Unadjusted comparison of heart rate variability measures by apnea-hypopnea index categories in the SAGIC cohort

Variable	Mean ± Standard Deviation				p-value*
	AHI<5	5≤AHI<15	15≤AHI<30	AHI≥30	
Time-domain parameters					
meanNN, ms	871 ± 123	897 ± 134[a, g, j]	866 ± 123[l]	836 ± 137[f]	**<0.0001**‡
SDNN, ms	48.36 ± 25.88	44.78 ± 24.59	41.48 ± 21.98[d, l]	35.86 ± 19.48[f]	**<0.0001**‡
SDANN1, ms	23.78 ± 19.34	22.32 ± 18.20	20.72 ± 15.25[l]	7.01 ± 12.76	**<0.0001**‡
CVNN	0.06 ± 0.03	0.05 ± 0.03[b, j]	0.05 ± 0.02[d, k]	0.04 ± 0.02[f]	**<0.0001**‡
RMSSD, ms	29.51 ± 17.16	26.68 ± 16.15[a, g, j]	23.65 ± 14.74[d, l]	20.55 ± 12.79[f]	**<0.0001**‡
pNN50, %	10.1 ± 13.1	8.1 ± 12.1[a, j]	6.4 ± 10.7[d]	4.7 ± 8.5[f]	**<0.0001**
pNNl20	0.58 ± 0.23	0.61 ± 0.22[a, h, j]	0.67 ± 0.23[d, k]	0.71 ± 0.23[f]	**<0.0001**
Frequency-domain parameters					
5-min TP, ms^2	312 ± 372	271 ± 365[b, i]	260 ± 292[c]	208 ± 247[f]	**<0.0001**‡
LF, ms^2	82 ± 92	67 ± 86[a]	67 ± 96[c]	60 ± 79[f]	**0.0039**
HF, ms^2	39 ± 49	30 ± 42[b, i]	24 ± 29[d]	22 ± 29[f]	**<0.0001**
LF/HF	3.2 ± 3.1	3.6 ± 3.6	4.1 ± 4.1	3.9 ± 4.4	0.178‡
LF nu	0.66 ± 0.18	0.68 ± 0.19	0.69 ± 0.20	0.68 ± 0.19	0.293
HF nu	0.34 ± 0.18	0.32 ± 0.19	0.31 ± 0.20	0.32 ±0.19	0.293
LF/P	0.29 ± 0.14	0.29 ± 0.15	0.28 ± 0.15	0.29 ± 0.15	0.3511
HF/P	0.16 ± 0.13	0.15 ± 0.14	0.14 ± 0.14[d]	0.15 ± 0.13	0.0031‡
Non-linear dynamic parameters					
Shannon entropy	2.1 ± 0.5	2.0 ± 0.5[j]	1.9 ± 0.5[d, l]	1.8 ± 0.5[f]	**<0.0001**
Fwshannon	2.8 ± 0.5	2.7± 0.5[a, g, j]	2.6 ± 0.6[d]	2.5 ± 0.5[f]	**<0.0001**
Forbword	32 ± 11	34 ± 11[b, j]	36±11[d]	38 ±10[f]	**<0.0001**
Renyi4	1.71 ± 0.48	1.65 ± 0.48	1.60 ± 0.50[c, j, l]	1.46 ± 0.48[f]	**<0.0001**
Fwrenyi025	3.23 ± 0.42	3.13 ± 0.44[d, j]	3.06 ± 0.45[d]	3.00 ± 0.43[f]	**<0.0001**
Fwrenyi4	2.05 ± 0.60	1.97 ± 0.57[j]	1.88 ± 0.60[d, k]	1.76 ± 0.57[f]	**<0.0001**
Wsdvar	1.29 ± 0.58	1.19 ± 0.59[a, j]	1.17 ± 0.63[c, k]	1.05 ± 0.55[f]	**<0.0001**
Wpsum02	0.54 ± 0.27	0.60 ± 0.27[b, j]	0.62 ± 0.28[d, l]	0.68 ± 0.234[f]	**<0.0001**
Wpsum13	0.15 ± 0.15	0.13 ± 0.13[i]	0.13 ± 0.14[k]	0.10 ± 0.11[f]	**0.0004**
Plvar20	0.19 ± 0.27	0.20 ± 0.28[d, h, j]	0.29 ± 0.32[k]	0.36 ± 0.34[f]	**<0.0001**

P-values in **bold showed** statistically significances after domain-specific Bonferroni correction; *p-value from analysis of variance (ANOVA) test comparing parameters among OSA severity groups; ‡Analysis performed on natural log transformed outcome; [a]P<0.05 (mild vs. control); [b]P<0.0083 (mild vs. control); [c]P<0.05 (moderate vs. control); [d]P<0.0083 (moderate vs. control); [e]P<0.05 (severe vs. control); [f]P<0.0083 (severe vs. control); [g]P<0.05 (mild vs. moderate); [h]P<0.0083 (mild vs. moderate); [i]P<0.05 (mild vs. severe); [j]P<0.0083 (mild vs. severe); [k]P<0.05 (moderate vs. severe); [l]P<0.0083 (moderate vs. severe); meanNN: mean value of normal to normal (NN) interval time series; SDNN: standard deviation of normal to normal (NN) interval; SDANN1: standard deviation of the average of 1-minute NN intervals; CVNN: ratio of SDNN divided by meanNN; RMSSD: square root of the mean squared differences of consecutive NN intervals; pNN50: percentage of NN > 50 ms counts divided by the total number of all NN intervals; pNNl20: percentage of NN intervals differences<20ms; 5-min TP: total power over five minutes; LF power: power of low frequency range; HF power: power of high frequency range; LF/HF: ratio of low frequency and high frequency power; LF nu: normalized low frequency power; HF nu: normalized high frequency power; Fwshannon: frequency of word distribution of Shannon entropy; Forbword: forbidden word; Renyi4:Renyi entropy with a weighting coefficient of α=4; FwrenyiX: frequency of shannon entropy of word distribution using a weighting coefficient of α (α=025,4); Wpsum02: relative portion of words consisting only of the symbols '0' and '2'; Wpsum13: relative portion of words consisting only of the symbols '1' and '3'; Wsdvar: standard deviation of the word sequence; Plvar20: portion of low-variability patterns in the NN interval time series (< 20 ms).

Seven features (meanNN, SDNN, SDANN1, CVNN, RMSSD, pNN50, and pNNl20) of time-domain HRV were presented. Unadjusted analyses demonstrated a significant reduction in all time-domain metrics with increased disease severity ($p<0.0001$; **Table 2**). After included clinical and technological covariates in adjustment model, differences in all variables remained significant ($p\leq0.005$; Table 3). For each HRV parameter, severe OSA patients had lower values in comparison with other groups (**Table 3**). In between-group comparisons, meanNN showed significant differences among each AHI groups. These results suggest that patients with more severe OSA tend to have lower time-domain HRV measures.

Six features (5-minute TP, LF, HF, LF/HF, LF nu, HF nu, LF/P and HF/P) of frequency-domain parameters were reported. Differences in 5-minute TP ($p<0.0001$), LF ($p=0.0039$), HF ($p<0.0001$) and HF/P ($P<0.0031$) were found across all AHI categories in unadjusted analyses. However, after controlling clinical and technological covariates, no differences in these measurements among AHI groups were observed (**Table 3**).

For ten non-linear HRV parameters (ShanEn, FwshanEn, Forbword, Renyi4, Fwrenyi025 and Fwrenyi4, wpsum02, wpsum13, Wsdvar, and Plvar20), there was an overall decrease of dynamic HRV complexity in patients with higher degree of AHI when compared to those without OSA. Severe OSA patients had significantly lower values in ShanEn, Renyi4, Fwshannon, Fwrenyi025, Fwrenyi4, Wsdvar, and Plvar20 and higher values in Forbword, wpsum02 and Plvar20. Results were significantly different in both unadjusted and adjusted comparisons (**Table 3**).

Table 3 Adjusted comparison of heart rate variability measures by apnea-hypopnea index categories in the SAGIC cohort

Variable	Covariate adjusted mean and 95% confidence interval[†]				p-value
	AHI<5	5≤AHI<15	15≤AHI<30	AHI≥30	
Time-domain metrics					
meanNN, ms	877 (864, 890)	892 (879, 905)[h, j]	859 (841, 877)[k]	836 (820, 851)[f]	<0.0001[‡]
SDNN, ms	46.19 (43.77, 48.60)	45.07 (42.53, 47.61) [j]	43.36 (40.01, 46.70) [l]	37.40 (34.55, 40.25) [f]	0.0001[‡]
SDANN1, ms	22.77 (20.97, 24.56)	22.24 (20.36, 24.12) [j]	21.50 (19.01, 23.98) [k]	17.98 (15.87, 20.10) [f]	0.0051[‡]
CVNN	0.052 (0.050, 0.055)	0.050 (0.048, 0.053)[j]	0.050 (0.046, 0.053)[k]	0.044 (0.041, 0.047)[f]	0.0030[‡]
RMSSD, ms	27.98 (26.35, 29.60))	27.08 (25.37, 28.78) [j]	24.85 (22.60, 27.09) [c,k]	21.51 (19.59, 23.42) [f]	<0.0001[‡]
pNN50, %	9.2 (8.0, 10.4)	8.4 (7.2, 9.7)[j]	7.2 (5.6, 8.9)	5.1 (3.7, 6.5)[f]	0.0008
pNNl20	0.60 (0.58, 0.62)	0.60 (0.58, 0.62)[g, j]	0.65 (0.62, 0.68)[c]	0.69 (0.66, 0.72)	<0.0001
Frequency-domain metrics					
5-min TP, ms^2	276 (242 310)	278 (242, 314)	289 (242, 337)	233 (193, 273)	0.126[‡]
LF, ms^2	71 (63, 80)	69 (60, 79)	76 (63, 88)	67 (56, 77)	0.726
HF, ms^2	33 (29, 38)	32 (27, 36)	28 (23, 34)	25 (20, 30)	0.098
LF/HF	3.3 (2.9, 3.7)	3.6 (3.2, 4.0)	4.1 (3.5, 4.6)	3.8 (3.3, 4.3)	0.240[‡]
LF nu	0.66 (0.64, 0.68)	0.67 (0.65, 0.69)	0.69 (0.67, 0.72)	0.68 (0.66, 0.71)	0.280
HF nu	0.34 (0.32, 0.36)	0.33 (0.31, 0.35)	0.31 (0.28, 0.33)	0.32 (0.29, 0.34)	0.280
LF/P	0.28 (0.27, 0.30)	0.29 (0.27, 0.31)	0.28 (0.26, 0.30)	0.30 (0.28, 0.32)	0.582
HF/P	0.16 (0.14, 0.17)	0.16 (0.14, 0.17)	0.14 (0.12, 0.16)	0.16 (0.13, 0.16)	0.392[‡]
Non-linear dynamic metrics					
Shannon entropy	2.0 (2.0, 2.1)	2.0 (2.0, 2.1)[j]	2.0 (1.9, 2.1)[l]	1.8 (1.8, 1.9)[f]	<0.0001
Fwshannon	2.7 (2.6, 2.7)	2.7 (2.6, 2.8)[j]	2.6 (2.5, 2.7)[k]	2.5 (2.4, 2.6)[f]	0.0001
Forbword	33 (32, 33)	34 (33, 35)[j]	35 (33, 36)	37 (35, 38)[f]	0.0006
Renyi4	1.65 (1.60, 1.70)	1.67 (1.61, 1.72)[j]	1.65 (1.58, 1.72)[l]	1.49 (1.44, 1.55)[f]	0.0001
Fwrenyi025	3.17 (3.13, 3.21)	3.16 (3.12, 3.21)[j]	3.11 (3.05, 3.17)	3.03 (2.98, 3.08)[f]	0.0007
Fwrenyi4	1.98 (1.92, 2.03)	2.00 (1.94, 2.06)[j]	1.94 (1.85, 2.02)[l]	1.79 (1.72, 1.86)[f]	0.0001
Wsdvar	1.22 (1.16, 1.28)	1.21 (1.15, 1.27)[i]	1.23 (1.15, 1.31)[l]	1.08 (1.02, 1.15)[f]	0.0133
Wpsum02	0.56 (0.55, 0.60)	0.59 (0.56, 0.61)[j]	0.59 (0.55, 0.62)[l]	0.67 (0.64, 0.70)[f]	0.0002
Wpsum13	0.14 (0.12, 0.15)	0.13 (0.12, 0.15)	0.14 (0.13, 0.16)[l]	0.11 (0.09, 0.13)[e]	0.0394
Plvar20	0.22 (0.19, 0.25)	0.20 (0.17, 0.23)[j, g]	0.27 (0.23, 0.31)[k]	0.33 (0.29, 0.36)[f]	<0.0001

P-values in bold showed statistically significances after domain-specific Bonferroni correction; [†]Least squares mean and 95% confidence interval, adjusted for age, gender, BMI, race/ethnicity, site, comorbidities, ECG sampling rate and time from end of ECG recording to sleep onset; [‡]Analysis performed on natural log transformed outcome; [a]P<0.05 (mild vs. control); [b]P<0.0083 (mild vs. control); [c]P<0.05 (moderate vs. control); [d]P<0.0083 (moderate vs. control); [e]P<0.05 (severe vs. control); [f]P<0.0083 (severe vs. control); [g]P<0.05 (mild vs. moderate); [h]P<0.0083 (mild vs. moderate); [i]P<0.05 (mild vs. severe); [j]P<0.0083 (mild vs. severe); [k]P<0.05 (moderate vs. severe); [l]P<0.0083 (moderate vs. severe); meanNN: mean value of normal to normal (NN) interval time series; SDNN: standard deviation of normal to normal (NN) interval; SDANN1: standard deviation of the average of 1-minute NN intervals; CVNN: ratio of SDNN divided by meanNN; RMSSD: square root of the mean squared differences of consecutive NN intervals; pNN50: percentage of NN > 50 ms counts divided by the total number of all NN intervals; pNNl20: percentage of NN intervals differences<20ms; 5-min TP: total power over five minutes; LF power: power of low frequency range; HF power: power of high frequency range; LF/HF: ratio of low frequency and high frequency power; LF nu: normalized low frequency power; HF nu: normalized high frequency power; Fwshannon: frequency of word distribution of Shannon entropy; Forbword: forbidden word; Renyi4:Renyi entropy with a weighting coefficient of α=4; FwrenyiX: frequency of shannon entropy of word distribution using a weighting coefficient of α (α=025,4); Wpsum02: relative portion of words consisting only of the symbols '0' and '2'; Wpsum13: relative portion of words consisting only of the symbols '1' and '3'; Wsdvar: standard deviation of the word sequence; Plvar20: portion of low-variability patterns in the NN interval time series (< 20 ms).

3.3 HRV metrics among OSA severity groups in obesity-stratified analyses

We also performed statistical interaction tests between the HRV variables and obesity status to formally evaluate whether the observed relationships are modified by obesity. Unadjusted and adjusted results are shown in **Table 4** and **5**. Those results indicate that in both obese and non-obese subjects, time domain and non-linear measurements of HRV during pre-sleep wakefulness remained significantly related to increasing AHI severity, with a reduction in the global HRV metrics and less complex HRV parameters in patients with more severe condition even after adjusted for relevant covariates (**Table 5**). In addition, obesity status was linked to a shift to sympathetic enhancement as manifested by a higher LF and LF/HF, which was not seen in lean patients. Overall, these results donated adequate evidence in support of the hypothesis that HRV measures are associated with OSA regardless of obesity.

Table 4 Unadjusted comparison of heart rate variability measures in patients with and without obstructive sleep apnea stratified by obesity

Variable	Interatciton p-value	Covariate Adjusted Mean and 95% Confidence Interval† in Non-obese individual				p-value†	Covariate Adjusted Mean and 95% Confidence Interval† in obese individual				p-value†
		AHI<5	5≤AHI<15	15≤AHI<30	AHI≥30		AHI<5	5≤AHI<15	15≤AHI<30	AHI≥30	
Timed domain metrics											
meanNN‡, ms	0.873	877(864, 892)	907 (887, 927)[a, j]	880 (851, 909)	861 (838, 885)	**0.0164**	849 (824, 875)	883 (863, 905)[j]	857(832, 881)[k]	822 (80, 840)	**0.0002**
SDNN‡, ms	0.198	49.3 (46.7, 51.9)	44.8 (41.2, 48.4)[a, j]	39.3 (33.7, 44.8)[d]	35.8 (31.3, 40.2)[f]	<0.0001	45.5 (41.1, 49.9)	44.7 (41.0, 48.4)[j]	42.9 (38.6, 47.1)[l]	35.9 (32.8, 39.0)[f]	0.0003
SDANN1‡, ms	0.163	24.3 (22.3, 26.2)	22.8 (20.1, 25.5)[j]	19.9 (15.8, 24.0)[c]	17.5 (14.2, 20.9)[f]	0.0001	22.2 (19.1, 25.3)	21.8 (19.2, 24.3)[j]	21.2 (18.3, 24.2)[l]	16.7 (14.6, 18.9)[e]	0.0084
CVNN‡	0.314	0.06 (0.05, 0.06)	0.05 (0.05, 0.05)[a, i]	0.04 (0.04, 0.05)	0.04 (0.04, 0.05)[f]	**<0.0001**	0.05 (0.05, 0.05)	0.050 (0.047, 0.054)[j]	0.049 (0.045, 0.054)[K]	0.043 (0.040, 0.046)[f]	**0.0028**
RMSSD‡, ms	0.236	30.0 (28.3, 31.7)	25.0 (23.7, 28.3)[b, j]	21.9 (18.3, 25.5)[d]	20.7 (17.8, 23.6)[f]	<0.0001	28.0 (25.0, 31.0)	27.5 (25.0, 30.0)[j]	24.8 (21.9, 27.7)[l]	20.5 (18.3, 22.6)[f]	<0.0001
pNN50, %	0.466	10.3 (09.1, 11.6)	0.07.5 (5.8, 9.3)[a, i]	5.0 (2.3, 7.7)[d]	4.7 (2.5, 6.9)[f]	<0.0001	9.5 (7.3, 11.7)	8.8 (7.0, 10.6)[j]	7.3 (5.2, 9.4)	4.7 (3.2, 6.2)[f]	0.0006
pNNl20	0.414	0.56 (0.53, 0.58)	0.62 (0.58, 0.65)[a, g, j]	0.69 (0.63, 0.74)[d]	0.71 (0.67, 0.75)[f]	<0.0001	0.59 (0.55, 0.64)	0.50 (0.56, 0.63)[g, j]	0.66 (0.62, 0.71)[c, k]	0.72 (0.69, 0.75)[f]	<0.0001
Frequency domain metrics											
5-min TP‡, ms2	**0.040**	320.5 (284.7, 356.3)	268.0 (218.8, 317.3)[b, i]	191.1 (115.3, 267.0)[d]	164.3 (103.0, 225.7)[f]	**<0.0001**	287.0 (222.6, 351.4)	275.4 (221.7, 329.1)	303.6 (241.9, 365.4)	231.2 (185.9, 276.5)	0.288
LF Power, ms2	**0.0011**	88.3 (78.7, 97.9)	66.7 (53.6, 79.9)[a]	46.2 (25.9, 66.4)[d]	46.2(29.8, 62.6)[f]	**<0.0001**	63.3 (46.4, 80.2)	67.5 (53.4, 81.6)	79.9 (63.7, 96.1)	66.4 (54.5, 78.3)	0.490
HF Power, ms2	0.190	39.8 (35.1, 44.6)	25.8 (19.3, 32.3)[b]	20.2 (10.2, 30.2)[d]	24.7 (16.6, 32.8)[f]	**0.0001**	36.7 (29.8, 43.6)	33.8 (28.0, 39.5)[j]	26.1 (19.5, 32.8)[c]	21.2 (16.3, 26.1)[f]	**0.0006**
LF/HF‡	**0.0003**	3.4 (3.0, 3.8)	3.8 (3.2, 4.3)	3.5 (2.7, 4.3)	3.2(2.6, 3.9)	0.086	2.7 (1.9, 3.5)	3.5 (2.8, 4.1)[i]	4.5 (3.8, 5.3)[d, g]	4.2 (3.6, 4.7)[f]	**0.0011**
LF nu	**0.0006**	0.675 (0.655, 0.695)	0.694 (0.666, 0.721)	0.673 (0.631, 0.715)	0.638 (0.604, 0.672)	0.102	0.624 (0.587, 0.661)	0.656 (0.625, 0.687)[j]	0.706 (0.671, 0.742)[d, g]	0.697 (0.671, 0.723)[f]	**0.0022**
HF nu	**0.0006**	0.325 (0.305, 0.345)	0.306 (0.279, 0.334)	0.327 (0.285, 0.369)	0.362 (0.328, 0.396)	0.102	0.376 (0.339, 0.413)	0.344 (0.313, 0.375)[i]	0.294 (0.258, 0.329)[d, g]	0.303 (0.277, 0.329)[f]	**0.0022**
LF/P	0.0208	0.30 (0.29, 0.32)	0.29 (0.27, 0.31)	0.26 (0.23, 0.29)	0.28 (0.25, 0.30)	0.0737	0.26 (0.23, 0.29)	0.28 (0.26, 0.31)	0.29 (0.26, 0.31)	0.30 (0.28, 0.32)	0.1997
HF/P‡	0.0024	0.15 (0.14, 0.17)	0.14 (0.12, 0.16)	0.15 (0.12, 0.18)	0.18 (0.15, 0.20)	0.2472	0.18 (0.16, 0.21)	0.16 (0.14, 0.18)[h]	0.13 (0.10, 0.15)[d]	0.13 (0.12, 0.15)[f]	0.002
Non-linear dynamic metrics											
Shannon entropy	0.122	2.09 (2.04, 2.13)	1.99(1.92, 2.06)[a, j]	1.87 (1.76, 1.98)[d]	1.79 (1.70, 1.88)[f]	**<0.0001**	1.98(1.88, 2.08)	2.00 (1.92, 2.08)[j]	1.97(1.88, 2.06)[l]	1.80 (1.73, 1.87)[f]	**0.0004**
Fwshannon	0.418	2.77 (2.71, 2.82)	2.64 (2.56, 2.72)[a, g, j]	2.48 (2.36, 2.60)[d]	2.45 (2.35, 2.55)[f]	**<0.0001**	2.72 (2.62, 2.83)	2.69(2.60, 2.77)[j]	2.60 (2.50, 2.70)[k]	2.46 (2.39, 2.54)[f]	**0.0001**
Forbword	0.268	31.2 (30.1, 32.4)	34.9 (33.3, 36.5)[b, i]	37.7 (35.2, 40.2)[d]	37.8 (35.8, 39.8)[f]	**<0.0001**	32.3 (30.2, 34.3)	33.6(31.8, 35.3)[j]	34.9 (32.9, 36.9)	37.3 (35.8, 38.8)[f]	**0.0003**
Renyi4	0.058	1.73 (1.68, 1.79)	1.64 (1.57, 1.71)[a, j]	1.53 (1.42, 1.64)[d]	1.45 (1.36, 1.54)[f]	**<0.0001**	1.62 (1.52, 1.71)	1.66(1.58, 1.74)[j]	1.65 (1.56, 1.74)[l]	1.47 (1.40, 1.54)[e]	**0.0008**
Fwrenyi025	0.1966	3.25 (3.20, 3.29)	3.11 (3.05, 3.18)[b, g, i]	2.98 (2.88, 3.08)[d]	2.98 (2.90, 3.06)[f]	<0.0001	3.20 (3.11, 3.28)	3.16 (3.09, 3.23)[j]	3.10 (3.02, 3.19)	3.01 (2.95, 3.06)[f]	0.0008
Fwrenyi4	0.8245	2.05 (1.98, 2.11)	1.94 (1.85, 2.03)[j]	1.83 (1.69, 1.96)[d]	1.76 (1.65, 1.87)[f]	<0.0001	2.04 (1.93, 2.15)	2.00 (1.90, 2.09[j]	1.91 (1.80, 2.02)[k]	1.77 (1.69, 1.85)[f]	0.0001
Wsdvar	0.3026	1.31 (1.24, 1.37)	1.19 (1.11, 1.27)[a, i]	1.10 (0.96, 1.23)[d]	1.01 (0.91, 1.12)[f]	<0.0001	1.23 (1.12, 1.35)	1.20 (1.10, 1.29)[j]	1.22 (1.11, 1.33)[k]	1.07 (0.99, 1.14)[e]	0.0317
Wpsum02	0.3907	0.53 (0.51, 0.56)	0.61 (0.57, 0.64)[b, j]	0.65 (0.59, 0.71)[d]	0.70 (0.65, 0.75)[f]	<0.0001	0.56 (0.51, 0.61)	0.59 (0.54, 0.63)[j]	0.60 (0.55, 0.64)[k]	0.68 (0.64, 0.71)[f]	0.0006
Wpsum13	0.5257	0.15 (0.13, 0.16)	0.13 (0.11, 0.15)[j]	0.12 (0.09, 0.16)	0.09 (0.07, 0.12)[f]	=0.0026	0.14 (0.11, 0.17)	0.13 (0.11, 0.15)	0.14 (0.12, 0.17)[k]	0.11 (0.09, 0.13)	0.1222
Plvar20	0.741	0.03 (0.03, 0.03)	0.02 (0.01, 0.03)[g, j]	0.01 (0.001, 0.02)[d]	0.01 (0.003, 0.02)[f]	=0.0007	0.04 (0.03, 0.05)	0.03 (0.02, 0.04)[g, j]	0.02 (0.01, 0.03)[k]	0.01 (0.004, 0.02)[f]	0.0003

Statistically significant p-values **in bold** were presented after domain-specific Bonferroni correction; †p-value from analysis of variance (ANOVA) test comparing measures among OSA severity groups; ‡Analysis performed on natural log transformed outcome; aP<0.05 (mild vs. control); bP<0.0083 (mild vs. control); cP<0.05 (moderate vs. control); dP<0.0083 (moderate vs. control); eP<0.05 (severe vs. control); fP<0.0083 (severe vs. control); gP<0.05 (mild vs. moderate); hP<0.0083 (mild vs. moderate); iP<0.05 (mild vs. severe); jP<0.0083 (mild vs. severe); kP<0.05 (moderate vs. severe); lP<0.0083 (moderate vs. severe); meanNN: mean value of normal to normal (NN) interval time series; SDNN: standard deviation of normal to normal (NN) interval; SDANN1: standard deviation of the average of 1-minute NN intervals; CVNN: ratio of SDNN divided by meanNN; RMSSD: square root of the mean squared differences of consecutive NN intervals; pNN50: percentage of NN > 50 ms counts divided by the total number of all NN intervals; pNNl20: percentage of NN intervals differences<20ms; 5-min TP: total power over five minutes; LF power: power of low frequency range; HF power: power of high frequency range; LF/HF: ratio of low frequency and high frequency power; LF nu: normalized low frequency power; HF nu: normalized high frequency power; Fwshannon: frequency of word distribution of Shannon entropy; Forbword: forbidden word; Renyi4:Renyi entropy with a weighting coefficient of α=4; FwrenyiX: frequency of shannon entropy of word distribution using a weighting coefficient of α (α=025,4); Wpsum02: relative portion of words consisting only of the symbols ‘0’ and ‘2’; Wpsum13: relative portion of words consisting only of the symbols ‘1’ and ‘3’; Wsdvar: standard deviation of the word sequence; Plvar20: portion of low-variability patterns in the NN interval time series (< 20 ms).

Table 5 Adjusted comparison of heart rate variability metrics in patients with and without obstructive sleep apnea stratified by obesity

Variable	Interatciton p-value	Covariate Adjusted Mean and 95% Confidence Interval†				p-value†	Covariate Adjusted Mean and 95% Confidence Interval†				p-value†
		AHI<5	5≤AHI<15	15≤AHI<30	AHI≥30		AHI<5	5≤AHI<15	15≤AHI<30	AHI≥30	
Time domain metrics											
meanNN, ms	0.5116	886 (872, 901)	897 (878, 916)	866(837, 895)	861 (835, 887)[f]	0.1154	858 (833, 884)	887 (866, 907)[g, j]	850 (827, 873)[k]	819 (800, 837)[e]	0.0001
SDNN‡, ms	0.224	47.5 (44.7, 50.2)	45.4 (41.9, 48.9)[i]	42.3 (36.8, 47.7)	38.2 (33.3, 43.1)[f]	**0.013**	42.9 (38.3, 47.5)	43.9 (40.2, 47.7)[j]	43.8 (39.6, 48.0)[k]	37.3 (33.9, 40.6)	**0.012**
SDANN1‡, ms	0.203	23.7 (21.6, 25.8)	22.8 (20.1, 25.5)[i]	20.8 (16.6, 25.0)	18.6 (14.7, 22.4)[f]	**0.041**	21.1 (17.8, 24.3)	21.2 (18.5, 23.8)	21.6 (18.6, 24.6)	17.5 (15.1, 19.9)	0.056
CVNN‡	0.5373	0.053 (0.050, 0.056)	0.050 (0.047, 0.054)	0.049 (0.043, 0.054)	0.044 (0.039, 0.049)[f]	0.0451	0.049 (0.044, 0.054)	0.050 (0.046, 0.053)[j]	0.051 (0.046, 0.055)[k]	0.045 (0.041, 0.048)[f]	0.1713
RMSSD‡, ms	0.154	28.4 (26.7, 30.2)	26.8 (24.5, 29.1)[i]	24.0 (20.4, 27.5)[c]	22.6 (19.4, 25.8)[f]	**0.008**	26.0 (22.8, 29.2)	27.2 (24.6, 29.7)[j]	25.5 (22.6, 28.4)[k]	21.3 (19.0, 23.7)[e]	**0.002**
pNN50, %	0.554	9.3 (7.9, 10.6)	8.2 (6.4, 9.9)	6.5 (3.8, 9.2)	5.8 (0.03.4, 8.3)	0.102	8.3 (5.9, 10.6)	8.6 (6.7, 10.5)	7.8 (5.7, 9.9)	5.2 (3.5, 6.9)	0.060
pNNl20	0.3083	0.59 (0.56, 0.61)	0.60 (0.57, 0.63)[i]	0.65 (0.60, 0.70)[c]	0.67 (0.62, 0.72)[f]	0.0177	0.63 (0.59, 0.68)	0.60 (0.56, 0.64)[g, j]	0.65 (0.61, 0.69)[c, k]	0.70 (0.67, 0.73)[e]	0.0031
Frequency-domain metrics											
5-min TP‡, ms2	0.128	290.0 (251.7, 328.2)	285.8 (236.5, 335.1)[i]	236.9 (160.9, 312.9)	196.3 (127.4, 265.1)[f]	0.045	238.1 (171.2, 305.0)	258.6 (204.6, 312.5)	322.1 (261.2, 382.9)	257.5 (209.0, 306.0)	0.159
LF Power, ms2	**0.009**	78.1 (68.1, 88.2)	71.9(59.0, 84.9)	61.6 (41.7, 81.6)	57.8 (39.8, 75.9)	0.264	51.1(33.8, 68.4)	64.9 (51.0, 78.9)	84.9 (69.2, 100.7)[d]	71.6 (59.0, 84.1)	0.042
HF Power, ms2	0.253	33.9 (28.9, 38.8)	30.7 (24.3, 37.1)	27.9 (18.0, 37.7)	29.5 (20.6, 38.4)	0.710	31.1 (23.9, 38.3)	32.9 (27.1, 38.7)	28.9 (22.4, 35.5)	23.1 (17.9, 28.3)	0.119
LF/HF‡	**0.009**	3.6 (3.2, 4.0)	3.6(3.0, 4.1)	3.4 (2.6, 4.2)	3.1 (2.4, 3.9)	0.533	2.7 (1.9, 3.6)	3.5 (2.8, 4.2)	4.5 (3.7, 5.2)[d]	4.2 (3.6, 4.8)[e]	0.017
LF nu	**0.014**	0.675 (0.653, 0.696)	0.683 (0.656, 0.711)	0.676 (0.633, 0.719)	0.654 (0.615, 0.692)	0.680	0.631 (0.593, 0.670)	0.659 (0.628, 0.690)	0.701 (0.666, 0.736)[c]	0.694 (0.666, 0.722)[e]	0.030
HF nu	**0.014**	0.325 (0.304, 0.347)	0.317 (0.289, 0.344)	0.324 (0.281, 0.367)	0.346 (0.308, 0.385)	0.680	0.369 (0.330, 0.407)	0.341 (0.310, 0.372)	0.299 (0.264, 0.334)[c]	0.306 (0.278, 0.334)[e]	0.030
LF/P	0.1197	0.29 (0.28, 0.31)	0.29 (0.27, 0.31)	0.27 (0.24, 0.31)	0.30 (0.27, 0.33)	0.7351	0.26 (0.22, 0.29)	0.29 (0.26, 0.31)	0.28 (0.26, 0.31)	0.30 (0.28, 0.32)	0.1966
HF/P‡	0.0175	0.15 (0.13, 0.16)	0.15 (0.13, 0.17)	0.15 (0.12, 0.19)	0.17 (0.14, 0.20)	0.6599	0.17 (0.15, 0.20)	0.16 (0.14, 0.18)	0.13 (0.11, 0.16)	0.14 (0.12, 0.16)	0.0587
Non-linear dynamic metrics											
Shannon entropy	0.162	2.04 (1.99, 2.09)	2.01 (1.94, 2.08)[i]	1.94 (1.84, 2.05)	1.85 (1.75, 1.94)[f]	**0.009**	1.92 (1.82, 2.01)	2.00 (1.91, 2.08)[j]	2.00 (1.90, 2.08)[l]	1.82 (1.75, 1.90)	**0.006**
Fwshannon	0.444	2.69 (2.63, 2.75)	2.69 (2.61, 2.77)[i]	2.58 (2.47, 2.70)	2.52 (2.42, 2.63)[e]	0.041	2.62 (2.51, 2.73)	2.69 (2.61, 2.78)[j]	2.64 (2.54, 2.73)[k]	2.49 (2.41, 2.57)	**0.011**
Forbword	0.347	32.8 (31.6, 34.0)	33.8 (32.3, 35.4)	35.5 (33.1, 37.8)	36.3 (34.1, 38.4)[e]	0.048	34.2 (32.0, 36.3)	33.6 (31.9, 35.3)	34.2 (32.27, 36.1)	36.7 (35.2, 38.3)	0.061
Renyi4	0.0802	1.69 (1.63, 1.74)	1.67 (1.60, 1.73)	1.60 (1.50, 1.71)	1.51 (1.41, 1.60)[f]	0.0189	1.55 (1.45, 1.65)	1.66 (1.58, 1.74)[j]	1.67 (1.58, 1.77)[l]	1.48 (1.41, 1.56)[e]	0.0031
Fwrenyi025	0.2391	3.18 (3.14, 3.23)	3.16 (3.10, 3.22)[i]	3.07 (2.98, 3.17)[c]	3.04 (2.96, 3.126)	0.031	3.11 (3.03, 3.20)	3.16 (3.09, 3.23)[j]	3.13 (3.05, 3.21)[k]	3.03 (2.97, 3.09)[f]	0.0583
Fwrenyi4	0.8171	1.97 (1.91, 2.04)	1.99 (1.91, 2.08)[i]	1.92 (1.79, 2.05)	1.84 (1.72, 1.96)	0.1881	1.94 (1.83, 2.06)	2.01 (1.91, 2.10)[j]	1.95 (1.85, 2.06)[k]	1.78 (1.70, 1.86)[f]	0.0058
Wsdvar	0.5482	1.24 (1.18, 1.31)	1.22 (1.13, 1.30)	1.19 (1.07, 1.32)	1.08 (0.96, 1.20)	0.1516	1.16 (1.04, 1.27)	1.19 (1.10, 1.28)[j]	1.25 (1.15, 1.36)[k]	1.09 (1.01, 1.18)[e]	0.1366
Wpsum02	0.5969	0.57 (0.54, 0.60)	0.59 (0.55, 0.62)[i]	0.60 (0.55, 0.66)	0.66 (0.61, 0.72)	0.0317	0.60 (0.55, 0.66)	0.59 (0.55, 0.63)[j]	0.58 (0.53, 0.63)[k]	0.66 (0.62, 0.70)[f]	0.0349
Wpsum13	0.8624	0.14 (0.12, 0.16)	0.13 (0.11, 0.15)	0.14 (0.11, 0.17)	0.10 (0.07, 0.13)	0.1698	0.13 (0.10, 0.15)	0.12 (0.10, 0.14)	0.15 (0.12, 0.17)	0.12 (0.10, 0.14)	0.2548
Plvar20	0.3084	0.20 (0.16, 0.23)	0.19 (0.15, 0.24)[i]	0.27 (0.21, 0.34)	0.31 (0.25, 0.37)	0.0067	0.28 (0.22, 0.35)	0.21 (0.15, 0.26)[j]	0.27 (0.22, 0.33)[k]	0.34 (0.30, 0.39)[f]	0.0031

Statistically significant p-values **in bold** were presented after domain-specific Bonferroni correction; †p-value from analysis of variance (ANOVA) test comparing measures among OSA severity groups; ‡Analysis performed on natural log transformed outcome; aP<0.05 (mild vs. control); bP<0.0083 (mild vs. control); cP<0.05 (moderate vs. control); dP<0.0083 (moderate vs. control); eP<0.05 (severe vs. control); fP<0.0083 (severe vs. control); gP<0.05 (mild vs. moderate); hP<0.0083 (mild vs. moderate); iP<0.05 (mild vs. severe); jP<0.0083 (mild vs. severe); kP<0.05 (moderate vs. severe); lP<0.0083 (moderate vs. severe); meanNN: mean value of normal to normal (NN) interval time series; SDNN: standard deviation of normal to normal (NN) interval; SDANN1: standard deviation of the average of 1-minute NN intervals; CVNN: ratio of SDNN divided by meanNN; RMSSD: square root of the mean squared differences of consecutive NN intervals; pNN50: percentage of NN > 50 ms counts divided by the total number of all NN intervals; pNNl20: percentage of NN intervals differences<20ms; 5-min TP: total power over five minutes; LF power: power of low frequency range; HF power: power of high frequency range; LF/HF: ratio of low frequency and high frequency power; LF nu: normalized low frequency power; HF nu: normalized high frequency power; Fwshannon: frequency of word distribution of Shannon entropy; Forbword: forbidden word; Renyi4:Renyi entropy with a weighting coefficient of α=4; FwrenyiX: frequency of shannon entropy of word distribution using a weighting coefficient of α (α=025,4); Wpsum02: relative portion of words consisting only of the symbols ‘0’ and ‘2’; Wpsum13: relative portion of words consisting only of the symbols ‘1’ and ‘3’; Wsdvar: standard deviation of the word sequence; Plvar20: portion of low-variability patterns in the NN interval time series (< 20 ms).

3.4 Relationship between HRV parameters and continuous AHI

To compare differences in HRV measurements among OSA severity groups, we evaluated the association with continuous AHI. Consistent with the results of comparisons among AHI groups, rising AHI was significantly related to lower values of all time-domain parameters in adjusted analyses, including meanNN (rho=-0.12, p<0.0001), SDNN (rho=-0.11, p<0.0001), SDANN1 (rho=-0.09, p=0.002), CVNN (rho=-0.09, p=0.0013), RMSSD (rho=-0.15, p<0.0001), pNN50 (rho=-0.11, p=0.0001), and pNNl20 (rho=0.14, p<0.0001). Similarly, more severe OSA have a close association with a loss of HRV complexity, showing negative correlations with ShanEn (rho=-0.11, p=0.0001), Fwshannon (rho=-0.11, p=0.0001), Renyi4 (rho=-0.09, p=0.0012), Fwrenyi025 (rho=-0.01, p=0.0001), Fwrenyi4 (-0.10, p=0.0003), Wsdvar (rho=-0.06, p=0.0354), and positive correlations with Forbword (rho=0.12, p<0.0001), Wpsum02 (rho=0.10, p=0.0007) and Plvar20 (rho=0.12, p<0.0001) in analyses of adjustment. No statistically significant correlations between AHI and frequency-domain parameters were found.

3.5 Association between HRV measures and Epworth Sleepiness Scale

To perform an exploratory analysis on the relationship between HRV and sleepiness, we also tested whether HRV during pre-sleep wakefulness correlated with the subjective sleepiness evaluated by the Epworth Sleepiness Scale (ESS). In covariates adjusted analyses, higher ESS was significantly related with meanNN (rho=-0.10, p=0.0018), SDNN (rho=-0.07, p=0.040), lower RMSSD (rho = -0.10, p=0.004), Renyi4 (rho=-0.08, p=0.0189), pNNl20 (rho=0.08, p=0.0237), lower Fwshannon (rho = -0.09, p=0.005), ShanEn (rho=-0.08, p=0.021), Fwrenyi4 (rho=-0.10, p=0.0043). Correlations were in line with other time domain and non-linear dynamic measurements, although results did not reach statistical significance after Bonferroni correction. Thus, results provided evidence regarding some correlations between ESS and HRV measures during wakefulness, even though they were not as strong as seen with AHI**.**

4. Discussion

4.1 Summary

This study provides further evidence of the existence of cardiovascular abnormalities and autonomic dysfunction in OSA patients even during the wake period without the presence of sleep apnea. Our results show a significant relationship between simple 5-minute ECG heart rate variability during pre-sleep wakefulness and OSA severity. OSA patients demonstrate significantly lower values of temporal HRV parameters and more regular dynamic HRV patterns compared to non-OSA patients after controlling relevant covariates. Our results suggested AHI severity is an independent contributor to a reduction in the variability and complexity of heartbeat time series, particularly in patients with severe OSA. Furthermore, our subgroup analyses show that there is sympathetic hyperactivity in more severe OSA among obese patients with higher LF and LF/HF compared to those who are non-obese. Current data has confirmed that many HRV measures during ambulatory wakefulness associate with OSA severity within small sample sizes.[9, 10, 16] Our study overcomes the limitation of published research and extends the research topic by using a board of OSA samples and comprehensive HRV measurements.

Multiple HRV measurements during pre-sleep wakefulness are employed to capture HRV patterns and address the observed relationships in this study. The wake HRV analyses allows a cost-effective method to be carried out in existing clinical settings. Moreover, interpreting results of sleep HRV in patients with OSA is challenging due to influences from different sleep stages, respiratory events, arousal and hypoxia.[13, 14, 23] Analyses focus on the carryover effect of OSA on the autonomic nervous system in quiet wake condition can avoid overnight physiological heterogeneities. Importantly, our data demonstrates meaningful associations with wake HRV metrics and their potential utility of HRV into clinical practices.

4.2 Time-domain HRV analysis

We found a significant decrease in overall time-domain HRV measures, quantified by meanNN,

CVNN, SDNN and SDANN1 among groups with different levels of OSA. This is consistent with previous findings on OSA during wake period without presence of OSA.[9, 10, 16] RMSSD and pNN50 are related to parasympathetic tone. pNNl20 is highly related to diminished beat-to-beat intervals as SDANN<20 ms is shown in more severe OSA. Those measures provide insight to visually lower variation of interbeat intervals. Both meanNN and CVNN distinguish mild, modest, and severe OSA even after adjustment. Notably, previous studies reported cut-off points for increased mortality risk. Reduced SDNN (e.g. <40ms) or RMSSD (e.g. <25ms) could be an indicator for cardiovascular risk stratification.[24, 25]

4.3 Frequency-domain HRV analysis

With respect to frequency-domain analysis in our study, the results showed that there is a relationship between AHI severity and unadjusted spectral measures but not after multivariable adjustment within the entire samples. However, exploratory analyses suggested that obesity status may modify the association between measures and OSA severity. Differences in LF and LF/HF indicate that a shift of sympathetic dominance occurs among obese patients with more severe OSA.

The results of the sympathetic and parasympathetic activity are less consistently found in frequency-domain analysis compared to time-domain analysis. The inconsistent findings in spectral HRV measures in OSA are possibly due to methodological concerns. The most controversial finding was the significant differences in the sympathetic and vagal outflow among different studies. In the secondary analyses, stratified by obesity, the observed differences are consistent in sympathetic overactivity in severe obese OSA patients with other studies, suggesting that, in particular, obesity status may cover changes in autonomic control in OSA.[26] Interestingly, Gula et al. suggested that changes in autonomic activity are disproportional to OSA severity, demonstrating elevated sympathetic predominance and discordant autonomic imbalance from mild to moderate OSA, compared to blunted responses in severe OSA.[27] Our data also show a tendency of less sympathetic hyperactivity in severe OSA compared to moderate OSA. This nonlinear relationship between impaired autonomic control and OSA severity is an open question. More evidence in support of this idea is needed in order to study its clinical implication.

4.5 Non-linear HRV analysis

Quantifying of the dynamics of cardiac rhythm is crucial to identify univocal and morbid HRV patterns. To the best of our knowledge, this study is the first to investigate the association between non-linear wake HRV and OSA severity. Complexity analysis is able to derive non-linear features of cardiac beat-to-beat variability compared to traditional time- and frequency-domain analysis.[7, 20] Shannon entropy and Renyi entropy are markers for regularity and predictability of HRV.[28] Conventional linear HRV analyses cannot capture the nonlinearity of heartbeat as cardiovascular system is a complex system.[29] Stein et al. suggested patients with similar overall variability exhibit a wide range of nonlinear HRV patterns, which help identify pathological conditions.[29] Thus, non-linear HRV is being increasingly explored as a tool to investigate cardiac autonomic regularity based on information theory and chaotic dynamics.

In this study, we compared differences in nonlinear dynamics among various AHI severity groups using a variety of symbolic dynamic parameters. We validated that those symbolic measures and were able to identify complexity of cardiac autonomic modulation in different levels of OSA severity. Entropy measures system regularity and predictability. Lower entropy indicates more system order and less randomness. We used Shannon Entropy, Renyi Entropy, and their relative symbolic word distribution frequency, to provide a potential physiological interpretation of what reduced entropy measures imply in this study. The decreased Renyi4 suggested that the dynamic of severe OSA is more likely to be predictable, showing heart rate control is less complex. Entropy measures, Fwshannon and Fwrenyi constantly decreased in more severe OSA. Symbolic dynamics provide intuitive insight of erratic behavior of heart rate time series.[30] RR sequences in more severe OSA become more regular with higher values of Forbword, Wpsum02, and Plvar20, and lower values of Wsdvar and Wpsum13. Those symbolic dynamic measures transfer numerous conditional probabilities into a wide range of regularity measurements. Compared to time-domain measures, entropy and symbolic measures appear to be more sensitive and effective to identify OSA severity with more significance between groups. Further studies on nonlinear HRV analysis are needed to facilitate its proper utilization, potential application, and medical interpretation in diagnostic and predictive uses.

4.6 Impact of impaired HRV

Impaired autonomic function and abnormal HRV patterns have previously been demonstrated in small-scale studies using short-term HRV analysis during pre-sleep wakefulness in OSA patients.[9, 10, 16, 31, 32] Current findings showed that several mechanisms may initially be involved in autonomic dysfunction in OSA. Chemoreceptors and baroreceptors play an important role in modulating autonomic input to the heart.[33] In addition, a negative intrathoracic pressure due to inspiratory effort against an occluded airway and arousal in OSA may activate pulmonary stretch receptors, which contributes to a fall in cardiac output.[34] Recurrent vicious circles of these nocturnal cardiovascular changes may cause chronic autonomic nervous system stimulation, possibly damaging baroreflex sensitivity and reset stimulation threshold.[35] The magnitude of bradycardia alters proportionally with the degree of hypoxia. Heart rate decrease leads to bradycardia during apnea due to increased vagal activity during apnea, while heart rate increase leads to tachycardia at the apnea due to elevated sympathetic tone or vagal withdrawal.[15, 23] As a result, those sleep apnea relative changes such as hypoxia, arousal, and pleural pressure swings may lead to sympathetic overactivity, parasympathetic withdrawal, impaired baroreflex sensitivity, discordant autonomic imbalance, or the combination of these changes mediated via the chemoreflex, baroreflex and mechanical reflexes. Those chronic autonomic impairments may be not only manifested during sleep but also persist in the daytime.

The predictive and prognostic value of HRV for use in cardiac risk stratification have been investigated in other populations, such as the patients after myocardial infarction and the elderly.[36, 37] Reduced HRV is in relation to risk and incidence of adverse cardiovascular outcomes. In a meta-analysis, Hillebrand et al demonstrated a 1% drop in HRV is related to approximately a 1% raise in fatal and non-fatal cardiovascular events in individuals without recognized CVD.[38] However, the associations of impaired HRV and long-term cardiovascular sequelae in patients with OSA is unknown. Sankari et al. used total and sleep RR intervals index (RRDI), computed by the number of RRI dips divided by total recording time and sleep time, respectively to investigate the role of overnight alternation in heartbeats in CV risk in OSA patients from the Wisconsin Sleep Cohort.[39] Their findings suggested that the increased dips in sleep RRI are related to CVD onset with hazard

ratio of 1.21 per 10-unit increment in RRDI. Patients with greater total RRDI are at higher risk in elevated incidence of CVD and mortality with a 7.4 hazard ratio. Whether HRV measures could be a good indicator of different CV risk phenotypes in OSA requires more investigation.

4.7 Strengths and limitations

There are several strengths in this study. It not only evaluated mathematical properties, but also discussed further clinical indication of HRV in OSA using time-domain, frequency-domain and nonlinear HRV measures. It provides better understanding of a clear relationship between OSA severity and HRV when patients are during wakefulness prior to sleep onset. Our study used a large OSA clinical cohort with a wide range of AHI severity across multiple centers. Thus, our results are more generalizable to patients in authentic clinical practices worldwide. Finally, we introduced standard 5-minute HRV measurements during wakefulness immediately prior to polysomnography. which is a simple method to translate to clinical settings.

However, there are also some limitations. First, our data was obtained from an observational study, which was not able to evaluate the causal relationship between HRV and OSA severity, or the associations between the lower HRV in patients with more severe OSA and the higher risk of new-onset cardiovascular diseases. Secondly, we recorded 5-minute ECG data preceding the sleep study. Whether our results could directly generalize to the daytime wake state needs further validation. Third, specific medications on the management of hypertension, cardiovascular diseases, diabetes and high cholesterol were not included as covariates in adjusted models, which might underestimate the impact of OSA severity on HRV and reduce the sympathetic tone. Fourth, a variety of ECG sampling rate were used among different SAGIC sites. We used sampling rate ≥128Hz due to its acceptability for HRV analysis.[40] To diminish any sampling rate bias, ECG sampling rate was included as a covariate in statistical models. Finally, we did not use paced breathing to control the tidal volume and respiratory rate for each participant because the impact of respiration on HRV is controversial. Instead, we instructed patients to breathe spontaneously in a relaxing supine position. This protocol is easy to implement in different sleep laboratories and allows to carry out standardized protocol of wake HRV measurements at normal breathing without

cooperation of patients. Therefore, our findings should still be clinically applicable.

5. Conclusion

HRV measures during wakefulness prior to sleep have a significant association with incremental severity of OSA, showing a loss of the global HRV and less complex HRV dynamics among more severe and obese patients after adjustment for relevant confounding factors. These observed changes are in agreement with worse cardiac autonomic regulation in patients with the most severe OSA. Acute and recurrent OSA related changes during sleep have cumulative impacts on autonomic nervous system activity. Thus, the HRV characteristics may provide valuable hidden information on cardiovascular pathophysiology and autonomic function in OSA simply based on ECG data. However, evidence concerning whether comprehensive HRV metrics, in combination with other features of OSA, may identify cardiac autonomic dysfunction and improve cardiovascular risk stratification with OSA is still limited. To more precisely characterize individual patients with OSA, further research on the role of HRV in developing cardiac phenotypes of OSA and predicting their cardiovascular outcomes is needed.

6. References

1. Senaratna CV, Perret JL, Lodge CJ, Lowe AJ, Campbell BE, Matheson MC, Hamilton GS, Dharmage SC. Prevalence of obstructive sleep apnea in the general population: A systematic review. Sleep Med Rev 2017;34:70-81.
2. Nieto FJ, Young TB, Lind BK, Shahar E, Samet JM, Redline S, D'Agostino RB, Newman AB, Lebowitz MD, Pickering TG. Association of sleep-disordered breathing, sleep apnea, and hypertension in a large community-based study. Sleep Heart Health Study. Jama 2000;283(14):1829-36.
3. Mehra R, Benjamin EJ, Shahar E, Gottlieb DJ, Nawabit R, Kirchner HL, Sahadevan J, Redline S. Association of nocturnal arrhythmias with sleep-disordered breathing: The Sleep Heart Health Study. Am J Respir Crit Care Med 2006;173(8):910-6.
4. Yaggi HK, Concato J, Kernan WN, Lichtman JH, Brass LM, Mohsenin V. Obstructive sleep apnea as a risk factor for stroke and death. N Engl J Med 2005;353(19):2034-41.
5. Pedrosa RP, Drager LF, Gonzaga CC, Sousa MG, de Paula LK, Amaro AC, Amodeo C, Bortolotto LA, Krieger EM, Bradley TD, Lorenzi-Filho G. Obstructive sleep apnea: the most common secondary cause of hypertension associated with resistant hypertension. Hypertension 2011;58(5):811-7.
6. Dempsey JA, Veasey SC, Morgan BJ, O'Donnell CP. Pathophysiology of sleep apnea. Physiol Rev 2010;90(1):47-112.
7. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation 1996;93(5):1043-65.
8. Dissanayake HU, Bin YS, Ucak S, de Chazal P, Sutherland K, Cistulli PA. Association between autonomic function and obstructive sleep apnea: A systematic review. Sleep Med Rev 2021;57:101470.
9. Narkiewicz K, Montano N, Cogliati C, van de Borne PJ, Dyken ME, Somers VK. Altered cardiovascular variability in obstructive sleep apnea. Circulation 1998;98(11):1071-7.
10. Wang W, Tretriluxana S, Redline S, Surovec S, Gottlieb DJ, Khoo MC. Association of cardiac autonomic function measures with severity of sleep-disordered breathing in a community-based sample. J Sleep Res 2008;17(3):251-62.
11. Iber C, Ancoli-Israel S, Chesson AL, Quan S. The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications. Westchester, IL: American Academy of Sleep Medicine 2007.
12. Ucak S, Dissanayake HU, Sutherland K, de Chazal P, Cistulli PA. Heart rate variability and obstructive sleep apnea: Current perspectives and novel technologies. J Sleep Res 2021:e13274.
13. Penzel T, Kantelhardt JW, Lo CC, Voigt K, Vogelmeier C. Dynamics of heart rate and sleep stages in normals and patients with sleep apnea. Neuropsychopharmacology 2003;28 Suppl 1:S48-53.
14. Szollosi I, Krum H, Kaye D, Naughton MT. Sleep apnea in heart failure increases heart rate variability and sympathetic dominance. Sleep 2007;30(11):1509-14.
15. Guilleminault C, Connolly S, Winkle R, Melvin K, Tilkian A. Cyclical variation of the heart rate in sleep apnoea syndrome. Mechanisms, and usefulness of 24 h electrocardiography as a screening technique. Lancet 1984;1(8369):126-31.
16. Park DH, Shin CJ, Hong SC, Yu J, Ryu SH, Kim EJ, Shin HB, Shin BH. Correlation between the severity of obstructive sleep apnea and heart rate variability indices. J Korean Med Sci 2008;23(2):226-31.
17. Balachandran JS, Bakker JP, Rahangdale S, Yim-Yeh S, Mietus JE, Goldberger AL, Malhotra A. Effect of mild, asymptomatic obstructive sleep apnea on daytime heart rate variability and impedance cardiography measurements. Am J Cardiol 2012;109(1):140-5.
18. Pan J, Tompkins WJ. A real-time QRS detection algorithm. IEEE Trans Biomed Eng 1985;32(3):230-6.
19. Akselrod S, Gordon D, Ubel FA, Shannon DC, Berger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. Science 1981;213(4504):220-2.
20. Wessel N, Voss A, Malberg H, Ziehmann C, Voss HU, Schirdewan A, Meyerfeldt U, Kurths J. Nonlinear analysis of complex phenomena in cardiological data. Herzschrittmachertherapie und Elektrophysiologie 2000;11(3):159-173.
21. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. (1474-547X

(Electronic)).
22. Qin H, Keenan BT, Mazzotti DR, Vaquerizo-Villar F, Kraemer JF, Wessel N, Tufik S, Bittencourt L, Cistulli PA, de Chazal P, Sutherland K, Singh B, Pack AI, Chen NH, Fietze I, Gislason T, Holfinger S, Magalang UJ, Penzel T. Heart rate variability during wakefulness as a marker of obstructive sleep apnea severity. Sleep 2021;44(5).
23. Somers VK, Dyken ME, Clary MP, Abboud FM. Sympathetic neural mechanisms in obstructive sleep apnea. J Clin Invest 1995;96(4):1897-904.
24. Bigger JT, Jr., Fleiss JL, Steinman RC, Rolnitzky LM, Kleiger RE, Rottman JN. Correlations among time and frequency domain measures of heart period variability two weeks after acute myocardial infarction. Am J Cardiol 1992;69(9):891-8.
25. Jarczok MN, Koenig J, Wittling A, Fischer JE, Thayer JF. First Evaluation of an Index of Low Vagally-Mediated Heart Rate Variability as a Marker of Health Risks in Human Adults: Proof of Concept. J Clin Med 2019;8(11).
26. Grassi G, Facchini A, Trevano FQ, Dell'Oro R, Arenare F, Tana F, Bolla G, Monzani A, Robuschi M, Mancia G. Obstructive sleep apnea-dependent and -independent adrenergic activation in obesity. Hypertension 2005;46(2):321-5.
27. Gula LJ, Krahn AD, Skanes A, Ferguson KA, George C, Yee R, Klein GJ. Heart rate variability in obstructive sleep apnea: a prospective study and frequency domain analysis. Ann Noninvasive Electrocardiol 2003;8(2):144-9.
28. Henriques T, Ribeiro M, Teixeira A, Castro L, Antunes L, Costa-Santos C. Nonlinear Methods Most Applied to Heart-Rate Time Series: A Review. Entropy (Basel) 2020;22(3).
29. Stein PK, Domitrovich PP, Hui N, Rautaharju P, Gottdiener J. Sometimes higher heart rate variability is not better heart rate variability: results of graphical and nonlinear analyses. J Cardiovasc Electrophysiol 2005;16(9):954-9.
30. Guzzetti S, Borroni E, Garbelli PE, Ceriani E, Della Bella P, Montano N, Cogliati C, Somers VK, Malliani A, Porta A. Symbolic dynamics of heart rate variability: a probe to investigate cardiac autonomic modulation. Circulation 2005;112(4):465-70.
31. Aydin M, Altin R, Ozeren A, Kart L, Bilge M, Unalacak M. Cardiac autonomic activity in obstructive sleep apnea: time-dependent and spectral analysis of heart rate variability using 24-hour Holter electrocardiograms. Tex Heart Inst J 2004;31(2):132-6.
32. Wiklund U, Olofsson BO, Franklin K, Blom H, Bjerle P, Niklasson U. Autonomic cardiovascular regulation in patients with obstructive sleep apnoea: a study based on spectral analysis of heart rate variability. Clin Physiol 2000;20(3):234-41.
33. Somers VK, Mark AL, Abboud FM. Interaction of baroreceptor and chemoreceptor reflex control of sympathetic nerve activity in normal humans. J Clin Invest 1991;87(6):1953-7.
34. Bonsignore MR, Marrone O, Insalaco G, Bonsignore G. The cardiovascular effects of obstructive sleep apnoeas: analysis of pathogenic mechanisms. Eur Respir J 1994;7(4):786-805.
35. Carlson JT, Hedner JA, Sellgren J, Elam M, Wallin BG. Depressed baroreflex sensitivity in patients with obstructive sleep apnea. Am J Respir Crit Care Med 1996;154(5):1490-6.
36. Kleiger RE, Miller JP, Bigger JT, Jr., Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. Am J Cardiol 1987;59(4):256-62.
37. Tsuji H, Venditti FJ, Jr., Manders ES, Evans JC, Larson MG, Feldman CL, Levy D. Reduced heart rate variability and mortality risk in an elderly cohort. The Framingham Heart Study. Circulation 1994;90(2):878-83.
38. Hillebrand S, Gast KB, de Mutsert R, Swenne CA, Jukema JW, Middeldorp S, Rosendaal FR, Dekkers OM. Heart rate variability and first cardiovascular event in populations without known cardiovascular disease: meta-analysis and dose-response meta-regression. Europace 2013;15(5):742-9.
39. Sankari A, Ravelo LA, Maresh S, Aljundi N, Alsabri B, Fawaz S, Hamdon M, Al-kubaisi G, Hagen E, Badr MS, Peppard P. Longitudinal effect of nocturnal R-R intervals changes on cardiovascular outcome in a community-based cohort. BMJ Open 2019;9(7):e030559.
40. Voss A, Wessel N, Sander A, Malberg H, Dietz R. Requirements on sampling rate in Holter systems for analysis of heart rate variability. Clin Sci (Lond) 1996;91 Suppl:120-1.

Statutory Declaration

"I, Hua Qin, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic Altered Autonomic Function in Patients with Obstructive Sleep Apnea during Wakefulness/Veränderte autonome Funktion bei Patienten mit obstruktiver Schlafapnoe im Wachzustand, independently and without the support of third parties, and that I used no other sources and aids than those stated.
All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me."

Date

Signature

Declaration of your own contribution to the publications

Hua Qin contributed the following to the below listed publications:

Publication: Qin H, Keenan BT, Mazzotti DR, Vaquerizo-Villar F, Kraemer JF, Wessel N, Tufik S, Bittencourt L, Cistulli PA, de Chazal P, Sutherland K, Singh B, Pack AI, Chen NH, Fietze I, Gislason T, Holfinger S, Magalang UJ, Penzel T. Heart rate variability during wakefulness as a marker of obstructive sleep apnea severity. Sleep. 2021 May 14;44(5):zsab018. https://doi.org/10.1093/sleep/zsab018

Contribution (please set out in detail):
I was responsible for study concept and design under the supervision of Prof. Dr. Thomas Penzel. I prepared the statistical analysis plan and discussed the plan with statistician Mr. Brendan T. Keenan. I was responsible for analysis and interpretation of data based on the discussion with statistician Mr. Brendan T. Keenan and physicists Mr. Jan F. Kraemer, and PD.Dr.Niels Wessel. I prepared the manuscript draft. Tables 1, 2, 3, 4, S1, S2, S3 and Figure 1 were created on the basis of my statistical evaluation. I contributed to critical revision of the manuscript for important intellectual content and responded to reviewers'comments. I am the linkage of Interdisciplinary Center of Sleep Medicine, Charité-Universitätsmedizin Berlin to major international sleep centers, such as University of Philadelphia (Dr. A. Pack), University of Sydney (Dr. P. Cistulli), and all members of the Sleep Apnea Global Interdisciplinary Consortium (SAGIC) group, on the physiological signal processing and heart rate variability analysis project.

_____

Signature, date and stamp of first supervising university professor / lecturer

_____

Signature of doctoral candidate

Publication

Qin H, Keenan BT, Mazzotti DR, Vaquerizo-Villar F, Kraemer JF, Wessel N, Tufik S, Bittencourt L, Cistulli PA, de Chazal P, Sutherland K, Singh B, Pack AI, Chen NH, Fietze I, Gislason T, Holfinger S, Magalang UJ, Penzel T. Heart rate variability during wakefulness as a marker of obstructive sleep apnea severity. Sleep. 2021 May 14;44(5):zsab018. https://doi.org/10.1093/sleep/zsab018

Curriculum Vitae

“Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.”

Publications

1. Qin H, Keenan BT, Mazzotti DR, Vaquerizo-Villar F, Kraemer JF, Wessel N, Tufik S, Bittencourt L, Cistulli PA, de Chazal P, Sutherland K, Singh B, Pack AI, Chen NH, Fietze I, Gislason T, Holfinger S, Magalang UJ, Penzel T. Heart rate variability during wakefulness as a marker of obstructive sleep apnea severity. Sleep. 2021 May 14;44(5):zsab018. doi: 10.1093/sleep/zsab018.
2. Qin H, Chen C, Steenbergen N, Cheng Y, Penzel T. Time-dependence and comparison of regional and overall anthropometric features between Asian and Caucasian populations with obstructive sleep apnea: a cumulative meta-analysis. J Thorac Dis. 2021 Mar;13(3):1746-1759. doi: 10.21037/jtd-20-1799.
3. Qin H, Steenbergen N, Glos M, Wessel N, Kraemer JF, Vaquerizo-Villar F, Penzel T. The Different Facets of Heart Rate Variability in Obstructive Sleep Apnea. Front Psychiatry. 2021 Jul 22;12:642333. doi: 10.3389/fpsyt.2021.642333.
4. Luo YM, Qiu ZH, Wang Y, He BT, Qin H, Xiao SC, Luo YM, Steier J, Moxham J, Polkey MI. Absence of dynamic hyperinflation during exhaustive exercise in severe COPD reflects submaximal IC maneuvers rather than a nonhyperinflator phenotype. J Appl Physiol (1985). 2020 Mar 1;128(3):586-595. doi: 10.1152/japplphysiol.00695.2018.

Abstracts

1. H. Qin; B.T. Keenan; D.R. Mazzotti et al. Heart rate variability during wakefulness as a marker of obstructive sleep apnea severity. Journal of Sleep Research, Volume 29, Issue S1, P 200. Special Issue: Abstracts of the 25th Congress of the European Sleep Research Society, 22-24 September 2020, Virtual Congress.
2. H. Qin; C.X. Chen; N. Steenbergen; Y. Cheng; T. Penzel. Effect of obesity on anthropometric features between Asian and Caucasian population with obstructive sleep apnea: a cumulative meta-analysis. Journal of Sleep Research, Volume 29, Issue S1, P 256. Special Issue: Abstracts of the 25th Congress of the European Sleep Research Society, 22-24 September 2020, Virtual Congress.
3. Hua Q, Senrui W, Yuan W, Yuanming L. Effect of Lung Volumes on Esophageal Pressure and Diaphragm EMG. American Journal of Respiratory and Critical Care Medicine

2018;197:A5879.

4. Yingmei L, Hua Q, Baiting H et al. Neural respiratory drive generated by inspiratory capacity maneuver could be further increased by inhalation of CO2. American Journal of Respiratory and Critical Care Medicine 2016;193:A5967
5. Luo YM, Zhi Hui Q, Bai Ting H, Hua Q, Yingmei L, Lian Z, Michael IP. The inspiratory capacity (IC) maneuver may underestimate dynamic hyperinflation in patients with COPD during exhaustive exercise. American Journal of Respiratory and Critical Care Medicine 2016;193:A5966.
6. Yingmei Luo, Hua Qin, Baiting He, Lian Zhou, Zhihui Qiu, John Moxham, Michael Iain Polkey, Yuanming Luo. Inspiratory capacity generated by neural respiratory drive could be further increased by inhalation of CO2. European Respiratory Journal 2016 48: PA2250.

Presentations

1. Global sleep medicine opportunities in China. World Sleep 2021 Virtual Meeting, 24-25 June, World Sleep Society. Oral Presentation
2. Heart rate variability during wakefulness as a marker of obstructive sleep apnea severity. The 3rd Congress of Asian Society of Sleep Medicine, 14-17 May, 2021, Beijing, China. Oral Presentation
3. International Sleep Research Training Program in Charité Universitätsmedizin Berlin. The 12th Symposium of Chinese Sleep Research Society, 09-11 October, 2020, Guangzhou, China. Oral Presentation
4. Heart rate variability during wakefulness as a marker of obstructive sleep apnea severity. The 25th Congress of the European Sleep Research Society, 22-24 September 2020, Virtual Congress. Poster Presentation
5. Effect of obesity on anthropometric features between Asian and Caucasian population with obstructive sleep apnea: a cumulative meta-analysis. The 25th Congress of the European Sleep Research Society, 22-24 September 2020, Virtual Congress. Poster Presentation
6. Effect of Lung Volumes on Esophageal Pressure and Diaphragm EMG. The American Thoracic Society International Conference 2018, San Diego, CA, American. Poster Presentation

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