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CLINICAL RESEARCH ARTICLE

Autonomic nervous function and low-grade inflammation in children with sleep-disordered breathing

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BACKGROUND: The objective of the study was to assess the relationship between autonomic nervous function and low-grade inflammation in children with sleep-disordered breathing.

METHODS: We enrolled habitually snoring children aged 3–14 years for overnight polysomnography (PSG) and high-sensitivity C-reactive protein (hsCRP) measurement. Low-grade inflammation was defined as hsCRP >1.0 mg/L to <10.0 mg/L. An electrocardiogram recording was extracted from PSG. Heart rate variability was analyzed using time and frequency domain methods.

RESULTS: In total, 190 children were included, with 61 having primary snoring (PS), 39 mild obstructive sleep apnea (OSA), and 90 moderate-to-severe OSA. The average RR interval displayed a significant decline, whereas the low frequency/high frequency (LF/HF) ratio showed an increasing tendency in children with PS, mild OSA, and moderate-to-severe OSA. Mean RR was mainly influenced by age and the apnea hypopnea index (AHI) (all $P < 0.01$). AHI was an independent risk factor for the altered LF/HF ratio at all sleep stages except N3 stage (all $P < 0.05$). In the wake stage, low-grade inflammation was an independent risk factor of altered LF/HF ratio ($P = 0.014$).

CONCLUSIONS: Autonomic nervous function was impaired in children with OSA. The sympathetic–vagal balance was influenced by low-grade inflammation in the wake stage, whereas it was only affected by AHI when falling asleep.

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IMPACT:

- We found that autonomic nervous function was impaired in children with OSA.
- We found that there was a negative correlation between systemic inflammation and autonomic nervous function in children with SDB only at wake stage.
- A negative association between systemic inflammation and autonomic nervous function was demonstrated in children in this study. Furthermore, altered LF/HF ratio maybe a good indicator of autonomic nervous dysfunction in children as it only correlated with the SDB severity, not with age.

INTRODUCTION

Pediatric sleep-disordered breathing (SDB), which encompasses the spectrum of primary snoring (PS), upper airway resistance syndrome, and obstructive sleep apnea (OSA), is a frequent condition affecting up to 7.45% of children.[1] The mechanisms underlying SDB in children are multiple and involve anatomic, craniofacial, and neuromuscular components.[2] The major implications of SDB reside in its morbid consequences, including the occurrence of cardiovascular sequelae, such as dysfunction of the autonomic nervous system (ANS), elevated systolic and diastolic blood pressure, and endothelial dysfunction.[3,4]

Heart rate variability (HRV) is used to assess the function of the ANS and some studies have revealed a negative relationship between HRV and inflammation assessed using C-reactive protein (CRP) in patients with OSA.[5,6] However, the participants in most past studies were adults, in whom there is a strong confounding influence of other cardiovascular risk factors. Therefore, a pediatric population is a better cohort in which to evaluate a possible association between inflammation and ANS function. An augmented sympathetic response, represented by altered HRV and increased low frequency/high frequency (LF/HF) ratio, have been attributed to the cardiovascular consequences of OSA.[7]

High-sensitivity CRP (hsCRP), an important circulating marker of inflammation, is increased in children with SDB and is correlated with disease severity measures, such as hypoxemia and sleep fragmentation, even after adjusting for the degree of obesity.[8–10] HsCRP is currently considered a robust and reliable marker for cardiovascular morbidity in adult patients with OSA.[11,12] Previous

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studies have reported that circulating levels of CRP in children with SDB are decreased in most patients after treatment,[13,14] thereby confirming the causative link between systemic inflammation and OSA.

In this study, we hypothesized that ANS function is impaired in children with OSA and that the level of hsCRP is an independent risk factor of ANS function after adjusting for age, sex, and body mass index (BMI) adjusted for age and sex (BMI *Z* score).

METHODS

Patients

Children aged 3–14 years who presented to Beijing Children's Hospital sleep center for evaluation of habitual snoring and underwent overnight polysomnography (PSG) from 1 April 2016 to 31 August 2018 were enrolled in this study. Exclusion criteria were acute or chronic inflammatory disorders, liver disease, kidney disorders, history of upper airway operation, craniofacial abnormalities, and comorbidities such as Down syndrome, Crouzon syndrome, and Pierre Robin sequence. Written informed consent and assent were obtained from parents or children (for children aged >8 years). The study was approved by the Ethics Committee of Beijing Children's Hospital.

Physical examination

Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Body weight was measured to the nearest 0.1 kg with a digital scale. Height and weight were converted to a BMI *Z* score to adjust for age and sex.[15]

Tonsil size is typically evaluated using the 0–4^+ scale described by Brodsky et al.[16], with tonsillar hypertrophy being defined as grades 3^+ and 4^+.[17] A fibro-laryngoscopic examination of the adenoids was performed by an ear, nose, and throat physician prior to the sleep study. The degree of adenoid obstruction was documented as a percentage obstruction of the measured distance between the anterior and posterior surfaces of the nasopharynx, with adenoid hypertrophy being defined as a percentage >50%.[18]

Polysomnography

A standard overnight PSG was performed using a Compumedics E-series (Compumedics, Australia), Alice 5 (Respironics, Murrysville, PA), or SOMNO screen Plus PSG+V5 system (SOMNO medics GmbH, Germany). During monitoring, children were accompanied by one of their parents to the same room. Monitoring lasted >7 h for each child. The following parameters were measured: six-channel electroencephalograph with bilateral frontal, central, and occipital leads; electrooculography; electromyography with submental electrodes; electrocardiogram (ECG); airflow measured through the nose via both nasal pressure cannula and thermistor; and respiratory effort measured using thoracic and abdominal inductive plethysmography. Oxygen saturation was measured with pulse oximetry via a finger probe. Two technicians and one pediatrician trained in sleep medicine, who were unaware of the children's clinical features, interpreted the PSG. Sleep stages and respiratory events were scored on the basis of criteria in the American Academy of Sleep Medicine manual.[19] The apnea hypopnea index (AHI) was defined as the number of apneas and hypopneas per hour. The obstructive apnea index (OAI) was defined as the number of obstructive apneas per hour. The arousal index and respiratory arousal index were defined as the number of arousals and those related to respiratory events per hour. The oxygen desaturation index (ODI) was defined as the number of ≥3% arterial oxygen desaturations per hour. The diagnosis of OSA was made based on the presence of an AHI >5 or OAI >1 episodes/hour of total sleep time. Mild OSA was defined as an AHI >5 to <10 or OAI >1 to <5 episodes/hour and moderate-to-severe OSA was defined as an AHI ≥10 or an OAI ≥5 episodes/hour.[20]

HRV analysis

The ECG sampling frequency setting, RR interval sequence editing, and time and frequency domain analysis methods involved in the HRV analysis followed the standard published by Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.[21] The sampling frequency of the ECG recording used in this study was 256 Hz. Each record was checked manually to rule out visual artifacts and arrhythmia before further analysis. A continuous ECG recording was extracted from the obtained and cleaned recording. After performing 5–35 Hz band-pass filtering on the ECG, the RR interval was determined using the "jqrs" complex detection method. All detected RR interval time series were carefully checked for artifacts, ectopic beats, or outliers. Thus, in this study, the RR intervals have the same meaning as NN intervals. In PSG, the sleep stage of each 30-s epoch was obtained, including W, N1, N2, N3, and R. Wake data included wake before sleep onset and the awake period during sleep. The RR intervals belonging to the same sleep stage were spliced together in chronological order so as to obtain the RR time series of each sleep stage during the entire night. Subsequently, the overall RR time series of each sleep stage was divided into multiple 5-min RR sequences (portions <5 min were discarded), which were used for the HRV analysis in this study. An average of 96 5-min epochs was segmented for each child. For HRV time and frequency domain analysis, the averaged HRV indices of all 5-min RR sequences within each sleep stage were taken as the final result.

Time domain analysis. Five time domain indices were derived: the mean of RR intervals (RR mean), standard deviation of RR intervals (SDNN), root mean square of successive RR interval differences (RMSSD), standard deviation of the average RR intervals for each 5-min segment (SDANN), and percentage of successive RR intervals that differed by >50 ms (pNN50).

Frequency domain analysis. First, the original RR interval was interpolated at 4 Hz, and then the HRV spectrum was calculated with fast Fourier transform using a Hamming window. The power density was calculated by integrating the power spectral density into the very LF (0.003–0.04 Hz) and the adjusted LF (0.04–0.20 Hz) and HF (0.20–0.45 Hz) components by considering that the breathing rate in children is faster than that in adults.[22] The ratio of LF-to-HF power (LF/HF) was also calculated.

The SDNN indicates the total tension of sympathetic and vagal tones, and its reduction is interpreted as enhanced sympathetic activity or reduced vagal activity. RMSSD, pNN50, and HF mostly reflect the parasympathetic tone, whereas the LF/HF ratio reflects the sympathetic–vagal balance. LF reflects the combination of sympathetic and parasympathetic nervous system influences, mainly modulated by sympathetic nervous system activity.[4]

Laboratory analyses

Blood was drawn in the morning from each child for high-sensitivity assessment of plasma CRP levels after PSG evaluation; samples were stored at 4 °C for no more than 48 h. HsCRP was measured using immune turbidimetry (QuikRead 101, Cat. No. 06040, Orion Diagnostica Oy, UK); the sensitivity is 0.1 mg/L. Low-grade inflammation was defined as hsCRP level >1.0 mg/L and <10.0 mg/L.[23]

Statistical analysis

All statistical analysis was performed using the JMP software, version 11.0 (SAS Institute, Cary, NC). Kolmogorov–Smirnov test was used to determine normality of the data distribution. Continuous variables are presented as mean ± standard deviation or median (25th percentile, 75th percentile), depending on whether the distribution was normal or skewed. Differences among groups were tested using analysis of variance or Wilcoxon signed-rank test. Categorical variables are expressed as frequency and chi-squared test was used for comparisons among groups. Multiple linear regression mediation models were conducted to determine whether the effect of SDB on HRV was mediated by hsCRP. Two-tailed $P < 0.05$ was considered to be indicative of statistical significance.

RESULTS

Three children with hsCRP ≥10 mg/L were excluded from the analysis owing to acute systemic inflammation. In total, 190 children met the inclusion criteria and completed the study, with 61 having PS, 39 mild OSA, and 90 moderate-to-severe OSA. The demographic characteristics, history of snoring, condition of adenoid tonsillar hypertrophy, and hsCRP levels of all children are presented in Table 1. Children in these three groups were similar with respect to age. The proportion of boys was higher in the group with moderate-to-severe OSA than that in the PS group ($P < 0.01$). Children with moderate-to-severe OSA had greater BMI *Z* score than those in the

Table 1. Demographic characteristics, history of snoring, and the level of hsCRP of all children.

	PS $n = 61$	Mild OSA $n = 39$	Moderate–severe OSA $n = 90$	Z/χ^2	*P* value
Age, years	5.9 (4.6, 8.4)	5.7 (4.8, 7.8)	6.0 (4.8, 7.6)	0.084	0.960
Gender (M), *n* (%)	36 (59.0%)	24 (61.5%)	72 (80.0%)**	9.186	0.010
BMI *Z* score	0.7 (−0.3, 1.4)	0.7 (−0.8, 1.4)	1.6 (−0.1, 2.3)**##	11.849	0.003
Clinical data					
History of snoring, median (25th, 75th)	12 (8.5, 30)	24 (12, 36)	24 (12, 36)	0.476	0.788
TH[a], *n* (%)	52 (92.8%)	35 (97.3%)	79 (90.8%)	0.581	0.775
AH[a], *n* (%)	47 (94.0%)	32 (97.1%)	74 (93.8%)	1.406	0.495
Low-grade inflammation, *n* (%)	16 (26.2%)	11 (28.2%)	33 (36.7%)	2.096	0.350

Low-grade inflammation (hsCRP >1.0 mg/L to <10.0 mg/L).
PS primary snoring, *BMI* body mass index, *TH* tonsil hypertrophy, *AH* adenoid hypertrophy.
Compared with PS, *$P < 0.05$, **$P < 0.01$; compared with mild OSA, #$P < 0.05$, ##$P < 0.01$.
[a]There is a small amount of missing data.

Table 2. Polysomnographic characteristics of all children.

	PS $n = 61$	Mild OSA $n = 39$	Moderate–severe OSA $n = 90$	*Z*	*P* value
TST, min	495.0 (453.3, 521.3)	487.5 (462.5, 501.5)	478.8 (452.3, 508.5)	2.880	0.237
SE, %	88.0 (80.3, 93.0)	86.8 (80.8, 92.9)	87.6 (81.9, 91.2)	0.091	0.956
N1, %	7.0 (5.1, 9.9)	7.1 (4.7, 11.9)	6.9 (4.7, 12.0)	0.028	0.986
N2, %	49.8 (45.3, 53.5)	50.3 (45.9, 52.4)	49.1 (43.4, 53.1)	0.710	0.701
N3, %	22.9 (18.9, 26.2)	24.7 (21.6, 27.4)	24.2 (20.6, 30.9)	5.183	0.075
R, %	20.0 (17.9, 22.5)	17.4 (14.6, 20.4)**	17.5 (14.8, 19.8)**	14.945	0.001
AHI, h^{-1}	2.4 (1.5, 3.5)	6.7 (5.4, 8.0)**	21.9 (16.4, 36.5)**##	159.183	<0.001
OAI, h^{-1}	0 (0, 0.3)	0.8 (0.2, 1.6)**	7.0 (3.1, 15.1)**##	118.176	<0.001
ODI, h^{-1}	0.6 (0.2, 1.2)	3.2 (1.2, 4.6)**	18.3 (11.4, 33.6)**##	141.286	<0.001
RArI, h^{-1}	1.4 (0.7, 2.3)	4.3 (2.9, 5.3)**	8.1 (4.0, 13.1)**##	96.258	<0.001
Mean SpO_2, %	98 (98, 98)	98 (98, 98)	97 (97, 98)**##	57.162	<0.001
SpO_2 nadir, %	93 (91, 95)	91 (89, 93)**	82 (75, 87)**##	98.937	<0.001
ODI90%, %	0 (0, 0)	0 (0, 0.1)**	0.6 (0, 3.2)**##	83.823	<0.001

TST total sleep time, *SE* sleep efficiency, *N1%, N2%, N3%, R%* percentage of NREM 1, 2, 3 and R sleep of TST, *AHI* apnea hypopnea index, *OAI* obstructive apnea index, *ODI* oxygen desaturation index, *RArI* respiratory arousal index, *ODI90%* percentage time spent with O_2 saturation <90%.
Compared with PS, *$P < 0.05$, **$P < 0.01$; compared with mild OSA, #$P < 0.05$, ##$P < 0.01$.

PS and mild OSA groups (all $P < 0.01$). There was no significant difference in terms of the history of snoring and rate of adenoid and tonsillar hypertrophy among the three groups (all $P > 0.05$). There was also no significant difference in terms of the percentage of low-grade inflammation among groups ($P = 0.350$), which was 26.2, 28.2, and 36.7% in children with PS, mild OSA, and moderate-to-severe OSA, respectively (Table 1).

Table 2 shows the PSG characteristics of children in all three groups. The percentage of R sleep in both the mild and moderate-to-severe OSA groups was lower than that in the PS group (all $P < 0.001$). As expected, the respiratory parameters (AHI and OAI), blood oxygen parameters [ODI, SpO_2 nadir, and ODI90% (percentage time spent with O_2 saturation <90%)], and respiratory arousal index were significantly different in pairwise comparisons, except for the mean oxygen saturation, which was not statistically different between the PS and mild OSA groups.

Table 3 illustrates the HRV characteristics of all children in the three groups. The average RR interval (RR mean) displayed a significant declining tendency from children with PS to those with mild OSA and to those with moderate–severe OSA. The RR mean was shorter during the entire night and during the wake stage and each sleep stage in the moderate-to-severe OSA group, as compared with that in the PS group (all $P < 0.01$). The mean RR was shorter in the moderate-to-severe OSA group than in the mild OSA group during the wake, N1, N2, and R sleep stages (all $P < 0.05$). The SDANN was lower in the mild OSA group than that in the PS group during the entire night ($P < 0.05$), and the PNN50 was lower in the moderate-to-severe OSA group than that in the PS group during N2 stage ($P < 0.01$).

The LF/HF ratio displayed a significant increasing tendency in the order of PS, mild OSA, and moderate-to-severe OSA groups. The LF/HF ratio was greater during the whole night and during each sleep stage in children with moderate-to-severe OSA in comparison with that in children with PS (all $P < 0.05$). The LF/HF ratio was greater in children with moderate-to-severe OSA than in those with mild OSA during N2 sleep stage ($P < 0.05$); this ratio was greater in children with mild OSA than in those with PS during the whole night and the N1 and R sleep stages (all $P < 0.05$).

As PSG variables, such as AHI, OAI, and ODI, were correlated with each other, only AHI was incorporated into the regression equation as a measure of disease severity to avoid multi-collinearity. Multiple stepwise regression of the RR mean and LF/HF ratio in different sleep stages showed that the RR mean was mainly influenced by age and AHI (all $P < 0.01$). The AHI was an

Table 3. Heart rate variability at different sleep stages of all children.

		PS $n = 49$	Mild OSA $n = 23$	Moderate–severe OSA $n = 48$	*F/Z*	*P* value
W	RR mean, ms	**640.9 ± 59.6**	**644.3 ± 80.3**	**606.2 ± 75.7**$^{**\#}$	**3.731**	**0.027**
	SDNN, ms	89.9 ± 24.7	91.6 ± 41.7	84.7 ± 27.7	0.563	0.571
	RMSSD, ms	57.9 ± 27.0	55.1 ± 27.1	51.9 ± 27.2	0.502	0.607
	SDANN, ms	38.6 ± 13.4	38.2 ± 29.7	38.1 ± 18.0	0.011	0.989
	pNN50, %	18.3 ± 11.1	18.5 ± 12.5	14.7 ± 11.4	1.412	0.248
	LF, ms^2	7900.5 (4683.4, 11,511.7)	12,046.2 (2837.2, 26,283.1)	6305.1 (3174.9, 17,103.8)	1.360	0.506
	HF, ms^2	3711.7 (1340.9, 8119.9)	4305.3 (1160.4, 9876.2)	2048.4 (1161.4, 7764.1)	0.956	0.620
	LF/HF	2.2 (1.4, 4.1)	2.5 (1.9, 4.7)	2.6 (1.8, 3.6)	1.121	0.571
N1	RR mean, ms	**720.8 ± 70.7**	**709.6 ± 87.3**	**665.0 ± 81.4**$^{**\#}$	**6.434**	**0.002**
	SDNN, ms	109.4 ± 30.1	108.9 ± 36.7	102.5 ± 27.0	0.710	0.494
	RMSSD, ms	71.8 ± 28.0	68.0 ± 31.1	63.9 ± 26.3	0.974	0.381
	SDANN, ms	37.0 ± 14.5	33.7 ± 12.0	35.0 ± 16.1	0.436	0.647
	pNN50, %	31.5 ± 13.7	28.7 ± 16.0	25.0 ± 13.7	2.483	0.088
	LF, ms^2	8256.1 (4688.9, 15,780.2)	12,370.6 (6521.7, 26,117.9)	14,118.4 (6512.9, 20,299.6)	4.067	0.131
	HF, ms^2	4207.6 (1744.3, 9214.6)	4590.9 (1626.7, 10,913.8)	3537.7 (1363.3, 7687.1)	0.794	0.672
	LF/HF	**2.1 (1.4, 3.4)**	**3.4 (2.3, 4.6)***	**3.6 (2.5, 5.1)****	**17.245**	**<0.001**
N2	RR mean, ms	**776.1 ± 83.5**	**751.7 ± 99.3**	**701.2 ± 86.7**$^{**\#}$	**8.574**	**<0.001**
	SDNN, ms	92.0 ± 26.6	83.7 ± 33.5	91.2 ± 23.9	0.822	0.442
	RMSSD, ms	75.1 ± 32.6	64.9 ± 36.3	64.6 ± 26.4	1.620	0.202
	SDANN, ms	45.3 ± 15.6	39.3 ± 14.6	44.7 ± 13.2	1.434	0.242
	PNN50, %	**41.0 ± 18.3**	**33.4 ± 21.4**	**31.1 ± 15.4****	**3.911**	**0.023**
	LF, ms^2	30,630.8 (15,376.9, 42,425.1)	23,868.1 (11,981.0, 41,836.6)	33,956.9 (19,885.1, 56,670.3)	4.931	0.085
	HF, ms^2	34,891.4 (20,073.2, 57,554.8)	23,640.8 (13,822.2, 50,552.8)	18,869.5 (10,121.3, 42,534.8)	5.871	0.053
	LF/HF	**0.7 (0.6, 1.2)**	**0.9 (0.7, 1.3)**	**1.4 (1.0, 2.9)**$^{**\#}$	**20.280**	**0.002**
N3	RR mean, ms	**765.1 ± 81.8**	**736.4 ± 105.0**	**695.5 ± 85.5****	**7.624**	**0.001**
	SDNN, ms	76.5 ± 25.8	69.3 ± 31.5	75.2 ± 23.2	0.622	0.539
	RMSSD, ms	70.4 ± 31.3	63.7 ± 39.8	61.6 ± 30.5	0.925	0.399
	SDANN, ms	40.9 ± 19.4	33.9 ± 18.6	43.1 ± 17.6	1.953	0.146
	pNN50, %	42.0 ± 20.3	34.8 ± 24.6	33.3 ± 19.4	2.315	0.103
	LF, ms^2	4975.3 (2144.8, 9134.4)	3500.5 (1948.1, 5938.8)	6223.1 (2531.9, 11,428.2)	2.475	0.290
	HF, ms^2	18,399.9 (6974.3, 27,448.9)	8211.2 (4711.1, 19,705.0)	8589.9 (3873.5, 21,930.9)	2.984	0.225
	LF/HF	**0.3 (0.2, 0.5)**	**0.3 (0.2, 0.5)**	**0.4 (0.3, 0.9)***	**7.162**	**0.028**
R	RR mean, ms	**713.4 ± 72.2**	**691.8 ± 88.1**	**650.1 ± 86.0**$^{**\#}$	**7.468**	**0.001**
	SDNN, ms	75.4 ± 23.1	70.3 ± 27.2	76.5 ± 25.4	0.501	0.607
	RMSSD, ms	54.0 ± 25.6	46.9 ± 30.4	51.6 ± 26.3	0.552	0.577
	SDANN, ms	32.7 ± 13.6	32.4 ± 13.7	34.6 ± 13.1	0.321	0.726
	pNN50, %	23.1 ± 15.5	18.5 ± 16.7	18.5 ± 13.9	2.848	0.062
	LF, ms^2	6636.2 (3393.6, 12,213.1)	6910.1 (3751.3, 10,548.7)	9105.1 (3771.7, 13,115.9)	1.557	0.459
	HF, ms^2	6226.8 (2720.2, 11,581.5)	3517.4 (1651.6, 7072.7)	3732.3 (1297.0, 8286.8)	3.665	0.160
	LF/HF	**1.3 (0.9, 1.8)**	**1.6 (1.2, 4.1)***	**1.8 (1.3, 2.9)****	**12.978**	**0.002**
Whole night	RR mean, ms	**738.4 ± 71.2**	**712.6 ± 91.7**	**674.3 ± 78.5****	**8.153**	**<0.001**
	SDNN, ms	101.9 ± 28.0	92.0 ± 34.9	95.9 ± 23.1	1.159	0.317
	RMSSD, ms	68.1 ± 27.9	60.1 ± 32.7	60.4 ± 25.2	1.120	0.330
	SDANN, ms	**62.4 ± 19.9**	**51.8 ± 17.2***	**57.2 ± 14.4**	**3.121**	**0.048**
	pNN50, %	33.6 ± 15.2	27.2 ± 18.0	26.4 ± 13.9	2.942	0.057
	LF, ms^2	72,564.9 (42,968.3, 109,889.6)	76,377.6 (47,707.6, 107,301.9)	101,125.8 (42,344.4, 134,019.1)	1.284	0.526
	HF, ms^2	87,956.5 (41,419.3, 136,611.4)	67,659.4 (34,969.5, 132,183.3)	49,580.0 (24,502.1, 98,778.0)	3.535	0.171
	LF/HF	**1.0 (0.6, 1.4)**	**1.3 (0.8, 1.7)***	**1.4 (1.0, 2.9)****	**15.961**	**<0.001**

The results of indices with statistically significance are bold presented.
W wake stage, *N1, N2, N3, R* non-rapid eye movement sleep stage 1, 2, 3 and rapid eye movement sleep stage, *RR mean* the mean of RR intervals, *SDNN* standard deviation of RR intervals, *RMSSD* root mean square of successive RR interval differences, *SDANN* standard deviation of the average RR intervals for each 5-min segment, *pNN50* the percentage of successive RR intervals that differed by >50 ms, *LF* the power spectral density of 0.04–0.20 Hz, *HF* the power spectral density of 0.20–0.45 Hz.
Compared with PS, $^{*}P < 0.05$, $^{**}P < 0.01$; compared with mild OSA, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.

independent risk factor for the altered LF/HF ratio at all sleep stages except for the N3 stage (all $P < 0.05$). During the wake stage, low-grade inflammation was an independent risk factor of a high LF/HF ratio ($P = 0.014$; Table 4).

DISCUSSION

The results of this study support our original hypothesis that altered sleep-related HRV patterns characterize ANS dysregulation in individuals with OSA. In this study, we demonstrated that the autonomic nervous function at night was impaired in children with OSA, including accelerated heart rate (decreased RR mean) and disturbance of the sympathetic–vagal balance. Heart rate during the wake stage and all sleep stages was mainly affected by age and AHI. The sympathetic–vagal balance was influenced by low-grade inflammation during the wake stage. Falling asleep it was only affected by AHI after adjusting for age, sex, and BMI *Z* score. We also found that the percentage of low-grade inflammation showed an increasing value trend, with the lowest value in the PS group and highest value in the moderate-to-severe OSA group, although with no statistical significance.

OSA and systemic inflammation

Previous studies have shown that hsCRP levels are increased in adults and children with SDB and are correlated with disease severity, after adjusting for the degree of obesity.[8–10] A main mechanism that mediates inflammation in OSA involves intermittent hypoxemia, which may produce oxygen free radicals.[24] As the characteristic feature of OSA, recurrent cycles of intermittent hypoxia and reoxygenation can promote extensive activation of various inflammatory cells as well as induce hypoxia-sensitive transcription factor hypoxia-inducible factor-1 (HIF-1) and the subsequent activation of inflammatory pathways. It has been proven that HIF-1-dependent inflammation has a principal role in various cardiovascular consequences in patients with OSA.[25] Furthermore, other studies have reported that circulating levels of CRP decrease in most children with SDB after treatment,[13,14] thereby confirming the causative link between systemic inflammation and OSA. In our study, the percentage of low-grade inflammation, defined as hsCRP >1.0 mg/L to <10.0 mg/L, showed an upward trend with the aggravation of disease, although without statistical significance. The reason for this may be the relatively small sample size. Additionally, a previous study demonstrated that the diurnal variation differed between OSA and controls, with children who had OSA showing higher levels of proinflammatory cytokines in the morning than in the afternoon and higher acute phase reactants in the afternoon. The explanation for this finding is that the morning rise in proinflammatory cytokines stimulates the production of acute phase reactants, which, based on their kinetics, peak several hours after the stimulus is applied.[26] This may also be a reason for the negative statistics in our research.

Table 4. Multiple stepwise regression of RR mean and LF/HF ratio at different sleep stages.

			Beta	R^2	*P* value
W	RR mean	Age	0.592		<0.001
		AHI	−0.266		<0.001
		Model		0.412	<0.001
	LF/HF	Low-grade inflammation	0.224		0.014
		Modal		0.042	0.014
N1	RR mean	Age	0.466		<0.001
		AHI	−0.382		<0.001
		Modal		0.354	<0.001
	LF/HF	AHI	0.292		0.001
		Model		0.077	0.001
N2	RR mean	Age	0.390		<0.001
		AHI	−0.439		<0.001
		Model		0.336	<0.001
	LF/HF	AHI	0.511		<0.001
		Model		0.255	<0.001
N3	RR mean	Age	0.335		<0.001
		AHI	−0.426		<0.001
		Model		0.283	<0.001
R	RR mean, ms	Age	0.481		<0.001
		AHI	−0.404		<0.001
		Model		0.386	<0.001
	LF/HF	AHI	0.398		<0.001
		Modal		0.151	<0.001
Whole night	RR mean	Age	0.432		<0.001
		AHI	−0.418		<0.001
		Model		0.352	<0.001
	LF/HF	AHI	0.462		<0.001
		Model		0.207	<0.001

Age, sex, AHI, BMI *Z* score, and low-grade inflammation were added to multiple linear regression models.

OSA and autonomic nervous dysfunction

Currently, there is substantial evidence to support the existence of adverse ANS consequences that span several functional domains such as the sympathetic and parasympathetic nervous systems and their balance in children with SDB.[27–30] The present study further confirmed the results of previous studies, as illustrated by a significantly decreased RR mean among children with moderate-to-severe OSA as compared with the RR mean in children with PS during the wake stage and all sleep stages. The gradual decreasing trend in the RR interval from the PS to the moderate–severe OSA groups suggested a relationship between heart rate and OSA severity. Previous studies have shown that obesity is associated with an increased risk for ANS dysfunction.[31] Linear regression model analysis confirmed that the AHI, an indicator of OSA severity, was an independent risk factor for heart rate after adjusting for age, sex, and BMI *Z* score. Moreover, age was revealed to be a negative influencing factor for the heart rate in our study, which is consistent with the findings reported by Sacrey et al.[32]

The balance of sympathetic and vagal activity was impaired in children with moderate-to-severe OSA when compared with that in the PS group at all sleep stages. The upward trend in the LF/HF ratio from the PS to the moderate–severe OSA groups at all sleep stages suggested a connection between sympathetic–vagal balance and disease severity. Linear regression model analysis confirmed that AHI was an independent risk factor for the altered LF/HF ratio in the N1, N2, and R sleep stages and during the whole night after adjusting for age, sex, and BMI *Z* score. At the N3 sleep stage, AHI did not influence the balance of sympathetic and vagal activity. The reason may be that the respiratory system is relatively stable and sleep apneas are least likely to occur during the N3 sleep stage. N3 is also the period when parasympathetic activity is dominant and the ANS is relatively immune to external adverse influences. The effect of OSA on children's HRV was observed mainly in the N1, N2, and R stages. The negative effect of OSA on HRV in children found in this study is consistent with the findings of a previous study.[4] Combined with the results of linear regression analysis, we believe that the LF/HF ratio is a good indicator of autonomic nervous function because it was only correlated with the severity of OSA, which is consistent with Flevari's research,[7] suggesting that this ratio might serve as a screening tool for OSA.

Relationship among SDB, low-grade inflammation, and ANS dysfunction

On the basis of the published associations between HRV and CRP in adults,[5,6] we hypothesized that hsCRP may be a viable candidate for risk stratification of ANS dysfunction in children. From our results, sympathetic–vagal balance was negatively correlated with low-grade inflammation level at the wake stage. This finding was consistent with those of a meta-analysis that affirmed the negative association between systemic inflammatory markers, such as hsCRP and ANS function.[6] However, the precise mechanistic link between the ANS and systemic inflammation remains unclear. Some researchers have hypothesized that the ANS modulates the immune system and is supported by anti-inflammatory effects mediated by vagal activity under conditions of pathogen invasion and tissue injury.[33] Other studies have shown that the induction of proinflammatory activity in humans reduces HRV,[34] supporting the notion of a bidirectional link between inflammation and ANS function. In the current work, sympathetic–vagal balance was affected only by the AHI when falling asleep, after adjusting for age, sex, and BMI *Z* score. Upon sleep onset, sympathetic tone decreases and cardiac parasympathetic activity/vagal tone increases.[35] This suggests that the balance of autonomic function is primarily affected by OSA rather than inflammation levels during the night when parasympathetic activity predominates.

There were some limitations in our study. First, we did not recruit children from the community as controls. Second, a small sample in the mild OSA group is a limitation of our study, which may account for the fact that no difference was found in the percentage of low-grade inflammation among children. Third, because of the lower sensitivity set in our instrument, it could not detect CRP levels <1.0 mg/L. Thus, hsCRP had to be analyzed as categorical data rather than as continuous data.

CONCLUSIONS

Our study findings showed that autonomic nervous function was impaired in children with OSA, and heart rate was mainly affected by age and OSA severity. The sympathetic–vagal balance was influenced by low-grade inflammation in the wake stage, whereas it was only affected by the AHI when falling asleep. The level of systemic inflammation in children with SDB did not affect the balance of the sympathetic and parasympathetic systems during sleep. An altered LF/HF ratio during sleep is a good indicator of autonomic nervous dysfunction in children because it was only correlated with the severity of OSA and not with age.

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AUTHOR CONTRIBUTIONS

Z.X. and X.C. are both responsible for the content of the manuscript and the integrity of the data analysis. Y. Wu contributed to experimental procedures performed during the study, data analysis and interpretation, and writing the manuscript. L.T. contributed to HRV analysis. P. Wu, D.M., and Y.T. contributed to experimental procedures performed during the study and grammar corrections. Z.X. contributed to study conception and design, data analysis and interpretation, and revised and approved the manuscript. X.C. contributed to study conception and design and approved the manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Written informed consent and assent were obtained from parents and children (for children aged >8 years).

ADDITIONAL INFORMATION

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