Respiratory sinus arrhythmia during sleep in children with upper airway obstruction

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SUMMARY

Upper airway obstruction during adulthood is associated with cardiovascular morbidity; cardiovascular consequences of childhood upper airway obstruction are less well established. This study aimed at investigating the effect of childhood upper airway obstruction on respiratory sinus arrhythmia as a measure of cardiac vagal modulation during night-time sleep. Overnight polysomnography was conducted in 40 healthy children (20 M; age: 7.5 ± 2.6 years; body mass index percentile: $60.7 \pm 26.4\%$) and 40 children with upper airway obstruction (24 M; age: 7.5 ± 2.7 years; body mass index percentile: $65.8 \pm 31.9\%$). We used the phase-averaging technique to compute respiratory sinus arrhythmia amplitude and phase delay. To study sleep stage effects and the effect of upper airway obstruction, respiratory sinus arrhythmia was measured during all artefact-free sleep episodes, and after exclusion of respiratory events. A significant increase in respiratory sinus arrhythmia amplitude and phase delay was observed during stage 4 sleep as compared with rapid eye movement sleep in both groups (amplitude: controls = 0.10 ± 0.03 versus 0.07 ± 0.02 s, $P < 0.01$, respectively, and upper airway obstruction = 0.07 ± 0.03 versus 0.05 ± 0.03 s, $P < 0.05$, respectively; phase delay: controls = 3.1 ± 0.1 versus 3.0 ± 0.1 rad, $P < 0.05$, respectively, and upper airway obstruction = 3.13 ± 0.04 versus 3.04 ± 0.08 rad, $P < 0.01$, respectively). A significant association between respiratory sinus arrhythmia and apnea/hypopnea index was observed during stage 2 sleep in children with upper airway obstruction. Compared with healthy controls, a significant decrease in respiratory sinus arrhythmia amplitude during stage 2 sleep was observed in children with upper airway obstruction (0.09 ± 0.03 versus 0.06 ± 0.03 s, $P < 0.05$). However, this difference was not apparent when respiratory events were excluded from analysis. Importantly, respiratory sinus arrhythmia showed a strong negative correlation with body mass index. In conclusion, night-time respiratory sinus arrhythmia in children is sleep stage dependent and normal during quiet sleep in children with relatively mild upper airway obstruction.

INTRODUCTION

Respiratory sinus arrhythmia (RSA) is a physiological pattern arising from cardiorespiratory interactions, and is characterized by heart rate accelerations/decelerations that occur in phase with inhalation and exhalation during the respiratory cycle. RSA is vagally mediated, arising as a result of inputs to cardiac vagal neurons from both the central pattern generator (Neff *et al.*, 2003), and from peripheral airways and lung stretch receptors (Taha *et al.*,

1995). RSA results in high-frequency (HF) oscillations of heart rate, which can be quantified using power spectral analysis of heart rate variability (HRV; Baharav *et al.*, 1995). However, phase domain analysis of respiration and heart rate measures temporal association between both, and has been suggested as a better measure of RSA (Kotani *et al.*, 2008). Although the physiological significance of RSA is not yet completely understood, it has been suggested that RSA serves to enhance pulmonary gas exchange thereby improving the energy efficiency of pulmonary circulation (Hayano *et al.*, 1996). Furthermore, basal RSA reflects the status of the parasympathetic nervous system at rest and provides an appropriate index of vagal activity (Taha *et al.*, 1995). Importantly, there is strong clinical evidence demonstrating that reduced RSA is a prognostic indicator for cardiac morbidity and mortality (Moser *et al.*, 1994).

Upper airway obstruction (UAO) is characterized by repetitive partial or complete closure of the upper airways during sleep that causes alterations in the functioning of the cardiorespiratory system, and affects between 1% and 4% of children (Lumeng and Chervin, 2008). In adults, the association between UAO and increased cardiovascular morbidity is now well recognized (McNicholas and Bonsignore, 2007). While the mechanisms leading to cardiovascular morbidity are likely to be multifactorial, frequent arousals triggered by episodes of UAO are believed to play a key role via repetitive sympathetic nervous system activation and destabilization of cardiorespiratory control (Pack and Gislason, 2009). Emerging evidence now suggests that children with UAO may also be at increased risk of developing cardiovascular disease (O'Driscoll *et al.*, 2009).

Previous studies have shown that non-rapid eye movement sleep (NREM) is associated with an increase in the HF component of HRV, as compared with wakefulness and rapid eye movement sleep (REM) in children (Baharav *et al.*, 1995) and adults (Vanoli *et al.*, 1995), indicative of increased RSA. Sleep deprivation, on the other hand, causes an excessive activation of cardiovascular and sympathetic nervous activity that adversely affects RSA, as evident from a decrease in HRV during nocturnal sleep in healthy adults (Zhong *et al.*, 2005). In adults with sleep-disordered breathing, decreased HF power of daytime HRV and its inverse correlation with apnea/hypopnea index was reported (Hilton *et al.*, 2001). Reduced HF power of HRV has also been demonstrated in obese adolescents and adults, indicative of reduced RSA (Rabbia *et al.*, 2003).

Given the well-established associations between reduced vagal cardiac modulation, sleep-disordered breathing and obesity in adolescence and adulthood, and the negative implications for cardiac health, the aim of this study was to investigate cardiac autonomic function during night-time sleep in children with UAO, by evaluating RSA. We hypothesized that RSA is reduced in children with UAO and inversely related to body mass index (BMI).

MATERIALS AND METHODS

Participants

The study conformed to the principles outlined in the Declaration of Helsinki, and was approved by the Human Ethics Committee, Women's and Children's Hospital (WCH), Adelaide. Parental consent and child assent were obtained from all participants.

Fifty-three healthy children and 54 children with UAO were enrolled in this study. Among the healthy children (controls), none was reported to snore regularly or was taking medication that would affect sleep architecture or cardiorespiratory physiology. The children with UAO were those who had a history of frequent snoring and were scheduled for adenotonsillectomy for suspected UAO, as diagnosed by an experienced paediatric otorhinolaryngologist at WCH, but had no other medical condition associated with hypoxia or sleep fragmentation. Thirteen of the 53 control children and 14 of the 54 children with UAO were excluded either due to poor signal quality, or significantly greater age and lower socioeconomic status. The age and BMI z-score of the remaining subjects (40 controls and 40 with UAO) ranged 3–12.9 years (7.7 $\pm$ 2.6 years) and −1.7–2.3 (0.3 $\pm$ 0.8), respectively. The percentage of males was 50% ($n = 20$) in the control group and 60% ($n = 24$) in the UAO group.

Overnight polysomnography (PSG)

Overnight PSG was conducted without sedation or sleep deprivation using the S-series sleep system (Compumedics, Australia). Each subject was continuously monitored via infrared camera by a paediatric sleep technician who also documented several observations of sleep behaviour.

For sleep staging and arousal scoring standard surface electrodes were applied to the face and scalp, including two-channel electroencephalograms (C3-A2 and C4-A1), left and right electrooculograms and a submental electromyogram. Respiratory frequency was monitored using chest and abdominal respiratory inductance plethysmography bands. Sleep stages were assigned to consecutive 30-s epochs according to standard rules (Rechtschaffen and Kales, 1968). The calculation of apnea–hypopnea index (AHI) and scoring of different artefacts including movement during sleep has been described in detail in our previous studies (Baumert *et al.*, 2010, 2011).

Electrocardiogram (ECG) and respiratory analysis

The ECG signal (lead II) was sampled at 500 Hz, and algorithms of the libRASCH library (www.librasch.org) were used to detect ECG R-wave peaks. The R-R time series obtained from the time-points of the R-peaks were visually scanned for artefacts and, if necessary, manually edited.

The abdominal respiratory inductance band, digitized at 25 Hz, was used in this study for respiratory timing analysis.

The signal was low-pass filtered at 0.5 Hz using a zero-phase forward and reverse digital filter. Inspiratory onset, used to compute the breath-to-breath time series, was determined as the zero-crossing of the first derivative of the respiratory signal. The phases of the respiratory signal were calculated using the Hilbert transform.

Phase-averaged RSA analysis

Respiratory sinus arrhythmia is usually assessed as the magnitude of R-R interval changes related to respiration. It has been suggested, however, that the temporal association between the respiratory phase and R-R interval changes in the ECG may also play an important role in the phenomenon of RSA. It has been shown, for example, that postural change affects both the amplitude and phase of the RSA pattern, and hence the study of both the RSA parameters would be useful for understanding the dynamic mechanism of RSA (Kotani *et al.*, 2008). In our study, the pattern of RSA was evaluated by selective averaging of R-R interval changes from multiple respiratory cycles over the respiratory phase. For all *n* respiratory cycles, the R-R intervals in each respiratory cycle were interpolated into 50 data points using cubic spline interpolation (Gilad *et al.*, 2005). The 50 data points for each of the *n* respiratory cycles corresponds to 2π (Fig. 1) – the origin in the figure being the expiratory onset of respiration. The overall RSA pattern was obtained by taking the average of all the RSA patterns for *n* respiratory cycles (Fig. 1). For the purpose of our analysis we defined two RSA parameters: RSA amplitude (calculated by taking the difference between the maximum and the minimum peaks of the overall RSA pattern); and phase delay (defined as the respiratory phase at the point of maximum overall RSA).
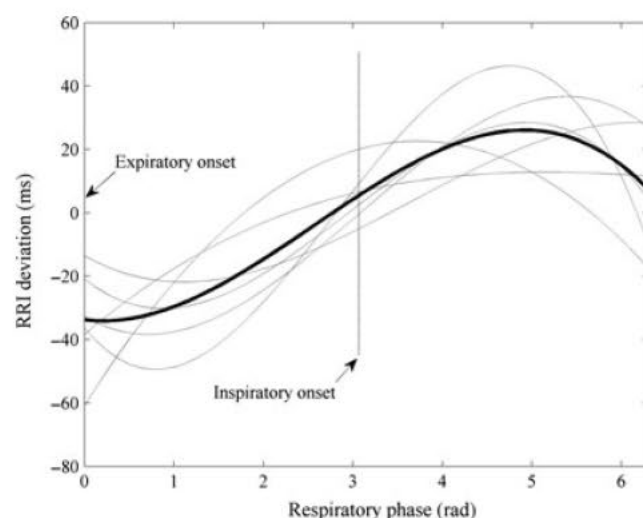

Figure 1. Phase-averaged RSA plot during an episode of stage 2 sleep of a subject for six respiratory cycles clustered together (thin grey lines), and the overall RSA (thick black line) obtained by averaging the clustered RSA. RRI, R-R interval.

Artefacts such as movement can potentially confound the results, and hence all 30-s epochs containing artefacts as well as the artefact-free epoch immediately before and after the artefact-epoch were excluded from the analysis. Furthermore, epochs containing scored arousals were excluded due to known cardiorespiratory effects (Baumert *et al.*, 2010, 2011).

The RSA parameters were calculated for every epoch of awake (AW), stages 2 and 4 of NREM sleep as well as REM sleep, and subsequently averaged for each sleep stage and child. Stages 1 and 3 were omitted from the analysis due to transitional character. In the children with UAO, two separate analyses were conducted: (i) inclusion of all artefact-free epochs, applying the exclusion criteria described above; and (ii) inclusion of all artefact-free epochs with the additional exclusion of any epochs containing UAO-related events (apneas, hypopneas, respiratory arousals) as well as the one epoch before and after the respiratory events. Such events, if any, were also excluded during data analysis of the control group. The former analysis provides an overall assessment of RSA in children with UAO, while the latter allows distinguishing between baseline RSA in these children and the acute effects of respiratory events.

Statistical analysis

Statistical software SPSS version 18.0 and GraphPad Prism version 5.01 for Windows (San Diego California, USA; www.graphpad.com) were used for statistical analysis. Significant associations between variables across all children were determined using Pearson's correlation coefficients and linear stepwise regression models. The RSA amplitudes and phase delays of sleep stages 2 and 4 were log-transformed to achieve normal distribution of data across the groups. One-way analysis of variance (ANOVA) for repeated measurements was used to test for differences in RSA between sleep stages. Differences in RSA between groups were studied using analysis of covariance (ANCOVA). *Post hoc* analysis was performed using Tukey's multiple comparison test. Data are expressed as mean $\pm$ SD. All *P*-values are two-tailed with statistical significance determined at $\alpha = 0.05$.

RESULTS

PSG findings

As reported previously (Baumert *et al.*, 2010, 2011), there were no significant differences in sleep architecture, spontaneous arousals and periodic leg movement index between the two groups. In children with UAO, baseline PSG confirmed the presence of respiratory abnormalities: a significantly higher AHI; elevated respiratory arousals; increased frequency of arterial oxygen saturation (SpO_2) desaturations; and a significantly lower mean SpO_2 nadir compared with healthy controls (Table 1).

Table 1 Demographic data and overnight PSG findings in controls and children with UAO

	Control (n = 40)	*UAO* (n = 40)
Age (years)	7.5 ± 2.6	7.5 ± 2.7
Gender, *n* males (%)	20 (50%)	24 (60%)
BMI percentile (%)	60.7 ± 26.4	65.8 ± 31.9
Socioeconomic status	994.9 ± 93.0	976.9 ± 93.9
TST (min)	446.9 ± 37.3	425.6 ± 59.5
Stage 2 sleep (% TST)	44.2 ± 6.9	42.4 ± 6.2
Stage 4 sleep (% TST)	25.6 ± 4.6	27.7 ± 5.7
REM sleep (% TST)	20.6 ± 3.9	19.7 ± 5.7
REM latency (min)	92.8 ± 22.0	85.8 ± 31.2
Movement time (min)	9.1 ± 4.9	8.7 ± 4.5
Periodic limb movement	3.3 ± 4.9	4.9 ± 7.3
index (median, range)	(1.2, 0 19.6)	(1.3, 0 27.2)
Spontaneous arousal index	9.4 ± 2.7	8.4 ± 2.4
Respiratory arousal index	0.4 ± 0.4	3.2 ± 4.2**
(median, range)	(0.3, 0 1.7)	(1.1, 0 17.0)
SpO$_2$ nadir	92.9 ± 1.9	90.6 ± 5.7*
SpO$_2$ desaturation index	0.8 ± 0.8	5.1 ± 9.4**
(median, range)	(0.8, 0 4.9)	(1.3, 0 53.1)
AHI (median, range)	0.1 ± 0.2	5.0 ± 9.0**
	(0.1, 0 0.9)	(0.9, 0 49.8)

AHI, apnea hypopnea index; BMI, body mass index; REM, rapid eye movement; SpO$_2$: arterial oxygen saturation; TST, total sleep time; UAO, upper airway obstruction.
*$P < 0.05$.
**$P < 0.0001$.

Effect of sleep stage on R-R intervals in controls and children with UAO

There was a significant shortening in the average R-R interval during REM sleep as compared with stage 4 sleep in healthy controls (0.61 ± 0.1 versus 0.69 ± 0.1 s, $P < 0.05$), as well as in children with UAO independent of whether respiratory events were included (0.60 ± 0.1 versus 0.67 ± 0.1 s, $P < 0.05$) or excluded in the analysis (0.60 ± 0.1 versus 0.67 ± 0.1 s, $P < 0.05$; Fig. 2). Wakefulness in controls and UAO children was associated with significantly shorter R-R intervals compared with other sleep stages (Fig. 2). There was, however, no significant difference in R-R intervals for respective sleep stages between the groups. Also, there was no significant effect of BMI on R-R intervals in any sleep stage.

Effect of sleep stage on respiratory intervals in controls and children with UAO

A statistically significant shortening in respiratory interval was observed during stage 4 compared with stage 2 sleep in both the groups (controls: 1.85 ± 0.2 versus 1.90 ± 0.2 s, $P < 0.05$; UAO including respiratory events: 1.94 ± 0.2 versus 2.05 ± 0.2 s, $P < 0.05$; UAO excluding respiratory events: 1.97 ± 0.2 versus 2.06 ± 0.2 s, $P < 0.05$; Fig. 3). In addition, there was a significant shortening in respiratory interval during stage 4 sleep compared with REM sleep, and between wakefulness and stage 2 sleep in controls (Fig. 3). The average respiratory interval during stages 2 and 4 sleep in controls was significantly shorter compared with those of the comparable sleep stage in children with UAO, independent of the inclusion/exclusion of respiratory events (Fig. 3). Lastly, there was no significant effect of BMI on respiratory intervals in any sleep stage.

Effect of age, gender and BMI on RSA in children

Scatter plots of RSA and age revealed four outliers, which were removed from correlation and regression analyses. The RSA parameters showed no significant correlation with age or gender (Table 2). However, inverse correlations between RSA amplitude and BMI were observed in all sleep stages. Using the stepwise linear regression model on all 80 children, we confirmed significant inverse associations between RSA and BMI across all sleep stages, the strongest being observed during stage 4 sleep (amplitude: $F_{1,76} = 20.03$, $P < 0.0001$; phase delay: $F_{1,76} = 12.67$, $P < 0.01$; Table 3).
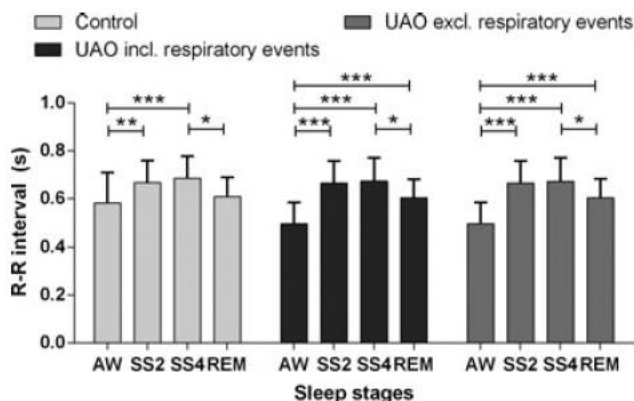

Figure 2. Group and sleep stage effects on R-R interval in controls and upper airway obstruction (UAO) children including and excluding respiratory events. *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$. AW, awake; REM, rapid eye movement; SS, sleep stage.
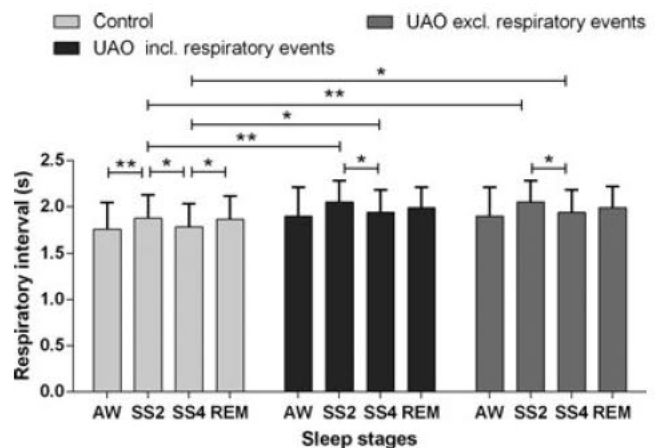

Figure 3. Group and sleep stage effects on respiratory interval in controls and upper airway obstruction (UAO) children including and excluding respiratory events. *$P < 0.05$, **$P < 0.01$. AW, awake; REM, rapid eye movement; SS, sleep stage.

Table 2 Correlation between RSA and demographic, respiratory and PSG parameters as a function of sleep stage in controls and UAO children (combined)

SS	RSA	RR r	RR P	Resp r	Resp P	Age r	Age P	BMI r	BMI P	Gender r	Gender P	AHI r	AHI P
AW	Mag	0.19	0.05	**0.50**	**0.00**	−0.19	0.10	−0.15	0.05	−0.05	0.34	−0.07	0.17
	Ph	**0.22**	**0.03**	0.05	0.33	−0.04	0.38	−0.02	0.26	−0.09	0.22	−0.05	0.35
SS2	Mag	**0.32**	**0.00**	**0.43**	**0.00**	−0.09	0.26	**−0.38**	**0.00**	−0.19	0.05	**−0.22**	**0.02**
	Ph	**0.38**	**0.00**	0.02	0.42	0.10	0.19	−0.12	0.11	−0.05	0.33	−0.16	0.08
SS4	Mag	**0.28**	**0.01**	**0.44**	**0.00**	−0.04	0.35	**−0.46**	**0.00**	−0.22	0.06	**−0.30**	**0.00**
	Ph	**0.30**	**0.00**	0.01	0.48	0.05	0.33	**−0.23**	**0.03**	0.07	0.28	**−0.19**	**0.04**
REM	Mag	**0.43**	**0.00**	**0.28**	**0.01**	−0.04	0.37	**−0.29**	**0.01**	−0.25	0.13	**−0.22**	**0.03**
	Ph	**0.28**	**0.01**	0.08	0.24	−0.10	0.19	−0.09	0.14	0.01	0.45	**−0.37**	**0.00**

AHI, apnea hypopnea index; AW, Awake; BMI, body mass index; Mag, magnitude; Ph, phase delay; REM, rapid eye movement; Resp, respiratory interval; RR, ECG beat-to-beat interval; RSA, respiratory sinus arrhythmia; SS, sleep stage.
Bold numbers represent statistically significant associations.

Table 3 Significant association of different measures with RSA parameters for different sleep stages in controls and UAO children (combined), as observed using linear regression model

	RR F	RR β	Resp F	Resp β	BMI F	BMI β
RSA magnitude						
AW						
SS2	$F_{1,74}$ 5.99	0.23*	$F_{1,76}$ 17.42	0.42***	$F_{1,75}$ 18.72	−0.38***
SS4	$F_{1,74}$ 7.99	0.26**	$F_{1,75}$ 20.85	0.44***	$F_{1,76}$ 20.03	−0.46***
REM			$F_{1,75}$ 15.96	0.32**	$F_{1,76}$ 17.18	−0.34**
RSA phase delay						
AW						
SS2	$F_{1,75}$ 4.79	0.26*			$F_{1,76}$ 7.68	−0.30**
SS4					$F_{1,76}$ 12.67	−0.38**
REM	$F_{1,75}$ 4.47	0.22*			$F_{1,76}$ 12.29	−0.33**

AW, awake; BMI, body mass index; REM, rapid eye movement; Resp, respiratory interval; RR, ECG beat-to-beat interval; RSA, respiratory sinus arrhythmia; SS, sleep stage; AW, awake.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.0001$.

Effect of sleep stage on RSA

Due to the finding that respiratory intervals were significantly different between the two groups and that BMI correlated inversely with RSA, all subsequent statistical analyses of RSA co-varied for respiratory rate and BMI. The mean amplitude of RSA showed a strong association with sleep stage in both the groups, and was significantly increased during stage 4 sleep compared with wakefulness and REM sleep (controls: 0.10 ± 0.03 versus 0.06 ± 0.03 s, $P < 0.0001$ and 0.07 ± 0.02 s, $P < 0.01$, respectively; and UAO with respiratory events: 0.08 ± 0.03 versus 0.05 ± 0.03 s, $P < 0.05$ and 0.06 ± 0.03 s, $P < 0.05$, respectively; Fig. 4). In addition, a significant increase in RSA phase delay was observed during stage 4 sleep compared with both wakefulness and REM sleep in both the groups (controls: 3.10 ± 0.08 versus 2.89 ± 0.09 rad, $P < 0.0001$ and 3.03 ± 0.06 rad, $P < 0.05$, respectively; and UAO with respiratory events:

3.13 ± 0.04 versus 2.91 ± 0.09 rad, $P < 0.0001$ and 3.04 ± 0.08 rad, $P < 0.01$, respectively; Fig. 5).

Comparison of RSA between controls and children with UAO

Compared with controls, the amplitude of RSA was significantly lower in the children with UAO during stage 2 sleep when including UAO events in the analysis (0.09 ± 0.02 versus 0.06 ± 0.02 s, respectively, $F_{1,76} = 4.50$, $P < 0.05$; Fig. 4). However, the amplitude of RSA in the UAO group differed significantly when comparing data with and without abnormal respiratory events (0.06 ± 0.02 versus 0.08 ± 0.03 s, $P < 0.05$, respectively). Significant between-group differences in RSA amplitude were no longer seen when only analysing epochs of quiet normal breathing (Fig. 4). Furthermore, no significant differences in RSA phase delay were observed between healthy controls and
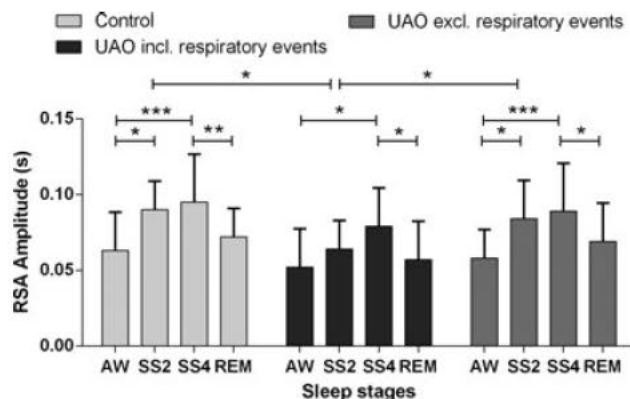
Figure 4. Group and sleep stage effects on the amplitude of respiratory sinus arrhythmia (RSA) in controls and upper airway obstruction (UAO) children including and excluding respiratory events. The values shown in the figure correspond to the estimated marginal mean and SD obtained from the corrected ANCOVA model. *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$. AW, awake; REM, rapid eye movement; SS, sleep stage.
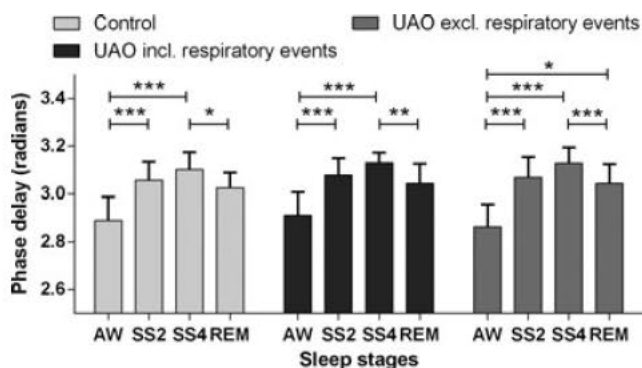

Figure 5. Group and sleep stage effects on phase delay of RSA in controls and upper airway obstruction (UAO) children including and excluding respiratory events. The values shown in the figure correspond to the estimated marginal mean and SD obtained from the corrected ANCOVA model. *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$.

children with UAO after excluding respiratory events (Fig. 5).

Furthermore, a significant difference in RSA amplitude was observed between controls and children with UAO (including respiratory events) during stage 4 sleep ($F_{1,76} = 6.88$, $P < 0.05$). However, there was a significant interaction between respiratory interval and subject group during stage 4 sleep ($F_{2,72} = 12.31$, $P < 0.05$), showing a shorter interval for controls compared with children with UAO (both including and excluding respiratory events). This suggests that the modulatory effect of respiratory rate on RSA was not the same for both the groups, and thus the between-group difference in RSA amplitude during stage 4 sleep should be interpreted with caution. No significant difference was observed in phase delay between the two groups during stage 4 sleep. In addition, there were no significant between-group differences in RSA parameters during REM sleep.

Relationship between AHI and RSA in children

Because AHI was zero for most children in the control group and thus would potentially confound any linear regression analysis, we studied the effect of AHI on RSA by developing a linear regression model separately for the 40 children with UAO, taking the data that include respiratory events into account. A significant negative association was observed between RSA amplitude and AHI during stage 2 sleep ($F_{1,37} = 5.85$, $\beta = -0.38$, $P < 0.05$). In addition, RSA phase delay showed a significant positive association with AHI during stage 2 sleep ($F_{1,37} = 4.64$, $\beta = 0.31$, $P < 0.05$). Confirming the regression results obtained with the whole data set including all children, BMI was also predictive of RSA amplitude and phase (amplitude: $F_{1,38} = 18.28$, $\beta = -0.57$, $P < 0.0001$; and phase: $F_{1,38} = 4.79$, $\beta = -0.28$, $P < 0.05$) in the group of children with UAO. No significant association between RSA and AHI was observed during REM sleep.

DISCUSSION

To our knowledge this is the first study to investigate RSA during sleep in children with UAO in comparison to healthy children. The major findings of this study are as follows: (1) A strong association between RSA and sleep stage in healthy children and in children with UAO, where the amplitude and phase delay of RSA are highest during stage 4 sleep. (2) Importantly, RSA amplitude and phase delay are inversely related to BMI. (3) Although we observed a significant decrease in RSA during stage 2 sleep in children with UAO as compared with healthy controls, these between-group differences in RSA are primarily a result of acute respiratory events and do not indicate a general loss of RSA as they disappear when respiratory events are excluded from analysis.

Effect of sleep stage on cardiorespiratory patterns

Sleep is associated with changes in cardiac autonomic control (Bonnet and Arand, 1998). In line with previous studies in children and adults, the R-R interval was significantly lengthened during stage 4 sleep compared with REM sleep in children with or without UAO (Baharav *et al.*, 1995; Vanoli *et al.*, 1995). Also, there was a significant lengthening in respiratory interval in both groups of children during stage 2 sleep when compared with stage 4 sleep, which is consistent with previous findings in children (Carskadon *et al.*, 1978).

Suppression of cardiac efferent vagal tone together with an increase in sympathetic neural outflow may explain the decrease in RSA during REM sleep in healthy children and those with UAO. Sympatho-vagal balance varies throughout sleep stages from NREM to REM sleep, with a transition from predominant vagal tone during stages 3 and 4 sleep to sympathetic dominance during REM sleep (Buchheit *et al.*, 2004). In particular, stage 4 sleep is characterized by high ECG stationarity and regular respiratory patterns (Buchheit

et al., 2004). The presence of high parasympathetic tone during stage 4 sleep contributes to the prominence of RSA during this sleep stage compared with all other sleep stages, and is in line with earlier studies in children and adults (Baharav *et al.*, 1995; Vanoli *et al.*, 1995).

Effect of UAO on RSA

Reduced HF power was previously reported in adults with sleep apnea/hypopnea syndrome as compared with matched controls (Hilton *et al.*, 2001). In our study, the analysis of RSA showed a reduction in RSA in children with UAO in stage 2 sleep in the presence of respiratory events. Importantly, no differences were found when periods of respiratory events were removed from the analysis, suggesting that there was no systemic reduction in RSA in our children with UAO and thus vagal cardiac modulation was normal. However, the correlation between AHI and RSA indicates that there might be an effect of UAO on RSA, but because the UAO was mild in most children, the effect may not have been reflected in the between-group comparison. In support of our findings, Chaicharn *et al.* (2009) found no significant changes in vagal cardiac modulation in children with obstructive sleep apnea syndrome applying autonomic reflex tests. Brief withdrawal of vagal tone paralleled by increased sympathetic nerve activity during the arousal period may therefore account for the decrease of RSA that we observed prior to excluding UAO-related events (Baumert *et al.*, 2011). In addition, changes in the respiratory waveform may have contributed to the reduction in RSA.

Effect of BMI on RSA

We observed a strong negative association between BMI and RSA, which confirms the finding by El-Sheikh *et al.* (2007), who reported an inverse correlation between BMI and vagal cardiac modulation in children. Although there is a strong association between obstructive sleep apnea and BMI in adolescents and adults (Ferguson *et al.*, 1995), this relationship is not apparent in young children (Kohler *et al.*, 2009). This would suggest that diminished RSA in children with UAO is primarily a consequence of increased BMI. Pronounced RSA is indicative of healthy vagal heart rate modulation and considered to have a cardioprotective effect. Reduced RSA is well documented in patients with heart failure or after myocardial infarction (Kleiger *et al.*, 1987). Reduced RSA in children with high BMI may therefore be a harbinger of future cardiovascular morbidity.

Effect of age on RSA

Aging is associated with changes in sympatho-vagal balance, with a significant increase in sympathetic nervous system outflow to the cardiovascular system in old age (De Meersman, 1993). According to Marshall and Stevenson-Hinde (1998), daytime RSA increased with age in children between 4.5 and 7 years, while another study of children between 8

and 17 years old reported no significant change in RSA (Salomon, 2005). In our study, using cross-correlation analysis, we observed a lack of correlation between age and RSA, suggesting that RSA during sleep is independent of age in children aged between 3 and 12.9 years.

Gender effects on RSA

There were no significant differences in the amplitude and phase delay of RSA between males and females. This is in contrast to adults where decreases in vagal tone and increases in sympatho-vagal balance have been observed in men compared with women during nocturnal sleep (Valladares *et al.*, 2008). Thus, the gender differences of RSA may emerge following pubertal development.

Limitations

We used strict inclusion criteria and carefully selected sleep episodes for our data analysis. In the regression analysis we developed separate models for each sleep stage under consideration. Although this approach provides somewhat redundant results, it allowed us to verify associations among several parameters. Because we were unable to include healthy children (AHI = 0) in the regression model of AHI analysis, only 40 children were included in this subanalysis of RSA predictors. In addition, due to the strong association between the factor (groups) and covariate (respiratory interval) during stage 4 sleep, the differences in RSA between the two groups could not be assessed. The age range of the participants included in this study was relatively wide. Developmental changes in RSA could have occurred, although correlation analysis did not demonstrate a direct linear association between both. Furthermore, this study uses the old scoring rules for the scoring of sleep and associated events.

CONCLUSION

In summary, night-time RSA in children is affected by the stage of sleep. Mild UAO has no significant effect on baseline levels of RSA, despite some acute effects of respiratory events. However, an increase in BMI adversely affects RSA in children, indicative of early autonomic dysfunction, which may be a precursor of cardiovascular disease. In future studies it would be interesting to investigate RSA in more detail, for example, during specified segments within each sleep stage.

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CONFLICT OF INTERESTS

The authors have declared that no conflict of interests exists.

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