Catecholamine Alterations in Pediatric Obstructive Sleep Apnea: Effect of Obesity

Ayelet B. Snow, MD, Abdelnaby Khalyfa, PhD, Laura D. Serpero, PhD, Oscar Sans Capdevila, MD, Jinkwan Kim, PhD, Muhamad O. Buazza, BSc, and David Gozal, MD*

Summary. Study Objectives: Obstructive sleep apnea (OSA) elicits increased sympathetic activity in adults and increased urinary catecholamines. Moreover, urinary catecholamine excretion is altered in obese patients. We hypothesized that morning urine catecholamine levels would be correlated with the severity of obstructive sleep apnea and degree of obesity in children. Methods: Children referred to the pediatric sleep center for habitual snoring underwent overnight polysomnography, and the first morning voided urine sample was collected. Urinary concentrations of norepinephrine, epinephrine and dopamine were measured and corrected for creatinine levels. In a subset of children, blood samples were drawn and gene expression of catecholamine-relevant genes analyzed by quantitative real-time PCR. Results: One hundred fifty-nine children were recruited and completed the protocol. Children with OSA had significantly higher urinary norepinephrine and epinephrine levels, but not dopamine, compared to habitual snorers (norepinephrine: 40.1 ± 24.7 ng/mg creatinine vs. 31.6 ± 16.2 ng/mg creatinine, $P < 0.01$; epinephrine: 6.4 ± 10.5 ng/mg vs. 4.5 ± 0.5 ng/mg, $P < 0.01$). There was a positive correlation between norepinephrine and epinephrine values and polysomnographic indices, but no effect of obesity on catecholamine levels. In addition, expression of several of the major genes involved in synthesis and transport of catecholamines, as well as in selected receptors were compatible with increased bioavailability of catecholamines. Conclusions: In children with OSA, morning urinary norepinephrine and epinephrine levels are significantly higher than those without OSA, and correlate with the severity of the disease. Gene expression patterns are in agreement with such findings. Urine catecholamine levels do not appear to be influenced by the presence of obesity. Thus, altered sympathetic activity in OSA patients appears to occur independently of the presence of obesity. **Pediatr Pulmonol.** © 2009 Wiley-Liss, Inc.

Key words: autonomic nervous system; sympathetic; catecholamines; hypoxia; sleep apnea.

BACKGROUND

Obstructive sleep apnea (OSA) is a relatively prevalent disorder among both adults and children.[1–4] Over the last decades, the association between OSA and cardiovascular morbidities has been extensively studied. Indeed, OSA will increase the risk for systemic hypertension,[5,6] promote ischemic heart disease and the propensity for stroke,[7] and lead to changes in cardiac structure and function.[8] Furthermore, OSA may also lead to chronic systemic inflammation and endothelial dysfunction along with significant metabolic disturbances.[1,8–11] The mechanisms underlying the association between OSA and cardiovascular disease are not fully understood. Obstructive respiratory events can result in gas exchange abnormalities, that is, intermittent hypoxia and hypercapnia, as well as sleep fragmentation and exaggerated intrathoracic pressure swings. All of these events, either alone or in combination, have been shown to promote the formation of reactive oxygen species, increase oxidative stress, and elicit sustained activation of the sympathetic autonomic nervous system.[12] As a corollary to the latter, elevated catecholamines levels were reported in the serum and urine of adult patients diagnosed with OSA.[13–16]

The relative wealth of research data on OSA and cardiovascular morbidity in adults is unfortunately not

Division of Pediatric Sleep Medicine and Kosair Children's Hospital Research Institute, Department of Pediatrics, University of Louisville, Louisville, Kentucky 40202.

Grant sponsor: NIH; Grant number: HL-65270; Grant sponsor: Children's Foundation Endowment for Sleep Research; Grant sponsor: Commonwealth of Kentucky Challenge for Excellence Trust Fund

*Correspondence to: David Gozal, MD, Professor and Chair, Department of Pediatrics, Physician-in-Chief, Comer Children's Hospital, The University of Chicago, 5721 S. Maryland Avenue, MC 8000, Suite K-160, Chicago, IL 60637.E-mail: dgozal@peds.bsd.uchicago.edu

Received 13 July 2008; Revised 27 October 2008; Accepted 28 October 2008.

DOI 10.1002/ppul.21015
Published online in Wiley InterScience (www.interscience.wiley.com).

matched by a similar extent of information in children. Since the initial study by Marcus et al.[17] reporting that OSA children had higher sleep and wake diastolic blood pressure compared with habitually snoring children, several other groups of investigators have identified heightened prevalence of elevated systemic blood pressure in children with OSA.[18–24] Along with such findings, evidence for altered sympathetic tonic activity and increased sympathetic reactivity were recently reported in children with OSA[25–27] suggesting that similar to adult patients with OSA, the presence of OSA in children will elicit substantial changes in autonomic nervous system regulation, and more particularly increased sympathetic outflow and reduced parasympathetic tone. Increased sympathetic activity could be directly and invasively measured using microneurographic recordings of sympathetic fibers in the extremities[12] or can alternatively be assessed through measurement of catecholamine levels in either the serum or urine. We therefore hypothesized that the previous evidence supporting increased sympathetic activity in children with OSA would be further confirmed by increased secretion of urinary catecholamines in a severity-dependent manner. Because obesity and enlarged adenotonsillar tissues are the two major risk factors for OSA in children,[28] we further posited that obese children with OSA may display increased levels of urinary catecholamines compared to non-obese children with OSA of similar severity.

METHODS AND PATIENTS

Patients

Consecutive snoring children referred to the University of Louisville Pediatric Sleep Medicine Center for evaluation of sleep disordered breathing were recruited to the study.

Exclusion criteria included the presence of chronic illnesses, genetic disorders, neuromuscular diseases, craniofacial abnormalities and the presence of acute infection, or treatment with any medications that may potentially alter catecholamine metabolism, such as methylphenidate and other psychostimulants, or corticosteroids.

The study was approved by the institutional human study review committee. Parental consent and child assent for children $\geq$7 years of age, in the presence of a parent, were obtained.

Patients voided before bedtime to ensure that the first morning urine void would reflect the cumulative changes in overnight sympathetic activity.

Night Time Polysomnography (NPSG)

A standard overnight multichannel polysomnographic evaluation was performed at the University of Louisville Pediatric Sleep laboratory. Children were studied for up to 12 hr in a quiet, darkened room with an ambient temperature of 24°C with a parent or guardian present. No drugs were used to induce sleep.

The following parameters were measured: chest and abdominal wall movements assessed by inductance plethysmography, heart rate assessed by electrocardiography, and air flow monitored by sidestream end-tidal capnography, which also provided breath-by-breath assessments of end-tidal carbon dioxide levels (BCI SC-300; Menomonee Falls, WI), nasal pressure, and an oronasal thermistor. Arterial oxygen saturation (SPO_2) was assessed by pulse oximetry (Nellcor N 100; Nellcor Inc., Hayward, CA), with simultaneous recording of the pulse waveform. Bilateral electroculograms, eight channels of the electroencephalogram, chin and anterior tibial electromyograms, and analog output from a body-position sensor (Braebon Medical Corp, Ogdensburg, NY) were also monitored. All measures were digitized with a commercially available polysomnographic system (Stellate, Montreal, Canada). Tracheal sounds were monitored with a microphone sensor (Sleepmate, Midlothian, VA), and a digital, time-synchronized video recording was obtained.

Sleep architecture was assessed by standard techniques.[29] The proportion of time spent in each sleep stage was expressed as percentage of the total sleep time (TST). Awakenings were defined as sustained arousal lasting for $\geq$15 sec. The apnea index was defined as the number of episodes of apnea per hour of TST. Central, obstructive, and mixed apneic events were counted. Obstructive apnea was defined as the absence of airflow with continued chest wall and abdominal movements for the duration of at least two breaths.[30–32] Hypopnea was defined as a decrease in nasal flow of $\geq$50% with a corresponding decrease in SPO_2 of $\geq$4% and/or arousal.[30,32] The obstructive apnea/hypopnea index (AHI) was defined as the number of episodes of obstructive apnea and hypopnea per hour of TST. Children with AHI values of $\geq$1 episode per hour of TST but $<$5 episodes/hr of TST were considered to have mild OSA, whereas children with AHI values of $\geq$5 episodes/hr of TST were considered to have OSA. Control children were defined as children with AHI values of $<$1 episode/hr of TST. For blood draw-based gene expression studies, consecutive children (10 obese and 10 non-obese) fulfilling OSA criteria (i.e., obstructive AHI $\geq$ 5/hrTST) were selected, after which, matching healthy controls were identified.

The mean SPO_2, as measured by pulse oximetry in the presence of a pulse waveform signal void-of-motion artifact, and the SPO_2 nadir were recorded.

Arousals were expressed as the total number of arousals per hour of sleep time (arousal index), and were defined as recommended by the American Sleep Disorders Association Task Force report[33,34] and included

respiratory-related (occurring immediately following an apnea, hypopnea, or snore), technician-induced, and spontaneous arousals.

BMI *z* Score Calculation

Height and weight were recorded for each child. Body mass index *z* score was calculated using an online BMI z score calculator provided by the CDC (http://www.cdc.gov/epiinfo/). Children with BMI z score values greater than 1.67 were considered as obese.[35]

Urine Samples

Subjects urinated prior to the sleep study and a first morning urine sample was collected immediately after completing the overnight polysomnographic evaluation. Children who urinated during the night were excluded from the study. Urine was stored shortly after collection in a −80°C freezer until assay

Urinary epinephrine, norepinephrine and dopamine were measured using a solid phase competitive enzyme linked immunosorbent assay (ELISA) (Tri-Cat EIA, Alpco diagnostics, Salem, NH). The ELISA sensitivity for epinephrine is 0.33 ng/ml; for norepinephrine 1.33 ng/ml and for dopamine 0.83 ng/ml, with cross-reactivity ranges between 14% and 20%. The interassay and intra-assay coefficients of variability were 13.2% and 15% for epinephrine, 8.5% and 16.1% for norepinephrine, and 15.9% and 9.5% for dopamine. All samples were assayed in duplicates and values were retained if they were within 10% of each other.

Urinary creatinine level was measured for each sample. Catecholamine levels were corrected for corresponding urine creatinine concentration.

Blood Samples

Following the sleep study, fasting peripheral blood samples were drawn from a total of 20 children with OSA (10 obese and 10 non-obese) and 20 age, gender, BMI, and ethnicity-matched controls within the first hour after awakening in vacutainer tubes containing EDTA (Becton Dickinson, Franklin Lakes, NJ). Total RNA was extracted from the peripheral leukocytes in the blood samples using PAXgene Mini columns and DNase treated (Qiagen, Valencia, CA), according to the manufacturer protocol. The RNA quality and integrity were determined using the Eukaryote Total RNA Nano 6000 LabChip assay (Agilent Technologies) on the Agilent 2100 Bioanalyzer using Agilent's RNA Integrity Number software.[36] All RNA samples showed $A_{260/280}$ ratios between 1.9 and 2.1. RNA samples were quantified by measuring $A_{260\ nm}$ on a UV/Vis spectrophotometer (ND-1000, NanoDrop Technologies, Wilmington, DE). The yield of RNA from peripheral blood leukocytes varies, but typically, the amount of RNA isolated revolves around 2–3 μg from 2.5-ml whole blood. The RIN is a software tool that scans the peaks of RNA electropherograms for RNA intactness. All the purified samples were stored at −80°C until further analyses.

Real-Time RT-PCR

Quantitative real time RT-PCR analyses were performed for a set of genes involved in catecholamine synthesis or in its biological activity using sets of specific primers and the ABI PRISM 7500 System (Applied Biosystems, Foster City, CA). The genes examined included tyrosine hydroxylase (NM_199292), dopamine β hydroxylase (NM_000787), monoamine oxidases A (NM_000240) and B (NM_000898), monooxygenase, DBH-like 1 (MOXD1), transcript variant 2 (NM_015529), catechol-*O*-methyltransferase (COMT), transcript variant MB-COMT (NM_000754), catechol-*O*-methyltransferase domain containing 1 (COMTD1; NM_144589), adrenergic α1A receptor (NM_033304), α1B receptor (NM_000679), α1D receptor (NM_000678), α2B receptor (NM_000682), and β2 receptor (NM_000024). cDNA synthesis was performed using a High-Capacity cDNA Archive Kit (Applied Biosystems). Housekeeping gene, ribosomal 18S rRNA, was used as a reference gene to normalize the expression ratios for the gene of interest. One microgram of total RNA from OSA and control group samples was used to generate cDNA templates for RT-PCR, and TaqMan® Master Mix Reagent Kit (Applied Biosystems) was used to amplify and quantify each transcript of interest in 25 μl reactions. Triplicate PCR reactions were performed in 96-well plates for each gene in parallel with the 18S rRNA. The steps involved in the reaction program included: the initial step of 2 min at 50°C; denaturation at 95°C for 10 min, followed by 45 thermal cycles of denaturation (15 sec at 95°C) and elongation (1 min at 60°C). The expression values were obtained from the cycle number (C_t value) using the Biosystems analysis software. All the genes of interest and 18S rRNA were performed in triplicates to determine the C_t-diff. These C_t values were averaged andthe difference between the 18S C_t (Avg) and the gene of interest C_t (Avg) was calculated (C_t-diff). The relative expression of the gene of interest was analyzed using the $2^{-\Delta\Delta C_t}$ method.[37]

Data Analysis

Data are presented as mean ± SD unless stated otherwise. Data were assessed for kurtosis and confirmed as being normally distributed. Multiple analyses of variance were applied for analysis of the variables. Pearson correlation tests were performed to assess potential relationships between catecholamine levels and polysomnographic indices. Data were analyzed by using SPSS

14.0 (SPSS, Chicago, IL). A P value of 0.05 was considered to achieve statistical significance.

RESULTS

Overall, 159 consecutive habitually-snoring children (57% male) with a mean age of 6.9 ± 1.2 years (range 4–16 years) were recruited into the study. Of these, 81 children (65% male) had evidence of OSA. OSA and non-OSA subjects were further subdivided into obese and non-obese subgroups according to their individual BMI-z scores.

Except for significant differences in BMI z score and severity of respiratory disturbance, there were no significant differences in age, gender, ethnicity, total sleep time and sleep stages distribution between groups. The characteristics of the subjects included in each of the four subgroups are described in Table 1.

Catecholaminergic Gene Expression in Peripheral Blood Leukocytes

There were no significant differences among any of the catecholamine-related gene expression among non-obese and obese children. However, significant differences emerged when 20 children with OSA were compared to age-, gender-, ethnicity-, and BMI-matched controls (Table 2). Indeed, the following genes exhibited increases in their expression: tyrosine hydroxylase: 7.57 ± 1.78-fold; $P < 0.0001$; dopamine β hydroxylase: 1.88 ± 0.28-fold; $P < 0.01$; monoamine oxidase A: 3.4 ± 0.8; $P < 0.0001$; monooxygenase, DBH-like 1 (MOXD1), transcript variant 2: 19.3 ± 4.9; $P < 0.0001$; adrenergic α1A receptor: 16.8 ± 4.6-fold; $P < 0.00001$; adrenergic α2B receptor: 3.47 ± 4.9; $P < 0.0001$ (Table 2). In contrast, decreases in the expression of catechol-*O*-methyltransferase (COMT) (0.23 ± 0.04; $P < 0.0001$), transcript variant MB-COMT (0.05 ± 0.02; $P < 0.0001$), and β2 adrenergic receptor (0.13 ± 0.03; $P < 0.0001$) emerged (Table 2). No significant changes occurred in catechol-*O*-methyltransferase domain containing 1 (COMTD1) and in adrenergic receptors α1B receptor and α1D (Table 2).

Urinary Catecholamines

Urinary norepinephrine and epinephrine levels in children with OSA were significantly higher when compared to children without OSA (norepinephrine: 40.1 ± 24.7 ng/mg creatinine in OSA vs. 31.6 ± 16.1 ng/mg creatinine in controls; $P < 0.01$; epinephrine: 6.4 ± 10.5 ng/mg creatinine in OSA vs. 4.5 ± 0.5 ng/mg creatinine in controls; $P < 0.01$). Analysis of variance failed to demonstrate any significant effect of obesity on urinary catecholamines levels. However, significant correlation emerged between norepinephrine levels and AHI, SPO_2 nadir, and respiratory arousal index ($r = 0.43$; $P < 0.01$; $r = -0.24$; $P < 0.01$; $r = 0.22$; $P < 0.01$, respectively). In addition, epinephrine levels were positively correlated with AHI ($r = 0.23$; $P < 0.01$), but did not show any significant association with either SPO_2 nadir or arousal index (Fig. 1A–D).

TABLE 1— Demographic and Polysomnographic Characteristics of 159 Children Included in the Study

	Non-obese AHI < 1 (n = 40)	Obese AHI < 1 (n = 38)	Non-obese AHI > 1 (n = 37)	Obese AHI > 1 (n = 44)	*P* value
Age (years)	6.7 ± 0.7	6.9 ± 0.7	6.7 ± 0.8	7.1 ± 2	NS
Gender (% males)	50	54	59	60	NS
% African American	27%	34%	29%	34%	NS
BMI z score	−0.015 ± 1.29	2.35 ± 0.41	0.27 ± 0.87	2.46 ± 0.49	<0.05
TST (min)	475.6 ± 48	471.5 ± 34.5	481.9 ± 40	469.9 ± 56	NS
Sleep efficiency (%)	88.7 ± 8.9	89.9 ± 5.2	91.1 ± 6.3	88.8 ± 9.9	NS
NREM sleep (%TST)					
Stage 1	6.8 ± 4.3	7.3 ± 4.8	7.9 ± 8.4	7.1 ± 4.5	NS
Stage 2	44.8 ± 8.5	45.5 ± 7	44 ± 6.7	42.5 ± 8.4	NS
Stage 3 + 4	27.5 ± 7.8	33.1 ± 34	26.5 ± 7.4	28.4 ± 8.7	NS
REM sleep (%TST)	18.9 ± 5.1	22 ± 16.9	23.9 ± 17.2	17.4 ± 5.8	NS
OAHI (/hrTST)	0.34 ± 0.3	0.4 ± 0.3	6 ± 8.2	10 ± 11.3	<0.05
SPO_2 Nadir (%)	94 ± 2.3	92.1 ± 6.2	90.3 ± 4.1	83.6 ± 9.4	<0.05
TAI (/hrTST)	6.6 ± 2.9	8.3 ± 4.2	11.2 ± 7.4	11.6 ± 6.7	<0.05
$P_{ET}CO_2 > 50$ mmHg (%TST)	0.01 ± 0.03	0	0.01 ± 0.06	3.4 ± 13.5	NS
Norepinephrine (ng/mg)	32.6 ± 18.6	30.5 ± 13.4	36.8 ± 18.5	42.9 ± 28.8	NS
	31.6 ± 16.1		40.1 ± 24.7		<0.05
Epinephrine (ng/mg)	5.4 ± 6	3.7 ± 2.5	6.1 ± 6.7	6.6 ± 13	NS
	4.5 ± 0.5		6.4 ± 10.5		<0.05
Dopamine (ng/mg)	528.9 ± 286.4	508.4 ± 284.9	502.1 ± 257.6	485.4 ± 198	NS
	518.9 ± 284		493 ± 226.1		NS

BMI, body mass index; NS, not significant; TST, total sleep time; NREM, non-rapid eye movement; REM, rapid eye movement; OAHI, obstructive apnea hypopnea index; SPO_2, oxygen saturation measured by pulse oxymetry; TAI, total arousal index; $PETCO_2$, end tidal carbon dioxide tension.

TABLE 2— Catecholaminergic Gene Expression in Peripheral Blood Leukocytes of Children With OSA and Matched Control Children

	OSA/control, Mean ± SE (range)	*P* value
Up-regulated genes		
Tyrosine hydroxylase	7.57 ± 1.78 (4.78–22.8)	<0.0001
Dopamine β hydroxylase	1.88 ± 0.28 (1.33–2.76)	<0.01
Monoamine oxidase A	3.4 ± 0.8 (2.77–9.62)	<0.0001
Monooxygenase, DBH-like 1 (MOXD1), transcript variant 2	19.3 ± 4.9 (15.8–39.6)	<0.0001
Adrenergic α1A receptor	16.8 ± 4.6 (10.8–23.5)	<0.00001
Adrenergic α2B receptor	3.47 ± 4.9 (1.78–14.7)	<0.0001
Down-regulated genes		
Catechol-*O*-methyltransferase (COMT)	0.23 ± 0.04 (0.09–0.45)	<0.0001
Transcript variant MB-COMT	0.05 ± 0.02 (0.01–0.08)	<0.0001
β2 adrenergic receptor	0.13 ± 0.03 (0.07–0.19)	<0.0001
Unchanged expression		
Catechol-*O*-methyltransferase domain containing 1 (COMTD1)	1.03 ± 0.12 (0.89–1.22)	NS
Adrenergic receptor α1B	0.97 ± 0.07 (0.91–1.17)	NS
Adrenergic receptor α1D	0.95 ± 0.02 (0.92–1.02)	NS

NS, not significant.
Gene expression is indicated in fold change compared to controls after correction for corresponding housekeeping gene in quantitative PCR reaction (see Methods and Patients Section for more details).

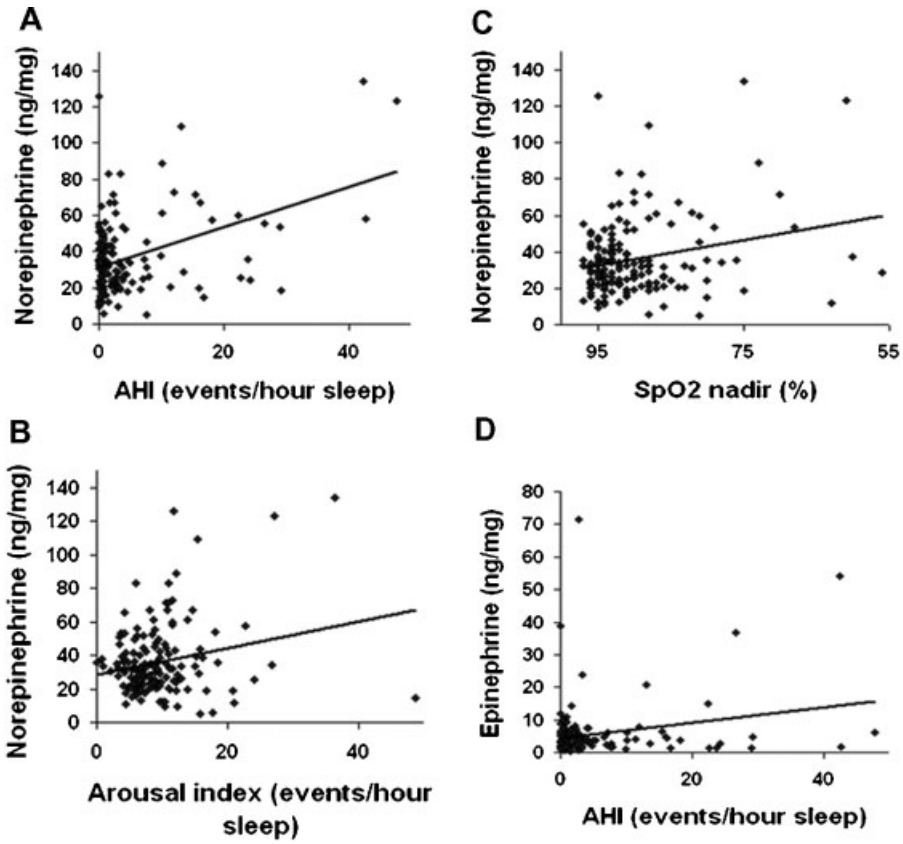

Fig. 1. Scatterplots of norepinephrine urine levels (corrected for creatinine) as a function of AHI (panel A), SPO_2 nadir (panel B), and arousal index (panel C) for the cohort (n = 159), as well as for epinephrine and AHI (panel D). Linear regression lines are shown and were all statistically significant ($P < 0.01$).

DISCUSSION

In this study, we show that urinary epinephrine and more particularly norepinephrine levels are significantly higher in children diagnosed with sleep disordered breathing when compared to habitually snoring children with overall normal polysomnographic evaluations. In addition, norepinephrine levels correlated significantly with nocturnal oxygen saturation nadir and the magnitude of respiratory events induced sleep fragmentation. Furthermore, OSA is associated with substantial alterations in the transcriptome of some of the major genes involved in production and transport of catecholamines, as well as in eliciting changes in the expression of some of the main catecholamine receptors. These findings suggest that OSA constitutes an independent risk factor for induction of increased sympathetic tone,[27] and thus may contribute to the pathogenesis of systemic hypertension in this disease.

It has now become well recognized that OSA is a fairly frequent condition in children, affecting 2–3% of all preschool and school-aged children.[1,3,4,38–44] Moreover, in recent years the association between cardiovascular morbidities and OSA in children has emerged, and includes thus far increased risk for systemic hypertension,[18,19,21,45] changes in cardiac left ventricular geometry and function that exhibit severity-dependent relationships.[25,46,47]

The mechanisms related to the developing alterations in blood pressure and cardiac structure are most probably a combination of several overlapping and parallel pathogenic processes that are activated or exaggerated in the presence of OSA. Such processes may include onset and propagation of endothelial dysfunction and atherogenesis,[48] increased systemic inflammation and oxidative stress and activation of vascular adhesion molecules,[49–53] and perhaps most importantly the sustained activation of increased sympathetic tone as well as enhanced sympathetic reactivity.[26,27]

While mechanistic studies are lacking in children, extensive research has been conducted on the relationships between OSA and cardiovascular morbidities and hypertension in the adult population.[5,6,54,55] The extensive body of literature available clearly supports a predominant effect of OSA, particularly via intermittent hypoxia, in the induction and maintenance of net increases in sympathetic activity both during sleep and wakefulness, the latter correlating with gradual elevations in systemic blood pressure over time.[56–58]

In an effort to obtain easier correlates of sympathetic activation than direct sympathetic nerve recordings, plasma and urine catecholamine levels where examined, and showed treatment-reversible increases in norepinephrine levels in adults with OSA when compared to adults with habitual snoring.[59–61] Furthermore, Kanstrup et al.[62] reported that following exposures to acute hypoxia, transient increments in plasma epinephrine and norepinephrine levels occurred, and that the kinetics of norepinephrine decrements were slower than those of epinephrine. In agreement with the more rapid disappearance of epinephrine, additional studies in adults with OSA could not find increases dopamine and epinephrine in the presence of OSA.[13–16] To the best of our knowledge, no data are currently available regarding catecholamine levels in children with OSA, although a preliminary communication has been recently published by Kaditis et al. in this Journal.[63]

Catecholamines are derived from the amino acid tyrosine. Tyrosine is generated from phenylalanine by the action of phenylalanine hydroxylase, but may be directly ingested from dietary protein as well. Tyrosine is then further metabolized in the adrenal medulla and other components of the sympathetic nervous system through a variety of tightly regulated enzymatic processes to release catecholamines. In the present study, we show that expression of several of the genes underlying both the synthesis and catabolism of catecholamines is altered by the presence of OSA in children, and we would surmise that such changes would predict increased overall bioavailability of catecholamines. In addition, the parallel increased expression of specific catecholaminergic receptors, such as adrenergic α1A and α2B receptors, along with decreases in β2 adrenergic receptor expression would further suggest that specific cardiovascular targets would be effected and likely to promote systemic blood pressure elevations.

Based on such findings, we speculated that urinary catecholamines measured from the first voided urine following an overnight sleep study would likely represent the mean cumulative level of autonomic activity throughout the night, and as such would reflect the potential effects of sleep apnea on global autonomic nervous system activity. However, special attention was also given to the occurrence of obesity as a possible confounding factor influencing sympathetic activity. Indeed, obesity is now recognized as a low grade systemic inflammatory disorder,[64,65] and is associated with an increased risk for hypertension as well as for OSA.[66–68] Furthermore, obesity has been associated with increased urinary and plasma levels of norepinephrine.[69–72] However, in the current study we found no evidence for an increase in urinary catecholamines in obese children without OSA, no differences in gene expression within catecholaminergic pathways among obese and non-obese children, and no significant association between urinary catecholamine levels and the degree of obesity. While the discrepancies between our current findings and those previously reported for obese children are unclear, we should emphasize that earlier studies failed to assess for the presence of sleep apnea in their cohorts. Furthermore, Li

et al.[24] have recently demonstrated elevated diurnal and nocturnal blood pressure among children diagnosed with OSA, independently of obesity, thereby suggesting that the contribution of OSA to catecholaminergic pathways and subsequent induction of blood pressure elevations is probably small. The possibility exists that OSA and obesity in children do not induce a measurable change in blood pressure, by virtue of highly competent compensatory mechanisms, which may require more severe disease or longer duration of disease until decompensation occurs. While we did not conduct concomitant measurements of blood pressure in our cohort we propose that assessment of urinary catecholamine changes in children may provide a sensitive reflection on the activation of upstream mechanisms that may ultimately lead to the systemic hypertension and other adverse cardiovascular outcomes recently identified in children with OSA.[18–21,25,45,48,49]

In summary, we have shown that children with OSA are at risk for having increased morning urinary norepinephrine and to a lesser extent epinephrine levels, which are not influenced by the presence of obesity, and that appear to reflect up-regulation of several genes involved in the production and metabolism of these amines. Urinary catecholamines were also associated with the severity of OSA, such as the apnea–hypopnea index, SPO_2 nadir, and arousal index. Future longitudinal studies may allow for characterization of the relationships between the changes in sympathetic activation in childhood OSA, the effects of treatment on these measures, and ultimately the long-term impact of such alterations on cardiovascular function later in life.

ACKNOWLEDGMENTS

This study was supported by NIH grant HL-65270, The Children's Foundation Endowment for Sleep Research, and by the Commonwealth of Kentucky Challenge for Excellence Trust Fund.

REFERENCES

1. Kaditis AG, Finder J, Alexopoulos EI, Starantzis K, Tanou K, Gampeta S, Agorogiannis E, Christodoulou S, Pantazidou A, Gourgoulianis K, et al. Sleep-disordered breathing in 3,680 Greek children. Pediatr Pulmonol 2004;37:499–509.
2. Blunden S, Lushington K, Lorenzen B, Wong J, Balendran R, Kennedy D. Symptoms of sleep breathing disorders in children are underreported by parents at general practice visits. Sleep Breath 2003;7:167–176.
3. O'Brien LM, Holbrook CR, Mervis CB, et al. Sleep and neurobehavioral characteristics of 5- to 7-year-old children with parentally reported symptoms of attention-deficit/hyperactivity disorder. Pediatrics 2003;111:554–563.
4. Montgomery-Downs HE, O'Brien LM, Holbrook CR, Gozal D. Snoring and sleep-disordered breathing in young children: subjective and objective correlates. Sleep 2004;27:87–94.
5. Nieto FJ, Young TB, Lind BK, et al. Association of sleep-disordered breathing, sleep apnea, and hypertension in a large community-based study. Sleep Heart Health Study. JAMA 2000; 283:1829–1836.
6. Noda A, Okada T, Hayashi H, Yasuma F, Yokota M. 24-hour ambulatory blood pressure variability in obstructive sleep apnea syndrome. Chest 1993;103:1343–1347.
7. Dyken ME, Somers VK, Yamada T, Ren ZY, Zimmerman MB. Investigating the relationship between stroke and obstructive sleep apnea. Stroke 1996;27:401–407.
8. Kraiczi H, Caidahl K, Samuelsson A, Peker Y, Hedner J. Impairment of vascular endothelial function and left ventricular filling: association with the severity of apnea-induced hypoxemia during sleep. Chest 2001;119:1085–1091.
9. Ip MS, Lam KS, Ho C, Tsang KW, Lam W. Serum leptin and vascular risk factors in obstructive sleep apnea. Chest 2000;118: 580–586.
10. Punjabi NM, Shahar E, Redline S, Gottlieb DJ, Givelber R, Resnick HE. Sleep-disordered breathing, glucose intolerance, and insulin resistance: the Sleep Heart Health Study. Am J Epidemiol 2004;160:521–530.
11. Yokoe T, Minoguchi K, Matsuo H, et al. Elevated levels of C-reactive protein and interleukin-6 in patients with obstructive sleep apnea syndrome are decreased by nasal continuous positive airway pressure. Circulation 2003;107:1129–1134.
12. Somers VK, Dyken ME, Clary MP, Abboud FM. Sympathetic neural mechanisms in obstructive sleep apnea. J Clin Invest 1995; 96:1897–1904.
13. Coy TV, Dimsdale JE, Ancoli-Israel S, Clausen J. Sleep apnoea and sympathetic nervous system activity: a review. J Sleep Res 1996;5:42–50.
14. Dimsdale JE, Coy T, Ancoli-Israel S, Mills P, Clausen J, Ziegler MG. Sympathetic nervous system alterations in sleep apnea. The relative importance of respiratory disturbance, hypoxia, and sleep quality. Chest 1997;111:639–642.
15. Elmasry A, Lindberg E, Hedner J, Janson C, Boman G. Obstructive sleep apnoea and urine catecholamines in hypertensive males: a population-based study. Eur Respir J 2002;19:511–517.
16. Marrone O, Riccobono L, Salvaggio A, Mirabella A, Bonanno A, Bonsignore MR. Catecholamines and blood pressure in obstructive sleep apnea syndrome. Chest 1993;103:722–727.
17. Marcus CL, Greene MG, Carroll JL. Blood pressure in children with obstructive sleep apnea. Am J Respir Crit Care Med 1998; 157:1098–1103.
18. Enright PL, Goodwin JL, Sherrill DL, Quan JR, Quan SF. Blood pressure elevation associated with sleep-related breathing disorder in a community sample of white and Hispanic children: the Tucson Children's Assessment of Sleep Apnea study 19. Arch Pediatr Adolesc Med 2003;157:901–904.
19. Kohyama J, Ohinata JS, Hasegawa T. Blood pressure in sleep disordered breathing. Arch Dis Child 2003;88:139–142.
20. Kwok KL, Ng DK, Cheung YF. BP and arterial distensibility in children with primary snoring. Chest 2003;123:1561–1566.
21. Amin RS, Carroll JL, Jeffries JL, et al. Twenty-four-hour ambulatory blood pressure in children with sleep-disordered breathing. Am J Respir Crit Care Med 2004;169:950–956.
22. Zintzaras E, Kaditis AG. Sleep-disordered breathing and blood pressure in children: a meta-analysis. Arch Pediatr Adolesc Med 2007;161:172–178.
23. Amin R, Somers VK, McConnell K, et al. Activity-adjusted 24-hour ambulatory blood pressure and cardiac remodeling in children with sleep disordered breathing. Hypertension 2008;51: 84–91.
24. Li AM, Au CT, Sung RY, et al. Ambulatory blood pressure in children with obstructive sleep apnoea—A community based study. Thorax 2008;63:803–809.

25. Aljadeff G, Gozal D, Schechtman VL, Burrell B, Harper RM, Ward SL. Heart rate variability in children with obstructive sleep apnea. Sleep 1997;20:151–157.
26. Baharav A, Kotagal S, Rubin BK, Pratt J, Akselrod S. Autonomic cardiovascular control in children with obstructive sleep apnea. Clin Auton Res 1999;9:345–351.
27. O'Brien LM, Gozal D. Autonomic dysfunction in children with sleep-disordered breathing. Sleep 2005;28:747–752.
28. Capdevila OS, Kheirandish-Gozal L, Dayyat E, Gozal D. Pediatric obstructive sleep apnea: complications, management, and long-term outcomes. Proc Am Thorac Soc 2008;5:274–282.
29. Rechtschaffen A KA. A manual of standardized terminology, techniques and scoring systems for sleep stages of human subjects. Washington, DC: National Institutes of Health; 1968. National Institutes of Health Publication 204. Ref Type: Generic.
30. Standards and indications for cardiopulmonary sleep studies in children. American Thoracic Society. Am J Respir Crit Care Med 1996;153:866–878.
31. Marcus CL, Omlin KJ, Basinki DJ, et al. Normal polysomnographic values for children and adolescents. Am Rev Respir Dis 1992;146:1235–1239.
32. Montgomery-Downs HE, O'Brien LM, Gulliver TE, Gozal D. Polysomnographic characteristics in normal preschool and early school-aged children. Pediatrics 2006;117:741–753.
33. EEG arousals: scoring rules and examples: a preliminary report from the Sleep Disorders Atlas Task Force of the American Sleep Disorders Association. Sleep 1992;15:173–184.
34. Mograss MA, Ducharme FM, Brouillette RT. Movement/arousals. Description, classification, and relationship to sleep apnea in children. Am J Respir Crit Care Med 1994;150:1690–1696.
35. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, et al. CDC growth charts: United States. Adv Data 2000;8:1–27.
36. Khan-Farooqi HR, Prins RM, Liau LM. Tumor immunology, immunomics and targeted immunotherapy for central nervous system malignancies. Neurol Res 2005;27:692–702.
37. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001;25:402–408.
38. Corbo GM, Forastiere F, Agabiti N, et al. Snoring in 9- to 15-year-old children: risk factors and clinical relevance. Pediatrics 2001;108:1149–1154.
39. Ersu R, Arman AR, Save D, et al. Prevalence of snoring and symptoms of sleep-disordered breathing in primary school children in Istanbul. Chest 2004;126:19–24.
40. Ferreira AM, Clemente V, Gozal D, et al. Snoring in Portuguese primary school children. Pediatrics 2000;106:E64.
41. Montgomery-Downs HE, Gozal D. Sleep habits and risk factors for sleep-disordered breathing in infants and young toddlers in Louisville, Kentucky. Sleep Med 2006;7:211–219.
42. Rosen CL, Larkin EK, Kirchner HL, et al. Prevalence and risk factors for sleep-disordered breathing in 8- to 11-year-old children: association with race and prematurity. J Pediatr 2003;142:383–389.
43. Tang JP, Rosen CL, Larkin EK, et al. Identification of sleep-disordered breathing in children: variation with event definition. Sleep 2002;25:72–79.
44. Urschitz MS, Guenther A, Eitner S, et al. Risk factors and natural history of habitual snoring. Chest 2004;126:790–800.
45. Marcus CL, Greene MG, Carroll JL. Blood pressure in children with obstructive sleep apnea. Am J Respir Crit Care Med 1998;157:1098–1103.
46. Amin RS, Kimball TR, Bean JA, et al. Left ventricular hypertrophy and abnormal ventricular geometry in children and adolescents with obstructive sleep apnea. Am J Respir Crit Care Med 2002;165:1395–1399.
47. Amin RS, Kimball TR, Kalra M, et al. Left ventricular function in children with sleep-disordered breathing. Am J Cardiol 2005;95:801–804.
48. Gozal D, Kheirandish-Gozal L, Serpero LD, Sans CO, Dayyat E. Obstructive sleep apnea and endothelial function in school-aged nonobese children: effect of adenotonsillectomy. Circulation 2007;116:2307–2314.
49. O'Brien LM, Serpero LD, Tauman R, Gozal D. Plasma adhesion molecules in children with sleep-disordered breathing. Chest 2006;129:947–953.
50. Kheirandish-Gozal L, Capdevila OS, Tauman R, Gozal D. Plasma C-reactive protein in nonobese children with obstructive sleep apnea before and after adenotonsillectomy. J Clin Sleep Med 2006;2:301–304.
51. Tauman R, Ivanenko A, O'Brien LM, Gozal D. Plasma C-reactive protein levels among children with sleep-disordered breathing. Pediatrics 2004;113:e564–e569.
52. Tauman R, O'Brien LM, Gozal D. Hypoxemia and obesity modulate plasma C-reactive protein and interleukin-6 levels in sleep-disordered breathing. Sleep Breath 2007;11:77–84.
53. Verhulst SL, Van HK, Schrauwen N, et al. Sleep-disordered breathing and uric acid in overweight and obese children and adolescents. Chest 2007;132:76–80.
54. Lavie P, Herer P, Hoffstein V. Obstructive sleep apnoea syndrome as a risk factor for hypertension: population study. BMJ 2000;320:479–482.
55. Peppard PE, Young T, Palta M, Skatrud J. Prospective study of the association between sleep-disordered breathing and hypertension. N Engl J Med 2000;342:1378–1384.
56. Ringler J, Basner RC, Shannon R, et al. Hypoxemia alone does not explain blood pressure elevations after obstructive apneas. J Appl Physiol 1990;69:2143–2148.
57. Smith ML, Niedermaier ON, Hardy SM, Decker MJ, Strohl KP. Role of hypoxemia in sleep apnea-induced sympathoexcitation. J Auton Nerv Syst 1996;56:184–190.
58. Prabhakar NR, Fields RD, Baker T, Fletcher EC. Intermittent hypoxia: cell to system. Am J Physiol Lung Cell Mol Physiol 2001;281:L524–L528.
59. Hoy LJ, Emery M, Wedzicha JA, et al. Obstructive sleep apnea presenting as pseudopheochromocytoma: a case report. J Clin Endocrinol Metab 2004;89:2033–2038.
60. Nelesen RA, Yu H, Ziegler MG, Mills PJ, Clausen JL, Dimsdale JE. Continuous positive airway pressure normalizes cardiac autonomic and hemodynamic responses to a laboratory stressor in apneic patients. Chest 2001;119:1092–1101.
61. Roche F, Court-Fortune Pichot V, et al. Reduced cardiac sympathetic autonomic tone after long-term nasal continuous positive airway pressure in obstructive sleep apnoea syndrome. Clin Physiol 1999;19:127–134.
62. Kanstrup IL, Poulsen TD, Hansen JM, et al. Blood pressure and plasma catecholamines in acute and prolonged hypoxia: effects of local hypothermia. J Appl Physiol 1999;87:2053–2058.
63. Kaditis AG, Alexopoulos EI, Damani E, Hatzi F, Chaidas K, Kostopoulou T, Tzigeroglou A, Gourgoulianis K. Urine levels of catecholamines in Greek children with obstructive sleep-disordered breathing. Pediatr Pulmonol 2009;44:38–45.
64. Ford ES, Galuska DA, Gillespie C, Will JC, Giles WH, Dietz WH. C-reactive protein and body mass index in children: findings from the Third National Health and Nutrition Examination Survey, 1988–1994. J Pediatr 2001;138:486–492.
65. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Low-grade systemic inflammation in overweight children. Pediatrics 2001;107:E13.

66. Gozal D, Wang M, Pope DW, Jr. Objective sleepiness measures in pediatric obstructive sleep apnea. Pediatrics 2001;108:693–697.
67. Kheirandish-Gozal L, Sans CO, Kheirandish E, Gozal D. Elevated serum aminotransferase levels in children at risk for obstructive sleep apnea. Chest 2008;133:92–99.
68. Tauman R, Gozal D. Obesity and obstructive sleep apnea in children. Paediatr Respir Rev 2006;7:247–259.
69. Corry DB, Tuck ML. Obesity, hypertension, and sympathetic nervous system activity. Curr Hypertens Rep 1999;1:119–126.
70. Esler M, Rumantir M, Wiesner G, Kaye D, Hastings J, Lambert G. Sympathetic nervous system and insulin resistance: from obesity to diabetes. Am J Hypertens 2001: 14:304S–309S.
71. Sowers JR, Whitfield LA, Catania RA, et al. Role of the sympathetic nervous system in blood pressure maintenance in obesity. J Clin Endocrinol Metab 1982;54:1181–1186.
72. Tuck ML, Sowers JR, Dornfeld L, Whitfield L, Maxwell M. Reductions in plasma catecholamines and blood pressure during weight loss in obese subjects. Acta Endocrinol (Copenh) 1983; 102:252–257.