Metabolic Alterations and Systemic Inflammation in Obstructive Sleep Apnea among Nonobese and Obese Prepubertal Children

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***Rationale*: Obstructive sleep apnea (OSA) has been associated with a higher prevalence and severity of the metabolic syndrome in adult patients, even after controlling for obesity. In contrast, OSA in prepubertal children does not appear to correlate with the magnitude of such metabolic derangements.**

***Objectives*: To further establish the potential mechanistic role of OSA in metabolic regulation in prepubertal children.**

***Methods*: Fasting glucose, insulin, C-reactive protein, apolipoprotein B, and serum lipid concentrations were determined during the initial polysomnographic diagnosis of OSA and 6–12 months after adenotonsillectomy in both obese and nonobese children.**

***Measurements and Main Results*: Sixty-two children with OSA (37 obese and 25 nonobese), age 7.40 ± 2.6 years (mean ± SD) completed the study. After adenotonsillectomy, significant improvements in apnea–hypopnea index and sleep fragmentation occurred, particularly among nonobese children. In nonobese children, adenotonsillectomy was associated with mild increases in body mass index *z* scores, no changes in either fasting glucose or insulin, significant increases in high-density lipoprotein and reciprocal decreases in low-density lipoprotein, and reductions in plasma C-reactive protein and apolipoprotein B levels. In obese children, adenotonsillectomy did not result in body mass index or glucose changes, but was associated with marked improvements in all other measures.**

***Conclusions*: OSA does not appear to induce insulin resistance in nonobese pediatric patients but seems to play a significant role in obese patients. The significant improvements in lipid profiles, C-reactive protein, and apolipoprotein B after adenotonsillectomy in the two groups suggest a pathogenic role for OSA in lipid homeostasis and systemic inflammation independent of the degree of adiposity.**

Keywords: obstructive sleep apnea; inflammation; obesity; serum lipids; diabetes

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Whether pediatric obstructive sleep apnea (OSA) is associated with metabolic dysfunction independently from obesity remains unclear.

What This Study Adds to the Field

OSA does not appear to induce insulin resistance in nonobese pediatric patients but seems to play a significant role in obese patients. The significant improvements in lipid profiles, C-reactive protein, and apolipoprotein B after adenotonsillectomy in the two groups suggest a pathogenic role for OSA in lipid homeostasis and systemic inflammation independent of the degree of adiposity.

Obstructive sleep apnea syndrome (OSA) is now recognized as a frequent medical condition in children, with an estimated prevalence of 2–3% (1–7). Although OSA in adults has been associated with increased risk for cardiovascular morbidities, it is only more recently that nocturnal elevation of systemic blood pressure and sustained diurnal hypertension (8–10) and severity-dependent changes in left ventricular geometry and function (11), as well as abnormal endothelial function (12), have been recognized in children with OSA. In addition, sustained sympathetic activation (13, 14) and systemic inflammation and platelet–leukocyte–endothelial interactions leading to initiation and propagation of atherogenesis-related processes have all been identified in children with OSA (15–17).

The incidence of childhood obesity has been increasing steadily in Western countries, with prevalence rates ranging from 7% up to 22% in various countries (18). Such elevated figures are predicted to impose major health-related adverse outcomes, particularly on the development of insulin resistance, type 2 diabetes, and cardiovascular morbidity (19, 20). "Metabolic syndrome" is a known risk factor for cardiovascular disease in adults and refers to the clustering of insulin resistance, dyslipidemia, hypertension, and obesity. Elevation of fasting insulin levels and increased body mass index (BMI) during childhood are the strongest predictors of metabolic syndrome in adulthood (21), possibly through the combination of altered insulin signaling and adrenocortical function, induction of inflammation and endothelial and vascular dysfunction, abnormal cardiac autonomic regulation, and aberrant hormonal output (22, 23). Moreover, insulin resistance in childhood is associated with increased risk for later cardiovascular morbidity and mortality (24, 25). Taken together, these data support the hypothesis that the metabolic disturbances, which are known to be associated with increased risk for cardiovascular disease, start developing in early childhood. Thus, early identification of insulin resistance and obesity may provide an opportunity for early intervention so as to minimize the risk of adult cardiovascular disease. Studies that assessed the contribution of OSA to metabolic disturbances in a large cohort of snoring prepubertal children suggested that insulin resistance and dyslipidemia seem to be determined primarily by the degree of body adiposity rather than by the severity of sleep-disordered breathing (26–28). This is in contrast with adolescent children or with obese children, in whom OSA appears to magnify the underlying contributions of obesity to metabolic derangements (29–32).

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To better understand the contribution of OSA to metabolic regulation in prepubertal children, a prospective study of obese (OB) and nonobese (NOB) children with OSA was conducted and the levels of glucose (Glu), insulin (Ins), C-reactive protein (CRP), apolipoprotein B (ApoB), and serum lipid concentrations were determined before and after surgical tonsillectomy and adenoidectomy (T&A).

METHODS

Consecutive prepubertal children who were evaluated from June 2006 until September 2006 at the Kosair Children's Hospital Sleep Medicine Center (Louisville, KY) for habitual snoring, and were polysomnographically diagnosed with moderate to severe OSA (*see below* for criteria), were invited to participate in the study, at which time blood was drawn after an overnight fast. The study was approved by the University of Louisville (Louisville, KY) Human Research Committee. Parental informed consent and child assent, in the presence of a parent, were obtained. Children were excluded when they had any chronic medical condition, were receiving medications that are known to affect glucose homeostasis or serum lipids, had any psychiatric diagnoses, or had any genetic or craniofacial syndromes. Children who underwent adenotonsillectomy (T&A) for OSA were also invited to return within 6–12 months for a second overnight polysomnographic assessment and a blood draw the next morning. The last subject enrolled in the study completed his participation in August 2007.

Body Mass Index

Height and weight of each child were determined by standard techniques. BMI was then calculated (body mass/height^2) and was expressed as BMI *z* score, using an online BMI z score calculator (Epi Info, a computer software package developed by the Centers for Disease Control and Prevention; *see* http://www.cdc.gov/epiinfo/). Children with BMI *z* score values exceeding 1.20 were classified as fulfilling the criteria for overweight/obesity (33).

Overnight Polysomnography

A standard overnight multichannel polysomnographic evaluation was performed in the sleep laboratory as previously described (34). No drugs were used to induce sleep. The following parameters were measured: chest and abdominal wall movement by inductance plethysmography, heart rate by electrocardiogram, and air flow triply monitored with a nasal pressure cannula, a thermistor, and a sidestream end-tidal capnograph that also provided breath-by-breath assessment of end-tidal carbon dioxide levels (BCI SC-300; Menomonee Falls, WI). Arterial oxygen saturation measured by pulse oximetry (Sp_{O_2}) was assessed (Nellcor N 100; Nellcor Inc, Hayward, CA), with simultaneous recording of the pulse waveform, and was recorded with a 3-second averaging routine. The bilateral electrooculogram, eight channels of 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 system (Rembrandt; MedCare Diagnostics, Amsterdam, The Netherlands). Tracheal sound was monitored with a microphone sensor (Sleepmate, Midlothian, VA), and a digital time-synchronized video recording was performed. Sleep architecture was assessed by standard techniques, as previously reported (34); briefly, obstructive apnea was defined as the absence of airflow with continued chest wall and abdominal movement for duration of at least two breaths. Hypopneas were defined as a decrease in nasal flow of greater than 50% with a corresponding decrease in Sp_{O_2} of at least 4% and/or terminated by a 3-second electroencephalogram arousal. The obstructive apnea–hypopnea index (OAHI) was defined as the number of apneas and hypopneas per hour of total sleep time (TST). Children with an OAHI less than 2 per hour of TST were considered to have normal respiratory patterns during sleep, whereas children with an AHI of at least 2 per hour of TST were considered to have OSA. The mean oxygen saturation, as measured by pulse oximetry (Sp_{O_2}) in the presence of a pulse waveform signal void of motion artifact, and the Sp_{O_2} nadir were recorded. Because criteria for arousals have not yet been developed for children, arousals were defined as recommended by the American Sleep Disorders Association Task Force report, using the 3-second rule and/or the presence of movement arousal (35, 36).

Blood Assays

For every child, a complete blood count, and fasting serum levels of glucose, insulin, lipid profile, CRP, and ApoB were obtained after blood collection in the morning after the initial diagnostic sleep study, and in the morning after the follow-up sleep study performed within 6–12 months after surgical treatment of OSA by T&A.

Serum insulin level was measured with a commercially available radioimmunoassay kit (Coat-A-Count Insulin; Diagnostic Products, Inc., Los Angeles, CA). This method has a detection level of 1.2 μIU/ml and exhibits linear behavior up to 350 μIU/ml, with intraassay and interassay coefficients of variability of 3.1 and 4.9%, respectively. The plasma glucose level was measured with a commercial kit based on the hexokinase–glucose-6-phosphate dehydrogenase method (Flex reagent cartridges; Dade Behring, Newark, DE). Insulin resistance was assessed on the basis of the fasting insulin/fasting glucose ratio (Ins/Glu ratio).

Serum lipids including total cholesterol, high-density lipoprotein (HDL) cholesterol, calculated low-density lipoprotein cholesterol, and triglycerides (TGs), were assessed with Flex reagent cartridges (Dade Behring).

Serum high-sensitivity CRP concentrations were measured within 2 to 3 hours of collection, using the Flex reagent cartridge (Dade Behring), which is based on a particle-enhanced turbidimetric immunoassay technique. This method has a detection level of 0.05 μg/ml and exhibits linear behavior up to 255 μg/ml, with intraassay and interassay coefficients of variability of 9 and 18%, respectively.

Apolipoprotein B serum levels were measured by immunoturbidimetry (Roche Diagnostics, Mannheim, Germany). Samples were always assayed in duplicate, and the mean values were retained if they were within 10% of each other. The assay demonstrated a linearity range of 2.11–264 mg/dl with intra- and interindividual coefficients of variation of 2.8 and 8.6%, respectively.

Data Analysis

Data are presented as means ± SE unless otherwise indicated. Comparisons of demographics according to group assignment were made with independent *t* tests or analysis of variance followed by post hoc comparisons, with *P* values adjusted for unequal variances when appropriate (Levene's test for equality of variances), or χ^2 analyses with the Fisher exact test (dichotomous outcomes). Correlations between changes in polysomnographic and metabolic variables were determined by linear regression, followed by calculation of Pearson correlation coefficients. Pre- and posttreatment variables were compared with paired *t* tests within OB and NOB groups, and by two-way analyses of variance for repeated measures followed by post hoc tests for comparisons between OB and NOB, with adjustments being made for the severity of OSA across groups by controlling for OAHI. All *P* values reported are after post hoc adjustments and are two tailed with statistical significance set at less than 0.05.

RESULTS

A total of 81 children of 97 potential candidates initially agreed to participate in the study, and of these, 62 subjects completed both phases of the protocol (25 NOB and 37 OB). Their demographic and polysomnographic characteristics before and after T&A are shown in Table 1 for the two groups. There were no significant differences in either demographic characteristics or polysomnographic findings between the children completing all phases of the protocol and those who did not. The major reason for noncompletion was lack of willingness to repeat the sleep study after T&A.

Outcomes of Tonsillectomy and Adenoidectomy

OB subjects were slightly older than NOB subjects ($P < 0.04$), and had no changes in their BMI after T&A compared with significant increases in BMI *z* scores in the NOB group after

TABLE 1. DEMOGRAPHIC AND POLYSOMNOGRAPHIC CHARACTERISTICS OF OBESE AND NONOBESE CHILDREN WITH OBSTRUCTIVE SLEEP APNEA BEFORE AND 6–12 MONTHS AFTER UNDERGOING TONSILLECTOMY AND ADENOIDECTOMY

	NOB ($n = 25$)			OB ($n = 37$)			*P* Value for
	Pre	Post	P_1 Value	Pre	Post	P_2 Value	NOB vs. OB*
Age, yr (range)	6.6 ± 0.5 (3–11)	7.3 ± 0.5 (3–12)	—	7.9 ± 0.4 (3–12)	8.7 ± 0.4 (3–12)	—	<0.04
Sex, F/M	10:15	—	—	14:18	—	—	NS
Ethnicity, % AA	32.0			48.6			NS
BMI, *z* score	−0.02 ± 0.20	0.47 ± 0.21	<0.04	2.47 ± 0.08	2.46 ± 0.08		<0.01
History of asthma, n	3			5			NS
Total sleep time, min	473 ± 8	469 ± 9	NS	448 ± 10	425 ± 12	NS	NS
Sleep efficiency, %	90.4 ± 1.2	91.7 ± 1.5	NS	87.3 ± 1.5	86.3 ± 2.5	NS	NS
Latency to sleep onset, min	16.9 ± 2.8	22.1 ± 3.2	0.075	11.9 ± 2.0	18.4 ± 2.2	<0.01	<0.01
NREM sleep, % TST							
Stage 1	8.9 ± 2.5	5.8 ± 1.0	0.068	10.2 ± 1.3	7.6 ± 1.7	0.076	NS
Stage 2	56.1 ± 4.9	43.9 ± 2.1	<0.001	50.3 ± 2.8	40.2 ± 3.8	<0.001	NS
Stage 3	9.4 ± 1.4	7.4 ± 1.2	<0.05	8.6 ± 1.2	12.5 ± 1.3	<0.04	<0.01
Stage 4	19.5 ± 1.4	23.3 ± 1.5	<0.05	16.1 ± 1.7	20.4 ± 2.1	<0.01	NS
Latency to REM sleep, min	118.2 ± 12.9	149.2 ± 15.1	<0.01	123.9 ± 16.9	177.2 ± 17.5	< 0.01	NS
REM, % TST	14.9 ± 1.2	20.3 ± 2.1	<0.001	14.6 ± 1.7	19.2 ± 2.1	<0.001	NS
OAHI, per h TST (range)	12.9 ± 1.3 (3.8–28.9)	1.9 ± 0.2 (0.2–4.3)	<0.0001	19.2 ± 2.9 (5.6–78.1)	5.5 ± 0.7 (0.3–17.1)	<0.0001	<0.01
Mean Sp_{O_2}, %	94.5 ± 0.5	95.1 ± 0.4	NS	92.4 ± 0.7	93.8 ± 0.3	NS	NS
Nadir Sp_{O_2}, %	82.5 ± 1.3	90.1 ± 0.6	<0.0001	72.6 ± 3.1	85.3 ± 1.1	<0.0001	<0.01
TAI, per h TST	14.9 ± 3.0	9.1 ± 1.2	<0.01	14.0 ± 1.5	8.3 ± 0.7	<0.001	NS
RAI, per h TST	6.2 ± 0.8	2.9 ± 0.6	<0.01	6.1 ± 1.0	2.8 ± 0.6	<0.001	NS
Peak $P_{ET_{CO_2}}$, mm Hg	56.9 ± 1.3	50.8 ± 1.4	<0.001	57.8 ± 1.5	53.6 ± 1.1	< 0.001	NS

Definition of abbreviations: AA = African American; BMI = body mass index; F = female; M = male; NOB = nonobese; NREM = non–rapid eye movement; NS = not significant; OAHI = obstructive apnea–hypopnea index; OB = obese; P_1 and P_2 = two-tailed paired *t* test comparisons within NOB and OB groups, respectively; $P_{ET_{CO_2}}$ = end-tidal carbon dioxide tension using capnography; RAI = respiratory arousal index; TAI = total arousal index; TST = total sleep time.

* Comparisons between OB and NOB were assessed by two-way analysis of variance for repeated measures followed by Tukey post hoc procedures.

surgery ($P < 0.01$). The interval periods between the initial sleep study and the follow-up sleep study was 8.4 ± 1.8 months in the NOB group and 7.9 ± 1.7 months in the OB group (*P* value, not significant). T&A led to significant and overall similar improvements in sleep latency and increased latency to REM sleep onset, as well as increases in the percentage of time spent in both slow wave sleep and REM sleep in both OB and NOB groups. However, T&A was more likely to normalize OAHI (i.e., OAHI < 2/h TST) or to reduce OAHI to the mild severity status (i.e., <5/h TST) among the NOB children compared with the OB children. Indeed, 15 of 25 NOB children had an OAHI less than 1.0 (60%) compared with 9 of the 37 OB children (odds ratio, 4.67; 95% confidence interval, 1.37–16.4; *P* value after Mantel-Haenszel correction, $P < 0.01$). Similarly, whereas all NOB children had a post-T&A OAHI less than 5/hour TST, 15 of the 37 OB children had an OAHI greater than 5/hour TST ($P < 0.0003$). Nadir Sp_{O_2}, total and respiratory arousal indexes, and peak end-tidal CO_2 levels were markedly improved after T&A in both groups, except for Sp_{O_2}, which was less improved after T&A in the OB group compared with the NOB group ($P < 0.01$). Of note, there were no significant differences between OB and NOB groups regarding the time elapsed between the two sleep studies or between T&A and the follow-up overnight polysomnogram.

In NOB children, T&A was associated with no changes in either Ins, Glu, or Ins/Glu (*P* value not significant; Table 2). In contrast, significant improvements emerged in both Ins and Ins/Glu in OB children in the absence of parallel changes in BMI after their surgery, even when controlling for OAHI, as the indicator of the severity of OSA (Table 2; $P < 0.001$ vs. NOB). Total cholesterol levels remained unaltered in NOB children and in OB children, a mild, albeit significant improvement was observed after surgery (Table 2). Notably, low-density lipoprotein (LDL), HDL, and LDL/HDL were markedly improved after T&A in both NOB and OB patients, and this effect was significantly more pronounced in the NOB group. TGs were improved only in the OB group. ApoB serum levels were remarkably reduced after T&A in both groups, and the effect was slightly greater in the NOB children (Table 2). Similarly, CRP levels, which were higher in OB children before T&A ($P < 0.01$), decreased along with the T&A-induced improvements in OSA, and these reductions in CRP were more prominent in the NOB children (Table 2). There were no discernible differences in any of the hematologic parameters except for a reduction of platelet counts in NOB children after T&A (Table 2).

Correlational Analyses between Sleep and Serum Metabolic Measurements

Table 3 illustrates some of the pertinent associations between changes in respiratory disturbances and sleep fragmentation in relation to the corresponding changes in glycemic and lipid findings. In general, sleep fragmentation was associated primarily with altered insulin sensitivity as evidenced by Ins/Glu, whereas OAHI and nadir Sp_{O_2} displayed stronger associations with lipid disturbances, even after adjusting for age and BMI *z* score (Table 3).

Subanalysis Based on Outcomes of Tonsillectomy and Adenoidectomy

To examine whether resolution of OSA was associated with improved metabolic and inflammatory outcomes, the OB and NOB cohorts were subdivided into those who demonstrated an OAHI less than 2/hour TST after T&A (i.e., resolved OSA) and into those with residual OSA (i.e., OAHI ⩾ 2/h TST). Among NOB subjects, those with residual OSA after T&A had more severe disease at diagnosis ($P < 0.01$; Table 4), but the overall degree of improvement in OAHI was similar after surgery. Normalization of polysomnographic abnormalities with T&A was associated with significant reductions of LDL and HDL cholesterol as well as ApoB and CRP, whereas the occurrence of residual OSA was accompanied by parallel residual abnormalities in the serum levels of these measures (Table 4). Similar findings emerged for HDL, LDL, ApoB, and CRP among OB children, in whom, as mentioned above, a disproportionate number of patients failed to normalize breathing patterns during

TABLE 2. METABOLIC, INFLAMMATORY, AND HEMATOLOGIC CHANGES IN OBESE AND NONOBESE CHILDREN WITH OBSTRUCTIVE SLEEP APNEA BEFORE AND 6–12 MONTHS AFTER UNDERGOING TONSILLECTOMY AND ADENOIDECTOMY

	NOB ($n = 25$)			OB ($n = 37$)			*P* Value for NOB vs. OB*
	Pre	Post	P_1 Value	Pre	Post	P_2 Value	
Hgb, g/dl	13.2 ± 0.2	13.2 ± 0.2	NS	13.6 ± 0.2	13.5 ± 0.2	NS	NS
WBC, × 1,000/μl	6.9 ± 0.4	6.8 ± 0.5	NS	6.8 ± 0.4	7.3 ± 0.4	NS	NS
Neutrophils, %	45.1 ± 3.8	41.6 ± 3.9	NS	45.8 ± 1.5	47.2 ± 2.0	NS	NS
Lymphocytes, %	41.1 ± 3.8	44.4 ± 3.7	NS	39.2 ± 1.6	37.5 ± 1,9	NS	NS
Monocytes, %	8.5 ± 0.6	9.0 ± 0.5	NS	10.2 ± 0.7	9.4 ± 0.6	NS	NS
Eosinophils, %	3.2 ± 0.5	3.4 ± 0.5	NS	3.4 ± 0.6	4.6 ± 0.7	NS	NS
Basophils, %	1.2 ± 0.1	1.4 ± 0.2	NS	1.5 ± 0.1	1.4 ± 0.1	NS	NS
Platelets, × 1,000/μl	351 ± 20	304 ± 14	<0.01	318 ± 14	313 ± 11	NS	<0.001
Glu, mg/dl	83.9 ± 1.9	86.4 ± 1.7	NS	89.8 ± 1.1	89.9 ± 1.3	NS	NS
Ins, μIU/ml	8.8 ± 2.1	8.7 ± 1.4	NS	26.2 ± 1.9	20.4 ± 1.1	<0.001	<0.001
Ins/Glu	0.10 ± 0.02	0.10 ± 0.02	NS	0.29 ± 0.03	0.21 ± 0.03	<0.001	<0.0004
TG, mg/dl	76.6 ± 7.4	76.2 ± 7.1	NS	104.4 ± 8.0	89.7 ± 8.1	<0.01	<0.002
Total cholesterol, mg/dl	157.8 ± 5.2	154.4 ± 6.1	NS	171.3 ± 6.3	164.3 ± 7.1	<0.01	<0.02
LDL, mg/dl	92.0 ± 5.1	66.0 ± 2.3	<0.0001	117.6 ± 4.9	91.3 ± 4.3	<0.01	<0.004
HDL, mg/dl	44.6 ± 2.8	64.2 ± 3.6	<0.0001	37.8 ± 1.3	51.7 ± 2.7	<0.01	<0.004
LDL/HDL	2.25 ± 0.2	1.13 ± 0.1	<0.0001	3.26 ± 0.3	2.10 ± 0.3	<0.0001	<0.004
ApoB, mg/dl	102.2 ± 5.4	56.3 ± 2.9	<0.00001	96.1 ± 3.1	62.5 ± 3.4	<0.001	<0.02
CRP, μg/ml	4.0 ± 0.9	1.1 ± 0.2	<0.0001	6.1 ± 1.0	2.4 ± 0.6	<0.001	<0.02

Definition of abbreviations: ApoB = apolipoprotein B; CRP = C-reactive protein; Glu = glucose; HDL = high-density lipoprotein; Hgb = hemoglobin; Ins = insulin; LDL = low-density lipoprotein; NOB = nonobese; OAHI = obstructive apnea–hypopnea index; OB = obese; TG = triglycerides; WBC = white blood cell count.

* Comparisons between OB and NOB were assessed by two-way analysis of variance for repeated measures followed by Tukey post hoc procedures, and were further adjusted for pre–tonsillectomy and adenoidectomy (surgical tonsillectomy and adenoidectomy) obstructive apnea–hypopnea index.

sleep after T&A (Table 5). However, OB children also showed differences in insulin sensitivity as a function of whether OSA was resolved or not, with most improvements in glycemic control being achieved when respiratory abnormalities were abrogated by T&A (Table 5).

DISCUSSION

In the present study, we show that OSA exerts significant effects on lipid homeostasis, and systemic inflammation, and that in the presence of underlying obesity the disease also affects glycemic regulation through incremental changes in insulin sensitivity that are independent of the adiposity index. These findings support the concept that the gas exchange abnormalities and sleep disturbance that characterize OSA will adversely affect serum lipid concentrations in a proatherogenic fashion, and promote inflammatory responses as evidenced by the reversibly increased CRP concentrations. Furthermore, although the effect of OSA on insulin sensitivity is undetectable in NOB children, in the presence of obesity there appears to be an interaction between increased adiposity and OSA to promote and amplify the insulin resistance associated with obesity in the absence of OSA.

Before we discuss the potential implications of our findings, several methodologic issues deserve comment. First, although 32 potential subjects of the 97 children either chose not to participate or failed to return for a post-T&A assessment, analysis of their demographic and polysomnographic characteristics did not reveal any specific differences between the nonparticipants and the 65 children who comprise this report. Of note, the representation of African Americans among the two subgroups in the study was higher than the known ethnic distribution in the city of Louisville, but is compatible with the higher prevalence of OSA among this ethnic group (1, 37). We used both fasting insulin levels and Ins/Glu ratios to examine the magnitude of insulin sensitivity. These measures have been successfully and reliably used in many previous studies, including those from our own laboratory (26). We consistently sampled all of the study participants in the morning after a sleep study, and thereby ensured standard fasting collection procedures as well as identical timing in relation to their sleep period. Thus, we did not examine whether OSA imposes any acute effects on homeostatic glycemic control during sleep, particularly in obese children, in whom the potential effects of OSA emerged as significant in the present study. In addition, lack of physical activity as well as dietary differences could account, at least in part, for the higher Ins/Glu ratios found in obese children (38, 39). Nevertheless, it is unlikely that such factors played a major role in the differential effects of OSA treatment in OB and NOB children, particularly considering that BMI remained unchanged in the OB cohort after treatment. Of course, although BMI remained unchanged, it is possible that the distribution of fat between the subcutaneous and visceral compartments may have changed. We did not specifically measure visceral fat, nor did we assess the potential relationships between visceral fat mass and metabolic and inflammatory alterations associated with obesity and OSA. These interactions may ultimately be of great relevance and will certainly warrant future studies, particularly when considering the putative differential roles played by these two adipose tissues in metabolic function, both in the context of obesity and in the presence of OSA (40–42). A major additional limitation of this study involves the absence of a control group that would demonstrate the stability of metabolic measures over time, and also the absence of a group undergoing adenotonsillectomy in the absence of OSA, so as to demonstrate that surgery per se was not the reason for the metabolic changes described herein.

Outcomes of Tonsillectomy and Adenoidectomy

This prospective cohort study showed that T&A resulted in OSA resolution rates that were significantly better in NOB children compared with OB children. These findings were anticipated overall, because we and others have clearly shown that the presence of obesity is associated with poorer sleep and respiratory outcomes after T&A in the context of OSA (37, 43). However, it is worthy of mention that the presence of residual OSA was markedly more frequent in OB children, and for both BMI-defined groups there was a correlation between the presurgical polysomnographic degree of respiratory disturbance and the likelihood of normalization of breathing during sleep after T&A (data not shown; 37).

TABLE 3. UNADJUSTED AND ADJUSTED ASSOCIATIONS BETWEEN CHANGES IN POLYSOMNOGRAPHIC MEASURES AND CORRESPONDING CHANGES IN SERUM METABOLIC MARKERS IN 62 PREPUBERTAL CHILDREN WITH OBSTRUCTIVE SLEEP APNEA BEFORE AND AFTER ADENOTONSILLECTOMY

Metabolic Marker	Polysomnographic Measure	Unadjusted r^2	Adjusted r^2	*P* Value
Insulin/glucose	OAHI	0.0169		NS
	nSp_{O_2}	0.0049		NS
	TAI	0.0381	0.0361	<0.04
	RAI	0.2304	0.2116	<0.001
Triglycerides	OAHI	0.0036		NS
	nSp_{O_2}	0.0009		NS
	TAI	0.0004		NS
	RAI	0.0009		NS
Total cholesterol	OAHI	0.0016		NS
	nSp_{O_2}	0.0004		NS
	TAI	0.0009		NS
	RAI	0.0025		NS
LDL	OAHI	0.1489	0.1089	<0.001
	nSp_{O_2}	−0.0675	−0.0484	<0.01
	TAI	0.0036		NS
	RAI	0.0049		NS
HDL	OAHI	−0.1261	−0.0961	<0.001
	nSp_{O_2}	0.0174	0.0144	<0.05
	TAI	0.0016		NS
	RAI	0.0016		NS
LDL/HDL	OAHI	0.1978	0.1681	<0.001
	nSp_{O_2}	−0.1144	−0.0961	<0.001
	TAI	0.0049		NS
	RAI	0.0036		NS
CRP	OAHI	0.0851	0.0736	<0.001
	nSp_{O_2}	−0.0942	−0.0641	<0.01
	TAI	0.0144		0.08
	RAI	0.0364	0.0289	<0.04
ApoB	OAHI	0.2446	0.1764	<0.0001
	nSp_{O_2}	−0.1338	−0.1024	<0.001
	TAI	0.0388	0.0324	0.04
	RAI	0.0978	0.0729	<0.004

Definition of abbreviations: CRP = C-reactive protein; HDL = high-density lipoprotein; LDL = low-density lipoprotein; nSp_{O_2} = nadir oxyhemoglobin saturation; NS = not significant; OAHI = obstructive apnea–hypopnea index; RAI = respiratory arousal index; TAI = total arousal index.

All coefficients of correlation and *P* values are adjusted for age and BMI *z* score.

Effect of Tonsillectomy and Adenoidectomy on Glycemic Control

Assessment of the changes in Glu, Ins, and Ins/Glu revealed dichotomous responses for OB and NOB children. Indeed, we did not find any changes in any of these measures for NOB children, independent of whether their underlying OSA was completely resolved, or whether there was polysomnographic evidence of some mild degree of respiratory disturbance after surgery (Tables 2 and 3). In contrast, when obesity was present, we found that not only was there an improvement in fasting morning Ins and Ins/Glu levels for the whole OB cohort (Table 2), but also the improvements were dependent on the magnitude of the reduction of OAHI, and these improvements were possible even in the absence of any BMI changes (Table 4). Our findings are not only in close agreement with previous studies supporting the presence of a putative relationship between OSA and insulin resistance among obese children (29, 30, 44, 45), but further stress the importance of including an intervention arm with the appropriate number of subjects, so as to validate the assumptions that were formulated in previous studies regarding potential associations between OSA and insulin resistance in children (32).

Murine models of sleep apnea appear to provide partial indirect validity to our present observations as well. In a series of elegant studies by Polotsky and colleagues, when obese mice were exposed to an intermittent hypoxic profile that attempted to mimic the oxyhemoglobin desaturations seen in severe OSA during sleep, exacerbation of insulin resistance became apparent (46). However, these investigators have also more recently reported on the occurrence of insulin resistance even among lean mice exposed to this rather severe OSA-like oxygenation profile, and have also shown that the alterations in glycemic control were independent of any associated changes in autonomic nervous system tone (47). Taken together, these studies would suggest that the severity of OSA that is likely to elicit measurable alterations in glucose homeostasis would be lesser among OB children, and rather unlikely to be observed in NOB children, particularly considering that the range of oxyhemoglobin desaturations used in mice would be rarely encountered in clinical pediatric practice.

Effect of Tonsillectomy and Adenoidectomy on Serum Lipid Profile and ApoB

Compared with published normative data in pediatric populations (48, 49), overall lipid levels measured in the present cohort were frequently above the 95th percentile for age and sex before T&A. For example for LDL cholesterol, 5 of 25 of the NOB children and 24 of the 37 OB children had elevated LDL cholesterol levels in the morning after the diagnostic sleep study. In contrast, none of the 25 NOB subjects ($P < 0.03$) and only 14 of the OB children ($P < 0.03$) had post-T&A LDL cholesterol concentrations that exceeded their corresponding 95th percentile normative values (48, 49). Reciprocal effects of similar magnitude were noted for HDL cholesterol (data not shown). Thus, surgical removal of hypertrophic tonsils and adenoids that leads to improvements in OSA severity is accompanied by substantial improvements in lipid homeostasis, even in the absence of BMI changes. Indeed, and in contradistinction to the effects of T&A on Ins and Ins/Glu, which were restricted to OB children, marked improvements in HDL and LDL cholesterol emerged, and led to overall reductions in fasting total cholesterol levels. Thus, the present study demonstrates for the first time in a pediatric population that the presence of OSA adversely affects lipid metabolism by increasing LDL cholesterol, and by reducing HDL cholesterol fractions, a phenomenon that was previously postulated either as an association analysis (27–31), or based on an intervention study that involved only a small number of patients (32). Furthermore, if HDL cholesterol is indeed dysfunctional as proposed (50), then the atherogenic effects of OSA should be even more pronounced (*see below*).

The mechanisms underlying the alterations in lipid metabolism are thus far unclear, although some of the involved pathways have begun to unravel. In a murine model of intermittent hypoxia during sleep that mimicks severe OSA, substantial increases in total and LDL cholesterol occurred in both lean and obese mice, and appeared to be mediated, at least in part, by the concomitant upregulation of hepatic stearoyl-CoA desaturase-1, a critical enzyme of lipid biosynthesis (51–53).

Similar to the serum lipid changes described heretofore, ApoB levels were markedly altered in the presence of OSA, and reversed toward normal levels as the severity of OSA was reduced. This observation occurred in all children independent of their obesity status, even if the effect was more pronounced among NOB children. ApoB is a large amphipathic protein that is intimately involved in the assembly and metabolism of LDL cholesterol (54). Epidemiologic studies have clearly established that elevated levels of ApoB-containing lipoproteins in humans are associated with increased incidence of cardiovascular disease (55, 56). However, there is a paucity of studies on the implications and normative range of serum ApoB levels in pediatric populations (57–59). In a study on 93 control children and 104 obese children with a mean age of approximately 13 years, mean

TABLE 4. OBSTRUCTIVE APNEA–HYPOPNEA INDEX AND METABOLIC AND INFLAMMATORY CHANGES IN NONOBESE CHILDREN WITH OBSTRUCTIVE SLEEP APNEA WITH OR WITHOUT RESOLUTION OF SLEEP-DISORDERED BREATHING AFTER TONSILLECTOMY AND ADENOIDECTOMY

	NOB1: OSA Resolved after T&A ($n = 15$)			NOB2: Residual OSA after T&A ($n = 10$)			P Value for
	Pre	Post	P_1 value	Pre	Post	P_2 value	NOB1 vs. NOB2*
OAHI, per h TST	10.2 ± 1.2	1.2 ± 0.2	<0.001	16.9 ± 2.4	3.1 ± 0.2	<0.001	NS
Glu, mg/dl	85.4 ± 1.9	85.7 ± 2.4	NS	82.5 ± 4.0	87.3 ± 1.7	NS	NS
Ins, μIU/ml	8.2 ± 1.2	9.6 ± 1.7	NS	10.1 ± 5.2	7.5 ± 1.8	NS	NS
Ins/Glu	0.10 ± 0.02	0.10 ± 0.02	NS	0.11 ± 0.05	0.11 ± 0.03	NS	NS
TG, mg/dl	70.4 ± 7.4	75.4 ± 7.2	NS	86.5 ± 15.8	77.23 ± 13.8	NS	NS
Total cholesterol, mg/dl	156.4 ± 6.6	138.5 ± 5.5	<0.01	154.5 ± 8.8	178.3 ± 8.8	<0.01	<0.01
LDL, mg/dl	90.3 ± 7.5	61.2 ± 2.3	<0.0001	87.3 ± 6.2	73.1 ± 3.5	<0.04	<0.01
HDL, mg/dl	44.6 ± 2.8	74.5 ± 3.7	<0.001	45.5 ± 5.6	48.6 ± 3.5	NS	<0.01
LDL/HDL	2.14 ± 0.3	0.94 ± 0.1	<0.0001	2.17 ± 0.3	1.43 ± 0.1	<0.01	<0.01
ApoB, mg/dl	98.0 ± 8.2	48.9 ± 2.8	<0.0001	103.7 ± 7.2	66.5 ± 3.9	<0.001	<0.01
CRP, μg/ml	3.4 ± 1.0	0.4 ± 0.1	<0.0001	5.2 ± 1.7	1.9 ± 0.3	<0.001	<0.01

Definition of abbreviations: CRP = C-reactive protein; HDL = high-density lipoprotein; LDL = low-density lipoprotein; NS = not significant; OAHI = obstructive apnea–hypopnea index; OSA = obstructive sleep apnea; T&A = surgical tonsillectomy and adenoidectomy; TG = triglyceride.

P_1 and P_2 represent two-tailed paired t test comparisons within NOB group that had resolution of OSA (NOB1) or did not have resolution of OSA (NOB2) after T&A, respectively.

* Comparisons between NOB1 and NOB2 were assessed by two-way analysis of variance for repeated measures followed by Tukey post hoc procedures.

serum ApoB concentrations were not predictive of early atherosclerosis changes assessed by carotid artery ultrasound (59). Our results appear to substantiate these findings, because those NOB and OB children who had complete resolution of their OSA after T&A had similar serum ApoB levels (Tables 3 and 4). Notwithstanding, our study clearly shows that OSA significantly modifies the kinetics of ApoB secretion and catabolism, and that by virtue of the functional role of this apolipoprotein, these changes seem to adversely affect LDL cholesterol, and thus exacerbate the theoretical risk for atherosclerosis in these patients. Although data on children are lacking in this regard, a study in adults with OSA demonstrated that correction of OSA was associated with reversal of early atherosclerotic changes (60). Furthermore, our laboratory has also shown that vascular dysfunction is frequently present even among nonobese children with OSA (12).

Inflammatory Changes in Pediatric OSA

Serum CRP concentrations were elevated in both NOB and OB children with OSA, and normalized proportionately to the improvements in OSA with T&A, and also as a function of whether obesity was present. These findings were overall not surprising, particularly when considering the rather consistent data emanating from published studies on CRP in pediatric OSA (15, 16, 61, 62). In this context, OSA was found to increase systemic inflammatory markers including IL-6 and CRP levels, and to induce reciprocal changes in antiatherogenic cytokines such as IL-10 (63). Moreover, the occurrence of increased CRP levels appears to serve as a predictor of neurobehavioral morbidity among nonobese children with OSA (64). Current results would suggest that the coincidence of OSA and obesity would further potentiate these abnormalities.

Conclusions

The present study provides compelling evidence that OSA in children adversely affects several of the components associated with the metabolic syndrome. Our findings not only extend those reported for a cohort of adolescents (31), but also provide the only available information to date regarding potential interactions between OSA, obesity, and biochemical markers of metabolic dysfunction and atherogenesis before and after treatment. The close association between OSA, obesity, and metabolic dysfunction, and the current evidence suggesting the presence of interacting pathophysiologies, would lend support to the development of screening and interventional strategies aiming

TABLE 5. OBSTRUCTIVE APNEA–HYPOPNEA INDEX AND METABOLIC AND INFLAMMATORY CHANGES IN OBESE CHILDREN WITH OBSTRUCTIVE SLEEP APNEA WITH OR WITHOUT RESOLUTION OF SLEEP-DISORDERED BREATHING AFTER TONSILLECTOMY AND ADENOIDECTOMY

	OB1: OSA Resolved after T&A ($n = 9$)			OB2: Residual OSA after T&A ($n = 28$)			P Value for
	Pre	Post	P_1 Value	Pre	Post	P_2 Value	NOB1 vs. NOB2*
OAHI, per h TST	10.7 ± 2.1	1.1 ± 0.2	<0.001	21.9 ± 3.7	6.8 ± 0.8	<0.001	<0.03
Glu, mg/dl	90.4 ± 1.7	89.2 ± 2.7	NS	89.6 ± 1.3	89.9 ± 1.5	NS	NS
Ins, μIU/ml	24.8 ± 2.6	17.1 ± 2.9	<0.02	26.6 ± 2.4	21.5 ± 1.2	<0.04	NS
Ins/Glu	0.27 ± 0.03	0.18 ± 0.02	<0.01	0.30 ± 0.02	0.25 ± 0.02	<0.05	<0.03
TG, mg/dl	108.4 ± 21.4	78.6 ± 8.8	<0.01	103.1 ± 8.3	93.3 ± 10.3	NS	<0.01
Total cholesterol, mg/dl	167.2 ± 6.3	142.6 ± 7.2	<0.01	172.6 ± 7.8	158.0 ± 4.98	<0.01	NS
LDL, mg/dl	110.3 ± 5.2	77.0 ± 6.9	<0.0001	119.1 ± 5.8	95.9 ± 4.9	<0.04	<0.01
HDL, mg/dl	39.6 ± 1.4	62.2 ± 4.8	<0.001	37.2 ± 1.6	48.3 ± 2.9	<0.01	<0.01
LDL/HDL	3.0 ± 0.2	1.2 ± 0.2	<0.001	3.3 ± 0.3	2.2 ± 0.2	<0.01	<0.01
ApoB, mg/dl	90.1 ± 5.0	49.1 ± 2.0	<0.0001	98.0 ± 3.8	67.9 ± 3.7	<0.001	<0.01
CRP, μg/ml	6.3 ± 1.3	0.9 ± 0.2	<0.0001	5.9 ± 1.0	2.9 ± 0.7	<0.001	<0.01

For definition of abbreviations, *see* Table 4.

P_1 and P_2 represent two-tailed paired t test comparisons within OB group who had resolution of OSA (OB1) or did not have resolution of OSA (OB2) after T&A, respectively.

* Comparisons between OB1 and OB2 were assessed by two-way analysis of variance for repeated measures followed by Tukey post hoc procedures.

to reduce the anticipated long-term adverse consequences associated with these disorders.

Conflict of Interest Statement: D.G. is on the National Speaker Bureau of Merck Company. O.S.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. L.K.-G. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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