SLEEP-DISORDERED BREATHING IN CHILDREN

Adipokines in Children With Sleep Disordered Breathing

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Study Objective: Associations between SDB, the metabolic syndrome, and circulating levels of adipokines have emerged in adults but have not been examined in snoring children, who, in contradistinction to adults, display insulin resistance and lipid abnormalities as a function of adiposity rather than SDB. Therefore, we aimed to examine associations between circulating adipokines levels, insulin resistance, and measures of SDB in children.

Design: Prospective study.

Setting: Polysomnographic evaluation and assessment of plasma levels of leptin, adiponectin, resistin, glucose, insulin, and CRP.

Participants: 130 children (mean age 8.2±2.8 years; 39% obese) were studied.

Measurements and Results: Log adiponectin levels were lower in obese than nonobese children (3.8±0.31 vs 4.0±0.30 corresponding to 8,381.4±8,441.0 ng/ml vs 12,853.2±7,780.2 ng/ml, P<0.0001) and were inversely correlated with BMI Z scores (r = -0.47, P<0.0001) but not with log AHI. Log leptin concentrations were higher in the obese group than the nonobese group (4.2±0.32 vs 3.4±0.57 corresponding to 19,542.6±13,643.6 vs 5,875.5±8,425.7 pg/ml, P<0.0001), correlated with BMI Z scores (r = 0.64, P<0.0001), and were significantly lower in children with AHI ≤1/hr than children with AHI> 1/hr (P = 0.006) and in children with SpO2 nadir ≥90% than children with SpO2 nadir <90%, even after controlling for BMI Z score (P<0.03). No significant differences were found in log resistin levels as a function of obesity or AHI. Significant correlations between log adiponectin levels and log Insulin/Glucose (I/G) ratios (<0.28, P = 0.006) and between log leptin levels and log I/G ratios (r = 0.66, P<0.0001) emerged.

Conclusions: In close agreement with the absence of a measurable effect of SDB on insulin resistance in children, circulating adipokines levels are primarily attributable to the ponderal index. However, SDB and associated hypoxemia may contribute to the elevation of leptin levels.

Keywords: Obstructive sleep apnea, leptin, adiponectin, resistin, insulin resistance, metabolic syndrome

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INTRODUCTION

IN RECENT YEARS, IT HAS BECOME INCREASINGLY EVIDENT THAT SIMILAR TO ADULTS, SLEEP DISORDERED BREATHING (SDB) IN CHILDREN IS ASSOCIATED WITH CARDIOVASCULAR MORBIDITY. One of the known risk factors for cardiovascular disease is the “metabolic syndrome” which designates the presence of insulin resistance, dyslipidemia, hypertension, and obesity. Although the association between SDB and the metabolic syndrome in adult patients appears to be independent of obesity, there are conflicting results regarding the reversibility of the metabolic disturbances following treatment with CPAP. In a cohort of snoring children, insulin resistance and lipid dysregulation were found to be primarily determined by obesity, and SDB played a minimal, if any, role in the occurrence of such metabolic abnormalities. These findings have been confirmed in a study of Greek children.

Disclosure Statement
This is not an industry supported study. Dr. Gozal serves on the national speakers’ bureau for Merck Inc. Drs. Tauman, Serpero, Capdevila, O’Brian, Goldbart, and Kheirandish-Gozal have indicated no conflicts of interest.

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Recent discoveries of fat-secreted substances, such as leptin, adiponectin, and resistin, which, in addition to local tissue effects also serve endocrine roles, have launched renewed interest and insights into our understanding of the complex relations/interactions between SDB, obesity, and the metabolic syndrome. Leptin, an adipocyte-derived hormone regulating energy expenditure and food intake is readily found in the circulation, and its levels appear to be determined by the degree of obesity as well as by the severity of SDB, particularly hypoxemia. Indeed, plasma leptin levels will decrease significantly following treatment with weight loss or CPAP, the latter independently from changes in BMI. These findings suggest that SDB is an independent contributing factor to serum leptin levels. Interestingly, leptin may directly affect respiratory control mechanisms, and intermittent hypoxia, such as found in SDB, has been shown to induce insulin resistance via disruption of leptin pathways.

Adiponectin is another novel adipocyte-derived hormone with anti-inflammatory, anti-atherogenic, and insulin-sensitizing properties. Reduced plasma adiponectin levels increase the risk for cardiovascular morbidity, insulin resistance, and “paradoxically” also increase the risk for obesity. Previous studies concerning plasma adiponectin levels in adults with SDB have revealed conflicting results. In contrast to leptin and adiponectin, the role of resistin in humans is still unclear. Although initially described as a fat tissue-derived hormone that increased insulin resistance (thereby its name), more recent studies have failed to demonstrate any association between resistin levels and insulin resistance. Of note, Harsch and colleagues recently reported a significant, albeit weak correlation between plasma resistin levels and insulin resistance in obese patients with SDB. However, while insulin resis-
tance improved with CPAP treatment, there were no concomitant changes in plasma resistin levels.\(^ {22}\)

Based on the cumulative evidence pointing towards a potential association between SDB, the metabolic syndrome, and plasma concentrations of several adipokines in adults, we examined the relative contributions of SDB and obesity on plasma adipokines levels in snoring children. We further assessed potential relationships between these adipokines, C-Reactive Protein (CRP) as a marker of inflammation, and glucose homeostasis.

**METHODS**

One hundred and thirty children consecutively evaluated for snoring and suspected SDB at the Kosair Children’s Hospital Sleep Medicine Center were studied. The study was approved by the University of Louisville Human Research Committee. Parental informed consent and child assent, in the presence of a parent, were obtained. Children were excluded if they had any chronic medical conditions, were receiving medications known to affect glucose homeostasis, or if they had any genetic or craniofacial syndromes.

A standard overnight multichannel polysomnographic evaluation was performed in the sleep laboratory as described previously.\(^ {7}\) Sleep architecture was assessed by standard techniques, as reported. Briefly, obstructive apnea was defined as the absence of airflow with continued chest wall and abdominal movement of at least 2 breaths duration. Hypopneas were defined as a decrease in nasal flow of \(\geq 50\%\) with a corresponding decrease in \(\mathrm{SpO}_2\), of \(\geq 4\%\) and/or termination by a 3-second EEG arousal. The obstructive apnea/hypopnea index (AHI) was defined as the number of apneas and hypopneas per hour of total sleep time (TST).

Children with an AHI>1 but \(<5/\) hr TST were considered to have mild SDB, while children with \(\mathrm{AHI} \geq 5/\) hr were considered to have SDB. To further examine the role of hypoxemia, children were divided according to their \(\mathrm{SpO}_2\) nadir into 3 groups: children with \(\mathrm{SpO}_2\) nadir \(\geq 90\%\) (mild hypoxemia), children with \(\mathrm{SpO}_2\) nadir of \(80-89\%\) (moderate hypoxemia), and children with \(\mathrm{SpO}_2\) nadir \(<80\%\) (severe hypoxemia). 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.

**Body Mass Index**

Height and weight were obtained using standard techniques from each child. BMI was then calculated (body mass/height\(^2\)). BMI Z scores for age and sex were determined based on Centers of Disease Control and Prevention growth charts.\(^ {23}\) Children with BMI Z scores exceeding 1.65 were classified as fulfilling the criteria for obesity.

**Blood Samples**

Blood for insulin and glucose levels was drawn the morning after the sleep study after an overnight fast. Plasma insulin levels were measured using a commercially available radioimmunoassay kit (Coat-A-Count Insulin, Diagnostic Products Inc.). This method has a detection level of 1.2 \(\mu\)IU/ml, and exhibits linear behavior up to 350 \(\mu\)IU/ml, with intra-assay and inter-assay coefficients of variability of 3.1% and 4.9%, respectively. Plasma glucose levels were measured using a commercial kit based on the hexokinase-glucose-6-phosphate dehydrogenase method (Flex Reagent Cartridges, Dade Behring, Newark, DE). Insulin resistance was assessed using the fasting insulin/fasting glucose ratio (I/G ratio).

Plasma CRP was measured within 2-3 hours after collection using the Flex reagent Cartridge (Date Behring, Newark, DE), which is based on a particle enhanced turbidimetric immunoassay technique. This method has a detection level of 0.05 mg/dl, and exhibits linear behavior up to 255 mg/dl, with intra-assay and inter-assay coefficients of variability of 9% and 18%, respectively.

Plasma samples were also obtained from the blood sample, and stored at \(-80^\circ\)C until assayed. Plasma levels for adiponectin were determined using a commercially available ELISA kit following the manufacturer recommendations (B-Briedge International Sunnyvale, CA). This method has a detection level of 23.4 pg/ml, and exhibits linear behavior up to 12 ng/ml, with intra-assay and inter-assay coefficients of variability of 5.2% and 5.3%. For leptin ELISA (Assay Design Inc., Ann Arbor, MI) detection level was 25.5 pg/ml, the linearity range was between 100 and 50,000 pg/ml with intra-assay and inter-assay coefficients of variability of 7.5% and 4.0%, respectively. Finally, plasma resistin levels using a commercially available ELISA (Biovendor [Alexis], San Diego, CA), with linearity range of 1.9 ng/ml to 16.3 ng/ml, and intra-assay and inter-assay coefficients of variability of 3.6% and 6.8%, respectively. All samples were assayed in duplicate along with duplicate calibration curves. The average of both values was retained unless they differed by \(>20\%\), in which case the assay was repeated.

All assays were performed by investigators who were unaware of the polysomnographic findings. Plasma adiponectin, leptin, and resistin levels were not normally distributed, and therefore logarithmic transformation was applied for subsequent analyses.

**Data Analysis**

Data are presented as means ± SD unless otherwise indicated. All analyses were conducted using SPSS software (version 11.5; SPPS Inc., Chicago, IL). Comparisons of demographics according to group assignment were made with independent t-tests or analysis of variance (ANOVA) followed by post hoc comparisons. Post hoc comparisons were performed using the least significant difference (LSD) when equal variances were assumed, and Dunnett’s test when equal variances were not assumed; P values were adjusted for unequal variances when appropriate (Levene’s test for equality of variances), or chi square (\(\chi^2\)) analyses with Fisher’s Exact Test (dichotomous outcomes). Correlations were performed using linear regression, followed by calculation of Pearson correlation coefficients. All P values reported are 2-tailed with statistical significance set at \(<0.05\). Odds ratio (OR) and 95% confidence intervals (CI) are presented.

**RESULTS**

One hundred and thirty children (54% males), aged 1-17 years (mean 8.2±2.8 years) participated in the study. Of these, 43 children were found to have SDB, 42 children had mild SDB, and 45 children were in the control (CO) group. Subject characteristics including glucose and insulin levels are shown in Table 1. There were no significant differences in age and sex among the 3 groups.
However, a significantly higher proportion of obese children were found in the SDB group than CO. As anticipated, significant differences were found among the 3 groups in AHI, arousal index, and SpO2 nadir, and a significant negative correlation between SpO2 nadir and AHI (r = -0.70, P<0.0001) was found for the entire cohort. Subject characteristics, including glucose and insulin levels after dividing the cohort based on the presence or absence of obesity, are shown in Table 2. Significantly higher insulin levels were found in obese children than nonobese children (P = 0.01).

**Adiponectin**

Log plasma adiponectin levels were significantly lower in the obese group than the nonobese group (3.8±0.31 vs 4.0±0.30, corresponding to 8381.4±5841.0 vs 12853.2±7780.2 ng/ml respectively; P<0.0001, Figure 1A). A significant negative correlation was found between log plasma adiponectin levels and corresponding BMI Z score (r = -0.70, P<0.0001, Figure 1B). No significant differences in log plasma adiponectin levels were found between children with SDB compared to children with mild SDB or with controls. Similarly, no significant differences were found in log plasma adiponectin levels after subdividing our cohort according to SpO2 nadir values. No significant correlations were found between log plasma adiponectin levels and log AHI, SpO2 nadir, and/or arousal index. Of note, a significant negative correlation was found between log plasma adiponectin levels and corresponding I/G ratios (r = -0.28, P = 0.006, Figure 1C). The OR for high I/G ratio (I/G ratio>0.1) in subjects with low plasma levels of adiponectin compared to subjects with high plasma levels of adiponectin was 3.6 (95% CI: 1.5-8.7, P = 0.004). A significant negative correlation was found between log plasma adiponectin levels and log plasma CRP concentrations (r = -0.41, P = 0.001). This correlation persisted after controlling for BMI Z score (r = -0.35, P = 0.01).

**Leptin**

Log plasma leptin levels were significantly higher in the obese children group than the nonobese group (4.2±0.32 vs 3.4±0.57 ng/ml; P<0.0001).

Figure 1—A. Log adiponectin levels in 51 obese children and 79 nonobese children. Log plasma adiponectin levels were significantly lower in the obese group than the nonobese group (P<0.0001). B. Scatterplot of log adiponectin levels plotted against log BMI Z score in 130 children. Linear regression line is shown (r = -0.47, P<0.0001). C. Scatterplot of log individual fasting I/G ratios plotted against corresponding log adiponectin levels in 130 children. Linear regression lines are shown (see text for details).
corresponding to 19,542.6±13,643.6 vs 5,875.5±8,425.7 pg/ml, respectively; P = 0.0001, Figure 2A), and a significant association was found between log plasma leptin levels and BMI Z scores (r = 0.64, P = 0.0001). Furthermore, log plasma leptin levels were significantly lower in control children (3.4±0.6 corresponding to 7005.9±8842.5 pg/ml) compared with either of the 2 severity-defined SDB groups (3.9±0.58 corresponding to 14737.0±14870.6 pg/ml, P = 0.009; CO vs SDB, and 3.8±0.58 corresponding to 12040.2±12904.4 pg/ml, P = 0.05; CO vs mild SDB, Figure 2B). A significant positive correlation was found between log plasma leptin levels and log AHI (r = 0.27, P = 0.002, Figure 2C).

Sub-analysis of the nonobese children also revealed significant positive correlation between log plasma leptin level and log AHI (r = 0.27, P = 0.002, Figure 2C).

To examine the potential contribution of hypoxemia to leptin levels, children were subdivided according to their SpO2 nadir into 3 severity groups. Significantly lower log plasma leptin levels occurred in “minimal hypoxemia” group (3.5±0.56 corresponding to 7149.9±9717.9 pg/ml) compared to either “moderate hypoxemia” group (3.9±0.58 corresponding to 14737.0±14870.6 pg/ml, P = 0.009; CO vs SDB, and 3.8±0.58 corresponding to 12040.2±12904.4 pg/ml, P = 0.05; CO vs mild SDB, Figure 2B). A significant positive correlation was found between log plasma leptin levels and BMI Z score using univariate analysis of variance.

Further, when stepwise linear regression analysis was performed for prediction of plasma leptin levels using BMI Z score, AHI, SpO2 nadir, and age as covariates, only BMI Z score, AHI, and age were retained in the model and accounted for 49% of the variance (P<0.0001). As with adiponectin, a significant positive correlation was found between log plasma leptin levels and log I/G ratios (r = 0.66, P <0.0001, Figure 2E). The OR for high I/G ratio (I/G ratio >0.1) in subjects with high plasma leptin levels compared to subjects with low plasma leptin levels was 13.9 (95% CI: 5.4-35.6, P <0.0001). A significant correlation was found between log plasma leptin levels and log plasma CRP concentrations (r = 0.41, P = 0.0001). This correlation persisted after controlling for BMI Z score (r = 0.33, P = 0.002).

Resistin

No significant differences were found in log plasma resistin levels among obese and nonobese children. Similarly, no differences emerged in resistin concentrations in children with SDB and controls. No correlations were found between log plasma resistin levels and BMI Z score, AHI, or SpO2 nadir, and no significant correlation emerged between log resistin levels and I/G ratios.

DISCUSSION

This is the first study conducted in snoring children that examines plasma concentrations of 3 major adipokines that have been suggested as important contributors to the regulation of energy homeostasis and insulin resistance. The differential responses of these 3 adipokines to the presence of SDB and obesity are intriguing, and they are vastly different from similar studies conducted in adult patients.10,11,19,20,22 In children, plasma adiponectin levels...
were found to associate only with obesity, while plasma leptin levels correlated with both obesity and the severity of the disease; no relationships emerged between plasma resistin levels and obesity or SDB severity. Furthermore, insulin resistance correlated positively with plasma leptin levels and negatively with plasma adiponectin levels. Plasma adiponectin and leptin levels were also found to correlate with plasma CRP concentrations.

**Leptin**

Both obesity and SDB severity contributed to the elevations of plasma leptin levels in snoring children. Plasma leptin levels were significantly elevated in children with SDB independently of obesity. Moreover, plasma leptin levels were significantly lower in children with minimal hypoxemia than in children with more pronounced/significant hypoxemia (Figure 2D). These findings support previous studies in adults showing that patients with SDB have significantly higher leptin levels compared to weight-matched subjects without SDB, and that treatment with CPAP may lead to significant reductions in leptin concentrations. There was no significant difference in leptin level between moderate and severe hypoxemia. This may be accounted by the fact that we divided our severity groups using the SpO2 nadir, rather than use a more cumulative index of desaturation. Such approach could have dampened potentially existing differences in oxygenation between the 2 SDB groups.

Animal studies have clearly demonstrated that hypoxia induces increases in both leptin gene expression (via nuclear binding and transcriptional activation of HIF-1α transcription factor) and in plasma leptin levels. Moreover in leptin-deficient mice, both up-regulation of leptin and leptin replacement protected against the development of glucose intolerance and insulin resistance during intermittent hypoxia. Thus, the elevation of leptin levels in children with SDB may represent an important compensatory mechanism aiming to minimize metabolic dysfunction and preserve glucose homeostasis. In addition, leptin prevents respiratory depression in obesity and may affect respiratory control mechanisms. Therefore, it is possible that the elevation of plasma leptin levels in SDB may reflect a respiratory compensatory mechanism to the alveolar hypoventilation induced by increased upper airway resistance.

In addition to its now well-defined role in energy balance, leptin has also pro-inflammatory properties, with the role of leptin in the modulation of immune response and inflammation becoming increasingly evident. Leptin circulating levels increase during infection and inflammation, stimulate cytokine activation and immune cell proliferation, and appear to be involved in inflammatory bowel and joint disorders. Recent evidence further suggests that leptin is involved in several aspects of cardiovascular morbidity, including ventricular hypertrophy, systemic hypertension, and angiogenesis, with increased leptin levels being associated with decreased arterial distensibility and with increased CRP levels, independently of measures of adiposity. The association between plasma leptin levels and CRP concentrations in the present study supports such previous reports.

Increased sympathetic activity and hypertension are present in both adults and children with SDB. It has been suggested that SDB-induced sympathetic activity may lead to alteration in leptin expression, and that these relationships between autonomic nervous system recruitment and leptin pathways may explain the almost immediate reduction in leptin levels and concomitant improvements in sympathetic tone following 24 hours of treatment with CPAP. On the other hand, leptin can induce increased sympathetic activity, as well as elevated heart rate and blood pressure. In the present study we did not measure blood pressure, nor did we assess for other measurements of sympathetic tone, and such studies will be clearly needed in the near future. Supporting previous reports, we also found a positive correlation between log plasma leptin levels and insulin resistance. It is possible that insulin plays an important role in regulating plasma leptin concentrations; however, it is also possible that leptin plays a role in modulating the action of insulin.

In a study by Yannakoula and colleagues a significant association between energy/macronutrient intake and plasma leptin concentrations emerged. This confounder will have to be incorporated in future studies, since, although fasting leptin measurements were performed in the present study, we did not control for diet during the days preceding blood sample draws. Previous reports have shown diurnal changes in circulating leptin. However, blood was obtained from our children only in the morning; therefore we cannot comment on circadian changes in plasma adipokine concentrations.

To summarize our findings on leptin, higher leptin levels were present among children with SDB and support our previous report on increased CRP levels in children with SDB. Thus, SDB in childhood appears to impose an independent risk for development of subclinical inflammation ultimately promoting cardiovascular morbidity.

**Adiponectin**

The present study confirmed previous findings linking obesity and insulin resistance to low plasma adiponectin concentrations in children, as well as in adults. Adiponectin is the most abundant adipose tissue-specific protein, and is exclusively expressed and secreted from the adipocytes. Similar to insulin resistance and dyslipidemia, low adiponectin levels do not seem to correlate with SDB severity in snoring children. Adiponectin plays a protective role against atherosclerosis, and reduced plasma adiponectin levels have been linked to the presence of endothelial inflammatory responses, the presence of coronary artery disease, dyslipidemia, and insulin resistance. In agreement with previous studies, we found an association between plasma adiponectin levels and insulin resistance measures, supporting the notion that adiponectin may have an insulin-sensitizing effect. Moreover, the negative correlation found in our study between plasma adiponectin levels and plasma CRP concentrations independently from obesity confirms the previous observations. Despite growing evidence suggesting that increased CRP levels are determined at least in part by SDB severity in children, we did not find any evidence supporting a role for SDB in reducing plasma adiponectin concentrations. One possible explanation may reside in the independent actions of CRP and adiponectin on the vascular wall. Alternatively, it is possible that much larger sample sizes will be needed to reveal the putative, albeit small size effects of SDB on plasma adiponectin levels. Finally, and in agreement with previous studies, we could not identify any association between plasma resistin concentrations and SDB severity and/or obesity.

Of note, our study cohort consisted of an otherwise typical referral-based pediatric population requiring clinical evaluation for...
suspected SDB. A large proportion of children (39%) were obese, compared to the 17% obesity prevalence in the metropolitan Louisville. In the current study we were unable to assess body fat distribution, particularly estimates of visceral and subcutaneous fat; patterns of fat distribution are an important factor contributing to the metabolic syndrome. In addition, we did not specifically assess pubertal stages, known to be another important determinant of insulin resistance. However, since the groups were closely matched for age and for male:female ratios, we believe that such similarities reflect close matching for pubertal stage distribution among the groups.

In summary, SDB appears to contribute to the magnitude of circulating leptin concentrations in children. However, as a whole, circulating adipokine levels are primarily linked to obesity in snoring children.

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