Angiopoietin-2 and Soluble Tie-2 Receptor Plasma Levels in Children with Obstructive Sleep Apnea and Obesity

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Objective: Obstructive sleep apnea (OSA) is a prevalent condition, especially in children with obesity, and is associated with increased risk for metabolic syndrome (MetS). Angiopoietins have been identified as potential biomarkers of endothelial dysfunction and MetS. In adults, angiopoietin-2 (Ang-2) and its soluble receptor (sTie-2) are associated with diabetes, hypertension, and obesity and could be increased in children with OSA and obesity, particularly those with evidence of cardiometabolic alterations.

Methods: One hundred twenty-six children (7.4 ± 2.0 years) were consecutively recruited and underwent overnight polysomnography, as well as endothelial function and BMI z score assessments and a fasting blood draw the morning after the sleep study. In addition to lipid profile, glucose and insulin levels, and homeostatic model assessment of insulin resistance (HOMA-IR), Ang-2 and sTie-2 concentrations were determined.

Results: Children with obesity and OSA had significantly elevated plasma Ang-2 and sTie-2 levels compared to corresponding controls with and without obesity. Furthermore, endothelial function (Tmax) and HOMA-IR were linearly and independently associated with Ang-2 and sTie-2 levels. In a small subset of children (n = 14), treatment of OSA by adenotonsillectomy resulted in reductions of Ang-2 and sTie-2 (P < 0.01).

Conclusions: Ang-2 and sTie-2 plasma levels are increased in pediatric OSA and obesity, particularly when endothelial dysfunction or insulin resistance is detectable, and appear to decrease upon OSA treatment.

Introduction

The prevalence of obesity in children has clearly increased all over the world, and such trends are further reflected by the increased emergence of cardiometabolic diseases in the pediatric age range (1-4). Among the obesity-related morbidities, insulin resistance and endothelial dysfunction (ED) are early manifestations and carry adverse prognosis if left untreated. Conversely, the presence of elevated BMI z scores in the overweight-obesity range does not necessarily indicate the presence of cardiometabolic dysfunction, and as such, identification of candidate biomarkers would be desirable for early detection and intervention (5,6).

Another highly prevalent pediatric condition, affecting around 3% to 4% of children, that is associated with increased risk for cardiometabolic dysfunction is obstructive sleep apnea (OSA) (7). This disease is characterized by recurring episodes of increased upper airway resistance during sleep, intermittent oxygen desaturation, and hypercapnia, as well as sleep fragmentation (8,9). The mechanisms underlying the association between cardiometabolic dysfunction and OSA remain unclear, even though both systemic low grade inflammation and oxidative stress pathways appear to be involved (10).

Angiopoietins are an important group of endothelial growth factors that modulate angiogenesis and vascular remodeling. Although the
Various angiopoietins bind to the tyrosine kinase receptor Tie-2, they exhibit contextually divergent biological functions. As such, when angiopoietin-1 (Ang-1) binds to Tie-2, it will enhance endothelial cell survival and promote angiogenesis (11-13). In contrast, angiopoietin-2 (Ang-2) operates as a competitor inhibitor of Ang-1-Tie-2 binding. Ang-2, an approximately 70-kDa glycoprotein, is expressed in vascular endothelial and smooth muscle cells (13), as well as in several other cell types, where it promotes leukocyte adhesion to the vascular endothelium and extravasation (11-13). Not surprisingly, Ang-2 and soluble Tie-2 (sTie-2) are elevated in adult patients with metabolic syndrome (14), as well as in a proportion of adults with obesity (15,16), with Ang-2 apparently being primarily correlated with vascular dysfunction and atherosclerosis (17,18) and sTie-2 being associated with obesity and dyslipidemia (19).

We hypothesized that the presence of OSA or obesity in children would be associated with increased plasma levels of Ang-2 and sTie-2, particularly among those children presenting evidence of ED or insulin resistance.

**Methods**

The research protocol was approved by the University of Chicago (protocol 09-115-B) human research ethics committee. Informed consent was obtained from the parents, and age-aptropriate assent was also obtained from the children. Children (4-11 years of age) were recruited from the sleep and ear, nose, and throat clinics at Comer Children’s Hospital at the University of Chicago, as well as by advertisement in the community. Children found to be hypertensive or using antihypertensive drug therapies were excluded (n = 6). Furthermore, children with overt diabetes (fasting serum glucose ≥120 mg/dL; n = 9) with a craniofacial, neuromuscular, or defined genetic syndrome and children on chronic anti-inflammatory therapy (n = 12) or with any known acute or chronic illness were also excluded. In addition, children placed on psychostimulants were not tested (n = 18).

**Overnight polysomnographic studies**

All children underwent standard nocturnal polysomnography evaluation as previously described (20), with assessment of eight standard electroencephalogram channels, bilateral electrooculogram, electro- myogram, two-lead electrocardiogram, oronasal airflow measurement using thermistor, nasal pressure transducer, end-tidal CO₂, chest and abdominal movement by respiratory inductance plethysmography, and pulse oximetry, including pulse waveform using a commercially available data acquisition system (Polysmith; Nihon Kohden America Inc., Irvine, California). The nocturnal polysomnography studies were scored as per the 2012 American Association of Sleep Medicine guidelines for the scoring of sleep and associated events (21). The proportion of time spent in each stage of sleep was calculated as a percentage of total sleep time (TST). A respiratory event was scored as an obstructive apnea if it was associated with a >90% fall in signal amplitude for >90% of the entire event compared to the baseline amplitude, the event lasted for at least two breaths, and there was continued or increased respiratory effort throughout the period of the event. A mixed apnea was scored if there was absent inspiratory effort in the initial part of the event, followed by resumption of inspiratory effort before the end of the event. A central apnea was scored if there was absent respiratory effort throughout the duration of the event, and the event lasted for at least two missed breaths and was associated with an arousal/awakening or a ≥3% desaturation. A hypopnea was scored if the event was associated with a ≥50% fall in amplitude of the nasal pressure transducer, lasted at least two breaths, and was associated with an arousal/awakening or ≥3% desaturation. The obstructive apnea-hypopnea index (AHI) was calculated as the number of apneas and hypopneas per hour of TST. Arousals were classified as either spontaneous or respiratory, and corresponding indices were computed.

The diagnosis of OSA was defined by the presence of AHI ≥2/h of TST. Control children were nonsnorers who had AHI <2/h of TST.

**Endothelial function measurements**

Endothelial function was assessed in a fasted state in the morning, using the hyperemic test after cuff-induced occlusion of wrist arteries, as previously described (22,23). In brief, a laser Doppler sensor (PeriFlux System 5000, Perimed, Järfalla, Sweden) was placed over the volar aspect of the hand at the first finger distal metacarpal surface, and the hand was secured and immobilized (24). Once cutaneous blood flow readings became stable, a cuff placed at the forearm and connected to a computer-controlled air pressure source was inflated to suprasystolic pressures, and blood flow signal declined to undetectable levels. The cuff was rapidly deflated, and the laser Doppler measured hyperemic responses. The time to maximal regional blood flow after occlusion release (Tmax) is representative of the postocclusion hyperemic response, an index of NO-dependent endothelial function (25). A Tmax greater than 45 seconds was considered as indicative of abnormal endothelial function (26) and represented three standard deviations (SD) above the mean. The previously examined interobserver and interobserver variability of the test is 8.9% and 13.8%, respectively.

According to our recruitment strategies, four distinctly different groups of children were identified: controls; nonobese and either non-snorers or snoring children with normal polysomnographic tests; children with obesity, i.e., BMI ≥ score ≥ 1.65, and with either normal polysomnographic tests or evidence of OSA; and nonobese snoring children with abnormal polysomnographic findings confirming the presence of OSA.

**Plasma assays**

Blood samples were drawn into either EDTA-containing tubes (purple top) or tubes without any additive. Samples were centrifuged within 30 minutes at 3,000g for 10 minutes, and plasma or serum were separated and either analyzed immediately or kept at −80°C. High-sensitivity C-reactive protein (hsCRP) was measured within 2 to 3 hours after collection using the FLEX Reagent Cartridge (Date Behring, Newark, Delaware), which is based on a particle-enhanced turbidimetric immunosay technique. Serum levels of lipids, including total cholesterol, high-density lipoprotein (HDL) cholesterol, calculated low-density lipoprotein (LDL) cholesterol, and triglycerides, were also assessed with a FLEX Reagent Cartridge. Plasma insulin levels were measured using a commercially available radioimmunoassay kit (Coat-A-Count® Insulin, Cambridge Diagnostic Products, Inc., Fort Lauderdale, Florida). Plasma glucose levels were measured using a commercial kit based on the hexokinase-glucose-6-phosphate dehydrogenase method (FLEX Reagent Cartridges). Insulin resistance was then assessed using the
homeostatic model assessment of insulin resistance (HOMA-IR) equation (fasting insulin × fasting glucose × 405) (27). In addition, plasma samples were frozen at −80°C until assay.

**Ang-2 and sTie-2 plasma assays**

Circulating levels of plasma Ang-2 and sTie-2 were assayed using commercially available Quantikine® human ELISA kits (R&D Systems, Minneapolis, Minnesota). The intra- and interassay coefficients of variation were 6.5% and 9.1%, respectively, for Ang-2 and 4.4% and 6.5%, respectively, for Tie-2. The assays were performed according to the manufacturer’s instructions.

**Statistical analysis**

All analyses were conducted using either SPSS Statistics® software (version 19.0; IBM Corp., Armonk, New York) or STATA®, and data are presented as mean ± SD. Children were subdivided into four groups based on the presence or absence of obesity (i.e., BMI ≥ score > 1.65) and OSA. 

A priori assumptions on the presence of differences in Ang-2 and sTie2 levels between children with and without OSA were formulated such as to allow for 80% power and a two-sided confidence level at 0.05 and indicated the need for 86 to 112 subjects in the cohort. Significant differences between groups were analyzed using two-way analysis of variance (ANOVA). If the data were not normally distributed, they were logarithmically transformed (i.e., AHI, hsCRP, respiratory arousal index). Pearson correlation analyses and linear regression analyses were conducted to examine potential associations between BMI, sleep variables, lipid profiles, log hsCRP, Tmax, and HOMA-IR and plasma concentrations of Ang-2 or sTie-2. To explore potential causal pathways in our data, we developed three logistic regression models with incremental complexity. First, we generated a simple model that was adjusted only for age. Then, a second model was adjusted for BMI z score, and the third model was adjusted for BMI z score and sleep variables. Finally, a model was constructed by adjusting for demographics, anthropometrics, sleep measures, and plasma levels of metabolic and inflammatory markers simultaneously, i.e., a fully adjusted model. All variables associated with Ang-2 or sTie-2 (P < 0.05) in one model were included in the next modeling steps, except when the information contained in two or more variables was very similar (colinear), in which case only one variable was included in the next modeling step. We also calculated the attributable Ang-1 or sTie-2 change fraction, which corresponds to the proportion of Ang-1 or sTie-2 change that could be explained causally of the associations and elimination of the various confounding factors by using the allogit command in STATA (28) on the logistic regression framework, as such an approach enables taking into account potential confounders. All P values reported are two-tailed with statistical significance set at <0.05.

**Results**

A total of 124 children fulfilling eligibility entry criteria completed all phases of the assessments and also provided a fasting blood sample after the sleep study. However, 34 additional children refused to participate in the study (14 parents declined to participate altogether and 20 parents were not willing to participate in the blood draw portion of the study). The demographic and polysomnographic characteristics of these 34 children were similar to those of the cohort, which are shown in Table 1.

In general, there were no significant differences in age, gender, or ethnicity across the four subgroups. However, children with obesity exhibited higher BMI z scores, as well as higher HOMA-IR, serum lipids, and hsCRP, and reduced HDL cholesterol levels (Table 2). In addition, the proportion of children with obesity with evidence of ED (endothelial dysfunction) (Tmax ≥ 45 s) was significantly higher than in nonobese children (54.4% vs. 13.2%, P < 0.001). Similarly, children with OSA had significantly higher HOMA-IR, LDL cholesterol, and hs-CRP concentrations and lower HDL cholesterol levels (Table 2). Furthermore, ED was more likely to occur in the presence of OSA (OSA vs. no OSA: 46.0% vs. 10.9%, P < 0.001).

Primary sleep disturbance measures clinically used to characterize the severity of OSA were not significantly different in children with obesity and nonobese children with OSA, although children with obesity exhibited a trend toward higher AHI and oxygen desaturation index 3% and lower nadir peripheral capillary oxygen saturation values. Similarly, there were no differences in sleep architecture measures among children with obesity and nonobese children without OSA (Table 1).

Children with obesity but without OSA had higher Ang-2 and sTie-2 levels than nonobese children without OSA (P < 0.01; Table 2). Similarly, nonobese children with OSA also exhibited higher Ang-2 and sTie-2 plasma concentrations compared to nonobese controls (P < 0.01; Table 2). However, children with obesity and OSA did not exhibit higher Ang-2 or sTie-2 levels compared to children with obesity but without OSA (P > 0.05; Table 2).

Individual HOMA-IR values were significantly associated with either BMI z score (r²: 0.18, P < 0.01) or with AHI (r²: 0.16, P < 0.01), but not with age, as illustrated by Pearson bivariate correlation analyses (Figure 1). Similarly, Tmax values were also significantly associated with BMI z scores (r²: 0.13, P < 0.01) and with AHI (r²: 0.30, P < 0.001), but not with age (Figure 1). In addition, no evidence of significant associations emerged between age, BMI z score, or AHI and total cholesterol, LDL, and HDL cholesterol levels. However, a very strong association emerged between HOMA-IR and Tmax (r²: 0.44, P < 0.0001).

In order to estimate potential associations between Ang-2 or sTie-2 plasma levels, polysomnographic measures, and metabolic (HOMA-IR) or endothelial function (Tmax) indices, we also performed Pearson correlation analyses. As shown in Figure 2, Ang-2 plasma levels were significantly associated with BMI z score (r²: 0.10, P < 0.01), AHI (r²: 0.22, P < 0.001), and particularly with Tmax (r²: 0.57, P < 0.001) and HOMA-IR (r²: 0.29, P < 0.001). Similarly, sTie-2 levels were significantly correlated with BMI z score (r²: 0.22, P < 0.001), AHI (r²: 0.12, P < 0.01), Tmax (r²: 0.34, P < 0.001), and particularly with HOMA-IR (r²: 0.59, P < 0.0001). Ang-2 levels were also inversely correlated with HDL (r²: 0.12, P < 0.01 and r²: 0.08, P < 0.01) but not with LDL or total cholesterol.

To further explore independent predictors of Ang-2 and sTie-2 levels, we performed stepwise multiple regression analyses for OSA severity with age and BMI z score included as potential confounders. In the first model (adjusted only for age), AHI was independently associated with Ang-2 and sTie-2 levels (Table 3, standardized coefficients: 0.490 and 0.306, P < 0.05). In the second model (adjusted for age and BMI z score), the severity of OSA accounted...
for 21.0% and 13.4% of the variance in Ang-2 and sTie-2, respectively. In addition, in the context of iterative variations on model 2, BMI z score contributed approximately 13.2% of the variance in Ang-2 and 24.3% of the variance in sTie-2 levels after adjusting for age and OSA severity (Table 3). However, when HOMA-IR was included in models, the proportion of variability in Ang-2 explained by AHI was reduced to 11.0, though AHI remained a statistically significant predictor (standardized coefficient: 0.382, P < 0.01). However, AHI was no longer a significant predictor (standardized coefficient: 0.382, P < 0.01) despite a strong positive relationship between HOMA-IR and AHI (r²: 0.413, P < 0.001).

### Table 1: Demographic, polysomnographic, and endothelial function findings among children with and without obesity or OSA

|                          | Nonobesity with OSA (n = 38) | Nonobesity without OSA (n = 30) | Obesity with OSA (n = 40) | Obesity without OSA (n = 16) |
|--------------------------|-------------------------------|-------------------------------|--------------------------|-------------------------------|
| Age (y)                  | 7.2 ± 2.4                     | 7.3 ± 1.5                     | 7.2 ± 1.9                | 7.3 ± 2.7                    |
| Gender (male, %)         | 50.0                          | 53.3                          | 57.5                     | 50.0                          |
| Ethnicity (African American, %) | 71.1                          | 70.0                          | 72.5                     | 62.5                          |
| BMI z score              | 0.34 ± 0.36b                 | 0.28 ± 0.81b                 | 2.57 ± 0.58a            | 2.32 ± 0.62b                 |
| OSA (y)                  | 2.8                           | 2.3                           | 3.2                      | 2.5                           |
| Total sleep duration (min) | 484.1 ± 53.1                | 471.2 ± 56.3                 | 487.2 ± 51.8             | 469.2 ± 55.3                 |
| Stage 1 (%)              | 7.0 ± 3.3c                   | 4.2 ± 3.1c                   | 8.2 ± 5.1d              | 4.8 ± 3.7d                   |
| Stage 2 (%)              | 38.3 ± 7.8                   | 38.4 ± 10.9                  | 40.3 ± 8.9               | 38.4 ± 13.5                  |
| Stage 3 (%)              | 36.7 ± 12.8c                 | 47.1 ± 11.4c                 | 39.6 ± 13.5             | 40.0 ± 14.2                  |
| REM sleep (%)            | 18.3 ± 8.1c                  | 25.0 ± 7.5c                  | 17.8 ± 8.6d             | 24.2 ± 12.2d                 |
| Sleep latency (min)      | 20.2 ± 15.6a,c               | 30.7 ± 13.6b,c              | 15.2 ± 13.0a,d          | 24.6 ± 17.2b,d              |
| REM latency (min)        | 119.7 ± 49.6a,c              | 139.5 ± 57.7b,c             | 103.8 ± 48.7a,d         | 138.7 ± 66.6b,d             |
| Total arousal index (events/h TST) | 23.4 ± 12.4c               | 8.9 ± 8.3c                   | 27.4 ± 15.2d            | 12.5 ± 9.6d                  |
| Respiratory arousal index (events/h TST) | 7.2 ± 3.2a,c                | 0.2 ± 0.2c                   | 8.9 ± 4.7a,d            | 0.6 ± 0.3d                   |
| Obstructive apnea-hypopnea index (events/h TST) | 18.9 ± 8.1c                 | 0.4 ± 0.1c                   | 20.9 ± 9.4d            | 0.6 ± 0.3d                   |
| SpO2 nadir (%)           | 81.7 ± 7.8c                  | 95.1 ± 0.7c                  | 76.9 ± 8.2d             | 93.1 ± 1.8d                  |
| ODI3%                    | 17.4 ± 8.0c                  | 0.2 ± 0.1c                   | 20.3 ± 8.8d             | 0.5 ± 0.2d                   |

All data are expressed as mean ± SD.
aNonobesity with OSA versus obesity with OSA: P < 0.01.
bNonobesity with OSA versus obesity without OSA: P < 0.01.
cNonobesity with OSA versus nonobesity without OSA: P < 0.01.
dNonobesity with OSA versus obesity OSA: P < 0.01.
eObesity with OSA versus nonobesity without OSA: P < 0.01.

### Table 2: Lipid profile, HOMA-IR, hsCRP, Ang-2, and sTie-2 plasma levels in children with and without obesity or OSA

|                          | Nonobesity with OSA (n = 38) | Nonobesity without OSA (n = 30) | Obesity with OSA (n = 40) | Obesity without OSA (n = 16) |
|--------------------------|-------------------------------|-------------------------------|--------------------------|-------------------------------|
| Total cholesterol (mg/dL) | 148.8 ± 21.7a,c             | 143.8 ± 18.3a,c             | 159.3 ± 27.2a,d          | 153.9 ± 31.6b,d             |
| HDL cholesterol (mg/dL)  | 50.1 ± 9.4a,c                | 63.3 ± 10.7a,c              | 48.8 ± 10.4a,d          | 50.4 ± 13.6a,b,d           |
| LDL cholesterol (mg/dL)  | 90.8 ± 18.7a                 | 89.7 ± 14.0a                 | 101.5 ± 26.8a,d         | 95.8 ± 29.0a,b,d           |
| Triglycerides (mg/dL)    | 75.4 ± 31.6a                 | 75.3 ± 27.9a                | 96.8 ± 34.1b           | 104.7 ± 59.4a,b            |
| HOMA-IR                  | 1.7 ± 0.6a,c                 | 1.2 ± 0.3c,c                | 2.9 ± 0.7a,d            | 2.3 ± 0.4a,d               |
| Log hsCRP (mean actual levels) | 0.27 ± 0.19c                | -0.05 ± 0.07b,c            | 1.28 ± 0.36a,d          | 0.44 ± 0.32a,b,d           |
| Ang-2 (ng/mL)            | 1.28 ± 0.33a,c              | 0.78 ± 0.25b,c             | 1.48 ± 0.27a           | 1.37 ± 0.28b              |
| sTie-2 (ng/mL)           | 11.2 ± 3.3a,c                | 8.9 ± 1.7b,c               | 14.8 ± 2.7a            | 15.2 ± 2.1b                |

aNonobesity with OSA versus obesity with OSA: P < 0.01.
bNonobesity without OSA versus obesity without OSA: P < 0.01.
cNonobesity with OSA versus nonobesity without OSA: P < 0.01.
dNonobesity with OSA versus obesity without OSA: P < 0.01.
eData were not normally distributed and were log-transformed.

Ang-2, angiopoietin-2; HDL, high-density lipid cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipid cholesterol; OSA, obstructive sleep apnea; sTie-2, soluble Tie-2.
predictor of sTie-2 with HOMA-IR in the model (P = 0.242). Similarly, due to the shared variance between Tmax and AHI ($r^2$: 0.298, $P < 0.001$) when Tmax was included in the model, AHI was no longer a significant predictor of Ang-2 ($P = 0.130$) or sTie-2 ($P = 0.681$). In this comprehensive and adjusted model, and using a statistical approach that allows multiple risk factors to be taken into account (28), OSA severity accounted for 13% of the variance in Ang-2 levels (standardized coefficient: 0.184, $P < 0.01$) and for 8% of the variance in sTie-2 levels (standardized coefficient: 0.184, $P < 0.05$), with BMI z score accounting for 36% for Ang-2 and 38% of the variance for sTie-2 (standardized coefficient: 0.387, $P < 0.001$ and 0.402, $P < 0.001$, respectively). There were no apparent

Figure 1 Scatterplots of Tmax and homeostatic model assessment of insulin resistance (HOMA-IR) versus age, BMI z score, and obstructive apnea-hypopnea index (AHI) in children with and without obesity or obstructive sleep apnea. 

(A) $r^2 = 0.133$, $P < 0.001$; (B) $r^2 = 0.299$, $P < 0.001$; (C) $P > 0.05$; (D) $r^2 = 0.190$, $P < 0.001$; (E) $r^2 = 0.169$, $P < 0.001$; (F) $P > 0.05$. 
Figure 2 Scatterplots of angiopoietin-2 (Ang-2) and soluble Tie-2 plasma levels versus BMI z score, obstructive apnea-hypopnea index (AHI), Tmax, and homeostatic model assessment of insulin resistance (HOMA-IR) in children with and without obesity or obstructive sleep apnea. (A1) $r^2 = 0.10, P < 0.01$; (A2) $r^2 = 0.22, P < 0.001$; (A3) $r^2 = 0.57, P < 0.001$; (A4) $r^2 = 0.29, P < 0.001$; (B1) $r^2 = 0.22, P < 0.001$; (B2) $r^2 = 0.12, P < 0.01$; (B3) $r^2 = 0.34, P < 0.001$; (B4) $r^2 = 0.59, P < 0.001$. 
TABLE 3 Multivariate stepwise regression analyses between sleep measures, HOMA-IR, Tmax, and Ang-2 or sTie-2 plasma levels

| Ang-2 plasma levels | S Tie-2 plasma levels |
|---------------------|-----------------------|
|                     | Standardized coefficient | P value | Standardized coefficient | P value |
| Age                 | -0.130 | 0.147 | -0.002 | 0.982 |
| BMI z score         | 0.373 | < 0.001 | 0.490 | < 0.001 |
| AHI                 | 0.490 | < 0.001 | 0.306 | < 0.001 |
| HOMA-IR             | 0.468 | < 0.001 | 0.690 | < 0.001 |

*Data were log-transformed; data for age, gender, and race were not adjusted. Data for BMI z score shown after adjusting for age only, and after adjusting for all sleep measures. All other data shown after controlling for age and BMI z score. AHI, obstructive sleep apnea hypopnea index; Ang-2, angiopoietin-2; HOMA-IR, homeostatic model assessment of insulin resistance; sTie-2, soluble Tie-2.

Discussion

This study shows that both children with obesity and children with OSA exhibit significantly higher Ang-2 and sTie-2 plasma levels when compared to healthy controls. However, a ceiling effect for both Ang-2 and sTie-2 levels emerges if obesity and OSA are concurrently present. Significant linear bivariate associations were detected between Ang-2 plasma levels and BMI z score and the degree of respiratory disturbance during sleep as indicated by the AHI. However, particularly prominent associations between Ang-2 and sTie-2 emerged with Tmax and HOMA-IR, but neither total, LDL, or HDL cholesterol nor hsCRP levels exhibited any significant correlation. Finally, in a small subset of children with obesity who underwent adenotonsillectomy, follow-up assessments revealed significant decreases in AHI as well as in Ang-2 and sTie-2 plasma concentrations after treatment. Taken together, these findings suggest that assessment of Ang-2 and sTie-2 plasma levels may provide reliable indicators for children at risk for cardiometabolic dysfunction in the context of either obesity or OSA.

Several methodological considerations and study limitations deserve comment. First, blood collection procedures were carefully standardized to coincide with an overnight fast after the overnight sleep study. Furthermore, the overall sleep duration in the night before the blood sample draw was available from the polysomnogram, such that we could ascertain that differences in sleep duration were not present among the four subgroups and could have potentially accounted for the findings. Second, to reduce potential sources of variability, all ELISA assays were performed concomitantly. Finally, nonsnoring children with and without obesity were recruited from the community rather than from clinical referral populations. As far as potential limitations, we should point out that the cohort included in the study as well as the group of children who were evaluated before and after treatment was relatively small, and therefore future validation studies in more extensive and potentially age- and ethnicity-diverse communities would be required to enable more accurate assessments of the predictive ability of Ang-2 and sTie-2 to serve as biomarkers of cardiometabolic dysfunction in the pediatric age range. Also, the roles of food intake and physical activity and of single-nucleotide polymorphisms in the variance of Ang-2 and sTie-2 were not evaluated (19,29).

We are not aware of any published studies that have examined Ang-2 or sTie-2 as potential indicators of cardiometabolic risk in children. In a recent population-based study conducted in northeast Germany in adults (14), both of these analytes were cross-sectionally associated with the metabolic syndrome. Globally similar findings were also reported in another large US-based cohort that evaluated the impact of diet-induced weight loss and exercise on angiogenic factors, whereby significant improvements in Ang-2 levels emerged after weight reduction (16), further attesting to the potential responsiveness of Ang-2 to treatment. The evidence linking Ang-2 and sTie-2 with cardiovascular and metabolic morbidity is further buttressed by the fact that Ang-2 levels were significantly higher in adult patients with type 2 diabetes who had evidence of microvascular angiopathy, and that Ang-2 levels were also strongly correlated with HOMA-IR (30). In another study in diabetic patients, Ang-2 levels were significantly and independently elevated among subjects with insulin therapy, diabetic polyneuropathy, and diabetic macroangiopathy, while sTie-2 was independently associated with hemoglobin A1c, insulin levels, and HOMA-IR (31). Based on our current findings and aforementioned studies, it would appear that the potential role of assessing Ang-2 and sTie-2 as indicators of cardiometabolic risk can be expanded to at-risk children. Although the mechanisms underlying the putative link between OSA and plasma levels of Ang-2 and sTie-2 remain unclear at this stage, it is very likely that the systemic oxidative stress and inflammation that accompany the presence of OSA and obesity may alter the transcriptional and translational regulation of these important molecules, whose roles in the microcirculation and metabolic function may reflect the interdependency between Ang-2 and sTie-2 and inflammatory mediators both systemically and at the tissue level (32-35).

We should also remark that in addition to changes in Ang-2 and sTie-2 levels with treatment, we found significant improvements in hsCRP, a finding that has now been reported on multiple occasions and further proposed as a biomarker for residual OSA after T&A (36,37). Furthermore, the anticipated effects of T&A on HOMA-IR are relatively small, a finding that is further recapitulated herein (38).

In summary, we have shown that children with obesity or those with OSA manifest elevated plasma levels of Ang-2 and sTie-2, particularly when such children exhibit evidence of insulin resistance or ED. Furthermore, declines in Ang-2 and sTie-2 levels occur after treatment of OSA. Thus, assessment of these markers may permit future identification of children with obesity or OSA who may be at risk for cardiometabolic dysfunction.

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