CHEST

Original Research

SLEEP DISORDERS

# Endothelial Dysfunction in Children Without Hypertension

## Potential Contributions of Obesity and Obstructive Sleep Apnea

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***Background:*** **Endothelial dysfunction can develop in the context of both obesity and obstructive sleep apnea (OSA) in children. However, the potential interactions between OSA and obesity have not been defined.**

***Methods:*** **Children who were prepubertal and nonhypertensive were recruited. Endothelial function was assessed in a morning fasted state, using a modified hyperemic test involving cuff-induced occlusion of the radial and ulnar arteries, and blood was drawn for assessment of myeloid-related protein 8/14 (MRP8/14) levels using a commercial enzyme-linked immunosorbent assay. Overnight polysomnography defined the presence of OSA or absence of OSA (NOSA) in subjects investigated for sleep-disordered breathing. Anthropometric measurements were performed to assign subjects to obese (OB) and nonobese (NOB) categories.**

***Results:*** **Fifty-four children with OSA who were obese and nonobese (mean age, 7.90 ± 0.26 years; mean BMI *z*-score, 1.70 ± 0.3; obstructive apnea-hypopnea index [OAHI], 7.36 ± 1.09) were compared with 54 children without OSA who were obese and nonobese (mean age, 8.26 ± 0.24 years; mean BMI *z*-score, 1.41 ± 0.18; OAHI, 0.86 ± 0.07). Of those subjects, 62.5% of the OB-OSA category, 38.7% of the OB-NOSA category, and 20.0% of the NOB-OSA category had evidence of endothelial dysfunction, compared with 0.0% of the NOB-NOSA category ($P < .01$). The degree of endothelial dysfunction in all groups was associated with circulating MRP8/14 levels ($r = 0.343$, $P < .001$).**

***Conclusions:*** **Both obesity and OSA can independently increase the risk for endothelial dysfunction, and the concurrent presence of both markedly increases such risk. Although the mechanisms underlying endothelial dysfunction remain unclear, a potential role for MRP8/14 as an inflammatory biomarker of endothelial dysfunction is suggested.** ***CHEST 2012; 141(3):682–691***

**Abbreviations:** DBPi = diastolic BP index; hrTST = hour of total sleep time; hsCRP = high-sensitivity C-reactive protein; MRP8/14 = myeloid-related protein 8/14; OAHI = obstructive apnea-hypopnea index; OSA = obstructive sleep apnea; PSG = polysomnography; PU = perfusion unit; SBPi = systolic BP index; Tmax = time to peak regional blood flow response postocclusion release

The presence of cardiovascular disease in children is usually related to congenital aberrations in the embryologic development of the cardiovascular system. In fact, acquired cardiovascular disease through environmental risk factors such as diabetes or obesity is not typically observed during early childhood. However, in the context of the obesity epidemic and the increases in the prevalence of cardiovascular disease in the United States,[1-5] it has become imperative to identify acquired cardiovascular disease as early as possible, so as to foster early preventative and treatment strategies.

Obesity is a prominent risk factor for the development of cardiovascular disease, diabetes mellitus, dyslipidemia, hypertension, and their constellation as the metabolic syndrome.[3] In fact, both the American Heart Association and the American Diabetes Association have identified obesity as a "major, modifiable risk factor."[6] Furthermore, there is now incremental evidence suggesting that the complications of obesity begin in early childhood. Hypercholesterolemia, dyslipidemia,[4,7] insulin resistance, and type 2 diabetes mellitus[8,9] have been all described as significant comorbidities associated with pediatric obesity.

Furthermore, although systemic hypertension in children is rare, with an estimated prevalence at 2% to 5%,[10] obesity has been shown to increase the risk of hypertension in children.[11,12] We have recently shown that obesity in children is associated with an increased risk for the development of endothelial dysfunction prior to the onset of hypertension.[13] Indeed, using a modified hyperemic test after cuff-induced occlusion of the radial and ulnar arteries, we demonstrated that significant delays occur in the vascular reperfusion kinetics among children who were obese and nonhypertensive and who did not suffer from either diabetes or obstructive sleep apnea (OSA).[13] The exclusion of OSA[13] is particularly relevant, considering that obesity markedly increases the risk for sleep-disordered breathing.[14-16]

OSA is a highly prevalent condition in children, in which intermittent occlusion of the upper airway during sleep leads to recurrent oxyhemoglobin desaturations, elevated $CO_2$ levels, sleep fragmentation, and reduced sleep efficiency. Similar to obesity, OSA is associated with multifaceted derangements in metabolic and cardiovascular function, including endothelial dysfunction.[17-19] Indeed, we have previously shown the presence of overall treatment-reversible endothelial dysfunction among children aged 6 to 9 years old who were nonobese and nonhypertensive and in whom OSA was diagnosed using polysomnography (PSG),[20] furthering the notion that OSA is also a potential cause of endothelial dysfunction in young children.

In addition, we have demonstrated the association of atherogenic markers, namely myeloid-related protein 8/14 (MRP8/14), with endothelial dysfunction[21] and further demonstrated the upregulation of MRP8/14 that occurs in the context of pediatric OSA.[22] MRP8/14, a major calcium-binding protein, is primarily expressed in cells of myeloid origin, namely monocytes and neutrophils.[23,24] Following activation of phagocytic activity, MRP 8 and MRP 14 combine to form the MRP8/14 complex, which then translocates to the cytoskeleton and plasma membrane, where it is secreted.[23] This is an early event during the process of *trans*-endothelial migration and the interaction of activated neutrophils and monocytes with the endothelium, and is, therefore, believed to play a significant role in the regulation of atherogenesis by cells of myeloid origin.[25,26]

Based on the aforementioned observations, we hypothesized that the risk for altered endothelial function would be further accentuated by the coexistence of obesity and OSA in children, when compared with either of these conditions in isolation. Thus, the aim of the current study was to assess endothelial function in children who were obese and nonobese, with and without OSA. We further assessed potential relationships between endothelial dysfunction, OSA, and obesity through examination of systemic biomarkers such as MRP8/14.

## Materials and Methods

The study was approved by the University of Louisville Human Research Committee (protocol #474.99), and informed consent was obtained from the legal caregiver of each participant. Consecutive children who were healthy and prepubertal (ages 4-12 years) from the community who agreed to participate in a sleep and neurocognitive research study at the University of Louisville Pediatric Sleep Medicine Center were recruited to investigate endothelial function. Subjects were recruited from November 2007 until September 2008. All participants underwent baseline overnight PSG and measurement of endothelial function followed by a fasting blood draw in the morning.

### *Anthropometry*

Children were weighed using the InBody 320 scale (Biospace), which employs the Direct Segmental Multi-frequency Bioelectrical Impedance Analysis method, and therefore enables assessment of the percentage of body fat and determination of individual impedance indices for all limbs. This feature allows for computation of an estimate of body fat percentage and its truncal distribution. The InBody 320 scale has been validated in children with excellent correlation to underwater weighing and body dual-energy x-ray absorptiometry scans.[27] Their height (to 0.1 cm) was measured using a stadiometer (Holtain). The BMI was calculated and the BMI *z*-score was computed using US Centers for Disease Control and Prevention 2000 growth standards (www.cdc.gov/growthcharts) and online software (www.cdc.gov/epiinfo). A BMI *z*-score $>1.65$ ($>$95th percentile) was considered as fulfilling the obesity criteria.

### *Sphygmomanometry*

All children had arterial BP measured noninvasively using an automated mercury sphygmomanometer (Welch Allyn) at the brachial artery and a guidelines-defined appropriate cuff size on the nondominant arm.[28] Two consecutive BP measurements were made while children lay supine with the head of the bed elevated to 45°. A nonstandard posture of BP measurements was chosen in order to mimic the posture used during endothelial function

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testing, which was both comfortable for children and best minimized arm motion artifact during measurement. BP measurements were made in the evening prior to commencement of nocturnal PSG. Systolic BP and diastolic BP indices (SBPi and DBPi, respectively) were calculated by dividing the average systolic and diastolic pressure by the respective 95th percentile for BP using National Heart, Lung and Blood Institute guidelines (www.nhlbi.nih.gov/guidelines/hypertension/child_tbl.htm), computed for age, sex, and height. Hypertension was defined when the SBPi or DBPi was $> 1$.

*Overnight PSG*

PSG was conducted and scored as previously reported.[13] Central, obstructive, and mixed apneic events were counted. Obstructive apnea was defined as the absence of airflow with continued chest wall and abdominal movement for a duration of $\geq 2$ breaths. Hypopneas were defined as a decrease in oronasal flow of $\geq 50\%$ for two or more consecutive breaths with a corresponding decrease in oxyhemoglobin saturation of $\geq 3\%$ and/or an arousal. The obstructive apnea-hypopnea index (OAHI) was defined as the number of obstructive apneas and hypopneas per hour of total sleep time. Arousals were defined according to the American Academy of Sleep Medicine Scoring Manual.[29] OSA was defined by the presence of an OAHI score $\geq 2$ per hour of total sleep time (hrTST).

*Endothelial Function*

Endothelial function was assessed using a modified hyperemic test after cuff-induced occlusion of the radial and ulnar arteries by placing the cuff over the wrist. All testing was performed upon awakening from the sleep study in the morning to ensure that children were in a fasted state. A laser Doppler sensor (Periflux 5000 System integrated with the PF 5050 Pressure Unit, Perimed) was applied over the volar aspect of the hand at the first finger distal metacarpal surface, and the hand was gently immobilized. This site was chosen as an area in order to minimize the effects of motion artifact and was also found to have a density of skin capillary blood flow that was of appropriate magnitude for detection.

Children lay supine with the head of the bed elevated at 45°. Once cutaneous blood flow over the area became stable, the pressure within an inflatable cuff placed at the forearm and connected to a computer-controlled manometer was raised to 200 mm Hg for 60 s, during which blood flow was reduced to undetectable levels. An occlusion time of 60 s was chosen from a large array of preliminary experiments, aiming to establish the reliability of this procedure while minimizing discomfort to the child and thus prevent motion and invalidation of the test. Furthermore, we used a computer-controlled pressure release to allow for consistent deflation times. The cuff was rapidly deflated, and the laser Doppler measured hyperemic responses. The maneuver was repeated twice at 10-min intervals between trials to ensure the return to baseline perfusion. The average of both maneuvers was then computed for subsequent analyses.

Laser Doppler determines the magnitude of perfusion at rest (resting flow), at occlusion (biologic zero flow), and at peak postflow occlusion (Fig 1). The reproducibility of the laser Doppler flowmetry using the Periflux system has been described previously.[30,31] While detection of microvascular perfusion varies by child according to the density of capillary blood vessels, thickness of skin, and other factors, all measurements are extrapolated to baseline perfusion before cuff occlusion, and analysis of reperfusion kinetics is based on time measurements. Furthermore, based on the variability of the perfusion characteristics of each child, which are further affected by the location of the laser Doppler sensor, we found that the magnitude of perfusion was quite variable, such that there was considerable variability in the baseline perfusion flow, peak flow, and biologic zero measurements. Accordingly, only variables that are solely based on kinetics, such as the time to peak regional blood flow response postocclusion

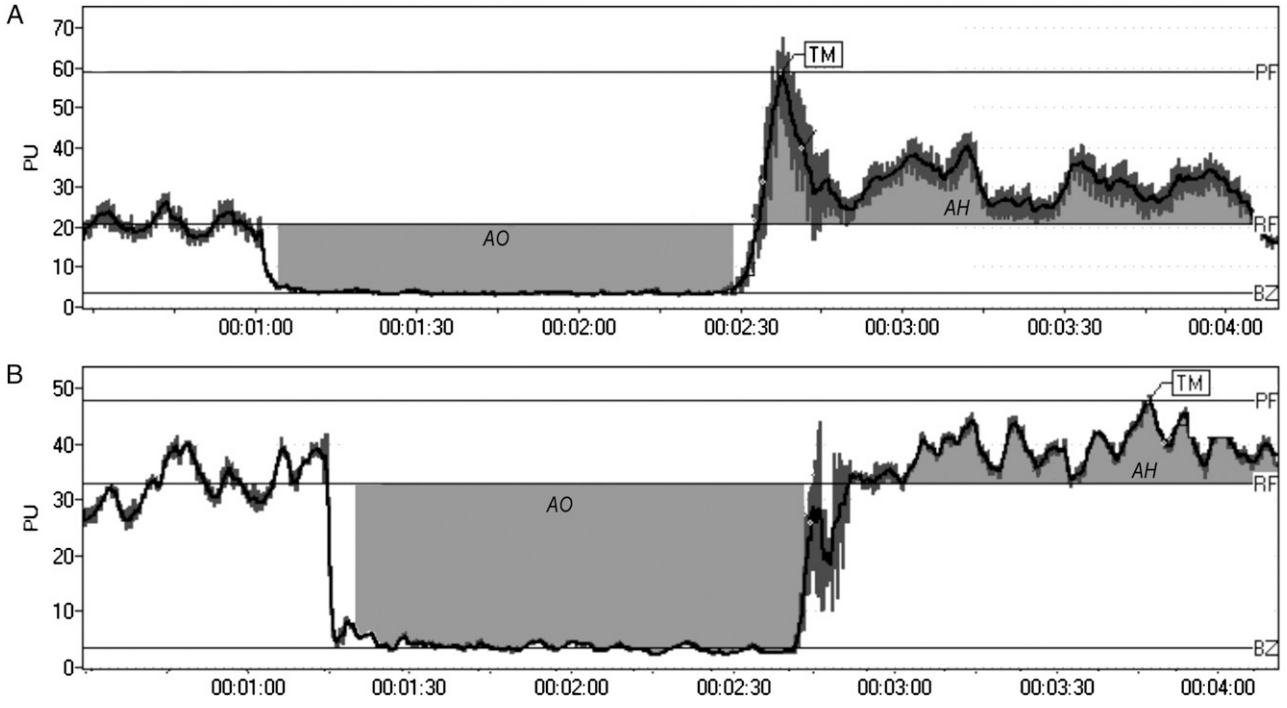


FIGURE 1. Results of representative cuff occlusion tests. A, In a child with normal endothelial function. B, In a child with abnormal endothelial function. AH = area of hyperemia; AO = area of occlusion; BZ = biologic zero; PF = peak flow; PU = perfusion unit; RF = rest flow; TM = time to peak flow following occlusion.

release (Tmax), emerged as highly reproducible and were therefore selected as the primary outcomes. Commercially available software (Perimed) enabled unbiased estimates of the Tmax, which is considered representative of the postocclusion hyperemic response and an index of endothelial function.[32]

### *Blood Tests*

Fasting blood samples were drawn by venipuncture in the morning immediately after endothelial function testing and nocturnal PSG. Blood was sent for routine laboratory testing, including tests for high-sensitivity C-reactive protein (hsCRP), glucose, insulin, and serum lipid concentrations, using standard laboratory techniques. In addition to standard blood testing, assays were performed for biomarkers of atherogenesis. IL-20, an inflammatory cytokine shown to be involved in angiogenesis and atherogenesis,[33] was found in plasma using an assay for IL-20 (DL200; R&D Systems). Of note, the assay has a reported sensitivity of 7.51 pg/mL, a linearity range of 94% to 98%, and intraassay and interassay coefficients of variability of 6.2% and 9.0%, respectively. Plasma MRP8/14, an important calcium-binding protein involved in phagocytosis, is closely linked to inflammation within the vascular endothelial wall.[34,35] As a corollary to these observations, MRP8/14 is linked to atherosclerosis and serves as a useful biomarker of cardiovascular disease.[36,37] Hence, assays were performed for MRP8/14 using commercial enzyme-linked immunosorbent assay kits (ALPCO Diagnostics and R&D Systems, respectively). MRP8/14 has a sensitivity of 0.4 μg/mL and 0.15 pg/mL, respectively. The interassay and intraassay of coefficients of variability for MRP8/14 is 4.8%.

### *Exclusion Criteria*

All children with hypertension (with either an SBPi or DBPi > 1) or using antihypertensive therapies were excluded. Furthermore, children with diabetes (fasting serum glucose ≥ 120 mg/dL), children with a craniofacial, neuromuscular, or defined genetic syndrome, and children on long-term antiinflammatory therapy or with any known acute or chronic illness were excluded. Children using sympathomimetic agents such as psychostimulants were excluded as well.

### *Data Analysis*

Results are presented as means ± SE, unless stated otherwise. All numerical data were subjected to statistical analysis using independent Student *t* tests or analyses of variance followed by post-hoc tests (Tukey) as appropriate. $\chi^2$ analysis was performed on categorical data concerning demographic characteristics of groups of subjects who were obese and nonobese. Pearson correlation testing was conducted to establish associations between several study parameters, including circulating biomarkers and the study's primary outcome, the Tmax. Univariate and multivariate linear regression analyses were used to estimate the prediction of endothelial function by including all the following independent variables in the model: age, sex, BMI *z*-score, race, and various polysomnographic measures. In addition, stepwise multivariate regression analyses were conducted while treating various Tmaxs as a dependent variable in relation to the OAHI and other covariates. Finally, canonical correlation analyses were performed to explore the relationships between sets of variables. Statistical analyses were performed using SPSS software (version 18.0; SPSS Inc) or StatSoft software (STATISTICA version 8.0, 2008; StatSoft, Inc). For all comparisons, a two-tailed $P < .05$ was considered to define statistical significance.

**Table 1—*Demographic Characteristics of 171 Children Evaluated for Endothelial Function***

| Demographic Factors | Subjects (N = 171) |
|---|---|
| Age, y | 8.2 ± 0.1 (4-13) |
| Sex | |
| Male | 96 (56) |
| Female | 75 (44%) |
| Ethnicity | |
| White | 101 (59) |
| Black | 53 (31) |
| Hispanic | 9 (5) |
| Other | 8 (5) |
| Obesity | |
| Nonobese (BMI *z*-score < 1.65) | 78 (46) |
| Obese (BMI *z*-score ≥ 1.65) | 93 (54) |
| OSA severity | |
| Normal (OAHI < 2/hrTST) | 117 (68) |
| Mild OSA (OAHI ≥ 2/hrTST but < 5/hrTST) | 33 (20) |
| Moderate to severe OSA (OAHI ≥ 5/hrTST) | 21 (12) |

Age expressed in mean ± SE, age range reported in parentheses. All other demographic factors reported in total values, with percentages expressed in parentheses. hrTST = hour of total sleep time; OAHI = obstructive apnea-hypopnea index; OSA = obstructive sleep apnea.

## Results

A total of 171 children meeting the inclusion criteria were studied (Table 1). Of the 171 children, 93 children (54%) met the criteria for obesity. A summary of other demographic data is provided in Table 1. PSG identified 117 children (68%) with normal sleep studies (OAHI < 2/hrTST). Of the 54 remaining children (32%) identified with OSA, 33 children (20%) were found to have mild OSA (OAHI ≥ 2/hrTST but < 5/hrTST), and 21 children (12%) were identified with moderate to severe OSA, with an OAHI ≥ 5/hrTST.

To avoid potential confounders introduced by age, sex, or race differences, we matched, based on these parameters, 54 of the 117 children with normal sleep studies to the 54 children with fulfilling polysomnographic criteria of OSA. A summary of the two matched groups is presented in Table 2. No significant differences emerged in age, sex, the proportion of children who were obese, BMI *z*-score, or the percentage of body fat among children with OSA when compared with the matched group of children without OSA (Table 2). Conversely and as expected from the group allocation criteria, significant differences were present in polysomnographic measures (Table 2, e-Table 1).

### *Endothelial Function Testing*

Children without OSA and children with mild or moderate to severe OSA were then stratified according to their obesity status. Analysis of the cutaneous laser-Doppler measures did not reveal any significant

**Table 2—*Demographic Summary of 54 Children With OSA Matched to 54 Control Subjects NOSA***

| Demographic Factors | NOSA (n = 54) | OSA (n = 54) | P Value |
|---|---|---|---|
| Age, y | 8.3 ± 0.2 | 7.9 ± 0.3 | NS |
| Sex | | | NS |
| Male | 36 | 32 | |
| Female | 18 | 22 | |
| Race | | | NS |
| White | 37 | 31 | |
| Black | 14 | 17 | |
| Biracial | 2 | 2 | |
| Hispanic | 1 | 4 | |
| Obesity | | | NS |
| Nonobese | 23 | 18 | |
| Obese | 31 | 36 | |
| BMI, kg/m² | 22.60 ± 0.92 | 23.57 ± 0.92 | NS |
| BMI *z*-score | 1.41 ± 0.18 | 1.70 ± 0.17 | NS |
| Lean body mass, kg | 27.8 ± 7.4 | 26.1 ± 7.5 | NS |
| Body fat mass, kg | 14.4 ± 11.5 | 15.7 ± 11.6 | NS |
| Body fat, % | 28.8 ± 15.0 | 32.7 ± 13.0 | NS |
| OAHI, events/h | 0.86 ± 0.49 | 7.36 ± 8.00 | < .001 |
| RAI, events/h | 0.41 ± 0.45 | 2.44 ± 2.13 | < .001 |
| $O_2$ saturation nadir, % | 91.0 ± 3.8 | 85.6 ± 9.4 | < .001 |
| Mean $P_{ETCO_2}$, mm Hg | 42.0 ± 3.8 | 42.5 ± 3.5 | NS |
| Mean systolic BP, mm Hg | 106.5 ± 10.3 | 106.5 ± 11.9 | NS |
| Mean SBPi | 0.91 ± 0.08 | 0.91 ± 0.07 | NS |
| Mean diastolic BP, mm Hg | 62.1 ± 7.5 | 64.2 ± 8.5 | NS |
| DBPi | 0.80 ± 0.09 | 0.84 ± 0.09 | NS |

Sex, race, and obesity expressed as total numbers. All other data expressed in mean ± SE. DBPi = diastolic BP index; NOSA = without obstructive sleep apnea; NS = nonsignificant; $O_2$ = oxygen; $P_{ETCO_2}$ = pressure of end-tidal carbon dioxide; RAI = respiratory arousal index; SBPi = systolic BP index. See Table 1 legend for expansion of the other abbreviations.

differences in resting blood flow, biologic zero flow during occlusion, and peak flow during postocclusion hyperemia (Table 3) among any of the six subgroups of children. At any level of OSA severity (none to moderate-severe), obesity was associated with significant prolongations in the Tmax (Fig 2, Table 3). The presence of OSA also resulted in progressive increases in the Tmax in a severity-dependent fashion (Fig 2, Table 3) in both groups: obese and nonobese. Accordingly, the absence of both obesity and OSA resulted in the shortest time to reperfusion peak flow (Tmax = 24.5 ± 1.3 s), while the presence of obesity and moderate to severe OSA resulted in the greatest time to peak reperfusion flow (Tmax = 60.1 ± 5.6 s, $P < .01$). Furthermore, endothelial dysfunction, defined as a Tmax cutoff value of ≥ 45 s, was absent among children who were nonobese without OSA, whereas the majority (62.5%) of children who were obese with OSA has Tmax values above the cutoff.

Although the SBPi was significantly increased at all levels of OAHI severity in children who were obese when compared with children who were nonobese with a similar OAHI the SBPi did not differ across OSA severity categories (Table 3). However, the DBPi was significantly greater in children who were obese with moderate to severe OSA compared with children who were obese without OSA, and also compared with children who were nonobese with mild OSA (Table 3).

In the univariate regression model, age was found to significantly correlate with Tmax ($r = 0.333$, $P < .01$) (Table 4). In addition, the Tmax was significantly associated with the BMI *z*-score ($r = 0.347$, $P < .01$) as well as with body fat mass and percentage of body fat. The OAHI was also significantly associated with the Tmax ($r = 0.401$, $P < .01$), and both the respiratory arousal index and oxygen saturation nadir were also significantly associated (Table 4). The Tmax was significantly associated with several serum lipids, including total cholesterol, total triglycerides, low-density lipoprotein, and very-low-density lipoprotein levels, and also with fasting insulin and with hsCRP and MRP8/14 concentrations.

After adjusting for age, BMI *z*-score, and OAHI, only MRP8/14 remained significantly associated with the Tmax ($r = 0.21$, $P < .05$) (Table 5). Furthermore, when replacing the OAHI with MRP8/14 in the multivariate model, the OAHI also remained significantly and strongly associated with the Tmax ($r = 0.364$, $P < .001$). In contrast, the BMI *z*-score was not significantly associated with the Tmax after adjusting for age, MRP8/14, and OAHI ($r = 0.188$, $P = .056$). In other words, the OAHI and MRP8/14 appear to contribute more important components of the variance of the Tmax than the BMI *z*-score (Fig 3).

The inclusion of body fat percentage instead of the BMI *z*-score as a marker of ponderosity revealed that the Tmax was strongly associated with the OAHI, even after adjustment for age and body fat percentage ($r = 0.357$, $P < .001$). After adjustment for age, OAHI, and body fat percentage, the correlation of MRP 8/14 with the Tmax was not statistically significant ($r = 0.153$, $P = .10$)

## Discussion

The major findings of the present study are to demonstrate the interrelationships between OSA and obesity on their adverse effect to a functional aspect of the microcirculation in children that is representative of endothelial integrity. Furthermore, this study illustrates the potential value of assessing the Tmax or MRP8/14 as reliable indicators of endothelial injury in the context of either obesity or OSA in children

Prior to discussing the potential implications of our findings, it is noteworthy to mention some of the strengths and potential limitations of this study. First

**Table 3—*BP and Perfusion Kinetic Measures of Endothelial Function in Children Who Are Prepubertal Stratified According to OSA Severity and Obesity Status***

| OSA Index | Obesity Status | SBPi | DBPi | Resting Flow (PU) | Biologic Zero (PU) | Peak Flow (PU) | Tmax (s) |
|---|---|---|---|---|---|---|---|
| OAHI < 2/hrTST (n = 54) | NOB (n = 23) | 0.86 ± 0.01 | 0.80 ± 0.02 | 21.1 ± 2.8 | 4.3 ± 0.5 | 49.1 ± 6.6 | 24.5 ± 1.3 |
| | OB (n = 31) | 0.94 ± 0.01[a] | 0.81 ± 0.02 | 22.6 ± 2.6 | 3.0 ± 0.2 | 41.7 ± 3.6 | 40.6 ± 2.9[b] |
| OAHI ≥ 2/hrTST | NOB (n = 13) | 0.85 ± 0.02[c] | 0.80 ± 0.03 | 24.1 ± 4.4 | 3.6 ± 0.6 | 46.0 ± 8.3 | 34.8 ± 3.7 |
| but < 5/hrTST (n = 33) | OB (n = 20) | 0.92 ± 0.01[b,d] | 0.83 ± 0.01 | 23.5 ± 4.1 | 3.7 ± 0.4 | 43.0 ± 8.4 | 47.1 ± 4.5[a] |
| OAHI ≥ 5/hrTST (n = 21) | NOB (n = 5) | 0.86 ± 0.01 | 0.79 ± 0.04 | 19.2 ± 3.2 | 4.2 ± 1.3 | 38.5 ± 7.9 | 43.8 ± 14.2 |
| | OB (n = 16) | 0.96 ± 0.01[a, e, f] | 0.89 ± 0.02[b, d,g] | 21.6 ± 3.7 | 3.0 ± 0.5 | 33.7 ± 5.4 | 60.1 ± 5.6[a,c,e] |

All data expressed in mean ± SE. NOB = nonobese; OB = obese; PU = perfusion unit; Tmax = time to peak regional blood flow response post-occlusion release. See Table 1 and 2 legends for expansion of the other abbreviations.

[a]$P < .01$ compared with NOB, OAHI < 2/hrTST.

[b]$P < .05$ compared with NOB, OAHI < 2/hrTST.

[c]$P < .01$ compared with OB, OAHI < 2/hrTST.

[d]$P < .05$ compared with NOB, OAHI ≥ 2 but < 5/hrTST.

[e]$P < .01$ compared with NOB, OAHI ≥ 2 but < 5/hrTST.

[f]$P < .05$ compared with NOB, OAHI ≥ 5/hrTST.

[g]$P < .05$ compared with OB, OAHI < 2/hrTST.

and foremost, the implementation of nocturnal PSG allowed for accurate assessment of the potential severity-dependent effect of OSA on endothelial function and its interactions with obesity. Second, the use of laser Doppler approaches for assessment of vascular responses following cuff-induced arterial occlusion permits the determination of kinetic characteristics of postischemic blood flow responses, and it does not only have a good degree of reproducibility in children and adults, but has also clinical implications in older patients.[30-32] Of note, neither arterial tonometry nor sonographic assessment of brachial or carotid flow responses have been critically validated in children, and the optimal maneuvers for critical determination of endothelial function remain to be delineated. Although our selection of the arterial occlusion time was set at 60 s rather than 5 min as is typically used in most ischemic challenges in the context of adult

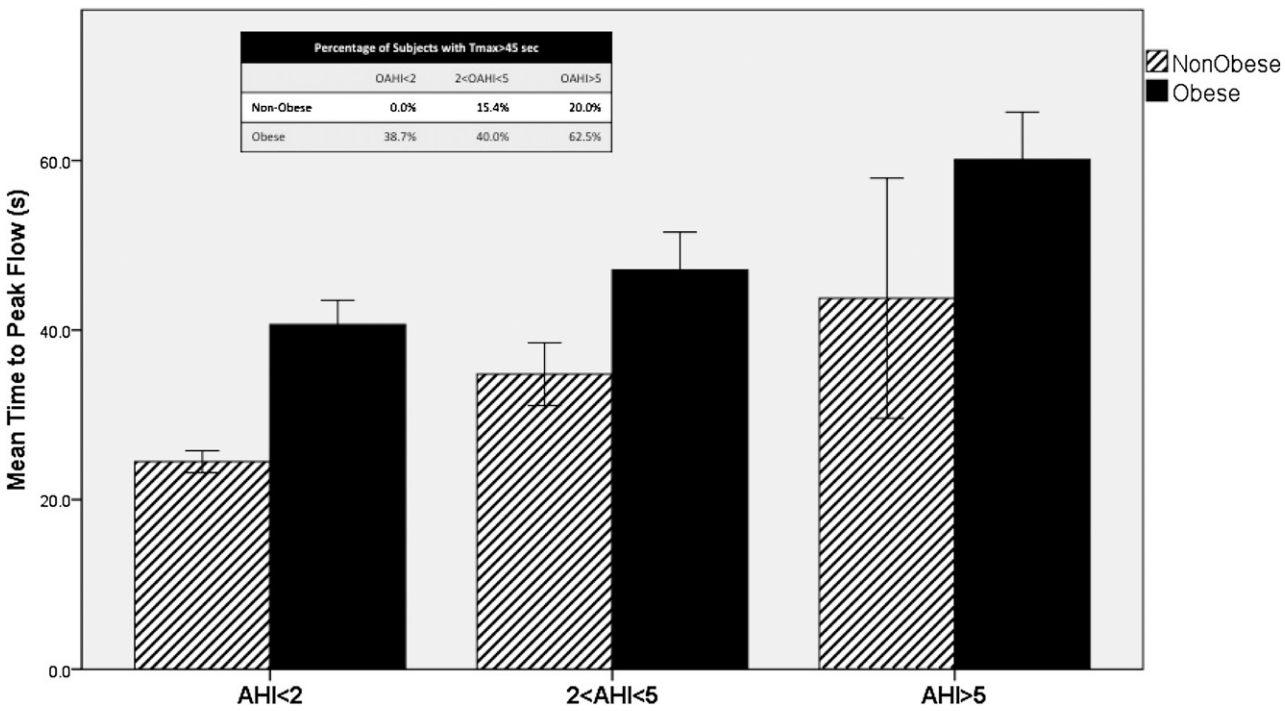


FIGURE 2. Graph shows endothelial function in children who are obese and nonobese, with and without obstructive sleep apnea (OSA). AHI = apnea-hypopnea index; OAHI = obstructive apnea-hypopnea index; Tmax = time to peak regional blood flow response postocclusion release.

**Table 4—*Univariate Coefficients of Correlation Between Time to Tmax and Indices of Obesity, OSA, and Circulating Biomarkers***

| Characteristic | Pearson Correlation | *P* Value |
|---|---|---|
| Age | 0.333 | < .01 |
| BMI | 0.422 | < .01 |
| BMI *z*-score | 0.347 | < .01 |
| Lean body mass | 0.301 | < .01 |
| Body fat mass | 0.464 | < .01 |
| Percentage of body fat | 0.508 | < .01 |
| Basal metabolic rate | 0.302 | < .01 |
| OAHI | 0.401 | < .01 |
| RAI | 0.545 | < .01 |
| $O_2$ saturation nadir | −0.304 | < .01 |
| Mean $P_{ETCO_2}$ | −0.02 | NS |
| Total cholesterol | 0.351 | < .01 |
| Total triglycerides | 0.243 | < .05 |
| LDL | 0.312 | < .01 |
| VLDL | 0.364 | < .05 |
| HDL | 0.022 | NS |
| Glucose | 0.057 | NS |
| Insulin | 0.406 | < .01 |
| hsCRP | 0.227 | < .05 |
| MRP8/14 | 0.343 | < .01 |

HDL = high-density lipoprotein; hsCRP = high-sensitivity C-reactive protein; LDL = low-density lipoprotein; MRP8/14 = myeloid-related protein 8/14; VLDL = very-low-density lipoprotein. See Table 1-3 legends for expansion of the other abbreviations.

studies, such a shorter occlusion time was preferred based on the priority of ensuring child comfort during testing and thus preventing movement and invalidation of a large proportion of the tests. However, we took substantial precautions during the early stages of the development and validation of this approach to ensure that the anticipated robust response of the nonarteriosclerotic vasculature would indeed be observable and reproducible in young children following the shorter occlusion time. As mentioned, all studies were conducted in the fasting state at the same time of the day to further minimize any variability introduced by differences in meal content and timing relative to the testing and also to ensure that

**Table 5—*Coefficients of Correlation Between Time to Tmax and Circulatory Biomarkers After Adjustment for Age, BMI, and OAHI***

| Biomarker | β In | *P* Value | Partial Correlation |
|---|---|---|---|
| Total cholesterol | 0.167 | NS | 0.193 |
| Total triglycerides | 0.041 | NS | 0.047 |
| LDL | 0.136 | NS | 0.158 |
| VLDL | 0.162 | NS | 0.212 |
| HDL | 0.094 | NS | 0.114 |
| Glucose | 0.063 | NS | 0.076 |
| Insulin | 0.177 | NS | 0.188 |
| hsCRP | 0.024 | NS | 0.028 |
| MRP8/14 | 0.21 | .02 | 0.236 |

β In = beta coefficient of correlation. See Table 1-3 legends for expansion of abbreviations.

circadian variation in endothelial function would not play a role in our findings. Here, we report for the first time, to our knowledge, that chronologic age appears to impose a significant effect on the Tmax, further emphasizing the importance of matching children according to some demographic characteristics such as age, sex, and race. Furthermore, we excluded children who had received a diagnosis of diabetes or elevated fasting insulin/glucose, as either diabetes or prediabetes can be associated with endothelial dysfunction.[38] Notwithstanding, larger cohorts are needed to further confirm the findings reported herein and to enable comparisons with the several adult cohort studies that have thus far examined the link between cardiovascular disease and OSA.[39,40] Furthermore, such expansive studies in children should enable potential associations with other markers of subclinical cardiovascular disease, such as systemic biomarkers, arterial tonometry, or intima-media thickness in larger arteries.

Both obesity and OSA emerged as significant and independent risk factors of endothelial dysfunction in children who were prepubertal, with OSA clearly showing a relatively large severity-dependent effect size on the Tmax. Conversely, the strength of the adjusted association between the Tmax and the BMI *z*-score was much weaker and displayed only a tendency toward significance, thereby reinforcing the need for much larger cohorts to conclusively determine the relative contribution of obesity to vascular dysfunction. Of note, a recent study in 51 children who were obese demonstrated that OSA exacerbates sonographically determined existing abnormalities in flow-mediated dilation of the brachial artery and the incremental elastic modulus of the common carotid artery.[41] However, these investigators did not concurrently examine children who were nonobese, such that comparisons between this study and the present one are not possible. Our hypothesis suggesting that obesity and OSA interact to exacerbate the risk of endothelial dysfunction was confirmed. Indeed, the proportion of children whose Tmax was > three standard deviations beyond the normative mean found in healthy children (ie, > 45 s) increased with each degree of OSA severity and increased with the presence of isolated obesity, but was maximal (62%) when both OSA and obesity were concomitantly present.

Although the precise mechanisms mediating endothelial dysfunction in children are yet to be elucidated, examination of previously identified candidate biomarkers of atherogenesis in children could provide some insights into some of the pathways activated by obesity or OSA that ultimately lead to endothelial dysfunction. We have shown previously that hsCRP is increased in the context of OSA in children, even those who are nonobese, and that such increases are

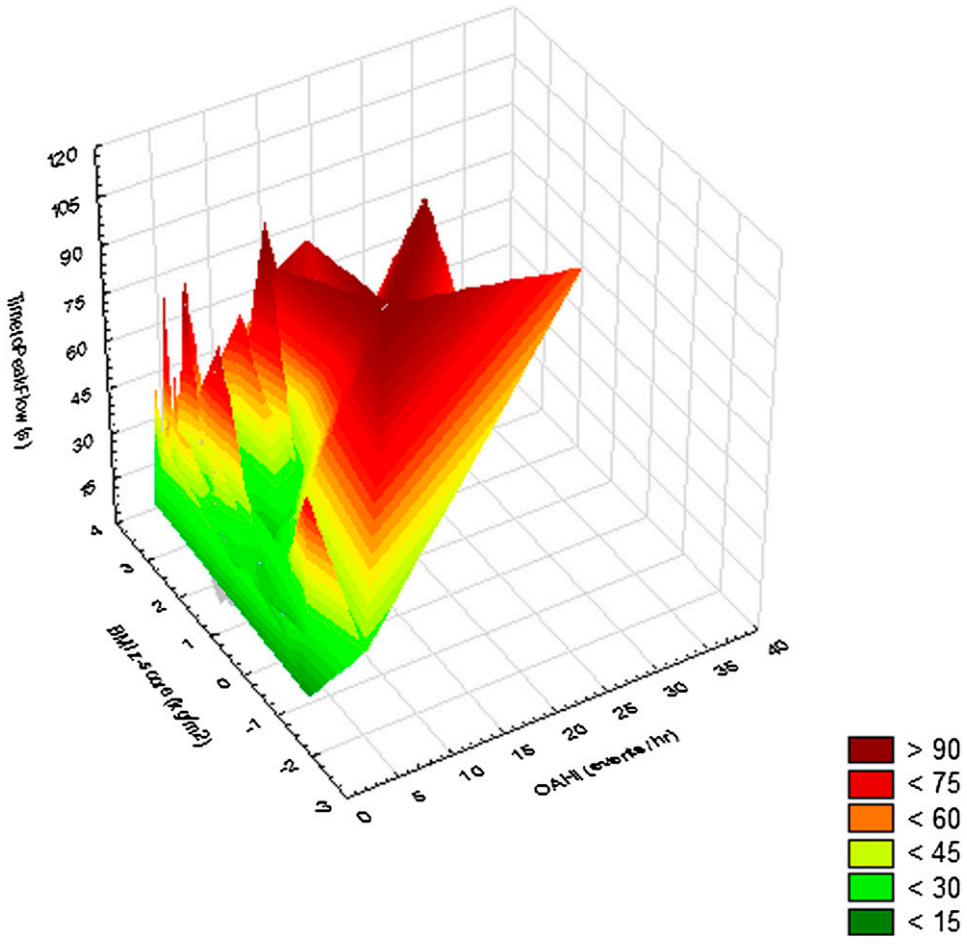


FIGURE 3. Wafer graph illustrates the associations between time to peak flow, BMI *z*-score, and OAHI. See Figure 2 legend for expansion of the abbreviations.

reversed upon treatment.[42-44] Thus, the assumption linking OSA to inflammation, with the latter mediating end-organ injury, appears justified. We have also shown that MRP8/14 levels are increased in pediatric OSA,[22] that such increases are exacerbated by the presence of obesity,[21,22] and that increases in MRP8/14 concentrations are highly associated with endothelial dysfunction.[21] In the present study, MRP8/14 plasma levels, but not hsCRP levels, emerged as the sole inflammatory marker associated with endothelial dysfunction after adjusting for age, BMI *z*-score, and OAHI. Furthermore, if the model was instead adjusted for MRP8/14, only the OAHI, and not the BMI *z*-score, remained significantly associated with the Tmax. Although the BMI *z*-score is routinely used as a clinical marker of obesity, anthropometric measures enabling the determination of body-fat percentage revealed that the OAHI was independently associated with the Tmax, but that MRP8/14 reflected the coordinated contributions of both the severity of OSA and the severity of obesity. Therefore, the current findings suggest that the effects of the OAHI and obesity on endothelial dysfunction may involve MRP8/14-related pathways. More importantly, MRP 8/14 levels emerge as a useful surrogate reporter of OSA- and obesity-mediated endothelial injury. This assumption is not far-fetched, however, considering the known functions of MRP8/14. MRP8/14 has been shown to regulate vascular inflammation and to contribute to the biologic responses to vascular injury,[45-47] and it is significantly correlated with both hsCRP and IL-6 circulating levels in children.[21] Therefore, plasma MRP8/14 levels may not only provide a reliable marker of OSA, but serve also as a reporter of the magnitude of the inflammatory response in the context of OSA and obesity.

We should remark that given the overall large variance in the associations described heretofore, other important modifiers of the Tmax may be operational. In this context, the potential role played by the magnitude of the autonomic sympathetic outflow activity and the recruitment of endothelial progenitor cells among children with OSA and those with obesity and the presence of endothelial dysfunction merits further investigation[48,49] In addition, the presence of endothelial dysfunction in OSA may be reflective of

a broader morbid phenotype involving other end organs, such as the CNS.[50]

## Conclusions

In summary, both OSA and obesity appear to independently confer increased risk for endothelial dysfunction in children who are prepubertal, and such individual effects are magnified by the coexistence of two. Furthermore, MRP8/14 morning plasma levels emerge as a solid surrogate indicator of endothelial dysfunction. Based on these findings, further studies assessing the reversibility of these processes and their long-term implications for the cardiovascular system are clearly needed and justified.

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*Dr Bhattacharjee:* contributed to study design, subject recruitment, vascular function testing, polysomnography scoring, preliminary data analyses, manuscript drafting, and editing and approval of the final manuscript.
*Dr Kim:* contributed to biomarker assays (enzyme-linked immunosorbent assays) and approval of the manuscript.
*Dr Alotaibi:* contributed to vascular function testing, recruiting subjects, and approval of the manuscript.
*Dr Kheirandish-Gozal:* contributed to recruiting subjects, performing vascular function studies, and approval of the manuscript.
*Dr Capdevila:* contributed to polysomnography scoring and approval of the manuscript.
*Dr Gozal:* contributed to the initial conceptual framework and preliminary data analysis, provided financial support, and edited the final manuscript.
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