Plasma Adhesion Molecules in Children With Sleep-Disordered Breathing*

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Study objectives: **To determine whether childhood sleep-disordered breathing (SDB) is associated with elevated levels of plasma adhesion molecules.**
Design: **Prospective, observational study.**
Setting: **Sleep Medicine Center of Kosair Children's Hospital.**
Participants: **Thirty-nine children with SDB (apnea-hypopnea index [AHI] > 5/h), 47 children with mild SDB (AHI 1 to 5/h), and 42 healthy control subjects (AHI < 1/h).**
Measurements and results: **One hundred twenty-eight children underwent a standard polysomnographic assessment with a blood draw the following morning. Plasma levels of CRP and the adhesion molecules intercellular adhesion molecule (ICAM)-1 and P-selectin were measured. No differences were observed in ICAM-1 levels among the groups; however, obese children had higher ICAM-1 levels than nonobese children (425.0 ± 123.0 ng/mL vs 375.6 ± 107.1 ng/mL, $p = 0.04$) [mean ± SD]. P-selectin levels were significantly higher in the SDB group (84.0 ± 52.2 ng/mL) and the mild SDB group (89.3 ± 49.9 ng/mL) when compared to control subjects (49.5 ± 22.3 ng/mL; $p < 0.001$ for both groups). Furthermore, P-selectin correlated with AHI ($r = 0.32$, $p < 0.001$), respiratory arousal index ($r = 0.27$, $p = 0.002$), and nadir of oxygen saturation as measured by pulse oximetry ($r = -0.19$, $p = 0.038$). Plasma CRP levels were found to correlate with P-selectin even after controlling for BMI ($r = 0.20$, $p = 0.05$). No correlations were found between CRP and ICAM-1.**
Conclusions: **Children with SDB have plasma elevations of P-selectin, a marker of platelet activation, lending support to the premise that inflammatory processes are elicited by SDB in children, and may contribute to accelerated risk for cardiovascular morbidity. In contrast, elevations in ICAM-1 are primarily associated with obesity rather than SDB.**
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Key words: adhesion molecules; atherosclerosis; sleep-disordered breathing

Abbreviations: AHI = apnea-hypopnea index; BMI = body mass index; CRP = C-reactive protein; ICAM = intercellular adhesion molecule; SDB = sleep-disordered breathing; Spo_2 = oxygen saturation as measured by pulse oximetry; TST = total sleep time

Sleep-disordered breathing (SDB) in adults is now widely recognized as an important and independent risk factor for cardiovascular morbidity, including hypertension, ischemic heart disease, and cerebrovascular accidents.[1–5] One of the potential mechanisms linking the strong association between

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SDB and cardiovascular morbidity is that SDB-induced hypoxic stress modulates the expression of circulating inflammatory mediators and may lead to accelerated atherogenesis. C-reactive protein (CRP), an important serum marker of inflammation, has emerged as one of the most powerful independent predictors of risk for cardiovascular morbidity.[6,7] Indeed, CRP may participate in atheromatous lesion formation through leukocyte activation and endothelial dysfunction.[8] Adhesion of circulating leukocytes to endothelial cells is considered one of the initial steps in the pathogenesis of atherosclerosis,[9] and this process is mediated by cellular adhesion molecules.[10] Elevated levels of adhesion molecules such as intercellular adhesion molecule (ICAM)-1, and vascular cell adhesion molecule-1, and E-selectin have been reported in adults with SDB[11,12] and are correlated with the severity of the disease. P-selectin, another member of the selectin family of molecules that is expressed on the surface of activated platelets, is hypothesized to play a role in the initiation of atherogenesis, and is viewed as a circulating biomarker affording prediction of adverse cardiovascular events.[13,14]

Evidence is now emerging for the presence of cardiovascular perturbations in children with SDB. For example, alterations in autonomic function,[15–17] BP control,[18–21] and left ventricular function[22,23] have all been reported. Furthermore, elevations in circulating CRP levels have also been described in children with SDB and correlate with the severity of disease.[24] While this finding has not been confirmed in another study,[25] since CRP appears to contribute to the pathophysiology of atherogenesis in a process that is mediated by adhesion molecules, we hypothesized that children with SDB would have increased levels of two potential biomarkers of cardiovascular pathology, namely ICAM-1 and P-selectin, compared to children without SDB.

Materials and Methods

Children were recruited to this study from two sources: children undergoing clinical evaluation for suspected SDB who were referred to the Kosair Children's Hospital Sleep Medicine and Apnea Center in Louisville, KY, and those children participating in a larger community-based study in Louisville, KY. The latter community group provided control children. All children were recruited between January and August of 2004. All subjects underwent a standard overnight polysomnographic evaluation and a blood draw at 7 AM the following morning. The study was approved by the Institutional Review Board of the University of Louisville, and parental consent and child assent in the presence of a parent or legal caretaker were obtained.

A standard overnight multichannel polysomnographic evaluation was performed in the sleep laboratory. Children were studied for up to 12 h in a quiet, darkened room with an ambient temperature of 24°C in the company of one of their parents. No drugs were used to induce sleep. The following parameters were measured: chest and abdominal wall movement by respiratory impedance or inductance plethysmography, heart rate by ECG, air flow by oronasal thermistor, sidestream end-tidal capnography that also provided breath-by-breath assessment of end-tidal carbon dioxide levels (BCI SC-300; BCI; Menomonee Falls, WI), and a nasal pressure transducer (ProTech Services; Mukilteo, WA). Oxygen saturation as measured by pulse oximetry (Spo_2) was also assessed (Nellcor N 100; Nellcor; Hayward, CA), with simultaneous recording of the pulse waveform. The bilateral electro-oculogram, eight channels of EEG, chin and anterior tibial electromyograms, and analog output from a body position sensor (Braebon Medical Corporation; Ogdensburg, NY) were also monitored. All measures were digitized using a commercially available polysomnography system (Rembrandt; MedCare Diagnostics; Amsterdam, the Netherlands). Tracheal sound was monitored with a microphone sensor (Sleepmate; Midlothian, VA), and a digital time-synchronized video recording was made.

Sleep architecture was assessed by standard techniques.[26] Arousals were defined as recommended by the American Sleep Disorders Association Task Force report[27] using the 3-s rule and/or the presence of movement arousal.[28] Arousals were classified as two types: spontaneous arousals and respiratory arousals. The mean Spo_2 in the presence of a pulse waveform signal void of motion artifact, and the nadir Spo_2 were recorded. Obstructive apnea was defined as the absence of airflow with continued chest wall and abdominal movement for duration of at least two breaths.[29,30] Hypopneas were defined as a decrease in nasal flow of $\geq 50\%$ with a corresponding decrease in Spo_2 of $\geq 4\%$ and/or arousal.[30] The apnea-hypopnea index (AHI) was defined as the number of obstructive apneas and hypopneas per hour of total sleep time (TST). Children with an AHI ≥ 1 but < 5/h of TST were considered to have mild SDB, while children with AHI ≥ 5/ h of TST were considered to have SDB. Control children were defined as nonsnoring children with AHI < 1/h of TST. These children were recruited from the community study.

Plasma was collected and frozen at -80°C until analysis of adhesion molecules. Circulating levels of ICAM-1 and P-selectin were measured with commercially available kits (R&D Systems; Abington, UK). For ICAM-1, the sensitivity was 0.35 ng/mL and the intra-assay and interassay coefficients of variation were 2.5% and 1.8%, respectively. For P-selectin, the sensitivity was 0.5 ng/mL and the intra-assay and interassay coefficients of variation were 3.6% and 6.9%, respectively. In addition to adhesion molecules, a subgroup of children had plasma levels of CRP measured the morning following the sleep study. Plasma CRP was measured (Flex reagent Cartridge; Date Behring; Newark, DE) 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 interassay coefficients of variability of 9% and 18%, respectively.

Height and weight were obtained from each child immediately prior to the hookup for the overnight sleep study, and body mass index (BMI) was calculated and standardized for age and gender. Height was measured using a wall stadiometer accurate to the nearest 0.5 cm (Accustat-Stadiometer; Genentech; San Francisco, CA), and weight was measured in kilograms to one decimal place using digital scales. Children were considered obese if the standardized BMI was $>$ 95th percentile.[31]

Data Analysis

Data are presented as mean ± SD or mean and 95% confidence interval (CI) unless otherwise indicated. Statistical comparisons were made (SPSS version 13; SPSS; Chicago, IL). Comparisons of demographics according to group assignment were made with independent *t* tests or analysis of variance followed by *post hoc* comparisons, with p values adjusted for

unequal variances when appropriate (Levene test for equality of variances), or χ^2 analyses with Fisher exact test (dichotomous outcomes). Bivariate correlations between ICAM-1 and P-selectin levels with age, BMI, AHI, arousal indexes, and Spo_2 nadir were performed. Since BMI is a potential confounding variable, correlations were repeated while controlling for BMI. In addition, general linear modeling was performed to determine the group differences in adhesion molecules while controlling for BMI. Furthermore, analyses were repeated while excluding children with a BMI > 95th percentile in order to conclusively rule out the role of obesity. Lastly, regression analyses were performed using the adhesion molecules as dependent variables. All p values reported are two tailed, with statistical significance set at < 0.05.

Results

A total of 128 children (51% male) underwent polysomnographic evaluation with a morning blood draw. The mean age of the population was 6.9 ± 1.2 years (range, 4.0 to 10.0 years). Sixty percent of the samples were white, 37% were African American, and 4% were of other ethnic backgrounds. A total of 39 children were found to have SDB (AHI > 5/h), 47 children had mild SDB, and 42 children were classified as control subjects (AHI < 1/h). All of the control children were recruited from the community sample of children; no child clinically referred for evaluation of SDB was found to have an AHI < 1. Thirty-eight children (30%) children were obese. Table 1 shows the demographic results of each group. There were no significant differences in gender, age, or proportion of obesity between the groups, although the mean BMI of the SDB group was significantly higher than that of the control group (p = 0.012).

ICAM-1

Plasma ICAM-1 levels were significantly higher in the SDB group compared to the control group (421.7 ± 115.6 ng/mL vs 369.8 ± 116.4 ng/mL, respectively; p = 0.044). Children with mild SDB had mean ICAM-1 levels of 379.9 ± 105.9 ng/mL, which were not significantly different from either the SDB or control groups. No correlations were found between ICAM-1 and age, although ICAM-1 was found to correlate with BMI ($r = 0.29$, p = 0.02). Obese children had higher ICAM-1 levels than nonobese children (425.0 ± 123.0 ng/mL vs 375.6 ± 107.1 ng/mL, respectively; p = 0.04; Fig 1), and no differences in ICAM-1 levels were found in nonobese children with and without SDB. Further analysis using general linear modeling found that ICAM-1 levels were no longer different between SDB and control groups once BMI was controlled for. Furthermore, ICAM-1 levels were not found to correlate with AHI, arousal indexes, or Spo_2 nadir whether or not BMI was accounted for in the model. No differences in ICAM-1 levels were found between male and female patients or between African-American and white patients.

P-Selectin

Plasma P-selectin levels were significantly higher in the SDB group (84.0 ± 52.1 ng/mL) and mild SDB group (89.3 ± 49.9 ng/mL) compared to the control group (49.5 ± 22.3 ng/mL; $p < 0.001$ for both comparisons). No differences were found between the SDB and mild SDB groups. Figure 2 shows the mean and 95% CI for P-selectin values in each group. There were no correlations between age or BMI and P-selectin. Bivariate correlations showed that P-selectin was positively correlated with AHI ($r = 0.32$, $p < 0.001$; Fig 3) and respiratory arousal index ($r = 0.27$ p = 0.002; Fig 4). Furthermore, P-selectin was negatively correlated with Spo_2 nadir ($r = -0.19$, p = 0.038; Fig 5). These correlations persisted after controlling for BMI. No differences were found in P-selectin levels between male and

Table 1—*Demographics and Sleep Parameters for Children With SDB, Mild SDB, and Control Subjects

Variables	SDB (n = 39)	Mild SDB (n = 47)	Control Subjects (n = 42)
Age, yr	7.2 ± 1.8	6.7 ± 1.0	6.9 ± 0.6
Male gender, %	44	64	43
BMI	21.4 ± 7.2†	18.6 ± 5.7	17.5 ± 3.8
Obese	42	30	20
AHI	15.2 ± 11.1§	2.1 ± 1.2§	0.3 ± 0.3
Spontaneous arousal index	5.9 ± 4.3†	6.2 ± 4.6‡	9.5 ± 4.3
Respiratory arousal index	9.3 ± 11.7§	2.4 ± 2.1§‖	0.4 ± 0.8
Mean Spo_2	96.0 ± 2.8§	97.3 ± 1.2†‖	97.9 ± 0.5
Spo_2 nadir	80.3 ± 12.6§	90.8 ± 3.6§‖	93.8 ± 2.9

*Data are presented as mean ± SD or %.
†$p < 0.05$ compared to control subjects.
‡$p < 0.01$ compared to control subjects.
§$p < 0.001$ compared to control subjects.
‖$p < 0.001$ compared to SDB.

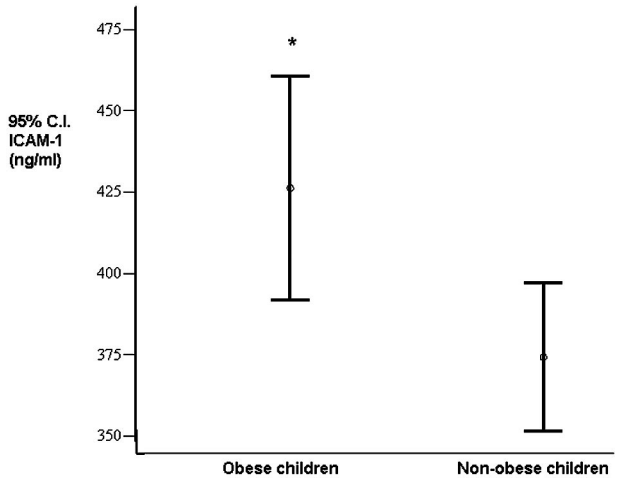

FIGURE 1. Comparison of ICAM-1 levels in obese and nonobese children; *p < 0.01.

female, obese and nonobese, or between African-American and white patients.

To further account for the potential role of obesity in the elevation of P-selectin levels, we repeated our analyses using only the nonobese children in the cohort (n = 90). P-selectin levels remained significantly elevated in the SDB group (81.9 ± 56.9 ng/mL) and mild SDB group (89.7 ± 46.1 ng/mL) compared to control subjects (48.1 ± 21.0 ng/mL; $p < 0.05$ between SDB and control subjects; $p < 0.001$ between mild SDB and control subjects). Furthermore, significant correlations persisted between P-selectin and AHI ($r = 0.45$, $p < 0.001$), P-selectin and respiratory arousal index ($r = 0.34$, $p = 0.001$), and P-selectin and Spo_2 nadir ($r = -0.19$, $p < 0.05$). No differences in mean ICAM-1 levels between the groups were found in this nonobese cohort, and no correlations emerged between ICAM-1 and any of the sleep measures.

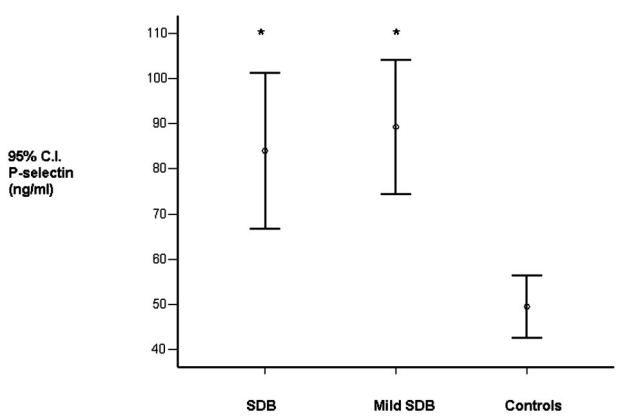

FIGURE 2. Comparison of P-selectin levels in SDB, mild SDB, and control children; *p < 0.001 compared to control group.

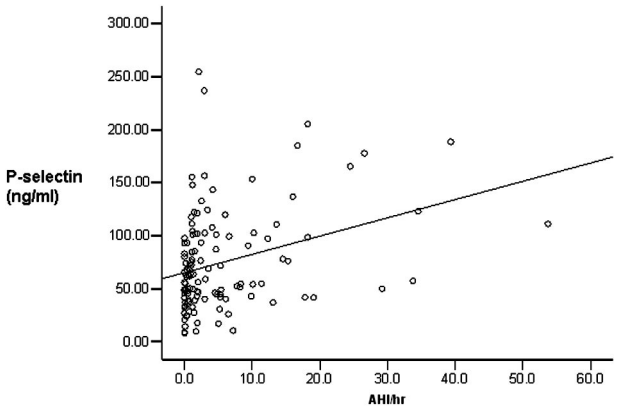

FIGURE 3. Correlation between P-selectin and AHI for the whole group ($r = 0.32$; $p < 0.001$).

CRP

A subgroup of 83 children (23 SDB, 29 mild SDB, and 31 control) also had plasma CRP levels measured. This subgroup comprised all children recruited to the study following the addition of CRP to the protocol. While there was a trend for increasing CRP with severity of disease, this did not reach statistical significance (0.32 ± 0.49 mg/dL vs 0.28 ± 0.76 mg/dL vs 0.15 ± 0.23 mg/dL for SDB, mild SDB, and control subjects, respectively). However, plasma CRP levels were found to correlate with P-selectin even after controlling for BMI ($r = 0.20$; $p = 0.05$). No correlations were found between CRP and ICAM-1.

Regression Analysis

Linear regression analysis was performed using both P-selectin and ICAM-1 as the dependent variable, with gender, BMI, and AHI as covariates. For P-selectin, only AHI predicted some of the variance

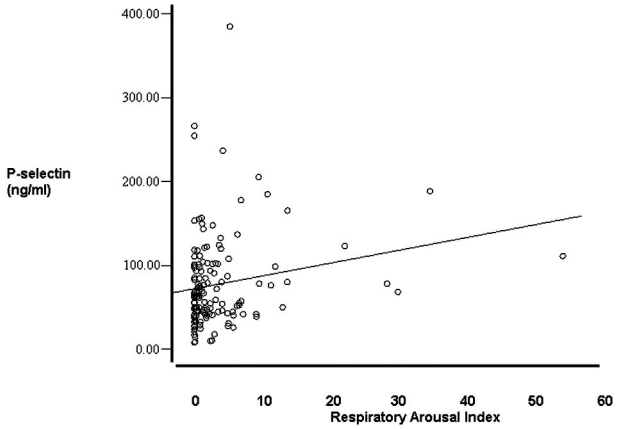

FIGURE 4. Correlation between P-selectin and respiratory arousal index for the whole group ($r = 0.27$; $p = 0.002$).

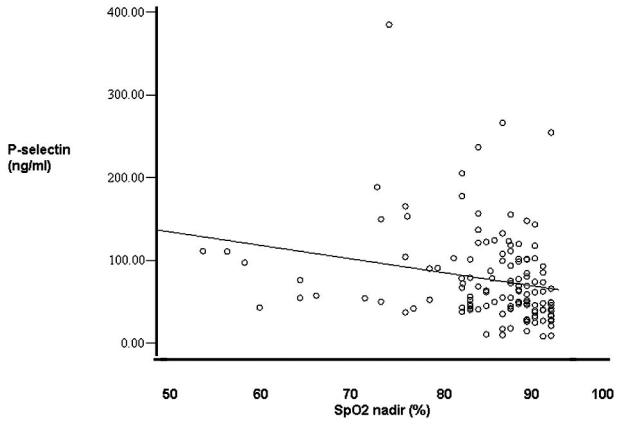

FIGURE 5. Correlation between P-selectin and SpO_2 nadir for the whole group ($r = -0.19$; $p = 0.038$).

(adjusted $R^2 = 8.2\%$; $p = 0.003$) after controlling for the other variables. In contrast, BMI predicted 8.4% (adjusted R^2) of the variance in ICAM-1 levels ($p < 0.001$).

DISCUSSION

This study conclusively demonstrates that children with SDB have elevated circulating levels of plasma P-selectin, a surface marker of platelet activation, and that P-selectin is correlated with components of SDB, namely the severity of oxyhemoglobin desaturation and the respiratory arousal index. Our current findings suggest that inflammatory processes associated with atherogenesis and leading to activation of P-selectin are elicited by SDB in children. Interestingly, although elevated ICAM-1 levels were found in obese children compared to nonobese children, no differences in ICAM-1 levels emerged between SDB and control groups, suggesting that in children elevations in ICAM-1 are significantly associated with obesity rather than with SDB *per se*.

Increased expression of platelet activation and adhesion molecules has been described in several studies[12,32–38] in adults with SDB, and has been postulated as a mechanism underlying the increased prevalence of cardiovascular morbidity in these patients.[39] The immediate physiologic consequences of SDB such as hypoxemia and sleep fragmentation can induce increased neural sympathetic discharge, which in turn may induce platelet activation, especially during sleep.[40] Similarly, the intermittent hypoxia associated with SDB cannot only promote increased sympathetic tone but can also promote free-radical formation, which leads to activation of transcriptional factors that up-regulate the expression of adhesion molecules.[41–44] Increased platelet activation markers have been found in patients with sickle-cell disease and were found to correlate with the severity of nocturnal hypoxemia.[45–46] Interestingly, we found that even children with mild SDB (AHI of 1 to 5/h) had significantly elevated P-selectin levels. This may suggest that even relatively mild alterations in gas homeostasis during sleep and disturbances in sleep continuity may exert profound effects on the expression of surface activation markers in circulating platelets, and could ultimately promote acceleration of the atherogenic process in susceptible children. Furthermore, we found a correlation between P-selectin and CRP levels, which supports our previous findings that SDB in children may be associated with acceleration of the atherosclerosis process in children.[24]

Platelet activation is enhanced by obesity in adults[47,48] and appears to correlate with serum cholesterol levels in obese children.[49] However, we failed to identify any correlation between BMI and P-selectin in our cohort, a finding that is in agreement with other published results by Ponthieux et al[47] in a group of children aged 4 to 17 years. Taken together, our results suggest that platelet activation may not be as strongly related to obesity in children as it appears to be in adults. Other studies[50,51] have also described an association between age and adhesion molecule levels in children; however, we did not find any correlation with age for either P-selectin or ICAM-1 in the present cohort.

It was somewhat surprising that significant differences in ICAM-1 levels did not occur in SDB children after controlling for BMI. Indeed, the currently available data in the adult literature shows that ICAM-1 circulating levels are elevated in patients with SDB,[11,12,34] and that such levels will be reduced following treatment with continuous positive airway pressure.[11] Furthermore, an *in vitro* study[52] has shown that hypoxia/reoxygenation induces an increase in the expression levels of adhesion molecules. *In vitro* studies[8,53] on human vascular endothelial cells have also demonstrated that CRP increases ICAM-1 expression in a dose-dependent fashion, and CRP has been found to be a positive determinant of ICAM-1 levels in children.[47] Nonetheless, we were unable to replicate the association between CRP and ICAM-1 in our sample. As potential confounders of ICAM-1 levels, adults may have coincident exposure to cigarette smoking and/or have subclinical cardiovascular disease, both of which may increase ICAM-1.[47,54,55] None of the children in our cohort reported smoking cigarettes or had documented cardiovascular disease. It is possible that soluble ICAM-1 levels in children are predominantly determined by the degree of obesity rather than by the severity of SDB. Alternatively, the more robust elevations in ICAM-1 observed in adults with SDB could be related to interactions between the duration of disease and overall SDB severity, both of which are likely to be less in pediatric patients.

Several limitations of this study deserve comment. Although the approach is widely used, circulating levels of soluble adhesion molecules may not reliably represent what is happening at the vascular tissue level, thereby necessitating more invasive assessments such as vascular biopsies. Alternatively, noninvasive vascular functional assessments may provide the opportunity to examine correlations with expression of circulating P-selectin and ICAM-1.[56–58] Such interventions are clearly beyond the scope of the present study. Although we found a trend for increasing CRP levels with increasing severity of disease, this relationship did not reach statistical significance. This may be due to the relatively small sample of children with CRP levels since we have previously shown a correlation between CRP and AHI.[24] Furthermore, while we found elevated levels of P-selectin in children with SDB, a cause-effect relationship cannot be conclusively demonstrated without a treatment arm, which was beyond the scope of this study. Additional studies measuring adhesion molecules before and after treatment are clearly required.

Notwithstanding such considerations, the apparent SDB-related increases in the expression of P-selectin in the plasma of children suggest the intriguing possibility that the occurrence of SDB during childhood may promote the onset and progression rate of atherosclerosis, particularly in risk-prone populations. Intriguingly, an association between apolipoprotein subtypes (specifically the ϵ4 allele) with both sleep apnea and atherogenesis has been noted.[59,60] Thus, it is possible that children at higher risk for the pathogenesis of atherosclerosis may sustain further aggravation of their underlying vascular disease if SDB develops and is left untreated. In support of such contention, a recent study[61] from our laboratory in mice deficient for apolipoprotein E revealed that intermittent hypoxia during sleep promoted the occurrence of dislipidemia and facilitated the generation of atherogenous plaques.

In summary, children with SDB display significant severity-dependent increases in the levels of circulating P-selectin, a biomarker of platelet activation that has been closely linked to atherosclerosis and cardiovascular risk. These biochemical alterations suggest that SDB induces an overall inflammatory response that ultimately modulates the activation of pathophysiologic processes underlying endothelial dysfunction and atheromatous plaque deposition, and that serial assessments of biomarkers such as P-selectin before and following SDB treatment may prove useful in identifying at-risk individuals.

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