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Myeloid Related Protein 8/14 levels in Children with Obstructive Sleep Apnoea

Jinkwan Kim[1], PhD, Rakesh Bhattacharjee[2], MD, Ayelet B. Snow[2], MD, Oscar Sans Capdevila[2], MD, Leila Kheirandish-Gozal[1], MD, David Gozal[1], MD.

[1]Departments of Pediatrics, University of Chicago, Chicago, IL

[2]Kosair Children's Hospital Research Institute, Department of Pediatrics, University of Louisville, Louisville, KY

Running Head: MRP 8/14 and pediatric OSA.

Corresponding Author: David Gozal, MD, Professor and Chair, Department of Pediatrics, Physician-in-Chief, Comer Children's Hospital, Pritzker School of Medicine, The University of Chicago, 5721 S. Maryland Avenue, MC 8000, Suite K-160 Chicago, IL 60637.

Phone: (773) 702-6205; FAX: (773) 702-4523; E-mail: dgozal@peds.bsd.uchicago.edu

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ABSTRACT

Background: Obstructive sleep apnoea (OSA) is common in children, and leads to multiple end-organ morbidities. Myeloid-related protein (MRP) 8/14 plays an important pathophysiological role in atherosclerosis, and plasma levels correlate with endothelial cell dysfunction. We hypothesized that MRP 8/14 levels would be altered with children with OSA.

Methods: 255 children (age: 7.6±1.5 years) were included after a sleep study and a morning blood sample. MRP 8/14 and IL-6 plasma levels were assayed using ELISA, and C-reactive protein by immunoturbidometry. Endothelial function was assessed as the hyperemic response after occlusion of the brachial artery.

Results: Plasma log MRP 8/14 levels showed AHI dose-dependent increases regardless of obesity. Moreover, log MRP 8/14 levels correlated with log AHI ($r=0.340$, $P<0.001$) after controlling for age and BMI z score, and with endothelial function. Children with highest MRP levels (>1.34 ug/ml) had 2.4 fold and 5.4 fold increased odds of mild, and of moderate to severe OSA, respectively, after adjusting for confounding variables.

Conclusions: Plasma MRP 8/14 levels are associated with pediatric OSA and may reflect increased risk for cardiovascular morbidity. The short-term and long-term consequences of elevated MRP 8/14 on cardiovascular function in the context of pediatric OSA remain to be defined.

KEY WORDS: Obstructive sleep apnoea, Myeloid related protein 8/14, Atherosclerosis, Inflammation, Endothelial dysfunction.

LIST OF ABBREVIATIONS

AHI: Obstructive Apnoea Hypopnoea Index

CRP: C-reactive protein

CVD: Cardiovascular Disease

MRP 8/14: Myeloid Related Protein 8/14

OSA: Obstructive Sleep Apnoea

INTRODUCTION

Obstructive sleep apnoea (OSA) is characterized by repeated events of partial and complete upper airway obstruction during sleep, which results in disruption of normal ventilation, hypoxemia, and sleep fragmentation. Increasing evidence from several lines of investigation strongly supports the concept that OSA in adults is pathophysiologically linked to cardiovascular diseases (CVD), such as hypertension, ischemic heart disease, and cerebrovascular disease [1, 2]. Similar to adult patients with OSA, pediatric OSA has been recently associated with a high risk of cardiovascular morbidities and metabolic dysfunction, particularly among obese children [3-6]. Increased generation of reactive oxygen species and systemic inflammatory responses related with hypoxia-reoxygenation events and to sleep fragmentation are mechanistically involved in acceleration and propagation of atherogenesis [7-9]. However, the mechanisms underlying the association between OSA and CVD are currently not fully understood.

Myeloid-related protein (MRP) 8 and MRP 14, are S100 proteins, and serve as important calcium-binding proteins in the process of phagocytosis. Indeed, non-covalently bound MRP 8/MRP 14 complexes are secreted by activated phagocytes under various inflammatory conditions [10-14]. As a corollary to these observations, MRP8/14 has been identified as an important predictor of cardiovascular disease [15, 16]. Indeed, MRP 8/14 protein complexes play an important role in atherosclerosis, and are closely correlated with inflammatory processes within the endothelial wall [17, 18]. We have previously shown that children with OSA, even non-obese, exhibit elevations in several systemic inflammatory markers that suggest the presence of increased risk for atherosclerosis [19-21]. Furthermore, we have recently shown that children with severe

OSA display reversible alterations in endothelial function, when the latter is determined using post-occlusion hyperemic response [22]. Not surprisingly, there is growing interest in identification of biomarkers that can serve as an early detection strategy of CVD risk factors, with the anticipation that timely interventions could reduce the risk for future cardiovascular events. Based on aforementioned considerations, we hypothesized that plasma MRP 8/14 levels would be elevated and serve as potential predictors of cardiovascular risk in children with OSA.

Subjects and Methods

Subjects

The study was approved by the University of Louisville Human Research Committee, and informed consent was obtained from the legal caregiver of each participant. Consecutive children with a diagnosis of OSA according to polysomnographic criteria and age between 5 and 10 years were invited to participate in the study. In addition, age- , gender-, and ethnicity-matched healthy non-snoring children without OSA who underwent overnight polysomnography were also invited to participate in the study. Children were excluded if they had known diabetes or pre-diabetes (http://www.diabetes.org/pre-diabetes/pre-diabetes-symptoms.jsp), any defined genetic abnormality or underlying systemic disease, or if they were within acute infectious processes. The diagnosis of children with mild and moderate to severe OSA was defined by the presence of an obstructive apnoea-hypopnoea index (AHI) $\geq$ 1 / hour of total sleep time and AHI $\geq$ 5 / hour of total sleep time, respectively. Control children had AHI < 1 / hour of total sleep time.

Anthropometry: Children were weighed in a calibrated scale to the nearest 0.1 kg and height (to 0.1cm) was measured with a stadiometer (Holtain, Crymych, UK). Body mass index (BMI) was calculated and BMI z-score was computed using CDC 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 obesity criteria.

Overnight Polysomnographic Evaluation

Children were studied for up to 12 hours 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 during the overnight sleep recordings: chest and abdominal wall movement by respiratory impedance or inductance plethysmography; heart rate by ECG; and air flow, which was triply monitored with a side-stream end-tidal capnograph that also provided breath-by-breath assessment of end-tidal carbon dioxide levels ($PETCO_2$; BCI SC-300, Menomonee Falls, Wis), a nasal pressure cannula, and an oronasal thermistor. Arterial oxygen saturation (SpO_2) was assessed by pulse oximetry (Nellcor N 100; Nellcor Inc, Hayward, Calif), with simultaneous recording of the pulse waveform. The bilateral electrooculogram, 8 channels of electroencephalogram, chin and anterior tibial electromyograms, and analog output from a body-position sensor (Braebon Medical Corp, Ogdensburg, NY) were also monitored. All measures were digitized with a commercially available polysomnography system (Sandman, Nellcor Puritan Bennett, Kanata, ON, Canada, or Stellate Instruments, Montreal, QC, Canada).. Tracheal sound was monitored with a microphone sensor (Sleepmate, Midlothian, Va), and a digital time-synchronized video recording was performed. All of the studies were initially scored by a certified technician and were then reviewed by a physician who was experienced in pediatric PSG and underwent training in an accredited fellowship program.

Sleep architecture was assessed by standard techniques [23]. 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 duration of at least 2

breaths [24, 25]. Hypopneas were defined as a decrease in oronasal flow of ≥50% with a corresponding decrease in SpO_2 of ≥4% or more and/or an arousal [25, 26]. The obstructive apnea hypopnea index (OAHI) was defined as the number of obstructive apneas and hypopneas per hour of total sleep time (TST). Arousals were identified as defined by the American Sleep Disorders Association Task Force report [26, 27].

Endothelial Function Tests

Endothelial function was assessed with a newly developed reactive hyperemic test after cuff-induced occlusion of the brachial artery [3, 22]. In brief, a laser Doppler sensor (Perimed AB, Periflux 5000 System integrated with the PF 5050 pressure unit, Järfälla, Sweden) was applied over the volar aspect of the hand at the second-finger distal metacarpal surface, and the hand was gently immobilized. Once cutaneous blood flow over the area was stable, the pressure within an inflatable cuff placed distal to the elbow and connected to a computer-controlled manometer was raised to 160 to 180 mm Hg for 60 seconds, during which blood flow was reduced to undetectable levels. To enable consistent deflation times, the cuff was deflated under computer control, and hyperemic responses were assessed. Time to peak blood flow following relief of occlusion was considered as representative of the post-occlusion hyperemic response.

Plasma IL-6, CRP and MRP 8/14 levels and Serum Lipids

Fasting blood samples were drawn by venipuncture in the morning after the sleep study. Blood samples were immediately centrifuged and frozen at -80 °C until assay. Plasma MRP 8/14 and IL-6 levels were measured using a commercial ELISA kits (ALPCO

Diagnostics, Salem, NH for MRP 8/14 and R&D systems, Minneapolis, MN for IL-6). MRP 8/14 and IL-6 assay have a sensitivity of 0.4 ug/ml and 0.15 pg/ml, respectively. The inter-assay and intra-assay of coefficients of variability for MRP 8/14 were 4.8% and 5.3%. For IL-6, the assay has an intra-assay coefficient of variability of 5.8%, and an inter-assay coefficient of variability of 8.2%. CRP was measured within 2 to 3 hours after collection using the Flex reagent Cartridge (Date Behring, Newark, DE), which is based on a particle-enhanced turbidimetric immunoassay technique. This method has a detection level of 0.05 mg/dl and exhibits linear behavior up to 255 mg/dl, with intra-assay and inter-assay coefficients of variability of 9% and 18%, respectively. Serum levels of lipids, including total cholesterol, high-density lipoprotein (HDL), calculated low-density lipoprotein, and triglycerides, were also assessed with a Flex reagent cartridge (Date Behring, Newark, DE)

Statistical Analysis

Data were expressed by mean±SD or mean±SE as indicated. Significant differences within groups were analyzed using ANOVA for continuous variables and chi-square tests for categorical variables. Bonferroni corrections were applied for multiple comparisons. The distribution of data was assessed by the Kolmogorov-Smirnov test. If the data were not normally distributed, data were logarithmically transformed. Because obesity would be expected to contribute to increase inflammatory markers, correlation and partial correlation analyses between AHI and inflammatory markers were performed after adjusting for age and BMI z score. We also performed univariate and stepwise multivariate linear regression treating MRP 8/14 levels as a dependent variable in relation

to AHI and other covariates. In addition, we used a logistic regression model to estimate odds ratio of OSA with its 95% confidence intervals after the population was sub-divided into groups based on tertile cut-points for the distribution of MRP 8/14 levels. After controlling for age, gender, race, BMI z score, we estimated odds ratios of OSA according to tertile of MRP 8/14 levels. Statistical analyses were performed using SPSS software (version 16.0; SPPS Inc., Chicago, Ill.). All p-values reported are 2-tailed with statistical significance set at <0.05.

RESULTS

Study Population

Two hundred thirty three children out of 255 eligible children who underwent NPSG studies were recruited and included in this study. The 23 children who refused to participate did not differ in any recognizable way from the participants. Based on the presence or absence of habitual snoring and AHI, 106 had mild OSA, 34 children had moderate to severe OSA, and 115 were control children. The demographic, polysomnographic, and biochemical characteristics are shown in Table 1. Mean age, gender and ethnic distribution were similar across the 3 groups ($p>0.05$). However, log CRP, log IL-6, and log MRP 8/16 levels showed significant group differences. Moreover, children with severe to moderate OSA (n=15, 41.9±24.4 sec) had delayed peak hyperemic responses compared to either control children (n=56, 36.8±21.1 sec) or those with mild OSA (n=67, 36.6±19.6 sec).

MRP levels according to severity of OSA based on the presence of Obesity

Log MRP levels were stratified according to the severity of OSA and the presence or absence of obesity (Figure 1). As shown in Figure 1, increases of log MRP 8/14 levels among groups based on AHI categories emerged, regardless of obesity. Moreover, moderate to severe OSA children had highest log MRP levels compared to those of controls of both obese and non obese children. (Controls vs. moderate to severe OSA in obese children: 0.02±0.29 vs. 0.20±0.18, $p<0.01$, in non obese children: -0.21±0.34 vs. 0.11±0.36, $p<0.01$)

MRP 8/14, hsCRP, and IL-6 in Children with OSA

To estimate potential associations between various inflammatory markers and polysomographic measures, we performed correlation analyses. A significant linear correlation between log MRP and log AHI ($r=3.40$, $p<0.001$; Figure 2) and inverse correlation with SaO2 nadir. ($r=-0.23$, $P<0.001$) emerged. Both log CRP and log IL-6 were also positively correlated with log AHI (n=112, $r=0.25$, $p<0.01$ and n=81, $r=0.28$, $p<0.05$, respectively) Furthermore, log MRP 8/14 levels were not only significantly correlated with BMI z score ($r=0.38$, $p<0.001$; Table 2), but also highly associated with log CRP (n=112, $r=0.63$, $p<0.001$; Table 2) and log IL-6 (n=81, $r=0.41$, $p<0.001$; Table 2). In addition, we performed partial correlation analysis with BMI z scores as a covariate, because obesity would be expected to contribute to increased levels of CRP and MRP 8/14 levels. Both log CRP and log MRP 8/14 levels were independently associated with log AHI, even after controlling for age and BMI z score. Furthermore, log MRP 8/14 levels showed slightly higher correlation coefficients with log AHI than those of log CRP ($r=0.29$, $p<0.001$ for log MRP 8/14, $r=0.24$, $p<0.05$ for log CRP, respectively.)

MRP 8/14 levels and Endothelial Function

Log MRP 8/14 levels were significantly positively correlated with post-occlusive hyperemic responses as a surrogate marker of endothelial function (n=138, $r=0.25$, $p<0.001$). However, this association did not persist after adjusting for age and BMI z score. Peak hyperemic responses were correlated with BMI z score (n=138, r=0.326,

$p<0.001$), but were not significantly correlated with lipid profiles and polysomnographic measuress ($p>0.05$).

Stepwise Multiple Regression Analysis in OSA Children

To examine independent predictors of MRP 8/14 levels in children, we performed a stepwise multiple regression analysis (table 3). The strongest predictors of MRP 8/14 morning concentrations were BMI z score ($p<0.001$) and AHI ($p<0.001$), which accounted for 24.6% of variance in MRP 8/14 level variance, after controlling for age, gender, and race.

Odd ratios for OSA according to tertiles of MRP 8/14 levels in Children.

In order to estimate odd ratio of OSA in relation to any given MRP 8/14 level, we performed logistic regression analysis. Table 4 presents univariate and multivariate odd ratios on the likelihood of OSA according to increasing tertiles of MRP 8/14 levels. In the univariate model, odd ratios of mild to moderate OSA (AHI $\geq$ 5) were 2.47 (95% CI, 1.26-4.85, $p<0.05$) for the second tertile of MRP 8/14 (0.706-1.34 ug/ml) and 7.68 (95% CI, 2.57-22.9, $p<0.01$) for the third tertile of MRP 8/14 (>1.34 ug/ml) using the lowest MRP 8/14 tertile level as reference. After adjusting for confounding factors such as age, gender, race, and BMI z score, children in highest tertile of MRP 8/14 levels had a 5.6 fold increased risk (95% CI, 1.64-17.1, $p<0.01$) for moderate to severe OSA compared to those whose MRP 8/14 levels were within the lower range.

DISCUSSION

In the present study, we found that both obese children and children with OSA have elevated plasma MRP 8/14 levels. Furthermore, MRP 8/14 levels are dose-dependently increased relative to the severity of OSA, even in non-obese children. Moreover, MRP 8/14 levels were not only highly correlated with CRP and IL-6, but were also correlated to endothelial function. Even after adjusting for potential confounding factors, both AHI and BMI were independently associated with MRP 8/14 levels. Moreover, children in the highest tertile of MRP 8/14 levels were at markedly higher risk (5.6 fold) for moderate to severe OSA.

Increasing evidence suggests the presence of increased risk for endothelial dysfunction and other adverse cardiovascular consequences in both adult and children with OSA [3, 7, 8, 28-30]. While the definite mechanisms are yet to be delineated, production of vasoactive substances such as endothelin1 by endothelial cells may be altered [31-33], along with reductions in nitric oxide availability, and potentially increases in circulating levels of endogenous inhibitors of nitric oxide synthase [34-36]. Two major, and to some extent overlapping mechanisms, namely increased generation and propagation of reactive oxygen species and amplification of inflammatory processes, may underlie such changes along with increasing the adherence of inflammatory mediators to endothelial cells and hypercoagulability [1, 5, 8, 19, 28, 37-40]. Hyperactivation and increased reactivity of the sympathetic system have been reported in children with OSA [41-43], and systemic blood pressure elevations are not only OSA-severity dependent [44-46], but are also associated with altered left ventricular geometry and contractibility [47]. Recently, we reported that children with SDB have elevated plasma IL-6 and P-selectin levels [39, 48].

Moreover, CRP, an important circulating marker of inflammation, is elevated in children with OSA and reduced after tonsillectomy [21, 49], even if not consistently [50, 51], suggesting that the determinants of CRP elevation in the presence of OSA can not be exclusively accounted for the severity of condition but are also dictated by other factors. Notwithstanding, the present study further confirms our previous findings. More importantly, both MRP 8/14 and CRP showed significant associations with AHI, even after adjustment for age and BMI z score, the latter being an important risk factor for cardiovascular morbidity [52]. The interactions between the severity of OSA, lifestyle patterns, environmental conditions, and genetically-driven individual susceptibility have are all likely involved in the magnitude of the inflammatory responses associated with OSA [3, 6, 28]. Therefore, more specific assessment of these factors as they relate to MRP 8/14 in the context of pediatric OSA will have to be investigated in the future.

MRP 8/14 is a major calcium binding protein and is primarily expressed in cells of myeloid origin, particularly in monocytes and neutrophils [11-13]. Upon phagocyte activation, MRP 8 and MRP 14 will form the MRP 8/14 complex, which translocates to the cytoskeleton and plasma membrane, where it is secreted [11, 12]. This is an early event during the process of transendothelial migration and interaction of MRP-expressing neutrophils and monocytes with the endothelium [53, 54]. However, the physiological roles of MRP 8/14 are not well characterized. Notwithstanding, elevated MRP 8/14 levels are useful biomarkers of disease activity, such as in rheumatoid arthritis and inflammatory bowel disease [55, 56]. MRP 8/14 levels may also play an important pathophysiological role in cardiovascular disease and in diabetic complications [15, 16,

18, 57, 58]. Indeed, Burkhartdt and colleagues [59] suggested that MRP 8/14 levels not only serve as reliable reporters on the state of inflammation in diabetic nephropathy, but also on the degree of microvascular dysfunction within the glomerular and retinal beds. Furthermore, Altwgg and colleagues [57] reported that MRP 8/14 is markedly expressed at the site of coronary occlusion by invading phagocytes. These investigators found that MRP 8/14 levels are increased in the systemic circulation well before elevation of other markers of myocardial damage, such as myoglobin, CK-MB, and troponin. Morrow and colleagues [15] also showed that patients with elevated levels of both MRP 8/14 and CRP have a 2-fold increased risk of cardiovascular death or of suffering a myocardial infarction compared to matched patients in whom these markers are not increased. Here, we observed that children with highest tertile MRP 8/14 levels had a 5.3 fold increase risk of moderate to severe OSA even after controlling for confounding factors. Moreover, plasma MRP 8/14 levels were significantly correlated with both CRP and IL-6 circulating levels. Therefore, plasma MRP 8/14 levels may not only provide a reliable marker of OSA and of the magnitude of the inflammatory response in the context of OSA, but may also be indicative of particular populations at increased risk for development of cardiovascular complications. However, this is the first report on the association between MRP 8/14 and OSA, and these initial observations will need to confirm by more extensive prospective interventional studies.

Some methodological considerations deserve comment. Firstly, the relative contribution of upper airway tissues such as tonsils and adenoids to the increases in inflammatory markers can not be ascertained [60]. Secondly, since MRP 8/14 can be highly expressed in different cell types [16, 61], it will be important to determine which cells populations

are more specifically involved the inflammatory responses in pediatric OSA, and account for the OSA-associated increases in MRP 8/14 levels. Thirdly, we can not exclude the possibility of existing contributions to MRP 8/14 levels by underlying metabolic dysfunction, particularly considering reports on elevated plasma levels of MRP 8/14 in the context of diabetes mellitus [58, 59]. However, all children included in this study did not have diabetes or any other systemic disease. Fourthly, there is some uncertainty as to the association between post-occlusive hyperemic responses and MRP 8/14 levels, and expanded studies in this regard are needed. Of note, we have previously uncovered that the presence of a strong family history of ischemic heart disease was significantly associated with persistence of endothelial dysfunction in children with OSA, even after treatment of OSA [22]. Unfortunately, we did not explore the cardiovascular family history in the present study. Finally, we did not assess the reversibility of MRP 8/14 elevations after effective treatment of OSA, nor did we examine the dynamic changes in MRP 8/14 levels in the course of the night.

In summary, children with OSA have elevated morning plasma MRP 8/14 levels which exhibit OSA severity-related dependencies, even among non-obese children. Additional studies will be needed to examine the intrinsic contributions to clinical practice of assessing MRP 8/14 levels in the context of evaluating children at risk for OSA.

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Table 1. General characteristics of children with OSA and healthy controls.

	Control (n=115)	Mild OSA (n=106)	Moderate to Severe OSA (n=34)
Age (years)	7.81±1.44	7.65±1.55	7.20±1.69
Gender (male, %)	55.7	60.4	61.8
Ethnicity (Caucasian, %)	64.3	61.3	55.9
BMI Z score	1.15±1.22**	1.32±1.39[&]	1.97±1.11[§]
Log AHI	-0.39±0.27** [0.40±0.27]	0.30±0.20[&] [2.23±1.02]	1.13±0.27[#] [16.60±11.05]
SaO2 Nadir (%)	92.2±5.2**	89.7±5.2[&]	81.1±9.4[§]
Respiratory Arousal Index (events/ hour)	5.2±7.3	6.9±7.8	8.3±5.2
Total Cholesterol (mg/dl)†	159.3±25.1*	168.2±27.2	181.3±44.8[#]
HDL cholesterol (mg/dl)†	50.1±10.2	52.6±11.6	49.0±12.7
LDL cholesterol (mg/dl)†	92.6±22.9*	100.2±22.6	110.1±37.5
Tryglycerides (mg/dl)†	82.7±43.1*	76.1±35.1	111.2±78.0
Peak hyperemic response (sec)†	36.8±21.1	36.6±19.6	41.9±24.4
Log IL-6‡	-0.22±0.51* [0.98±0.95 pg/ml]	-0.13±0.45 [1.16±1.26 pg/ml]	0.13±0.30[#] [1.78±1.63 pg/ml]
Log hsCRP‡	-0.17±0.41** [1.16±1.57 mg/dl]	-0.10±0.44 [1.51±2.35 mg/dl]	0.27±0.36[§] [2.54±2.02 mg/dl]
Log MRP 8/14	-0.11±0.34** [1.02±0.85 ug/ml]	-0.00±0.31[&] [1.27±0.87 ug/ml]	0.18±0.23[§] [1.73±0.92 ug/ml]

All data are expressed as mean±SD,

† These data were acquired in 134 children.

‡ IL-6 and hsCRP levels include 72 and 112 children, respectively.

*P<0.05, **P<0.01, Differences between three groups (ANOVA test)

[&]P<0.05, Controls vs. Mild OSA

[#]P<0.05, Controls vs. Moderate to severe OSA

[§]P<0.01, Controls vs. Moderate to severe OSA

Table 2. Correlation coefficients between CRP and MRP 8/14 level and various variables.

	Correlation coefficients							
	Unadjusted				Adjusted[#]			
	hsCRP[‡] (n=112)		MRP 8/14[‡] (n=255)		hsCRP (n=112)		MRP 8/14 (n=255)	
Variables	r-value	P-value	r-value	P-value	r-value	P-value	r-value	P-value
Age (years)	0.259**	0.006	-0.042	0.499	-	-	-	-
BMI z score	0.563**	<0.001	0.383**	<0.001	-	-	-	-
Apnea Hypopnea Index[‡] (events/ hour)	0.258**	0.006	0.340**	<0.001	0.242*	0.011	0.297**	<0.001
SaO2 Nadir (%)	-0.142	0.159	-0.236**	0.001	-0.018	0.864	-0.170**	<0.01
Total Cholesterol (mg/dl)†	0.049	0.609	0.126	0.147	0.043	0.663	0.100	0.253
HDL cholesterol (mg/dl)†	-0.113	0.241	-0.117	0.178	-0.076	0.437	-0.013	0.130
LDL cholesterol (mg/dl)†	0.048	0.622	0.135	0.121	0.002	0.984	0.076	0.384
Tryglycerides (mg/dl)†	0.194*	0.044	0.170*	0.049	0.221	0.022	0.133	0.128
Peak hyperemic response[‡] (sec)#	0.188	0.062	0.254**	0.003	-0.052	0.598	0.076	0.382
IL-6 (pg/ml)[‡]	-	-	0.412**	<0.001	-	-	0.448**	<0.001
hsCRP (mg/ml)[‡]	-	-	0.632**	<0.001	-	-	0.514**	<0.001

Data were adjusted for age and body mass index Z score.

† These data were included 112 children.

‡ These data were log-transformed

* P<0.05, **P<0.01

Table 3. Association of AHI and MRP 8/14 levels and covariates.

	MRP 8/14†					
	Univariate			Stepwise multivariate		
Independent Variables	Beta	SE	P-value	Beta	SE	P-value
Age	-0.25	0.01	0.67	-	-	-
Gender	0.001	0.03	0.92	-	-	-
Race	0.12	0.02	0.04	-	-	-
BMI z score	0.32	0.01	<0.001	0.31	0.01	<0.001
AHI†	0.27	0.03	<0.001	0.28	0.03	<0.001

† Data were log-transformed

Table 4. Logistic regression analysis on the association of OSA and MRP tertile levels in children.

Definition of Outcome	OSA, n	Tertile of MRP 8/14 [Range]		
		Univariate Odds Ratio (95% CI)		
		1st tertile [<0.705 ug/ml] n=84	2nd tertile [0.706-1.34 ug/ml] n=86	3rd tertile [>1.34 ug/ml] n=85
Mild OSA vs. Control	n=106	1.0	1.29 (0.68-2.43)	2.05 (0.63-6.6)
Moderate to severe OSA vs. Control	n=34	1.0	2.47 (1.26-4.85)*	7.68 (2.57-22.9)**
		Multivariate Odds Ratio (95% CI)†		
Mild OSA vs. Control	n=106	1.0	1.30 (0.68-2.51)	1.77 (0.52-5.93)
Moderate to severe OSA vs. Control	n=34	1.0	2.40 (1.16-4.95)**	5.30 (1.64-17.1)**

Logistic regression analysis was used to estimate odds ratios and 95% confidence intervals after the cohort was divided into 3 groups based on tertile cutpoints according to the distribution of MRP 8/14 for the whole cohort.

†Data were adjusted for age, gender, race, and body mass index Z score.

* P<0.05, **P<0.01

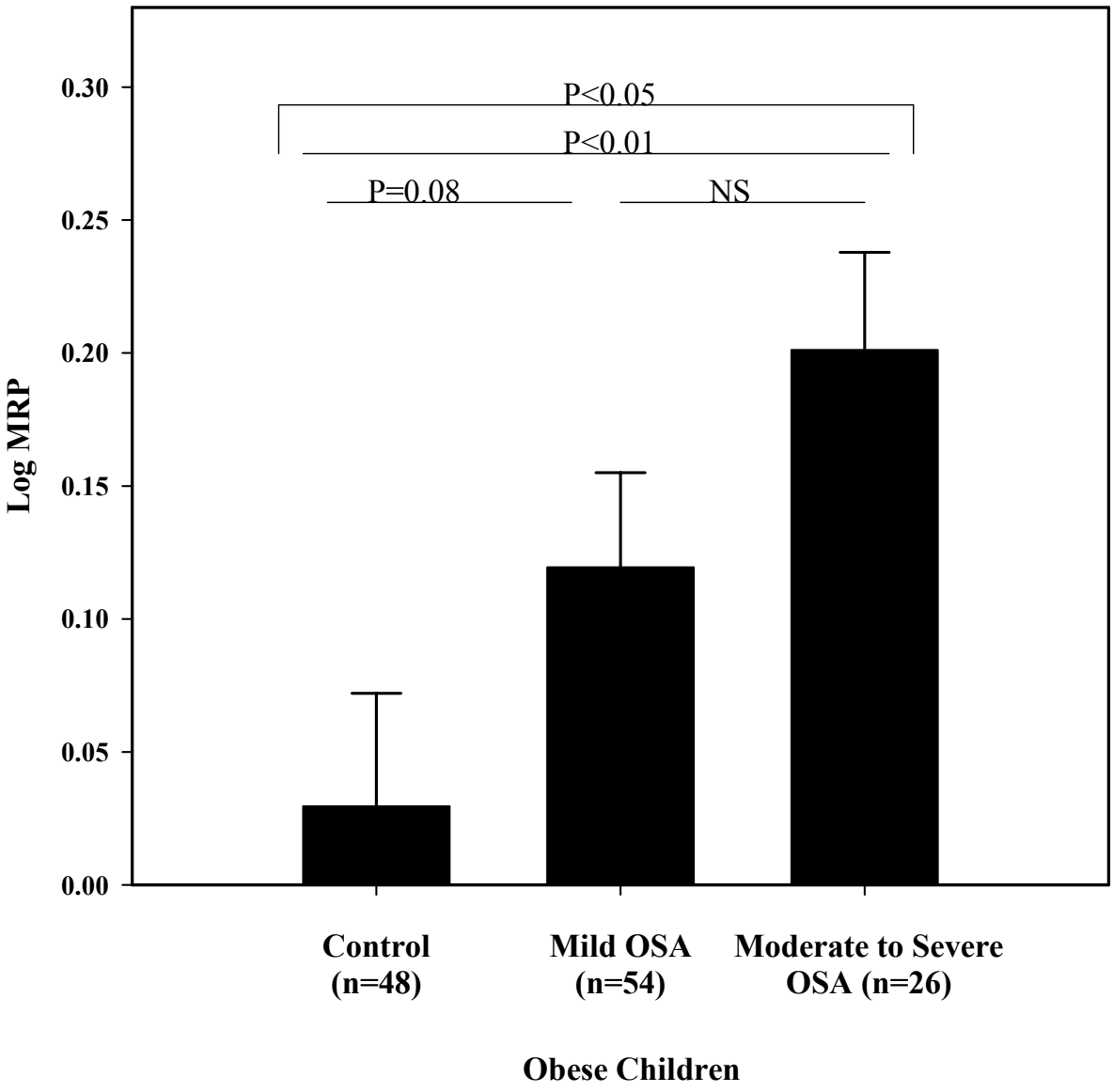
Legends to Figures

Figure 1. Log MRP levels in children with OSA and controls among non-obese and obese children. Obesity was defined as a body mass index Z score >1.65. Data are expressed as mean±SE.

Figure 2. Scatterplot of individual log MRP 8/14 levels plotted against corresponding log AHI levels on overnight polysomnography.

Figure 1.

A)


P<0.05
P<0.01
P=0.08
NS
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Log MRP
Control (n=48)
Mild OSA (n=54)
Moderate to Severe OSA (n=26)
Obese Children

B)

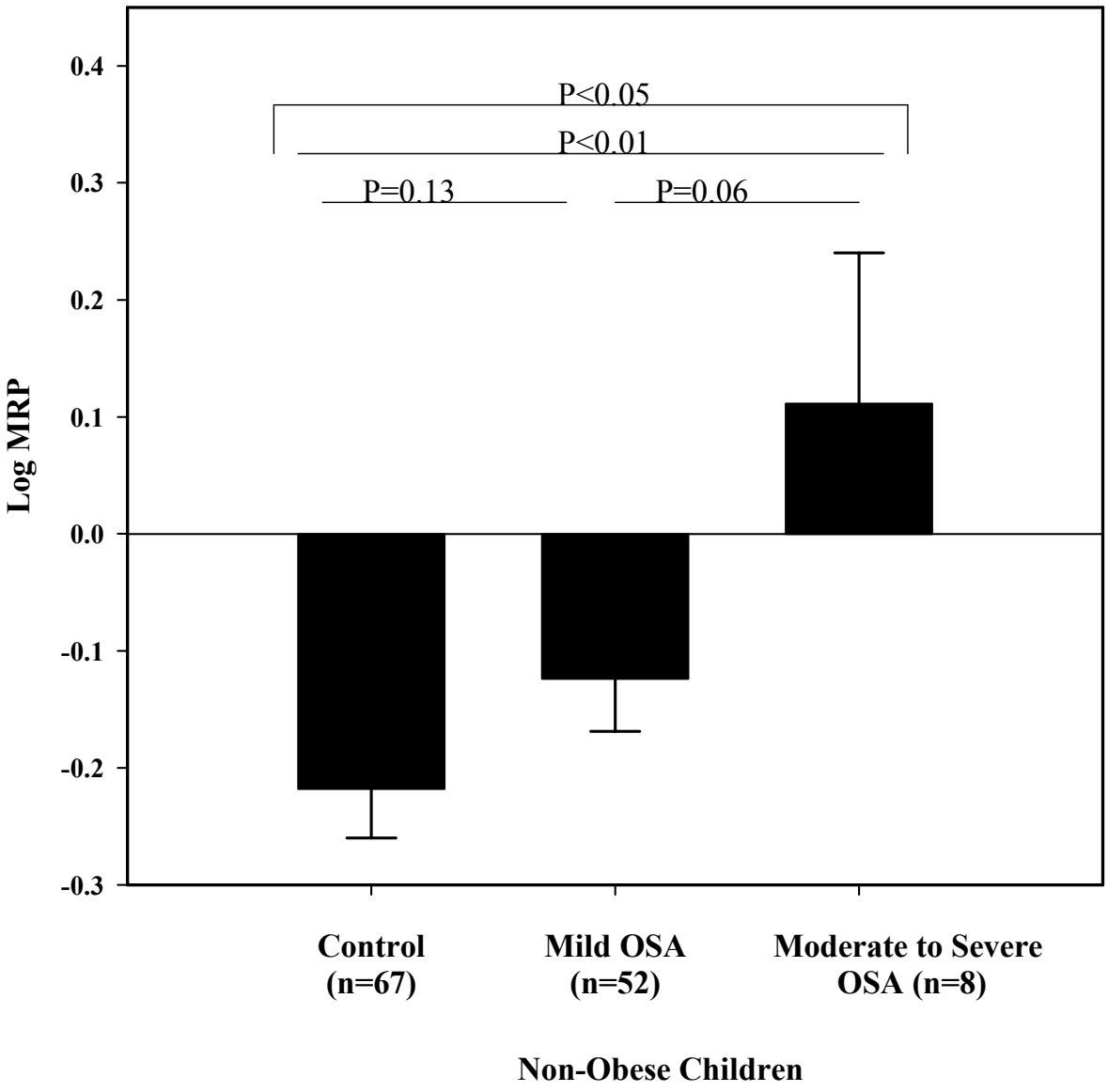

P<0.05
P<0.01
P=0.13
P=0.06
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
Log MRP
Control (n=67)
Mild OSA (n=52)
Moderate to Severe OSA (n=8)
Non-Obese Children

Figure 2.