Early Atherosclerotic Inflammatory Pathways in Children With Obstructive Sleep Apnea

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Abstract

Objective: To evaluate structural and functional carotid changes and inflammatory profiles in children with OSA (obstructive sleep apnea) and healthy controls.

Study design: Patients with OSA and matched controls (ages 5–13) were recruited. Pro-inflammatory cytokines and acute phase reactants were measured at 6:00 PM. Common carotid artery measures were determined using ultrasound. Confirmatory factor analysis (CFA) was used to determine subgroups of cytokines and their effects on carotid measures.

Results: Ninety-six patients participated (53 healthy controls, 43 patients with OSA). OSA was associated with increased pro-inflammatory cytokines (CD40L, IL6, and IL8) and high sensitivity C-reactive protein ($P < .05$ for all). One cytokine subgroup (IL-6 and IL-8) was

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negatively associated with markers of carotid function, indicating reduced arterial distensibility and increased stiffness (p<0.05 for three ultrasound measures); TNF-α had an opposing effect on carotid function compared with this cytokine subgroup (p<0.05 for two ultrasound measures). Linear regression demonstrated significant associations between TNF-α and two measures of carotid function (p<0.05 for each). Children with OSA did not have functional or structural carotid changes compared with controls.

Conclusion: OSA was not directly associated with structural and functional carotid changes but was associated with upregulation of key pro-inflammatory cytokines (sCD40L, IL-6, and IL-8). Together, IL-6 and IL-8 were associated with changes in carotid function. Longitudinal studies are needed to demonstrate that the inflammatory milieu observed in our population is a precursor of atherosclerosis in children.

Keywords

carotid intima media thickness; obstructive sleep apnea syndrome; pediatric OSA; cardiovascular disease; inflammation

In adults, obstructive sleep apnea (OSA) is associated with impaired blood pressure (BP) regulation,[1] stroke,[2] and impaired cardiac function, including heart failure.[3] Changes in carotid artery diameter can also occur.[4, 5] Despite what is known in adult patients who have suffered from years of untreated disease, the effects of OSA in pediatric patients are not clearly defined. Children with OSA do not typically develop overt hypertension but may have changes in autonomic function,[6, 7] abnormal baroreflex physiology,[8] and greater variability in BP.[9, 10] Although heart failure rarely occurs in children secondary to OSA, affected children may have subclinical left ventricular remodeling, hypertrophy,[11] or impaired ventricular function.[12]

To fully understand the evolution of cardiovascular sequelae, we must identify subclinical changes in children with OSA that precede atherosclerosis and associated underlying inflammatory processes. Children have changes in pulse transit time (PTT), a marker of vascular stiffness, secondary to OSA.[13] Furthermore, there is evidence of a delicate balance between opposing acute phase reactants and proinflammatory cytokines on PTT to maintain cardiovascular homeostasis.[13] However, the effects of OSA on carotid diameter and function have not been thoroughly investigated in children with OSA. In one study, children with adenotonsillar hypertrophy had increased carotid intima-media thickness (CIT) compared with controls but did not have altered arterial distensibility.[14] Another study found no association between CIT and apnea hypopnea index (AHI).[15] Further studies are indicated to elucidate the subclinical changes associated with OSA that lead to atherosclerotic disease. We hypothesized that children with OSA have increased pro-inflammatory biomarkers and acute phase reactants and that changes in carotid structure and function that occur in children with OSA are associated with specific inflammatory profiles and correlate to changes in groups of proinflammatory cytokines and acute phase reactants.

METHODS

Study participants ages 5 to 13 years were recruited to a prospective study through otolaryngology and pulmonary clinics. Healthy controls were recruited using advertisements at various other clinics at Cincinnati Children's Hospital Medical Center (CCHMC). Children in the control group had a normal examination, an AHI <1 / hr of sleep on an overnight polysomnogram (PSG) and no indication of sleep disturbances, such as snoring or gasping and choking while asleep. OSA was classified as follows: mild (obstructive AHI between 1 and 4.9 events / hour (E / hr)) and moderate to severe (obstructive AHI 5 E / hr). Exclusion criteria included: chronic or recurrent tonsillitis, chronic pulmonary conditions, cardiac diseases, neuromuscular disorders, developmental delay or genetic syndromes, chronic renal disease, endocrinological disorders, acute or chronic inflammatory conditions, a body mass index (BMI) z-score > 2.5, or if taking medication(s) that may affect the autonomic nervous system.

Participants recruited to this study overlap with a similar cohort of participants published previously evaluating changes in pulse transit time in children with OSA.[13] However, the total number of participants in the two studies differs. Furthermore, the data presented here were incorporated in an independent analysis. None of the data regarding carotid artery structure and function, or the correlations to the inflammatory markers, have been presented previously.

Study Design

Participants underwent PSG, BP measurements, and phlebotomy to determine serum cytokine and acute phase reactant levels. BP was determined through an average of three readings obtained with an automated oscillometric device after at least 5 minutes at rest in the sitting position. Height, weight, BMI and head and neck examinations were recorded. CIT measurements were obtained with PSG. Institutional review board approval was obtained. Personnel were blinded except for the scheduling coordinator.

Polysomnography

All PSGs were performed in a pediatric sleep laboratory overnight using a 16-channel computerized system (Grass Telefactor, Astro-Med Inc) with standard electrodes recorded electrocardiograms (EKGs). PSGs included: electroencephalograms, electrooculograms, electromyograms at various locations, nasal/oral airflow, end tidal carbon dioxide (CO_2) and pulse oximetry waveforms. Inductive plethysmography (Somnostar; SensorMedics Corp) monitored respiratory cycles. Finger arterial photoplethysmography (PortaPres; TNO-TPD Biomedical Instrumentation) continuously monitored BP during PSG. A pediatric sleep physician blinded from subject grouping reviewed and analyzed the PSGs.

Plasma Inflammatory Biomarkers

Plasma inflammatory biomarkers were evaluated as previously described.[13] In brief, seven milliliters of whole blood were collected at approximately 6 pm on the evening prior to the sleep study. A human multiplex cytokine assay (Linco Research Inc) was used to evaluate the samples for serum levels of the following inflammatory markers and acute

phase reactants: high-sensitivity C reactive protein (hs-CRP), serum amyloid A (SAA), monocyte chemoattractant protein (MCP)-1, interleukin (IL)-6 and 8, adiponectin, soluble cluster of differentiation (CD)-40 ligand (sCD40-L), and tumor necrosis factor-alpha (TNF-α). Cell supernatant was incubated with the antibody-coated bead mixture. Streptavidin-phycoerythrin was added and the sample incubated. The beads were washed and analyzed on a Luminex100 Platform (Luminex Corp). Cytokine concentrations were calculated from standard curves.

Carotid Artery Measurements

Study participants underwent a carotid ultrasound during the admission for the PSG. Measurements of the carotid were performed as previously described.[16] Carotid ultrasounds were performed by a registered vascular technologist blinded to study group allocation. High-resolution B-mode ultrasonography (Vivid 7 ultrasound, GE Healthcare) was used with a GE Vivid 7 ultrasound imaging system. For each study participant, bilateral carotid artery walls and segments were independently examined at continuous angles. The vascular technologist used a trace technique to obtain measurements for the maximum carotid thickness from leading edge (lumen-intima) to leading edge (media-adventitia). Three segments were imaged from both the right and left sides during peak systole and diastole. These were then averaged to determine the measurements for the common carotid artery, the carotid bulb at the bifurcation, and the internal carotid artery. M-mode measurements of the common carotid arteries were also performed to determine carotid stiffness.[17] Using 2-dimensional images of the carotid arteries, maximal and minimal lumen diameters were read. Calculations for carotid stiffness and distensibility included the Peterson modulus.[18] Carotid ultrasound measures are highly reproducible with coefficients of variation for repeat readings between 5.3% to 8.0%.[19]

Statistical Analyses

Sample sizes were calculated using the software package nQuery, version 6 (GraphPad Software DBA Statistical Solutions). The means for the measures of the CIT were used to calculate an effect size and to ascertain if power was 80% or greater. Based on pilot data, a sample size of 128, equally divided between control and SDB, achieves a power of 99%. The power was determined to be >80% given our final number of study participants.

Descriptive statistics were generated for demographics and sleep measures by OSA severity levels as mean ± standard deviation (SD) or frequency with percentage. The *Student t*-test, chi-square test, or Fisher exact test were used to determine associations between demographic and sleep measures. Medians for each PM cytokine were compared for control and OSA groups with the Wilcoxon signed rank sum test. The effects of PM cytokines and/or cytokine groups on measures of carotid intimal thickness (structure) and carotid stiffness (function) were estimated from confirmatory factor analysis (CFA). CFA included two phases: 1) grouping of cytokines, ie, the latent factor(s), and 2) development of a structural equation model with CFA to determine the effect of cytokines on measures of carotid intimal thickness (structure), carotid stiffness (function), luminal cross-sectional area (LCSA) and wall cross-sectional area (WCSA) (adjusting for age, BMI z score, sex, race,

and presence of OSA). All analyses were performed using SAS (version 9.4, SAS Institute Inc). A p-value of less than 0.05 was considered statistically significant.

RESULTS

The study sample included 96 participants, including 53 healthy controls (mean age 10.0±2.3 years), 18 with mild OSA (mean age 9.6±3.1 years), and 25 with moderate to severe OSA (mean age 8.6±2.1 years). There was no significant difference in age, BMI z score, or cholesterol or triglyceride levels between groups. (Table 1) There was a higher proportion of Black children in the OSA group compared with the control group. Children with OSA had a significantly higher AHI, obstructive AHI, and arousal index compared controls. No other significant differences in sleep study variables were identified for any of the study participants.

Inflammatory Markers and OSA

Except for adiponectin, levels of all plasma biomarkers were elevated in children with OSA compared with healthy participants. There were statistically significantly positive associations between OSA and pro-inflammatory cytokines including sCD40L, IL6, and IL8 after adjusting for age, race, sex, and BMI z score in linear regression models. There was also a positive association between OSA and hs-CRP in the adjusted analysis. (Table 2)

Factor analysis: Cytokine groups and carotid structure and function

As certain groups of cytokines have similar physiologic function, we used structural equation modeling with a confirmatory factor analysis to evaluate the effects of these groups on carotid structure and function. Using this model, two separate groups were identified. Group 1 was composed of the pro-inflammatory cytokines IL-6 and IL-8. Group 2 consisted of acute phase reactants SAA, hs-CRP and adiponectin. PM MCP-1 and TNF-a were not found to be associated with other cytokines through CFA and were thus evaluated individually for effects on the carotid arteries. There were positive associations between TNF-α and two measures of carotid function, peak diastolic mean and mean arterial lumen cross-sectional area. Group 1 cytokines (IL-6 and IL-8) were negatively associated with multiple markers of carotid function. Additionally, Group 1 cytokines were positively associated with Peterson common, indicating reduced arterial distensibility and increased stiffness. (Table 3) Conversely, TNF-α was positively associated with the common carotid artery diameter at peak systole. Group 2 cytokines were not significantly associated with measures of carotid structure or function. As we adjusted for age in our analysis, we did not specifically evaluate the changes of inflammatory markers across different age groups.

Cytokines (individually) and Carotid Structure and Function

Linear regression models adjusting for age, sex, race, BMI z-score and group (OSA vs control) demonstrated statistically significant associations between TNF-α and two measures of carotid function, peak diastolic mean (estimate: 0.0071, p=0.02) and mean arterial lumen cross-sectional area (estimate: 0.0053, p= 0.02). Using linear regression, plasma biomarkers were not significantly associated with carotid structure.

OSA and Carotid Structure and Function

Values depicting carotid artery structure and function are displayed in Table 4. There were no statistically significant differences for any measures of carotid structure or function between controls and children with OSA after adjusting for age, race, sex, and BMI z score.

DISCUSSION

OSA was not directly associated with structural and functional carotid changes in this sample of children. However, OSA was significantly associated with upregulation of key pro-inflammatory cytokines (sCD40L, IL-6, and IL-8) known to play a role in the evolution of atherosclerosis.[20–24] In CFA, which examined the collective effects of pro-inflammatory cytokines with similar physiologic function, the subgroup including IL-6 and IL-8 was associated with functional changes in the carotid arteries that precede atherosclerotic plaque development, including subtle changes in carotid stiffness and distensibility. TNF-alpha had an opposing effect on carotid function as measured by two ultrasound measures. Thus, although structural and functional cardiovascular changes were not yet apparent in children with OSA, they demonstrate upregulation in cytokines that are associated with cardiovascular sequelae (atherosclerosis) in adults with OSA.

Subgroups of plasma biomarkers are essential to consider in relation to structural and functional carotid changes in children with OSA. Our CFA determined that IL-6 and IL-8 behave similarly, and this subgroup (subgroup 1) was significantly associated with multiple indicators of altered carotid function. For example, there was a significant association between this subgroup with the Peterson common measure, indicating that even in the absence of observable physical changes in carotid artery structure, reductions in vessel distensibility occur. Similarly, the significant negative association between subgroup 1 and multiple other measures (common peak systolic, common peak diastolic, and mean luminal cross-sectional area) indicate impaired carotid distensibility, likely secondary to increased vessel wall stiffness. Given that pro-inflammatory cytokines function in a complex and dynamic milieu where a variety of compensatory forces are likely in play, our study provides valuable insight into the pathophysiology that likely predates atherosclerotic changes. These findings are consistent with our prior work showing that IL-6 and IL-8, in combination with other plasma biomarkers, have a shortening effect on PTT, another marker of atherosclerotic disease. Counterregulatory mechanisms involving other plasma biomarkers were also identified in this context.

Upregulation of proinflammatory cytokines among children with OSA is an important indicator of an evolving landscape. We identified significant, positive associations between IL-6, IL-8, sCD40, and hs-CRP in children with OSA compared with healthy controls. One prior study has identified dysregulation of these inflammatory pathways in children with OSA and the associated effects on the carotid arteries. Specifically, Ianuzzi et al noted that hs-CRP was increased in children with SDB; however, they attributed this change primarily to increased body weight.[15] Our study evaluated multiple plasma biomarkers and identified changes associated with OSA even when adjusting for age, sex, race, and BMI z-score. Consequently, we suspect that upregulation of these plasma biomarkers is occurring

secondary to OSA and is not due to obesity or other factors included in the adjusted analyses.

Our findings regarding the lack of overt structural and functional carotid changes in children with OSA must be considered in the context of the limited available literature in this area. One study by Ciftel et al identified a statistically significant increase in CIT in the setting of adenotonsillar hypertrophy; however, other variables related to vessel stiffness, elasticity, arterial strain and carotid distensibility did not differ significantly between patients with adenotonsillar hypertrophy and healthy controls.[14] Another study by Ianuzzi et al found no significant association between CIT and AHI or sleep disordered breathing.[15] In a study of obese nondiabetic adolescents, AHI was not associated with CIT; in this same study, BMI was positively correlated with CIT.[25] Collectively, our results and these previous studies indicate that changes in CIT are not consistently present in children with OSA, perhaps due to the relatively short duration of time children suffer from the consequences of OSA. Additional years of exposure to OSA are likely necessary before these functional and structural changes manifest clinically.

It is important to consider the findings of the present study in the context of the adult literature on OSA and atherosclerotic changes, particularly changes in CIT. Although a full review of the adult literature on this topic is beyond the scope of this study, a recent systematic review including 18 studies determined that, despite heterogeneity in studies, increased CIT was associated with OSA in adjusted analyses.[26] There was also a moderate correlation between AHI and CIT.[26] Multiple studies have also highlighted the importance of the pro-inflammatory cytokine IL-6 in relation to CIT.[20–22] IL-8 has also been found to be increased in patients with OSA,[23] and has been identified as a pro-inflammatory cytokine that predisposes to atherosclerotic changes.[27] TNF alpha and sCD40L have also been implicated in adult cardiovascular disease and are worthy of further study in children with OSA at risk for atherosclerosis.[28–31]

Our prospective clinical study offers significant insight into the association between OSA and inflammatory cytokines known to play a role in cardiovascular disease in adults. We evaluated multiple cardiovascular measures to comprehensively evaluate carotid structure and function. Specifically, the measures used have been utilized in adult studies examining cardiovascular changes associated with OSA.[32–36] Of note, there were not significant differences between the OSA and control groups with regard to cholesterol or triglycerides. By evaluating subgroups of plasma biomarkers grouped according to function, our study addressed a notable gap in the literature regarding the collective effects of key pro-inflammatory cytokines in children; IL-6 and IL-8 are particularly relevant to atherosclerotic disease in adults.

There are limitations to the present study that warrant discussion. Given the cross-sectional design of our study, we were unable to document changes over time among children in our study population. Additionally, we were unable to control for lifestyle, including diet and exercise, over the course of the study. Although specific age restrictions were followed for recruitment, pubertal stage was not evaluated in our study. This may have impacted the

outcomes of our measures. As we adjusted for age and sex in our analysis, we did not specifically evaluate distributions.

The natural history of vascular changes in children due to OSA is insidious yet essential to understand given the extensive morbidity associated with heart disease in adults. Children with OSA exhibit alterations in inflammatory pathways that may predate overt structural changes associated with atherosclerosis. Our findings are an important initial step to identifying biomarkers known to be associated with atherosclerosis in adults that may be useful in detecting pre-clinical cardiovascular changes in children with OSA. However, longitudinal studies are needed to demonstrate that the inflammatory milieu observed in our population is a precursor of atherosclerosis in children. Escalation of inflammatory processes in children with OSA, and counterregulatory processes, are not yet completely understood. Understanding the progression of atherosclerotic changes over time, in the context of obesity, other co-morbid conditions, growth trajectory and hormonal changes during puberty, is necessary to determine approaches that may prevent cardiovascular sequelae in adulthood.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations:

AHI	apnea hypopnea index
BMI	body mass index
BP	blood pressure
CFA	confirmatory factor analysis
CIT	carotid intima-media thickness
Hs-CRP	high-sensitivity C reactive protein
LCSA	luminal cross-sectional area
OSA	obstructive sleep apnea
PTT	pulse transit time
WCSA	wall cross-sectional area

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Table 1.

Demographic characteristics and sleep parameters for children recruited to the study.

	Controls (n=53)	OSA (n=43)
Age, mean±SD, years	10.0±2.3	9.0±2.6
Sex		
Male, n (%)	22 (42%)	16 (37%)
Race, n (%) [*]		
Black	13 (24%)	20 (47%)
Caucasian	37 (70%)	20 (47%)
Other	3 (6%)	3 (6%)
BMI, mean±SD	20.1±4.8	20.4±5.3
BMI Z score, mean±SD, kg/m^2	0.8±0.9	0.9±1.2
Total sleep time, min, mean±SD	522.8±41.4	528.6±36.8
Sleep efficiency, % mean±SD	81.4±8.6	80.8±8.0
% Time in Stage 1, mean±SD	3.1±1.2	3.0±1.2
% Time in Stage 2, mean±SD	46.2±7.3	45.9±6.3
% Time in Stage 3, mean±SD	1.9±0.8	1.8±0.6
% Time in Stage 4, mean±SD	29.3±7.3	27.8±6.0
Arousal index, mean±SD [*]	9.7±2.6	13.9±7.4
Obstructive index, mean±SD [*]	0.3±0.3	9.8±9.2
Peak end-tidal CO2, mean±SD	49.3±3.1	51.0±7.2
Obstructive hypopnea index, mean±SD	0.9±0.0	0.9±0.0
SBP, mm Hg, mean±SD	105.9±10.2	103.3±10.6
SBP %, mean±SD	65.5±25.9	61.3±28.0
DBP, mm Hg, mean±SD	60.5±6.8	59.2.3±6.3
DBP %, mean±SD	50.2±20.8	50.4±19.1
AHI, mean±SD [*]	0.8±1.4	10.3±9.1
% Avg Oxygen, REM, mean±SD	97.5±1.5	97.7±1.2
% Avg Oxygen, NREM, mean±SD	97.1±1.3	97.4±1.2
% time with CO2<50mmHg	50.0±35.7	55.7±35.6
Cholesterol Level	157.8±27.3	150.1±20.6
HDL	51.9± 11.8	47.6±10.6
LDL	89.7±26.7	88.1±23.2
Triglycerides	72.7±41.5	59.3±32.5

n=number, SD=standard deviation

BMI = Body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, AHI = apnea hypopnea index, HDL= high density lipoprotein, LDL= low density lipoprotein Other race includes Hispanic, Asian, mixed race and those specified as "Other" in the medical record

[*] $p<0.05$ comparing control vs. OSA groups

Table 2:

Associations between OSA and various biomarkers (log scale) adjusted for age, race, sex and body mass index (BMI) z score

	Controls (n=53) (IQR)	OSA (n=43) (IQR)	Group Estimate (OSA vs control) (SE)	Group estimate p
Adiponectin (μg/mL)	20.2 (13.4, 28.7)	18.8 (13.5, 29.6)	−0.05 (0.13)	0.66
SAA (μg/mL)	1.5 (1.0, 3.2)	2.1 (1.1, 4.9)	0.04 (0.24)	0.87
sCD40L (pg/mL) [*]	0.95 (0.35, 2.95)	3.14 (1.11, 9.18)	0.94 (0.30)	0.002
IL-6 (pg/mL) [*]	7.0 (2.2, 27.2)	18.1 (6.0, 54.7)	0.73 (0.33)	0.03
IL-8 (pg/mL) [*]	5.4 (2.0, 8.4)	9.0 (4.1, 23.1)	0.62 (0.24)	0.01
MCP-1 (pg/mL)	92.0 (77.1, 109.0)	101.0 (86.7, 123.6)	0.06 (0.08)	0.48
TNF-α (pg/mL)	3.9 (2.6, 5.9)	5.2 (3.2, 8.8)	0.10 (0.23)	0.67
CRP (μg/mL)	0.27 (0.21, 0.55)	0.45 (0.23, 1.42)	0.44 (0.21)	0.04

n=number, IQR=interquartile range; CRP = C-reactive protein; IL = interleukin; MCP-1 = monocyte chemoattractant protein; OSA = obstructive sleep apnea; TNF-α = tumor necrosis factor.

[*]
$p<0.05$

Table 3:

Coefficient estimates from confirmatory factor analysis with: age, BMI z score, sex, race, OSA status and PM cytokines (MCP-1, sCD40L, TNF-α, Group 1: (IL-6, IL-8), and Group 2: (SAA, CRP, Adiponectin)) (n=96)

	Composite Mean		Common Peak Systole		Common Peak Diastole		Peterson Common		Mean lumen cross-sectional area of the artery (LCSA)		Mean wall cross-sectional area (WCSA)	
	Estimate	p	Estimate	p	Estimate	p	Estimate	p	Estimate	p	Estimate	p
Age	0.3936	<.0001	0.5722	<.0001	0.4752	<.0001	0.0511	0.4413	0.4746	<.0001	0.6521	<.0001
BMIZ	0.0277	0.7575	0.2621	0.0090	0.1815	0.0354	-0.0746	0.2260	0.1887	0.0337	0.1280	0.1515
Female	0.0034	0.9696	-0.4568	<.0001	-0.2553	0.0036	0.2346	0.0002	-0.2649	0.0033	-0.0714	0.4347
Race (African American)	0.2773	0.0011	-0.0650	0.5133	-0.0495	0.5635	0.0597	0.3268	-0.0395	0.6548	0.2233	0.0104
OSA	-0.0429	0.6491	-0.0540	0.6121	-0.1154	0.2083	-0.0867	0.1822	-0.1294	0.1710	-0.0290	0.7598
MCP-1	0.1096	0.2149	0.0447	0.6555	-0.0358	0.6782	0.0643	0.2925	-0.0396	0.6567	0.0594	0.5050
sCD40L	0.2476	0.0043	0.0920	0.3623	0.0020	0.9819	-0.0006	0.9925	0.0152	0.9430	0.1957	0.0271
TNF-α	-0.0504	0.5811	0.1997	0.0539	0.2121	0.0165	-0.0636	0.3131	0.2211	0.0152	-0.0323	0.7251
Group 1 (IL-6, IL-8)	0.0760	0.4500	-0.2768	0.0142	-0.2221	0.0217	0.1499	0.0311	-0.2119	0.0338	0.0176	0.8619
Group 2 (SAA, CRP, Adiponectin)	0.0088	0.9305	-0.0535	0.6383	-0.0693	0.4795	0.0005	0.9946	-0.0657	0.5152	-0.0751	0.4660

Table 4.

Associations between OSA and markers ultrasound measures of carotid structure and function adjusted for age, race, sex and BMI z score

	Controls (n=53) (IQR)	OSA (n=43) (IQR)	Group Estimate (OSA vs no OSA) (SE)	Group estimate p
[a]Composite Mean (cm)	0.039 (0.036, 0.042)	0.040 (0.036, 0.044)	.0002 (.001)	0.83
[b]Common Peak Systole Mean (cm)	0.58 (0.54, 0.61)	0.55 (0.53, 0.59)	−.007 (.008)	0.37
[c]Common Peak Diastole Mean (cm)	0.48 (0.44, 0.50)	0.45 (0.43, 0.47)	−.012 (.007)	0.08
[d]Peterson Common (mmHg/cm)	966.45 (793.77, 1158.04)	896.20 (755.34, 1073.92)	−26.62 (50.41)	0.60
[e]Mean lumen cross-sectional area (LCSA) (cm^2)	0.18 (0.15, 0.20)	0.16 (0.14, 0.18)	−.0096 (.005)	0.07
[f]Mean wall cross-sectional area (WCSA) (cm^2)	0.07 (0.06, 0.08)	0.07 (0.06, 0.08)	−.0007 (.002)	0.78

n=number, IQR=interquartile range

[a]Composite Mean = Composite measurement of the mean of the R and L CCA distal far wall IMT measurement, R and L bifurcation far wall intima medial measurement, and R and L ICA proximal far wall intimal medical thickness measurement

[b]Common Peak Systole Mean = Mean of R and L CCA diameter at peak systole

[c]Common Peak Diastole Mean = Mean of R and L CCA diameter at end diastole

[d]Peterson Common:

$$\text{Mean of} \left[\frac{\text{Systolic BP} - \text{Diastolic BP}}{\left(\begin{array}{c}\text{(R CCA diameter peak systole} - \text{R CCA} \\ \text{diameter end diastole)} * \text{(mean R CCA} \\ \text{diameter peak systole)}\end{array}\right)} + \frac{\text{Systolic BP} - \text{Diastolic BP}}{\left(\begin{array}{c}\text{(L CCA diameter peak systole} - \text{L CCA} \\ \text{diameter end diastole)} * \text{(mean L CCA} \\ \text{diameter end systole)}\end{array}\right)} \right]$$

[e]Mean lumen cross-sectional area of common carotid $= \left(\pi(dD)^2\right)/4$; Mean diastolic diameter (dD) $= \frac{(RdD + LdD)}{2}$; LdD is left and RdD is right common carotid artery diastolic diameter

[f]Mean wall cross sectional area (WCSA) $= \pi\left(\frac{dD}{2} + IMT\right)$

R=right, L=left, CCA = common carotid artery, ICA = internal carotid artery