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Structural equation modeling of sleep apnea, inflammation, and metabolic dysfunction in children

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SUMMARY Obstructive sleep apnea (OSA), often concomitant with obesity, increases the risk for the metabolic syndrome. One mechanism that may participate in this association is upregulation of inflammatory pathways. We used structural equation modeling to assess the interrelations between childhood obesity, OSA, inflammation, and metabolic dysfunction. One hundred and eighty-four children (127 boys, mean age: 8.5 ± 4.1years) had height and weight measured, underwent overnight polysomnography and had fasting blood taken. The blood was analyzed for insulin, glucose, lipids, leptin, and cytokines [interferon (IFN)-γ, granulocyte macrophage–colony stimulating factor, interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, tumor necrosis factor-α]. Structural equation modeling (SEM) was used to evaluate associations between the outcomes of interest including hypoxia, arousal (related to respiratory and spontaneous), obesity, metabolic dysfunction, and inflammatory markers. Two cytokine factors and one metabolic factor were derived for the SEM. These factors provided good fit in the structural equation model (χ^2/df = 2.855; comparative fit index = 0.90, root mean squared error of approximation = 0.10) and all factor loadings were significantly different from zero ($P \leq 0.01$). Overall, our results indicate that while obesity (as measured by body mass index *z*-score) has a major influence on the metabolic dysfunction associated with OSA, arousal indices, and cytokine markers may also influence this association. Our results support the hypothesis that OSA is a contributor to the mechanisms that link sleep, systemic inflammation and insulin resistance, and show that the interrelations may begin in childhood.

KEYWORDS children, inflammation, metabolic syndrome, obstructive sleep apnea, structural equation model

INTRODUCTION

The term 'metabolic syndrome' is used to identify a clustering of risk factors that indicates an increased risk for morbidity and mortality associated with cardiovascular disease. Although there is some debate regarding which factors are obligatory and which cut-points are used in the definition of the syndrome, this most commonly refers to the clustering of obesity, hypertension, insulin resistance (type 2 diabetes), heart disease, and dyslipidemia (Eckel *et al*., 2005).

Independent associations have been demonstrated between obstructive sleep apnea (OSA) in adults and metabolic and cardiovascular complications (Caples *et al*., 2007; Kasasbeh *et al*., 2006). Similar associations have also been demonstrated in children (NG *et al*., 2005; Srinivasan *et al*., 2006); children

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with OSA have alterations in systemic blood pressure (Amin *et al*., 2004) and in echocardiographic variables (Amin *et al*., 2002, 2005). De la Eva *et al*. (2002) found a positive association between the severity of OSA and insulin resistance in obese children and Verhulst *et al*. (2007) found that oxygen saturation (mean and nadir) were independent significant predicators for the presence of metabolic syndrome in overweight children with sleep-disordered breathing.

Inflammation is a mechanism that may link OSA and cardiovascular disease (Parish and Somers, 2004; Ryan *et al*., 2005). Adults with OSA have higher levels of pro-inflammatory cytokines, tumor necrosis factor (TNF)-α, and interleukin (IL)-6 (Alberti *et al*., 2003; Vgontzas *et al*., 1997) which decrease following treatment for OSA (Kataoka *et al*., 2004; Ohga *et al*., 2003; Yokoe *et al*., 2003). Children with OSA also have higher levels of inflammation: Tauman *et al*. found raised levels of C-reactive protein (CRP), a strong marker of cardiovascular risk, in children with sleep-disordered breathing, compared with controls, with levels correlating with disease severity. CRP levels were significantly decreased 10–14 weeks after adenotonsillectomy in children with OSA (Kheirandish-Gozal *et al*., 2006).

Structural equation modeling is a comprehensive, flexible approach to modeling associations between variables. Unlike ANOVA or multiple regression techniques, which model individual observations, SEM permits exploration of complex interrelationships amongst a number of potentially inter-dependent variables. SEM usually starts with a hypothesis which is represented as a model. Measured (observed) and latent (unobserved) variables are 'factored' into the model and various models are tested for goodness of fit. It is important to note in SEM analysis that although the model may be a good fit to the data, this does not necessarily imply that it is the best model for the data or that causal associations exist (Bentler and Stein, 1992). The advantages of SEM include more flexible assumptions (even when variables are collinear) and the use of confirmatory factor analysis, a technique used when there are many variables which may be explained by a smaller number of factors. SEM analysis was initially established in the psychometric literature (Maccallum and Austin, 2000), but has since been applied to medical and physiological models (Yamanishi *et al*., 2007).

The aim of this study was to use SEM to better evaluate the complex interrelationships between obesity, OSA, inflammation, and metabolic dysfunction in children. The hypothesis that we modeled and tested was that an independent association exists between OSA and metabolic dysfunction in children, and that at least part of this association is explained by upregulation of inflammatory mediators.

METHODS

Study design

Subjects were recruited consecutively when they presented for evaluation of suspected OSA to the Read Sleep Unit, The Children's Hospital at Westmead, Sydney, Australia. All parents completed a questionnaire inquiring about the child's normal sleep routine, symptoms of sleep-disordered breathing, and family history. Exclusion criteria included the presence of genetic disorders, cerebral palsy, neuromuscular disorders, and children with acute infections. This study was approved by the Human Research Ethics Committee for The Children's Hospital at Westmead and written parental consent was obtained for all participants.

Anthropometric measures

Height and weight were obtained for each child. Height was measured to the nearest 0.1cm using a Harpenden Stadiometer (Holtain Ltd., Crymych, UK) and weight was measured in light clothing to the nearest 0.01 kg on digital scales. Body mass index (BMI; kg m^{-2}) and *z*-scores were calculated from age and gender specific reference values (Kuczmarski *et al*., 2000) For the purposes of this study, participants with a BMI *z*-score > 2 were considered obese.

Overnight polysomnography

Polysomnograms was performed on all children referred to the Sleep Unit for evaluation. Children were studied for up to 12 h in a quiet, darkened room in the company of a parent. The following parameters were measured: four channels of electroencephalogram, electrooculogram, submental, diaphragm, and abdominal electromyelograms. Respiratory variables included thoracic and abdominal wall movement (inductance plethysmography using Respitrace® (Non Invasive Monitoring Systems, Miami, FL, USA), nasal airflow using a pressure transducer attached to nasal prongs (No. 1615; Slater Labs, Arvin, CA, USA) and a thermister (Compumedics, Melbourne, Australia), oxyhemoglobin saturation (SpO_2; Ohmeda Biox, CO, USA), and transcutaneous carbon dioxide (TCM3; Radiometer, Copenhagen, Denmark). In addition, electrocardiogram and body position were recorded. All sleep study data were acquired on a digital data acquisition system (Compumedics S Series, Melbourne, Australia) and analyzed with COMPUMEDICS PROFUSION Software.

The sleep architecture was assessed using standardized techniques. Briefly, respiratory events were considered significant if they lasted ≥2 respiratory cycles (AAP and Pediatrics, A. A. O., 2002) and were accompanied with a $\geq 3\%$ SpO_2 desaturation and/or terminated by arousal. Obstructive apneas were defined as the cessation/reduction of airflow to $< 80\%$ of baseline with continuing or increasing effort (as measured by the Respitrace and/or diaphragm EMG). Hypopneas were defined as a decrease in airflow ≥50% of the baseline amplitude. Arousals (spontaneous, movement, respiratory, and sigh) were defined as changes in ≥2 independent channels, with at least a 10-s period of sleep prior to the change and a disturbance lasting for > 1 s. The primary outcome measure for sleep studies was the respiratory disturbance index (RDI), defined as the number of respiratory events per hour of

sleep time, to provide an indication of the severity of OSA. Children with an RDI ≥ 1 were considered to have OSA. Additional outcomes included in our model were the arousal indices, for spontaneous events and for those precipitated by an apnea or hypopnea (respiratory arousals). Arousal indices were also defined as the number (by type) of event per hour of sleep time.

Blood samples

A fasting blood sample was collected the morning following the sleep study at approximately 6:30 hours. Local anesthetic cream was applied to the cubital fossa and blood was drawn for measurement of insulin, glucose, lipid profile, and cytokines.

Metabolic markers

Insulin levels were measured by a commercially available assay (Linco Research Inc., St Charles, MO, USA) based on a radioimmunoassay, double antibody/ PEG technique. The lowest level of insulin that can be detected by this assay is 0.2 μU mL^{-1} (1.4 pmol/L) when using a 100 μL sample size with an intra-assay coefficient of variability of 3.2% and an inter-assay coefficient of 3.9%. Glucose profiles were measured on a Beckman CX5 high performance liquid chromatography automated analyzer (Beckman Instruments Inc., Fullerton, CA, USA). Glucose concentrations were measured using the glucose oxidase method with an analytical range of 0.3–38.8 mmol L^{-1} and intra- and inter-assay coefficients of variability of 1.6 and 1.3%, respectively. Plasma lipids including total cholesterol, HDL cholesterol, and triglycerides were assessed using Flex Reagent Cartridges (Dade Behring, Newark, DE, USA).

Inflammatory markers

Plasma was aliquotted and stored at −80º until required for cytokine assays. Cytokines were quantified using a multiplex bead-based assay (LiquiChip Human 10-Cytokine kit; Qiagen, Tokyo, Japan). Leptin was measured by a commercial available assay (Linco Research Inc, St Charles, MO, USA). Cytokine assays were performed for interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, granulocyte macrophage-colony stimulating factor (GM-CSF), interferon (IFN)-γ and tumor necrosis factor (TNF)-α. All cytokine assays were completed over 2 days to minimize inter-assay variability.

DATA ANALYSIS

Data were analyzed using SPSS for Windows version 13 and AMOS 4.0 statistical software (SPSS Inc. Chicago, IL, USA). A *P*-value of <0.05 was considered statistically significant.

Structural equation modeling analyses were conducted in three steps. First, exploratory factor analysis, using principal axis factoring and oblique rotation, was conducted on inflammatory and metabolic markers and sleep parameters. These are standard methods used in SEM to decrease the number of variables (http://www.statsoft.com/textbooks/stfacan). Measured (observed) and latent (unobserved) variables were examined for co-linearity, and related variables were combined into single 'factors', with individual factor loadings. Two criteria were used to determine the number of factors including eigen values >1 and the percent of variance accounted for by the factors $<70\%$ (http://www.statsoft.com/textbooks/stfacan).

Next, the factors were incorporated into a model using confirmatory factor analysis; the model was tested using goodness of fit tests to assess the overall fit of the model to the data. Various models were tested until the best fitting model was obtained. Finally, the model obtained from confirmatory factor analysis was incorporated into a structural equation model examining the links between BMI, sleep variables, and the cytokine and metabolic factors.

RESULTS

From 216 children recruited for this study, 184 children (69% male) had complete data for anthropometry, sleep studies, and all blood markers, allowing them to be included in the SEM. The mean age was 8.5 ± 4.1 years (range 1.5–17.9 years). Forty-two percent of the children were considered obese (BMI *z*-score >2.00). The mean AHI was 8.8 ± 16.9 h^{-1} (range 0–163.4); 25 (14%) children had an RDI <1 and 74 (40%) had an RDI≥5. The mean sleep efficiency was 79.7 ± 13.2%. The mean total arousal index was 13.1 ± 2.2 h^{-1}, with mean spontaneous and respiratory arousal indices being 4.8 ± 2.6 h^{-1} and 5.1 ± 8.8 h^{-1} respectively. Baseline SpO_2 was 96.4 ± 2.1% while SpO_2 nadir was 89.1 ± 6.6%. There were no differences in sleep parameters between males and females. The mean values for cytokine and metabolic markers are shown in Table 1.

Table 1 Summary data for inflammatory and metabolic markers

Inflammatory markers	*Mean ± SD* (n = *184*)
IFN-γ (pg mL^{-1})	13.8 ± 17.2
GM-CSF (pg mL^{-1})	106.8 ± 295.1
IL-1β (pg mL^{-1})	7.1 ± 10.9
IL-2 (pg mL^{-1})	4.4 ± 5.9
IL-4 (pg mL^{-1})	7.4 ± 9.4
IL-6 (pg mL^{-1})	8.2 ± 14.1
IL-8 (pg mL^{-1})	11.0 ± 22.8
IL-10 (pg mL^{-1})	6.2 ± 10.4
IL-12 (pg mL^{-1})	44.3 ± 118.8
TNF-α (pg mL^{-1})	6.8 ± 10.5
Leptin (ng mL^{-1})	9.9 ± 10.4
Metabolic markers	
Insulin (pmol L^{-1})	105.3 ± 79.5
Glucose (mmol L^{-1})	4.6 ± 0.7
Insulin/glucose ratio	22.5 ± 15.8
Triglycerides (mmol L^{-1})	1.0 ± 0.5
Cholesterol (mmol L^{-1})	4.6 ± 0.9
HDL cholesterol (mmol L^{-1})	1.4 ± 0.4

IFN, interferon; GM-CSF, granulocyte macrophage-colony stimulating factor; IL, interleukin; TNF, tumor necrosis factor.

Structural equation modeling

Exploratory factor analysis of the cytokine and metabolic marker variables produced three latent factors including two cytokine factors and one factor for the metabolic syndrome. Components of the first cytokine factor included IL-4, IL-6, IL-10, and IL-12, GM-CSF and IFN-γ. Components of the second cytokine factor included IL-1, IL-2, IL-4, IL-8, and TNF-α. Each of these factor loadings were statistically significant ($P < 0.05$). IL-4 and TNF-α were included in both cytokine factors, because they were significant in both factors. Cholesterol did not load significantly on the latent variables and was therefore modeled as an individual variable separate from inflammation and metabolic dysfunction in subsequent models.

These factors were incorporated into a confirmatory factor analysis to determine the fit of this model to the data. The best fitting model from confirmatory factor analysis was incorporated into a structural equation model examining the links between BMI *z*-score, sleep variables, inflammation, and the metabolic syndrome. The model provided good fit to the data (χ^2/df = 2.855; comparative fit index = 0.90, root mean squared error of approximation = 0.10) and all factor loadings (Table 2) were significantly different from zero ($P \leq 0.01$). A simplified version of the SEM is pictured (Fig. 1). The paths represented are only those that were found to have statistically significant path coefficients (not all of those tested). All analyses were controlled for age. The figure demonstrates which markers load on to their respective factors as well as the magnitude of factor loadings.

We re-ran the SEM model after forcing the cytokine factors into 'biological' (TH2, pro-, and anti-inflammatory) rather than 'statistically derived' clusters, but this model did not converge. This outcome suggests there is a problem with the biological model specified and for these data alternative models, such as the model derived from the exploratory factor analyses, may provide a better solution. This is further described in the discussion.

The major findings of this modeling analysis were that BMI showed a positive association with the metabolic variable and

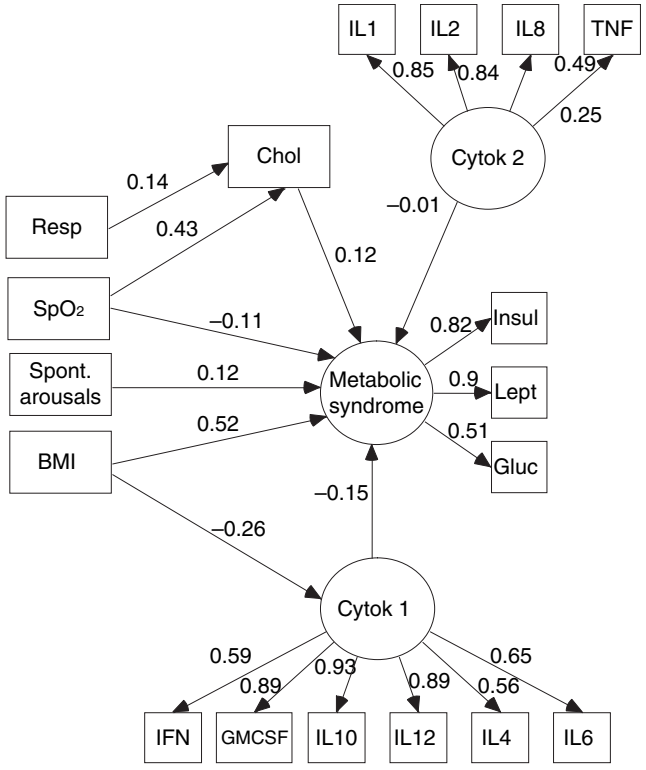

Figure 1. A simplified diagram of the structural equation modeling between childhood obstructive sleep apnea (OSA), metabolic syndrome and inflammation. Measured variables are shown as rectangles and latent variables/factors are shown as circles. The direction of the arrow indicates correlations and the path coefficient is shown next to the arrow. All associations shown in the figure are significant. However, for simplicity, not all significant correlations are shown in the figure. *Note*: Negative coefficients are expected for SpO_2 since lower oxygen saturation indicates more severe hypoxia. The negative correlations between 'Cytok. l'and other variables may reflect the anti-cytokine factors in this cluster of inflammatory markers. IL = interleukin, TNF = tumor necrosis factor, Insuli = insulin, Lept = leptin, cytok = cytokines (for factors), Chol = cholesterol, Resp = respiratory disturbance index, SpO2 = oxygen saturation, Spont arousals = spontaneous arousal index, BMI = body mass index, IFN = interferon, GM-CSF = granulocyte macrophage-colony stimulating factor.

Table 2 Confirmatory factor analysis standardized factor loadings

Cytokine factor 1		*Cytokine factor 2*		*Metabolic factor 1*	
Component (log transformed)	*Loading*	*Component (log transformed)*	*Loading*	*Component*	*Loading*
IFN-γ	0.59	IL-1	0.83	LogInsulin	0.91
GM-CSF	0.89	IL-2	0.85	LogLeptin	0.77
IL-10	0.93	IL-8	0.52	Glucose	0.59
IL-12	0.89	TNF-$\alpha^{\dagger}$	0.24		
IL-4†	0.56	IL-4†	0.42		
IL-6	0.64				
TNF-$\alpha^{\dagger}$	0.37				

IFN, interferon; GM-CSF, granulocyte macrophage-colony stimulating factor; IL, interleukin; TNF, tumor necrosis factor.
†Markers that loaded onto more than one factor.

a negative association with the cytokines. In terms of sleep variables, spontaneous arousals were predictive of the metabolic variable, but were not predictive of inflammation.

From the lipids we measured, only total cholesterol was included in the model. Respiratory arousals were predictive of cholesterol but not of the metabolic variable or inflammation. Cholesterol, in turn, was associated with the metabolic variable. SpO_2 was predictive of cholesterol and had a relation with the metabolic variable that demonstrated a trend towards significance ($P = 0.07$). The relation between SpO_2 and the metabolic variable was statistically significant if cholesterol was removed from the model ($P < 0.05$).

Of the cytokine factors (Cytokines 1 and 2), cytokines 1, but not 2, was associated with the metabolic factor in the model. Even if the path from cytokines 1 to 2 was removed from the analysis, cytokines 2 was not linked to the metabolic factor. Cytokines 1 was a significant predictor of Cytokines 2 ($\beta = 0.53$, $P < 0.001$, not pictured).

DISCUSSION

Using SEM in a cohort of children with and without OSA, results from our study indicate that while BMI has a major influence on metabolic abnormalities, arousal indices and cytokine markers also contribute to metabolic dysfunction. Direct associations were found between sleep parameters and metabolic dysfunction, but we found no direct associations between sleep and cytokine factors (as markers of inflammation).

Structural equation modeling

Structural equation modeling is a powerful statistical tool for building and testing models. It is a hybrid technique that encompasses general linear modeling, path analysis and regression, and the major advantage is that 'latent' (unobserved) variables can be incorporated into the model. However, SEM does have certain limitations. To date, there is no recommended method for power analysis to calculate appropriate sample size to test a hypothesis using this modeling technique. In general, a sample size 10 times that of the number of variables is recommended. Hence using this general rule, our study, with 184 children may be viewed as a pilot study, which needs to be confirmed in larger studies. Although, after reviewing the literature, Loehlin *et al.* concluded that in SEM analysis that uses two to four factors (such as ours) (http://www.utexas.edu/its/rc/tutorials/amos), 100 cases is sufficient (http://www.utexas.edu.its/rc/tutorials/stat/amos/) and previous published studies have used similar sized cohorts (Chan *et al.*, 2002).

Another limitation of SEM is that the statistical model chosen may not be the best-fitting model for the data and causal associations cannot be drawn. Although different models can be generated, the models need to be interpreted in light of the theoretical background of the topic, such that the final model is valid both statistically and theoretically (Joreskog and Sorbom, 1993). It is important to note that results from SEM analysis may be influenced by the sample, variables, time frame and the grouping of factors used in the analysis (Maccallum and Austin, 2000). Here we highlight some of the interesting findings from the present study.

Components of OSA

Physiological disturbances associated with OSA include arousal and desaturation (hypoxia). Our method of scoring apneas means that termination of apnea is always associated with desaturation, but not always with arousal. Because arousal from sleep is linked to dysregulation of inflammation, and studies in adults have shown that sleep deprivation is associated with reduced insulin responses to glucose, we made a distinction between arousal events and desaturation events (Shamsuzzaman *et al.*, 2003; Spiegel *et al.*, 1999). Although there is controversy about arousal definitions and their clinical significance (Ciftci *et al.*, 2004b), arousal is less consistently associated with the termination of apnea in children than adults (Scholle and Zwacka, 2001).

Minimum oxygen saturation showed the anticipated, negative correlation with metabolic markers although this may reflect other mechanisms for disturbed glucose metabolism or inflammation, including the increase in sympathetic activity associated with OSA (Punjabi *et al.*, 2004). In our respiratory analyses obstructive events were only marked if they terminated in desaturation or arousal and we acknowledge that this is a relatively conservative method of scoring respiratory events in pediatrics. Nonetheless, the association with minimum saturation was stronger than that for respiratory event indices. The frequency of spontaneous arousals correlated positively with lipid (cholesterol) levels, but negatively with metabolic dysfunction. The result is consistent with studies showing that effective treatment of OSA in adults is associated with a fall in cholesterol levels (Robinson *et al.*, 2004). This occurred despite the previous observations that the frequency of spontaneous arousals falls with increasing severity of OSA in children, and that arousal frequency does not correlate with markers of disturbed glucose metabolism in adults (Punjabi *et al.*, 2004; Tauman *et al.*, 2004).

Associations with metabolic dysfunction

Potential mediators of the relation between OSA and metabolic dysfunction include direct effects of hypoxemia on glucose regulation (Oltmanns *et al.*, 2004), elevations in sympathetic nerve activity (Somers *et al.*, 1995), or upregulation of inflammatory mediators (Arter *et al.*, 2004). Results from our study indicate three underlying factors, namely a metabolic factor and two cytokine factors. BMI z-score (as a measure of obesity) may mediate metabolic dysfunction and in severely obese children the prevalence of metabolic syndrome may be as high as 50% (Weiss *et al.*, 2004).

In our model, the metabolic factor included insulin, glucose, and leptin. We also found that OSA and BMI z-score was

associated with leptin. Leptin, a hormone produced by adipose tissue, regulates fat mass by decreasing food intake and increasing resting energy expenditure (Shamsuzzaman *et al*., 2003). Leptin binds to receptors in the hypothalamus that stimulate anorexigenic peptides, such as poopiomelanocortin and inhibit orexigenic peptides, including neuropeptide Y. Leptin levels are elevated in both obesity (Bao *et al*., 1997; Berenson *et al*., 1997) and OSA (Caro *et al*., 1996; Ulukavak Ciftci *et al*., 2005), with these levels decreasing following treatment for OSA (Chin *et al*., 1999; IP *et al*., 2000). The persistence of OSA and obesity, despite high leptin levels, may represent a leptin resistant state, although the molecular basis of this is yet to be determined (Ulukavak Ciftci *et al*., 2005).

Associations with inflammatory markers

It is important to recognize that the cytokine clustering used in this model was based on statistical compatibility, not current understanding of biological functions. In that context, whereas sleep variables are associated with metabolic dysfunction in children, our model suggests that the main connection between sleep apnea and inflammation is through the influence of BMI. Obesity (increasing BMI) was associated with worsening of metabolic markers but with reduced levels of the cytokine markers. Sleep variables were not related to the cytokine factors.

An alternative path showed that changes in metabolic function led to downstream changes in inflammatory markers. The two cytokine factors uncovered in the analysis suggest that in general the pro-inflammatory cytokines and the anti-inflammatory cytokines tended to load on to separate factors although there was some overlap. The cytokine 1 factor including anti-inflammatory cytokines IFN-γ, IL-10, and IL-12 was negatively related to BMI z-score. This may suggest that increasing BMI inhibits the anti-inflammatory cytokines with upregulation of some pro-inflammatory cytokines. Indeed, obesity in itself may be a low-grade inflammatory state. Adipose tissue can also have a substantial impact on systemic glucose homeostasis, insulin resistance, and vascular disorders through altered production and release of adipokines, such as leptin. Some cytokines are derived from adipocytes as well as other tissues (IL-6, IL-8). The mechanism for the disturbances in inflammatory mediators may relate to oxidative stress (Furukawa *et al*., 2004).

It has been suggested that the intermittent hypoxemia associated with OSA may also underlie inflammation by triggering the formation of reactive oxygen species (Dyugovskaya *et al*., 2002; Lavie, 2003) and in turn trigger the release of pro-inflammatory cytokines that affect metabolism (Pickup and Crook, 1998; Stumvoll and Haring, 2001). In the present study, where our measure of hypoxia was the SpO_2 nadir, we did not find an association between hypoxia and either of the cytokine factors. It remains possible that alternative measures of hypoxia, such as percentage of time with saturation levels below a given threshold, would yield an association with the cytokine factors.

Several cytokines (e.g., IL-6, IL-8, TNF-α) are also derived from adipocytes, and production of these cytokines is increased in obesity, particularly TNF-α and IL-6 (Vgontzas *et al*., 1997). Vgontzas *et al*. (2003) have shown that IL-6, TNF-α, leptin, and insulin levels are elevated in adult sleep apnea independent of obesity. In addition, visceral fat was the primary parameter linked with sleep apnea and blockade of TNF-α was associated with reduced sleepiness (Vgontzas *et al*., 2005). We found that BMI *z*-score was related to one of the cytokine factors but in our models the relations between obesity (BMI) and cytokine factors were complex. There was a negative association between BMI and cytokine 1 (which included a number of anti-inflammatory cytokines), although BMI showed a positive relation to the metabolic factor which was in turn related to the second cytokine factor.

Several recent studies have shown that IL-6, and TNF-α are elevated in adults with OSA (Ciftci *et al*., 2004a). We found a positive association between BMI and the cytokine factor included TNF-α, but a negative correlation between BMI and the cytokine factor that included IL-6. Ciftci *et al*.,(2004a) have shown that IL-6 and TNF-α correlated with RDI but not with BMI, which suggests that the link between cardiovascular morbidity and OSA may be explained by the coexistence of other cardiovascular risk factors (such as IL6 and TNF-α).

CONCLUSION

The markers of metabolic dysfunction we used, such as obesity, insulin resistance, hypertension, and dyslipidemia are known to be pathological risk markers for cardiovascular disease in young adults. The analysis that we presented here is novel in children. Exploration of associations between sleep apnea, metabolic dysfunction, and inflammation in children provided further support for the existence of a link between obesity and OSA in children, which is likely to increase the risk for future atherosclerosis and cardiovascular disease (Vgontzas *et al*., 2003). The results also support the hypothesis that different components of OSA may contribute to different elements of metabolic dysfunction. SEM provides useful exploration of complex associations in physiological systems.

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