Sleep-disordered breathing, systemic adipokine secretion, and metabolic dysregulation in overweight and obese children and adolescents

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Abstract

Objective: Obstructive sleep apnea (OSA) is common among overweight and obese children, and it is an independent risk factor for developing the metabolic syndrome. However, the mechanisms linking OSA and the metabolic syndrome are still unclear, but a role for adipose tissue dysfunction caused by intermittent hypoxia has been suggested. Therefore, the goal of this study was to investigate the relationship between OSA and systemic adipokine concentrations in overweight and obese children.

Study design: We included 164 overweight and obese children in a tertiary center and distributed them in groups based on their oAHI (111 controls, 28 mild OSA, 25 moderate-to-severe OSA). All subjects underwent polysomnography and a blood sample was taken to determine leptin, adiponectin, TNF alpha and IL-6 levels.

Results: No significant differences were found in adipokine levels between subjects with or without OSA. Leptin correlated with oxygen desaturation index (r=-0,17, p=0,03), adiponectin correlated with mean oxygen saturation (r=0,24, p=0,002) and with the percentage of sleep time with an oxygen saturation >95% (r=0,25, P=0,001). However, these associations did not persist after correction for adiposity. No correlations between IL-6 and TNF alpha, and OSA severity were found.

Conclusion: These results suggest that serum adipokine levels are mostly dependent on central obesity, while they are not influenced by OSA in an obese pediatric population.

Introduction

Childhood obesity has reached epidemic proportions worldwide and its prevalence is still increasing(1). It is recognized as one of the most important public health issues that is already associated with several health complications(2). It is known that obesity is associated with adipocyte hypertrophy and consequently inadequate blood flow to the cell, resulting in hypoxia at the level of the adipose tissue. Patients with hypertrophic adipose tissue are more likely to develop obesity-related comorbidities(3), and hypertrophy of the adipose tissue by itself is postulated to lead to a predominantly proinflammatory adipokine secretion pattern(4).

Obesity is an important risk factor for developing obstructive sleep apnea (OSA), which is defined by intermittent cycles of upper airway collapse associated with hypoxia and arousals during sleep. The prevalence of OSA in the general pediatric population is about 2-3%(5), whereas studies have shown a much higher prevalence between 13-59% in obese children and adolescents(6). OSA is an independent risk factor for acquiring different components of the metabolic syndrome (MetS), which is defined as the clustering of abdominal obesity, glucose intolerance, dyslipidemia and hypertension. Furthermore, OSA severity is associated with the degree of metabolic alterations(7, 8). This independent contribution of OSA has also been demonstrated for non-classical factors of the MetS such as non-alcoholic fatty liver disease (NAFLD)(9, 10). Intermittent hypoxia associated with OSA can result in oxidative stress and systemic inflammation, which are possible underlying mechanisms for the development of the MetS. However, the exact link between inflammation, oxidative stress, the MetS and OSA is still unclear. Adipose tissue could play an important role in the link between OSA and the MetS since it actively secretes adipokines, which have an important role in the metabolic regulation. Intermittent hypoxia of the adipose tissue could have an additional effect on the structure of the adipocytes, but also on the regulation of adipokine secretion.

Adiponectin, leptin, tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) are adipokines which previously have been studied for their role in OSA, with conflicting results in literature. Four studies

found elevated leptin levels in children with sleep-disordered breathing(11-14), while two other studies found no relationship between leptin and measures of sleep-disordered breathing(15, 16). Only two studies described a decrease of adiponectin with increasing OSA severity(13, 17). However, in the study by Tsaoussoglou et al., there was no significant difference between the overweight/obese controls and the overweight/obese group with OSA, suggesting that the difference they describe is mainly attributable to the effect of obesity, and not OSA. For IL-6 and TNF-α, the majority of studies didn't find any effect of sleep-disordered breathing(13, 16, 18-20). Only one study found elevated TNF-α levels in children with OSA(21), while another study found elevated IL-6 levels but no difference in TNF-α levels in children with OSA(22).

In view of these contradicting results, our goal was to evaluate the relationship between adipokine secretion in the serum, OSA and obesity in a pediatric and extensively phenotyped population, and link this to metabolic end-organ complications.

Materials and methods

1. Study population

In this prospective study, 164 consecutive overweight and obese children were recruited between June 2010 and August 2014 at the Pediatric Obesity Clinic of the Antwerp University Hospital. Children were excluded in case of infection, a chronic medical condition or genetic, neuromuscular or craniofacial syndromes. The ethics committee of the Antwerp University Hospital approved this study and informed consent was obtained from the patients and their parents.

2. Anthropometry

All measurements were performed in the morning, after an overnight fast with patients undressed. Height, weight, waist circumference and waist-to-hip ratio (WHR) were measured using standardized techniques by skilled personnel. Fat mass was measured with bioelectrical impedance analysis, using the Deurenberg formula for children(23). BMI was calculated as weight in kilograms over height in m² and was further analyzed as z-scores, using the Flemish growth study as a reference population(24). Overweight and obesity were defined according to the International Obesity Task Force criteria(25).

3. Blood sample

A fasting venous blood sample was drawn in the morning at the moment of admission.

3.1. Adipokines

Leptin, adiponectin, TNF-α and IL-6 were measured by means of commercially available ELISA-kits according to the provided laboratory guide (*Invitrogen, Life Technologies, Waltham, Massachusetts, USA* for leptin, adiponectin and TNF-α; and *R&D systems, Minneapolis, Minnesota, USA* for IL-6).

3.2. Metabolic measures

The following measures of metabolic dysregulation were determined by means of a chemiluminescence assay: glucose, insulin, c-peptide, total cholesterol, HDL-cholesterol and triglycerides (Dimesion Vista 1500, Siemens, Brussels, Belgium). HOMA-index (homeostasis model assessment) was calculated as the product of glucose (mg/dl) and insulin (μU/ml), divided by 405[26]. Area under the curve (AUC) was calculated with the trapezoidal rule for glucose, insulin and c-peptide at the following time points: time zero, 15 minutes, 30 minutes, 60 minutes, 120 minutes and 180 minutes. ALAT and ASAT were determined as markers for fatty liver disease by means of a kinetic UV- test (Beckman Coulter, Nyon, Switzerland).

4. Metabolic syndrome

The MetS was diagnoses when 3 or more of the following criteria were present:

1) Waist circumference ≥ 90th percentile adjusted for age and gender[27, 28].

2) Fasting glucose level ≥ 110 mg/dl.

3) Fasting triglyceride level ≥110 mg/dl.

4) Fasting HDL cholesterol level ≤ 40mg/dl.

5) Blood pressure ≥ 90th percentile adjusted for age and gender[29].

5. *Polysomnography*

All children underwent nocturnal polysomnography for at least 6 hours. The following variables were continuously measured and recorded by a computerized polysomnograph (Brain RT, OSG, Rumst, Belgium): electroencephalography (C4/Al and C3/A2); electrooculography; electromyography of anterior tibial and chin muscles; and electrocardiography. Respiratory effort was measured by respiratory inductance plethysmography and oxygen saturation by a finger probe connected to a pulse oximeter. Airflow was measured by means of a nasal pressure cannula and thermistor, and snoring was detected by means of a microphone at the suprasternal notch. All patients were

monitored on audio/videotape using an infrared camera. Respiratory events were scored according to the American Academy of Sleep Medicine guidelines(30).

The obstructive apnea–hypopnea index (oAHI) was defined as the average number of obstructive apneas and hypopneas per hour of sleep. Mild OSA was diagnosed by the presence of an oAHI between 2 and 5 and moderate-to-severe OSA was defined by an oAHI ≥ 5(31). The respiratory disturbance index (RDI) was calculated as the sum of the recorded apneas and hypopneas divided by the total sleep time. All desaturations of ≥3% from the baseline oxygen saturation were quantified and the oxygen desaturation index (ODI) was calculated as the total of desaturations divided by the total sleep time.

6. Statistical analysis

Statistical analysis was performed using SPSS 20.0 (SPSS, Chigaco, Illinois, USA). A previous study showed a significant difference in leptin concentrations between children with and without OSA(11). Based on that study, a sample size of 29 patients with OSA would be needed to achieve adequate statistical power (type I error rate of 5%, and a power goal of 90%). Normality was tested by the Kolmogorov-Smirnov test. Normally distributed data are presented as mean ± standard deviation. Skewed data are reported as median [range]. Patients were distributed in groups based on their oAHI. Groups were compared by means of χ^2, one-way ANOVA or the Kruskal-Wallis test. Correlations between adipokines, sleep parameters, and metabolic and end-organ complications were calculated using Pearson's or Spearman's correlation analysis as appropriate.

Linear regression analysis was performed in case of a significant correlation between adipokines and sleep parameters to determine if the correlation persisted after controlling for the degree of adiposity. Because the different measures of adiposity are highly intercorrelated, linear regression was done by the inclusion of one measure of adiposity with the highest univariate correlation coefficient for the respective outcome (BMI z-score, waist circumference, WHR, fat mass). For all analyses, $p < 0.05$ was considered statistically significant.

Results

1. Subjects' characteristics

A total of 164 overweight and obese children were included in this study with an average BMI of 30.4 kg/m² (range: 19.7-46.2 kg/m²), which corresponds to a mean z-score of 2.5 (range: 1.5-4.1). Mean age was 12 years (range: 5-17 years) and 37% of subjects were male. OSA was diagnosed in 53 children (32.3%), 28 subjects had mild OSA (17.1%) and 25 moderate-to-severe OSA (15.2%). The MetS was diagnosed in 52 patients (31.7%), of which 17 had OSA (10 mild OSA; 7 moderate-to-severe OSA). There is no difference in the prevalence of the MetS in children with and without OSA (p=0.8). Patient characteristics between the 3 groups (oAHI<2; 2<oAHI<5; oAHI≥5) are compared in Table 1. No significant difference in patient characteristics between groups was found except for WHR, which was significantly higher in the mild and moderate-to-severe OSA group. Sleep-related respiratory parameters were significantly different between groups as expected.

2. Adipokines

No differences in plasma adipokine levels were found between groups (table 1), although several correlations between adipokines and sleep-related respiratory parameters were found. Leptin correlated with ODI (r=-0,17, p=0,03) and there was a trend for a correlation between leptin and TST95 (r=-0,14, p=0,07). Adiponectin correlated with mean SaO_2 (r=0,24, p=0,002) and TST95 (r=0,25, p=0,001). No correlations between IL-6 and TNF-α, and measures for obstructive sleep apnea were found. Correlations between measures of adiposity and adipokines are shown in table 2.

No significant differences in IL-6, adiponectin and TNF-α were found between genders. However, leptin levels were significantly lower in male patients (p<0,001). Leptin (r=0,53, p<0,001), adiponectin (r=-0,34, p<0,01) and TNF-α(r=-0,17, p=0,03) were significantly correlated with age.

If adipokines correlated with both sleep-related respiratory parameters and measures of adiposity linear regression analysis was performed. The Spearman correlation between leptin and ODI (p=0,7)

did not remain significant after correction for fat mass. The correlations between adiponectin and mean SaO_2 (p=0,4), and adiponectin and TST95 (p=0,08) also did not remain significant after correction for waist circumference. A final model for adiponectin and leptin is presented in table 3.

No significant differences were found in patient characteristics between children with and without the MetS, except for waist circumference (p=0.01) and WHR (p=0.009). However, children with the MetS had a lower SaO_2 nadir compared to children without the MetS (p=0.004), and there was a trend for a higher ODI in children with the MetS (p=0.06). Differences in adipokine levels and metabolic parameters between children with and without the MetS are shown in table 4.

Discussion

No associations between OSA severity and the blood level of leptin, adiponectin, IL-6 and TNF-α were found in this clinical sample of overweight and obese children and adolescents. However, an association between measures of adiposity and all adipokines, except TNF-α was found.

As mentioned above in the introduction, a number of studies have found different results concerning the relationship between OSA and adipokines in children. Nevertheless, if we focus on the studies that only included overweight and obese children (12-16, 18), elevated leptin levels in children with OSA is the only significant result that was found in 3 studies. None of the studies found a relationship between OSA and adiponectin, IL-6 or TNF-α. The degree of obesity is an important confounder when studying adipose tissue-derived factors. The studies that did not distinguish between normal-weight and obese subjects(11, 17, 19-22) may not have excluded all covariates associated with obesity, which could be a possible explanation for the difference in results. Indeed, BMI was the only measure for obesity included in the analysis of these studies, despite the fact that this is not the best marker of abdominal obesity. This is a strong point of our study since we did an extensive phenotyping of obesity in our population by including several measures of adiposity. It is also possible that the direct effect of obesity could hide the independent effect of intermittent hypoxia, and this could explain why the results between obese and normal-weight populations differ. Severe central obesity appears to trigger an array of downstream effects that overwhelm those potentially caused by mild intermittent hypoxia, which could explain why we could not find any effect of OSA on the mediators studied in this obese pediatric population.

Not only studies concerning the relationship between OSA and adipokines in children have found contradicting results. Inconsistent results have also been observed when studying C-reactive protein in pediatric OSA(32-37). Furthermore, there is a clear phenotypic variability in the presence of morbidity in pediatric sleep-disordered breathing, since there are children who exhibit morbidity and those who do not, at any level of disease severity. This suggests, that next to the confounding factor

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of obesity, also other aspects could be of importance. Indeed, studies have indicated that genetic and environmental factors could have a role in the disease phenotype(38-40).

Animal studies have demonstrated that intermittent hypoxia influences several processes in adipose tissue(41, 42). However, these studies were performed in lean animals, and it is not known if intermittent hypoxia caused by OSA in childhood is severe enough to exacerbate this local tissue hypoxia in an obese population. Due to conflicting results in human studies, both in normal weight and obese patients, it is important to repeat these animal studies in an obese animal model. This could give us a better understanding of the effect of intermittent hypoxia on the adipose tissue and how it interacts with the confounding factor of obesity.

Even though we could not find any associations between adipokines and OSA in our population, we did find associations between OSA and metabolic end-organ complications, again confirming a role for sleep-disordered breathing in metabolic dysregulation. However the mechanisms linking OSA with metabolic dysregulation remain unclear. Other mechanisms have been suggested as the link between OSA and the MetS. Intermittent hypoxia and sleep fragmentation may alter sympathetic activity or influence endocrine and hypothalamic-pituitary-adrenal axes, or result in oxidative stress and inflammatory responses(43). On the other hand, adipokines still seem to have a role in the metabolic end-organ complications. In agreement with other reports(44-46), we found several correlations between adipokines and metabolic measures. Especially IL-6 and TNF-α seem to play a role in NAFLD(47-49). Studies have indicated that adiponectin could be a strong predictor for the presence of the MetS in children(50). This could also be the case in our population since adiponectin correlated with all components necessary for a diagnosis of the MetS. We could not find a difference in adiponectin levels between the children with and without the MetS, but there was a trend for a lower adiponectin in children with the MetS ($p=0.09$). It is possible that with inclusion of more patients, this trend will become significant.

Several study limitation need to be taken into considerations. First, this is a cross-sectional study design so we can't make any conclusion about a cause-and-effect relationship. Furthermore, the majority of our population consisted of subjects without OSA or with only mild OSA. However, this is a direct results of our methodology since we included all obese subjects and not only those suspected of sleep-disordered breathing, implying a representative sample of a pediatric obesity clinic.

In conclusion, we could not find an association between leptin, adiponectin IL-6 and TNF-α and OSA in our population of overweight and obese children and adolescents. All adipokines, except TNF-α, were associated with measures of adiposity, suggesting that central obesity is the determining factor in this relationship. Nevertheless, the relationship between adipokines, OSA and obesity remains complicated, with many confounding factors. Further research with emphasis on translational studies is therefore necessary.

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Table 1: Patient characteristics of subjects with a without obstructive sleep apnea. Results are presented as mean ± standard deviation or median [range]

BMI, body mass index; WHR, waist-to-hip ratio; FM, fat mass; oAHI, obstructive apnea–hypopnea index; RDI, respiratory disturbance index; SaO2, oxygen saturation; TST95, percentage of sleep time with an oxygen saturation >95%; ODI, oxygen desaturation index; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha

1: χ^2

2: one-way ANOVA

3: Kruskal-Wallis test

	oAHI < 2	2 < oAHI < 5	oAHI ≥ 5	p
N	111	28	25	
male/female	41/70	10/18	9/16	0,9 [1]
age (years)	12 [6-17]	11 [5-16]	13 [6-17]	0,1 [3]
length (m)	1,553 [1,140-1,830]	1,510 [1,070-1,770]	1,530 [1,140-1,855]	0,4 [3]
weight (kg)	71,2 [30,4-129,8]	69,2 [28,0-109,8]	78,8 [29,4-137,0]	0,6 [3]
BMI z-score	2,6 [1,5-3,5]	2,5 [1,7-3,7]	2,6 [1,9-4,1]	0,7 [3]
Waist circumference (cm)	92,4 ± 13,6	92,7 ± 14,5	95,6 ± 20,7	0,6 [2]
WHR	0,88 ± 0,06	0,92 ± 0,06	0,92 ± 0,06	*0,002* [2]
FM (%)	37,1 [25,7-60,4]	36,1 [27,3-42,8]	36,4 [31,9-53,1]	0,9 [3]
oAHI (events/h)	0,50 [0,00-1,90]	3,20 [2,00-4,90]	7,70 [5,30-130,30]	*<0,001* [3]
RDI (events/h)	0,80 [0,00-4,80]	4,40 [2,50-19,90]	8,95 [5,40-130,30]	*<0,001* [3]
<SaO2> (%)	97,3 [91,2-98,2]	96,9 [93,7-98,3]	96,6 [81,8-98,6]	*0,003* [3]
SaO2 nadir (%)	93,0 [84,0-100,0]	91,2 [83,0-100,0]	91,0 [35,6-100,0]	*0,003* [3]
TST 95 (%)	99,7 [25,6-100,0]	98,5 [22,3-100,0]	98,2 [26,1-100,0]	*0,006* [3]
ODI (events/h)	0,20 [0,00-8,40]	0,50 [0,00-17,60]	1,35 [0,00-4,60]	*0,001* [3]
Leptin (ng/ml)	47,56 [4,20-152,93]	44,41 [17,27-97,02]	50,34 [13,25-98,99]	0,9 [3]
IL-6 (pg/ml)	1,50 [0,40-10,04]	0,99 [0,49-8,97]	1,22 [0,85-5,90]	0,2 [3]
Adiponectin (μg/ml)	14,17 [2,47-38,72]	12,52 [7,09-27,30]	14,91 [0,10-62,44]	0,4 [3]
TNF-α (pg/ml)	3,19 [0,76-8,40]	3,28 [1,41-5,50]	3,29 [1,79-9,54]	0,8 [3]

Table 2: Spearman correlation analysis between measures of adiposity and adipokines. Significant Spearman correlation coefficients are shown.

BMI, body mass index; WHR, waist-to-hip ratio; FM, fat mass; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha; n.s., non-significant

*: p<0.05

§:
p<0.001

	BMI z-score	waist	WHR	FM
Leptin	0,32§	0,63§	n.s.	0,69§
IL-6	n.s.	0,20*	n.s.	0,25*
Adiponectin	n.s.	-0,34§	-0,15*	n.s.
TNF-α	n.s.	n.s.	n.s.	n.s.

Table 3: Linear regression model for leptin and adiponectin.

ODI, oxygen desaturation index; TST95, percentage of sleep time with an oxygen saturation >95%

		r	p
Leptin	ODI	0,013	0,9
	Gender	-0,2	0,02
	Fat mass	0,4	<0,001
	Age	0,35	<0,001
adiponectin	TST95	-0,15	0,07
	Age	-0,06	0,5
	Waist	-0,17	0,03

Table 4: Adipokine levels and metabolic measures between subjects with a without the MetS. Results are presented as mean ± standard deviation or median [range].

IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha; ASAT, aspartate aminotransferase ;ALAT, alanine aminotransferase; HOMA, homeostatic model assessment; AUC, area under the curve

	No MetS	MetS	p
N	112	52	
Leptin (ng/ml)	44,14 [4,20-152,93]	50,15 [13,25-129,73]	0,2
IL-6 (pg/ml)	1,18 [0,40-8,97]	1,79 [0,55-10,04]	*0,03*
Adiponectin (µg/ml)	15,13 [0,10-38,72]	12,51 [2,47-62,44]	0,09
TNF-α (pg/ml)	3,13 [0,76-6,26]	3,36 [1,41-9,54]	*0,04*
ASAT (U/l)	20 [8-79]	24 [12-75]	*0,03*
ALAT (U/l)	23 [10-62]	27 [16-185]	*0,002*
Triglycerides (mg/dl)	77 [36-422]	141 [30-397]	*<0,001*
Cholesterol (mg/dl)	156 [95-251]	163 [74-260]	0,3
HDL-cholesterol (mg/dl)	46 [24-71]	38 [24-53]	*<0,001*
Glucose (mg/dl)	84 [70-105]	85 [67-124]	0,3
Insulin (mU/l)	17,1 [5,0-111,6]	26,1 [9,6-103,0]	*<0,001*
C-peptide (nmol/l)	0,86 [0,39-2,82]	1,14 [0,58-2,64]	*<0,001*
HOMA-index	3,60 [1,02-25,35]	5,69 [0,00-21-36]	*0,001*
log AUC glucose	4,31 [4,14-4,45]	4,32 [4,23-4,71]	0,06
log AUC insulin	4,26 ± 0,29	4,37 ± 0,23	*0,05*
log AUC C-peptide	2,72 ± 0,15	2,78 ± 0,13	*0,03*