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LETTER TO THE EDITOR

SERUM RESISTIN LEVELS IN CHILDREN WITH PRIMARY SNORING

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Primary Snoring (PS) has been positioned at the milder end of the Sleep-Disordered Breathing severity continuum characterized by snoring and it is usually underestimated. PS is defined as snoring without apnea, frequent arousals, or gas exchange abnormalities and recent studies demonstrated that children with PS have increased blood pressure and reduced arterial distensibility. The association between adipokines and SDB has been recently investigated, though most of the studies were focused on OSAS where intermittent hypoxia characterizing the disease may lead to an inflammatory cascade and to the release of several adipokines, contributing to oxidative stress. Resistin, initially described as an adipokine increasing insulin resistance, has been recently identified as a novel important member of the cytokine family involved in the regulation of inflammation. The aim of our study was to investigate circulating resistin levels in normal weight children with PS. Sixty-five children of normal weight aged between 4 and 14 years of age were selected for habitual snoring. Children with positive polysomnography were excluded from the study. Serum resistin levels were detected in all children with PS. Thirty-three healthy non-snorer children with similar age, sex and BMI were selected as a control group. A significantly higher level of resistin was observed in patients with PS compared to the control group (4.67±1.91 ng/ml vs 3.98±1.58 ng/ml; p<0.01). Patients with inconclusive pulse oximetry showed significantly higher resistin levels than those with negative recordings (5.29±1.91 ng/ml *vs* 4.20±1.93 ng/ml; p<0.008). Moreover, there was a significant increasing trend between sieric adipokine level and the frequency of snoring (p<0.006). Our results suggest that systemic inflammation and oxidative stress may also play a significant role in the pathophysiology of PS.

Sleep Disordered Breathing (SDB) represents a broad spectrum of alterations, ranging from Primary Snoring (PS) to obstructive sleep apnea syndrome (OSAS), often underestimated by pediatricians.

Habitual Snoring (HS), usually defined as the presence of snoring at least three nights per week, shows an increased prevalence in children. It ranged from 4.9% to 17.1% in school-aged children in Western countries (1) and about 27% in Italian preschool-aged children (2), thus most children with HS have PS, defined as snoring without apnea, frequent arousals, or gas exchange abnormalities (3). Recent studies demonstrated that children with PS have increased blood pressure and reduced arterial

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distensibility (3, 4).

There are some controversies regarding the pathophysiological mechanisms of SDB in children, but recent studies correlate a fragmented sleep with the increase of oxidative stress mediators (5). Intermittent episodes of hypoxia, and particularly reoxygenation in HS and OSAS patients, seem to predispose to cell stress through the activation of a proinflammatory response (6). Elevated circulating levels of adipokines have been reported in adults and children with OSAS but studies focusing on children with PS are few (7, 8).

Resistin, initially described as an adipokine increasing insulin resistence (9), is associated with human obesity and has been recently identified as a novel important member of the cytokine family involved in the regulation of inflammation with potent regulatory function. Adipocytes, circulating monocytes and macrophages may be responsible for resistin production in humans (10).

The aim of our study was to investigate circulating resistin levels in normal weight children with PS.

MATERIALS AND METHODS

Between December 2012 and July 2013, at the Allergology and Immunology centre of the Pediatric Department of the hospital "Policlinico Umberto I" in Rome, 65 non-allergic children with HS (42 males) aged 4-14 years (mean age 8.96±3.14 years) were selected by an SDB validate questionnaire composed of 51 multiple choice items (11, 12). Snoring was investigated with the question: "Does your child snore?"; responses were rated on a 4-point rating scale (0=never, 1=occasionally, 2=frequently or 3=always). In particular, children defined as occasional snorers reported snoring at least 3 times per week, frequent snorers 4-6 times per week, always snoring every night. The questionnaire was completed by a single investigator who interviewed the patients. When necessary, parents were asked to help the child without interfering with his/her responses. Moreover, 33 healthy non-snorer children of similar age, sex and Body Mass Index (BMI), were selected as a control group from the same Paediatric Allergology and Immunology service for the comparison of serum resistin levels.

For each child a detailed clinical history was collected and a complete physical examination was performed. Height and weight were obtained using standard techniques and BMI was calculated as body weight (Kg) /height (m^2). BMI percentile for age and sex was determined based on the national cross-sectional growth charts (13).

Subjects with the following characteristics were excluded from the study: overweight or obesity (BMI > 85° percentile), craniofacial anomalies or a genetic syndromic disorder, chronic medical conditions, intercurrent upper respiratory tract or systemic infection within the previous 4 weeks, neuromuscular disorder or if they had previously undergone upper airway surgery.

The study was approved by the International Review Board of "Sapienza" University of Rome and was carried out with written parental informed consent.

Analysis of serum resistin

Blood samples were obtained between 6 and 9 a.m. after an overnight fast. After clotting at 4°C, the serum was separated by centrifugation and stored at -70°C until assay.

The serum resistin levels were measured using ELISA kits (BioVendor Laboratory Medicine inc. Brno, Czech Republic) according to the manufacturer›s instructions and expressed as ng/ml. Reference values in a group of healthy subjects were 3.98±1.58 ng/ml in our laboratory.

Nocturnal pulse oximetry and polysomnography

All children selected for HS underwent a nocturnal pulse oximetry and a polysomography. The pulse oximetry was performed according to Brouillette et al. (14) and Nixon et al. (15) with a motion-resistant pulse oximeter set for a 2-sec averaging time for hemoglobin saturation (SpO2) (RAD 5, Masimo, Irvine, CA, USA). In our centre parents were briefly instructed on how to perform oximetry and took the oximeter home for one night, returning instrument the next day. The pulse oximeter used a fixed 7-sec averaging time for pulse rate (PR) and stored in memory new SpO2 and PR values every 2 sec. Pulse oximetry data were extracted and analyzed with Profox Oximetry Software (Profox Associates, Escondido, CA, USA). The following parameters were evaluated: mean oxygen saturation (mean SpO2), lowest SpO2, number of SpO2 dips >4%/hr of study (DI4), mean, minimum, maximum PR and standard deviation of PR, and total effective recording time (TERT). Periods of oximetry recording were excluded from analysis if the oximeter quality signal indicated low signal IQTM (Masimo), low perfusion, unrecognized, defective or no sensor, interference, or ambient light. A recording was considered sufficient when the artifact-free recording time was at least 6 hours.

According to the Bruillette definitions and criteria, each oximetry was classified as positive, negative or non-conclusive (14). Children with at least 1 cluster, hence those with positive or non-conclusive recordings, were considered as experiencing intermittent hypoxia (16).

An overnight polysomnography (PSG) was carried

out on all children selected for HS to record the following parameters: six EEG channels (Fp1-A2, Fp2-A1, C3-A2, C4-A1, O1-A2, and O2-A1 electrode placement according to the international 10–20 system), left and right electrooculogram (EOG), chin electromyogram (EMG), electrocardiogram (ECG), electromyogram of left and right tibialis anterior muscles, nasal flow, thoracic and abdominal respiratory effort, body position and oxygen saturation. Obstructive apnea was defined as an absence of airflow with persistent respiratory effort for more than two baseline breaths, irrespective of the arterial oxyhemoglobin saturation changes. The obstructive apnea-hypopnea index (OAHI) was defined as the total number of apneic and hypopneic episodes per hour of sleep. Hypopnea was considered as a reduction of 50% or more in the amplitude of the airflow signal and it was only quantified if longer than two baseline breaths and associated with oxygen desaturations of at least 4% and/or arousals. A diagnosis of PS was given if OAHI was < 1 and SpO2 nadir was $\geq 90\%$, while a diagnosis of OSA if OAHI was ≥ 1 (17).

Children with OSA were excluded from the study.

Statistical analysis

Statistical analyses were performed using SPSS (Statistical Package of Social Sciences, Chicago, IL, USA) software version 19. Descriptive statistics were performed expressing continuous data as means with SDs, and categorical data were expressed by frequency and percentage. Comparisons were evaluated using a *t*-test, and a *chi*-square test while correlations between serum resistin levels and anthropometric variables were calculated with Pearson's correlation test. A *p*-value less than 0.05 was considered statistically significant.

RESULTS

Resistin and HS

Of the 65 children with HS, 11 received a diagnosis of OSA and were excluded from the study. A significantly higher level of resistin was observed in our sample of 54 primary snorer children (35 males: 65%) when compared to the control group (4.67±1.91 ng/ml *vs* 3.98±1.58 ng/ml; $p \leq 0.04$) (Fig. 1). Among children with PS, 36 pulse oximetries (67%) were negative while 18 (33%) were not conclusive; patients with non-conclusive pulse oximetry showed significantly higher resistin levels than those with negative recordings (5.29±1.91 ng/ml *vs* 4.20±1.93 ng/ml; $p<0.008$). Serum resistin levels showed no differences between the sexes in our sample (female: 4.41±1.75 ng/ml; male: 5.03±2.74 ng/ml). Resistin levels were not related with age and weight (data not shown; $p>0.05$).

Resistin and frequency of snore and apnea

According to the questionnaires, 16 children (30%) reported occasional snoring, 14 (26%) frequent

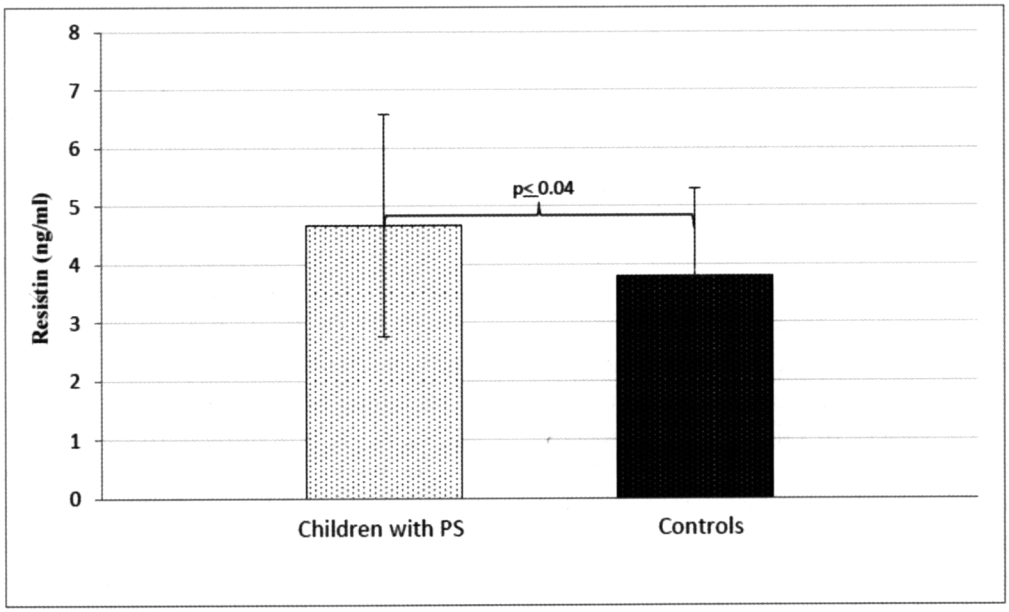

Fig. 1. *Children with Primary Snoring showed significantly higher resistin level when compared to the control group ($p<0.04$).*

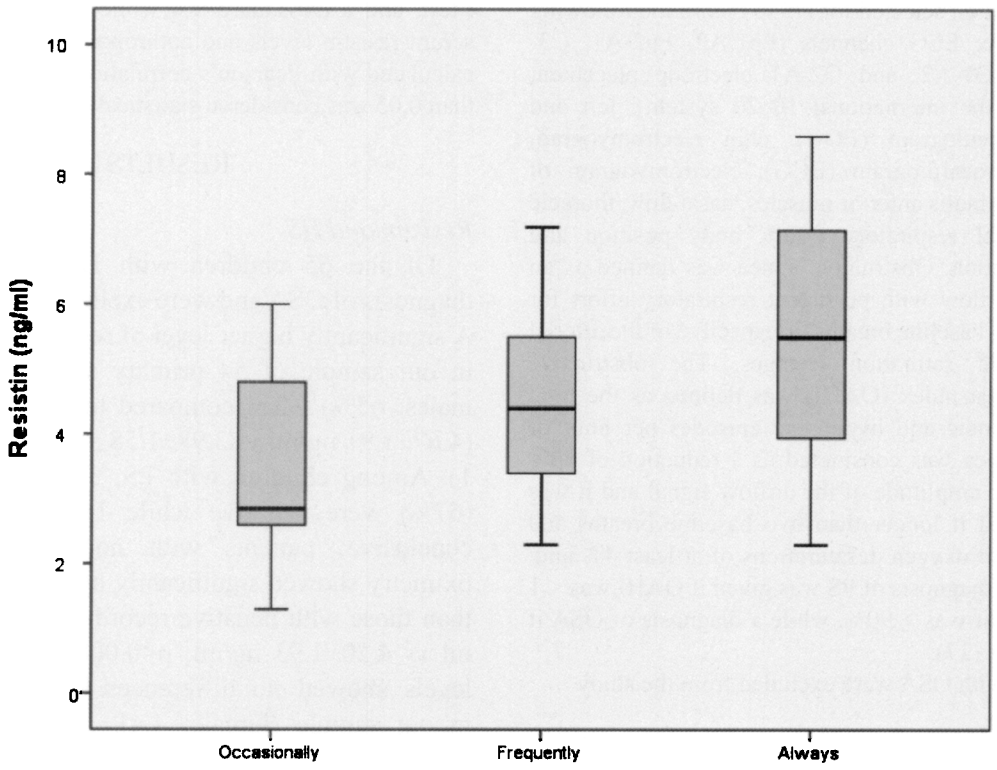

Fig. 2. *Increasing trend between resistin levels and frequency of snoring. The figure shows medians with interquartile ranges (25-75%).*

snoring and 24 (44%) always snoring. Children with occasional snoring had higher sieric resistin levels than those with frequent snoring but this difference was not statistically significant (3.39±1.42 ng/ml *vs* 4.50±1.68 ng/ml; $p<0.06$). Notably, the difference between occasional snoring and always snoring was statistically significant ($p<0.02$). Moreover, there is a significant increasing trend between serum adipokine level and the frequency of snoring ($p<0.006$) (Fig. 2).

DISCUSSION

PS is considered as the mildest of SDB disorders, but increasing evidence suggests that it may be associated with a variety of clinical consequences. As already demonstrated for OSAS, also PS is associated with a wide number of significant complications including cardiac remodeling and dysfunction, blood pressure elevation, neurobehavioral and neurocognitive impairments (3, 18-20). Furthermore, recently Li et al. (20) reported that one-third of children with PS progressed over a 4-year period to the development of OSA. The present study provides additional data supporting the hypothesis that PS should no longer be regarded as a completely benign entity.

Although the role played by resistin in inflammatory diseases still remains unclear, previous studies reported increased levels of this adipokine in patients affected by rheumatoid arthritis (21), rhinitis (22) and asthma (23). Recently, many authors investigated the association between adipokines and SDB even though most of the studies were focused on OSAS (24, 25). In this syndrome, the intermittent hypoxia (IH) characterizing the disease may lead to an inflammatory cascade and to the release of several adipokines contributing to oxidative stress (26, 27).

Our results demonstrate increased serum levels of resistin in children with PS when compared with controls. Adipokines are mainly released by fat tissue and, when measured in the circulation, they can pose a problem in identifying whether they are synthesized in obesity per se and/or because of HS. For this reason overweight and obese children were

excluded from the study (27).

The desaturation–reoxygenation sequence defined as IH is a typical pattern coupled with the majority of events characterized by repeated breathing cessation and it leads to oxidative stress with production of reactive oxygen species (ROS) (26). The increased levels of ROS contribute to the generation of systemic inflammation and to the production of adipokines (27). Notably resistin can further induce oxidative stress, thus creating a vicious circle of oxidative stress and inflammation (28).

Recently, Zhou et al. reported that severity of oxidative stress induced by IH is time-dependent and significantly correlated with the degree of IH (29). Although in primary snoring PSG parameters are considered under the international ranges to diagnose OSAS, it is reasonable that also mild episodes of IH might predispose to cell stress and determine an increased production of inflammatory mediators. In this perspective PS children with non-conclusive pulse oximetry showed higher levels of serum resistin when compared with those with negative pulse oximetry recording.

Furthermore, according to our knowledge, this is the first study underlying a correlation between serum resistin levels and the frequency of snoring. Many authors have already suggested that the recurrent vibration of the air column in the upper airway can induce mechanical trauma, promoting the development of an inflammatory response leading to the up-regulation of leukotriene receptor expression and possibly many other inflammatory mediators (30). This finding supports the role of validated questionnaires as an important instrument for the identification of children with SDB.

In conclusion, our results underline the importance of an early institution of the therapy and suggest that systemic inflammation and oxidative stress might play a significant role also in the pathophysiology of PS. Further studies are warranted to clarify whether the inflammatory mechanisms are a component or the cause of this apparently benign sleep disorder.

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