Serum apelin-12 level is elevated in schoolchildren with atopic asthma

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KEYWORDS
Apelin; Atopic asthma; Children; Obesity

Summary
Background: There are limited data on the role of adipokines in atopic asthma.
Aim: To determine serum level of apelin-12 (APE-12) in asthmatic children in relation to BMI and gender.
Methods: Serum APE-12 levels were measured using ELISA in 89 asthmatic children (61 boys and 28 girls, aged 7.0–17.0 years) and in 33 healthy children. Among examined asthmatics 59 (19 girls and 40 boys) had normal weight and 30 (9 girls and 21 boys) were obese.
Results: The mean serum levels of APE-12 were significantly (p < 0.001) higher both in obese (174.1 ± 5.9 pg/mL) and non-obese asthmatic children (171.0 ± 4.0 pg/mL) than in healthy children (130.6 ± 2.1 pg/mL), regardless of gender. No relationships between examined the adipokine level and asthma severity, spirometric parameters, degree of allergic sensitization, BMI, BMI-SDS were observed.
Conclusion: Increased serum level of APE-12 suggests that this adipokine may be implicated in the pathogenesis of childhood atopic asthma.

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Introduction

Obesity is associated with asthma in both adults and children, as shown by cross-sectional, case—control, longitudinal, and weight intervention studies. The exact mechanisms for the association between obesity and asthma are not known. The immunologic pathway invokes a possible role for pro-inflammatory and anti-inflammatory adipokines produced by adipose tissue. 1–5 Although adipose tissue produces over 50 adipokines, most reports concentrated on the role of pro-inflammatory leptin and anti-inflammatory adiponectin, which may affect asthma. However, recent data are inconclusive regarding the independent association between serum leptin or adiponectin and the risk of asthma. 6–8 Some findings suggest that leptin and adiponectin might be involved in the causal pathway between obesity and allergic sensitization and asthma in humans, 6,7 but other studies do not confirm this hypothesis. 5

It has been suggested that another adipokine, resistin, play a role in asthma pathogenesis and severity in adults. 9 Conversely, studies in children suggest that resistin may reduce risk of asthma. 10 Hence, it seems that other adipokines should be additionally explored in relation to asthma risk and asthma severity. 5

Apelin (APE) is a bioactive peptide identified as the endogenous ligand of the previously discovered “orphan” receptor named APJ, isolated by Tatemoto et al. from bovine stomach extracts in 1998. 11 Several different isoforms of APE have been identified. The predominant form of circulating APE is believed to be APE-36. However, also shorter C-terminal fragments with biological activity have been found, including APE-17, APE-16, APE-13 and APE-12. 12,13 It has been demonstrated that the biological activity of APEs is related inversely to the peptide length: thus, APE-12 is the most potent isoform. However, APE-11 and shorter peptides are inactive. 14

APE belongs to the adipokines group because its mRNA expression has been demonstrated in mature adipocytes and vascular stroma of fat tissue. 15,16 APE peptide expression has been also detected in other tissues, including brain cells, gastrointestinal tract, and osteoblasts. 17,18 APE and its receptor APJ constitute a signaling pathway best recognized as an important regulator of cardiovascular homeostasis. 19 APE suppresses the production of pro-inflammatory cytokines 20 and chemotactic activity. 21 This multifunctional apelinergic system is also highly expressed in pulmonary tissue, including bronchial and alveolar epithelial cells, and small pulmonary blood vessels. 22–24

The apelinergic system distribution over such a variety of tissues has suggested that it might play relevant roles in human physiology. The potential association between asthma and apelin has not been explored so far and remains obscure. Thus, the aim of our study was to analyze apelin serum level in children with allergic asthma in relation to BMI and gender. Comparison between serum levels of APE-12 in distinguishing children with atopic asthma from healthy was also performed.

Material and methods

The study group was comprised of randomly selected atopic asthma patients from 320 asthmatics who consecutive visited allergy outpatient clinics in Department Pediatric Silesian University of Medicine between January 2010 to December 2010. The study was approved by the Ethics Committee of the Medical University of Silesia in Katowice and written informed consent was obtained from children's parents.

Finally 89 children (61 boys and 28 girls, aged 7.0—17.0 years; mean age 11.34 ± 0.37 years) with stable atopic asthma were enrolled into study. The diagnosis of asthma, the assessment of severity, asthma management plan and asthma control level were established according to the GINA 2006 criteria. 25 Twelve children had intermittent, 56 had mild disease, and 32 had moderate persistent asthma. Children with severe asthma and asthma exacerbations were excluded from the study. Duration of the diseases ranged from 2 years to 6 years. During control visit children underwent spirometric assessment using LUNG TEST 1000 device (Poland) as previously described in details. 26 All asthmatic children had positive skin prick tests (SPTs) to ≥1 allergens. A positive SPT was defined as a mean diameter of at least 3 mm in the presence of negative diluent and positive histamine controls.

The degree of allergic sensitization was measured by the wheal size of SPTs.

All children had stable, well controlled asthma according to GINA 2006 criteria and no changes were made in chronic anti-inflammatory treatment within the previous 12 weeks. None of the patients reported respiratory tract or other infections at least 3 month prior to the study. Seventy-six children with mild or moderate asthma were treated with regularly inhaled corticosteroids (ICS) at a variable daily dose required to control disease symptoms.

At the time of evaluation, daily ICS doses ranged from 100 to 600 µg/day (mean daily dose: 246.76 ± 16.70 µg/day).

The control group consisted of 33 healthy children (20 boys and 13 girls) with normal weight matched for sex and age (aged 7.0—17.0 years; mean age 11.71 ± 3.79 years). Controls had negative history of allergic diseases with negative SPT results to a panel of aeroallergens (dust mite, mixed grass, or tree pollen; cat and dog; Allergopharma, Reinbek, Germany) and had normal level of total serum immunoglobulin (IgE). These control children without evidence of pulmonary or systemic inflammatory disease attended the outpatient pediatric clinic for non-immunological and non-inflammatory problems and they needed venous puncture.

Anthropometric measurements

Body mass index (BMI) (body weight [kg] divided by height [m²]) and standard deviation [SD] score for BMI (BMI-SDS) were calculated according to current Polish populational predicted values. 27 BMI-SDS was calculated according to formula: [current patient’s BMI (kg/m²) — BMI equal to 50th percentile (kg/m²)]/[BMI equal to 50th percentile (kg/m²) - BMI equal to 3rd percentile (kg/m²)]. Normal weight was defined as BMI-SDS between −2.0 and +2.0. Obesity was defined as BMI-SDS >2.0.

Laboratory assays

Blood samples for analyses were collected in the fasting state between 07:00 AM and 09:30 AM.
Serum APE-12 concentrations were determined, as previously described,\textsuperscript{28} using commercial human APE-12 enzyme immunoassay kits (Phoenix Pharmaceuticals Inc., Burlingame, CA) following the manufacturer’s instructions. Before the assay, serum samples were extracted to isolate analyzed peptides before assay. Buffer A (1% trifluoroacetic acid aqueous solution), buffer B (60% acetoni trile solution in 1% trifluoroacetic acid solution), and SEP-PAK C chromatographic columns (Waters Associates, Milford, MA) were used for extraction. The obtained extract was lyophilized and then dissolved in the assay buffer before analysis. The absorbance measurements for all samples were performed using the Quant Universal Microplate Spectrophotometer (BioTek Instruments Inc., Winooski, VT, USA) at 450 nm wavelengths. The sensitivity was 0.07 pg/mL for APE-12 kit; the intraassay coefficient of variance was 5% or less, and the extraassay coefficient of variance was 14%.

**Statistical analysis**

Statistical analysis was performed using Statistica 6.0 software (StatSoft Inc., Tulsa, OK). Normal data distribution was assessed using Shapiro–Wilk test, and the homogeneity of variance was assessed using Levene’s test. Comparisons between the examined groups were performed using the ANOVA and post hoc Tukey’s multiple comparison test for different sample sizes or Kruskal–Wallis test if data distribution was not normal. Correlations were analyzed by Spearman’s test if data distribution was not normal. All results were considered statistically significant at \( p < 0.05 \).

**Results**

Characteristics of the 89 subjects with asthma and the 33 healthy control subjects are presented in Table 1. Both groups were similar with regard to age and sex. Among studied asthma children 30 (9 girls and 21 boys) were obese (AO) and 59 (19 girls and 40 boys) had normal weigh, normal BMI and BMI-SDS within range of \(-2\) to \(+2\) (ANW). Obese asthmatic children (both girls and boys) had significantly higher \(( p < 0.001 \) BMI and BMI-SDS than healthy controls (boys and girls with BMI-SD within range \(-2\) to \(+2\)). Asthma was well controlled in studied children and the mean values of FEV\(_1\) and FVC in asthmatics did not differ from mean values obtained from control group. Mean values of IgE serum levels in all children were significantly higher \(( p < 0.001 \) ) than in control boys \((134.1 \pm 5.9\) pg/mL) and non-obese asthmatic children \((171.0 \pm 4.0\) pg/mL) than in healthy children \((130.6 \pm 2.1\) pg/mL) regardless of gender. After stratifying by gender, there was a significant higher mean APE-12 concentration \(( p < 0.01 \) ) in AO girls \((188.9 \pm 8.0\) pg/mL) and ANW girls \((186.2 \pm 5.9\) pg/mL) compared to girls from control group \((134.4 \pm 1.8\) pg/mL). In AO boys and ANW boys also significantly higher \(( p < 0.01 \) ) mean values of APE-12 \((167.8 \pm 5.9\) pg/mL and 163.9 \(\pm\) 3.5 pg/mL respectively) were observed than in control boys \((124.8 \pm 1.7\) pg/mL).

The mean APE-12 levels obtained in girls with asthma were significantly higher than in boys with asthma \((p < 0.001, P < 0.001\) respectively). In the control group, serum levels of APE-12 did not differ significantly between girls and boys (Table 2).

No significantly relationships between APE-12 levels and BMI or BMI-SDS were noticed in asthmatic and healthy children (data not shown).

The serum IgE levels correlated with a degree of sensitization measured by wheal size of SPTs \((r: 0.45, P = 0.005\) ). We did not find any correlation between APE-12 levels and lung function tests, allergic sensitization as well as severity of asthma (data not shown).

**Discussion**

In the present study we provided evidence that in asthmatic children, irrespective to BMI, serum profile of apelin (APE-12) is different from healthy ones. To best our knowledge for the first time we have demonstrated that APE-12 serum levels are increased in asthmatic children. However no relationships between serum apelin levels and allergic sensitization were found.

The origin of blood apelin and the regulations of its input into the bloodstream remain a subject of interest. One source of the plasma apelin may be overspill from the vascular and endothelial cells, but it also has been demonstrated that apelin is expressed and secreted by both human and mouse adipocytes. Apelin synthesis in adipocytes is stimulated by insulin, and plasma apelin level markedly increases in obesity associated with insulin resistance and hyperinsulinemia.\textsuperscript{15} Thus, increased apelin expression in adipose tissue could contribute to apelin plasma levels. Well known pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-\(\alpha\)), that has been implicated in many aspects of the airway pathology in asthma,\textsuperscript{29} may act as a direct up-regulator of apelin expression in adipocytes in both obese and lean humans. TNF-\(\alpha\) increases significantly apelin plasma levels when administered to mice by intraperitoneal injection.\textsuperscript{30}

The origin and the role of increased apelin levels in the serum of examined children with allergic asthma remain unclear. To date, there are no reports about the eventual influences of apelin on bronchial smooth muscle, bronchial contractility or hyperreactivity, or its potential role in allergic inflammation. In the human lungs APJ-LI was restricted to endothelial cells of small pulmonary vessels and lower levels to vascular smooth muscle of pulmonary vessels.\textsuperscript{22} Nevertheless recently, Feng et al\textsuperscript{23} noticed significantly elevated apelin expression in the bronchial epithelium after induction of acute pulmonary embolism in dogs. Apelin had also beneficial effect in neonatal rats with hyperoxia induced lung injury. Prophylactic apelin administration improved alveolarisation, reduced pulmonary fibrin deposition, inflammation and septum thickness via a nitric oxide synthase-dependent mechanism.\textsuperscript{24} If apelin has some beneficial effect in human asthma and if increased apelin plasma levels may be the defense mechanisms in childhood asthma are subjects of speculations and need further explorations. It is assumed that the overproduction of apelin in obesity could be a protective mechanism before
onset of an obesity-related disorder, such as hypertension or cardiovascular dysfunction. DeVisser et al. conclude that results of their recent study suggest the potential role of apelin administration for therapeutic intervention in humans suffering from chronic obstructive pulmonary disease or asthma. In the cardiovascular system apelin elicits endothelium-dependent, nitric oxide-mediated vasorelaxation and reduces arterial blood pressure. Addi- tionally apelin demonstrates potent and long-lasting positive inotropic activity being one of the most potent endogenous stimulators of cardiac contractility. 

In our study we found no linear relationship between BMI and apelin. Recently Reinehr et al. find a lack of association between apelin, insulin resistance, cardiovascular risk factors, and obesity in children. In Reinher et al. study apelin levels did not differ significantly between obese and lean children. Additionally apelin concentrations were not significantly related to age, gender, pubertal stage, SDS-BMI, body fat leptin. Also weight loss in obese children was not associated with a change in apelin concentrations. In our previous study APE-12 concentrations were related to BMI, but in larger group comprising 4 subgroups: girls (but not boys) with anorexia nervosa, girls with no otherwise specified eating disorders, healthy girls, and obese girls.

A possible limitation of our study was that it included children from a wide age range. Lack of subjects with previous study APE-12 concentrations were related to BMI, but in larger group comprising 4 subgroups: girls (but not boys) with anorexia nervosa, girls with no otherwise specified eating disorders, healthy girls, and obese girls.

### Table 1 Demographic characteristic in asthma and healthy children.

<table>
<thead>
<tr>
<th></th>
<th>Asthmatics with normal weight N = 59</th>
<th>Asthmatics with obesity N = 30</th>
<th>Healthy controls N = 33</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ANW (19 girls, 40 boys)</td>
<td>AO (9 girls, 21 boys)</td>
<td>H (13 girls, 20 boys)</td>
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<tr>
<td>Age [years]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girls</td>
<td>12.4 ± 0.6 (12.0, 4.5)</td>
<td>12.4 ± 2.6 (12.0, 7.5)</td>
<td>13.5 ± 1.2 (14.2, 3.7)</td>
</tr>
<tr>
<td>Boys</td>
<td>11.8 ± 0.5 (12.5, 5.7)</td>
<td>11.7 ± 1.3 (12.0, 3.0)</td>
<td>11.8 ± 1.6 (11.0, 4.0)</td>
</tr>
<tr>
<td>All</td>
<td>12.0 ± 0.82 (12.5, 4.6)</td>
<td>11.9 ± 1.2 (12.7, 6.0)</td>
<td>12.9 ± 1.0 (13.0, 4.0)</td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td></td>
<td></td>
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<tr>
<td>Girls</td>
<td>18.2 ± 1.15 (18.9, 4.1)</td>
<td>23.4 ± 2.0 (23.8, 5.1) b</td>
<td>18.8 ± 0.9 (19.1, 3.0)</td>
</tr>
<tr>
<td>Boys</td>
<td>18.4 ± 0.72 (18.3, 4.1)</td>
<td>25.2 ± 1.8 (25.8, 5.0) c</td>
<td>17.3 ± 1.1 (16.8, 2.4)</td>
</tr>
<tr>
<td>Total</td>
<td>18.3 ± 0.63 (18.3, 3.9)</td>
<td>24.6 ± 1.4 (24.8, 4.8) c</td>
<td>18.3 ± 0.8 (18.0, 3.0)</td>
</tr>
<tr>
<td>FVC [% predicted]</td>
<td></td>
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<td></td>
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<tr>
<td>Girls</td>
<td>87.3 ± 9.28 (87.0, 32.0)</td>
<td>95.8 ± 6.8 (98.0, 10.0)</td>
<td>98.7 ± 5.8 (102.0, 15.5)</td>
</tr>
<tr>
<td>Boys</td>
<td>87.2 ± 4.22 (85.0, 19.0)</td>
<td>93.3 ± 8.19 (100.0, 21.0)</td>
<td>90.8 ± 11.9 (87.5, 31.7)</td>
</tr>
<tr>
<td>Total</td>
<td>87.2 ± 4.185.0 (23.0, 23.0)</td>
<td>94.1 ± 5.9 (99.5, 20.0)</td>
<td>96.06 ± 5.62 (98.0, 19.0)</td>
</tr>
<tr>
<td>IgE [IU/mL]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girls</td>
<td>485.1 ± 269.3 (284.4, 503.0)</td>
<td>414.4 ± 344.4 (248.8, 292.0)</td>
<td>70.5 ± 39.4 (32.7, 115.7)</td>
</tr>
<tr>
<td>Boys</td>
<td>355.7 ± 105.8 (272.5, 402.3)</td>
<td>415.2 ± 161.4 (393.7, 396.0)</td>
<td>24.3 ± 16.2 (16.8, 31.7)</td>
</tr>
<tr>
<td>Total</td>
<td>398.6 ± 111.3 (280.0, 384.5) a</td>
<td>414.9 ± 150.7 (329.0, 404.0) a</td>
<td>50.7 ± 25.2 (30.0, 60.7)</td>
</tr>
</tbody>
</table>

Data are shown as: mean ± 1.96 SE (median, IQR i.e the interquartile range i.e. difference between the upper and lower quartiles).  

- a p < 0.01 in comparison with control group.  
- b p = 0.02 in comparison with control group and vs asthmatics with normal weight.  
- c p < 0.001 in comparison with control group and vs asthmatics with normal weight.

### Table 2 APE-12 serum concentration in asthmatic and healthy children.

<table>
<thead>
<tr>
<th></th>
<th>Asthmatics with normal weight N = 59</th>
<th>Asthmatics with obesity N = 30</th>
<th>Healthy controls N = 33</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ANW (19 girls, 40 boys)</td>
<td>AO (9 girls, 21 boys)</td>
<td>H (13 girls, 20 boys)</td>
</tr>
<tr>
<td>Apelin (pg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girls</td>
<td>186.2 ± 5.9 (188.0, 18.0) a, b</td>
<td>188.9 ± 8.0 (192.0, 16.0) b, b</td>
<td>134.4 ± 1.8 (135.6, 5.8)</td>
</tr>
<tr>
<td>Boys</td>
<td>163.9 ± 3.5 (166.0, 13.0) a</td>
<td>167.8 ± 5.9 (168.0, 8.0) a</td>
<td>124.8 ± 1.7 (123.5, 2.6)</td>
</tr>
<tr>
<td>All</td>
<td>171.0 ± 4.0 (170.0, 20.0) a</td>
<td>174.1 ± 5.9 (170.0, 20.0) a</td>
<td>130.6 ± 2.1 (131.1, 10.6)</td>
</tr>
</tbody>
</table>

Data are shown as: mean ± 1.96 SE (median, IQR i.e the interquartile range i.e. difference between the upper and lower quartiles).  

- a p < 0.001 in comparison with control group.  
- b p < 0.001 girls vs boys.
severe asthma and exacerbation of asthma may also be a drawback of this study. In our study only children with atopic asthma, but not with non-atopic asthma, were investigated. So we can’t state with certainty whether elevated serum apelin levels are characteristic for asthma itself or if this finding reflects at least partially atopic status per se. Another limitation of the study was gender bias. Atopic asthma is more prevalent in prepubescent and adolescent boys than in girls, so it was not surprising that in our cohort boys outnumbered girls. However, our study was performed on a specialty clinic sample to minimize the likelihood that children without asthma would be included, and to ensure that consecutive patients who visited allergy outpatient clinics would be enrolled. We did not estimate the BAL-concentration of apelin.

Conclusions

In atopic childhood asthma, increased apelin (APE-12) levels were observed independent of obesity. These findings suggest the potential association between apelin and atopic asthma. However, APE-12 has limited value as potential biomarker in estimation of atopic asthma severity and degree of allergic sensitization.

Although this was a cross-sectional study with a relatively small sample size, we believe that our data may be a basis for further studies evaluating the possible role of adipokines in childhood atopic asthma pathogenesis.

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Authors’ contributions

Edyta Machura: contributed substantially to the conception and design of this study, data acquisition and analysis, data interpretation, and writing, revising, and approval of this article for publication.

Katarzyna Ziora: contributed substantially to the conception and design of this study, and data analysis and interpretation, and revising and approving this article for publication.

Dariusz Ziora: contributed substantially to the conception and design of this study, data interpretation, and writing, revising, and approving this article for publication.

Elżbieta Świetochoławska: assisted with the data analysis and interpretation of this study, and revising, and approving this article for publication.

Helena Krakowczyk, and Franciszek Hakiewicz: contributed to data acquisition of this study and writing, revising, and approving this article for publication.

Alicja Kasparska-Zajęc: contributed to writing, revising, and approving this article for publication.

Conflicts of interest statement

Disclosure summary: The authors have nothing to disclose.

References


