Elevated vaspin and leptin levels are associated with obesity in prepubertal Korean children

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Abstract. Adipokines are associated with obesity. However, the relationships between adipokines, specifically vaspin, obesity, and obesity-related variables remain controversial, and only a few studies have been conducted which examines them in children. We investigated the relationships between obesity in prepubertal Korean children and three types of adipokines: vaspin, leptin, and visfatin. In this cross-sectional study, 168 nine-year-old boys and 176 nine-year-old girls participated in a school-based health examination program. Children were classified as overweight using the Korean Pediatric Society 2007 guidelines. Overweight boys and girls had higher leptin and vaspin levels than both boys and girls of normal weight, whereas only overweight boys had higher visfatin levels than normal weight boys. Leptin, visfatin and vaspin concentrations were correlated with obesity-related variables. A multiple logistic regression analysis showed that systolic blood pressure (SBP), total cholesterol (TC), alanine aminotransferase (ALT), homeostasis model assessment of insulin resistance (HOMA-IR), leptin, and vaspin were associated with an increased risk of being overweight, whereas high-density lipoprotein (HDL) cholesterol was associated with a decreased risk of being overweight. Elevated vaspin and leptin levels are associated with obesity in prepubertal Korean children.

Key words: Vaspin, Leptin, Children, Obesity

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improvements in hyperinsulinemia and blood chemistry related to insulin resistance [6, 7]. However, most obese individuals not only have higher circulating leptin levels than lean individuals but also are leptin-resistant, which complicates the function of leptin [5].

Visfatin/pre-B-cell colony-enhancing factor/nicotinamide phosphoribosyltransferase is an adipokine that mimics the action of insulin [8]. Visfatin is related to several insulin resistant conditions, such as obesity, metabolic syndrome, and dyslipidemia [9-14]. Elevated visfatin concentrations were correlated with body fat mass and visfatin levels decrease when weight loss occurs [13, 15].

Vaspin is a visceral adipose tissue-derived serpin. Human vaspin contains 395 amino acids and about 40% are homologous with α1 antitrypsin [16]. In obese mice, the administration of vaspin resulted in improved insulin sensitivity and glucose tolerance [16]. Some human studies have found that elevated vaspin concentrations are associated with higher body mass index (BMI) and impaired insulin sensitivity [17, 18]; however, other studies did not observe this relationship [19]. Several pediatric studies, but not all, illustrated possible relationships between vaspin, insulin sensitivity, and obesity [13, 20, 21]. Some reported that weight reduction decreased circulating vaspin levels [22-24], but others did not [13, 25].

The relationships between adipokines, specifically vaspin, and obesity are inconclusive and few studies have been conducted examining them in children. The aim of this study was to examine the relationships between vaspin, leptin, and visfatin and obesity and obesity-related variables in a homogeneous group of children.

Methods

Study subjects

Study participants consisted of 168 boys and 176 girls selected from the Korean Metabolic Disorders & Obesity Study in Elementary School Children (KMOSES). They were all nine years old and had previously participated in a school-based health examination program in 2007 and 2008. None of the children had histories of diabetes, hypertension, cardiovascular disease, endocrine disorders, or smoking. Written informed consent was obtained from their parents and the study protocol was approved by the Korea University Institutional Review Board, in accordance with the Declaration of Helsinki of the World Medical Association.

Anthropometric and laboratory measurements

All children wore light clothing and no shoes while anthropometric measurements were taken. Height and weight were estimated to the nearest 0.5 cm and 0.5 kg using an automated height-weight scale. BMI was calculated by the following formula: weight (kg) divided by the square of height (m). Waist circumference was measured at the midpoint of the lower border of the rib cage and the top of the lateral border of the iliac crest. The standard brachial cuff technique was used to measure blood pressure.

Serum was obtained only if the children had fasted overnight for more than eight hours. An autoanalyzer (LX20; Beckman Coulter, Fullerton, CA, USA) was used to measure fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) concentrations. A radioimmunoassay (Roche, Indianapolis, IN, USA) was used to analyze plasma insulin concentrations. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: fasting insulin (in µIU/mL) × fasting glucose (in mmol/L)/22.5 [26]. Serum leptin levels were measured by enzyme-linked immunosorbent assay (Alexis, Lausen, Switzerland), and intra- and interassay CVs were 4.2-7.6% and 4.4-6.7%, respectively. Serum visfatin levels were measured by enzyme-linked immunosorbent assay (AdipoGen, Seoul, Korea), and intra- and interassay CVs were 3.46-5.53% and 6.31-9.53%, respectively. Serum vaspin levels were measured by enzyme-linked immunosorbent assay (AdipoGen, Seoul, Korea), and intra- and interassay CVs were 1.31-3.85% and 3.27-9.06%, respectively.

Definition of obesity

According to the Korean Pediatric Society 2007 guidelines [27, 28], BMI between the 85th percentile and 95th percentile was defined as overweight and 95th percentile and above was defined as obesity. In this study, children with BMI greater than or equal the 85th percentile for age and sex were classified as overweight.

Statistical analysis

Results are expressed as means ± SDs. A t-test was used to compare the anthropometric and laboratory characteristics between normal-weight and overweight children. Pearson’s partial correlations were used to analyze the relationship between adipokines and other
obesity-related variables after adjusting for sex. FPG, TG, AST, ALT, insulin, HOMA-IR, leptin, and vaspin were not normally distributed and therefore were log-transformed before analysis. Multiple regression analyses were conducted in order to assess the relationship between leptin, visfatin, and vaspin with other variables. Backward elimination regression analysis was applied in the logistic regression analysis of variables related to being overweight. Sex, systolic blood pressure (SBP), diastolic blood pressure (DBP), FPG, TC, TG, HDL-C, ALT, HOMA-IR, leptin, visfatin, and vaspin were used as independent variables. p <0.05 was considered to be statistically significant. SPSS version 12.0 (SPSS Inc, Chicago, IL, USA) was used for the statistical analysis.

Results

Clinical and biochemical characteristics of the study populations are shown on Table 1. The percentage of overweight children was 48.8%. Overweight boys and girls had higher BMI, waist circumference, SBP, DBP, total cholesterol levels, triglyceride levels, ALT, insulin levels, HOMA-IR, leptin, and vaspin levels, but lower HDL-C levels than normal weight boys and girls. FPG was higher in overweight girls than in normal weight girls, whereas serum visfatin levels were higher in overweight boys than in normal weight boys. There were no differences between sexes in leptin levels (boys 8.02 ± 7.90; girls 8.64 ± 8.22 ng/mL; p=0.477) or visfatin levels (boys 0.25 ± 0.46; girls 0.22 ± 0.38 ng/mL; p=0.515), but visfatin levels were higher in boys than in girls (boys 11.57 ± 5.61, girls 10.08 ± 5.73 ng/mL; p=0.026). Overweight children had higher leptin, visfatin, and vaspin concentrations than normal weight children after adjusting for sex (Fig. 1).

Leptin levels were positively correlated with weight, BMI, waist circumference, SBP, DBP, FPG, TC, TG, ALT, insulin levels, and HOMA-IR, and were negatively correlated with HDL cholesterol levels. The visfatin levels were positively correlated with weight, BMI, and TG levels, whereas the vaspin levels were positively correlated with weight (r=0.140, p=0.009), BMI (r=0.171, p=0.002), and DBP (r=0.139, p=0.010) (Table 2).

Multiple linear regression analyses were performed to determine which variables affected leptin, visfatin,
Vaspin is a member of the serine protease inhibitor family, which has been found in the visceral white adipose tissue of Otsuka Long-Evans Tokushima fatty rats [16]. Vaspin is an adipokine that is related to obesity and impaired insulin sensitivity in both adults and children [17, 20]. We found that elevated vaspin levels are related to obesity in prepubertal Korean children after adjustment for sex, blood pressure, biochemical parameters, and other adipokines. Recent publication and vaspin levels. There was a significant relationship between leptin and sex, BMI, triglycerides, and ALT, and between visfatin to sex, FPG and TG. BMI was correlated with vaspin level after adjustment of sex, SBP, DBP and leptin (Table 3).

A multiple logistic regression analysis showed that SBP, TC, ALT, HOMA-IR, leptin, and vaspin were associated with an increased risk of being overweight, while HDL-C was associated with a decreased risk of being overweight (Table 4).

**Discussion**

Vaspin is a member of the serine protease inhibitor family, which has been found in the visceral white adipose tissue of Otsuka Long-Evans Tokushima fatty rats [16]. Vaspin is an adipokine that is related to obesity and impaired insulin sensitivity in both adults and children [17, 20]. We found that elevated vaspin levels are related to obesity in prepubertal Korean children after adjustment for sex, blood pressure, biochemical parameters, and other adipokines. Recent publication
Vaspin and leptin in young children

However, these previous studies also included relatively small numbers of participants with a wide age range, resulting in small, heterogeneous study samples [13, 21]. The present study had a larger sample size and all children were the same age. Some previous studies reported a sexual dimorphism in that serum vaspin levels were higher in females than males [17, 31]. In the present study, vaspin levels were not different between sexes in young children. This is in accordance with a previous study that found gender differences in serum reported that serum vaspin levels are related to insulin resistance, and rs77060950 at SERPINA12 was associated with higher serum levels [29]. In addition, reports stated that genetic variants in the vaspin gene might affect serum vaspin levels [30]. The recently published data is parallel with our data by means that vaspin plays an important role in childhood obesity. Our results, however, contradict those of previous studies showing that elevated vaspin concentrations are not related to obesity [13, 21]. This discrepancy may be partly explained by different population origins of study subjects, or different definitions of obesity in children. However, these previous studies also included relatively small numbers of participants with a wide age range, resulting in small, heterogeneous study samples [13, 21]. The present study had a larger sample size and all children were the same age. Some previous studies reported a sexual dimorphism in that serum vaspin levels were higher in females than males [17, 31]. In the present study, vaspin levels were not different between sexes in young children. This is in accordance with a previous study that found gender differences in serum

### Table 3 Results of multiple linear regression analyses to assess relationship between adipokines and other variables

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Independent variables</th>
<th>Unstandardized coefficients</th>
<th>Standardized coefficients</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin</td>
<td>Sex</td>
<td>0.072</td>
<td>0.108</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>0.040</td>
<td>0.461</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>0.001</td>
<td>0.138</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>ALT</td>
<td>0.004</td>
<td>0.130</td>
<td>0.009</td>
</tr>
<tr>
<td>Visfatin</td>
<td>Sex</td>
<td>-1.806</td>
<td>-0.146</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>FPG</td>
<td>-0.068</td>
<td>-0.133</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>0.021</td>
<td>0.166</td>
<td>0.003</td>
</tr>
<tr>
<td>Vaspin</td>
<td>BMI</td>
<td>0.004</td>
<td>0.175</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Independent variables were included in multiple linear regression analysis if p value was less than 0.10 in univariate linear regression analysis. Leptin was regarded as the dependent variable and height, BMI, SBP, DBP, FPG, TC, TG, HDL-C, ALT, HOMA-IR, visfatin, and vaspin as the independent variables. Visfatin was regarded as the dependent variable and BMI, FPG, TG, and leptin as the independent variables. Vaspin was regarded as the dependent variable and BMI, SBP, DBP, and leptin as the independent variables. Sex was included in all the analyses as an independent variable to adjust its effect and boys were regarded as a reference. Backward elimination regression analysis was applied. SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglyceride; HDL-C, HDL cholesterol; ALT, alanine aminotransferase; HOMA-IR, homeostasis model assessment of insulin resistance. Values for leptin and vaspin were log-transformed.

### Table 4 Multiple logistic regression analysis of variables related to being overweight

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>SE</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>0.031</td>
<td>0.009</td>
<td>1.03</td>
<td>1.01-1.05</td>
<td>0.000</td>
</tr>
<tr>
<td>TC</td>
<td>0.014</td>
<td>0.005</td>
<td>1.01</td>
<td>1.00-1.03</td>
<td>0.005</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-0.088</td>
<td>0.019</td>
<td>0.92</td>
<td>0.88-0.95</td>
<td>0.000</td>
</tr>
<tr>
<td>ALT</td>
<td>0.135</td>
<td>0.031</td>
<td>1.15</td>
<td>1.08-1.22</td>
<td>0.000</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.604</td>
<td>0.185</td>
<td>1.83</td>
<td>1.27-2.63</td>
<td>0.001</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.119</td>
<td>0.030</td>
<td>1.13</td>
<td>1.06-1.19</td>
<td>0.000</td>
</tr>
<tr>
<td>Vaspin</td>
<td>0.918</td>
<td>0.446</td>
<td>2.50</td>
<td>1.05-6.00</td>
<td>0.039</td>
</tr>
</tbody>
</table>

Backward elimination regression analysis was applied. Sex, SBP, DBP, FPG, TC, TG, HDL-C, ALT, HOMA-IR, leptin, visfatin, and vaspin were used as independent variables. SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglyceride; HDL-C, HDL cholesterol; ALT, alanine aminotransferase; HOMA-IR, homeostasis model assessment of insulin resistance; CI, confidence interval.
vaspin levels develop during pubertal progression in girls and are not present in prepubertal children [21]. Another study regarding vispin levels in prepubertal children revealed that there is no difference between the two sexes [13], suggesting that puberty may play a role in sexual dimorphism in vispin levels.

Leptin is a well-known adipokine that is produced by adipose tissue and functions as a component of a negative feedback loop that preserves body fat stores. Serum leptin concentrations are proportional to total body fat [5]. In this study, overweight children had higher serum leptin levels than normal weight children, which is consistent with the previous findings [32], and leptin was correlated with obesity-related variables. Furthermore, elevated leptin levels were associated with obesity after adjusting for obesity-related variables. In contrast to the previous studies [33], we did not observe sexual dimorphism in leptin levels in univariate analysis. However, leptin concentrations were higher in girls after adjusting for obesity-related variables (Table 3).

Visfatin is an adipocytokine found in visceral adipose tissue [8]. In children, elevated serum visfatin levels are associated with visceral adipose tissue area, obesity, and metabolic syndrome [10-13, 34]. Several studies have shown that visfatin is positively correlated with HOMA-IR in children [10, 35]. The present study did not report the same findings, but instead found that boys had higher visfatin levels than girls, which is in contrast to previous studies [9, 13]. This study included a large homogenous sample, which may explain the difference in results. One report stated that the boys had a higher visfatin level than girls (1.62 ± 1.36 ng/mL, 1.47 ± 1.26 ng/mL, respectively), which is consistent with our results. However, the differences were not significant ($p=0.527$) [34]. We also found that visfatin is associated with metabolic parameters that are related to obesity and metabolic syndrome, which is in accordance with earlier studies [9, 10, 12, 13, 34]. The fact that FPG was negatively correlated to visfatin after adjusting for sex, BMI, TG, and leptin suggests that visfatin has insulin-like effects [8].

The present study had several strengths, including its large sample size and the homogenous study sample in prepubertal children. However, this study does have its’ limitations. Firstly, since the study was a cross-sectional design, it was difficult to determine causality. Secondly, Tanner stage was not measured due to the nature of the school-based examination. We do not believe there would have been significant results associated with Tanner stage since the participants were not only young, but were also all the same chronological age. Thus, we do not believe these limitations affected the overall results of the study.

In conclusion, leptin, visfatin, and vaspin levels are correlated with obesity and obesity-related variables in early childhood. Moreover, elevated vaspin and leptin levels are associated with obesity in prepubertal Korean children after adjustment of sex, blood pressure, biochemical parameters, and other adipokines. Prospective studies should be performed in order to better define the roles of vaspin and other adipokines in children.

Acknowledgments

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Conflict of Interest

The authors have no conflict of interest.

Author Contributions

BJ Ko, GJ Cho, HS Park and SM Kim: designed the experiment; M Lee, GJ Cho, TG Hwang, and SM Kim: collected data; BJ Ko, K Han, HS Park and SM Kim: analyzed the data; BJ Ko, HS Park and SM Kim: wrote the manuscript; M Lee, JH Kim, SH Lee, HY Lee: provided significant advice and contributed to the discussion.

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