Clustering of Metabolic Syndrome Risk Factors Associated With Lifestyle Factors and Serum Leptin in Korean Children

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The present study investigated the relationships among metabolic risk factors, major lifestyle factors, and serum cytokines in a sample of Korean children. In a cross-sectional design, we studied a total of 275 children (130 boys and 145 girls) aged 12–13 years. Measured variables included anthropometrics, blood pressures (BP), VO₂\text{max}, physical activity (PA), dietary intakes, lipids, glucose, and insulin. We explored the extent to which dietary intakes, VO₂\text{max}, PA, and serum cytokines explained variance in a clustered risk score, which is a sum of Z scores for waist circumference, BP, TG, HDLC, and HOMA-IR, using a stepwise linear regression by blocks. VO₂\text{max}, vigorous PA (VPA), and leptin were independent predictors for the clustered risk score while adjusting for age and Tanner stage. Our findings suggest that the clustered risk score is associated not only with low levels of VO₂\text{max} and VPA, but also with elevated serum leptin in Korean children.

Metabolic syndrome, featuring of hyperinsulinemia, dyslipidemia, and hypertension, as well as low grade inflammation (6), can result in an increased risk of type 2 diabetes mellitus and cardiovascular disease, thereby contributing to increased mortality from these chronic diseases (28). A global epidemic of the metabolic syndrome already exists in the adult population, and the condition is now emerging in children and adolescents, especially in East Asia.

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Low levels of physical activity and high caloric intake are becoming more prevalent in Korea (17). Industrialization and the rapid growth of the technology sector have led to further decreases in workplace-based physical activity, along with simultaneously increasing physical inactivity as a consequence of watching TV and using personal computers and the internet. The National Health and Nutrition Survey reported in 2001 that compared with data reported in 1998 (15), Korean children have experienced significant increases in body weight and body mass index (BMI) along with increased risk factors (14). Although the exact etiology of metabolic syndrome is uncertain, various lifestyle factors, such as body fatness, low levels of physical activity and fitness, and a Westernized diet, likely contribute collectively to its development in children and adolescents in Korea.

Adipose tissue is a rich source of metabolically active molecules including leptin and vaspin (1). Leptin is a peptide produced by the obese (ob) gene of differentiated adipose tissues. This peptide plays a key role in regulating energy intake and energy expenditure, and thereby body fat stores (33). Obese individuals with unusually high leptin levels are resistant to leptin functions (leptin resistance), in much the same way that those with type 2 diabetes mellitus are resistant to insulin functions (insulin resistance). The relationships between serum leptin concentrations, body fatness, and insulin resistance have been established in adults (22), and in children and adolescents (11,12).

Visceral adipose tissue-derived serine protease inhibitor (vaspin) is a novel adipocytokine that may link obesity, insulin resistance, and type 2 diabetes mellitus (10). Vaspin cDNA was initially isolated from the visceral white adipose tissue of Otsuka Long-Evans Tokushima fatty (OLETF) rats, an animal model of abdominal obesity with type 2 diabetes mellitus (10). Vaspin expression was found to be associated with body fatness, insulin sensitivity, and diabetes in adults (8,32). Likewise, serum vaspin has also been associated with obesity and insulin resistance in children and adolescents (19,30).

Given that fatness and low levels of physical fitness and activity are highly modifiable major lifestyle risk factors for metabolic syndrome in adults, the aim of the current study was to investigate the potential associations among the clustering of metabolic risk factors, serum cytokines including leptin and vaspin, and lifestyle factors in a sample of Korean children.

**Methods**

**Participants**

At the beginning of the study, a total of 300 children aged 11–13 (132 boys and 168 girls) were recruited from elementary schools located in the city of Suwon, a northern province of South Korea. At the end of the study, 275 out of the 300 children aged 12 years (69 boys and 62 girls) and 13 years (61 boys and 83 girls) had completed all the measurements. Attrition was largely due to discomfort and technical problems related to wearing the accelerometer, as described below. Our Institutional Research Ethics Committee, in accordance with the Declaration of Helsinki of the World Medical Association, reviewed and approved the current study, and we obtained signed informed consent from all parents who agreed to allow their children to participate.
Anthropometric and Blood Pressure Measurements

Height and body mass were measured using a scale with an attached stadiometer (Jenix, Seoul, South Korea) and BMI was calculated as weight (kg) divided by height squared (m²). Waist circumference measurements were made using a cloth tape and were taken at the level of the umbilicus. Hip girth was measured as the horizontal circumference at the broadest part of the lower body, usually at the level of the trochanters. Percentage body fat was assessed using the X-scan bioelectrical body composition analyzer (Jawon Medical Co., Kyungsan, South Korea). Blood pressure (BP) was measured with an automated BP instrument (Jawon Medical Co., Kyungsan, South Korea) with the subject in a seated position, with the arm at heart level and resting on the armrest of a chair. Sexual maturation was assessed by home visits by a trained registered nurse using Tanner staging (23).

Assessments of Physical Activity and Cardiorespiratory Fitness

Routine physical activity (PA) was assessed with the Kenz Lifecorder EX, a uniaxial accelerometer (LC; Suzuken Co. Ltd, Nagoya, Japan). All children were asked to wear the device from the time they got up in the morning until they went to bed at night, except during bathing and showering, for the full 7-day data collection period. At the end of the seventh day, the LC was manually stopped by our research staff, and the recorded data were downloaded to a personal computer. The activity levels were categorized into one of nine activity classes (levels 1.0–9.0) based on PA energy expenditure (20). The nine activity levels were further classified into light PA (LPA), moderate PA (MPA), and vigorous PA (VPA). In addition, the LC assesses the number of cycles present in the acceleration signal and outputs this value as a measure of total PA (TPA) in steps count per day.

Maximal oxygen consumption (VO₂max) was used to quantify CRF and was measured with a graded treadmill walking and/or running Bruce protocol, as suggested by ACSM (2). Specifically, the speed of the treadmill was initially set at the subject’s comfortable speed (walking 1–1.7 mph) at a 10% grade, with speed and grade increased every 3 min until volitional exhaustion (average final speed of 4 ± 0.4 mph and average final grade of 15.5 ± 0.9%). Requirements to determine whether subjects reached their VO₂max by this protocol included at least 2 of the following 4 criteria (2): (i) leveling off of VO₂, (ii) rate of perceived exertion (RPE) greater than 17 using the original category scale, (iii) volitional exhaustion, and (iv) reaching age-predicted maximal heart rate. A plateau in VO₂ was defined as a change of < 2 mL/min/kg in VO₂ over the last 60 s of the test. The subjects were verbally encouraged to exercise to exhaustion during the test. All subjects tested to exhaustion, 97.8% had a respiratory exchange ratio (RER) > 1.0 and fulfilled the second criterion, and 80% of participants achieved 3 or all 4 of the criteria.

Blood Samples

Blood samples were drawn with the subjects in the supine position, following an overnight 10-hr fast. The fasting state was verbally confirmed by the subject before blood sampling. Fasting glucose, total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) levels were measured in duplicate by using the Ektachem DT-60 II analyzer (Johnson & Johnson Clinical Diagnostics,
Inc., Rochester, NY, USA). Fasting insulin was also measured in duplicate using a commercially-available enzyme-linked immunosorbent assay (ELISA) kit (ALPCO Diagnostics, Salem, NH, USA). The index of insulin resistance was assessed using the homoeostasis model of assessment for insulin resistance (HOMA-IR), as HOMA-IR = \([\text{fasting insulin (uU/ml)} \times \text{fasting glucose (mM)}]/22.5\). The coefficients of variation (CVs) for intra- and interassays were 4.3% and 6.8%, respectively, for insulin. Serum leptin and vaspin were measured using commercially available ELISA kits. The CVs for intra- and interassays were 3.2% and 4.4%, respectively, for leptin (R & D Systems, Minneapolis, MN, USA) and 2.6% and 5.3%, respectively, for vaspin (Adipogen, Songdo, Korea).

**Dietary Assessment**

Dietary intake was assessed using 3-day written food records. All subjects were provided with a dated diary and several practice sessions describing how to keep a record of the amount and types of food consumed (2 weekdays and 1 weekend day). To help them estimate portion size, the children were provided with sets of photographs. The children completed the records with their parents’ assistance. Their dietary intakes were reviewed by a registered dietitian and analyzed using CAN-PRO, a computer-based software package (version 2.0, the Korean Nutrition Society, Seoul, South Korea).

**Metabolic Syndrome Risk Score (or Clustered Metabolic Risk Score)**

A clustered risk score was computed as a sum of Z scores for metabolic risk factors, including waist circumference (WC), resting BP, TG, HDLC, and HOMA-IR, by sex. A Z score was computed for each component of the metabolic syndrome as the number of SD units from the sample mean after normalization of the variables, as suggested by Brage et al. (5), and was adjusted for age. The HDLC standardized value was multiplied by -1 to confer higher risk with increasing value for the purpose of calculating a Mets risk score. For resting systolic and diastolic BPs, the Z score of mean arterial pressure was used as the BP standardized value for the metabolic syndrome risk score.

**Statistical Analyses**

All variables were checked for normality and subjected to log10 transformation, if necessary, before statistical analyses. First, the upper (high-risk group) and lower (lower risk group) quartiles of the clustered risk score by sex were selected for comparisons of body fatness, CRF, dietary intake, accelerometer-based physical activity, fasting insulin levels, and serum cytokine values. This approach ensured that the analysis included some subjects who were obese, had low fitness, and were insulin resistant.

Analysis of covariance with Tanner scale as a covariate was used to compare any significant differences in the measured variables between boys and girls and between the low- and high-risk groups. Then we explored the extent to which major lifestyle factors along with serum cytokines explained variance in a clustered metabolic risk factor, which was measured as a sum of Z scores for WC, BP, TG, HDLC, and HOMA-IR, by using a multivariate stepwise linear regression by
blocks while adjusting for age and Tanner scale. In this stepwise regression analysis, potential predictors were entered by blocks as follows; block 1 = parameters of dietary intakes including caloric intake and proportions of macronutrients, block 2 = VO2max as an index of CRF, block 3 = parameters of accelerometer-based PA including LPA, MPA, VPA, and TPA; block 4 = serum levels of leptin and vaspin. All statistical analyses were performed using SPSS-PC for Windows (SPSS Inc., Chicago, IL, USA) and P values <0.05 were considered statistically significant.

Results

Table 1 contains data representing body fatness, CRF, dietary intake, and accelerometer-based physical activity in the low- and high-risk groups for boys and girls. Girls had higher percentage body fat than boys, while boys had higher values for BMI, waist circumference, WHR, and VO2max than girls. Based on low-, moderate-, and vigorous-physical activity and total physical activity (i.e., daily step count),

<table>
<thead>
<tr>
<th>Boys (n = 61)</th>
<th>Girls (n = 54)</th>
<th>P value for sex</th>
<th>P value for low- vs. high-risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk</td>
<td>High risk</td>
<td>Low risk</td>
<td>High risk</td>
</tr>
<tr>
<td>Age, years</td>
<td>12.4 ± 0.5</td>
<td>12.5 ± 0.5</td>
<td>12.5 ± 0.5</td>
</tr>
<tr>
<td>Tanner</td>
<td>1.1 ± 0.3</td>
<td>1.7 ± 0.9</td>
<td>1.6 ± 1.1</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>19.1 ± 3.2</td>
<td>22.9 ± 3.6</td>
<td>16.9 ± 1.9</td>
</tr>
<tr>
<td>BF, %</td>
<td>13.8 ± 6.7</td>
<td>19.8 ± 7.4</td>
<td>17.1 ± 4.4</td>
</tr>
<tr>
<td>WC, cm</td>
<td>67.7 ± 7.6</td>
<td>80.6 ± 10.2</td>
<td>62.9 ± 5.6</td>
</tr>
<tr>
<td>WHR</td>
<td>0.84 ± 0.06</td>
<td>0.90 ± 0.05</td>
<td>0.80 ± 0.05</td>
</tr>
<tr>
<td>VO2, ml/kg/min</td>
<td>43.4 ± 8.0</td>
<td>43.4 ± 8.9</td>
<td>39.4 ± 6.7</td>
</tr>
<tr>
<td>LPA, min/day</td>
<td>216 ± 154</td>
<td>129 ± 112</td>
<td>109 ± 28</td>
</tr>
<tr>
<td>MPA, min/day</td>
<td>99 ± 73</td>
<td>48 ± 38</td>
<td>41 ± 15</td>
</tr>
<tr>
<td>VPA, min/day</td>
<td>52 ± 45</td>
<td>17 ± 11</td>
<td>14 ± 9</td>
</tr>
<tr>
<td>TPA, steps/day</td>
<td>14597 ± 3793</td>
<td>12786 ± 2629</td>
<td>13063 ± 2180</td>
</tr>
<tr>
<td>CI, Kcal</td>
<td>1675 ± 363</td>
<td>1809 ± 355</td>
<td>1865 ± 294</td>
</tr>
<tr>
<td>CHO, %</td>
<td>67.2 ± 4.5</td>
<td>65.6 ± 3.4</td>
<td>67.6 ± 5.0</td>
</tr>
<tr>
<td>FAT, %</td>
<td>17.3 ± 2.3</td>
<td>16.5 ± 1.9</td>
<td>19.7 ± 5.4</td>
</tr>
<tr>
<td>PRO, %</td>
<td>13.3 ± 2.5</td>
<td>13.0 ± 2.4</td>
<td>13.8 ± 3.0</td>
</tr>
</tbody>
</table>
boys were more active than girls. Girls had a higher proportion of fat intake than boys, with no sex differences in any of other dietary intakes between boys and girls. As expected, mean values in body fatness variables, including BMI, percentage body fat, WC, and WHR were significantly higher in the high-risk group than in the low-risk group. The high-risk group had lower levels of physical activity than the low-risk group.

Table 2 presents data on the metabolic risk factors and serum cytokines in the low- and high-risk groups for boys and girls. Girls had significantly higher insulin and HOMA-IR values than boys, with no sex differences in any of the other metabolic risk factors measured in this study. As expected, metabolic risk factors, including blood pressure, blood lipoprotein lipid values, fasting glucose and insulin levels, and HOMA-IR, were significantly higher in the high-risk group than in the low-risk group. Serum leptin was significantly higher in the high-risk group than in the low-risk group. Serum vaspin was significantly higher in girls than in boys, and no significant group difference in vaspin was found between the low- and high-risk groups.

Table 3 presents a multivariate stepwise linear regression for the clustered metabolic risk factors in the total study sample. VO\textsubscript{2max}, VPA, and serum vaspin

### Table 2: Metabolic Risk Factors and Serum Cytokines in the Low- and High-Risk Groups (Upper and Lower Quartiles of Clustered Risk Score)

<table>
<thead>
<tr>
<th></th>
<th>Boys (n = 61)</th>
<th>Girls (n = 54)</th>
<th>P value for sex</th>
<th>P value for low-vs. high-risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low risk</td>
<td>High risk</td>
<td>Low risk</td>
<td>High risk</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>96.0 ± 7.2</td>
<td>112.4 ± 11.2</td>
<td>98.2 ± 8.2</td>
<td>106.0 ± 8.5</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>58.4 ± 5.4</td>
<td>66.8 ± 5.1</td>
<td>61.1 ± 6.8</td>
<td>66.7 ± 8.4</td>
</tr>
<tr>
<td>TC, mg/dl</td>
<td>170.7 ± 25.8</td>
<td>187.4 ± 51.3</td>
<td>167.2 ± 26.6</td>
<td>200.3 ± 50.0</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>53.1 ± 12.5</td>
<td>146.5 ± 65.2</td>
<td>75.8 ± 19.3</td>
<td>141.7 ± 50.0</td>
</tr>
<tr>
<td>HDLC, mg/dl</td>
<td>63.4 ± 11.7</td>
<td>43.9 ± 9.7</td>
<td>56.7 ± 11.3</td>
<td>40.4 ± 6.6</td>
</tr>
<tr>
<td>FBG, mg/dl</td>
<td>86.2 ± 13.2</td>
<td>97.4 ± 14.8</td>
<td>84.0 ± 11.6</td>
<td>103.3 ± 16.8</td>
</tr>
<tr>
<td>Insulin, uU/ml</td>
<td>4.1 ± 4.0</td>
<td>12.5 ± 5.9</td>
<td>6.9 ± 3.2</td>
<td>19.0 ± 10.6</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.8 ± 0.6</td>
<td>3.0 ± 1.4</td>
<td>1.4 ± 0.7</td>
<td>4.8 ± 2.7</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>4.0 ± 3.5</td>
<td>8.5 ± 5.4</td>
<td>3.5 ± 2.6</td>
<td>11.3 ± 7.0</td>
</tr>
<tr>
<td>Vaspin, ng/ml</td>
<td>1.8 ± 4.7</td>
<td>0.4 ± 0.6</td>
<td>0.3 ± 0.2</td>
<td>2.5 ± 0.8</td>
</tr>
</tbody>
</table>
were found to be significant predictors for the clustered metabolic risk score independent of age and Tanner stage, explaining its variance by approximately 44% in this study sample.

### Discussion

The sample examined in the current study included children with a wide range of characteristics; some were overweight/obese, had clustered metabolic risk factors, or low levels of physical activity, but all traits not necessarily were found in the same children. We compared lifestyle factors, including dietary intakes, physical activity, body fatness and fitness, and serum cytokines in the upper and lower quartiles of the clustered risk factor score (i.e., a sum of Z scores for WC, resting BP, TG, HDLC, and HOMA-IR) because this provided sufficient statistical power to observe any associations between exposures and outcomes (27).

In the study, we found that children who were in the upper quartile of the clustered risk factor score (the high-risk group) were physically more mature, heavier, and less active than children who were in the lower quartile of the clustered risk factor score (the low-risk group). These differences in lifestyle factors observed between the high- and low-risk groups appear to be major contributors to the clustering of metabolic risk factors in this study sample; the high-risk group had significantly higher values for BP, lipoprotein-lipids, fasting glucose, insulin, HOMR-IR, and serum leptin than the low-risk group. The multivariate regression analyses showed that VO_{2max}, VPA, and serum leptin were independent predictors for the clustering of metabolic risk factors. Thus, the current findings suggest that low levels of aerobic fitness and vigorous physical activity as well as elevated serum leptin are major risk factors for the clustering of metabolic risk factors in Korean children aged 12–13 yrs.

The current study findings are in line with previous studies reporting lifestyle factors, including aerobic fitness and vigorous physical activity, as major risk factors for metabolic syndrome in children and adolescents (3,9,16,24). In Asian children and adolescents, only a handful of studies have examined the associations between lifestyle factors and metabolic risk factors, reporting similar findings to those of the current study. In a Vietnamese survey study involving 693 high school students, Nguyen et al. (25) found that youth in the lowest accelerometer-based physical activity level (<43 min/day) had a 5 times higher chance of having metabolic syndrome.
than their counterparts in the highest physical activity level (>103 min/day). Both current and previous findings underscore the necessity for a lifestyle intervention designed to promote increased physical fitness and activity, especially, vigorous one, as a nonpharmacologic means of preventing and treating obesity, and thereby its related metabolic risk factors, early in life.

With respect to serum cytokines measured in this study, we found that only leptin was significantly higher in the high-risk group than in the low-risk group, and it was retained as an independent predictor for the clustering of metabolic risk factors in the stepwise regression analysis. In addition, partial correlations showed that the association between serum leptin and the clustered risk score remained significant even when controlling for age ($r = .645, p < .001$), Tanner stage ($r = .629, p < .001$), body fatness parameters including BMI, percent body fat, and WHR ($r = .334, p < .001$), CRF ($r = .567, p < .001$), physical activity parameters including LPA, MPA, VPA, and TPA ($r = .569, p < .001$), and dietary intake ($r = .611, p < .001$), respectively (data not shown). Thus, the current findings suggest that elevated serum leptin values may be an important and independent biomarker for the clustering of metabolic risk factors early in life.

In agreement with the current findings, circulating leptin has been found to be positively related to body fatness parameters (31) and fasting insulin and insulin resistance markers (11,18) in children. In a cross-sectional study involving 342 children aged 11–14 years, Steinberger et al. (29) found that serum leptin levels were significantly related to insulin and insulin sensitivity-to-lean body mass ratio in heavy children, and insulin in thin children, independent of adiposity. In a cross-sectional study, Park et al. (26) found that serum leptin values were highly related to several metabolic risk factors, such as overall and abdominal fatness, in Korean adolescents. Cambuli et al. (7) found that at baseline, mean serum adiponectin levels were significantly lower in overweight and obese children than in normal-weight children and were negatively associated with HOMA-IR in their study sample.

With respect to serum vaspin, we found that girls had significantly higher values for serum vaspin than boys. A recent study done by Körner et al. (19) also reported age- and sex- dependent differences in serum vaspin levels between boys and girls. On the other hand, both the clustered risk factor-based group analysis and the regression analyses showed that serum vaspin levels were not significantly associated with metabolic risk factors in the study sample. These current findings do not agree with those of previous studies. In a cross-sectional study involving 33 obese and 36 healthy children aged 11–16 yrs, Suleymanoglu et al. (30) found that obese children had significantly higher serum vaspin concentrations than nonobese children. In the same study, vaspin levels were found to be positively associated with BMI-SDS, TG, and insulin resistance markers. In a short-term lifestyle intervention study involving overweight/obese Korean children aged 11–13 yrs, Lee et al. (21) also found that at baseline, fasting insulin levels and HOMA-IR were positively related to serum vaspin levels and negatively related to serum adiponectin levels. The short-term intervention resulted in a significant decrease in serum vaspin concentrations along with improved HOMA-IR, perhaps as a compensatory response to increased insulin sensitivity.

Several explanations can be proposed for the inconsistencies between the present and previous findings. First, the associations between serum vaspin values and metabolic complications may be limited to studies involving overweight and/or obese children and adolescents (18). This question needs to be further addressed.
in a large scale study. Second, it may be possible that responses to acute exercise, including changes in serum cytokine levels, may not necessarily reflect adaptations to chronic exercise training (4). Whether the decreased vispakin reported by Lee et al. (21) reflect an acute response or a chronic adaptation to exercise training remains to be further investigated. Third, we found substantial individual variation in serum vaspin levels, even in children of normal weight and insulin sensitivity. In a recent KORA study, Kempf et al. (13) reported a significant association between vaspin SNP rs2236242 and type 2 diabetes mellitus. Thus, it is possible that the relationships between lifestyle factors and serum vaspin levels may differ depending on the vaspin genotype. Considering the cross-sectional nature of this study, however, we cannot provide further insight on this subject.

In summary, the current study investigated the possible relationships among the clustering of metabolic risk factors, major lifestyle factors, and serum cytokines in a sample of 275 Korean children aged 12–13 yrs. Group analyses based on the clustered risk factor score showed that the high-risk group had significantly higher values for BP, blood lipoprotein lipids, fasting glucose and insulin, HOMA-IR, and serum lepin than the low-risk group. A stepwise regression analysis showed that the clustering of metabolic risk factors in Korean children aged 12–13 years was associated not only with low levels of aerobic fitness and vigorous physical activity, but also with elevated serum lepin level. One clinical implication of the current findings is that promoting healthy lifestyles, including increased exercise and vigorous physical activity, should be implemented early in life to prevent metabolic risk factors from being clustered later in life in Korea and other Asian countries.

Acknowledgments

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Disclosures

The authors have no conflicts of interest to declare.

References


