Circulating Growth Arrest-Specific 6 Protein Is Associated With Adiposity, Systemic Inflammation, and Insulin Resistance Among Overweight and Obese Adolescents

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Context: Growth arrest-specific 6 (Gas6) is a vitamin K-dependent protein secreted by immune cells, endothelial cells, vascular smooth muscle cells, and adipocytes. Preclinical studies indicate that Gas6 and its receptors of the TAM (Tyro-3, Axl, Mer) family may be involved in the pathogenesis of obesity and its complications, including systemic inflammation and insulin resistance. Until now, little has been known about the clinical significance of the Gas6/TAM system in childhood obesity.

Objectives: This study aimed to determine whether circulating Gas6 and soluble Axl (sAxl) levels are associated with adiposity, inflammation, and insulin resistance status among Taiwanese adolescents.

Methods: Cross-sectional analyses using the data from the Taipei Children Heart Study-III were performed. A total of 832 adolescents (average age, 13.3 years) were included; they were divided into 3 groups: lean, overweight, and obese. Circulating Gas6 and sAxl levels, adiposity, inflammatory markers, and insulin resistance status were examined.

Results: Levels of circulating Gas6 and sAxl were significantly higher in overweight and obese adolescents than in the lean group (both \(P < .05\)). Circulating Gas6 levels were significantly positively correlated with body mass index Z-score (\(P = .045\)), waist circumference (\(P < .001\)), waist to hip circumference ratio (\(P < .001\)), body fat mass (\(P = .02\)), serum high-sensitivity C-reactive protein (\(P = .005\)), and tumor necrosis factor-\(\alpha\) levels (\(P = .039\)) among overweight and obese adolescents. The correlations remained significant after adjusting for age, gender, Tanner stage, smoking status, and drinking status. In addition, every 1 ng/mL increase in circulating Gas6 concentration corresponded to a 15% to 19% increase in the risk of developing insulin resistance among overweight and obese adolescents.

Conclusions: Circulating Gas6 levels are strongly associated with adiposity, inflammation, and insulin resistance status among overweight and obese adolescents. The potential role of the Gas6/TAM system in the initiation of childhood obesity and obesity-associated complications deserves further attention. (J Clin Endocrinol Metab 98: E267–E274, 2013)

Abbreviations: BMI, body mass index; BP, blood pressure; CV, coefficients of variation; DBP, diastolic BP; Gas6, growth arrest-specific 6; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; sAxl, soluble Axl; SBP, systolic BP; TAM, Tyro-3, Axl, Mer; TNF, tumor necrosis factor; WHR, waist-to-hip circumference ratio; WC, waist circumference.
Childhood obesity is a serious public health problem, and its worldwide prevalence has increased greatly during the past 3 decades (1). The increasing occurrence in children of disorders such as type 2 diabetes is believed to be a consequence of this obesity epidemic (1). Metabolic and cardiovascular complications of obesity in childhood are less common than in adulthood. However, recent longitudinal studies have demonstrated that obese children have an increased risk of developing metabolic and cardiovascular diseases in later life (2, 3).

In obese children, it is well established that insulin resistance is a fundamental aspect of the etiology of type 2 diabetes and is also linked to other pathophysiologic sequelae, including hypertension, dyslipidemia, and atherosclerosis (4). Over the past decade, the search for a potential unifying mechanism behind the pathogenesis of obesity-engendered insulin resistance has revealed a close relationship between adipose tissue inflammation and disturbances in insulin signaling (5). Adipose tissue inflammation is a consequence of obesity-associated pathologic adipose tissue expansion (6) and is characterized by increased infiltration of macrophages and other types of immune cells in adipose tissue, along with overproduction of proinflammatory cytokines (5, 7). Excess proinflammatory mediators, such as tumor necrosis factor (TNF)-α and IL-6, can interfere with insulin activity in adipose tissue, liver, and skeletal muscle, resulting in systemic insulin resistance (5). Although abnormal adipose tissue expansion and excess recruitment of immune cells into adipose tissue are now viewed as central to the pathogenesis of obesity-associated inflammation and insulin resistance, it is still unclear how these abnormalities arise and how they are mediated.

Growth arrest-specific 6 (Gas6) was cloned in 1988 and characterized in 1993 (8). The original observation that Gas6 is up-regulated in growth-arrested fibroblasts suggested a role in protection from certain cellular stresses, such as apoptosis (9). This antiapoptotic effect has been described in many cell types and experimental settings, not only deprived of growth factor but also treated with specific inducers of apoptosis such as TNF-α or β-amyloid peptide (10–12). Gas6 expression is widespread in many tissues, including immune cells, endothelial cells, vascular smooth muscle cells, and adipocytes (13–15). It was recognized as a secreted vitamin K-dependent protein because it interacted with receptor tyrosine kinases of the TAM (Tyro-3, Axl, Mer) family (8). Membrane-bound Axl can be shed from the cell membrane as a result of proteolysis, and Axl is therefore present in the circulation in a soluble form (sAxl) that consists of the extracellular region of the protein (16, 17). Gas6 and sAxl are present in the circulatory systems of both mice and humans and circulate bound to each other in a high-affinity complex (18). The Gas6/TAM system has been implicated in cell survival and proliferation, cell adhesion and migration, hemostasis, and inflammatory cytokine release (8, 19). Recently, the Gas6/TAM system was also found to be involved in mediating adipocyte survival and proliferation (20, 21). Experiments with mice fed a high-fat diet indicated that overexpression of Gas6/TAM signaling might enhance body-fat accumulation, but blocking the system could reduce body-fat mass and body weight (15, 22). In addition, some studies of transgenic mice found that Gas6/TAM signaling might recruit macrophages from the bone marrow into the adipose tissue, implying that Gas6/TAM signaling may play a potential role in the pathogenesis of adipose tissue inflammation (14, 23, 24). Interestingly, transgenic animals that ectopically express the TAM family of receptors also develop progressive obesity with elevated circulating TNF-α concentrations and severe systemic insulin resistance (25). This preclinical evidence indicates that the Gas6/TAM system likely represents an important pathogenic mechanism for obesity and obesity-associated inflammation and insulin resistance.

Recent human studies demonstrated that plasma Gas6 and sAxl concentrations are correlated with a number of inflammatory markers among adult patients with systemic inflammatory diseases (26, 27). Our previous human study also found that plasma Gas6 concentrations are associated with altered glucose tolerance and inflammation in middle-aged adults (28). However, little has been known about the clinical significance of the Gas6/TAM system in childhood obesity and obesity-associated inflammation and insulin resistance. Therefore, we addressed this issue by conducting a cross-sectional, community-based study to determine whether plasma Gas6 and sAxl levels are associated with adiposity, obesity-associated inflammation, and insulin resistance among Taiwanese adolescents.

Subjects and Methods

Study design and sampling

The Taipei Children Heart Study-III was an epidemiological survey that investigated obesity and cardiovascular disease risk factors among adolescents in Taipei during 2006. The sampling method and results have been described elsewhere (29). In brief, this survey was administered to junior high school students in Taipei to collect a representative distribution of demographic, lifestyle, and biochemical characteristics to measure their risk for cardiovascular disease. After multistage sampling, researchers randomly selected 1283 Taipei adolescents. Those with autoimmune diseases, cancers, or active infection and those taking medications known to interfere with insulin or glucose metabolism were excluded. Excluding any missing data, a total of 832 ado-
lescents (420 boys and 412 girls) of an average age of 13.3 (range, 12–15) years were included in the final analyses.

Data collection
The Ethical Committee of the Scientific Institute approved these studies and obtained informed consent from both parents and adolescents. All of the participants completed a structured questionnaire detailing their gender, age, puberty development, and lifestyle characteristics, including cigarette smoking and alcohol consumption. On the basis of their responses to the questionnaire, the subjects were divided into young adolescents who had never smoked, those who had smoked in the past, and those who currently smoke. The study divides alcohol consumption into 2 categories: currently drinks and never drinks. Survey questions on puberty included items regarding the development of the penis/testis and pubic hair for boys and breast enlargement and pubic hair for girls. Pubertal status was evaluated according to the criteria of Tanner (30).

Anthropometric measurements
Body weight was measured to the nearest 0.1 kg while the subjects were barefoot and wearing light indoor clothing. Body height was recorded to the nearest 0.1 cm. Waist circumference (WC) was measured at the midpoint between the inferior margin of the last rib and the crest of the ilium in a horizontal plane and was recorded to the nearest 0.1 cm. Hip circumference was measured at its widest point to the nearest 0.1 cm. The waist-to-hip circumference ratio (WHR) was also computed. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters and was expressed as a BMI Z-score using established guidelines (30). We defined overweight (≥85th but <95th BMI percentile) and obese (≥95th BMI percentile) using gender- and age-specific criteria from an expert panel on obesity in children and adolescents from Taiwan’s Department of Health. Segmental bioelectrical impedance analysis was applied to measure the percentage of body fat mass (BFM) to an accuracy of 0.1% (BC-418; Tanita, Tokyo, Japan). Blood pressure (BP) was measured from the right arm using an appropriate cuff size after subjects had rested for 10 minutes in a sitting position; the first and fifth Korotkoff sounds were recorded as the systolic BP (SBP) and diastolic BP (DBP), respectively. The BP was measured again after a 5-minute rest, and the average BP was used in the analysis.

Analytic methods
To reduce extraneous variation between subjects, we collected a 12-hour fasting blood sample only from those participants who had consumed their usual dietary pattern during the previous 3 days. Children who had recently attended a holiday or family celebration were contacted for a blood sample several weeks later. The biochemical assays, including serum lipid profiles and glucose levels, were performed within 2 weeks on blood samples stored at −4°C. Plasma was stored at −70°C until other biochemical markers were assayed.

Circulating Gas6 and sAxl concentrations
The Gas6 protein concentration was measured with a sandwich ELISA using a polyclonal mouse anti-human Gas6 antibody (R&D Systems, Lille, France) as a catcher and a biotinylated goat antiserum as a detector (R&D Systems), as described in our previous study (28). The method has been validated according to the Food and Drug Administration guidelines in a previous study (intra- and interassay CV of 6.5% and 8.5%, respectively; mean recovery on 10 patients of 97%; lower limit of quantification, 0.26 ng/mL) (32). Circulating sAxl concentrations were determined using a RayBio human ELISA kit (RayBiotech, Norcross, Georgia) that permits intra- and interassay CV of <10% and <12%, respectively. Briefly, this commercial kit employs an antibody specific for human Axl coated on a 96-well plate. Plasma or standards (100 μL) were added for 2 hours at room temperature. Washes were repeated, and 100 μL of prepared biotinylated antibody was added for 1 hour at room temperature with gentle shaking. Detection was performed with horseradish peroxidase-conjugated streptavidin. Both plasma Gas6 and sAxl concentrations were measured in duplicate.

Definitions of insulin resistance
Insulin resistance was defined based on a number of different thresholds, including HOMA-IR >3.16 (based on receiver-operator curve analysis) (33) HOMA-IR >4.18 (the upper quartile of HOMA-IR for all adolescents in the present study) (34), and HOMA-IR >6.08 (the upper 2.5 percentile based on non–log-transformed HOMA-IR for normal-weight adolescents with normoglycemia in the present study) (35).

Statistical methods
Descriptive results of continuous variables are expressed as mean ± SD. Before statistical analysis, the normal distribution...
and homogeneity of the variables were evaluated using the Levene test for quality of variance, and variables were then given a base logarithmic transformation if necessary. The parameters HOMA-IR, triglycerides, hsCRP, IL-6, and TNF-α were analyzed and tested for significance on a log scale. A one-way ANOVA using the Bonferroni test as a post hoc test was applied to determine differences in continuous variables between three groups. Relationships between variables were tested using Spearman rank-order correlations and partial correlation analysis after adjusting for various variables. Logistic regression analysis, with the development of insulin resistance as the dependent variable, was used to study the independent determinants of plasma Gas6 and sAxl levels. A two-sided P value < .05 was considered statistically significant. All statistical analyses were performed using PASW Statistics version 18.0 software (SPSS Inc, Chicago, Illinois).

Results

Anthropometric data for all adolescents

The mean age of the 832 adolescents enrolled in this study was 13.3 ± 0.9 years and was similar among all groups. Only 49 adolescents (46 boys and 3 girls) were prepubertal, and most girls (n = 386) had attained menarche. The study population was divided into 3 groups: lean, overweight, and obese (Table 1). By study design, obese adolescents showed significantly greater weight, BMI, BMI Z-score, WC, WHR, and BFM than the other 2 groups. Obese adolescents also had the highest SBP and DBP; these were significantly higher than those for lean adolescents but not overweight adolescents. However, the 3 groups did not differ with respect to age and Tanner stages.

Biochemistry variables for all adolescents

Fasting glucose levels were similar in the 3 groups of adolescents (Table 1). However, obese adolescents presented significantly higher fasting insulin levels and HOMA-IR than the other 2 groups. The overweight group had significantly higher HOMA-IR than the lean group. The obese group showed significantly higher triglycerides and LDL cholesterol levels and lower HDL cholesterol levels (without a difference in total cholesterol levels) as compared with the other 2 groups.

Overweight and obese adolescents presented significantly higher serum hsCRP, TNF-α, and IL-6 levels than the lean group. There was no difference in serum IL-6 levels between the overweight and obese adolescents. Moreover, overweight and obese adolescents had significantly higher plasma Gas6 and sAxl levels than the lean group. Plasma Gas6 levels in obese adolescents were significantly higher than those in overweight adolescents. However, there was no difference in plasma sAxl levels between obese and overweight adolescents. Among all ad-

| Table 1. Anthropometric and Biochemical Data Among All Adolescents<sup>a</sup> |
|-------------------------------|---|---|---|---|---|
|                              | Lean | Overweight | Obesity | P<sup>b</sup> | P<sup>c</sup> | P<sup>d</sup> |
| n (male/female)               | 517 (225/292) | 137 (74/63) | 158 (106/52) |             |             |             |
| Age, y                        | 13.3 ± 0.9 | 13.3 ± 0.9 | 13.2 ± 1.0 | 1.000      | 1.000      | 1.000      |
| Tanner stage                  | 3.1 ± 0.4 | 3.1 ± 0.5 | 3.0 ± 0.6 | 1.000      | 1.000      | 1.000      |
| BMI, kg/m<sup>2</sup>         | 19.5 ± 1.5 | 23.4 ± 0.9 | 27.8 ± 2.7 | <.001      | <.001      | <.001      |
| BMI Z-score                   | -0.4 ± 0.4 | 0.6 ± 0.4 | 1.7 ± 0.7 | <.001      | <.001      | <.001      |
| WC, cm                        | 72.5 ± 5.4 | 81.6 ± 5.0 | 91.8 ± 7.7 | <.001      | <.001      | <.001      |
| WHR                           | 0.79 ± 0.05 | 0.83 ± 0.05 | 0.86 ± 0.06 | <.001      | <.001      | <.001      |
| BFM, %                        | 21.8 ± 5.1 | 28.9 ± 6.2 | 34.1 ± 7.8 | <.001      | <.001      | <.001      |
| SBP, mm Hg                    | 114 ± 13   | 115 ± 15   | 119 ± 12   | .554       | <.001      | 0.044      |
| DBP, mm Hg                    | 69 ± 10    | 70 ± 10    | 72 ± 11    | .962       | <.001      | 0.059      |
| Glucose, mg/dL                | 92.3 ± 6.6 | 92.9 ± 6.5 | 93.5 ± 6.1 | .964       | 1.000      | 1.000      |
| Insulin, μU/mL                | 12.9 ± 6.4 | 14.4 ± 6.6 | 23.9 ± 11.4 | .122      | <.001      | <.001      |
| HOMA-IR<sup>c</sup>           | 3.0 ± 1.6 | 3.3 ± 1.6 | 5.6 ± 2.8 | .037       | <.001      | <.001      |
| Total cholesterol, mg/dL      | 170.7 ± 31.5 | 165.9 ± 29.1 | 171.5 ± 28.3 | .326      | 1.000      | 0.390      |
| Triglyceride, mg/dL           | 66.8 ± 28.7 | 72.4 ± 35.6 | 93.9 ± 49.8 | .404      | <.001      | <.001      |
| LDL cholesterol, mg/dL        | 93.2 ± 25.8 | 97.02 ± 6.4 | 107.3 ± 26.9 | .389     | <.001      | .002       |
| HDL cholesterol, mg/dL        | 52.4 ± 11.3 | 45.5 ± 10.0 | 41.0 ± 7.9 | <.001      | <.001      | <.001      |
| hsCRP, mg/L<sup>e</sup>       | 0.6 ± 1.1 | 0.8 ± 1.3 | 1.0 ± 1.2 | <.001      | <.001      | <.001      |
| TNF-α, pg/mL<sup>e</sup>      | 14.0 ± 3.0 | 35.2 ± 9.30 | 62.9 ± 15.9 | <.001      | <.001      | <.001      |
| IL-6, pg/mL<sup>e</sup>       | 3.0 ± 0.1 | 3.5 ± 5.8 | 3.5 ± 5.8 | <.001      | <.001      | 1.000      |
| Gas6, ng/mL                   | 12.3 ± 4.4 | 13.1 ± 3.6 | 13.9 ± 3.9 | .008       | <.001      | 0.005      |
| sAxl, ng/mL                   | 3.8 ± 1.0 | 4.6 ± 1.1 | 4.7 ± 1.3 | <.001      | <.001      | 1.000      |

<sup>a</sup> Data are expressed as mean ± SD.

<sup>b</sup> Overweight vs lean.

<sup>c</sup> Obesity vs lean.

<sup>d</sup> Obesity vs overweight.

<sup>e</sup> The logarithms of these variables were used for the analysis.
Gas6, sAxl, and adiposity among overweight and obese adolescents

Plasma Gas6 levels were significantly positively correlated with plasma sAxl levels, BMI Z-score, WC, WHR, and BFM among overweight and obese adolescents (Table 2). These correlations remained significant after adjusting for age, gender, Tanner stages, smoking status, and drinking status. Moreover, there was a direct correlation in plasma sAxl levels with WC and WHR (r = 0.120, P = .043; r = 0.144, P = .015, respectively). However, this correlation was abolished after adjusting for age, gender, Tanner stages, smoking status, and drinking status.

Gas6, sAxl, and inflammatory markers among overweight and obese adolescents

A significant positive correlation was observed between plasma Gas6 levels and serum hsCRP and TNF-α levels among overweight and obese adolescents (Table 2). Even after adjusting for all possible confounding factors including age, gender, puberty status, smoking/drinking status, and adiposity, the correlation between circulating Gas6 and TNF-α levels still remained significant (r = 0.207, P = .007). In addition, the Gas6/sAxl ratio is significantly associated with WC (r = 0.128, P = .032), WHR (r = 0.152, P = .011), serum hsCRP (r = 0.134, P = .024), and TNF-α levels (r = 0.303, P < .001) among overweight/obese adolescents, independent of age, gender, Tanner stages, smoking status, or drinking status.

Table 2. Spearman Correlation Coefficients Between Plasma Gas6 Levels, Adiposity, and Inflammatory Markers Among Overweight and Obese Adolescents

<table>
<thead>
<tr>
<th></th>
<th>Gas6</th>
<th>sAxl</th>
<th>BMI Z-score</th>
<th>WC</th>
<th>WHR</th>
<th>BFM</th>
<th>hsCRP *</th>
<th>TNF-α *</th>
<th>IL-6 *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 1a</td>
<td>Model 2b</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>r²</td>
<td>P</td>
<td>r²</td>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sAxl</td>
<td>0.235</td>
<td>&lt;.001</td>
<td>0.232</td>
<td>&lt;.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI Z-score</td>
<td>0.117</td>
<td>0.045</td>
<td>0.139</td>
<td>0.019</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WC</td>
<td>0.213</td>
<td>&lt;.001</td>
<td>0.191</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td>0.226</td>
<td>&lt;.001</td>
<td>0.227</td>
<td>&lt;.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BFM</td>
<td>0.135</td>
<td>0.020</td>
<td>0.197</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsCRP *</td>
<td>0.165</td>
<td>0.005</td>
<td>0.176</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α *</td>
<td>0.120</td>
<td>0.039</td>
<td>0.120</td>
<td>0.045</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 *</td>
<td>0.060</td>
<td>0.303</td>
<td>0.073</td>
<td>0.222</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* A direct correlation between Gas6 levels and other variables.

** Adjusting for age, gender, Tanner stages, smoking status, and drinking status.

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Table 3. Logistic Regression Analyses of Plasma Gas6 Concentrations on the Development of Insulin Resistance Among Overweight and Obese Adolescents

<table>
<thead>
<tr>
<th>Insulin Resistance</th>
<th>Model 1 OR (95% CI)</th>
<th>Gas6</th>
<th>Model 2 OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA-IR Cutoff *</td>
<td>3.16 66.6</td>
<td>1.15 (1.07–1.24)</td>
<td>1.18 (1.13–1.31)</td>
</tr>
<tr>
<td></td>
<td>4.18 43.9</td>
<td>1.15 (1.08–1.23)</td>
<td>1.20 (1.09–1.31)</td>
</tr>
<tr>
<td></td>
<td>6.08 23.3</td>
<td>1.19 (1.10–1.28)</td>
<td>1.25 (1.13–1.40)</td>
</tr>
</tbody>
</table>

Abbreviation: OR, odds ratio.

* Univariable analyses for an increase of 1 ng/mL in plasma Gas6 concentration.

** Adjusting for age, gender, Tanner stages, smoking status, and drinking status.

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Discussion

Although Gas6/TAM signaling is known to be involved in triggering systemic inflammation in diverse human diseases (such as infection, acute stroke, and acute coronary syndrome), this is, to our knowledge, the first clinical study to investigate the pleiotropic effects of Gas6 on insulin resistance.
childhood obesity. Our study showed that obese adolescents had higher levels of proinflammatory markers, Gas6, and sAxl as well as adverse insulin sensitivity status. Circulating Gas6 levels correlated positively with body adiposity and circulating proinflammatory cytokine levels and further predicted the insulin resistance status among overweight and obese adolescents.

In humans, the physiological increase of adipocyte numbers begins during childhood and adolescence and ends in early adulthood (36). Adipocyte numbers always remain constant via stable dynamics of adipocyte turnover during adulthood (36). The generation of adipocytes is thought to be the most important determining factor for body fat accumulation during childhood and adolescence. Therefore, identification of the mechanism regulating adipocyte numbers during childhood and adolescence is of great importance in preventing obesity in children. Our study has shown that plasma Gas6 levels were correlated with several adiposity variables, suggesting that the increased circulating concentrations of Gas6 are associated with the increased fat mass among adolescents. Our findings are supported by several previous experiments. In 1995, Shugart et al (21) found that Gas6 plays a pivotal role in enhancing 3T3-L1 adipocyte proliferation. Augustine et al (25) accidentally found that transgenic mice with overexpression of Gas6/TAM signaling exhibit progressive obesity. Moreover, adipose tissue is highly vascularized, and its growth depends on vascularization (38). The Gas6/TAM system is involved in vascularization, including the development of arterial aneurysms (39). Two experiments also demonstrated that using a Gas6 gene knockout or receptor antagonist to block Gas6/TAM signaling could reduce both the blood vessel density of adipose tissue and the body fat mass in mice, indicating that the Gas6/TAM system may impair adipose tissue development by modulating vascularization (15, 22). Together, this evidence points toward the possibility that the Gas6/TAM system might be involved in the pathogenesis of adipocyte proliferation and adipose tissue expansion during adolescence and may provide a novel target for pharmacological intervention in childhood obesity.

Obese children of all ages show evidence of a low-grade chronic inflammatory state (40). Obesity-associated inflammation appears to be central to the development of insulin resistance and atherosclerosis and may be important in the pathogenesis of other comorbid conditions (5). Not surprisingly, our findings also showed that overweight and obese adolescents in Taiwan have a more severe degree of inflammation and insulin resistance than do lean adolescents. Moreover, the current study showed that elevated circulating Gas6 levels are directly associated with the development of systemic inflammation and insulin resistance. Our findings provide new and important clinical evidence that circulating Gas6 protein may be involved in childhood obesity-associated inflammation and insulin resistance. However, our study did not find a significant correlation between circulating Gas6 and IL-6 levels. A previous study demonstrated that serum IL-6 levels can be influenced by pubertal changes of hormones in children (41). Martos-Moreno et al (42) also indicated that serum IL-6 levels are negatively correlated with serum testosterone and estradiol levels in pubertal children. Therefore, one might speculate that the differences in circulating sex hormones among adolescents confound the association between Gas6 and IL-6 levels. Further research investigating the relationship between circulating sex hormones, Gas6, and IL-6 concentrations is required to test our assumption.

It should be noted, however, that different results were obtained in our previous study on adult subjects with glucose intolerance. We demonstrated that hyperglycemia could inhibit Gas6/TAM signaling (28). Therefore, circulating Gas6 levels were reduced among adults with type 2 diabetes, even those who are obese (28). Meanwhile, our in vitro cell line study (unpublished data) also provided evidence that hyperglycemia can cause decreased production of Gas6 protein. These findings indicate that the Gas6/TAM system may have different stage-specific expression in the process leading from obesity to type 2 diabetes. We speculate that Gas6/TAM signaling may be activated in the early pathogenesis of obesity and its related comorbidity. However, once insulin resistance deteriorates and prediabetes or diabetes develops, GAS6/TAM signaling would be suppressed by hyperglycemia. Certainly, further longitudinal investigations are needed to clarify the pleiotropic effect of the Gas6/TAM system on metabolic diseases among children and adults.

Recently, Scroyen and colleagues (43) demonstrated that single Axl receptor deficiency had no significant effect on adipogenesis or adipose tissue development in mice because Axl deficiency can be partially compensated for by the other TAM family members (Tyro-3 and Mer) via Gas6. The findings clarified that Axl may not be the only TAM receptor through which Gas6 could modulate adipogenesis. It also partially explains our finding that there were no significant relationships between circulating sAxl levels and adiposity after adjusting for other confounding variables. Several studies demonstrated that plasma sAxl levels are correlated with inflammatory markers (such as hs-CRP, IL-6, and TNF-α) and strongly related to disease severity in sepsis, critical limb ischemia, and systemic lupus erythematosus (27, 39, 44). Interestingly, plasma sAxl levels among overweight/obese adolescents are higher than in lean adolescents. However, no direct correlation...
between plasma sAxl levels and circulating inflammatory makers was detected. The difference between our results and the previous observations may be explained by the unique nature of obesity-associated inflammation compared with other classical inflammatory paradigms (eg, infection and autoimmune disease). In addition, these findings also imply that sAxl may play 2 distinct roles in classical and obesity-associated inflammation. The potential relevant role of sAxl in obesity-associated inflammation has never been investigated and should be documented in future studies.

Despite these contributions, this study has certain limitations. First, because this was a cross-sectional study, interpretation of the results is limited. Therefore, it is difficult to prove causality or the direction of influence based on the findings. Further longitudinal studies are needed to confirm our results. Second, Tanner’s stage was not examined by our researchers but derived from a self-reported questionnaire, which may be prone to confounding effects. In addition, the data regarding cigarette smoking and alcohol consumption may be selectively underreported by adolescents because of concerns about punishment from their teachers or parents; hence, these results need to be interpreted with some caution.

In conclusion, our community-based study has demonstrated that overweight/obese adolescents have higher circulating Gas6 levels than do lean adolescents of comparable age and puberty status. Moreover, circulating Gas6 levels are positively correlated with body fat, inflammation severity, and insulin resistance status among overweight and obese adolescents. Our study provides the first clinical evidence demonstrating that circulating concentrations of Gas6 are closely associated with obesity and its related chronic inflammation and metabolic complications. The potential role of the Gas6/TAM system in the initiation of childhood obesity and obesity-associated complications deserves further attention.

Acknowledgments
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