Adipocytokine Levels in Children: Effects of Fatness and Training

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To investigate the effects of obesity and exercise training on plasma adipocytokines, a sample of 42 children (lean = 24, %BF = 17.8 ± 7.5%; obese = 18; %BF = 29.1 ± 9.3%; mean age = 12.4 ± 1.9 yrs), were divided into 4 age-matched for activity groups: lean inactive (n = 11), obese inactive (n = 9), lean active (n = 13) and obese active (n = 9). Active children participated in swimming training (≥1 year, ≥3 times/week, ≥1 h per session, covering a distance of 10,000–12,000 m per week). Obese individuals demonstrated greater visfatin levels (3.3 ± 1.3 ng/ml) than their lean counterparts (2.6 ± 1.1 ng/ml; p = .06) whereas adiponectin was significantly
lower in obese children (3.8 ± 1.9) than their lean counterparts (5.9 ± 2.7; \( p \leq .05 \)). Insulin and HOMA values were significantly greater in obese compared with lean children (\( p \leq .05 \)). Within obese individuals, active individuals had significantly lower visfatin levels (2.8 ± 1.2 ng/ml) compared with their inactive counterparts (3.8 ± 1.2 ng/ml; \( p \leq .05 \)). Resistin levels were comparable between groups (\( p > .05 \)). Childhood obesity elevates visfatin and lowers adiponectin levels whereas exercise training could reduce visfatin levels in obese children.

Obesity is a major global epidemic problem involving both children and adults. Sufficient epidemiological evidence indicates that overweight or obese children are at increased risk for becoming overweight or obese adults (24). The prevalence of childhood obesity has increased at an alarming rate in most western countries (6,25) and has been linked with diseases such as type 2 diabetes, dyslipidemia, hypertension, coronary heart disease and stroke that are typically seen in overweight and obese adults. Furthermore, one in four overweight children in the 6–12 yr age group has impaired glucose tolerance and 60% of these children have at least one risk factor for developing heart disease (4,5). It seems that cardiovascular disease risk factors tend to cluster in childhood and the metabolic cardiovascular syndrome is strongly associated with obesity even at this young age (12).

Adipose tissue is not considered a passive reservoir for energy storage anymore but a complex and highly active metabolic endocrine organ that secretes a large number of proteins that are collectively referred to as adipocytokines (20). Adipocytokines, i.e., leptin, adiponectin, resistin, visfatin, have numerous functions, including regulation of satiety, carbohydrate and lipid metabolism and insulin sensitivity. Visfatin is a newly discovered adipocytokine that is highly enriched in the visceral fat (37). Moreover, visfatin may play a role in the association between visceral obesity and increased metabolic syndrome since its levels are increased in obesity and type 2 diabetes (23). However, others found visfatin levels to be significantly lower in obese subjects compared with normal-weight controls (32). Similarly in children, some reports indicate that visfatin levels are elevated (2,17) whereas others suggested that visfatin is not likely to contribute significantly to the variation in BMI, glucose or insulin levels (23). To our knowledge there are only two studies on visfatin levels in children which reported a reduction in visfatin levels following aerobic exercise training in obese female adolescents (7,26).

Adiponectin appears to increase insulin sensitivity and possess anti-inflammatory and antiatherogenic properties (30). Plasma adiponectin levels are negatively correlated with obesity, insulin resistance and cardiovascular disease (8). Since exercise training improves insulin sensitivity and prevents type 2 diabetes development, might be speculated that exercise-induced improvement in insulin sensitivity may be mediated by circulating adiponectin (21). However, some authors reported no exercise effects on adiponectin levels in children (19,29).

Resistin is produced by white and brown adipose tissues and is elevated in obesity (1). It seems that resistin is involved in glucose homeostasis, lipid metabolism and insulin action (31). Based on its impact on insulin sensitivity, resistin has been proposed as a possible moderator of the pathogenic sequence associated with adipocyte-obesity-insulin resistance (22). Although previous studies link resistin with obesity and diabetes (40), exercise training has produced mixed results with some studies indicating no effect on resistin levels (18,39) while others report lower circulating resistin in overweight adolescents (18).
Due to dearth of a consensus on adipocytokines’ role on metabolism and their relationship to anthropometric and metabolic parameters in children, the purpose of the present investigation was to determine the effects of obesity and exercise training on plasma visfatin, adiponectin and resistin levels in children.

### Subjects and Methods

#### Volunteers

The total of 42 children (10 boys and 32 girls) volunteered. This sample (lean = 24; obese = 18; mean age= 12.4 ± 1.9 yrs, Table 1), was divided into 4 groups: lean inactive (LI, \(n = 11\)), obese inactive (OI, \(n = 9\)), according to the International Obesity Task Force criteria (10), lean active (LA, \(n = 13\)) and obese active (OA, \(n = 9\); Table 2). In the obese active group children that had BMI over their age- and sex-specific 95th percentile values and were engaged in systematic training were included. BMI percentile ranged from 13 to 77 for the lean inactive group and from 32 to 77 for the lean active group. Age ranged from 10 to 15 years old. Pubertal development was assessed by physical examination according to Tanner criteria (41). Participants were at Tanner stages II-IV. More specifically, three children in the LI group were in stage II and eight children in stage III, three children in the OI group were in stage II and six in stage III, two children in the group LA were in stage II, ten in stage III and one in stage IV, and two children in the group OA were in stage II, six in stage III and one in stage IV. The participants in the active groups were engaged in systematic swimming training (≥1 year, ≥3 times/week, ≥1 hr per session, covering a distance of 10,000–12,000 m per week). Mean training years were 4.4 ± 1.2 yrs. No acute or chronic illness, or medication were declared. A written informed consent was provided by all guardians after they were informed of all risks, discomforts and benefits involved. The procedures were in accordance with the principles of the Bioethics Committees of Democritus University of Thrace and University of Thessaly, Greece.

#### Table 1  Age, Physical Characteristics and Biochemical Variables in Lean and Obese Children (Mean± SD)

<table>
<thead>
<tr>
<th></th>
<th>Lean ((n = 24))</th>
<th>Obese ((n = 18))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>12.4 ± 2.2</td>
<td>12.3 ± 1.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>46.8 ± 11.8</td>
<td>57.8 ± 11.2*</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.6 ± 0.1</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>BMI (kg·m(^{-2}))</td>
<td>18.8 ± 1.7</td>
<td>23.1 ± 2.3*</td>
</tr>
<tr>
<td>% Body Fat</td>
<td>17.8 ± 7.5</td>
<td>29.1 ± 9.3*</td>
</tr>
<tr>
<td>Pubertal Stage</td>
<td>2.8 ± 0.5</td>
<td>2.7 ± 0.5</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>0.6 ± 0.2</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Insulin (mIU/ml)</td>
<td>10.1 ± 3.5</td>
<td>12.3 ± 3.7*</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>4.6 ± 0.6</td>
<td>4.8 ± 0.5</td>
</tr>
<tr>
<td>HOMA</td>
<td>2.1 ± 0.8</td>
<td>2.6 ± 0.9*</td>
</tr>
</tbody>
</table>

*Significantly different from the Lean group
Table 2  Physical Characteristics and Biochemical Variables in Lean and Obese Inactive and Active Children (Mean± SD)

<table>
<thead>
<tr>
<th></th>
<th>Lean Inactive (n = 11)</th>
<th>Lean Active (n = 13)</th>
<th>Obese Inactive (n = 9)</th>
<th>Obese Active (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>11.3 ± 1.2</td>
<td>14.2 ± 0.8</td>
<td>11.0 ± 1.1</td>
<td>13.6 ± 0.9</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.4 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>46.5 ± 7.2</td>
<td>55.5 ± 6.3</td>
<td>49.0 ± 8.2</td>
<td>66.6 ± 5.2</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>20.2 ± 3.7</td>
<td>14.2 ± 7.6</td>
<td>32.4 ± 5.6</td>
<td>26.2 ± 10.7</td>
</tr>
<tr>
<td>PA (METS/day)</td>
<td>37.4 ± 1.6*§</td>
<td>47.3 ± 2.4</td>
<td>33.2 ± 2.1*§</td>
<td>47.4 ± 1.1</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>5.5 ± 2.2</td>
<td>6.3 ± 2.6</td>
<td>3.5 ± 2.2*</td>
<td>4.1 ± 2.2</td>
</tr>
<tr>
<td>Visfatin (ng/ml)</td>
<td>2.8 ± 1.0</td>
<td>2.5 ± 1.2</td>
<td>3.8 ± 1.2*§</td>
<td>2.8 ± 1.2</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>0.66 ± 0.2</td>
<td>0.56 ± 0.3</td>
<td>0.61 ± 0.2</td>
<td>0.60 ± 0.2</td>
</tr>
<tr>
<td>Insulin (μIU/ml)</td>
<td>10.9 ± 3.3</td>
<td>9.5 ± 3.9</td>
<td>12.6 ± 3.6*#</td>
<td>11.9 ± 3.6</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>4.5 ± 0.5</td>
<td>4.6 ± 0.6</td>
<td>4.9 ± 0.6</td>
<td>4.7 ± 0.6</td>
</tr>
<tr>
<td>HOMA</td>
<td>2.2 ± 0.8</td>
<td>1.9 ± 0.9</td>
<td>2.8 ± 0.8*#</td>
<td>2.5 ± 0.8</td>
</tr>
</tbody>
</table>

*Significantly different from the Lean Inactive group. §Significantly different from the Lean Active group. *Significantly different from the Obese Active group

Experimental Design

Volunteers visited the data collection venue during mornings and in a fasting state, where they underwent body composition assessment followed by blood taking conducted by a trained phlebotomist.

Body Composition Assessment

Body mass was measured to the nearest 0.5 Kg (Seca 714, Seca Vogel and Halke GmbH & Co, Germany) with children lightly dressed and barefooted. Standing height was measured to the nearest 0.5 cm (Seca 714, Seca Vogel and Halke GmbH & Co, Germany). Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Obesity was defined according to the cut-off points for BMI recommended by the International Obesity Task Force criteria (24). More specifically, children with BMI over their age- and sex-specific 95th percentile values were defined as obese children and those with BMI <85 percentiles were considered as lean. Percent body fat was assessed through bioelectrical impedance (TANITA TBF-521, UK).

Physical Activity Assessment

Participants completed 4-day physical activity questionnaires (2 school days and two weekend days) designed to determine self-reported physical activity levels for children aged 10 years and older (3). The questionnaire measures four dimensions
of physical activity: a) physical activity at school, b) sport at school, c) physical activity during leisure time and d) sport during leisure time. In addition the questionnaire provides an objective scoring system and measure of physical activity in terms of: a) average daily energy expenditure (Kcal · kg⁻¹ · day⁻¹), b) time spent on moderate physical activity, c) time spent on hard and very hard activity, and d) bouts of “huff” and “puff” activity. The final score assigns the responder to one of the following categories: inactive, moderately active and active. The reliability and validity of this questionnaire have been tested for 10–14 year-old children in Greece and have been reported as ICC = 0.70 (p < .01; reliability) and r = .62 (p < .01; validity; 3).

Blood Analysis
From each participant 10ml blood sample was drawn with the subject sitting in a relaxed state. A portion (1 ml) of the blood sample was used for the assessment of the hematologic parameters which were analyzed in a Sysmex K-1000 (TOA Electronics, Japan) autoanalyzer. The rest (9 ml) of the blood sample remained at room temperature for 30 min and centrifuged at 1370g for 10 min at 4 C. The plasma obtained was stored in multiple aliquots at -70 °C until assayed. The assessment of insulin, adiponectin, resistin, and visfatin was performed through ELISA Kits (Phoenix CO., USA). Glucose was analyzed by enzymic spectrophotometric method using a reagent kit from Zafiropoulos P., Greece. The intra-assay CV for insulin, adiponectin, resistin, visfatin and glucose were 5.0, 4.1, 5.4, 4.8 and 3.8, respectively. The homeostasis model assessment (HOMA) was used as a surrogate measure of insulin resistance and was calculated as fasting insulin (mIU·mL⁻¹) x fasting glucose (mmol·L⁻¹)/22.5 (27).

Data Analysis
Two-way (2 × 2) analyses of variance were calculated for differences in the biochemical indices as a function of BMI (lean, obese) and exercise (active, inactive). An independent t test was run to determine differences due to adiposity and between genders. The SPSS version 15.0 was used for all analyses (SPSS, Inc., Chicago, IL). Data are presented as mean ± SD. The level of significance was set at p < .05.

Results
BMI was significantly higher (p < .05) in the obese compared with children in the lean group (Table 1). Obese children demonstrated higher visfatin values that approached significance (p = .06) compared with their lean counterparts (Figure 1A). It was also noticed that within lean individuals there was no difference between active and nonactive, whereas within obese individuals, active individuals had lower scores than inactive (Table 2). Furthermore, within active individuals insulin levels and HOMA values were not different between obese and lean children, whereas within inactive individuals obese had greater scores than lean ones (p ≤ .05, Table 2). In addition, obese children demonstrated significantly lower adiponectin (Figure 1B) and elevated insulin and HOMA levels (p ≤ .05) compared with the lean ones (Table 1). No significant differences were observed between the two groups for
resistin and glucose (Table 1). Finally, no significant interactions were observed for the remaining variables between active and inactive children in the obese and lean groups. An independent $t$ test that was run to determine differences between genders revealed significant differences between boys and girls for adiponectin, height and percent body fat (Table 3).

**Discussion**

The main purpose of the current study was to investigate the effects of obesity and exercise training on plasma visfatin, adiponectin and resistin levels in children. The results revealed that two (visfatin and adiponectin) of the three adipocytokines

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**Figure 1** — Visfatin (A) and adiponectin (B) levels in obese and lean children. ‡ Significant difference compared with Lean, $p = .06$ for visfatin, * Significant difference compared with Lean, $p < .05$ for adiponectin
Assessed were different in obese compared with lean children. Furthermore, exercise training resulted in lower visfatin levels in the obese active group indicating that regular exercise can modulate its concentration. In addition, insulin resistance, as assessed by HOMA, appeared to be elevated in obese children mainly due to higher insulin levels. Finally, gender had an effect on adiponectin levels since girls had higher mean adiponectin levels compared with boys.

Visfatin is a novel adipocytokine expressed by visceral adipose tissue. Although the concept of visfatin as an adiposity-derived insulin-mimetic factor is compelling, visfatin improves glucose tolerance and might play a role in the development of obesity-associated insulin resistance and type 2 diabetes (39). The current results showed that visfatin levels were significantly elevated in the obese children as compared with the nonobese children. This is in line with available reports suggesting that increased visfatin concentration may be associated with increased BMI and insulin resistance in obese children (13). Visfatin has also been found to be positively associated with BMI and percent body fat (37). It might be argued, therefore, that increased adiposity elevates visfatin levels in childhood. Exercise training resulted in lower visfatin levels in OA group yielding values similar with the ones of the LA children. This is supported by earlier findings indicating that aerobic exercise training (12-wks, four 40- to 50-min sessions per week with an energy expenditure of 300–400 kcal/d), combined with weight loss, may reduce plasma visfatin (40%) and insulin-resistance levels (7). Similarly visfatin levels were found to be significantly reduced following a 12-week aerobic exercise program in obese female adolescent (26). Matching information involving adults is also available. A significant decrease in visfatin levels of healthy women has been reported after a 12-week aerobic exercise and weight training program (9) whereas an aerobic exercise program for 12 weeks resulted in lower visfatin levels in men

Table 3 Age, Physical Characteristics and Biochemical Variables in Boys and Girls (Mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>Boys (n = 10)</th>
<th>Girls (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>12.7 ± 1.6</td>
<td>12.3 ± 2.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>56.3 ± 13.5</td>
<td>50.0 ± 12.3</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.65 ± 0.15</td>
<td>1.55 ± 0.14*</td>
</tr>
<tr>
<td>BMI (kg·m²)</td>
<td>20.5 ± 2.3</td>
<td>20.7 ± 3.1</td>
</tr>
<tr>
<td>% Body Fat</td>
<td>12.3 ± 8.7</td>
<td>25.9 ± 8.0*</td>
</tr>
<tr>
<td>PA (METS/day)</td>
<td>41.7 ± 6.8</td>
<td>41.7 ± 6.4</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>3.6 ± 1.7</td>
<td>5.5 ± 2.7*</td>
</tr>
<tr>
<td>Visfatin (ng/ml)</td>
<td>2.6 ± 1.0</td>
<td>3.0 ± 1.2</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>0.56 ± 0.2</td>
<td>0.61 ± 0.2</td>
</tr>
<tr>
<td>Insulin (μIU/ml)</td>
<td>10.8 ± 3.5</td>
<td>11.1 ± 3.8</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>4.7 ± 0.4</td>
<td>4.6 ± 0.6</td>
</tr>
<tr>
<td>HOMA</td>
<td>2.3 ± 0.8</td>
<td>2.6 ± 0.9</td>
</tr>
</tbody>
</table>

*Significantly different from Boys
and women; these changes were significantly correlated with changes in insulin and glucose (14). The results from the current study are in agreement with the results from the adult studies and indicate that systematic swimming training may lower visfatin levels in children, possibly due to changes in adiposity levels.

Our results coincide with earlier findings whereby lower adiponectin levels were noted in obese children as compared with their lean counterparts (16). Likewise, Park et al. (33) reported that adiponectin levels were lower in obese children, concluding that adiponectin may function as a biomarker of the onset of metabolic syndrome in childhood obesity. Cruz et al. (11) described a strong association with HOMA-IR in children 8–16 yr with type 2 diabetes and concluded that high adiponectin levels predict a lower prevalence of type 2 diabetes. It has been also found that adiponectin levels are approximately 25% higher in healthy overweight youth, compared with those with the metabolic syndrome (38). We found further that exercise training did not result in significant differences between inactive and active children. This is in agreement with previous reports advocating that, without concomitant weight loss or changes in body composition, exercise may have little to no effect on adiponectin levels (19,29). It seems that exercise training alone does not contribute significantly toward an elevation of adiponectin levels and changes in body composition are required to modulate adiponectin levels.

The physiological role of resistin on obesity and insulin resistance is unclear. We showed that resistin levels were not significantly altered in obese children. This finding is in accordance with several studies in pediatric research (35–37). In contrast, Rubin et al. (35) reported increased levels of resistin in obese children which were related to insulin resistance. The latter findings warrant further investigation on this matter. In agreement with previous reports, exercise training in the current study did not result in alterations of resistin levels in lean and obese children. In Kelly et al. (19) study, both resistin and body weight of obese children remained unaffected by exercise training despite an increase in aerobic capacity. Similarly, no differences in resistin levels were reported between active and inactive girls (36). On the other hand Jones et al. reported reduced levels of resistin due to exercise training in adolescents (18). Further research will clarify the effects of training on resistin levels in children.

The prevalence of insulin resistance in the youth has witnessed a significant rise lately (15). Elevated HOMA levels in the obese children suggest that obesity is an important cause of peripheral insulin resistance which may modulate the metabolic profile even at that young age. The current results are in line with published reports since HOMA levels were significantly elevated in the obese children. Furthermore, research indicates that physical activity and exercise training can improve insulin resistance (28). Results from this study support the previous findings since the obese active children did not have significantly different insulin and HOMA levels compared with lean inactive and active children. Therefore, physical activity can have a positive influence on insulin resistance which might be modulated, in part, by changes in adipocytokine levels.

Gender analysis revealed that there were significant differences in adiponectin, height and body fat. Analysis of the results revealed that girls had higher mean adiponectin levels compared with boys. Our results coincide with the results from other studies which indicate that gender differences exist as far as adiponectin is concerned (34).
In conclusion, adipocytokines levels may be used as biomarkers to assess the obesity-related health problems. It has to be noted that one of the limitations of the study is the low sample size. This limitation may partly explain why some of the expected differences (e.g., visfatin) were not evident in a more clear way. Additional prospective studies with larger sample of children are needed to clarify the results of the current study.

References


