Reversal of Obesity-Related Hypoadiponectinemia by Lifestyle Intervention: A Controlled, Randomized Study in Obese Adolescents

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Context: Hypoadiponectinemia and chronic subclinical inflammation in adults are associated with the development of diabetes and cardiovascular disease. The potential relationship between adiponectin and inflammation and its modulation by lifestyle intervention in the pediatric obese population remain unclear.

Objectives: The objectives were to investigate in adolescents 1) the relationship between adiponectin and obesity-related inflammatory factors, C-reactive protein, and IL-6; and 2) the effect of a lifestyle intervention on adiponectin and whether these effects are related to changes in inflammatory factors.

Research Methods and Procedures: Twenty-one obese and age-matched lean adolescents (age, 14–18 yr; Tanner stage, ≥IV) were studied cross-sectionally. Fifteen obese adolescents also underwent a randomized, controlled physical activity–behavior–diet-based lifestyle intervention for 3 months. Associations among adiponectin, fat mass, insulin resistance, and inflammatory factors at baseline as well as after the intervention were assessed.

A DIPOSE TISSUE, ONCE considered a metabolically passive fuel depot for energy substrate and insulation, has recently become apparent as a metabolically active tissue. It secretes multiple proteins (collectively called adipokines) into the circulation, which play important roles in the modulation of various biological functions. Among the adipokines, adiponectin is an adipose-specific gene product (1). It is an atypical adipokine because in contrast to the dramatic increase in plasma levels of all other adipokines, the circulating concentration of adiponectin is, paradoxically, decreased in obesity (2). There is increasing evidence indicating that adiponectin is involved in the pathogenesis of type 2 diabetes (3), and its role as a link between adipose tissue and the vasculature is becoming more evident (4–6).

The decline in circulating levels of adiponectin has been shown to coincide with the onset of insulin resistance and the development of diabetes in rhesus monkeys (7). A relationship between insulin sensitivity and adiponectin levels and gene expression in both rodents and obese humans (8–10) has also been reported. Plasma adiponectin concentrations are also significantly lower in patients with coronary artery disease than in matched control subjects (11), suggesting a possible association of reduced adiponectin in cardiovascular disease (CVD) in adults (3, 12). In a recent nested case-control study, Pischon et al. (13) found an association between high plasma adiponectin concentrations and lower risk of myocardial infarction in men. Additionally, studies in adiponectin-deficient mice have shown that adiponectin plays a protective role against insulin resistance and atherosclerosis in vivo (14). Because the circulating concentration of adiponectin is reported to be low in obesity, type 2 diabetes, and CVD, its concentration has been considered as a biological link between obesity and these related disorders. The role of adiponectin and its relationship to proinflammatory factors such as C-reactive protein (CRP), IL-6, and fibrinogen still remain unclear in the pediatric population.

Previous studies in adults have shown improvements in adiponectin concentration after weight reduction in obese adults with and without diabetes and/or CVD (15, 16). Studies have also reported that improving insulin resistance and reducing insulin levels with insulin-sensitizing agents markedly increase adiponectin concentrations, even in the absence of or after adjustment of changes in body weight (17). Although the response of adiponectin to exercise and/or physical activity in adults is mixed (15, 18), there is little information on circulating levels of adiponectin and its modulation by lifestyle-only changes in the pediatric population. Therefore, given the increasing rates of obesity in

Results: Plasma adiponectin concentration was lower (P < 0.001) in the obese vs. age-matched lean adolescents. Significant inverse relationships were observed between adiponectin and inflammatory factors, insulinemia, insulin resistance, and fat mass. Intervention produced a 34% increase in adiponectin concentration (P = 0.0004) despite negligible weight loss but with reductions in fat mass, hyperinsulinenia, insulin resistance, and inflammatory factors (all P < 0.01).

Conclusions: The data suggest that in adolescents, obesity-related hypoadiponectinemia is associated with subclinical inflammation, and a short-term lifestyle intervention augments adiponectin concentrations. These effects appear to be related to reductions in fat mass and inflammatory factors. Based on our current understanding of adiponectin physiology, reversal of hypoadiponectinemia in obese adolescents may protect against risks for cardiovascular disease and diabetes. (J Clin Endocrinol Metab 90: 6192–6197, 2005)
children, the escalation in obesity-related complications including CVD and diabetes, the presence of a subclinical inflammation early in life, and the potential for adiponectin to be a cardioprotective and antiinflammatory agent, the purpose of the current study in adolescents was to examine: 1) the relationship among adiponectin, indices of obesity, and factors of inflammation; and 2) the impact of a 3-month lifestyle-only intervention program on adiponectin concentration in relation to insulin resistance and inflammatory factors in a controlled, randomized intervention study.

Subjects and Methods

A total of 21 subjects, 15 obese and six lean adolescents (Table 1), were enrolled for the study. The study subjects were not pair matched, but the two groups were similar in age and maturity stage (Tanner stage ≥ IV). Staging was assigned by physical examination by a pediatrician and/or nurse practitioner according to the criteria of Tanner for breast development and pubic hair in females and genital development and pubic hair in males. Exclusion criteria included use of β-adrenergic blockers or steroids, active participation in any exercise activity of at least 20 min two times per week or more, or any diet program, tobacco use, alcohol and factors of inflammation; and 2) the impact of a 3-month lifestyle-only intervention program on adiponectin concentration in relation to insulin resistance and inflammatory factors in a controlled, randomized intervention study.

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Protocol

The study protocol was approved by the Nemours Children’s Clinic Research Review Committee and Baptist Medical Center/Wolfson Children’s Hospital Institutional Review Board. After explaining the study procedures and protocol to the participants and their parents at the time of physical examination, informed written consents were obtained before the study began. After fulfillment of recruitment inclusion/exclusion criteria, all subjects were asked to not change their eating habits and to maintain a diet history for at least three days before the baseline study. They were admitted to the Clinical Research Center (CRC) at Wolfson Children’s Hospital on the evening of the day before the study. Height and weight were measured to calculate body mass index (BMI) (kilograms/meters²). All body composition measurements were performed under the same conditions. During their stay at the CRC, body composition was assessed by dual-energy x-ray absorptiometry using a Hologic QDR 4500-A instrument (Waltham, MA).

All biochemical measurements were performed in duplicate using plasma samples collected after an overnight fast. Plasma concentrations of adiponectin were determined by using RIA (Linco Laboratories, St. Charles, MO). Determination of the circulating concentrations of CRP was performed using a high-sensitivity assay based on particle-enhanced immunonephelometry (20, 21). IL-6 concentration was measured using the standard two-antibody ELISA (22) with commercial antibody pairs and recombinant standards (from Endogen, Boston, MA). The data on the intervention program of the study. They were not actively monitored during the intervention phase. The participants cooled down for approximately 5 min by slow walking. One of these physical activity sessions each week was monitored at the clinic, and the family (at least one parent) also participated in these monitored sessions. The other days, the family monitored the physical activity portion of the intervention. The physical activity regimen was supplemented with other lifestyle changes that included caloric restriction by exchanging high-calorie snacks with low-calorie, low-fat snacks, cutting down meal portions and frequency of snack consumption, limiting sugar-based carbonated drinks, and limiting the duration of TV watching. In this study, we did not objectively monitor the diet-related lifestyle changes.

Obese control subjects were in usual care and were given advice for physical activity and diet but were not included in the specific intervention program of the study. They were not actively monitored during the 3-month intervention period unlike the subjects in the intervention group. For all obese subjects, the biological measurement protocol was repeated at 3 months. The lean control subjects were studied only at baseline.

Statistical analysis

Data are presented as mean ± sem. Nonnormally distributed values of CRP concentration were transformed logarithmically. Differences at baseline were analyzed using an independent-sample t test and a one-way ANOVA. Stepwise multivariate regression analysis with adiponectin as dependent variable was performed to identify the optimum model. Regression analysis with a step-type backward elimination procedure was used to determine the relative contribution of statistically significant predictors of adiponectin concentration. The data on the intervention studies were analyzed by Student’s t test and ANOVA for repeated measures. The level of significance was set at P < 0.05 for all analyses.

### TABLE 1. Physical characteristics of the subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Lean Age (yr)</th>
<th>Lean BMI (kg/m²)</th>
<th>Lean Body fat (%)</th>
<th>Obese Age (yr)</th>
<th>Obese BMI (kg/m²)</th>
<th>Obese Body fat (%)</th>
<th>Significance (lean vs. obese)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 (3 M + 3 F)</td>
<td>16.0 ± 0.4</td>
<td>21.3 ± 1.2</td>
<td>7 (4 M + 3 F)</td>
<td>15.9 ± 0.5</td>
<td>41.2 ± 4.2</td>
<td>22.5 ± 2.1</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>15.9 ± 0.5</td>
<td>21.3 ± 1.2</td>
<td>41.2 ± 4.2</td>
<td>15.6 ± 0.3</td>
<td>38.1 ± 3.1</td>
<td>45.5 ± 2.3</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are means ± sem. M, Male; F, Female; NS, not significant.
*P values, unpaired two-way test. The two obese groups (control and intervention) were not different. P values were considered for the lean group, and the two obese groups (obese control group and obese intervention group) were considered together.
All probability values are two-tailed. The calculations were performed using a standard statistical package, either Microsoft Excel or the SAS program.

**Results**

**Baseline data**

Table 1 shows the physical characteristics of the study subjects. We considered each group separately and analyzed the data. However, because the two obese groups were similar in all parameters they were considered as one obese group for baseline comparisons with the lean group (19, 20). Adiponectin concentration was significantly lower in the obese group in comparison with that in the lean control group (Fig. 1A; \( P < 0.001 \)). The adiponectin concentration in the lean group was 7.7 ± 0.6 mg/liter, whereas that in the obese group was 4.9 ± 0.4 mg/liter. Although adiponectin levels in females (5.10 ± 0.49 mg/liter) tended to be higher than those in males (4.23 ± 0.47 mg/liter), these differences did not reach statistical significance (\( P > 0.05 \)). Table 2 shows the association among body composition parameters, insulin concentration, HOMA-IR, the atherogenic potential (represented as LDL-C/HDL-C), and adiponectin. The relationships among adiponectin and factors of inflammation (IL-6 and CRP) at baseline are highlighted in Fig. 1, B and C.

Step-type multiple regression analysis based on a backward elimination criterion showed that, at a significance level of 0.05, the best-fitting model involved log CRP. About 43% of the variance in adiponectin between lean and obese subjects was explained by log CRP (\( R^2 = 0.43; F = 13.39; P = 0.001 \)). The lean control group was studied only once at baseline, and the data were used only for baseline comparison purposes.

**Intervention study data**

Figure 2 shows the changes in adiponectin concentration before and after intervention (Fig. 2A) and in controls (Fig. 2B), respectively. A mean increase of 34% in adiponectin concentration (from 4.44 ± 0.47 to 5.95 ± 0.49 mg/liter; \( P = 0.0002 \)) was observed in response to the lifestyle intervention. The adiponectin concentration showed a tendency to decrease (from 4.61 ± 0.67 to 4.11 ± 0.69 mg/liter; \( P = 0.07 \)) in the obese control group (mean decrease = 6.6%; \( P = 0.07 \)) after 3 months of usual care. The intervention produced a significant decrease in percent body fat (before, 45.5 ± 2.3 kg, and after, 39.2 ± 2.3 kg; \( P = 0.002 \)) and increase in percent bone-free lean mass (before, 51.9 ± 2.2, and after, 58.3 ± 2.2 kg; \( P < 0.05 \)). No apparent changes in body weight (before, 105.7 ± 5.2 kg, and after, 104.5 ± 5.3 kg; \( P = 0.15 \)) and/or BMI (before, 38.1 ± 2.09 kg/m\(^2\), and after, 37.5 ± 3.1 kg/m\(^2\); \( P = 0.13 \)) were observed. However, between-group (intervention vs. control) comparison showed a significant benefit (\( P <

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**TABLE 2.** Baseline studies: univariate correlation (r) among body fatness, insulin, HOMA-IR, atherogenic potential, IL-6, and circulating concentration of adiponectin

<table>
<thead>
<tr>
<th></th>
<th>Adiponectin (r)</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>BMI</td>
<td>−0.63</td>
<td>0.002</td>
</tr>
<tr>
<td>% Body fat</td>
<td>−0.62</td>
<td>0.002</td>
</tr>
<tr>
<td>Trunk fat mass</td>
<td>−0.65</td>
<td>0.001</td>
</tr>
<tr>
<td>% Nonbone lean mass</td>
<td>0.54</td>
<td>0.013</td>
</tr>
<tr>
<td>Insulin</td>
<td>−0.41</td>
<td>0.067</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>−0.65</td>
<td>0.002</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>−0.59</td>
<td>0.005</td>
</tr>
<tr>
<td>IL-6</td>
<td>−0.63</td>
<td>0.001</td>
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</table>

LDL/HDL represents atherogenic potential.

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Fig. 1. A, Baseline adiponectin concentration (milligrams/liter) in lean and obese adolescents. B and C, Relationship between adiponectin and log CRP and IL-6, respectively, in adolescents. Correlation coefficient (r) and P values are included in the figures. ×, Lean subjects; ○ and ●, obese intervention and control groups, respectively.
The current study shows that a modest lifestyle-only intervention increased adiponectin concentration in obese adolescents even without noticeable reduction in body weight and/or BMI. Although a direct role of adiponectin as an antiinflammatory agent is not apparent from this study, the facts that adiponectin and factors of inflammation are negatively correlated and lifestyle intervention induces an up-regulation of adiponectin concentration with concomitant but opposite changes in inflammatory factors suggest a potential regulatory role for adiponectin in the obesity-related subclinical inflammation present in adolescents.

Although the obesity–inflammation relationships in adults and children have been addressed by various previous studies (20, 25–27), the negative association between adiponectin concentration and inflammatory factors (such as CRP and IL-6) shown in this study is novel and intriguing. The relationship between adiponectin and the ratio of LDL to HDL, a measure of atherogenic potential (23), in the current study indicates that lower concentrations of adiponectin in the obese group compared with that in the lean group may contribute to a worsening of the lipoprotein characteristic of insulin-resistant obesity in adolescents. In agreement with previous studies (28–31), the data from the current study also show significant negative associations between adiponectin concentration and indices of obesity as well as HOMA-IR. Collectively, the baseline studies suggest a compromised or aggravated vascular defense in obese adolescents, and it appears that adiponectin plays a major role in the adipovascular axis. It is unclear whether hypoadiponectinemia at an early age in obese adolescents contributes directly to the future development of diabetes and/or CVD. Of note, hypoadiponectinemia was not only observed in obesity, type 2 diabetes, and CVD in both animal and human studies (3, 7, 11, 28, 32, 33) but has also been shown to predict cardiovascular events years in advance in a population without diagnosed CVD as well (13).

The apparent increase in adiponectin concentration in response to a moderate lifestyle-only intervention in obese adolescents with only negligible reductions in body weight and/or BMI in the current study is fascinating. This is contrary to some reports (15) but in agreement with studies showing an increase in adiponectin concentration in adults in response to moderate exercise only (18). A recent study has also shown an increase in adiponectin concentration with significant weight reduction in obese children (29). Of note, although the obese subjects in the current study did not show any apparent decrease in body weight and/or BMI, between-group comparisons after 3 months showed small but significant decreases in body weight and BMI. The percent body fat showed a substantial reduction, and percent bone-free lean mass (dual-energy x-ray absorptiometry) showed an increase. It also appears that the reduction in fat mass is predominantly from the trunk region. Because adiponectin is an adipose-specific molecule, the increase in its concentration in response to the intervention in the current study may, in part, be related to the reduction in fat mass. The decrease in fat mass and inflammatory factors (CRP and IL-6) before and after lifestyle intervention was also correlated with the restoration of adiponectin concentration relative to baseline values (Table 3). Gains in lean mass may also have contributed to the attenuation of insulin resistance and compensatory hyperinsulinemia.

### Table 3. Intervention studies: univariate correlation (r) between Δ increase in adiponectin and Δ changes in body fatness and circulating levels of inflammatory factors (CRP and IL-6)

| % Body fat | 0.62 |
| Trunk fat (DEXA) | 0.51 |
| CRP | 0.59 |
| IL-6 | 0.43 |

Δ, Change (increase or decrease).

<table>
<thead>
<tr>
<th>r&lt;sup&gt;a&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>P &lt; 0.05.</td>
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</table>
The results of step-type multiple regression analysis for determining an optimal model in the current study indicates that the circulating concentration of CRP is an important factor in the variance in adiponectin concentration and that adiponectin may play a protective role against CVD through reduction in subclinical inflammation in obese adolescents. These results imply that adiponectin may have a direct effect on obesity-related subclinical inflammation or vice versa in adolescents. It is unclear whether the inverse relationship between adiponectin and inflammatory factors and/or insulin resistance found in the current study is one of direct cause and effect. It is possible that these relationships may be mediated in part by insulin levels, by proinflammatory cytokines, or by changes in clearance of adiponectin. Evidently, future studies should address these issues more closely, and they need to be independently verified.

An important limitation of the current study is the less sophisticated measure of insulin sensitivity/resistance. The study was part of an investigation to understand the effect of lifestyle changes on protein metabolism in obese adolescents (19), and it involved the continuous infusion of stable isotopically labeled leucine and frequent blood sampling for 5 h under postabsorptive conditions. Because it required iv infusions and frequent blood sampling over at least an extra 3-h period, we could not perform the glucose clamp studies for directly quantifying insulin sensitivity/resistance in vivo. Nevertheless, it is likely that measurement of insulin sensitivity by direct methods would have, perhaps, produced better relationships between adiponectin and insulin sensitivity.

In conclusion, the present study suggests that obesity-related hypoadiponectinemia is associated with subclinical inflammation in adolescents and is reversible, at least in part, by a modest lifestyle intervention. Lifestyle-induced augmentation of adiponectin concentration along with attenuation of subclinical inflammation has important clinical implications and potentially provides a cardioprotective effect in the obese adolescents. Longer-term studies are needed to show whether the improvement observed in adiponectin concentration will eventually translate into a significant clinical benefit in regard to delaying and/or reversing diabetes and cardiovascular morbidity and mortality.

Acknowledgments
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