

Adiponectin in Youth

Relationship to visceral adiposity, insulin sensitivity, and β -cell function

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OBJECTIVE — Adiponectin is an adipose tissue protein that enhances insulin sensitivity and has antiatherogenic properties. The present study investigated the relationship of adiponectin levels in adolescents to 1) obesity and body fat distribution and 2) insulin sensitivity and the components of syndrome X.

RESEARCH DESIGN AND METHODS — Twenty-three normal-weight and 26 obese adolescents had fasting adiponectin, lipid profile, and proinsulin measurements performed. Hepatic and peripheral insulin sensitivity were assessed with constant-rate [6,6-²H₂]glucose infusion and a 3-h hyperinsulinemic-euglycemic clamp, respectively. Body composition was evaluated by dual-energy X-ray absorptiometry, and visceral adipose tissue (VAT) and subcutaneous adipose tissue were measured by computed tomography scan at the L₄-L₅ level.

RESULTS — Obese adolescents had ~50% lower adiponectin than normal-weight adolescents. Moreover, obese adolescents with high ($111.8 \pm 9.3 \text{ cm}^2$) versus low ($55.4 \pm 2.1 \text{ cm}^2$) VAT had lower adiponectin levels (6.2 ± 0.9 vs. $9.0 \pm 1.0 \text{ } \mu\text{g/ml}$, $P = 0.05$). Plasma adiponectin correlated positively with peripheral and hepatic insulin sensitivity ($r = 0.67$, $P < 0.001$ and $r = 0.54$, $P < 0.001$, respectively) and HDL ($r = 0.52$, $P < 0.001$) and negatively with fasting proinsulin and the proinsulin-to-insulin ratio ($r = -0.64$, $P < 0.001$ and $r = -0.43$, $P = 0.003$, respectively). In a multiple regression analysis, adiponectin, independently and together with BMI, explained 73% ($R^2 = 0.73$, $P < 0.001$) of the variance in insulin sensitivity. Adiponectin, but not adiposity, was the significant independent determinant of the proinsulin-to-insulin ratio ($R^2 = 0.18$, $P = 0.008$) and of HDL ($R^2 = 0.45$, $P < 0.001$).

CONCLUSIONS — In summary, hypo adiponectinemia in youth is a strong and independent correlate of insulin resistance, β -cell dysfunction, visceral adiposity, and syndrome X. The antidiabetogenic and antiatherogenic properties of adiponectin are evident early in life and compromised in youth-onset obesity.

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Adiponectin is an adipocytokine that is exclusively expressed and secreted from adipose tissue (1,2). Its levels are low in obesity (3,4) and increase after weight loss (5). Its role as a protective adipokine is suggested by low levels in states of insulin resistance, cardiovascular disease, and type 2 diabetes (4). In addition, it is believed to have anti-inflammatory and antiatherogenic properties (6). These studies imply that

adiponectin has an important role in the regulation of glucose metabolism and insulin resistance and that it might be involved in the pathogenesis of other components of syndrome X, including dyslipidemia and elevated blood pressure.

Given the increasing rates of obesity and its comorbidities in children (7), we hypothesized that the negative impact of low adiponectin levels on insulin sensitiv-

ity and the components of syndrome X could be a marker of youth-onset obesity. Therefore, the present study examined the relationship of adiponectin to 1) obesity and body fat distribution and 2) insulin sensitivity and the components of syndrome X in adolescents.

RESEARCH DESIGN AND METHODS

Twenty-three normal-weight and 26 obese but otherwise healthy white adolescents were studied, some of whom have been previously reported (8). The studies were approved by the Human Rights Committee of the Children's Hospital of Pittsburgh. Study participants were recruited through newspaper advertisements. Parental informed consent and child assent were obtained after a detailed explanation of the study was provided. Clinical characteristics of the study subjects are summarized in Table 1. All subjects were at Tanner stage II to V of puberty, as assessed by physical examination and confirmed by measurement of plasma testosterone in males, estradiol in females, and dehydroepiandrosterone sulfate in all (Table 1).

Clamp studies

Each participant underwent a hyperinsulinemic-euglycemic clamp after 10–12 h of overnight fasting. Fasting endogenous glucose production was measured with a primed constant-rate infusion of [6,6-²H₂]glucose ($0.198 \pm 0.008 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in normal-weight and $0.306 \pm 0.009 \text{ } \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in obese adolescents) (Isotech, Miamisburg, OH) from 7:30 to 9:30 A.M. (9,10). Blood was sampled at the start of the stable isotope infusion (–120 min) and every 10 min from –30 min to time zero (basal period) for determination of plasma glucose, insulin, and isotopic enrichment of glucose. Blood was sampled for fasting proinsulin at –30 and –10 min. Fasting turnover calculations were made during the last 30 min (–30 to time zero) of the basal 2-h infusion period. After the 2-h baseline period, insulin-mediated glucose metabolism and insulin sensitivity were evaluated during a 3-h hyperinsulinemic-euglycemic

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Abbreviations: DEXA, dual-energy X-ray absorptiometry; HGP, hepatic glucose production; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

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Table 1—Study subjects

	Normal weight	Obese	P
n (M/F)	23 (12/11)	26 (14/12)	
Age (years)	12.5 ± 0.4	13.3 ± 0.4	NS
Tanner			
II to III	16	16	
IV to V	7	10	
BMI (kg/m ²)	20.3 ± 0.8	35.2 ± 1.0	<0.001
Fat mass (kg)	11.2 ± 1.6	37.1 ± 1.7	<0.001
Percent body fat	22.4 ± 2.2	43.4 ± 1.2	<0.001
Total abdominal adipose tissue (cm ²)	151.4 ± 30.0	630.3 ± 32.4	<0.001
SAT (cm ²)	126.6 ± 26.0	542.1 ± 29.0	<0.001
VAT (cm ²)	24.8 ± 4.2	88.3 ± 7.9	<0.001
DHEAS (μmol/l)	2.9 ± 0.4	4.3 ± 0.5	NS
Estradiol (pmol/l)*	125.9 ± 19.8	275.3 ± 84.1	NS
Free testosterone (nmol/l)†	0.23 ± 0.05	0.16 ± 0.03	NS

Data are means ± SE. *Estradiol in females only; †free testosterone in males only. DHEAS, dehydroepiandrosterone sulfate.

clamp from 9:30 A.M. to 12:30 P.M. (9,10). Intravenous crystalline insulin (Humulin; Lilly, Indianapolis, IN) was infused at a constant rate of 40 mU · m⁻² · min in normal-weight and 80 mU · m⁻² · min in obese adolescents, as previously described by us (9,10) to suppress hepatic glucose production (HGP) during the clamp. Plasma glucose was clamped at 5.6 mmol/l with a variable-rate infusion of 20% dextrose based on arterialized plasma glucose determinations every 5 min. Fasting blood was obtained for determination of lipid profile, HbA_{1c}, and adiponectin.

Body composition

Body composition was determined by dual-energy X-ray absorptiometry (DEXA). Subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) were examined by a single-slice computed tomography scan at intervertebral space L₄-L₅, as we have previously described (10). Blood pressure was measured between 10:00 to 11:00 P.M. and 6:00 to 7:00 A.M., when, as before, the subjects were resting in the supine position in bed (9).

Biochemical measurements

Adiponectin (μg/ml) was measured using a commercially available radioimmunoassay kit (Linco Research). The intra- and interassay coefficients of variation were 3.6 and 9.3% for low and 1.8 and 9.3%, respectively, for high serum concentrations.

Plasma glucose was measured by the glucose oxidase method (Yellow Springs Instrument, Yellow Springs, OH), and the insulin concentration was determined by radioimmunoassay (10). HbA_{1c} was measured by high-performance liquid chromatography (A1c 2.2 Plus Glycohemoglobin Analyzer; Tosoh Medics). Plasma lipid levels were measured using the standards of the Centers for Disease Control and Prevention (9). Deuterium enrichment of glucose in the plasma was determined on a Hewlett-

Packard 5971 mass spectrometer (Hewlett-Packard, Palo Alto, CA) coupled with a 5890 series II gas chromatograph, as reported (9,10).

Fasting HGP was calculated during the last 30 min of the fasting 2-h isotope infusion period according to steady-state tracer dilution equations previously reported by us (9,10). In the fasting state, an index of hepatic insulin sensitivity was calculated as the inverse of the product of HGP and the fasting plasma insulin concentration (1,000/HGP × fasting plasma insulin), as described by Miyazaki et al. (11). Insulin-stimulated glucose disposal was calculated, during the last 30 min of the euglycemic clamp, to be equal to the rate of exogenous glucose infusion. Peripheral insulin sensitivity was calculated by dividing the glucose disposal rate by the steady-state clamp insulin level, as reported previously (9,12).

Statistical analyses

Statistical analyses were performed using Student's *t* test for two-group comparisons. Pearson or Spearman correlation analysis was used when applicable to examine bivariate relationships. To evaluate multivariate relationships, multiple regression analysis was applied. All statistical assumptions were met. Data are presented as means ± SE. *P* value ≤ 0.05 was considered statistically significant. Data on visceral adiposity were missing in

Table 2—Metabolic profile of normal-weight and obese white adolescents

	Normal weight	Obese	P
Fasting glucose (mmol/l)	5.4 ± 0.1	5.4 ± 0.1	NS
Fasting insulin (pmol/l)	122.4 ± 9.9	250.2 ± 18.0	<0.001
Adiponectin (μg/ml)	14.1 ± 1.2	7.3 ± 0.7	<.001
HGP (μmol/kg/min)	16.8 ± 0.6	13.8 ± 0.8	0.006
Hepatic insulin sensitivity (μmol · kg ⁻¹ · min ⁻¹ per pmol/l) ⁻¹	0.56 ± 0.04	0.34 ± 0.02	<0.001
Peripheral insulin sensitivity (μmol · kg ⁻¹ · min ⁻¹ per pmol/l)	8.8 ± 0.8	2.1 ± 0.2	<.001
Proinsulin-to-insulin ratio	0.14 ± 0.02	0.19 ± 0.02	.032
Cholesterol (mmol/l)	4.09 ± 0.17	4.68 ± 0.15	0.011
Triglycerides (mmol/l)	1.03 ± 0.09	1.63 ± 0.16	0.002
LDL (mmol/l)	2.30 ± 0.13	2.87 ± 0.13	0.004
HDL (mmol/l)	1.32 ± 0.06	1.08 ± 0.04	0.001
Cholesterol/HDL	3.2 ± 0.1	4.5 ± 0.2	<0.001
Morning systolic blood pressure (mmHg)	104.5 ± 1.6	115.8 ± 2.6	0.001
Morning diastolic blood pressure (mmHg)	58.7 ± 1.4	63.7 ± 1.5	0.019

Data are means ± SE.

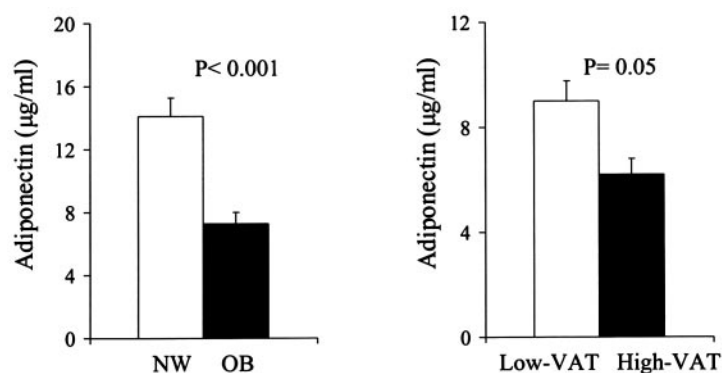


Figure 1—Fasting adiponectin concentrations in normal-weight (NW) and obese (OB) adolescents (left panel) and obese adolescents with low versus high VAT (right panel).

two obese subjects, one male and one female, and DEXA measurements were not possible in five subjects (four males and one female) with severe obesity whose weight exceeded the DEXA limit of 250 lbs.

RESULTS—The normal-weight and obese adolescents had similar age and pubertal stage distribution. The obese group had significantly higher BMI, fat mass, percent body fat, and abdominal adipose tissue (Table 1).

Fasting metabolic data and blood pressure

Both normal-weight and obese adolescents had normal fasting glucose and HbA_{1c} levels (5.14 ± 0.08 vs $5.03 \pm 0.09\%$). However, obese adolescents had significantly higher fasting insulin, proinsulin-to-insulin ratio, cholesterol, triglycerides, LDL, cholesterol-to-HDL ratio, and blood pressure and significantly lower HDL. HGP was lower in obese adolescents in the presence of higher fasting insulin levels (Table 2). Thus, hepatic insulin sensitivity was significantly lower. Adiponectin level was ~50% lower in obese adolescents (Fig. 1).

Visceral adiposity and adiponectin in obese adolescents

To assess the contribution of visceral adiposity to hypoadiponectinemia, the obese group was divided into low- and high-VAT groups. The low-VAT group had VAT within 2 SD above the mean and the high-VAT group had VAT >2 SD above the mean VAT of the normal-weight adolescents. The mean VAT +2 SD of normal-weight adolescents was 65.1 cm². Among the obese adolescents, there were 14 with high VAT ($111.8 \pm$

9.3 cm²) and 10 with low VAT (55.4 ± 2.6 cm², $P < 0.001$). The low- and the high-VAT groups did not differ significantly in BMI (34.0 ± 1.3 and 36.5 ± 1.9 kg/m²), fat mass (37.9 ± 3.2 and 37.7 ± 2.1 kg), and SAT (519.2 ± 39.2 and 558.4 ± 41.6 cm²). Adiponectin level, however, was lower in the high-VAT group (6.2 ± 0.9 vs. 9.0 ± 1.0 μg/ml, $P = 0.05$) (Fig. 1). There was no difference in adiponectin levels between males and females in the normal-weight group (15.6 ± 1.8 vs. 12.4 ± 1.3 μg/ml, $P = 0.2$), but in the obese adolescents, there was a tendency for adiponectin to be higher in females (9.1 ± 1.2 vs. 6.5 ± 0.8 μg/ml, $P = 0.07$). This could be related to the lower VAT in females than in males (68.6 ± 7.6 vs. 104.9 ± 11.5 cm², $P = 0.02$), despite similar total body fat mass (38.4 ± 2.3 vs. 35.7 ± 2.5 kg).

Peripheral insulin sensitivity

Plasma glucose during the last 30 min of the hyperinsulinemic-euglycemic clamp was similar in normal-weight and obese adolescents (5.6 ± 0.02 vs. 5.6 ± 0.03 mmol/l, respectively), while insulin concentration was higher in obese (638.7 ± 35.3 vs. $1,744.7 \pm 93.9$ pmol/l, $P < 0.001$). The rates of glucose disposal and insulin sensitivity were lower in obese adolescents (51.7 ± 3.9 vs. 32.7 ± 2.5 mmol · kg⁻¹ · min⁻¹, $P < 0.001$ and 8.8 ± 0.8 vs. 2.1 ± 0.2 μmol · kg⁻¹ · min⁻¹ per pmol/l, $P < 0.001$, respectively). In the obese group, insulin sensitivity was lower in the high- than low-VAT group (1.5 ± 0.1 vs. 2.6 ± 0.5 μmol · kg⁻¹ · min⁻¹ per pmol/l, $P = 0.03$).

Relationship of adiponectin to the components of syndrome X

Adiponectin levels correlated inversely with BMI ($r = -0.70$, $P < 0.001$), fat mass ($r = -0.73$, $P < 0.001$), percent body fat ($r = -0.66$, $P < 0.001$), VAT ($r = -0.67$, $P < 0.001$), and SAT ($r = -0.69$, $P < 0.001$). Adiponectin levels correlated positively with peripheral and hepatic insulin sensitivity ($r = 0.67$, $P < 0.001$ and $r = 0.54$, $P < 0.001$, respectively) and negatively with fasting insulin ($r = -0.66$, $P < 0.001$), proinsulin ($r = -0.64$, $P < 0.001$), and the proinsulin-to-insulin ratio ($r = -0.43$, $P = 0.003$) (Fig. 2). The positive relationship between adiponectin and peripheral insulin sensitivity persisted after controlling for percent body fat ($r = 0.48$, $P = 0.002$) and reached significance for hepatic insulin sensitivity ($r = 0.27$, $P = 0.098$). The inverse relationship between adiponectin and the proinsulin-to-insulin ratio persisted after controlling for percent body fat ($r = -0.33$, $P = 0.04$).

Adiponectin level correlated positively with HDL ($r = 0.52$, $P < 0.001$) and inversely with VLDL ($r = -0.47$, $P = 0.001$), cholesterol-to-HDL ratio ($r = -0.58$, $P < 0.001$), morning systolic ($r = -0.39$, $P = 0.01$) and diastolic ($r = -0.33$, $P = 0.03$) blood pressure, and evening systolic ($r = -0.43$, $P = 0.004$) and diastolic ($r = -0.34$, $P = 0.026$) blood pressure.

In a multiple regression analysis, with peripheral insulin sensitivity as the dependent variable, adiponectin independently (partial correlation 0.43, $P = 0.004$), and together with BMI, explained 73% of the variance in insulin sensitivity ($R^2 = 0.73$, $P < 0.001$ for both BMI and adiponectin in the model, and $R^2 = 0.67$, $P < 0.001$ for only BMI in the model). For hepatic insulin sensitivity, adiponectin ($P = 0.088$) and BMI ($P = 0.003$) together explained 44% ($R^2 = 0.44$, $P < 0.001$) of the variance. Adiponectin, but not BMI, was the significant independent determinant of the proinsulin-to-insulin ratio ($R^2 = 0.18$, $P = 0.008$) and HDL ($R^2 = 0.45$, $P < 0.001$). Adiponectin did not have a significant independent contribution to the variance in cholesterol, VLDL, or blood pressure. BMI and not adiponectin was a significant independent determinant of LDL ($R^2 = 0.19$, $P = 0.03$) and systolic ($R^2 = 0.39$, $P < 0.001$) and diastolic ($R^2 = 0.16$, $P = 0.049$) blood pressure.

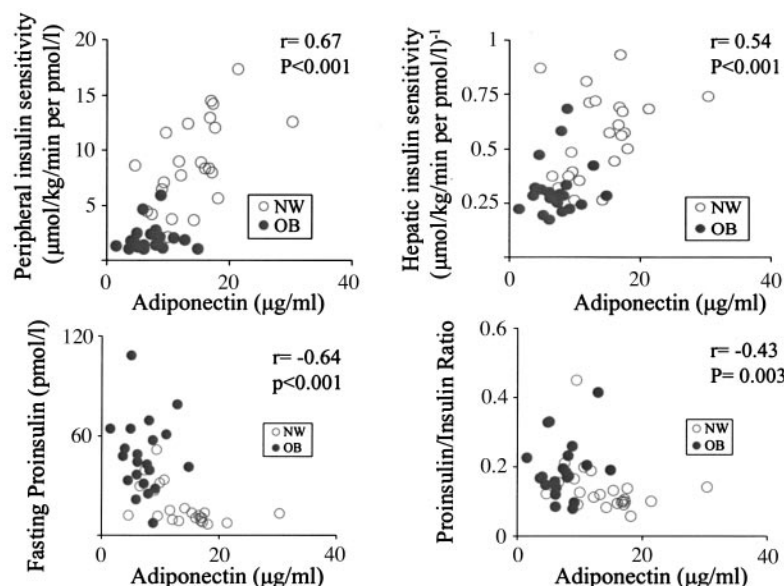


Figure 2—The relationship between adiponectin concentration and peripheral insulin sensitivity (upper left panel), hepatic insulin sensitivity (upper right panel), fasting proinsulin level (lower left panel), and proinsulin-to-insulin ratio (lower right panel) in normal-weight (NW) and obese (OB) adolescents.

CONCLUSIONS—Adiponectin is an adipocyte-specific plasma protein that modulates insulin resistance, dyslipidemia, and cardiovascular disease (13,14). The present study advances previous observations by demonstrating that 1) not only obesity, but particularly visceral adiposity, is associated with low adiponectin levels, 2) adiponectin is an important determinant of insulin sensitivity and HDL levels in children, and 3) adiponectin concentrations have an inverse relationship to the proinsulin-to-insulin ratio, a marker of β -cell failure, an observation that has not been investigated in the past.

In the adult literature, adiponectin levels have been reported to correlate inversely with BMI (3,13) and percent body fat (4,14) and to increase with weight loss (5). In Pima Indian children, plasma adiponectin concentrations inversely correlated with BMI and percent body fat and decreased with increasing adiposity over time (15). Similarly, in Hispanic and Asian-American children, adiponectin correlated inversely with BMI and fat mass (16). In a study of obese and non-obese adolescents, adiponectin levels were inversely related to percent body fat and muscle lipid content (17). The present study extends these findings by demonstrating that not only total body adiposity but also body fat topography is related to

alterations in adiponectin levels in youth. More specifically, our results demonstrate that in obese adolescents, visceral adiposity is associated with further decline in adiponectin levels. This is consistent with findings in adults showing an inverse relation between plasma adiponectin and waist-to-hip ratio (4,13,18). In agreement with these studies, *in vitro* data demonstrate that adiponectin secretion from human omental adipose cells correlates negatively with the subjects' BMI (19). Our results of lower adiponectin levels in visceraally obese adolescents differ from a prior publication where the authors found no correlation between adiponectin and visceral fat (17). This could be related to statistical power because our study has almost twice as many obese adolescents. Moreover, our study grouped the obese adolescents according to low and high VAT, whereas the prior study assessed correlations (17).

Our data also demonstrate that adiponectin is a strong and independent predictor of *in vivo* insulin sensitivity. Previous studies in Pima Indian, Hispanic, and Asian-American children demonstrated that plasma adiponectin levels correlate inversely with fasting insulin levels (15,16). Also, adiponectin levels were positively related to insulin-stimulated glucose metabolism in adolescents (17). In our study, adiponectin

levels correlated directly with both peripheral and hepatic insulin sensitivity. Moreover, in a multiple regression analysis, adiponectin independent of adiposity contributed to the variability in insulin sensitivity. This is consistent with the Pima Indian data showing that hypoadiponectinemia precedes the decline in insulin sensitivity independent of changes in percent body fat (14).

The observed inverse relationship between adiponectin and proinsulin-to-insulin ratio in the current study has not been previously reported in adult or pediatric subjects. Elevated proinsulin-to-insulin ratio is found in patients with type 2 diabetes (20) or impaired glucose tolerance (21) and correlates with the degree of islet cell dysfunction (22). Furthermore, increased proinsulin level is a marker of the future development of type 2 diabetes (23–25). Therefore, we postulate that hypoadiponectinemia may be used as a surrogate marker of β -cell dysfunction. In favor of such a postulate is the observation that in a prospective nested case-control study, low concentrations of adiponectin were associated with the future development of type 2 diabetes (26). Thus, hypoadiponectinemia may not only be a marker of insulin resistance but also β -cell dysfunction, both of which contribute to the pathophysiology of type 2 diabetes.

Adiponectin also has anti-inflammatory and antiatherogenic properties (6,27). Consistent with this, adiponectin levels are lower in patients with coronary artery disease (6,28). In diabetic and non-diabetic adults, as well as in children, adiponectin concentrations correlated inversely with triglycerides and positively with HDL levels (13,16,17,29). In our study, adiponectin levels correlated positively with HDL and inversely with triglycerides, VLDL, and cholesterol-to-HDL ratio. However, after controlling for adiposity, adiponectin remained to be a significant determinant of only HDL, one of the components of syndrome X.

In summary, the present study demonstrates that hypoadiponectinemia in youth is associated with 1) obesity, particularly visceral adiposity, 2) insulin resistance independent of adiposity, 3) β -cell dysfunction, reflected in elevated proinsulin-to-insulin ratio, and 4) some of the components of syndrome X. The low adiponectin levels observed in white adolescents with youth-onset obesity and

insulin resistance translates to an early loss of the antidiabetogenic and anti-atherogenic benefits of adiponectin. Thus, adiponectin may constitute an early biomarker to identify obese youth at high risk for the future development of diabetes and atherosclerosis.

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