

# Circulating Adiponectin Levels Are Reduced in Nonobese but Insulin-Resistant First-Degree Relatives of Type 2 Diabetic Patients

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**Adiponectin, one of the most abundant gene transcript proteins in human fat cells, has been shown to improve insulin action and is also suggested to exert antiatherogenic effects. We measured circulating adiponectin levels and risk factors for atherosclerosis in 45 healthy first-degree relatives of type 2 diabetic subjects (FDR) as well as 40 healthy control subjects (CON) without a known family history of diabetes. Insulin sensitivity ( $S_i$ ) was studied with the minimal model, and measurements of adiponectin, metabolic variables, inflammatory markers, and endothelial injury markers, as well as lipoprotein concentrations, were performed. FDR were insulin resistant ( $3.3 \pm 2.4$  vs.  $4.5 \pm 2.6 \times 10^{-4} \times \text{min}^{-1}$  per  $\mu\text{U/ml}$  [mean  $\pm$  SD],  $P < 0.01$ ), and their circulating plasma adiponectin levels ( $6.6 \pm 1.8$  vs.  $8.1 \pm 3.0 \mu\text{g/ml}$ ,  $P < 0.03$ ) were decreased. After adjustments for age in FDR, adiponectin levels were negatively correlated with fasting proinsulin ( $r -0.64$ ,  $P < 0.001$ ), plasminogen activator inhibitor (PAI)-1 activity ( $r -0.56$ ,  $P < 0.001$ ), fasting insulin ( $r -0.55$ ,  $P < 0.001$ ), and acute insulin response ( $r -0.40$ ,  $P < 0.05$ ); they were positively related to HDL cholesterol ( $r 0.48$ ,  $P < 0.01$ ) and  $S_i$  ( $r 0.41$ ,  $P < 0.01$ ). Furthermore, when adjusted for age, waist, and  $S_i$ , adiponectin was associated with HDL cholesterol and proinsulin, which explained 51% of the variation in adiponectin in multiple regression analyses in that group. In conclusion, circulating plasma adiponectin levels were decreased in nonobese but insulin-resistant FDR and, in addition, related to several facets of the insulin resistance syndrome (IRS). Thus, hypoadiponectinemia may be an important component of the association between cardiovascular disease and IRS. *Diabetes* 52:1182–1186, 2003**

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CON, control subjects; CRP, C-reactive protein; CVD, cardiovascular disease; FDR, first-degree relatives of type 2 diabetic subjects; IRS, insulin resistance syndrome; OGTT, oral glucose tolerance test; PAI, plasminogen activator inhibitor; PPAR, peroxisome proliferator-activated receptor;  $S_i$ , insulin sensitivity index; TNF, tumor necrosis factor.

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**F**orty percent of newly diagnosed type 2 diabetic patients exhibit macrovascular disease (1). Furthermore, obesity is a major risk factor for type 2 diabetes and cardiovascular disease (CVD). Adipose tissue was recently shown to express and secrete different hormones, cytokines, and metabolites that may play a role in the development of insulin resistance and atherosclerosis (2,3). These include tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-6, plasminogen activator inhibitor (PAI)-1, angiotensin II, leptin, and complement C3 (4).

A novel adipose-specific protein, adiponectin or Acrp30, was independently described by several groups (5–7). In humans, adiponectin is one of the most abundant gene transcript proteins in adipose cells, corresponding to ~0.01% of all proteins (8). Previous work demonstrated that insulin-resistant individuals with obesity, type 2 diabetes, or coronary artery disease have low adiponectin concentrations (9). However, when insulin action was enhanced by thiazolidinediones, synthetic ligands of the peroxisome proliferator-activated receptor (PPAR)- $\gamma$  receptor, adiponectin levels increased (10,11). Furthermore, adiponectin may be protective from the initiation of atherosclerotic lesions in human endothelial aortic cells, since adiponectin attenuates the expression of cellular adhesion molecules (12).

Recently, a close association between adiponectin levels and insulin sensitivity, obesity, serum triglycerides, and lipoprotein levels was shown in humans (13–17). In this study, our aim was to compare circulating plasma adiponectin levels and metabolic risk factors in first-degree relatives of type 2 diabetic patients (FDR) with those found in healthy control subjects (CON). Furthermore, the relationship between circulating adiponectin levels and risk factors for CVD was evaluated in this group.

## RESEARCH DESIGN AND METHODS

Forty-five FDR were recruited by advertisements in local newspapers and via questionnaires, and 40 CON were randomly selected among men in the county council register for Göteborg. Inclusion criteria were subjects with two first-degree relatives or one first-degree relative and two second-degree relatives with type 2 diabetes (grandparents, uncle, or aunt); male sex (to exclude variation in insulin sensitivity during the menstrual cycle); normal glucose tolerance; and no evidence of hypertension, endocrine disease, or obesity (BMI  $>30 \text{ kg/m}^2$ ) (Table 1). The control group consisted of subjects who did not have a known family history of diabetes but fulfilled the remaining criteria. FDR and CON were similar with respect to age ( $43 \pm 9$  vs.  $45 \pm 7$  years), cigarette smoking ( $3.7 \pm 6.8$  vs.  $5.7 \pm 10.9$  pack-years), and use

TABLE 1

Basic characteristics, metabolic variables, lipoprotein concentrations, inflammatory markers, endothelial injury markers, and other markers in relatives and control subjects

	Relatives	Control subjects	<i>P</i>
Sex	Male ( <i>n</i> = 45)	Male ( <i>n</i> = 40)	
Age (years)	43.1 ± 8.6	45.1 ± 6.9	
BMI (kg/m <sup>2</sup> )	25.8 ± 2.6	24.6 ± 2.6	<0.05
Waist circumference (cm)	94 ± 0.9	90 ± 0.9	<0.05
Body fat (kg)	19.5 ± 6.1	16.5 ± 6.6	<0.03
Blood pressure			
Systolic (mmHg)	128 ± 16	118 ± 9	<0.01
Diastolic (mmHg)	76 ± 6	73 ± 7	<0.05
Oral glucose tolerance test			
Fasting plasma proinsulin (pmol/l)	11 ± 7	9 ± 6	
Fasting plasma glucose (mmol/l)	4.9 ± 0.5	4.4 ± 0.3	<0.0001
Fasting plasma insulin (pmol/l)	54 ± 24	48 ± 30	
Plasma glucose at 120 min (mmol/l)	5.0 ± 1.2	4.0 ± 1.1	<0.0001
Plasma insulin at 120 min (pmol/l)	246 ± 174	168 ± 126	<0.05
<i>S<sub>i</sub></i> (×10 <sup>-4</sup> × min <sup>-1</sup> per μU/ml)	3.3 ± 2.4	4.5 ± 2.6	<0.01
Insulin secretion rate			
First-phase insulin response (0–10 min) (μU/min)	409 ± 305	412 ± 300	
Lipoprotein concentration			
Fasting serum total cholesterol (mmol/l)	4.9 ± 0.9	4.4 ± 1.2	
Fasting serum HDL cholesterol (mmol/l)	1.15 ± 0.23	1.15 ± 0.45	
Fasting serum LDL cholesterol (mmol/l)	3.2 ± 0.8	2.7 ± 0.9	<0.01
Fasting serum triglyceride (mmol/l)	1.26 ± 0.60	1.37 ± 1.20	
Fasting plasma free fatty acid (mmol/l)	0.48 ± 0.18	0.49 ± 0.23	
LDL size (nm)	26.0 ± 1.22	26.0 ± 1.20	
Inflammatory markers			
Serum C-reactive protein, (mg/l)	1.28 ± 2.04	0.76 ± 0.68	<0.1
Plasma fibrinogen (g/l)	2.55 ± 0.38	2.49 ± 0.52	
Endothelial injury markers			
Serum VCAM-1 (ng/ml)	438 ± 72	462 ± 76	
Serum ICAM-1 (ng/ml)	249 ± 50	233 ± 58	
Serum E-selectin (ng/ml)	56.8 ± 19.6	51.2 ± 16.4	
Other markers			
Serum adiponectin (μg/ml)	6.6 ± 1.8	8.1 ± 3.0	<0.03
Plasma PAI-1 (IU/ml)	19.4 ± 15.4	13.0 ± 14.7	<0.01

Data are mean ± SD. ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule.

of smoke-free tobacco (6.4 ± 11.4 vs. 2.3 ± 8.0 g/day, NS), respectively. All participants gave informed consent and the study was approved by the Ethical Committee of Göteborg University.

**Blood pressure, oral glucose tolerance test, insulin sensitivity, and acute insulin response.** Blood pressure was measured with a standard mercury sphygmomanometer on the right arm after the subjects had been resting in the supine position for at least 5 min. Mean values were determined from two independent measurements taken at 5-min intervals.

All subjects underwent a 75-g oral glucose tolerance (OGTT) test after having fasted overnight. Baseline samples were drawn from an antecubital vein and stored at -20° or -80°C until analysis.

Glucose was injected intravenously in an antecubital vein over a period of 60 s (0.3 g/kg body wt of 30% glucose) to measure the acute insulin response. Twenty minutes after the glucose injection, insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) was administered intravenously as a bolus of 0.03 units/kg. Blood samples were collected at 20 time points during the intravenous glucose tolerance test (IVGTT) at -5, -1, 2, 4, 6, 8, 10, 14, 19, 22, 30, 40, 50, 60, 90, 100, 120, 140, 160, and 180 min. Insulin sensitivity index (*S<sub>i</sub>*) was calculated using the Bergman MINIMOD computer program (18).

Body fat was calculated from the bioelectrical impedance method (BIA-103; RJL Systems, Detroit, MI) (19).

**Blood chemistry.** Glucose was analyzed in venous blood using an automatic glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH), and plasma insulin was analyzed with a standard radioimmunoassay having 40% cross-reactivity with proinsulin (Pharmacia, Uppsala, Sweden). Proinsulin was measured with the Mercodia Proinsulin ELISA (Mercodia AB, Uppsala, Sweden).

Lipid concentrations were determined with an automated Cobas Mira

analyzer (Hoffman-LaRoche, Basel, Switzerland) as previously reported (20,21), and LDL cholesterol was calculated according to Friedewalds formula (LDL cholesterol = total cholesterol - HDL cholesterol - 0.45 × triglyceride level [mmol/l]). LDL peak particle diameter was measured with gradient gel electrophoresis (22).

C-reactive protein (CRP) was measured with an immunoenzymometric assay for quantitative determination of human CRP (CRP IEMA Test; Medix Biochemica, Kauniainen, Finland), while serum intracellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM), and E-selectin were measured by the quantitative sandwich enzymatic immunoassay technique using Parameter kits (catalogue no. BBE 3, 1B, 2B; R&D Systems, Minneapolis, MN). The optical densities were read in a Perkin Elmer HTS 7000 Plus BioAssay fluorescent and absorbance microplate reader.

Fibrinogen and PAI-1 were analyzed using standard methods, and Apo E genotype was determined by a PCR technique. Adiponectin was analyzed by a quantitative immunoblotting technique (23).

**Statistical analysis.** For descriptive purposes, mean and SD were used. The Mann-Whitney *U* test was used for comparisons between the groups. To adjust for confounding variables when comparing the groups, logistic regression was used. All correlations were analyzed with Spearman's nonparametric correlation coefficient. To adjust for confounding variables in the correlation analysis, Spearman's partial nonparametric correlation coefficient was calculated. To select independent predictors, only variables with a univariate correlation (*P* < 0.1) were chosen. Then, a stepwise multiple regression analysis was used after transforming the dependent variable to normal distribution by calculating normal score using Blom's method (24).

All tests were two-tailed and conducted at 5% significance level. SAS 8.2 was used for statistical calculations.

TABLE 2  
Partial correlation coefficients ( $r$ ) between adiponectin and selected variables in the relatives ( $n = 45$ )

Parameter	Model 1	Model 2	Model 3
Fasting plasma insulin	-0.55*	-0.52*	-0.43†
Fasting plasma proinsulin	-0.64*	-0.61*	-0.56*
$S_i$	0.41†	0.34‡	—
Acute insulin response	-0.40‡	-0.34†	-0.26
HDL cholesterol	0.48†	0.43‡	0.39‡
PAI-1 activity	-0.56*	-0.54*	-0.53*

Data are  $r$ . Model 1 is adjusted for age; model 2 is adjusted for age and waist; model 3 is adjusted for age, waist, and  $S_i$ . \* $P < 0.001$ ; † $P < 0.01$ ; ‡ $P < 0.05$ .

## RESULTS

**Anthropometry, metabolic variables, and other markers.** The basic characteristics of the 85 participants are shown in Table 1. The FDR had higher BMI, waist circumference, body fat, and blood pressure levels. However, Apo E phenotype distribution was similar for the two groups (data not shown).

All participants had normal glucose tolerance, but the glucose concentrations during the OGTT were higher in the FDR as compared with the CON (Table 1). Furthermore, plasma insulin at 2 h was increased, and the FDR were also insulin resistant as determined with the Minimal Model computer program.

Lipoprotein concentrations, inflammatory markers, PAI-1, cellular adhesion molecules, and adiponectin levels are presented in Table 1. Comparisons between the groups showed that PAI-1 and LDL cholesterol levels were significantly higher in the FDR. Moreover, the FDR also had significantly lower adiponectin levels while CRP was higher, but this difference only reached borderline significance ( $P < 0.1$ ). The lower adiponectin levels in the relatives remained also when the small differences in BMI, body fat, and waist were adjusted for (data not shown). Thus, high propensity for type 2 diabetes is associated with lower adiponectin levels also when adjusted for body fat and of its localization.

**Adiponectin and relationships with other variables.** When adjusting for age, there were significant negative correlations between adiponectin and proinsulin ( $r -0.64$ ,  $P < 0.001$ ), PAI-1 activity ( $r -0.56$ ,  $P < 0.001$ ), fasting insulin ( $r -0.55$ ,  $P < 0.001$ ), and the acute insulin response ( $r -0.40$ ,  $P < 0.05$ ) in the FDR (Table 2). In addition, E-selectin showed a negative correlation of borderline significance ( $r -0.27$ ,  $P < 0.1$ ). We also found significant positive correlations between adiponectin and HDL cholesterol ( $r 0.48$ ,  $P < 0.01$ ) and  $S_i$  ( $r 0.41$ ,  $P < 0.01$ ). In the CON group, there were no significant correlations between adiponectin and proinsulin or HDL cholesterol, but otherwise essentially similar correlations appeared as compared with those found in the FDR (data not shown).

In stepwise multiple regression analyses with adiponectin as a dependent variable in the FDR, insulin, proinsulin, HDL cholesterol, and PAI-1 levels remained significant throughout partial correlation models adjusted for age, waist, and  $S_i$  and were entered as independent variables. The analyses excluded all variables, with the exception of HDL cholesterol (parameter estimate  $-0.05$ , SE 0.01,  $F$  value 12.6,  $P = 0.002$ ) and fasting plasma proinsulin

(parameter estimate 1.53, SE 0.43,  $F$  value 10.7,  $P = 0.002$ ), that remained significant explanatory variables of adiponectin, with a total explanatory power of 51%.

In the CON, a similar analysis revealed that fasting insulin (parameter estimate  $-0.12$ , SE 0.04,  $F$  value 7.7,  $P = 0.009$ ) explained 48% of the variability in the adiponectin concentration.

## DISCUSSION

The two salient findings of the present study are 1) circulating adiponectin levels are significantly lower in healthy individuals with high propensity for type 2 diabetes, adjusted for BMI, body fat, or waist circumference; and 2) adiponectin levels, when adjusted for age, are related to several key risk factors for CVD in the FDR group. These factors include both metabolic risk factors related to insulin sensitivity (like hyperinsulinemia, proinsulin, HDL cholesterol, and PAI-1) and cellular adhesion molecules (E-selectin). Thus, these data support experimental studies that adiponectin can improve insulin sensitivity and action in obese rodent models, as well as several observations suggesting that it exerts antiatherogenic effects (12,25–30).

In contrast, a recent study of FDR and CON found similar adiponectin levels in the two groups and no relationship between adiponectin and different measures of insulin sensitivity in the FDR group. However, the authors found significantly reduced levels of adiponectin mRNA in subcutaneous adipose tissue from FDR as compared with CON (31). We did not examine adiponectin mRNA in this study, but recent findings in our laboratory are consistent with lower adiponectin mRNA levels in nonobese insulin-resistant subjects (data not shown). This is a novel finding, although it is well established that plasma adiponectin levels are decreased in other insulin-resistant states, such as obesity and type 2 diabetes (9,13,15,32).

Interestingly, small insulin-sensitive adipocytes appear to secrete more adiponectin, since obese monkeys with hypercellular but small adipocytes have higher plasma adiponectin levels than obese monkeys with fat cell hypertrophy (33). By analogy, activation of PPAR- $\gamma$ , a transcription factor of key importance for adipocyte differentiation, also leads to increased adiponectin levels (34). Yu et al. (35) also recently reported that troglitazone, a synthetic PPAR- $\gamma$  ligand, increased plasma adiponectin levels in lean, obese, and type 2 diabetic subjects after 3 months. Moreover, adiponectin correlated with HDL cholesterol, which is in agreement with the present data as well as other recent studies (14,16,17). Taken together, these observations support the concept that the cellular expression of adiponectin is under the control of PPAR- $\gamma$  and that it is more closely related to insulin sensitivity than obesity.

In this study, we also found that adiponectin was inversely correlated with proinsulin. This was true after adjustment for age, waist circumference, and insulin sensitivity and has, to our knowledge, not been reported previously. The reason for this is unclear, but may be a consequence of the increased insulin levels and, initially, insulin resistance. However, other possibilities, including effects on the intracellular processing of insulin, cannot be

excluded. Hyperproinsulinemia has been shown to be a risk factor for cardiovascular disease (36), and this may also be related to lower adiponectin levels. Similarly, we found in the present study a strong correlation between circulating PAI-1 levels and adiponectin. PAI-1 is also a risk factor for CVD (37), and its secretion from the adipose tissue is increased by cytokines like TNF- $\alpha$  (38). Adiponectin has been shown to reduce TNF- $\alpha$  secretion as well as the TNF- $\alpha$ -induced expression of adhesion molecules in endothelial cells (12).

Taken together, our data lend further support to the hypothesis that adiponectin can exert antiatherogenic effects in humans.

In conclusion, adiponectin levels were significantly reduced in nonobese but insulin-resistant FDR with a high propensity for type 2 diabetes. In addition, an association was found between adiponectin, proinsulin, HDL cholesterol, and other facets of the insulin resistance syndrome. Thus, adiponectin may be an important mediator of the relationship between insulin resistance and atherosclerosis, and thus could be an important target for future diabetes therapy.

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