

Adiponectin Is Associated With Vascular Function Independent of Insulin Sensitivity

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OBJECTIVE — Adiponectin has been proposed to play important roles in the regulation of energy homeostasis and insulin sensitivity. In experimental studies, adiponectin has also been found to inhibit vascular smooth muscle cell proliferation. Decreased adiponectin levels have been described in patients with coronary artery disease, and circulating adiponectin predicts cardiovascular death in patients with renal failure. Because adiponectin appears to influence both insulin sensitivity and vessel wall physiology, we examined insulin sensitivity and vascular function in relation with circulating adiponectin.

RESEARCH DESIGN AND METHODS — We studied brachial artery vascular reactivity (high-resolution external ultrasound) and insulin sensitivity (minimal model) in 68 healthy subjects. Brachial artery vascular reactivity was also determined in 52 patients with altered glucose tolerance: 30 subjects with impaired fasting glucose (IFG) or glucose intolerance (GIT) and 22 patients with type 2 diabetes.

RESULTS — Circulating adiponectin concentration was significantly associated with insulin sensitivity ($r = 0.29$, $P = 0.02$) and with fasting serum triglycerides ($r = -0.29$, $P = 0.02$) in healthy subjects. In the latter, adiponectin levels were positively associated with arterial vasodilation in response to nitroglycerin (endothelium-independent vasodilation [EIVD], $r = 0.41$, $P = 0.002$) but not with flux-induced, endothelium-dependent vasodilation (EDVD) ($r = 0.007$, $P = \text{NS}$). In contrast, EIVD was not significantly associated with adiponectin in subjects with IFG, GIT, or type 2 diabetes ($r \leq 0.10$, $P = \text{NS}$). In a multiple linear regression analysis to predict EIVD in healthy subjects, age ($P = 0.012$), sex ($P = 0.042$), and adiponectin concentration ($P = 0.045$), but not BMI, insulin sensitivity, or fasting triglycerides, contributed to 39% of EIVD variance.

CONCLUSIONS — Serum adiponectin concentration appears to be significantly associated with vascular function in apparently healthy humans. This association seems to be independent of its link with insulin sensitivity.

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Increased adipose tissue mass is a major risk factor for metabolic disorders such as diabetes, hypertension, and atherogenic diseases (1,2). The knowledge of the factors involved in this relationship

will be of crucial importance in the prevention of cardiovascular complications. Adiponectin (also called Acrp30 or adipoQ in mice) is a 244–amino acid protein synthesized and secreted exclusively by

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Abbreviations: EDVD, endothelium-dependent vasodilation; EIVD, endothelium-independent vasodilation; GIT, glucose intolerance; IFG, impaired fasting glucose; sICAM, soluble intercellular molecule.

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the adipose tissue (3,4). It has been proposed to play important roles in the regulation of energy homeostasis and insulin sensitivity (5–9). Injection of adiponectin decreases plasma glucose levels by suppressing glucose production in the liver (5,6). Administration of globular adiponectin ameliorates insulin resistance in obese mice (8). In humans, adiponectin has been demonstrated to circulate in inverse proportion to the degree of insulin resistance (10–12). A 21% reduction in mean BMI was accompanied by a 46% increase in circulating adiponectin in a recent study, linking at a long-term regulation of adiponectin levels by changes in insulin sensitivity (13). Recent observations suggest pleiotropic insulin sensitizing effects of adiponectin in humans (14).

Heterozygous adiponectin-deficient mice showed mild insulin resistance, while homozygous adiponectin-deficient mice showed moderate insulin resistance and glucose intolerance (GIT) despite a body weight gain similar to that of wild-type mice. In addition to displaying major insulin-sensitizing properties, adiponectin may have putative antiatherogenic properties. Adiponectin null mice also developed twofold more neointimal formation in response to external vascular cuff injury than wild-type mice (15). Interestingly, globular adiponectin protected *ob/ob* mice from diabetes and apolipoprotein E-deficient mice from atherosclerosis (16).

In humans, decreased adiponectin was originally described in patients with coronary artery disease (17,18). More recently, the incidence of cardiovascular death was found to be higher in renal failure patients with low plasma adiponectin compared with those with higher plasma adiponectin levels (19).

Because adiponectin appears to influence both insulin sensitivity and vessel wall physiology, we hypothesized changes in vascular function that could be attributed to changes in circulating adiponectin concentrations. For this reason, we examined insulin sensitivity, vascular function, and adiponectin in apparently

healthy individuals. We also studied, for comparison, patients with impaired fasting glucose (IFG), GIT, and type 2 diabetes.

RESEARCH DESIGN AND METHODS

Consecutive subjects studied prospectively were included in this study. None of the subjects was taking any medication or had any evidence of metabolic disease other than obesity. All subjects were interviewed, and a medical history was recorded. All subjects were of Caucasian origin and reported that their body weight had been stable for at least 3 months before the study. Inclusion criteria were BMI (weight in kilograms divided by the square of height in meters) <40 kg/m², absence of any systemic disease, and absence of any infections in the previous month. All women were premenopausal and were studied in the follicular phase of the menstrual cycle.

Brachial artery vascular reactivity was also determined in a group of 52 patients with altered glucose tolerance and similar inclusion criteria: 30 subjects with IFG or GIT, and 22 patients with type 2 diabetes according to World Health Organization criteria. All these patients were of recent diagnosis after a standard oral glucose tolerance test and were studied before dietary treatment was given.

Informed written consent was obtained after the purpose, nature, and potential risks of the study were explained to the subjects. The subjects were instructed not to change their diet and exercise habits before the tests. The experimental protocol was approved by the hospital ethics committee.

Measurements

Each subject was studied in the research laboratory in fasting conditions. The room was quiet, lights were dimmed, and temperature was controlled at 23°C. BMI was calculated as weight (in kilograms) divided by height (in meters) squared. The subjects' waists were measured with a soft tape midway between the lowest rib and the iliac crest. The hip circumference was measured at the widest part of the gluteal region. Blood pressure was measured in the supine position on the right arm after a 10-min rest; a standard sphygmomanometer of appropriate cuff size was used, and the first and fifth phases were recorded. Values used in the analysis are the average of three readings taken at 5-min intervals.

Study of insulin sensitivity

Insulin sensitivity was measured in healthy subjects using the frequently sampled intravenous glucose tolerance test on a different day. In brief, the experimental protocol started between 8:00 and 8:30 A.M. after an overnight fast. A butterfly needle was inserted into an antecubital vein, and patency was maintained with a slow saline drip. Basal blood samples were drawn at -20 , -10 , and -5 min, after which glucose (300 mg/kg body wt) was injected over 1 min starting at time 0, and insulin (0.03 units/kg; Actrapid; Novo, Copenhagen, Denmark) was administered at time 20. Additional samples were obtained from a contra-lateral antecubital vein up to 180 min, as previously described (20).

Brachial artery vascular reactivity

High-resolution external ultrasound (128XP/10 mainframe with a 7.5-MHz linear array transducer; SSH-140A; Toshiba, Tokyo, Japan) was used to measure changes in brachial artery diameter. The lumen diameter of the artery was defined as the distance between the leading edge of the echo of the near wall-lumen interface to the leading edge of the far wall-lumen interface echo. All scans were electrocardiogram triggered and made coincident with the R wave (end of diastole). All images were recorded with S-VHS videotape (MD-830AG; Panasonic, Secaucus, NJ). Endothelial-dependent vasodilation (EDVD) was elicited by induced hyperemia following inflation of a pneumatic tourniquet placed around the forearm, distal to the scanned part of the artery, up to a pressure of 300 mmHg for 5 min and followed by sudden deflation. This maneuver is recognized to rise shear stress on the endothelial cells, which in turn release nitric oxide (NO)-producing vasodilation, which allows testing for endothelial function. EDVD is expressed as the percentage of change in the arterial diameter 1 min after hyperemia (21).

Endothelial-independent vasodilation (EIVD) is induced after sublingual administration of a 400- μ g metered dose of GTN, an exogenous NO donor (Solinitrina spray; Almirall Prodesfarma, Barcelona, Spain), and expressed as the percentage of change in the arterial diameter 3 min later. Reactive hyperemia is calculated as the percentage change between the maximum flow recorded in the first

15 s after cuff deflation and the flow during the resting scan (21).

A first scan was recorded after 10 min of resting in a quiet room in the supine position. Then the tourniquet was inflated for 5 min. A second scan was recorded over 90 s beginning 10 s before cuff deflation. After at least 10 more minutes of rest, a new control scan was recorded. A last scan was recorded from 2 min after GTN administration over 70 s.

All images were registered on S-VHS tape and later analyzed by two independent observers blinded to the randomization of the subject and the stage of the experiment. Each observer analyzed the arterial diameter for four cardiac cycles for each condition, and these measurements were averaged.

Before the initiation of the study, validation of this technique was performed through the evaluation of inter- and intraobserver reproducibility in 22 healthy subjects (12 men and 10 women, mean age 30.1 years [95% CI 27.1–33.2], BMI 22.6 kg/m² [21.3–23.8]). Measurements were performed by two observers (A and B). The intraclass coefficient of correlation of fixed effects between observers A and B was 0.90. Coefficient of variation (CV) between means obtained by observers A and B was 9%. The CV obtained by observer A was 3%. The repeatability (95% CI) was 0.27 mm (observer A). In observer B, the CV was 4%, with repeatability (95% CI) 0.39 mm. The study of the variability of the means by the same observer in 5 consecutive days showed a CV of 6% (observer A) and 2% (observer B).

Analytical methods

Serum glucose concentrations were measured in duplicate by the glucose oxidase method with the use of a Beckman Glucose Analyzer II (Beckman Instruments, Brea, CA). The CV was 1.9%. HbA_{1c} was measured by high-performance liquid chromatography with the use of a fully automated glycosylated hemoglobin analyzer system (L-9100; Hitachi, Tarrytown, NY).

Serum insulin levels during the frequently sampled intravenous glucose tolerance test were measured in duplicate by monoclonal immunoradiometric assay (IRMA; Medgenix Diagnostics, Fleunes, Belgium). Intra- and interassay CVs were similar to those previously reported.

Serum adiponectin concentrations

were measured by radioimmunoassay (Linco Research, St. Charles, MO). Samples were diluted 500 times before the assay. The sensitivity of this method is 2 ng/ml. The intra- and interassay CVs were <5%.

Soluble intercellular molecule (sICAM)-1 in citrated plasma samples was measured using a commercially available enzyme-linked immunosorbent assay kit (sICAM-1 ELISA; Bender-MedSystems Diagnostics, Vienna, Austria) according to the manufacturer's recommendations.

Statistical methods

The study power was calculated as follows: assuming an α risk of 0.05, a β risk of 0.10, and a unilateral test, 50 subjects were needed to detect a statistically significant correlation, with $r = 0.30$. A greater number of patients were included to further decrease the α and β risks. Descriptive results of continuous variables are expressed as means \pm SD. Before statistical analysis, normal distribution and homogeneity of the variances were tested. Parameters that did not fulfill these tests (triglycerides, insulin sensitivity, adiponectin, and endothelium-dependent and -independent vasodilation) were log transformed. The relations between variables were analyzed by unpaired *t* test, simple correlation (Pearson's *r*), and multiple regression in a stepwise manner. Levels of statistical significance were set at $P < 0.05$.

RESULTS— Anthropometric and biochemical characteristics of the healthy subjects are shown on Table 1. In this study, we included 68 subjects (14 women). Men and women were comparable in proportion of smokers, systolic and diastolic blood pressure, fasting glucose, lipids, and insulin sensitivity (Table 1). EDVD and the response to nitroglycerin were significantly greater in women than men, and adiponectin also tended to be higher among women (Table 1).

When all subjects were considered as a group, circulating adiponectin concentration was significantly associated with insulin sensitivity ($r = 0.29$, $P = 0.02$) (Fig. 1) and with fasting serum triglycerides ($r = -0.29$, $P = 0.02$). Adiponectin levels were also associated with arterial vasodilation in response to nitroglycerin (EIVD, $r = 0.41$, $P = 0.002$ (Fig. 1) but

Table 1—Anthropometric and biochemical variables of the healthy subjects

Variable	Men	Women	P
n	54	14	—
Age (years)	46.2 \pm 11.1	38 \pm 8.3	0.01
BMI (kg/m ²)	27.1 \pm 3.7	29.7 \pm 5.3	0.03
Smokers (n)	4	1	NS
Systolic blood pressure (mmHg)	122.7 \pm 11.1	110 \pm 10	0.06
Diastolic blood pressure (mmHg)	79.8 \pm 7.8	71.6 \pm 12.5	NS
Fasting glucose (mg/dl)	96 \pm 12	92.3 \pm 8.8	NS
HbA _{1c} (%)	4.5 \pm 0.4	4.4 \pm 0.3	NS
Total cholesterol (mg/dl)	193 \pm 36	193 \pm 30	NS
LDL cholesterol (mg/dl)	130 \pm 28.4	127 \pm 21	NS
HDL cholesterol (mg/dl)	48.2 \pm 10	49.4 \pm 14	NS
Triglycerides (mg/dl)	109.3 \pm 93	83.6 \pm 42	NS
Insulin sensitivity (min \cdot mU ⁻¹ \cdot l ⁻¹)	2.7 \pm 2.4	2.7 \pm 2.3	NS
EDVD (%)	3.4 \pm 4.9	8.8 \pm 4.4	0.003
EIVD (%)	16.1 \pm 6.8	24.5 \pm 5.6	0.001
Adiponectin (μ g/ml)	8.7 \pm 8.4	11.2 \pm 7.8	0.1

Data are means \pm SD unless noted otherwise.

not with flux-induced EDVD ($r = 0.007$, $P = NS$) (Fig. 2). Neither insulin sensitivity ($r = 0.10$, $P = 0.4$) nor fasting triglycerides ($r = -0.04$, $P = 0.6$) correlated significantly with EIVD. The relationship between EIVD and adiponectin persisted when only men were considered ($r = 0.34$, $P = 0.03$). In 25 consecutive healthy subjects (20 men), who did not differ significantly from the remaining subjects, adiponectin concentration tended to be associated with plasma sICAM-1 levels ($r = -0.38$, $P = 0.059$).

We constructed a multiple linear regression analysis to predict EIVD. In this model, age ($P = 0.012$), sex ($P = 0.042$), and adiponectin concentration ($P = 0.045$), but not BMI, insulin sensitivity, or fasting triglycerides, contributed to 39% of EIVD variance.

All subjects with IFG, GIT, or type 2 diabetes were men and slightly older than control subjects (Table 2). Adiponectin concentration was significantly decreased in type 2 diabetic in comparison with healthy control subjects ($P = 0.02$). In contrast to healthy subjects, adiponectin was not significantly associated with EIVD or EDVD either in patients with IFG, GIT, or type 2 diabetes or in all subjects as a whole (all $r < 0.10$, $P = NS$).

CONCLUSIONS— In vitro, adiponectin inhibited monocyte adhesion to endothelial cells and lipid accumulation in human monocyte-derived macrophages (22,23). In cultured human endo-

thelial cells, adiponectin downregulated expression of intracellular adhesion molecules (23). It could also show anti-inflammatory properties as suggested by the suppressive effect of adiponectin on phagocytic activity and lipopolysaccharide-induced tumor necrosis factor- α production in cultured macrophages (24). Adiponectin has been shown to inhibit tumor necrosis factor- α induction of nuclear factor κ B through activation of the cAMP-protein kinase A pathway (24). Adiponectin also seems to stimulate the production of NO in vascular endothelial cells in in vitro studies (25). According to this information, we hypothesized changes in vascular reactivity in relation with adiponectin.

We found that adiponectin was associated with vascular function in addition to its known effects on insulin sensitivity. Two recent studies have also evaluated endothelial dysfunction in relation with adiponectin. Shimabukuro et al. (26) evaluated forearm blood flow using plethysmography in 76 Japanese subjects without a history of cardiovascular disease or diabetes. They found positive associations between forearm blood flow and adiponectin. Sex distribution and the ages of the subjects were not reported. They studied the reproducibility of reactivity hyperemia in 28 healthy men aged 27 \pm 5 years. If we assume that this is the mean age of the total group, the findings in this study (26) were attributed to young subjects. In our study, we evaluated subjects

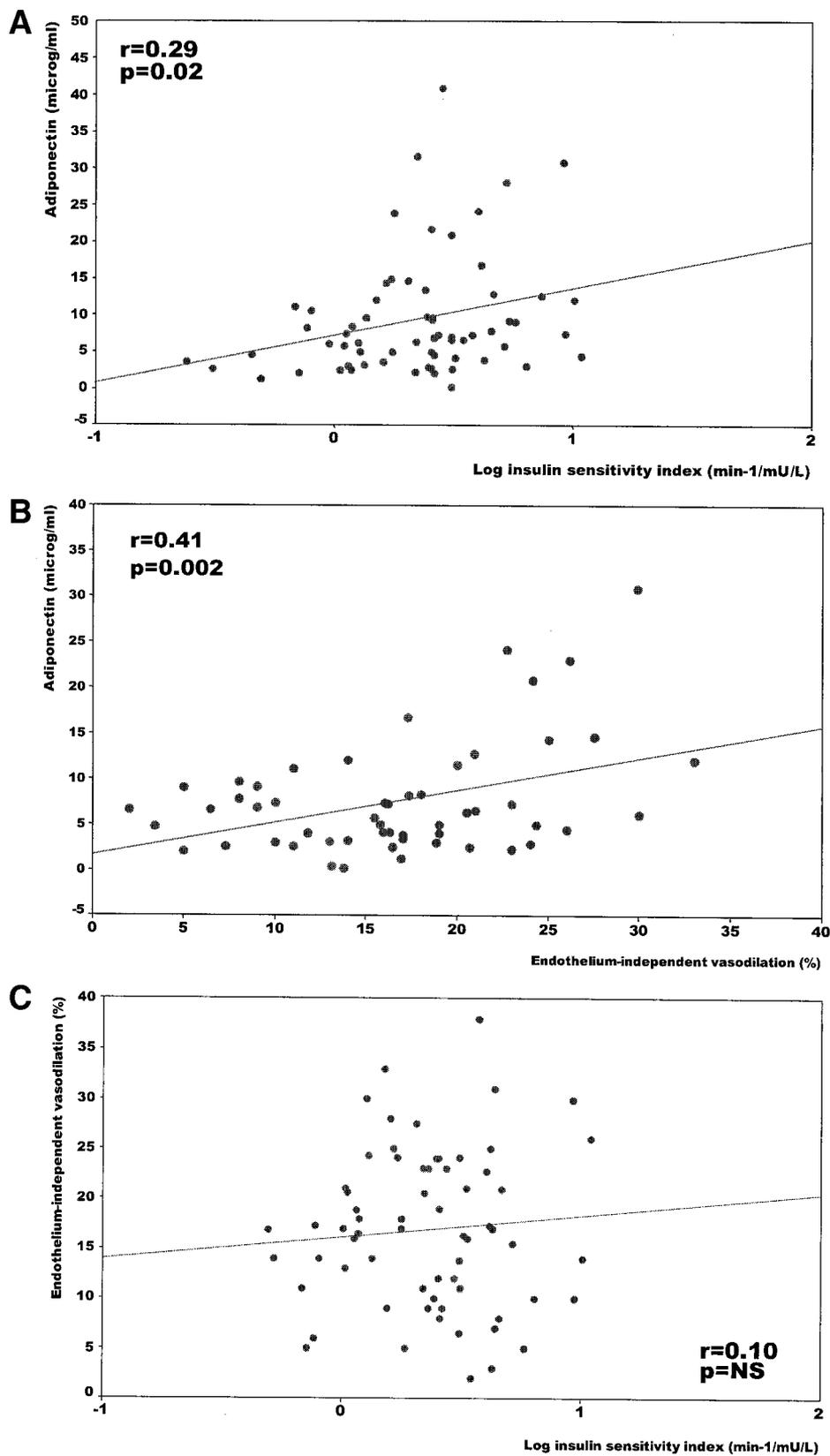


Figure 1—Linear correlation between circulating adiponectin concentration and log-transformed insulin sensitivity index (A) and EIVD (B) in apparently healthy subjects. The insulin sensitivity index was not significantly associated with EIVD (C) in these same subjects.

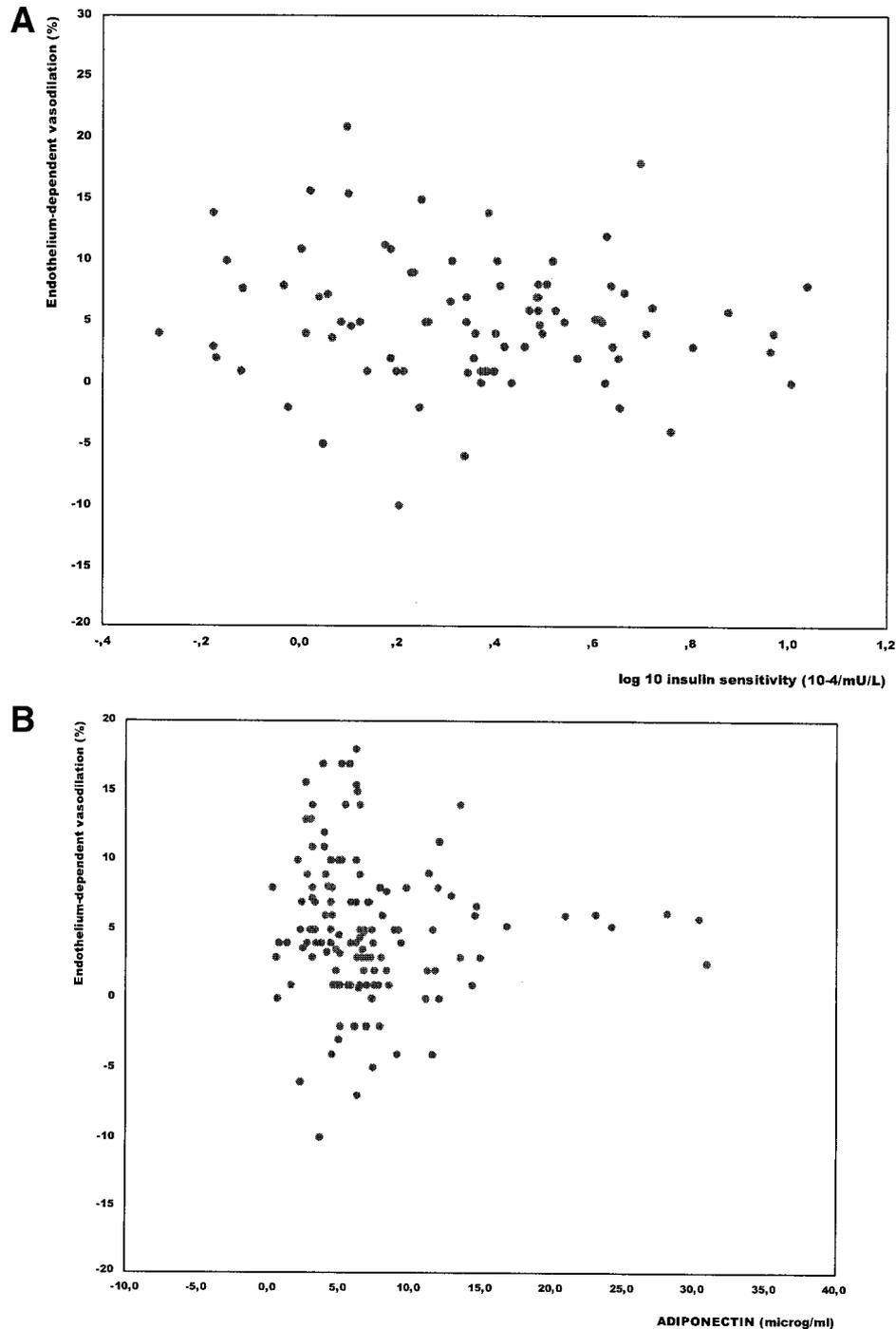


Figure 2—EDVD and insulin sensitivity (A) and circulating adiponectin (B) in healthy subjects.

of middle age, and we found significant associations between EDVD and adiponectin in the whole group of healthy subjects (including women) and when only men were considered in the analysis. In our study, women had significantly increased EDVD and EIVD and tended to display higher adiponectin concentrations than men (Table 1). Thus, a different sex dis-

tribution could explain the apparent discrepant results. Ouchi et al. (27) also reported a significant and positive association between adiponectin and EDVD among hypertensive patients. Again, sex distribution was not reported and was not considered as a potential confounding variable. It would be interesting to know the associations of EDVD and EIVD with

adiponectin according to sex in these studies.

The arterial smooth muscle response to nitroglycerin has been consistently demonstrated to be impaired in humans with risk factors for atherosclerosis and in diabetic patients (21,28,29). We observed increased nitroglycerin-induced vasodilation of forearm conduit vessels in

Table 2—Anthropometric and biochemical variables of patients with altered glucose tolerance

Variable	IFG/GIT	Diabetes	P
n (men)	30	22	—
Age (years)	56.2 ± 9.6	59.3 ± 10.8	NS
BMI (kg/m ²)	28.5 ± 3.4	30.3 ± 5.2	NS
Smokers (n)	2	2	NS
Systolic blood pressure (mmHg)	128.6 ± 14.9	142 ± 19	0.0097
Diastolic blood pressure (mmHg)	82.5 ± 8.7	82.8 ± 10.9	NS
Fasting glucose (mg/dl)	102.1 ± 11.8	138.5 ± 44.2	<0.0001
Total cholesterol (mg/dl)	221 ± 33	210 ± 29	NS
Triglycerides (mg/dl)	127.5 ± 99	162.4 ± 39	NS
EDVD (%)	4.7 ± 5.4	4.1 ± 4.4	NS
EIVD (%)	15.5 ± 8	13.7 ± 6.5	NS
Adiponectin (μg/ml)	6.5 ± 3.2	4.8 ± 3.5	NS

Data are means ± SD unless noted otherwise.

those apparently healthy subjects with the highest circulating adiponectin concentration. Vasodilation in response to nitroglycerin is mediated by vascular smooth muscle cells, which form part of the arterial wall structure. In those arteries with eccentric hypertrophy, with a thicker vascular wall, the poorly vascularized intimal layer is less easily reached by oxygen and other nutrients. As adiponectin inhibits vascular smooth muscle cell proliferation (15,30), we hypothesize that adiponectin leads to increased arterial distensibility in humans. However, when established cardiovascular risk factors were present, such as IFG, GIT, or type 2 diabetes, we observed no significant associations between endothelial or vascular dysfunction and adiponectin. It could be that once hypoadiponectinemia develops, as in type 2 diabetes, homeostatic mechanisms are lost.

Inflammatory processes are being increasingly recognized as important players in atherosclerosis (31). These inflammatory processes play a crucial role in the remodeling of the vascular wall after injury. In this sense, adiponectin suppressed the expression of different growth factors in the stimulated endothelial cells of an injured vascular wall and also suppressed the proliferation and migration of smooth muscle cells stimulated by these factors (30). Adiponectin null mice formed twofold more neointima in response to external vascular cuff injury than wild-type mice (15). In fact, in wild-type mice, adiponectin infiltrated rapidly into the subendothelial space of the vascular wall when the endothelial barrier of the arterial wall was injured by balloon

angioplasty (32). Adenovirus-mediated supplement of adiponectin improved the intimal thickening in adiponectin null mice to the wild-type level (30). In these studies, the protective effect of adiponectin seemed to be a direct consequence of adiponectin action on the vascular wall and/or macrophages rather than an indirect consequence of alteration of conventional atherosclerotic risk factors in vivo (15,30). Although null mice showed GIT and hypertriglyceridemia compared with wild-type mice, these metabolic abnormalities were unlikely to account for the increased neointimal formation (15).

In cultured human endothelial cells, adiponectin downregulated expression of intracellular adhesion molecules (18). We here show how adiponectin tended to be associated with circulating sICAM-1 in a subsample of subjects. In fact, circulating sICAM-1 concentrations have been described (33) to be independently associated with atherosclerosis and coronary artery disease.

Interestingly, subjects carrying a missense mutation in the adiponectin gene associated with hypoadiponectinemia exhibit the phenotype of the metabolic syndrome, including insulin resistance and coronary artery disease (34).

In summary, adiponectin concentration appears to be significantly associated with vascular dysfunction in apparently healthy humans. However, these observations are preliminary and cross-sectional. This association seems to be independent of the link of adiponectin with insulin sensitivity.

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