CLINICAL STUDY

Lower plasma adiponectin is a marker of increased intima-media thickness associated with type 2 diabetes mellitus and with male gender

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Abstract

Objective: We tested the extent to which altered plasma adipokine levels may contribute to the increased carotid artery intima-media thickness (IMT) associated with type 2 diabetes mellitus and with male gender, independently of conventional cardiovascular risk factors, insulin resistance, and plasma C-reactive protein (CRP).

Design: IMT (mean of three segments of both carotid arteries by ultrasonography), insulin resistance (homeostasis model assessment; HOMA_{ir}), plasma CRP, lipids, adiponectin, leptin, resistin, and tumor necrosis factor- α (TNF- α) were measured in 84 type 2 diabetic patients and 85 control subjects.

Results: In diabetic patients, IMT ($P < 0.001$), mean arterial pressure ($P < 0.001$), HOMA_{ir} ($P < 0.001$), plasma CRP ($P = 0.003$), triglycerides ($P = 0.037$), leptin ($P = 0.023$), resistin ($P = 0.003$), and TNF- α ($P = 0.003$) levels were higher, whereas high-density lipoproteins (HDL) cholesterol ($P < 0.001$) and adiponectin ($P < 0.001$) levels were lower compared with control subjects. Plasma adiponectin ($P < 0.001$) and leptin ($P < 0.001$) were substantially lower in men than in women. IMT was positively and independently associated with age ($P < 0.001$), diabetes ($P = 0.049$), and male gender ($P = 0.002$) in a multivariate regression model, not including other variables. Further analyses showed that IMT was positively related to age ($P < 0.001$) and plasma triglycerides ($P = 0.038$) and negatively to adiponectin ($P < 0.001$), without independent effects of diabetes, gender, and HOMA_{ir}.

Conclusions: Increased IMT in type 2 diabetes may in part be explained by lower plasma adiponectin and higher triglycerides, but not by leptin, resistin, and TNF- α . The gender effect on IMT is related to lower plasma adiponectin.

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Introduction

The increased cardiovascular risk in type 2 diabetes mellitus (1, 2) is reflected by a higher carotid artery intima-media thickness (IMT) (3–7), which is an established predictor of coronary heart disease and stroke in older subjects (8). This diabetes-associated increase in IMT is associated with conventional cardiovascular risk factors, such as central obesity, high blood pressure, and dyslipidemia, as well as with insulin resistance (3–7).

The pathogenic role of adipokines in insulin resistance, low grade inflammation and cardiovascular disease is increasingly recognized. Decreased plasma adiponectin levels have been documented in type 2 diabetes mellitus (9, 10). Plasma adiponectin levels are inversely related to insulin resistance (10–12), as well as to plasma C-reactive protein (CRP) (13). In contrast, plasma leptin is positively related to insulin resistance and adiposity (14, 15). Some studies have shown that plasma resistin is also higher in diabetes (16), although

the role of resistin in insulin resistance in humans is controversial (17). Both leptin and resistin are regarded to be pro-inflammatory adipokines, as judged by their associations with CRP (16, 18). High plasma levels of tumor necrosis factor- α (TNF- α), another pro-inflammatory adipokine, have been observed in diabetes as well, in association with insulin resistance (19), and its expression is negatively regulated by adiponectin (11).

Clinical end-point studies have suggested that low plasma adiponectin levels may predict coronary heart disease risk in both non-diabetic and diabetic subjects (20, 21). High plasma leptin was associated with incident coronary heart disease in some studies (22), but not in others (23). Furthermore, plasma resistin levels are increased in women with coronary heart disease (24) and may determine the severity of coronary artery calcification (25), whereas high plasma TNF- α levels may predict incident cardiovascular disease in men (26). In keeping with these observations, limited literature data raise the possibility that IMT may also be associated with plasma levels of adipokines. IMT is

inversely related to plasma adiponectin in adults and obese juveniles (27–29). IMT may also be positively associated with plasma leptin (30) and TNF- α levels (31). Importantly, it is unknown to what extent the increased IMT in type 2 diabetes is related to altered plasma levels of either adiponectin, leptin, resistin or TNF- α , independently of conventional risk factors, insulin resistance and plasma CRP.

The present study was carried out to test the hypothesis that the increased IMT in diabetes is related to diabetes-associated abnormalities in plasma adipokine levels. Since adiponectin, leptin and possibly also resistin and TNF- α are subject to a sexual dimorphism, with lower levels in men than in women (11, 32–35), we also questioned whether the expected association of higher IMT with male gender (36) was weakened or lost when plasma adipokine levels are taken into account.

Subjects and methods

The study was approved by the Medical Ethics Committee of the University Medical Center Groningen, and all participants provided written informed consent. Type 2 diabetic patients and control subjects were recruited by advertisement. Diabetes was diagnosed using glucose cut-off values as defined by the WHO. Medical history did not reveal recent illnesses and physical examination did not show pulmonary or cardiac abnormalities. Current smoking, the use of more than three alcoholic beverages per day, previous or current use of lipid lowering drugs, clinically manifest cardiovascular disease, renal function abnormalities (elevated serum creatinine and/or elevated urinary albumin excretion), urinary tract infection (positive urine dipstick for leukocytes or blood), thyroid disorders (abnormal thyroid-stimulating hormone levels, use of thyroxine or antithyroid drugs) and liver function abnormalities (transaminase levels >1.5 times the upper normal level) were exclusion criteria. Diabetic patients using insulin or thiazolidinediones were also excluded, but the use of antihypertensive medication was allowed. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Systolic and diastolic blood pressure was measured after 15 min rest with a sphygmomanometer in sitting position. Mean arterial pressure (MAP) was calculated as $1/3 \times \text{systolic} + 2/3 \times \text{diastolic}$ blood pressure. Homeostasis model assessment was taken as a measure of insulin sensitivity (homeostasis model assessment; HOMA_{IR}) using the equation: $\text{fasting plasma insulin} \times \text{glucose} / 22.5$ (37). All participants were studied after an overnight fast at the Laboratory of Vascular Medicine, while the diabetic patients did not take their usual medication before blood sampling.

The study comprised 84 Caucasian type 2 diabetics and 85 Caucasian control subjects. Gender distribution did not significantly differ between the groups ($P=0.69$).

In diabetic patients, median diabetes duration was 4.3 (interquartile range, 4.0–6.7) years. In addition to diet, 45 patients were treated with sulfonylureas and 42 with biguanides, either alone or in combination. One patient used acarbose. None of the control subjects and 36 diabetic patients used one or more antihypertensive drugs (mostly angiotensin-converting enzyme inhibitors, angiotensin II antagonists, diuretics and β -blockers, either as single therapy or in combination). None of the diabetic and three of the non-diabetic women used oral contraceptives ($P=0.34$).

Carotid IMT measurement

IMT of the carotid arteries was measured by ultrasonography in the supine position. Well-trained sonographers, not informed about subject disease state, scanned high-resolution B-mode ultrasound images (ACUSON 128 XP, Mountain View, CA, USA) with a 7.5 MHz linear array transducer. Three arterial wall segments in each carotid artery were imaged from a fixed lateral transducer angle at the far wall. The segments scanned were the segment 1 cm proximal to the carotid dilatation (common carotid artery), the segment between the carotid dilatation and carotid flow divider (carotid bulb) and a 1 cm segment distal to the flow divider (internal carotid artery). The scans were recorded on S-VHS tape and analyzed off-line by an independent image analyst, who was unaware of the subjects' characteristics. B-mode image analyses were digitized with a frame grabber (DT286 I; Data Translation, Inc., Marlboro, MA, USA). The image analysis software was developed using an algorithm as developed by Selzer *et al.* (38). The mean IMT over the six segments of both carotid arteries was calculated and was designated mean IMT. At a mean IMT of 0.80 mm, inter-sonographer variability amounted to 0.05 mm, with an image analyst variability of less than 0.03 mm, corresponding to a total coefficient of variation between 6.3 and 7.3%.

Laboratory measurements

Venous blood samples for measurement of lipids, apolipoproteins (apos), insulin, CRP and adipokines were collected into EDTA-containing tubes (1.5 mg/ml) after overnight fasting and were immediately placed on ice. Plasma was obtained by centrifugation at 1400 **g** for 15 min at 4 °C. Glucose was measured shortly after blood collection. Samples for other assays were kept frozen at -80 °C until analysis.

The high-density lipoprotein (HDL) fraction was separated by precipitation of apo B-containing lipoproteins with polyethylene glycol-6000. Plasma cholesterol, triglycerides and cholesterol in the HDL fraction were assayed by routine enzymatic methods (Roche/Hitachi cat. nos 11876023 and 11875540 respectively, Roche Diagnostics GmbH). Non-HDL cholesterol was calculated

as the difference of plasma total cholesterol–HDL cholesterol. Apo A-I and apo B were measured by immunoturbidimetry (Roche/Cobas Integra Tina-quant cat. nos 03032566 and 03032574 respectively, Roche Diagnostics GmbH).

Plasma total adiponectin, leptin, resistin and TNF- α were assayed using Luminex xMAP technology with commercially available kits, according to the manufacturer's instructions (Linco Research Inc., St Charles, MO, USA, Lincoplex panel A cat. no. HADK1-61K-A and panel B cat. no. HADK2-61K-B, purchased from Nuchilab Inc., Ede, The Netherlands). Validation experiments have shown that adiponectin, leptin, resistin and TNF- α levels, as measured with this technology, are strongly correlated ($r=0.81$ – 0.99) with assay results obtained by enzyme-linked immunoassays obtained from Linco Inc. (data provided by the manufacturer, (50, 51)).

Glucose was analyzed with an APEC glucose analyzer (APEC Inc., Danvers, MA, USA). Plasma insulin was measured with a microparticle enzyme immunoassay (AxSYM Insulin assay; Abbott Laboratories, Abbott Park, IL, USA). High sensitive-CRP was assayed by nephelometry with a threshold of 0.175 mg/l (BNII N; Dade Behring, Marburg, Germany). FFAs were assayed by a commercially available kit (Wako Chemicals, Neuss, Germany, cat. no. 999-75406). Glycated hemoglobin (HbA_{1c}) was measured by high-performance liquid chromatography (Bio-Rad; normal range 4.6–6.1%).

Statistical analysis

Data are given in medians (interquartile ranges) and were compared by Mann–Whitney *U*-tests. Differences in proportions of variables were determined by χ^2 -analysis. Multiple linear regression analysis with a subsequent backward procedure was used to disclose adjusted and independent relationships between variables. Because of skewed distribution, logarithmically

transformed values for HOMA_{ir}, triglycerides, leptin, resistin, adiponectin, TNF- α and CRP were used. Two-sided *P* values <0.05 were considered significant.

Results

As shown in Table 1, IMT, BMI, systolic and diastolic blood pressure, MAP, fasting glucose, HbA_{1c}, plasma insulin, HOMA_{ir} and plasma CRP levels were higher in diabetic compared with control subjects. When comparing all women with all men, IMT was clearly lower in women than in men. IMT was also lower in non-diabetic women than in non-diabetic men, but the gender difference in IMT just failed to reach significance in the diabetic group ($P=0.063$). Plasma insulin, HOMA_{ir} and CRP were higher in diabetic women than in diabetic men. CRP was higher than 10 mg/l in three diabetic patients and one control subject. After exclusion of these four subjects, CRP was still higher in diabetic than in the control subjects ($P=0.002$).

Plasma total cholesterol was slightly lower in diabetic patients compared with control subjects, but non-HDL cholesterol and apo B levels were not different (Table 2). As expected, plasma triglycerides were higher, whereas HDL cholesterol and apo A-I levels were lower in diabetic patients. HDL cholesterol and apo A-I levels were higher in all women compared with all men, as well in the diabetic and control groups separately. Plasma adiponectin was lower, whereas leptin, resistin and TNF- α levels were higher in diabetic patients. Plasma adiponectin and leptin levels were much higher in all women compared with all men, and these gender differences were also present in healthy subjects and diabetic patients separately. There was no significant gender difference with respect to plasma resistin and TNF- α , except for somewhat higher resistin levels in diabetic women.

Table 1 Clinical characteristics, mean carotid artery intima-media thickness (IMT) and plasma C-reactive protein in type 2 diabetic patients and control subjects.

	Type 2 diabetic patients (<i>n</i> =84)		Control subjects (<i>n</i> =85)		<i>P</i> value DM vs C	<i>P</i> value M vs F
	Male (<i>n</i> =52)	Female (<i>n</i> =32)	Male (<i>n</i> =46)	Female (<i>n</i> =39)		
Age (years)	60 (51–65)	60 (56–67)	57 (51–66)	50 (44–59)	0.062	0.059
IMT (mm)	0.88 (0.78–1.04)	0.80 (0.74–0.95)	0.84 (0.75–0.91)	0.72 (0.68–0.79) [†]	0.002	<0.001
BMI (kg/m ²)	27.0 (24.8–30.9)	29.7 (25.4–33.6)	25.8 (23.6–27.7)	24.7 (22.3–26.9)	<0.001	0.86
Systolic blood pressure (mmHg)	142 (123–155)	146 (133–167)	128 (118–139)	128 (118–146)	<0.001	0.44
Diastolic blood pressure (mmHg)	85 (78–92)	86 (82–96)	82 (78–88)	78 (72–90)	0.004	0.91
MAP (mmHg)	103 (95–111)	107 (100–117)	97 (92–105)	99 (87–110)	<0.001	0.65
Fasting glucose (mmol/l)	8.3 (7.4–9.3)	8.5 (7.2–11.0)	5.8 (5.1–6.2)	5.4 (5.0–6.1)	<0.001	0.12
HbA _{1c} (%)	6.6 (6.1–7.1)	6.6 (6.1–7.6)	5.3 (5.0–5.6)	5.2 (4.9–5.6)	<0.001	0.23
Plasma insulin (mU/l)	8.9 (5.6–12.9)	12.3 (9.2–17.7) [†]	6.9 (4.6–9.0)	5.8 (4.6–7.8)	<0.001	0.45
HOMA _{ir} (mU $\times$ mmol/(l ² $\times$ 22.5))	3.58 (2.11–5.54)	4.38 (3.08–7.74)*	1.65 (1.10–2.58)	1.42 (1.03–1.97)	<0.001	0.99
Plasma CRP (mg/l)	1.67 (0.67–3.89)	2.17 (1.45–5.36)*	0.94 (0.48–2.08)	1.47 (0.54–2.51)	0.003	0.061

Data in medians (interquartile ranges). DM, diabetes; C, control; M, male; F, female; BMI, body mass index; HOMA_{ir}, homeostasis model assessment; CRP, C-reactive protein; MAP, mean arterial pressure. * $P < 0.05$; [†] $P < 0.01$; [‡] $P < 0.001$ from male type 2 diabetic patients or male control subjects.

Table 2 Plasma lipids, apolipoproteins (apos), adiponectin, leptin resistin and tumor necrosis factor- α (TNF- α) levels in type 2 diabetic patients and control subjects.

	Type 2 diabetic patients (n=84)		Control subjects (n=85)		P value DM vs C	P value M vs F
	Male (n=52)	Female (n=32)	Male (n=49)	Female (n=39)		
Plasma cholesterol (mmol/l)	5.14 (4.46–5.75)	5.53 (5.05–6.13)*	5.71 (4.93–6.48)	5.47 (5.03–6.43)	0.021	0.12
Non-HDL cholesterol (mmol/l)	4.00 (3.37–4.78)	4.13 (3.72–4.57)	4.30 (3.42–5.12)	3.94 (3.51–4.49)	0.56	0.44
HDL cholesterol (mmol/l)	1.22 (1.06–1.45)	1.46 (1.20–1.73) [†]	1.54 (1.29–1.71)	1.88 (1.51–2.06) [‡]	<0.001	<0.001
Plasma triglycerides (mmol/l)	1.83 (0.96–2.47)	1.54 (1.21–2.01)	1.40 (0.97–2.10)	1.16 (0.87–1.72)	0.037	0.16
Plasma apo A-I (g/l)	1.25 (1.13–1.40)	1.50 (1.25–1.70) [‡]	1.37 (1.23–1.49)	1.52 (1.39–1.65) [‡]	0.003	<0.001
Plasma apo B (g/l)	0.89 (0.76–1.07)	0.94 (0.83–1.06)	0.99 (0.80–1.21)	0.86 (0.73–0.95)*	0.72	0.38
Plasma adiponectin (mg/l)	12.3 (9.0–18.8)	16.6 (13.3–28.1)*	16.3 (12.6–24.7)	37.4 (20.1–52.2) [‡]	<0.001	<0.001
Plasma leptin (μ g/l)	4.8 (3.3–10.7)	30.1 (17.6–34.4) [‡]	3.8 (2.4–5.8)	12.3 (5.9–26.9) [‡]	0.023	<0.001
Plasma resistin (μ g/l)	13.8 (9.3–19.8)	17.2 (12.4–24.7)*	11.5 (8.6–17.5)	11.8 (8.5–14.1)	0.003	0.56
Plasma TNF- α (ng/l)	3.9 (2.5–5.2)	3.5 (3.0–4.5)	3.1 (2.4–4.3)	3.0 (2.4–3.5)	0.003	0.27

Data in medians (interquartile ranges). DM, diabetes; C, control; M, male; F, female; HDL, high-density lipoproteins. * $P < 0.05$; [†] $P < 0.01$; [‡] $P < 0.001$ from male type 2 diabetic or male control subjects.

In diabetic patients treated with oral hypoglycemic drugs, HbA_{1c} (6.7 (6.3–7.6) %) was higher and plasma adiponectin levels were lower (13.4 (9.1–18.6) mg/l) than in patients treated with diet alone (HbA_{1c} 6.1 (5.4–6.6) %, $P < 0.001$; adiponectin 17.7 (12.9–30.8) mg/l, $P = 0.005$). In diabetic patients treated with antihypertensive drugs, MAP (107 (102–115) mmHg) was not significantly different, whereas plasma adiponectin was lower (11.5 (9.1–18.7) compared with patients not using antihypertensive drugs (101 (93–114) mmHg, $P = 0.09$); 15.7 (12.1–27.9) mg/l, $P = 0.04$).

In diabetic patients, age- and sex-adjusted IMT was positively related to HOMA_{ir} ($\beta = 0.22$, $P = 0.023$) and plasma triglycerides ($\beta = 0.27$, $P = 0.005$), and inversely with plasma adiponectin ($\beta = -0.27$, $P = 0.006$). IMT was not significantly related to MAP, as hemodynamic factor, BMI, plasma CRP, non-HDL cholesterol, HDL cholesterol, plasma leptin, resistin and TNF- α (all $P > 0.05$). In control subjects, age- and sex-adjusted IMT was associated positively with BMI ($\beta = 0.27$, $P = 0.002$), HOMA_{ir} ($\beta = 0.23$, $P = 0.015$), plasma triglycerides ($\beta = 0.16$, $P = 0.047$) and again inversely with plasma adiponectin ($\beta = -0.22$, $P = 0.031$). No significant relationships of IMT with the MAP, plasma CRP, other lipid variables, plasma leptin, resistin or TNF- α were found (all $P > 0.05$). In both groups, the relationship of IMT with plasma adiponectin remained significant (diabetic patients: $\beta = -0.22$, $P = 0.037$; control subjects: $\beta = -0.19$, $P = 0.045$) after adjustment for HOMA_{ir}. Furthermore, in both men and women, IMT adjusted for age and diabetic status was associated inversely with plasma adiponectin (men: $\beta = -0.25$, $P = 0.008$; women: $\beta = -0.35$, $P = 0.002$).

In order to disclose which variables could explain the higher IMT in diabetic patients and in men (Table 1), several multiple linear regression analysis models were made using the combined data from the diabetic and the control subjects (Table 3). In model 1, which only includes age, presence of diabetes and gender, it was

confirmed that in addition to age, IMT was positively and independently associated with diabetic state and with male gender. In model 2, which also includes other clinical factors (MAP, BMI, and HOMA_{ir}) and plasma CRP as possible determinants of IMT, it was found that the effect of the diabetic state on IMT was attributable to HOMA_{ir}, but not significantly to MAP, BMI or CRP. In this model, the independent effects of age and gender on IMT remained present. In model 3, which includes lipid variables, the relationship of IMT with diabetes was explained by plasma triglycerides, whereas the relationship of IMT with age and male gender again remained significant. Similar results were obtained in an alternative model, which includes plasma apo B and apo A-I levels instead of non-HDL cholesterol and HDL cholesterol (not shown). In this model, IMT was again associated with plasma triglycerides, without independent contributions from the presence of diabetes, plasma apo B or apo A-I levels (not shown). In model 4, the possible relationships with plasma adipokine levels were examined. In this model, IMT was only significantly related to age and plasma adiponectin without independent contributions from plasma leptin, resistin and TNF- α . In model 4, the effects of the diabetic state and gender on IMT were no longer significant. Finally, model 5 was made, which includes age, gender, the presence of diabetes as well as those variables from models 2–4 with which IMT was significantly associated. This analysis demonstrated that in addition to age, IMT was only independently related to plasma adiponectin and triglycerides. These relationships remained significant (adiponectin, $\beta = -0.18$, $P = 0.017$; triglycerides, $\beta = 0.14$, $P = 0.042$) when the use of oral glucose lowering drugs ($P = 0.44$) and of antihypertensive medication ($P = 0.17$) were accounted for. Similar results were obtained in a model including all variables used in models 1–4 (not shown). Taken together, these analyses thus disclosed that the higher IMT associated with diabetes was attributable to lower plasma adiponectin levels and higher triglyceride levels, and that the

Table 3 Multiple linear regression models showing statistical determinants of mean carotid artery intima-media thickness (IMT) in type 2 diabetic patients and control subjects ($n=169$).

	Model 1		Model 2		Model 3		Model 4		Model 5	
	β	P value	β	P value	β	P value	β	P value	β	P value
Age	0.45	<0.001	0.42	<0.001	0.47	0.001	0.49	<0.001	0.48	<0.001
			0.45	<0.001	0.49	<0.001	0.52	<0.001	0.52	<0.001
DM (yes vs no)	0.13	0.049	-0.01	0.86	0.09	0.22	0.05	0.51	-0.01	0.96
Gender (M vs F)	0.20	0.002	0.22	<0.001	0.18	0.01	0.11	0.51	0.15	0.031
			0.22	0.001	0.19	0.004				
MAP			0.10	0.16						
BMI			-0.02	0.81						
Ln HOMA _{ir}			0.23	0.015					0.11	0.22
			0.23	<0.001						
Ln CRP			-0.04	0.61						
Non-HDL cholesterol					-0.03	0.89				
Ln triglycerides					0.23	0.01			0.12	0.099
					0.23	<0.001			0.15	0.038
HDL cholesterol					-0.02	0.83				
Ln adiponectin							-0.29	0.002	-0.14	0.092
							-0.29	0.000	-0.23	0.001
Ln leptin							-0.01	0.87		
Ln resistin							-0.10	0.15		
Ln TNF- α							0.12	0.061		

β , standardized regression coefficient; DM, type 2 diabetes mellitus; F, female; M, male; MAP, mean arterial pressure. Independent statistical determinants ($P<0.05$) are shown in bold. Variables included in the models: Model 1: age, diabetes, and gender. Model 2: age, diabetes, gender, clinical variables, and C-reactive protein (CRP). Model 3: age, diabetes, gender, and lipid variables. Model 4: age, diabetes, gender, and adipokines. Model 5: age, diabetes, gender, and variables with which IMT is independently associated as derived from models 2–4.

higher IMT in men could be explained by lower plasma adiponectin levels.

Discussion

We documented higher IMT in type 2 diabetic patients, without clinically manifest cardiovascular disease and severe microvascular complications, compared with healthy control subjects. Plasma adiponectin levels were lower, whereas leptin, resistin and TNF- α levels were higher, coinciding with increased adiposity, insulin resistance, higher plasma triglycerides, lower HDL cholesterol and higher CRP levels in the diabetic group. The main new finding of this report is that the increased IMT in diabetes was found to be largely attributable to lower plasma adiponectin, to which IMT was related independently of conventional cardiovascular risk factors, insulin resistance and CRP. Furthermore, our study demonstrates that lower plasma adiponectin concentrations in men may largely account for the gender difference of IMT. No significant relationship of IMT with plasma levels of leptin, resistin or TNF- α was found.

The diabetic state is hallmarked by abnormalities in a cluster of strongly interrelated clinical and metabolic factors that are likely to contribute to increased atherosclerosis susceptibility (39–41). To determine the extent to which IMT was associated with plasma adipokine levels, independently of conventional risk factors, insulin resistance and low grade inflammation,

we categorized possible determinants of IMT into clinical variables, including HOMA_{ir} and CRP, lipid variables and plasma adipokine levels. Using this somewhat arbitrary approach, which has the advantage that insight is provided into the relative importance of the various factors to which IMT is related, we first found that IMT was associated with either insulin resistance (model 2) or plasma triglycerides (model 3), apart from its expected strong relationship with age and association with gender (36). The effect of mean arterial pressure on IMT did not reach significance. Notably, despite plasma CRP being considered to be a predictor of coronary heart disease risk (26, 42), there was no independent relation of IMT with this inflammatory marker, in keeping with a number of previous studies but in contrast to other reports ((43), overviewed in (44)). Furthermore, in the present study, IMT was unrelated to non-HDL cholesterol and plasma apo B levels. This is probably due to the exclusion of subjects using lipid-lowering drugs, which was done in order to obviate effects of such treatment on IMT and plasma CRP. This selection resulted in preferential participation of diabetic patients with a relatively favorable lipid profile. Of the adipokines measured in our study, IMT was only independently related to plasma adiponectin (model 4), at least suggesting that the relationship of IMT with plasma adiponectin is much stronger than that with leptin, resistin and TNF- α . The effect of the diabetic state on IMT disappeared when either insulin resistance (model 2) or plasma triglycerides (model 3) was taken into account, concordant with previously

described effects of these diabetes-associated abnormalities on IMT (5, 7). Importantly, both in the diabetic and control subjects separately and in the combined subjects (model 5), IMT was found to be independently related to plasma adiponectin but not to insulin resistance. The inverse relationship of plasma adiponectin with IMT agrees with several other studies (27–29, 45, 52), although in men this relationship was weakened after adjustment for cardiovascular risk factors (52). In women with variable degrees of glucose tolerance, the relationship of IMT with plasma adiponectin disappeared after adjustment for plasma insulin (45). In our study, IMT was inversely related to plasma adiponectin in both sexes, whereas this relationship was only found in men in another report (52). In Japanese type 2 diabetic patients, IMT was unrelated to plasma adiponectin (31). The reason for this apparent discrepancy is not clear, but may be related to different patient selection, e.g. inclusion of patients with complications, differences in drug treatment, e.g. insulin use, or to genetic differences.

As expected, plasma adiponectin was lower in men compared with women (11, 34, 35), and this gender difference is most likely attributable to androgen rather than to estrogen/progestagen status (46). In the current survey, this gender dimorphism of plasma adiponectin was present irrespective of diabetic status, whereas an earlier study showed no difference in this adipokine between diabetic men and women (34). Notably, an independent gender effect on IMT remained present in our models accounting for insulin resistance (model 2) and plasma triglycerides (model 3) and disappeared only in models that included plasma adiponectin (models 4 and 5). Thus, the present report demonstrates for the first time that lower plasma adiponectin levels, at least in part, explain the higher IMT in men compared with women, and shows that this relationship is independent of insulin resistance and lipid levels.

Our cross-sectional study has possible limitations due to the selection of patients with relatively low plasma lipids, the use of oral blood glucose lowering and antihypertensive medication by many diabetic patients, and the predominant inclusion of middle-aged subjects. Plasma adiponectin was lower in patients receiving medical treatment for glycemic control and high blood pressure, whereas in these patients HbA_{1c} was higher and blood pressure was not lower compared with patients treated with diet alone. This suggests that the use of oral glucose lowering and antihypertensive drugs is associated with adverse consequences of diabetes, rather than with treatment effects *per se*. Therefore, it is difficult to draw conclusions about the possible confounding effect of these medications on the relationship of IMT with plasma adiponectin, although this association remained present after adjustment for medication. Finally, we measured plasma total adiponectin, rather than its high molecular form,

which may be regarded as the most active form of this adipokine.

An increasing body of experimental and clinical evidence supports the possibility that adiponectin may be implicated in the pathogenesis of insulin resistance and in the development of atherosclerosis (47, 48). Adiponectin also plays a role in the metabolism of HDL and triglyceride-rich lipoproteins (12, 49). Recent intervention studies have demonstrated that regression of IMT by thiazolidinedione treatment coincides with an increase in plasma adiponectin (53, 54). The present study agrees with the contention that lower plasma adiponectin levels may be involved in enhanced subclinical atherosclerosis associated with type 2 diabetes mellitus. Obviously, our cross-sectional study cannot provide evidence for a causal relationship.

In conclusion, increased IMT in type 2 diabetes mellitus is associated with lower plasma adiponectin and higher triglycerides. The gender effect on IMT is related to lower plasma adiponectin levels in men.

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