Clustering of Insulin Resistance With Vascular Dysfunction and Low-Grade Inflammation in Type 2 Diabetes

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Vascular dysfunction, low-grade inflammation, insulin resistance, and impaired fibrinolysis have each been reported to be present in type 2 diabetes, but their relationships, and the role of obesity, have not been investigated. We measured insulin sensitivity (euglycemic clamp), forearm blood flow responses to graded local acetylcholine (Ach) and sodium nitroprusside (SNP) infusions, plasma concentrations of tumor necrosis factor (TNF)-α, interleukin (IL)-6, von Willebrand factor (vWF), plasminogen activator inhibitor (PAI)-1, tissue plasminogen activator (tPA), and high-sensitivity C-reactive protein (hs-CRP) in 81 diabetic patients. When patients were stratified by insulin resistance, more severe insulin resistance was associated ($P < 0.05$) with overweight, central fat distribution, hypertension, and dyslipidemia (with similar sex distribution, age, fasting plasma glucose, and HbA_{1c}). With regard to vascular function, both endothelium-dependent (Ach) (−22, −40, and −52%; $P < 0.0001$) and -independent (SNP) (−3, −7, and −27%; $P < 0.02$) vasodilatation were progressively reduced across insulin resistance tertiles. In multivariate analysis, inflammatory markers (IL-6, hs-CRP, and TNF-α) were independently associated with insulin resistance and fasting glycemia, fibrinolytic markers PAI-1 and tPA with insulin resistance and central fat distribution, and vascular indexes (vWF, Ach, and SNP vasodilation) with insulin resistance and obesity or cytokines (TNF-α or IL-6). In type 2 diabetes, insulin resistance is associated with vascular dysfunction/damage, impaired fibrinolysis, and low-grade inflammation independently of obesity and poor glycemic control. *Diabetes* 55:1133–1140, 2006

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Ach, acetylcholine; EGP, endogenous glucose production; ELISA, enzyme-linked immunosorbent assay; FBF, forearm blood flow; FFA, free fatty acid; hs-CRP, high-sensitivity C-reactive protein; IL, interleukin; L-NMMA, L-monomethyl-*N*-arginine; PAI, plasminogen activator inhibitor; SNP, sodium nitroprusside; TNF, tumor necrosis factor; tPA, tissue plasminogen activator; vWF, von Willebrand factor.

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Insulin resistance is generally regarded as relevant to cardiovascular risk (1). Prospective studies, mostly in nondiabetic subjects (2–7), have provided evidence in support of this concept. In large population samples, insulin resistance and associated metabolic abnormalities have been estimated to be present in 60–80% of patients with type 2 diabetes (8,9). As type 2 diabetes per se is a powerful cardiovascular risk factor, neither insulin resistance nor associated metabolic abnormalities have clearly proven to be strong risk modifiers in diabetic patients, possibly in part because insulin resistance has not been measured directly (8–12).

Vascular dysfunction, inflammation, and impairment in fibrinolysis are established cardiovascular risk factors. In type 2 diabetes, however, no study has evaluated whether insulin resistance, when measured directly, clusters with these abnormalities. In normal (13) and obese (14) subjects and in patients with essential hypertension (15), the vascular response to endothelium-dependent vasodilators has been reported to be unrelated to insulin resistance. Previous studies in type 2 diabetes (Table 1) have generally found an impairment in endothelium-dependent vasodilatation; endothelium-independent dilatation, however, has been variably reported to be reduced or unaffected. More importantly, no previous study has simultaneously measured insulin resistance, and no correlation has been found between endothelial dysfunction of small forearm arteries and degree of metabolic control, disease duration, and other classical cardiovascular risk factors. One study (16) has reported an association between vascular dysfunction and elevated bood pressure and another (17) an association with abundance of small dense LDL cholesterol particles. Vascular endothelial dysfunction has been described in first-degree relatives of diabetic patients, among whom it was more prominent in those with insulin resistance (18). Studies in transgenic animals indicate that vascular endothelial dysfunction can be induced by disrupting the intracellular insulin signaling pathway (19) and, conversely, that an insulin-resistant phenotype can be induced by experimental deprivation of endothelial nitric oxide (NO) synthase (20). Taken together, these findings suggest that in type 2 diabetes, insulin resistance and vascular endothelial dysfunction may be linked with one another.

With regard to low-grade inflammation, ex vivo studies (21) have shown that insulin has anti-inflammatory properties and that these are blunted in insulin-resistant indi-

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TABLE 1
Studies of vascular function of resistance arteries in patients with type 2 diabetes

Year	First author (ref. no.)	Patients/control subjects (n)	Vascular response	Tested covariates	Significant covariates (r value)
1992	McVeigh GE (35)	29/21	Ach (−), SNP (−)	DD, FPG, A1C, LDL	—
1996	Williams SB (36)	21/23	Ach (−), SNP (=)	DD, FPG, A1C, LDL, HDL, TG, BMI, FPI	—
1996	Watts GF (37)	29/18	Ach (−), SNP (−)	NA	NA
1998	Hogikyan RV (38)	17/20	Ach (−), SNP (=)	DD, FPG, A1C, LDL, HDL, TG, BMI, FPI, S_i, MAP	—
1999	Makimattila S (17)	30/12	Ach (−), SNP (=)	DD, FPG, A1C, LDL, HDL, TG, BMI, MAP, AER, LDL size, TRAP	LDL size ($r = 0.49$)
2000	Heitzer T (39)	23/12	Ach (−), SNP (=)	NA	NA
2000	Chowienczyk PJ (40)	10/10	Ach (−), SNP (=)	NA	NA
2000	Preik M (16)	22/20	Ach (−), SNP (−)	A1C, AER, MAP	MAP ($r = -0.65$)
2001	Mather KJ (50)	44/0	Ach, SNP	DD, FPG, A1C, LDL, HDL, TG, BMI	—
2002	van Etten RW (51)	23/21	Ach (−), SNP (−)	NA	NA

(−), attenuated response vs. control subjects; (=), normal response vs. control subjects; AER, albumin excretion rate; DD, duration of diabetes; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HDL, HDL cholesterol; LDL, LDL cholesterol; MAP, mean arterial blood pressure; NA, not available; S_i, intravenous glucose tolerance test–derived insulin sensitivity index; TG, fasting triglycerides; TRAP, total reducing ability of the plasma. Ach may also refer to metacholine.

viduals. Moreover, in obese subjects, evidence of low-grade inflammation is present only in the insulin-resistant subgroup (22). Experimentally induced low-grade inflammation in healthy individuals causes vascular endothelial dysfunction (23) and impairs glucose homeostasis (24).

The present study was undertaken to establish whether insulin resistance in type 2 diabetes signals the presence of vascular dysfunction (endothelial and/or smooth muscle) and low-grade inflammation. To this end, we simultaneously obtained multiple measurements of vascular function and of serum markers of vascular activation and inflammation in a large group of type 2 diabetic patients in whom insulin resistance was directly measured by the clamp method.

RESEARCH DESIGN AND METHODS

Eighty-one type 2 diabetic patients were recruited at three centers (London, Pisa, and Naples); 25 nondiabetic subjects matched for sex, age, and BMI served as the control group. Exclusion criteria were BMI ≥35 kg/m^2; clinically significant renal or hepatic disease, anemia, diabetic retinopathy or symptomatic neuropathy, cardiac failure (New York Heart Association grades III and IV), angina pectoris, or recent myocardial infarction; severe uncontrolled hypertension; and current treatment with nitrates, anticoagulants, calcium channel blockers, or vitamins.

After the screening visit, patients began a 4-week wash-out period from antidiabetic oral agents, after which they all underwent 24-h ambulatory blood pressure monitoring on 1 day and both the vascular and metabolic test procedures on the subsequent day. Concomitant treatments (lipid-lowering and antihypertensive agents) were not assumed on the study day. The study was approved by the ethics review committee of each participating institution.

Vascular reactivity (by the perfused forearm technique) and insulin sensitivity (by the euglycemic-hyperinsulinemic clamp technique) were measured during a single study session. To reduce between-center variability, the procedures were carefully standardized and the same strain-gauge plethysmograph (EC4; Hokanson, Bellevue, WA) and test chemicals were used in all centers. A teflon cannula (20G) was inserted percutaneously into the brachial artery of the nondominant arm under local anesthesia (2% lidocaine) and used for drug infusion, intra-arterial blood pressure/heart rate monitoring, and blood sampling. Another catheter was inserted into an antecubital vein for systemic insulin and glucose infusion. A baseline sample for high-sensitivity C-reactive protein (hs-CRP), interleukin (IL)-6, tumor necrosis factor (TNF)-α, von Willebrand factor (vWF), HbA_{1c} (A1C), plasminogen activator inhibitor (PAI)-1 antigen, and tissue plasminogen activator (tPA) antigen was taken (in diabetic patients only), and thereafter a primed (0.05 mg/kg)-continuous (0.05 mg · min^{-1} · kg^{-1}) intravenous infusion of [6,6-^{2}H]glucose was started (time −180 min) and maintained until the end of the clamp. Once forearm blood flow (FBF) had stabilized (at least 30 min after cannulation), FBF was measured at the end of each of five 5-min steps of intra-arterial acetylcholine (Ach) infusion (0.15, 0.45, 1.5, 4.5, and 15 μg · min^{-1} · dl^{-1} of forearm tissue) and at the end of three 5-min steps of intra-arterial infusion of sodium nitroprusside (SNP) (1, 2, and 4 μg · min^{-1} · dl^{-1}). Forearm volume was measured from the water displacement induced by the immersion of the forearm in a graduated cylinder. Three blood samples were taken to measure plasma [6,6-^{2}H]glucose enrichment, glucose, insulin, and free fatty acid (FFA) concentrations at 10, 5, and 1 min before the start of the clamp (time 0) using a primed-continuous (40 mU/min per m^2) infusion of exogenous insulin. Glucose infusion was started only when the plasma glucose concentration reached 6.0 mmol/l. Blood samples for [6,6-^{2}H]glucose enrichment, insulin, and FFA were taken every 20 min thereafter. In the diabetic patients, FBF was also measured at time 0, 60, and 120 min and then repeated at 125 min after a 5-min intra-arterial infusion of L-monomethyl-*N*-arginine (L-NMMA) at the rate of 100 μg · min^{-1} · dl^{-1}.

Assays. Plasma and infusate [6,6-^{2}H]glucose enrichment determinations were done in the same laboratory (Pisa). Plasma [6,6-^{2}H]glucose enrichment was measured in arterialized blood samples following deproteinization using cold methanol as previously described (25). All samples from the same study were processed and assayed in the same run. A1C, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, insulin, FFAs, and routine blood chemistry were assayed at a central laboratory. Samples were stored at −70°C. PAI-1 antigen was measured using an enzyme-linked immunosorbent assay (ELISA), detecting PAI-1 complexed to tPA as well as free active and latent PAI-1 (Tint-Elize PAI-1; Biopool, Umeå, Sweden), with intra- and interassay coefficients of variation (CVs) of 2.0 and 6.8%, respectively. IL-6 and TNF-α were measured using high-sensitivity commercial ELISA kits (Quantikine HS;

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TABLE 2
Clinical characteristics

	Control subjects	P*	Type 2 diabetic patients			P†
			Group IS	Group I	Group IR	
Center (London/Pisa/Naples)	0/25/0	—	6/16/5	9/17/2	15/10/1	NS
Women (n)	5	NS	5	3	5	NS
Age (years)	54 ± 2	0.036	56 ± 2	59 ± 1	59 ± 2	NS
BMI (kg/m^2)	27.7 ± 0.7	NS	26.9 ± 0.5	27.6 ± 0.6	30.2 ± 0.6	<0.001
WHR	0.94 ± 0.02	NS	0.92 ± 0.02	0.95 ± 0.01	0.95 ± 0.01	0.048
Diabetes duration (years)	—	—	6 ± 1	7 ± 1	5 ± 1	NS
FPG (mmol/l)	5.2 ± 0.4	<0.001	9.9 ± 0.4	9.7 ± 0.5	10.8 ± 0.5	NS
A1C (%)	—	—	7.8 ± 0.2	7.7 ± 0.2	8.0 ± 0.2	NS
M value (μmol · min^{-1} · kg FFM^{-1})	41.1 ± 3.2	<0.001	45.3 ± 1.5	27.5 ± 0.8	16.5 ± 0.7	<0.001
FPI (pmol/l)	79 ± 10	<0.001	80 ± 11	94 ± 13	96 ± 8	0.018
Fasting FFA (μmol/l)	428 ± 38	NS	419 ± 31	425 ± 35	587 ± 51	0.005
Total cholesterol (mmol/l)	5.00 ± 0.17	NS	4.98 ± 0.18	4.91 ± 0.22	5.13 ± 0.16	NS
HDL cholesterol (mmol/l)	1.19 ± 0.09	NS	1.22 ± 0.05	1.11 ± 0.06	1.12 ± 0.04	0.092
Triglycerides (mmol/l)	1.27 ± 0.12	0.005	1.38 ± 0.15	2.12 ± 0.52	2.33 ± 0.34	0.054
Fibrate treatment (n)	0	<0.001	1	4	3	NS
24-h MBP (mmHg)	93 ± 3	NS	94 ± 3	92 ± 3	93 ± 3	NS
Hypertension (n)	10	NS	6	12	14	0.050
Smokers (n)	5	NS	6	4	5	NS

Data are means ± SE. Group IS, insulin-sensitive type 2 diabetic subjects; group I, type 2 diabetic subjects with intermediate insulin sensivity; group IR, insulin-resistant type 2 diabetic subjects. *P value for control subjects vs. all type 2 diabetic subjects (ANOVA for continuous variables and χ^2 test for frequencies); †P value for group IS vs. group I vs. group IR (ANOVA for continuous variables and χ^2 test for frequencies). FFM, fat-free mass; MBP, mean blood pressure; WHR, waist-to-hip ratio.

R&D Systems, Oxon, U.K.). The IL-6 assay had a detection limit of 0.09 pg/ml and intra- and interassay CVs of 5.3 and 9.2%, respectively. The TNF-α assay had a detection limit of 0.1 pg/ml and intra- and interassay CVs of 6.9 and 8.4%, respectively. CRP was measured with a high-sensitivity assay (High Sensitive UL-CRP; Wako Chemical, Neuss, Germany). vWF (Dako, Glostrup, Denmark) and tPA (Imulyse; Biopool, Umeå, Sweden) were both measured by ELISA with intra- and interassay CVs of 2.8 and 4.7% for vWF and 7.0 and 10.0% for tPA, respectively.

Calculations. All plethysmographic trace recordings were centrally analyzed by the same investigator (A.N.), who was blinded to the metabolic results. FBF values were expressed as both absolute values (milliliters per minute per deciliter of forearm tissue) and percent changes from baseline. Since a slight but consistent drop in intra-arterial blood pressure was observed in some patients during the highest local SNP infusion, conductance (i.e., blood flow × 100/mean blood pressure) was used for the calculation of the percentage of SNP-induced increments above baseline. The effect of L-NMMA was calculated as percent change with respect to the 120-min FBF value.

Insulin sensitivity was calculated as the mean glucose infusion rate between 80 and 120 min into the clamp, corrected for the concomitant plasma glucose changes, and expressed per kilogram of fat-free mass (26). In the fasting state, glucose rate of appearance (i.e., endogenous glucose production [EGP]) was calculated as the ratio of [6,6-^{2}H]glucose infusion rate to the plasma [6,6-^{2}H]glucose enrichment at the end of the 180-min period of tracer equilibration. During the clamp, glucose rate of appearance was calculated using a two-compartment model (27). Steady-state EGP during the clamp was obtained as the difference between the mean glucose rate of appearance during the last 40 min and the mean exogenous glucose infusion during the same time period. Lean body mass was estimated by Hume's formula (28).

Statistical analysis. Data are expressed as means ± SE unless otherwise indicated. Between-group differences in mean values were analyzed by ANOVA (for continuous variables), Mann-Whitney or Kruskal-Wallis tests (for triglycerides and cholesterol levels), or χ^2 test (for proportions); adjustment for covariates was carried out by ANCOVA. Post hoc comparisons were analyzed by Fisher's test. Differences in dose-response curves between groups were tested by two-way ANOVA for repeated measures. Simple and multiple stepwise regression analysis were performed by standard methods.

RESULTS

As a group, diabetic patients were similar to control subjects with respect to age, sex distribution, degree of obesity and body fat distribution, prevalence of hypertension, and smoking habits (Table 2). Despite lipid-lowering treatment, diabetic patients had higher triglycerides than control subjects. Insulin sensitivity averaged 30 μmol · min^{-1} · kg fat-free mass^{-1}, with an eightfold range (9–74 μmol · min^{-1} · kg fat-free mass^{-1}), and was 29% lower ($P <$ 0.001) than in control subjects (41 ± 3 μmol · min^{-1} · kg fat-free mass^{-1}). When patients were stratified into tertiles of insulin sensitivity, the more sensitive ones (group IS) showed an M value similar to control subjects, whereas the patients with intermediate (group I) or low (group IR) insulin sensitivity showed average reductions of 33 and 60%, respectively. The more insulin-resistant patients had a higher degree of overall and central adiposity, higher serum triglyceride, and lower HDL cholesterol concentrations, as well as a higher prevalence of hypertension and current antihypertensive treatment, while sex distribution, age, disease duration, fasting glucose, A1C, serum total cholesterol, and smoking habits were similar (Table 2). In diabetic patients, insulin resistance was associated with fasting hyperinsulinemia, increased plasma FFA concentrations (Table 2) and EGP rates both in the fasting state (group IS 15.7 ± 0.3, group I 15.2 ± 0.5, and group IR 17.2 ± 0.8 μmol · min^{-1} · kg fat-free mass^{-1}; $P <$ 0.04 by ANOVA) and during the clamp (1.7 ± 1.1, 2.1 ± 0.6, and 5.3 ± 0.9 μmol · min^{-1} · kg fat-free mass^{-1}; $P <$ 0.04 by ANOVA). After controlling for BMI, only EGP remained closely associated with insulin resistance.

Baseline FBF was similar in the three diabetic groups (2.7 ± 0.3 ml · min^{-1} · dl^{-1}) and increased during the clamp by 35% on average ($P <$ 0.001). This increase was more rapid (already present at time 60 min) in group IS, and the percent change above baseline was progressively less pronounced across tertiles of insulin resistance (+43 ± 7, +36 ± 5, and +24 ± 9%; $P <$ 0.01 for the interaction term by ANOVA). The infusion of L-NMMA at the end of the clamp produced a similar degree of vasoconstriction in the three groups (group IS 20 ± 3, group I 15 ± 4, and group IR 22 ± 4%; P = NS).

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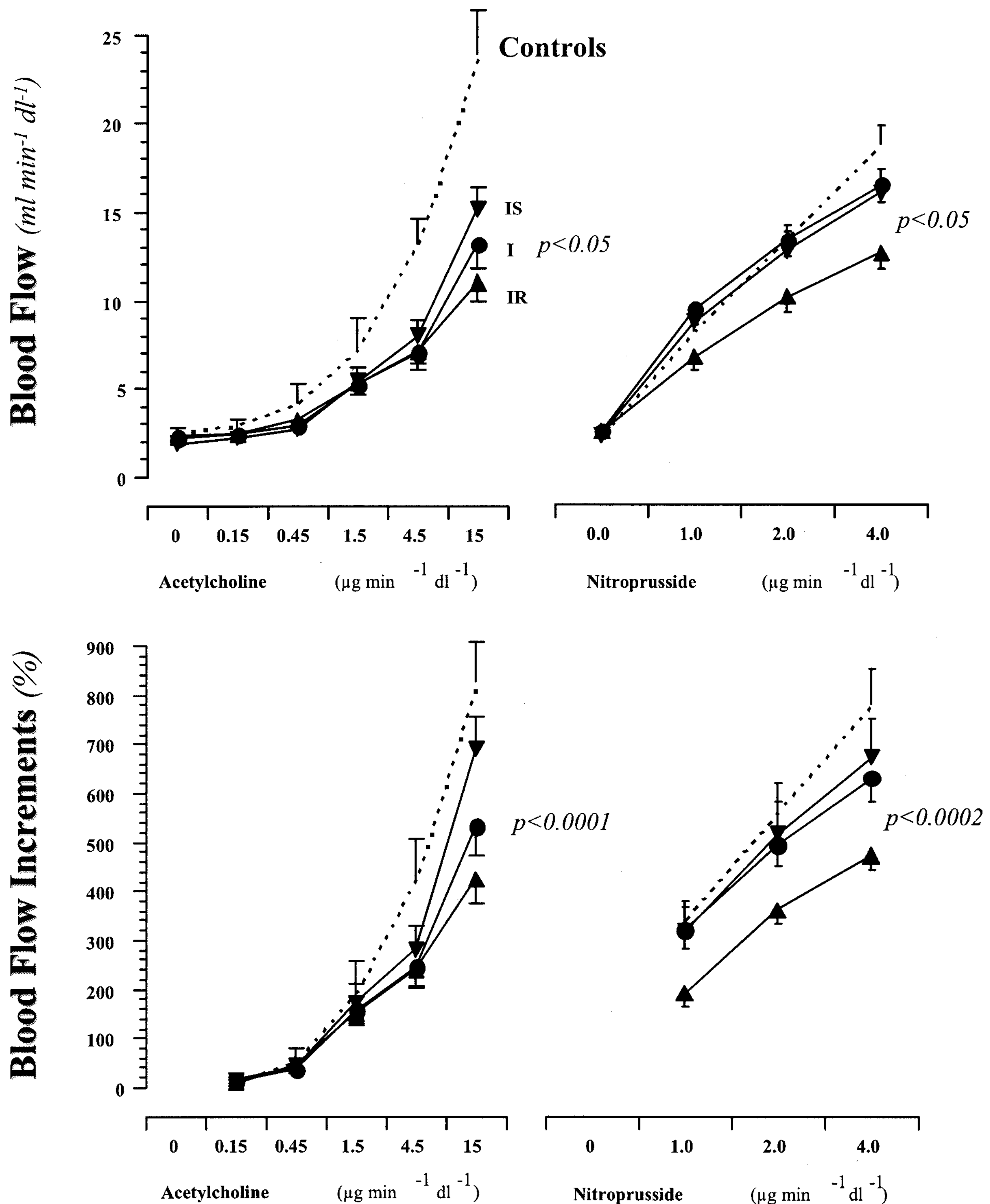

FIG. 1. FBF response (absolute values and percent increment above baseline) to the graded intra-arterial infusion of Ach and SNP in control subjects (Controls) and diabetic patients startified into tertiles of insulin sensitivity. *P* values indicate the statistical significance of the difference across tertiles, as estimated by two-way ANOVA for repeated measures. Data are means ± SE.

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Diabetic patients showed a reduction in endothelium-dependent (Ach) relaxation (Fig. 1) that was more evident when the vascular response was expressed as absolute values ($P < 0.0001$) than as percent increments from baseline ($P < 0.002$ by ANOVA) due to a slightly higher baseline FBF in control subjects (2.6 ± 0.2 vs. 2.1 ± 0.1 ml $\cdot$ min^{-1} $\cdot$ dl^{-1}; $P = 0.045$). The maximal Ach response was already reduced in group IS (−22%, $P < 0.04$) and progressively deteriorated in groups I (−40%, $P < 0.001$) and IR (−52%, $P < 0.001$). Combined, patients also showed a reduced response to SNP that was particularly low in the more insulin-resistant patients ($P < 0.05$ by Fisher's test). When normalizing the maximal Ach response to the maximal SNP response (Ach-to-SNP ratio), groups I and IR had a similar reduction in the Ach-to-SNP ratio (0.89 ± 0.09 and 0.88 ± 0.10, respectively) compared with both group

TABLE 3
Vascular and inflammatory markers by tertiles of insulin sensitivity

	Group IS	Group I	Group IR	*P*
PAI-1 (ng/ml)	10 ± 3	17 ± 3	26 ± 3	0.002
vWF (%)	87 ± 14	125 ± 13	149 ± 16	0.016
tPA (μg/l)	2.3 ± 1.02	5.4 ± 1.4	7.2 ± 1.7	0.061
Homocysteine (μmol/l)	11.1 ± 1.0	10.2 ± 0.8	11.4 ± 1.0	NS
TNF-α (pg/ml)	1.6 ± 0.3	1.6 ± 0.3	2.5 ± 0.2	0.020
IL-6 (pg/ml)	1.8 ± 0.3	1.9 ± 0.2	2.8 ± 0.3	0.005
hs-CRP (mg/l)	0.92 ± 0.15	1.50 ± 0.24	1.60 ± 0.27	0.052

Data are means ± SE. *P* values by ANOVA.

IS (1.28 ± 0.17, $P < 0.03$ by ANOVA and $P < 0.05$ by Fisher's test) and control subjects (1.34 ± 0.11, $P < 0.05$ by Fisher's test).

In the whole dataset, all measured markers, with the exception of homocysteine, showed a progressive increase across degree of insulin resistance (Table 3).

The relationships between circulating markers or vascular parameters and clinical/metabolic variables were sought in univariate association first and then in multivariate models including all significant univariate associations (Table 4). In general, serum inflammatory, fibrinolytic, and endothelial markers were intercorrelated. With regard to clinical/metabolic parameters, the inflammatory markers (IL-6, hs-CRP, and TNF-α) were independently associated with fasting glycemia (but not A1C) and insulin resistance. The fibrinolytic markers (PAI-1 and tPA) were both independently associated with central fat distribution; only PAI-1 was related to insulin resistance. Endothelial dysfunction/damage (as the response to Ach and serum vWF, respectively) and endothelium-independent vasodilatation (as the response to SNP) were significantly linked with insulin resistance, in association with an obesity index (BMI or waist-to-hip ratio) or a cytokine (TNF-α or IL-6).

TABLE 4
Correlation analysis

	Age, sex	DD, A1C, FPG	HT, TG	LDL, HDL, LDL/HDL	BMI, WHR	*M*	FPI, FFA	IL-6, TNF-α	Stepwise *r*
IL-6	NS, **0.34**	NS, NS, **0.28**	**0.34**, NS	NS, NS, NS	*0.20*, NS	**−0.44**	*0.20*, **0.24**	—, **0.40**	M (0.42), HT (0.49), FPG (0.54)
hs-CRP	NS, NS	NS, NS, **0.39**	*0.19*, NS	NS, NS, NS	*0.23*, NS	**−0.32**	*0.20*, *0.20*	**0.50, 0.40**	FPG (0.46), FPI (0.52), M (0.55)
TNF-α	NS, NS	NS, NS, *0.20*	NS, NS	NS, NS, NS	NS, NS	*−0.20*	NS, *0.19*	**0.40**, —	FPG (0.26), M (0.30)
PAI-1	*0.23*, NS	NS, NS, NS	*0.21*, NS	NS, NS, NS	NS, *0.22*	**−0.48**	NS, **0.41**	**0.52, 0.44**	IL-6 (0.51), M (0.59), FFA (0.62), WHR (0.64)
tPA	NS, NS	NS, NS, NS	NS, NS	NS, NS, NS	NS, NS	**−0.29**	NS, **0.37**	**0.38, 0.45**	TNF-α (0.37), WHR (0.49)
vWF	NS, NS	NS, NS, NS	NS, NS	NS, NS, NS	**0.26**, NS	**−0.36**	NS, *0.32*	**0.29**, NS	M (0.35), BMI (0.40)
Ach	NS, NS	NS, NS, NS	NS, NS	NS, NS, NS	NS, *0.20*	**0.43**	NS, NS	NS, **−0.31**	M (0.40), TNF-α (0.46), WHR (0.50)
SNP	NS, NS	NS, NS, NS	NS, NS	NS, NS, NS	*−0.19*, NS	**0.26**	NS, NS	**−0.27**, NS	M (0.28), IL-6 (0.33)
Ach-to-SNP	NS	NS, NS, NS	NS, NS	NS, NS, NS	NS, NS	**0.34**	NS, NS	NS, *−0.23*	M (0.32), TNF-α (0.36)

Dependent variables are listed vertically, and covariates are listed horizontally. Cells show univariate correlation coefficients (significant in bold, $P < 0.05$ for $r > 0.24$ and $P < 0.005$ for $r > 0.31$; borderline significant in italics, $P < 0.10$). The column to the far right gives the multivariate correlation coefficients as selected by the stepwise procedure. DD, duration of diabetes; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HDL, HDL cholesterol; HT, hypertension; LDL, LDL cholesterol; LDL/HDL, LDL-to-HDL cholesterol ratio; TG, serum triglycerides >1.7 mmol/l or fibrate treatment; WHR, waist-to-hip ratio.

DISCUSSION

Our group of type 2 diabetic patients was severely insulin resistant. Relative to sex-, age-, and BMI-matched nondiabetic control subjects, these patients had, on average, a 29% lower insulin sensitivity, with tertile mean *M* values corresponding to the 3rd, 18th, and 45th percentiles of 387 nondiabetic men and women matched for age and BMI (selected from the European Group for the Study of Insulin Resistance database) (29). As a clinical phenotype, insulin resistance was associated with overweight, central body fat distribution, hypertension, and dyslipidemia. The metabolic phenotype of insulin-resistant patients was characterized by hyperinsulinemia, endogenous glucose overproduction (and reduced sensitivity of this process to the effect of insulin), and enhanced lipolysis (as indexed by raised plasma FFA concentrations in the presence of hyperinsulinemia). As a vascular phenotype, insulin resistance was accompanied by a reduction in both endothelium-dependent and non–endothelium-dependent vasodilatation, impaired insulin-induced vasodilatation, and elevated plasma levels of markers of endothelial damage (vWF), low-grade inflammation (hs-CRP, TNF-α, and IL-6), and impaired fibrinolysis (PAI-1 and tPA). This complex of metabolic and vascular abnormalities was evident despite ongoing treatment of hypertension and dyslipidemia and was independent of diabetes duration and glycemic control.

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Obesity is a strong amplifier of insulin resistance (30); furthermore, epidemiologic and experimental data in nondiabetic subjects suggest a role of obesity and insulin resistance in inducing both low-grade inflammation (31–33) and vascular dysfunction (34). To determine the relative role of obesity and insulin resistance in the observed cluster of abnormalities, we carried out an extensive correlation analysis including all relevant confounders (Table 4). In our type 2 diabetic patient cohort, an independent impact of obesity (as the BMI or waist-to-hip ratio) was evident for the fibrinolytic parameters (PAI-1 and tPA) and for the parameters of endothelial dysfunction/damage (Ach-mediated vasodilatation and serum vWF), whereas insulin resistance was associated with virtually all measured functions (all the inflammatory markers, PAI-1 for the fibrinolytic markers, and all indexes of vascular dysfunction/damage) even after controlling for obesity. In addition, in all stepwise regression models, insulin resistance was the dominant correlate when obesity was a significant covariate. Thus, our results indicate a wider and stronger impact of insulin resistance than obesity on cardiovascular risk factors in patients with type 2 diabetes.

The vascular dysfunction observed in our diabetic patients was graded across severity of insulin resistance: for mild degrees of insulin resistance, only the endothelium was affected, whereas the patients with severe insulin resistance also showed abnormal vascular smooth muscle function. This interplay may explain why nonendothelial dysfunction has been found by some (35–37) but not other (17,38–41) previous studies in type 2 diabetic patients and is in accord with experimental studies demonstrating abnormal platelet and smooth muscle function in patients with insulin resistance (42).

With regard to endothelial dysfunction, previous studies in smaller groups of patients have indirectly suggested a link with insulin resistance by reporting that neither metabolic control nor the serum lipid profile or blood pressure predicted vascular endothelial dysfunction, whereas small dense LDL concentration, a variable that typically clusters with insulin resistance (17), did predict vascular endothelial dysfunction. To our knowledge, the present study is the first to report a direct association between insulin resistance and vascular endothelial dysfunction in type 2 diabetes, whereas such an association has not been observed in patients with obesity (14) or essential hypertension (15) or in normal subjects (13). This suggests a unique sensitivity of the endothelium of diabetic patients to the detrimental effect of insulin resistance; therefore, it is not surprising that treatment with tiazolidinediones has been shown to induce a concomitant amelioration of vascular endothelial function in type 2 diabetic (43) but not obese (14) patients.

The impaired vasodilatory ability was also evident in response to the clamp experiment, in which more insulin-sensitive subjects showed an earlier and relatively more pronounced blood flow increment, implying a relatively better preserved vascular response to systemic insulin infusion. The similar response to the subsequent L-NMMA infusion strongly suggests that this difference is not determined by an altered NO bioavailability; it must involve other pathways (β-adrenergic activation, hyperpolarization, intracellular calcium) or tissues (smooth muscle cells). This finding is not in contrast with the reduced endothelium-dependent response to pharmachologic doses of Ach; in fact, this tool explores vasodilatation close to maximal, elicited through muscarinic receptor–mediated pathways. Indeed, in our dataset, the correlation between the two different vascular responses (clamp/L-NMMA and Ach or SNP) were modest (r values <0.40) or not statistically significant.

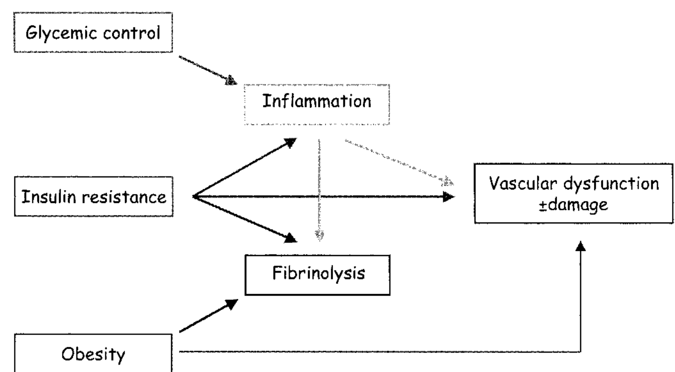

FIG. 2. Summary of the results of the multivariate analysis of the data in type 2 diabetic patients (see Table 4 and text for further explanation).

The mechanisms whereby insulin resistance may cause vascular dysfunction are multiple (44), but their relative importance in vivo is still undetermined. Raised circulating levels of molecules with toxic effects on the endothelium, such as asymmetric dimethylarginine and homocysteine, have been proposed as mediators (45–47). We did not find differences in plasma homocysteine levels between insulin-sensitive and -resistant patients (Table 3). Similarly, although acute large (two- to fourfold) elevations in circulating FFAs can impair vascular endothelial function (48), the lack of independent association between plasma FFA levels and the vascular responses in our data suggests that chronic milder elevations have a minor impact. Inflammation, on the other hand, did contribute to vascular dysfunction, but this effect was additive to that of insulin resistance. Of interest is that glycemic control was not an independent correlate of vascular dysfunction but was clearly related to all indexes of inflammation.

Higher levels of vWF were found in the insulin-resistant patients, and multivariate analysis showed that obesity (in the form of BMI) made a separate contribution to raise vWF concentrations. The shedding into the plasma of this endothelial molecule is considered an early marker of endothelial damage, but there is no evidence that raised vWF levels act as a mechanism of disease.

In conclusion, the presence of severe insulin resistance not only identified type 2 diabetic patients with multiple metabolic abnormalities but also those with endothelium-dependent and non–endothelium-dependent dysfunction, endothelial activation, impaired fibrinolysis, and low-grade inflammation. Furthermore, our multivariate analysis (summarized in Fig. 2) showed that vascular dysfunction and damage result from the action of three distinct metabolic defects (hyperglycemia, insulin resistance, and obesity) either directly or through two additional factors (low-grade inflammation and impaired fibrinolysis). The arrows in Fig. 2 illustrate the pattern of independent (from confounders and one another) associations emerging from the data. While cause-effect relationships cannot be inferred with certainty, the scheme does resonate with findings of experimental studies and does offer plausible working hypetheses. Thus, insulin resistance may affect vascular tissues directly, e.g., by modulating cellular ion

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metabolism and/or intracellular signaling pathways that cross talk with NO production or other vasomotor stimuli (49).

Of clinical relevance is that although most diabetic patients are insulin resistant, they are far from homogeneous for the insulin resistance trait: its severity varies widely between patients, and it is independent of disease duration and degree of metabolic control. The close link between vascular dysfunction and insulin resistance suggests that interventions directed at attenuating the metabolic defect, particularly if associated with weight reduction, could elicit multiple beneficial consequences for the vasculature.

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