The Effects of Troglitazone, an Insulin-Sensitizing Agent, on the Endothelial Function in Early and Late Type 2 Diabetes: A Placebo-Controlled Randomized Clinical Trial

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Activation of the peroxisome proliferator-activator receptor γ (PPARγ) improves insulin resistance and glycemic control in patients with diabetes. As PPARγ is expressed in the endothelial cell, we have investigated the effect of troglitazone, a PPARγ activator, on the endothelial function in people with type 2 diabetes in a 12-week, prospective, randomized, double-blinded clinical trial. We studied 87 type 2 diabetic patients who were divided into 3 groups. Group A consisted of 27 patients with recently diagnosed diabetes and no clinical manifestations of macrovascular disease; group B, 29 patients with long-term diabetes and no clinically evident macrovascular disease; and group C, 31 diabetic patients with documented macrovascular disease (cardiovascular, cerebrovascular, or peripheral vascular disease). High-resolution ultrasound images were used to measure the flow-mediated dilation (FMD, endothelium-dependent) and nitroglycerin-induced dilation (NID, endothelium-independent) in the brachial artery. Laser Doppler perfusion imaging was used to measure vasodilation in the forearm skin in response to iontophoresis of 1% acetylcholine (Ach, endothelium-dependent) and 1% sodium nitroprusside (NaNP, endothelium-independent). The plasma concentrations of von Willebrand factor (vWF), soluble intercellular adhesion molecule (sICAM), and soluble vascular cell adhesion molecule (sVCAM) were also measured as indicators of endothelial cell activation. The FMD improved in the troglitazone-treated patients in group A (7.72 ± 3.4 *v* 5.27 ± 2.0, *P* < .05 [exit visit *v* baseline, percent of increase in brachial artery diameter, mean ± SD]). The fasting insulin level also improved in this group (15.6 ± 10 *v* 19.7 ± 10, *P* < .05) and was strongly correlated to changes in FMD (*r* = -.73, *P* < .01). No changes were found in the FMD or the fasting insulin levels in the troglitazone-treated patients in groups B or C. The NID was not changed by troglitazone treatment in any of the 3 groups. Also, no differences were found in the microcirculation reactivity measurements or in the biochemical markers of endothelial dysfunction in all 3 groups. A small, but significant, improvement of the FMD was found in placebo-treated patients in group B, probably related to the low FMD levels at baseline in the patients (5.40 ± 3.0 *v* 4.36 ± 2.4, *P* < .05). We concluded that troglitazone treatment for 12 weeks improved endothelial function in the macrocirculation of patients with recently diagnosed type 2 diabetes and no clinical evidence of macrovascular disease. This improvement was strongly associated with the improvement of fasting plasma insulin concentrations.

PEOPLE WITH TYPE 2 diabetes have a high risk of developing micro and macrovascular complications, including coronary heart disease (CHD), the leading cause of death in this population.[1] The impact of CHD has recently been emphasized by data showing that the inherent risk for cardiovascular mortality in individuals with diabetes without a prior myocardial infarction, is as high as that in individuals without diabetes with a prior myocardial infarction.[2] The main recognized factors associated with the increased risk of macrovascular disease in diabetes include, dyslipidemia, hypertension, obesity, and coagulation/fibrinolysis abnormalities.

Endothelial dysfunction has been considered a key element in the development of atherosclerosis and has also been found to be associated with insulin resistance.[3,4] We previously reported that impaired vascular reactivity and endothelial activation occurs not only in type 2 diabetes, but even in those at risk for diabetes, representing earlier stages of the insulin resistance syndrome.[5] Recent evidence also suggests that hyperinsulinemia/insulin resistance are independent risk factors for the genesis of CHD and atherosclerosis.[6,7] As a result of the above, the prevailing opinion today is that the endothelial dysfunction that is associated with the metabolic syndrome is a contributing factor for the increased risk for cardiovascular disease, not only in diabetes, but also in the prediabetic stage.[8] Therefore, targeting insulin resistance can become an attractive approach not only to treating hyperglycemia, but also to reducing the risk for CHD.

The thiazolidinediones (TZDs) represent a unique class of drugs that directly reduce insulin resistance by enhancing insulin action in peripheral tissues.[9] Preliminary evidence also indicates that they can improve some of the cardiovascular risk factors associated with the insulin resistance syndrome, and that they have a direct effect in the vasculature.[10] However, initial studies that evaluated the effect of troglitazone, the only approved TZD for clinical practice at the time this study was initiated, on the vasculature of subjects with diabetes or insulin resistance have yielded conflicting results.[11-13]

The main objective of the present study was to investigate the effect of troglitazone on the vasculature of diabetic patients. To this end, we have studied type 2 diabetic patients with recently diagnosed diabetes, patients with long-term type 2

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diabetes without macrovascular disease, and patients with diabetes and macrovascular disease. The main reason for selecting these 3 groups is that we hypothesized that improvement of the endothelial function was more likely to occur in patients with recently diagnosed typed 2 diabetes without any serious microvascular and macrovascular disease as long-term diabetes may lead to nonreversible endothelial impairment.

SUBJECTS AND METHODS

Subjects

A total of 87 type 2 diabetic patients, age 25 to 70 years, were included in the study. The participants were divided in 3 groups based on duration of diabetes and the presence of macrovascular complications. The first group (A) consisted of 27 subjects with type 2 diabetes, diagnosed within the last 3 years and no evidence of macrovascular disease as described below. The second group (B) consisted of 29 subjects with type 2 diabetes diagnosed more than 3 years before participation in the study and no evidence of macrovascular disease. The third group (C) included 31 subjects with type 2 diabetes diagnosed more than 3 years before participation in the study and evidence of macrovascular disease. The decision to study the above 3 groups and the selection criteria for each group were determined prospectively before the study was initialized.

Diabetes was defined according to the recommendations of the American Diabetes Association (ADA) Expert Committee on the Classification and Diagnosis of Diabetes.[8] Macrovascular disease was diagnosed by the presence of a history of coronary artery disease, including information on positive exercise stress tests, unequivocal electrocardiogram (EKG) changes consistent with ischemia, prior coronary artery bypass grafts and/or coronary angioplasty, or a history of carotid artery disease requiring endarterectomy, or peripheral vascular disease requiring lower extremity bypass surgery.

To avoid confounding factors known to affect endothelial function and/or glucose metabolism, the following exclusion criteria were applied to subjects in all groups: smoking any amount of cigarettes during the previous 6 months, subjects with cardiac arrhythmia, congestive heart failure (CHF), recent stroke, chronic renal disease, macroalbuminuria (expressed as albumin/creatinine ratio > 300 μg/mg), severe dyslipidemia (triglycerides > 600 mg/dL or cholesterol > 300 mg/dL), or any other serious chronic disease requiring active treatment. Subjects were also excluded if they were on any of the following medications: glucocorticoids, antineoplastic agents, psychoactive agents, bronchodilators, or any thiazolidenedione. In addition, participants in groups A and B were excluded from the study if taking any type of antihypertensives, lipid-lowering agents, or insulin. Due to the known potential hepatotoxicity of the study drug, a particularly careful review of this effect was performed with all participants, and only individuals without any history of liver disease and with completely normal liver function tests, including liver enzymes, were allowed to participate in the study.

The protocol was approved by the ethics committee or institutional review board at each center, and all participants gave written informed consent. Volunteers for the study were recruited through local advertisement at the Joslin Diabetes Center and The Beth Israel Deaconess Medical Center in Boston. Most subjects were patients followed at these centers. All selected volunteers met the inclusion and exclusion criteria described above.

Methods

Interested volunteers were asked to attend the Joslin Clinical Research Center to perform the initial clinical and laboratory evaluations. Subjects were studied at all visits following an overnight fast and a 24-hour period of abstinence from alcohol and vigorous exercise. Participants were asked not to take their diabetes medications (sulfonylureas or metformin) for 12 hours before any of the studies. Those participants taking insulin (9 subjects in group C only) were asked to omit the rapid-acting insulin the morning of each visit. A general physical examination was performed by a study physician. The diagnosis of proliferative retinopathy was made on the basis of clinical examination or a history of previous retinal laser treatment. The systolic and diastolic blood pressure readings were recorded to the nearest 2 mm Hg as the mean of 2 measurements with the subjects seated. Subjects' weight, height, waist/hip ratio, and body mass index (BMI) were also obtained.

Blood samples were drawn from an antecubital vein with a 19-gauge needle without venous stasis. Plasma glucose, total serum cholesterol, triglycerides, liver function tests, electrolytes, blood urea nitrogen (BUN), and creatinine were measured using the Synchron CX analyzer (Beckman Systems, Oxford, CT), whereas high-density lipoprotein (HDL) serum cholesterol was measured directly (Sigma Diagnostics, St Louis, MO). Low-density lipoprotein (LDL) cholesterol was calculated from these results. A general urinalysis was also performed. The glycosylated hemoglobin (HbA_{1c}) (normal range, 4% to 6%) was determined in whole blood using ion-exchange high-performance liquid chromatography (HPLC). Plasma insulin was measured using the radioimmunoassay (RIA) method. von Willebrand Factor (vWF) (Asserachrom; American Bioproducts, Parsippany, NJ), soluble vascular cell adhesion molecule (sVCAM) and soluble intercellular adhesion molecule (sICAM) (R&D Systems, Minneapolis MN) were measured in plasma in duplicate using the enzyme-linked immunosorbent assay (ELISA) method.

Vascular Reactivity Tests

All eligible participants returned for a second visit to the Microcirculation Laboratory at the Beth Israel Deaconess Medical Center to perform the vascular reactivity tests. All measurements were performed in the morning while the subjects were in a fasting state. The investigators who performed the vascular reactivity measurements were blinded to the medical history of the subjects. These studies were performed in a temperature-controlled room (24°C to 26°C) and after a 30-minute acclimatization period.

The vascular reactivity of the forearm skin microcirculation was evaluated by laser Doppler perfusion imaging measurements before and after the iontophoresis of acetylcholine chloride (Ach, endothelium-dependent vasodilation) and sodium nitroprusside (SNP, endothelium-independent vasodilation) as previously described.[14] The reproducibility of the technique has been previously reported by our group.[14] The coefficient of variation of the baseline measurement was 14.1% and during maximal hyperemic response after the iontophoresis, 13.7%.

To assess the endothelium-dependent reactivity in the macrocirculation, the flow-mediated brachial artery dilation (FMD) was measured by using a high-resolution ultrasound with a 10.0 MHz linear array transducer and an HDI Ultramark 9 system (Advanced Technology Laboratories, Bothel, WA). Reactive hyperemia is produced by inflating a pneumatic tourniquet distally to the brachial artery to 50 mm Hg above the systolic pressure for 5 minutes and then deflating it. This protocol is described in detail elsewhere.[15] Endothelium-independent vasodilation in the macrocirculation was assessed by studying brachial artery diameter changes 3.5 minutes after the administration of 400 μg sublingual nitroglycerine (nitroglycerine-induced dilation [NID]). This test was performed 15 minutes after the reactive hyperemia test and after obtaining a new baseline reading.

After the baseline clinical and laboratory evaluations, participants in all 3 groups were randomized to either 600 mg troglitazone treatment (three 200 mg tablets every morning) or corresponding placebo. The randomization procedure was performed in a double-blind fashion, and the codes were kept unmasked until the end of the study.

Table 1. Baseline Characteristics of the Three Groups

Group	Early Diabetes, No Complications (A)	Late Diabetes, No Complications (B)	Late Diabetes, Macrovascular Disease (C)
No.	27	29	31
Age (yr)*	55.7 ± 8.9	56.4 ± 10	63.1 ± 5.4
Gender (M/F)	13/14	20/9	24/7
Diabetes duration (yr)†	1.3 ± 0.9	10.3 ± 9.3	12.1 ± 8.0
BMI	33.5 ± 5.5	30.4 ± 4.3	31.2 ± 5.3
Systolic BP (mm Hg)	135.2 ± 17	129.1 ± 16	141 ± 18
Diastolic BP (mm Hg)	80.9 ± 9	80.2 ± 9	80.8 ± 8
Fasting plasma glucose (mg/dL)	149 ± 32	177 ± 53	166 ± 46
Plasma insulin‡ (μU/ML)	21 ± 13	16 ± 8	21 ± 12
HbA_{1c}	7.5 ± 1.2	7.9 ± 1.5	7.8 ± 1.2
Total cholesterol* (mg/dL)	212 ± 33	215 ± 36	183 ± 42
HDL-cholesterol (mg/dL)	51 ± 16	46 ± 8	44 ± 11
LDL-cholesterol* (mg/dL)	129 ± 39	133 ± 29	103 ± 35
Triglycerides (mg/dL)	155 ± 72	205 ± 113	195 ± 98
Antihypertensive treatment (%)			28 (90)
Beta blockers			7
ACE inhibitors			5
Diuretic			1
Calcium antagonists			2
Combination			14
Aspirin* (%)	9 (33)	7 (24)	22 (71)
Diabetes treatment (%)			
Diet	12 (44)	2 (7)	2 (6)
Oral agents	15 (56)	27 (83)	20 (65)
Insulin			9 (29)
Proliferative retinopathy	0 (0)	5 (17)	10 (32)

NOTE. Data are mean ± SD.

Abbreviations: BP, blood pressure; ACE, angiotensin-converting enzyme.

*A and B *v* C, $P < .01$; †A *v* B and C, $P < .001$, B *v* C, ‡$P < .01$.

Participants were asked to return for 3 more monthly visits for the completion of the study. They were also asked to continue with their same diabetes medications and dosages and were encouraged to continue with their usual meal plan and physical activity level. In case problems with diabetes control were encountered, any modification to the diabetes management was recorded.

During the first monthly visit, blood tests for HbA_{1c}, glucose, insulin, alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin, and alkaline phosphatase were taken and the vascular reactivity in the micro- and macrocirculation was measured. During the second visit, liver function tests were obtained. During the third visit (exit visit), the same blood tests as at the baseline visit were performed along with the vascular reactivity measurements in the micro- and macrocirculation.

Data Analysis

The Minitab statistical package (Minitab, State College, PA) for personal computers was used for the statistical analysis. The study was designed to detect a 50% improvement in the vascular reactivity measurements at a β level of 0.80 and α level of 0.05 in each group separately. A 2-tailed comparison was assumed. The analysis was performed using a paired *t* test to compare baseline data and changes in all variables at each study point within each group. The *t* test was used to compare the baseline characteristics between those receiving active treatment and those receiving placebo in all groups, while the analysis of variance (ANOVA) test was used to compare differences among the 3 groups. Correlation between variables was tested using both univariate and multivariate analyses (Pearson correlation analysis and multiple stepwise regression analysis). The results are presented as mean ± SD.

Corrections for Multiple Comparisons

We tested whether the number of significant results for a single group was consistent with chance or not. There are 18 separate comparisons (see Tables 3 and 4) for each of the 6 groups (A, B, or C, treated or placebo). With 18 significance tests and a nominal $\alpha = 0.05$, 2-sided, one would expect 0.9 significant results per group ($0.9 = 18 \times 0.05$), and, if results for different variables are independent, then the number of significant tests would follow a Poisson distribution. With an expected number of 0.9, the probabilities of observing a specific number of statistical results are (with expected probability in parentheses): 0 (0.407), 1 (0.366), 2 (0.165), 3 (0.049), 4 or more (0.013).

We observed no significant results in placebo group A and 2 significant results in placebo groups B and C, both of which are consistent with chance. In contrast, in group A, we observed 6 significant (probability of 6 or more significant results: 0.00034), suggesting to us that these results are real findings.

RESULTS

Characteristics of the Study Groups

A total of 87 subjects were randomized into the 3 groups, 27 patients with early diabetes were included in group A, 29 with long-term diabetes in group B, and 31 with diabetes and macrovascular disease in group C. Details about the demographic characteristics of the 3 study groups are shown in Table 1. In

Table 2. Baseline Measurements of Vascular Reactivity and Biochemical Markers of Endothelial Function in All Three Groups

Group	Early Diabetes, No Complications (A)	Late Diabetes, No Complications (B)	Late Diabetes, Macrovascular Disease (C)
Flow-mediated dilation (% of increase over baseline)	5.14 ± 2.1	5.17 ± 2.3	4.68 ± 1.3
Nitroglycerin-induced dilation (% of increase over baseline)*	14.58 ± 5.3	14.24 ± 5.2	11.3 ± 4.1
Acetylcholine-induced skin vasodilation (% of increase over baseline)	37 ± 28	41 ± 32	26 ± 25
Sodium nitroprusside skin-induced vasodilation (% of increase over baseline)	37 ± 27	42 ± 28	34 ± 32
Von Willebrand factor (%)	102 ± 54	92 ± 38	102 ± 52
ICAM (ng/mL)	281 ± 64	271 ± 68	282 ± 62
VCAM (ng/mL)	521 ± 93	569 ± 142	559 ± 138

NOTE. Data are mean ± SD.
*A and B *v* C, $P < .01$.

short, patients in group C were older when compared with the other 2 groups ($P < .001$). The fasting insulin levels were higher in group C when compared with group B ($P < .01$), while the total cholesterol and LDL were lower in group C compared with groups A and B ($P < .01$). Results about the baseline measurements of vascular reactivity and biochemical markers of endothelial function are given in Table 2. The nitroglycerin-induced vasodilation was lower in group C compared with the other 2 groups ($P < .05$), but all other vascular reactivity measurement were similar in all 3 groups.

Participants in all groups were randomized to either active or placebo treatment. There were no statistically significant differences in any of the baseline characteristics between those individuals assigned to the active treatment and those receiving placebo in any of the 3 groups (Tables 3 and 4). The results of the interim visit 1 month after treatment initiation showed no major differences compared with the exit visit results and are not shown.

Results in Patients With Early Diabetes and No Macrovascular Complications (Group A)

The main results on the endothelium-dependent and independent vasodilatory responses in the macro- and microcirculation in this group are shown in Table 4. A significant improvement in the flow-mediated dilation (FMD) in the brachial artery diameter was found in the troglitazone-treated patients after 3 months of treatment ($P < .05$) (Fig 1). In contrast, no significant changes in the brachial artery dilation were seen in response to nitroglycerin. No significant changes were seen in the endothelium-dependent and independent responses in the skin microcirculation or in the various markers of endothelial activation. Troglitazone-treated patients showed an increase in weight and total cholesterol and a reduction in HbA_{1c}, insulin, and systolic and diastolic blood pressure levels (Table 3).

A significant correlation was found in the troglitazone-treated patients in the difference between baseline and exit visit FMD and fasting insulin levels ($r = -.73$, $P < .01$, Fig 2). Significant correlations were also found between the FMD change and the baseline BMI ($r = .79$, $P < .01$) and total cholesterol at baseline ($r = .72$, $P < .02$). Stepwise regression analysis showed that BMI and the change in the insulin levels between baseline and exit visits were the 2 factors that significantly contributed to the FMD change during the same visits and could explain 80% of the variation.

Results in Patients With Long-Standing Diabetes Without Macrovascular Complications (Group B) and With Macrovascular Complications (Group C)

The results of group B and C are presented in Tables 3 and 4. In summary, no improvements in vascular reactivity in the micro- and macrocirculation or in the plasma levels of adhesion molecules and vWF in response to troglitazone were found in these groups. Patients in the placebo arm of B and C groups showed a slight improvement in FMD of the brachial artery after 3 months of treatment, but this reached statistical significance only in group B ($P < .05$). No significant correlations were found in the placebo-treated patients in group B between the change in FMD and any other baseline measurement.

Troglitazone-treated patients in both groups B and C had a significant decrease in fasting plasma glucose, HbA_{1c}, and hematocrit (Table 3). In addition, patients in group B showed an increase in total cholesterol, while patients in group C showed an increase in body weight.

Follow-Up and Side Effects

A total of 14 subjects did not complete the study. Nine people voluntarily withdrew from the study (1 in group A, 5 in group B, and 3 in group C). Two troglitazone-treated patients in group C developed an elevation of liver enzymes 3 times above the upper limit of normal after the second month of treatment and were withdrawn from the study. In both cases, the liver enzymes returned to normal a few weeks after discontinuation of the study medication. Two patients taking placebo, 1 in group B and 1 in group C, developed an acute coronary event that required hospitalization and were discontinued from the study, while 1 troglitazone-treated patient in group B developed a stroke and was also withdrawn from the study. Compliance with the study medications was above 90% in all groups.

DISCUSSION

The main finding of this study is that troglitazone improved the FMD in the brachial artery (endothelium-dependent vaso-

Table 3. Results of Changes in Demographic Characteristics after a 3-Month Treatment Period in Troglitazone- or Placebo-Treated Patients Who Completed the Study in All Three Groups

		Group A		Group B		Group C	
		Active (n = 11)	Placebo (n = 14)	Active (n = 10)	Placebo (n = 12)	Active (n = 11)	Placebo (n = 13)
Age (yr)		54 ± 10	58 ± 7	55 ± 9	58 ± 11	64 ± 6	62 ± 4
Weight (kg)	Baseline	97 ± 11	97 ± 18	86 ± 16	89 ± 14	94 ± 15	93 ± 17
	Exit	98 ± 12	96 ± 16	87 ± 15	89 ± 14	96 ± 15	95 ± 16
	P	.223	.321	.261	.859	.012	.134
Systolic BP (mm Hg)	Baseline	138 ± 18	133 ± 22	124 ± 16	134 ± 14	135 ± 17	136 ± 13
	Exit	129 ± 11	125 ± 15	127 ± 16	130 ± 17	136 ± 14	130 ± 10
	P	.035	.070	.0556	.512	.938	.231
Diastolic BP (mm Hg)	Baseline	82 ± 11	80 ± 6	79 ± 10	79 ± 8	79 ± 11	82 ± 8
	Exit	76 ± 6	78 ± 6	76 ± 6	79 ± 9	77 ± 7	77 ± 7
	P	.037	.371	.230	1.000	.485	.027
Fasting glucose (mg/dL)	Baseline	134 ± 22	162 ± 36	184 ± 54	185 ± 49	164 ± 39	156 ± 51
	Exit	126 ± 28	167 ± 66	148 ± 42	191 ± 42	133 ± 41	179 ± 52
	P	.416	.637	.028	.523	.075	.113
HbA_{1c}	Baseline	7.0 ± 0.9	7.9 ± 1.4	8.4 ± 1.7	7.7 ± 1.4	7.4 ± 1.0	8.2 ± 1.4
	Exit	6.1 ± 1.1	7.9 ± 1.8	7.0 ± 1.3	7.8 ± 1.1	6.7 ± 0.5	8.4 ± 1.4
	P	.021	.926	.007	.765	.042	.441
Insulin (uU/dL)	Baseline	19.7 ± 10	21.7 ± 15	15.0 ± 11	17.5 ± 7	18.1 ± 12	25.3 ± 13
	Exit	15.6 ± 10	19.4 ± 14	14.1 ± 8	16.5 ± 4	28.9 ± 18	21.3 ± 6
	P	.040	.434	.756	.631	.154	.412
Total	Baseline	205 ± 33	212 ± 32	209 ± 44	211 ± 24	174 ± 50	194 ± 36
	Exit	227 ± 35	209 ± 35	235 ± 57	220 ± 28	179 ± 42	190 ± 37
	P	.050	.575	.020	.073	.429	.321
LDL-C (mg/dL)	Baseline	118 ± 39	129 ± 39	131 ± 31	128 ± 23	97 ± 36	115 ± 35
	Exit	127 ± 38	132 ± 30	144 ± 51	130 ± 12	107 ± 43	113 ± 25
	P	.445	.689	.306	.802	.143	.635
HDL-C (mg/dL)	Baseline	54 ± 22	48 ± 12	44 ± 7	46 ± 8	47 ± 15	41 ± 4
	Exit	53 ± 13	48 ± 10	49 ± 7	53 ± 26	47 ± 13	41 ± 4
	P	.909	.808	.051	.333	.908	.846
Triglycerides	Baseline	150 ± 72	146 ± 71	177 ± 134	198 ± 67	151 ± 72	210 ± 97
	Exit	184 ± 98	164 ± 87	207 ± 188	213 ± 99	147 ± 80	193 ± 123
	P	.077	.323	.225	.483	.805	.505
Hematocrit	Baseline	41 ± 3	43 ± 4	45 ± 3	43 ± 4	42 ± 4	45 ± 4.2
	Exit	40 ± 3	43 ± 4	42 ± 4	42 ± 4	40.3 ± 3	44 ± 4
	P	.117	.479	.005	.354	.018	.155

NOTE. Data are mean ± SD.

dilation) in patients with recently diagnosed type 2 diabetes, whereas it had no effect in patients with type 2 diabetes of longer duration with or without clinically evident macrovascular complications. The observed improvement in the endothelial function showed a strong correlation with the reduction that was seen in the fasting insulin levels. In addition, troglitazone treatment had no effect on the vascular reactivity of the skin microcirculation or the plasma levels of adhesion molecules and vWF in any of the study groups.

Troglitazone is an agonist for the peroxisome proliferator-activated receptor γ (PPARγ) and was originally introduced for improving glycemic control of type 2 diabetic patients.[16] In addition to this, there is experimental evidence to suggest that troglitazone can affect the vasculature through various mechanisms. The main findings include a reduction of insulin resistance, plasminogen activator inhibitor (PAI)-1 activity, angiotensin II type 1 receptor expression, ICAM, VCAM, and plasma E-selectin concentrations and LDL oxidation.[17-20]

Our results suggest that the main beneficial action on the endothelial function in human diabetes is exerted through the improvement of insulin resistance, as reflected by a reduction in fasting plasma insulin levels. Thus, a very strong correlation was observed between the improvement in endothelial function and the reduction in insulin levels in the patients with recently diagnosed type 2 diabetes. Further support to this point is provided by the fact that the failure to improve the endothelial function in the patients with long-term diabetes with and without macrovascular disease was associated with a failure of troglitazone to reduce the fasting insulin levels during the 12-week study period.

Insulin is known to have a direct vasodilatory effect mediated through the nitric oxide pathway, while recent data have demonstrated an independent association between insulin resistance and atherosclerosis.[21-24] In the insulin-resistant state, the ability of insulin to stimulate nitric oxide production in the endothelium is diminished. In addition to this, the chronic compensatory hyperinsulinemia may be detrimental to the vasculature, as it can stimulate the migration and growth of the

Table 4. Results of Changes in Vascular Reactivity After a 3-Month Treatment Period in Troglitazone- or Placebo-Treated Patients Who Completed the Study in all Three Groups

		Group A		Group B		Group C	
		Active (n = 11)	Placebo (n = 14)	Active (n = 10)	Placebo (n = 12)	Active (n = 11)	Placebo (n = 13)
FMD	Baseline	5.3 ± 2.0	5.1 ± 2.4	6.1 ± 1.8	4.4 ± 2.4	4.9 ± 1.9	4.6 ± 1.0
	Exit	7.7 ± 3.4	5.7 ± 3.1	6.4 ± 2.5	5.4 ± 3.0	4.9 ± 1.0	5.5 ± 2.4
	P	.038	.526	.601	.046	.991	.106
NID	Baseline	12.7 ± 2.9	16.1 ± 6.6	15.7 ± 5.2	14.9 ± 5.1	14.2 ± 3.1	11.0 ± 3.0
	Exit	12.7 ± 6.6	18.3 ± 5.5	14.7 ± 3.9	12.7 ± 3.7	14.4 ± 4.4	11.8 ± 4.8
	P	.994	.235	.506	.225	.879	.544
Ach	Baseline	46 ± 21	32 ± 31	44 ± 31	33 ± 35	26 ± 31	28 ± 19
	Exit	37 ± 30	27 ± 18	36 ± 22	28 ± 25	27 ± 24	18 ± 21
	P	.283	.600	.574	.591	.763	.323
SNP	Baseline	41 ± 34	33 ± 22	50 ± 31	41 ± 29	41 ± 43	27 ± 20
	Exit	50 ± 32	35 ± 23	36 ± 27	42 ± 27	28 ± 31	28 ± 29
	P	.284	.734	.144	.919	.203	.869
sICAM (ng/mL)	Baseline	269 ± 81	284 ± 43	273 ± 91	268 ± 57	269 ± 55	285 ± 55
	Exit	250 ± 79	284 ± 49	270 ± 66	288 ± 56	260 ± 58	269 ± 62
	P	.218	.988	.824	.001	.143	.038
sVCAM (ng/mL)	Baseline	515 ± 96	517 ± 93	576 ± 163	560 ± 123	480 ± 96	580 ± 147
	Exit	538 ± 174	514 ± 97	558 ± 132	563 ± 113	448 ± 56	556 ± 161
	P	.467	.840	.252	.854	.158	.107
vWF (%)	Baseline	95 ± 40	105 ± 66	97 ± 39	81 ± 41	83 ± 40	110 ± 68
	Exit	106 ± 63	109 ± 63	93 ± 41	97 ± 41	71 ± 34	105 ± 50
	P	.396	.786	.620	.199	.293	.618

NOTE. Data are mean ± SD.

Abbreviations: FMD, flow-mediated dilation; NID, nitroglycerin-induced dilation; Ach, acetylcholine; % of increase over baseline; SNP, sodium nitroprusside, % of increase over baseline for all measurements.

smooth muscle cells and increase the production of PAI-1, which attenuates fibrinolysis.[25,26]

In the present study, we also found no change in the nitroglycerin-induced vasodilation, a measure of endothelium-independent vasodilation that depends on vascular smooth muscle cell response, in all 3 groups. These results indicate that troglitazone acted mainly on the endothelial cell rather than on the smooth muscle cell. No changes were also observed in our studied population regarding the microvascular reactivity in the skin microcirculation. As troglitazone seems to exert its beneficial effects mainly by reducing insulin resistance, its failure to have any effect on the microcirculation should not be surpris-

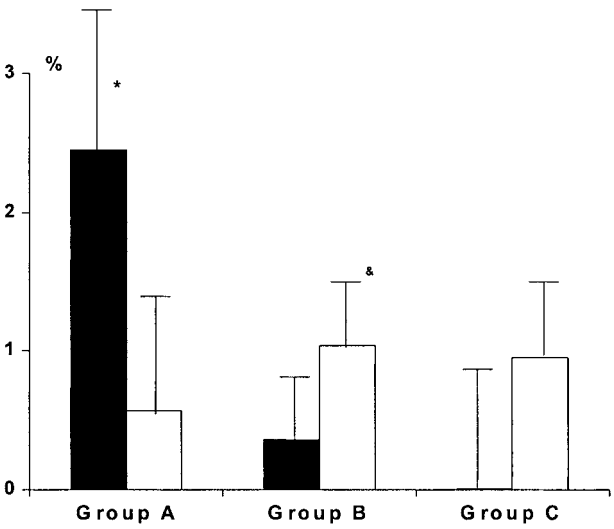

Fig 1. The increase (difference between exit and baseline visits) in the FMD in troglitazone (■) and placebo-treated patients (□). A significant increase was found in the troglitazone-treated patients with early type 2 diabetes (group A, *$P = .038$). In addition, a small, but significant, increase was noticed in the placebo-treated patients with long duration type 2 diabetes without macrovascular disease (group B, &$P = .046$). No changes were found in the diabetic patients with macrovascular disease (group C).

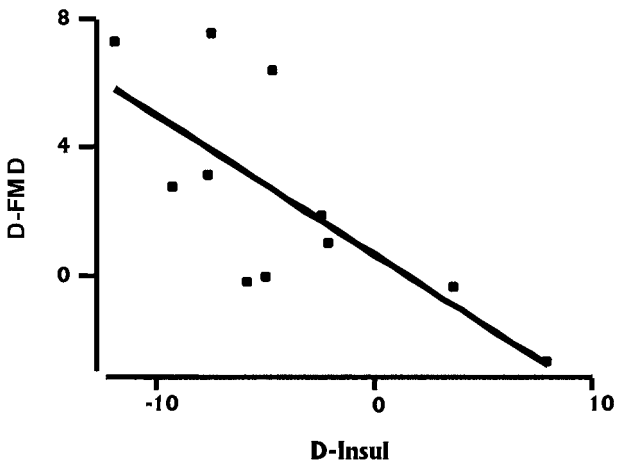

Fig 2. A significant correlation ($r = .730$, $P < .01$) was found between the increase in FMD and reduction of fasting insulin levels in the troglitazone-treated patients with early diabetes (group A).

ing. Finally, no changes were seen in the biochemical markers of endothelial activation, such as VCAM-1, ICAM-1, and the vWF. The reasons for this are not clear, but the possibility that a longer duration of treatment may be required before any beneficial changes are observed cannot be excluded.

Based on the above, we believe that the present study provides novel information about the effect of troglitazone on the endothelial function and the population that is more likely to benefit from it. Thus, a clear picture emerges that troglitazone can be beneficial to the endothelium only in the early stages of diabetes, in which clear improvement in the insulin resistance characterized by a significant reduction in fasting insulin can be obtained.

A significant decrease in HbA_{1c}, systolic and diastolic blood pressure, and insulin concentrations along with a significant increase in weight and total cholesterol was seen in troglitazone-treated patients in group A, effects already known to be associated with this medication.[9,10] In group B, the main effects of troglitazone were a reduction in fasting plasma glucose and HbA_{1c} and an increase in total cholesterol, while in group C, an increase in the weight associated with lowering of HbA_{1c} was present. In addition, a reduction of the hematocrit was noticed in both groups. Previous studies have shown that water retention and therefore, hemodilution, cause this hematocrit reduction.

An unexpected improvement was found in the endothelial function of the placebo-treated patients in all groups and reached statistical significance in group B. The exact reasons for this finding are not clear. The techniques used to evaluate flow-mediated vasodilation are well established and have a very satisfactory coefficient of variation (less than 5% in our laboratory) and cannot be responsible for the observed results. The slight improvement in all groups is probably related to bias in data evaluation, as the radiologist (R.S.) who interpreted the data was aware of the timing of the examination despite the fact that she was unaware about the group and the treatment each participant was allocated. The troglitazone-related water retention (best seen by the reduction of the hematocrit) may also be a contributing factor to the finding that the actively treated groups B and C did not show a similar improvement. Finally, regarding the improvement in the placebo-treated patients in group B, we believe that it is also related to the fact that these patients tended to have lower baseline measurements compared with other groups, probably related to a hyperglycemia-related dehydration.

The present study has its limitations. The main one is probably the lack of more detailed insulin resistance measurements. However, we believe that the strong correlation observed between changes in insulin levels and FMD make the extraction of the proper conclusions possible despite the lack of detailed insulin resistance measurements. Further studies that properly assess insulin resistance will be required to confirm the present findings. The main reason for this design was that at the beginning of the study, it was felt that possible troglitazone beneficial effects would be derived by the direct action of troglitazone on the endothelial and smooth muscle cells (e.g., reduction in CAMs) rather through an improvement in insulin resistance. We also speculated that as vitamin E activity is inherited in the troglitazone molecule, the medication might exert a considerable antioxidant action and improve the endothelial function by reducing the oxidative stress irrespectively of the insulin resistance status. As such an action was not apparent in the present study and troglitazone acted mainly through improving insulin resistance, further studies will be required to evaluate any potential additional benefit by treatment with antioxidants. Finally, another limitation is that a control group was not included in this study to compare the vascular reactivity measurements of the studied diabetic groups. However, previous work from our unit indicates that the endothelial function in all diabetic groups was considerably lower when compared with healthy subjects.[5,14,27]

In conclusion, in the present study we have shown that troglitazone improved the FMD in the brachial artery (endothelium-dependent vasodilation) in patients with early type 2 diabetes, and this improvement was strongly associated with the improvement of fasting plasma insulin concentrations. In contrast, it produced no improvement in those with late diabetes with and without macrovascular complications. Finally, troglitazone did not have any effect on the skin vascular reactivity or the plasma levels of adhesion molecules and vWF in any of the study groups.

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