Addition of Pioglitazone and Ramipril to Intensive Insulin Therapy in Type 2 Diabetic Patients Improves Vascular Dysfunction by Different Mechanisms

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OBJECTIVE — We examined the relationship between glycemic control, vascular reactivity, and inflammation in type 2 diabetic subjects.

RESEARCH DESIGN AND METHODS — Thirty subjects with type 2 diabetes were initiated on intensive insulin therapy (continuous subcutaneous insulin infusion [$n = 12$] or multiple daily injections [$n = 18$]) and then randomized to either pioglitazone (PIO group;45 mg/day), ramipril (RAM group; 10 mg/day), or placebo (PLC group) for 36 weeks. Euglycemic-hyperinsulinemic clamp was used to quantify insulin resistance, and plethysmography was used to assess changes in forearm blood flow (FBF) after *1*) 5 min of reactive hyperemia and *2*) brachial artery infusion of acetylcholine (7.5, 15, and 30 μg/min) and sodium nitroprusside (3 and 10 μg/min).

RESULTS — The decreases in A1C (~9.0–7.0%) and fasting plasma glucose (~190–128 mg/dl) were equal in all groups. In the PIO group, glucose disposal increased from 3.1 to 4.7 mg $\cdot kg^{-1} \cdot min^{-1}$, and there was a greater decrease in plasma triglycerides (~148 vs. 123 mg/dl) and free fatty acids (~838 vs. 595 mEq/l) compared with the RAM or PLC groups ($P < 0.05$). Plasma adiponectin doubled with pioglitazone treatment (6.2 ± 0.7 to 13.1 ± 1.8 μg/ml), while endothelin-1 decreased only with ramipril treatment (2.5 ± 0.2 to 1.1 ± 0.2 pg/ml) ($P < 001$). The increase in FBF during reactive hyperemia (215%) and acetylcholine (from 132 to 205%, 216 to 262%, and 222 to 323%) was greater in the PIO versus RAM or PLC groups. In contrast, FBF during sodium nitroprusside treatment was greater in the RAM group (141–221% and 218–336%) compared with the PIO or PLC groups (all $P < 0.05$).

CONCLUSIONS — Addition of pioglitazone or ramipril to intensive insulin therapy in type 2 diabetes further improves vascular dysfunction. Pioglitazone enhances endothelial-mediated vasodilation, whereas ACE inhibition enhances endothelial-independent vasodilation. These different vascular effects, combined with the observation that pioglitazone decreases free fatty acids and triglycerides and increases adiponectin, while ramipril reduces endothelin-1, suggest that different mechanisms underlie the vascular responses.

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Atherosclerotic cardiovascular disease affects >80% of type 2 diabetic patients and accounts for a substantial increase in morbidity and mortality (1,2). The clustering of risk factors, including hyperglycemia, hypertension, dyslipidemia, and visceral obesity, can only partially explain the two- to fourfold higher frequency of cardiovascular events reported in diabetic patients (3). Recent evidence (4) suggests that insulin resistance, vascular endothelial dysfunction, and inflammation play critical roles in the development of atherosclerosis. Thus, interventions aimed at improving insulin resistance and vascular dysfunction and inflammation may provide additional benefits to diabetic subjects at risk for cardiovascular disease over and beyond normalization of conventional risk factors (5).

Intensive insulin therapy to maintain near normoglycemia has been associated with a decrease in long-term morbidity (6) and enhanced survival in selected diabetic (7) and nondiabetic (8) hospitalized patients. More recently, a reduction in cardiovascular events has also been demonstrated in type 1 diabetic patients treated aggressively with insulin for >10 years (9). In addition, the use of thiazolidinediones (TZDs) (10) and agents that inhibit the angiotensin system (11,12) have been shown to result in improved cardiovascular outcomes in diabetic subjects; TZDs also improve dyslipidemia and enhance insulin sensitivity. Of note, both TZDs and ACE inhibitors enhance insulin signal transduction and increase phosphoinositol-3 kinase/Akt activity in vitro, which would be expected to augment nitric oxide synthetase activity (13), thereby improving endothelial dysfunction. Previous studies (14–16) have confirmed that vascular dysfunction and

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Abbreviations: ARB, angiotensin II receptor blockade; CSII, continuous subcutaneous insulin infusion; FBF, forearm blood flow; hsCRP, high-sensitivity C-reactive protein; IL, interleukin; MDII, multiple daily insulin injection; TZD, thiazolidinedione.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

inflammation are attenuated with TZD therapy in diabetic subjects, though most studies could not discern whether these metabolic effects have a direct impact on vascular structure and function and whether these are mediated by the reduction in glycemia or some other action of TZDs. In some studies (16,17), amelioration of vascular dysfunction and reduction in inflammatory biomarkers was shown to occur independently of glycemic control. This finding, along with the observation that abnormal vascular and inflammatory responses are improved with ACE-blocking agents (14–17), supports the concept that vascular dysfunction cannot be attributed solely to elevated plasma glucose concentrations.

To gain insight into the pathophysiologic and molecular mechanisms responsible for endothelial dysfunction, we examined vascular reactivity and markers of inflammation in patients with type 2 diabetes who were intensively treated with insulin and subsequently randomized to receive either pioglitazone (PIO group; 45 mg/day), ramipril (RAM group; 10 mg/day), or placebo (PLC group) for 36 weeks, in a double-blind study.

RESEARCH DESIGN AND METHODS

— Thirty adult Mexican-American patients with type 2 diabetes who required insulin therapy (A1C >8.0% despite optimized oral agent therapy) were recruited from the outpatient clinic at the Texas Diabetes Institute in San Antonio, Texas. The protocol was approved by the institutional review board of the University of Texas Health Science Center at San Antonio, and before the study all subjects provided informed voluntary written consent. Patients on insulin combination therapy with metformins, sulfonylureas, and/or meglitinides were included, but those on TZDs were excluded. Patients taking ACE inhibitors or angiotensin II receptor blockade (ARB) agents were switched to α-methyl dopa, and the dose was adjusted to reestablish blood pressure (<130/80 mmHg) control before they were enrolled in the study. The ACE inhibitor/ARB therapy was discontinued for at least 2 months before the study, and other medications were allowed only if the subject was stable for at least 3 months.

Subjects reported to the Clinical Research Center after a 10-h overnight fast, and blood samples for measurement of fasting plasma glucose, lipids, A1C, and urine for the determination of the microalbumin-to-creatinine ratio were obtained. Three consecutive sitting blood pressure measurements were recorded with an automated arm cuff. Patients then were enrolled in a 3-day comprehensive diabetes education and nutritional program conducted at the Texas Diabetes Institute. Patients were eligible for either therapy and were allowed to select between insulin therapy using multiple daily insulin injection (MDII) or continuous subcutaneous insulin infusion (CSII). MDII consisted of a basal-bolus program with four daily insulin injections using a combination of insulin glargine (sanofi-aventis) at bedtime plus premeal insulin aspart (Novo Nordisk). CSII was implemented using the Medtronic/Minimed ($n = 6$) or the Animas ($n = 6$) pump using basal infusion and premeal boluses of insulin aspart (Novo Nordisk). Before randomization, patients received a euglycemic-hyperinsulinemic clamp and vascular studies.

Euglycemic-hyperinsulinemic clamp

Insulin sensitivity was quantitated with the euglycemic-hyperinsulinemic (80 mU/m^2 per min) clamp technique (18), which is sufficient to suppress endogenous glucose production by >90% (19). The rate of exogenous glucose infusion was averaged during the last 60 min of the insulin clamp to provide a measure of insulin-stimulated total body glucose metabolism (*M*). Before the start of the insulin clamp, baseline blood samples were obtained from the arterialized hand vein for the determination of plasma concentrations of free fatty acids, adiponectin, endothelin-1, adhesion molecules (vascular cell adhesion molecules and intercellular adhesion molecules), high-sensitivity C-reactive protein (hsCRP), tumor necrosis factor-α, interleukin (IL)-6, lipids (total/HDL/LDL cholesterol and triglycerides), and lipid particle size.

Vascular studies

Endothelial function was evaluated in all patients using venous occlusion plethysmography (Hokanson, Bellevue, WA). Changes in resting forearm blood flow (FBF) following *1*) 5 min of transient ischemia and *2*) brachial artery infusion of acetylcholine and sodium nitroprusside were documented by venous occlusion plethysmography. In the morning, after an overnight fast and a 30-min rest in the supine position, five measurements of FBF were performed to establish the baseline value. An arm cuff then was inflated above the patient's systolic blood pressure for 5 min. Changes in FBF were documented at 5-s intervals after the arm cuff was released, and a mean value was calculated. Then, an 18-gauge catheter (Arrow International, Erding, Germany) was introduced into the brachial artery of the nondominant arm under local lidocaine anesthesia. After 60 min of rest, test substances were infused into the brachial artery according to a previously published technique (14,20). In brief, the initital 6-min infusion of sodium nitroprusside (3 μg/min) was followed by an increase to 10 μg/min for an additional 6 min. After the line was cleared, acetylcholine was infused at a constant rate of 7.5 μg/min for 8 min and then increased to 15 and 30 μg/min for 6 min in sequence. FBF was measured five times during the last 3 min of each intrabrachial infusion period, and a mean value was calculated. Baseline FBF was expressed as ml/100 ml of forearm tissue per minute using a software program (Hokanson) and expressed as a percent changes from basal. At the end of the experiment, the arterial line was removed and compression pressure was applied to the artery. All patients were contacted within 24 h and 1 week after the study to ascertain that there had been no complications.

Randomization

Upon successful completion of the baseline studies, patients were randomized to pioglitazone (PIO group; 45 mg/day), ramipril (RAM group; 10 mg/day), or placebo (PLC group) for 36 weeks, in a double-blind fashion. Pioglitazone was started at the dose of 15 mg daily and then increased to 30 mg daily at week 2 and to 45 mg daily at week 4. Ramipril was started at the dose of 5 mg daily and increased to 10 mg daily, as tolerated, at 2 and 4 weeks. Placebo tablets were added to match the other treatment regimen. Participants were contacted by phone at least weekly during the first 2 months. Insulin dose was adjusted according to the University of Texas Hospital protocol to achieve the following pre-established glycemic goals: fasting and premeal capillary blood glucose values between 80 and 120 mg/dl, 2-h postmeal glucose values <160 mg/dl, and bedtime glucose levels <140 mg/dl. If the premeal glycemic goal range was not attained, patients were instructed to supplement their usual insulin dose with an additional 1, 2, or 3 units if the capillary blood glucose measurement was >120, >150, or >180 mg/dl, respectively. If the capillary blood glucose measurement was <80 mg/dl,

the calculated premeal insulin dose was reduced by 1–2 units. If the fasting blood glucose concentration was >80 and <120 mg/dl for a minimum of 3 consecutive days, the insulin basal dose and the basal infusion rate were adjusted accordingly by ~10% daily.

Follow-up plan

All patients were asked to return for office visits at 2- to 4-week intervals during the first 3 months and every 2 months thereafter for the remainder of the 9-month study period. During each visit, compliance with the assigned therapeutic regimen was ascertained, self-monitored glucose values were reviewed, and routine blood and urine samples were obtained. The hyperinsulinemic clamp, vascular studies, and measurements of markers of endothelial damage and inflammation were repeated at 36 weeks (study termination)

Laboratory analyses

Plasma glucose concentration was measured using the glucose oxidase method with the Beckman Glucose Analyzer, and tritiated glucose-specific activity was determined in barium/zinc deproteinized plasma samples. A1C was measured by affinity chromatography (Biochemical Methodology, Drower 4350; Isolab, Akron, OH), plasma insulin (Diagnostic Products, Los Angeles, CA) and plasma free fatty acid concentration by enzymatic colorimetric quantification (Wako Chemicals, Neuss, Germany), adiponectin by commercially available radioimmunoassay (Linco, St. Charles, MO), and plasma CRP, vascular cell adhesion molecules, intercellular adhesion molecules, tumor necrosis factor-α, IL-6, and endothelin-1 by measured by enyme-linked immunosorbent assays (Linco). Lipid profile and particle size were determined by nuclear magnetic resonance spectroscopy (Liposcience, Raleigh, NC).

Statistics

The sample size was estimated on the basis of our previous vascular data and powered to demonstrate a difference between the mean basal value increase of 50% and the alternate mean increase of 75% between groups in FBF during reactive hyperemia and following acetylcholine and sodium nitroprusside brachial artery infusions before and after the interventions. A total of 10 subjects per group was derived from a two-sided test with significance levels $\alpha = 0.05$ and a power of $1\text{-}\beta = 0.90$, using a mean SD of ±30%. Changes in metabolic (fasting plasma glucose; free fatty acids; A1C; total, LDL, and HDL cholesterol and triglyceride concentrations; and in insulin-mediated glucose disposal) and inflammatory biomarkers (hsCRP, adiponectin, endotheln-1, vascular cell adhesion molecules, tumor necrosis factor-α, and IL-6) and in vascular parameters (percent change in FBF above basal during reactive hyperemia and acetylcholine-stimulated and sodium nitroprusside-stimulated vasodilation) were analyzed in the intensively treated diabetic patients (MDII and CSII together) assigned to each oral therapy and between groups using ANOVA with repeated measures. Statistical significance ($P < 0.05$) was considered when the within-group variability was inferior to the intergroup variability. Univariate analyses were performed between vascular responses (reactive hyperemia, acetylcholine, and sodium nitroprusside vasodilation) and A1C values, and multiple regression analyses (simultaneous regression) were conducted between the metabolic and inflammatory biomarkers and the vascular parameters listed above as continuous variables in a stepwise regression. Statistical significance was considered for P value <0.05. All statistical analyses were performed using a Sigma Stat software program.

RESULTS — Baseline characteristics showed patients of comparable sex (n = ~6 female subjects; $n = 4$ male subjects), mean age (~46 years), duration of disease (6.2–8.4 years), BMI (~31–33 kg/m^2), waist circumference (101–104 cm), blood pressure (~130/70 mmHg), insulin dose (~1.2 units · kg^{-1} · day^{-1}), mean A1C (~9.0%), and fasting plasma glucose (~190 mg/dl). The use of oral antidiabetes medications (sulfonylureas, meglitinides, and metformin) was similar in all three groups. Nearly one-half of the patients was taking a statin and one-third was on antihypertensive therapy, with α-methyl dopa substituted for ACE inhibitors ($n = 15$) and ARB ($n = 2$). After 36 weeks of insulin therapy, mean body weight increased in all patients, by 1.7 kg in the PLC group, 3.2 in the RAM group, and 4.4 kg in the PIO group (all $P < 0.01$ vs. baseline). The average daily insulin requirement remained unchanged in the PLC group (~1.2 units/kg) and decreased similarly in the RAM and PIO groups to 1.0 ± 0.2 units/kg ($P < 0.05$). Except for the discontinuation of sulfonylureas and meglitinides (to avoid hypoglycemia), all other medications (including metformin) were maintained stable in all patients during the entire study period.

Insulin therapy using either MDII or CSII was well tolerated by patients, and there were no withdrawals. During the 36 weeks of observation, 14 patients (6 in the PLC, 4 in the PIO, and 4 in the RAM groups) had 33 hypoglycemic episodes (0.32 patients per year), defined as symptomatic hypoglycemia requiring glucose ingestion. Mechanical malfunction of the insulin pump was documented on four occasions, and patients had to temporarily (<2 days) interrupt the insulin pump therapy and substitute multiple daily injections. One patient developed an abdominal skin infection, which was resolved with oral antibiotic therapy and local treatment. Three patients in the PIO and one in the RAM group developed mild peripheral edema that did not require therapy.

Metabolic and inflammatory parameters

Patients in all three groups showed a substantial improvement in their metabolic control over the 9-month period, with a reduction in fasting plasma glucose concentration to 123–128 mg/dl and decrease in mean A1C to ~7.0% ($P < 0.01$) (Table 1). Plasma free fatty acid (838 ± 84 to 595 ± 65 mEq/l) and triglyceride (148 ± 17 to 123 ± 11 mg/dl) concentrations decreased significantly ($P < 0.01$ vs. baseline and vs. PLC and RAM groups) after 9 months of pioglitazone therapy. In the PIO group, although plasma LDL concentration did not change (107 ± 7 vs. 105 ± 12 mg/dl), both HDL concentration (45 ± 3 to 51 ± 3 mg/dl) and LDL particle size (20.2 ± 0.5 to 21.2 ± 0.8 nm) increased significantly ($P < 0.05$ vs. placebo and ramipril). Plasma adiponectin levels more than doubled (6.2 ± 0.7 to 13.1 ± 1.8 μg/ml) with pioglitazone therapy ($P < 0.001$ vs. baseline and vs. placebo and ramipril) and remained unchanged in the PLC and RAM groups. Plasma levels of endothelin-1 (from 2.5 ± 0.2 to 1.1 ± 0.2 pg/ml) and IL-6 (from 3.6 ± 0.3 to 2.4 ± 0.2) decreased significantly with the addition of ramipril to intensive insulin therapy ($P < 0.05$ vs. baseline and vs. placebo and pioglitazone). After 36 weeks, M was significantly enhanced by ~50% in the insulin therapy group that received pioglitazone (3.1–4.7 mg · kg^{-1} · min^{-1}; $P < 0.01$ vs. baseline and vs. the PLC and RAM groups). The

Table 1—*Changes in metabolic parameters, markers of endothelial dysfunction, and inflammation in patients with type 2 diabetes after 36 weeks of intensive insulin therapy in combination with placebo, pioglitazone, or ramipril*

	Placebo		Pioglitazone		Ramipril	
Parameters	Pre	Post	Pre	Post	Pre	Post
Fasting plasma glucose (mg/dl)	196 ± 19	128 ± 19	190 ± 14	123 ± 13	189 ± 11	126 ± 6
Fasting plasma insulin (μU/ml)*	16 ± 5	28 ± 9	14 ± 4	24 ± 7	11 ± 4	16 ± 10
A1C (%)	9.2 ± 0.4	7.2 ± 0.1	9.0 ± 0.7	6.9 ± 0.3	9.1 ± 0.5	7.0 ± 0.2
Free fatty acids (mEq/l)	778 ± 54	695 ± 88	838 ± 84	595 ± 65†	753 ± 64	705 ± 73
Total cholesterol (mg/dl)	195 ± 9	180 ± 8	176 ± 9	175 ± 16	190 ± 12	199 ± 13
LDL cholesterol (mg/dl)	121 ± 8	115 ± 7	107 ± 7	105 ± 12	117 ± 8	122 ± 10
HDL cholesterol (mg/dl)	49 ± 4	46 ± 3	45 ± 3	51 ± 3†	50 ± 4	51 ± 7
VLDL cholesterol (mg/dl)	113 ± 24	93 ± 19	109 ± 16	88 ± 15	120 ± 28	92 ± 10
Triglycerides (mg/dl)	146 ± 15	132 ± 18	148 ± 17	123 ± 11†	145 ± 12	142 ± 17
Adiponectin (μg/ml)	8.9 ± 1.3	9.1 ± 1.1	6.2 ± 0.7	13.1 ± 1.8†	7.1 ± 0.2	8.6 ± 0.3
hsCRP (mg/l)*	2.5 ± 0.3	2.1 ± 0.2	1.7 ± 0.2	1.1 ± 0.2	1.5 ± 0.2	1.1 ± 0.1
Tumor necrosis factor-α (mg/l)	1.8 ± 0.2	1.3 ± 0.1	1.6 ± 0.1	1.3 ± 0.2	1.9 ± 0.4	1.6 ± 0.3
IL-6 (pg/ml)	2.4 ± 0.2	2.1 ± 0.3	3.7 ± 0.7	2.9 ± 0.6	3.6 ± 0.3	2.4 ± 0.2‡
VCAM (ng/ml)	574 ± 33	522 ± 38	556 ± 38	487 ± 32	590 ± 40	529 ± 32
ICAM (ng/ml)	278 ± 9	269 ± 8	247 ± 11	235 ± 10	257 ± 25	242 ± 29
Endothelin-1 (pg/ml)	1.8 ± 0.2	2.0 ± 0.3	2.3 ± 0.3	2.1 ± 0.4	2.5 ± 0.2	1.1 ± 0.2‡

Data are means ± SD. *$P < 0.05$ vs. baseline for all groups; †$P < 0.05$–0.01 pioglitazone vs. baseline; ‡$P < 0.05$ ramipril vs. baseline.

increments in *M* in the placebo (4.5 vs. 4.9 mg · kg^{-1} · min^{-1}) and ramipril (3.2 vs. 3.6 mg · kg^{-1} · min^{-1}) groups were not statistically significant.

Vascular reactivity

Vascular reactivity was assessed by the changes in FBF during reactive hyperemia and during intrabrachial infusion of acetylcholine and sodium nitroprusside (Fig. 1). Basal FBF was comparable in all groups: 2.4–2.6 ml · 100 ml^{-1} · min^{-1}. The percent rise in FBF following 5 min of brachial artery occlusion was similar (~30–40% above baseline) in all groups before the start of insulin/drug therapy (Fig. 1*A*). After 36 weeks of therapy, the percent increase in FBF during reactive hyperemia was significantly ($P < 0.05$) greater in the PIO (215%) compared with the PLC (170%) and RAM (181%) groups. Pioglitazone-treated patients achieved the highest absolute increase in FBF during reactive hyperemia (5.2 ml · 100 ml^{-1} · min^{-1}) compared with the PLC (4.3 ml) and RAM (4.2 ml) groups (both $P < 0.05$). The percent rise in FBF in the RAM group during acetylcholine infusion was 139–181% (7.5 μg/min), 147–196% (15 μg/min), and 193–230% (30 μg/min), respectively, and did not differ from the PLC group (Fig. 1*B*). The percent increase in FBF with acetylcholine infusion was greater with pioglitazone compared with both placebo and ramipril (132–205% [7.5 μg/min], 216–262% [15 μg/min], and 222–323% [30 μg/min]) (all $P < 0.05$–0.01). In contrast, endothelial-independent sodium nitroprusside–stimulated vasodilation (Fig. 1*C*) was significantly greater in the RAM group ($P < 0.05$) with percent increments of 141–221% (3 μg/min) and 218–336% (10 μg/min). Percent change in FBF in the PLC (151–182% at 3 μg/min and 237–265% at 10 μg/min) and the PIO (138–184% at 3 μg/min and 230–257% at 10 μg/min) groups were small and did not reach statistical significance.

Multivariate analyses were performed between the metabolic and inflammatory biomarkers and vascular continuous variables. Positive correlations between the change in A1C and reactive hyperemia ($P = 0.04$) and between fasting plasma glucose ($P < 0.01$) and insulin-mediated glucose disposal ($P < 0.05$) and acetylcholine-stimulated vasodilation (30 μg/min) were obtained, indicating that glycemic control and insulin sensitivity were closely associated with vascular reactivity. In multiple regression analyses, there were also strong negative correlations between changes in plasma triglyceride concentration ($P = 0.04$) and in plasma levels of hsCRP ($P < 0.02$) with sodium nitroprusside–stimulated (10 μg/min) vasodilation and positive correlations between plasma HDL cholesterol and both low (3 μg/min) and high (10 μg/min) sodium nitroprusside–stimulated vasodilation in all subjects, which imply also that there was a role for plasma lipid concentrations in vascular responses. Among the inflammatory parameters, plasma adiponectin concentration showed an independent strong positive correlation with acetylcholine-stimulated vasodilation (30 μg/min) ($P < 0.001$), whereas plasma endothelin-1 levels showed a negative correlation with sodium nitroprusside–induced vasodilation (3 μg/min) ($P = 0.04$).

CONCLUSIONS — In this study, we have demonstrated that the addition of either the insulin sensitizer pioglitazone or the ACE-blocking agent ramipril further improves vascular dysfunction and markers of inflammation independent of glycemic control. Our data indicate that pioglitazone primarily enhances endothelial-mediated, whereas ramipril augments endothelial-independent, vasodilation. These different vascular effects, combined with the observation that pioglitazone increases the vasodilator adiponectin concentration, while ramipril reduces the vasoconstrictor endothelin-1 levels, suggest that different and complementary mechanisms underlie the observed improvements in vascular reactivity. In agreement with previous studies, pioglitazone therapy also was accompanied by a reduction in plasma fatty acids and triglyceride concentrations, both of which may play a role in the correction of vascular dysfunction (14–16).

It should be emphasized that intensive insulin therapy alone in the PLC

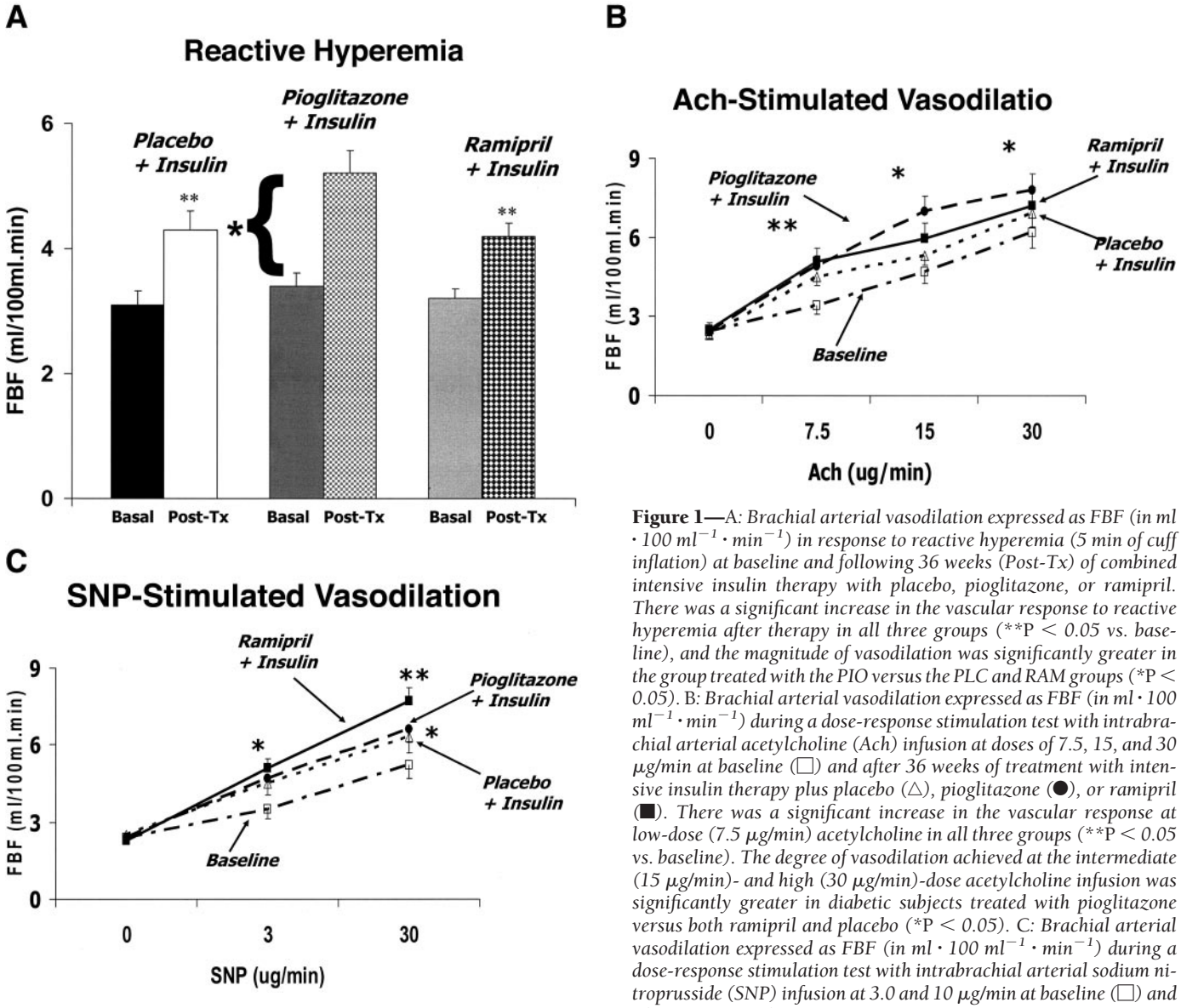

Figure 1—A: *Brachial arterial vasodilation expressed as FBF (in ml · 100 ml^{-1} · min^{-1}) in response to reactive hyperemia (5 min of cuff inflation) at baseline and following 36 weeks (Post-Tx) of combined intensive insulin therapy with placebo, pioglitazone, or ramipril. There was a significant increase in the vascular response to reactive hyperemia after therapy in all three groups (**P < 0.05 vs. baseline), and the magnitude of vasodilation was significantly greater in the group treated with the PIO versus the PLC and RAM groups (*P < 0.05).* B: *Brachial arterial vasodilation expressed as FBF (in ml · 100 ml^{-1} · min^{-1}) during a dose-response stimulation test with intrabrachial arterial acetylcholine (Ach) infusion at doses of 7.5, 15, and 30 μg/min at baseline (□) and after 36 weeks of treatment with intensive insulin therapy plus placebo (△), pioglitazone (●), or ramipril (■). There was a significant increase in the vascular response at low-dose (7.5 μg/min) acetylcholine in all three groups (**P < 0.05 vs. baseline). The degree of vasodilation achieved at the intermediate (15 μg/min)- and high (30 μg/min)-dose acetylcholine infusion was significantly greater in diabetic subjects treated with pioglitazone versus both ramipril and placebo (*P < 0.05).* C: *Brachial arterial vasodilation expressed as FBF (in ml · 100 ml^{-1} · min^{-1}) during a dose-response stimulation test with intrabrachial arterial sodium nitroprusside (SNP) infusion at 3.0 and 10 μg/min at baseline (□) and after 36 weeks of treatment with intensive insulin therapy plus placebo (△), pioglitazone (●), or ramipril (■). There was a significant increase in the vascular response at the low (3 μg/min) and high (10 μg/min) sodium nitroprusside dose in all three groups (*P < 0.005 vs. baseline). The degree of vasodilation achieved at the high sodium nitroprusside dose was significantly greater in diabetic subjects treated with ramipril versus both placebo and pioglitazone (**P < 0.05).*

group was effective in decreasing the circulating inflammatory biomarker hsCRP and that this decrease was equivalent to that seen with the addition of pioglitazone or ramipril. Moreover, endothelial-mediated vasodilation, measured by *1*) the percent increase in FBF during reactive hyperemia and during the low (7.5 μg/min) acetylcholine infusion and 2) endothelial-independent vasodilation following both low (3.0 μg/min)- and high (10.0 μg/min)-dose sodium nitroprusside infusion were improved in the PLC group, and there was a significant correlation between glycemic control and vascular reactivity in all groups. These findings indicate that in addition to attenuating inflammation intensive insulin therapy improves vascular endothelial dysfunction. The independent association between the decrease in plasma hsCRP levels and the improvement in vasodilation support the concept that intensive insulin therapy improves vascular endothelial dysfunction, in part, via attenuation of the inflammatory response. The possibility that acute glycemic fluctuations, induced by insulin therapy, interfere with vascular reactivity is not ruled out, however, since these may not be adequately reflected in the A1C values (21). The relationship between hyperglycemia and vascular reactivity previously has been examined in both diabetic (22) and nondiabetic subjects (8) and, in the presence of hyperglycemia, endothelial-mediated vascular dilation is impaired (23,24). Correction of hyperglycemia, regardless of the therapy, has been shown to

enhance endothelial-dependent vasodilation (24–26), and intensive insulin therapy is believed to reduce the formation of reactive oxygen and nitrogen species in tissue (22,24,26) and macrophages (27), thus minimizing the inflammatory response.

Our findings indicate that additional mechanisms, beyond glycemic control and more directly related to changes in insulin resistance and vascular reactivity, must play an important role in the improvement in endothelial dysfunction observed with these agents. There is increasing evidence that in order to prevent/retard atherosclerotic cardiovascular complications in type 2 diabetic patients, the underlying defects of both insulin resistance and endothelial dysfunction need to be addressed (5). Enhanced insulin sensitivity consistently has been demonstrated with pioglitazone (14,19), although the beneficial effect of ramipril on insulin sensitivity is more controversial (28), and in a recent trial (28) the diabetes conversion rate was unaffected. Both pioglitazone and ramipril have been shown to improve vascular reactivity (16,28,29). In the present study, a 50% increment in insulin-mediated glucose disposal was demonstrated during the hyperinsulinemic clamp in the pioglitazone-treated group. The modest nonsignificant increase in insulin sensitivity in the ramipril-treated group is consistent with previously reported results (30). We observed a highly significant correlation between the improvements in peripheral insulin resistance and in endothelial-mediated vasodilation, suggesting that enhanced insulin sensitivity, induced by pioglitazone, contributes to the improvement in endothelial-mediated vasodilation. However, previous studies by us (14) and others (15,16) also have shown that pioglitazone can improve endothelial dysfunction independent of changes in blood glucose levels. Thus, it is likely that the beneficial effects of TZDs on vascular function are mediated by *1*) direct effects on the vascular endothelium, *2*) improved glycemic control, and *3*) enhanced insulin-mediated glucose metabolism.

Pioglitazone did not improve endothelial-independent vasodilation beyond that observed with intensive insulin therapy alone. In contrast, ramipril significantly increased endothelial-independent vasodilation above that observed with insulin alone. The improvement in sodium nitroprusside–stimulated vasodilation (an index of endothelial-independent nitric oxide–mediated vasorelaxation) was closely correlated with the reduction in plasma endothelin-1 levels. Following pioglitazone treatment for 36 weeks, plasma adiponectin levels doubled, and the increase correlated closely with improved endothelial-dependent vasodilation. Based on these findings, we speculate that increased levels of the vasodilator adiponectin (31) play a role in mediating the improvement in vascular reactivity following pioglitazone, while a decrease in the vasoconstrictor endothelin-1 (32) levels contributes to the enhanced vascular function observed with ramipril. These complementary mechanisms suggest that combined TZD/ACE inhibitor therapy may be especially beneficial in improving vascular health in type 2 diabetic patients.

Our experiments are limited by the inherent difficulty of clinical observations performed in diabetic patients who are being treated with multiple drugs. The effects of prior treatment with agents that interfere with the angiotensin system were minimized by switching all ACE inhibitor/ARB–treated patients to α-methyldopa, as is done routinely in pregnant women. The number of diabetic patients receiving metformin therapy was similar in all three groups ($n = 3–4$), and we did not observe any differences in any parameters in patients treated with and without metformin. Statins were used in four to six patients in each group, but the dose of statin therapy was stable for at least 6 months before the study commencement, and the statin dose was not changed during the study. Although no clear difference has been reported regarding the effect of intensive therapy provided with an insulin pump versus multiple insulin injections, it must be recognized that our groups were heterogeneous with respect to this modality of therapy. Nevertheless, each of the three treatment groups contained equal number of individuals treated with CSII and MDII. Other limitations of our study are the absence of a group receiving pioglitazone combined with ramipril and the period of observation (36 weeks) in the present study that is relatively short, considering that these patients have had diabetes for 6–8 years. Whether our observations would continue to be observed over longer periods of therapy and translate into significant clinical benefits remain to be determined.

In conclusion, the addition of pioglitazone or ramipril to intensive insulin therapy in type 2 diabetes further improves vascular dysfunction. Pioglitazone enhances endothelial-mediated, whereas ACE inhibition enhances endothelial-independent vasodilation. These different vascular effects, combined with the observation that pioglitazone decreases free fatty acids and triglycerides and increases adiponectin, while ramipril reduces endothelin-1, suggest that different mechanisms underlie the vascular responses.

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