

Effects of Pioglitazone on Endothelial Function, Insulin Sensitivity, and Glucose Control in Subjects With Coronary Artery Disease and New-Onset Type 2 Diabetes

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OBJECTIVE — About one of five patients with coronary artery disease (CAD) suffers from previously unknown, predominantly postprandial type 2 diabetes. In the process of atherogenesis and the subsequent increased cardiovascular mortality of diabetic patients, endothelial dysfunction is suspected to play an important role, and it is observed in diabetic as well as insulin-resistant states. Thus, the aim of our study was to investigate the effect of pioglitazone on endothelial dysfunction, insulin sensitivity, and glucose control in newly detected type 2 diabetic patients with CAD.

RESEARCH DESIGN AND METHODS — We investigated 42 patients (39 men and 3 women, age 60.25 ± 7.5 years, HbA_{1c} $6.1 \pm 0.5\%$) with manifest CAD and newly detected type 2 diabetes. A randomized, double-blind, placebo-controlled, parallel study with pioglitazone (30 mg/day for 12 weeks) was performed. At study entry and end, we performed an oral glucose tolerance test and measurements of endothelial dysfunction by photoplethysmographic pulse wave analysis.

RESULTS — Endothelial dysfunction was severely impaired at baseline in both groups. After 12 weeks, endothelial dysfunction was significantly better in the pioglitazone group (change of reflection index 6.5 ± 5.1 vs. $1.6 \pm 2.9\%$, $P = 0.002$) compared with placebo. Insulin sensitivity, as assessed by homeostasis model assessment (2.20 ± 1.62 vs. 3.61 ± 1.87 , $P = 0.01$), or the change of insulin sensitivity index from baseline to study end (0.021 ± 0.023 vs. $-0.003 \pm 0.012 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ per pmol/l , $P = 0.0001$) and β -cell function (57.42 ± 49.86 vs. $21.78 \pm 18.54 \text{ mU/l per mmol/l}$, $P = 0.0014$) significantly improved in the pioglitazone group, with no change observed after placebo.

CONCLUSIONS — Pioglitazone improves endothelial dysfunction independently from the observed benefits on insulin sensitivity and β -cell function in patients with newly diagnosed type 2 diabetes and CAD.

Diabetes Care 29:1039–1045, 2006

Patients with type 2 diabetes are at substantially increased risk for atherosclerotic diseases (1). This is particularly the case in secondary prevention in patients with manifest coronary artery

disease (CAD) (2). In patients with stable CAD, ~30% suffer from known diabetes and another 20% from undiagnosed diabetes that can only be detected by an oral glucose tolerance test (OGTT) because of

its predominantly postprandial nature (3). This subset of patients exhibits increased vascular risk not far from that of established diabetic patients (4).

In the process of accelerated atherogenesis, endothelial dysfunction, by means of reduced synthesis or bioavailability of nitric oxide (NO), is believed to play a crucial role (5). Loss of NO's vasoactive effects predisposes the vessel wall toward vasoconstriction, leukocyte and platelet adhesion, and proinflammatory changes. Endothelial dysfunction can be detected in vivo by measurement of NO-mediated vasodilation, and several techniques have been successfully established (6).

Improving endothelial function in patients at high vascular risk has become an emerging concept in vascular medicine. Endothelial dysfunction depends on the number and expression of vascular risk factors, such as smoking (7), hypercholesterolemia (8), or diabetes (9), and it can be improved at least partially by treatment of those risk factors (7,10,11). In addition, preliminary evidence suggests that treatment-induced improvement of endothelial function identifies subjects who face a greater benefit from therapy (12).

Preliminary evidence suggests that glitazones, a new class of antidiabetic drugs that primarily act via amelioration of insulin resistance, are able to improve endothelial function (13–15). In addition, a recent publication of the Prospective Pioglitazone Clinical Trial in Macrovascular Events (PROactive) (16) supports the evidence of vasoprotective effects of glitazones in the secondary prevention of vascular diseases. In the current study, we examined the effects of pioglitazone on endothelial function, insulin resistance, and glucose control in newly detected type 2 diabetic patients with angiographically established CAD.

RESEARCH DESIGN AND METHODS

A total of 42 patients with stable CAD and recently detected type 2 diabetes, diagnosed by an OGTT, were recruited from our cardiology divi-

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Received for publication 15 November 2005 and accepted in revised form 31 January 2006.

Abbreviations: CAD, coronary artery disease; HOMA, homeostasis model assessment; MAPK, mitogen-activated protein kinase; NF- κ B, nuclear factor- κ B; OGTT, oral glucose tolerance test; PI, phosphoinositol; RI, reflection index; TZD, thiazolidinedione.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

DOI: 10.2337/dc05-2226

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sion. Type 2 diabetes was diagnosed according to World Health Organization criteria (17), by a 2-h postload venous full blood glucose value >10.0 mmol/l. Presence of CAD was defined as at least one stenosis $\geq 50\%$ in one coronary artery in a recent coronary angiography. Patients with any of the following criteria were excluded: diabetes by history or fasting blood glucose >6.1 mmol/l, acute coronary syndrome or cerebrovascular event within the previous 8 weeks, heart failure according to NYHA (New York Heart Association) III and IV criteria, uncontrolled hypertension ($>165/100$ mmHg), HbA_{1c} (A1C) $>7\%$, BMI >35 kg/m², aspartate aminotransferase or alanine aminotransferase above three times the upper limit of normal, serum creatinine >2.5 mg/dl, new onset of statin or ACE inhibitor treatment within the previous 8 weeks, or known hypersensitivity or intolerance to pioglitazone.

We performed a randomized, double-blind, placebo-controlled, parallel, prospective study. On the day of enrollment, endothelial function was assessed in the morning after an overnight fast, and subsequently a 3-h OGTT was performed. Insulin and glucose were measured before and 15, 30, 60, 120, and 180 min after ingestion of 75 g glucose. Patients were treated with 30 mg pioglitazone or pioglitazone-matched placebo once daily for 12 weeks. After the treatment period, they underwent the same investigation as on the day of enrollment. The study protocol was approved by the local ethics committee, and all participants gave written informed consent before inclusion in the study.

Endothelial function testing

Endothelial function was assessed by pulse wave analysis, detected by finger photoplethysmography (Micro Medical, Rochester, Kent, U.K.) (8,9). Changes of reflection index (RI) after salbutamol administration (ΔRI_{Salb}) have been established as a test of endothelial vasodilatory function (9) that depends on endothelial NO formation (8). Changes of RI after inhalative glyceroltrinitrate (ΔRI_{GTN}) describe endothelium-independent vasodilation to assess vascular smooth muscle cell function (8).

Three measurements were taken for baseline, RI_{GTN} was measured 3 and 5 min after application of glyceroltrinitrate (0.4 mg), and the average was taken as RI_{GTN} (ΔRI_{GTN} = % change from baseline). After 20 min, a dose of 600 μ g sal-

butamol was given via inhalation. At 10 and 15 min after salbutamol application, digital volume pulses were detected and the average taken as RI_{Salb} , (ΔRI_{Salb} = % change from baseline).

Insulin sensitivity and β -cell function

We assessed insulin sensitivity by homeostasis model assessment (HOMA), calculated as (fasting plasma glucose [mmol/l] \times fasting serum insulin [μ U/ml])/22.5 (18), as well as by the insulin sensitivity index (S_i), calculated as $0.222 - 0.00333 \times \text{BMI} - 0.0000779 \times \text{Ins}_{120} - 0.000422 \times \text{age}$, as suggested by Stumvoll et al. (19). β -Cell function was quantified as the ratio of the incremental insulin to glucose responses over the first

30 min during the OGTT on the one hand ($\Delta I_{30}/\Delta G_{30}$) (20) and by first- and second-phase insulin secretion index on the other hand (19): first phase = $1.283 + 1.829 \times \text{Ins}_{30} - 138.7 \times \text{Gluc}_{30} + 3.772 \times \text{Ins}_0$; second phase = $286 + 0.416 \times \text{Ins}_{30} - 25.94 \times \text{Gluc}_{30} + 0.926 \times \text{Ins}_0$.

Lab methods

Blood samples were taken after endothelial function testing. Blood glucose was determined from sodium fluoride-anticoagulated blood by the hexokinase method; insulin was measured in serum by enzyme-linked immunosorbent assay (EIA 1825; DRG Diagnostics, Marburg, Germany). All other laboratory param-

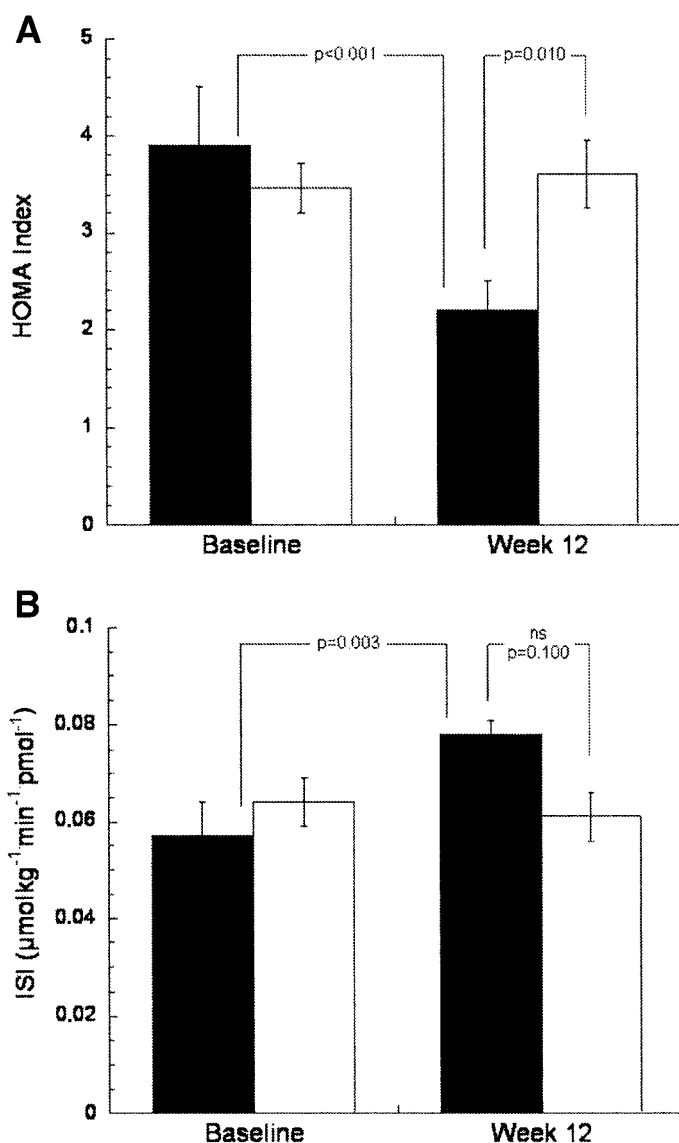


Figure 1—Insulin resistance expressed as HOMA index (A) and S_i (B) before and after treatment with either pioglitazone (■) or placebo (□). Error bars represent SE. ISI, S_i .

Table 1—Glucometabolic and lipid parameters before and after treatment with placebo or pioglitazone

	Placebo (n = 21)		Pioglitazone (n = 21)		P*
	Week 0	Week 12	Week 0	Week 12	
A1C (%)	6.1 ± 0.5	5.9 ± 0.4	6.1 ± 0.6	6.1 ± 0.5	NS
Fasting blood glucose (mmol/l)	5.89 ± 0.72	5.83 ± 0.94	6.22 ± 0.89	6 ± 1.11	NS
Mean blood glucose (mmol/l)†	9.67 ± 1.39	9 ± 1.44	10.17 ± 1.72	9.22 ± 1.05	NS
Fasting insulin (mU/l)	13.7 ± 6.6	14.0 ± 6.6	13.5 ± 11.0	8.4 ± 6.3	0.005
Mean insulin (mU/l)†	45.4 ± 21.0	45.2 ± 19.9	50.4 ± 39.5	29.0 ± 16.0	0.002
β-Cell function (mU/l per mmol/l)	18.36 ± 9.54	21.78 ± 18.54	25.56 ± 13.86	57.42 ± 49.86	0.014
First-phase insulin secretion (pmol/l)	788 ± 494	774 ± 507	635 ± 503	418 ± 345	0.0116
Second-phase insulin secretion (pmol/l)	512 ± 99	501 ± 76	498 ± 132	431 ± 73	0.0043
Total cholesterol (mmol/l)	4.97 ± 0.73	5.17 ± 0.83	5.04 ± 1.25	4.99 ± 1.09	NS
LDL cholesterol (mmol/l)	2.16 ± 0.52	2.39 ± 1.01	2.78 ± 1.27	2.86 ± 1.30	NS
HDL cholesterol (mmol/l)	1.38 ± 0.44	1.43 ± 0.39	1.35 ± 0.26	1.56 ± 0.31	NS
ΔHDL cholesterol (mmol/l)	0.05 ± 0.13		0.23 ± 0.08		<0.0001
Triglycerides (mmol/l)	2.9 ± 1.46	2.72 ± 1.55	1.85 ± 0.75	1.43 ± 0.65	0.0016
Δtriglycerides (mmol/l)	0.12 ± 1.36		0.39 ± 0.45		NS

Data are the means ± SD. *Pioglitazone week 12 vs. placebo week 12; †during the OGTT.

ters were determined in our routine local lab.

Statistics

Statistical analyses were computed with JMP IN software (version 5, release 5.1.2; SAS, Vienna, Austria). Analyses were performed by use of the Wilcoxon rank-sum test (for group comparisons) or the Wilcoxon signed-rank test (for within-group comparison). Univariate linear regression analyses were computed to identify possible predictors of improved endothelial function. All identified possible variables ($P < 0.1$) were forced into a linear model with or without therapy as an additional variable. The level of significance was set at $P < 0.05$; unless indicated, all data represent the means ± SD.

RESULTS— We recruited 42 patients (93% male) aged 60.3 ± 7.5 years with a BMI of 28.2 ± 4.1 kg/m² and a waist-to-hip ratio of 0.97 ± 0.05 . The mean blood pressure was 134 ± 21 mmHg over 79 ± 12 mmHg. There were no significant differences between the pioglitazone and placebo group at baseline.

Endothelial function

Before the start of medication, ΔRI_{Salb} in both groups was substantially impaired with no significant difference (3.3 ± 4.8 vs. 1.6 ± 3.6 , $P = NS$) between the groups (Fig. 1). Also, no difference in ΔRI_{GTN} between the two groups (14.7 ± 6.3 vs. 13.5 ± 8.5 , $P = NS$) was observed. After treatment a significantly higher ΔRI_{Salb} in the pioglitazone group versus

the placebo group was measured ($P = 0.002$) (Fig. 1), whereas there was no change in the endothelium-independent parameter ΔRI_{GTN} (13.3 ± 7.6 vs. 12.9 ± 8.3 , $P = NS$). Consequently, the change of ΔRI_{Salb} from baseline to study end was significantly higher in the pioglitazone-treated group (3.3 ± 3.1 vs. 0.1 ± 3.5 , $P = 0.01$).

Metabolic parameters

As shown in Table 1, A1C did not change, and neither did fasting blood glucose in both groups. In contrast, fasting insulin significantly decreased in the pioglitazone group compared with the placebo group.

During the OGTT, mean insulin concentration was significantly lower after pioglitazone, whereas mean glucose during the OGTT was not different from placebo. HOMA index improved in the pioglitazone group and was significantly better at study end than in the placebo group ($P = 0.01$) (Fig. 2A). S_i improved after pioglitazone (Fig. 2B); absolute values, however, missed statistical significance, but baseline to end difference was significantly higher in the pioglitazone group (0.021 ± 0.023 vs. -0.003 ± 0.012 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ per pmol/l with pioglitazone vs. placebo, $P = 0.0001$). β -Cell function ($\Delta I_{30}/\Delta G_{30}$) significantly

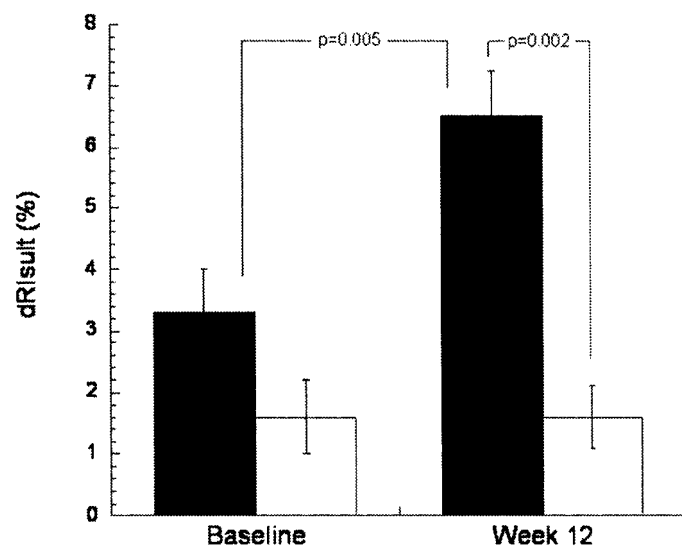


Figure 2—Endothelium-dependent vasodilation (ΔRI_{Salb}) before and after treatment with either pioglitazone (■) or placebo (□). Error bars represent SE. ΔRI_{Salb} .

Table 2—Univariate regression analysis of possible factors predicting improved endothelial function after completion of the study listed by strength of the association

Parameter	F ratio	r ²	P
BMI	16.8	0.30	0.0002
ΔHDL	16.41	0.29	0.0002
Δtriglycerides	7.59	0.16	0.008
HOMA at baseline	6.34	0.14	0.016
Waist-to-hip ratio	3.66	0.11	0.07
Endothelial function at baseline	3.41	0.08	0.07
LDL	1.88	0.05	0.18
Total cholesterol	1.02	0.02	0.32
HDL	1.02	0.02	0.32
Triglycerides	0.65	0.02	0.42
ΔHOMA	0.32	0.01	0.57
Age	0.16	0.01	0.69
Model 1	6.29	0.59	0.0004
Model 2	21.09	0.86	<0.0001

Subsequently, all parameters with a $P < 0.1$ were forced into a model without (model 1) or with (model 2) therapy as additional variable.

improved and insulin secretion expressed by first- and second-phase secretion rates decreased through the study in the pioglitazone group only.

A significantly lower level of triglycerides in the pioglitazone group at study end was evident. The change in triglycerides showed a trend in favor of pioglitazone without statistical significance. HDL, in contrast, increased significantly by pioglitazone treatment (Table 1).

Predictors of improved endothelial function

Univariate regression analysis was performed to identify predictors for improved endothelial function (Table 2). With those parameters and therapy as an additional variable forced into the model, 86% of the improvement of endothelial function could be explained, and therapy, change of triglycerides, and endothelial function at baseline were identified as independent parameters within that model. If the model was recalculated without therapy as a variable, a highly significant proportion of the improvement (59%) could still be predicted.

CONCLUSIONS— Our study, for the first time, indicates that pioglitazone in contrast to placebo significantly improves endothelial function as well as insulin resistance in patients with newly diagnosed type 2 diabetes and established CAD. In addition, our data provide evidence that the improvement of endothelial function is independent of any effects on insulin resistance or glycemia. It is, to

the best of our knowledge, the first study to investigate a glitazone in diabetic patients with manifest CAD with respect to endothelial function as well as insulin resistance.

Improved endothelial function

Several recent studies in either healthy subjects (15) or diabetic patients (13,21) indicate that thiazolidinediones (TZDs) are able to improve endothelial dysfunction. No study, however, has investigated a glitazone in the high-risk population with manifest CAD and diabetes.

In the above-mentioned studies, flow-mediated dilation (6) of the brachial artery was used to assess endothelial function. We used pulse wave analysis after inhalation of salbutamol, a technique that provides a reliable measure of NO-dependent vasodilation (8,9) and that has been shown to represent impaired endothelial function in diabetic patients (9). Moreover, a strict correlation exists between flow-mediated dilation and pulse wave analysis over the entire range of endothelial function (22). In our trial, vasodilator response to inhaled salbutamol almost doubled from baseline to study end after pioglitazone treatment, even if the response was still far from normalization. Chwienczyk et al. (9) determined endothelial vasodilator response to 400 μ g of inhaled albuterol in healthy subjects with a Δ RI (means \pm SE) of $11.8 \pm 1.8\%$. Dose-finding studies (H. Pressl, S. Heher, T.C.W., unpublished observations) indicated that a dose of 600 μ g is best suited for the evaluation of endothelial func-

tion, with a Δ RI_{salb} of $18 \pm 3\%$ in healthy subjects.

Almost all patients in our study (91%) were concomitantly treated with a statin but still exhibited pronounced endothelial dysfunction. It is noteworthy that we (23) and others (24) have shown that statins do not improve endothelial dysfunction in patients with diabetes, in contrast to their effect in almost all other risk populations (10,11,25). Despite this, statins reduce cardiovascular events in diabetic patients (26). We therefore suggest that no structural alterations in the vessel wall preclude improvements of endothelial function in patients with diabetes but that differences in the molecular mechanisms of action between statins and glitazones are responsible for such discrepant effects observed. This suggestion is further supported by the observation that endothelium-independent nitroglycerin-induced vasodilation was not influenced by pioglitazone.

Possible direct effects of pioglitazone in the vessel wall

Peroxisome proliferator-activated receptor γ as well as insulin receptors are expressed in endothelial cells, both playing a pivotal role in the maintenance of vascular homeostasis (27,28). Insulin acts via two pathways. Through the phosphoinositide (PI) 3-kinase pathway, insulin stimulates NO production (28). Through the mitogen-activated protein kinase (MAPK) pathway, insulin stimulates proatherogenic effects, such as endothelin-1 production and induction of plasminogen activator inhibitor-1 or adhesion molecules, such as E-selectin and vascular cell adhesion molecule-1. Jiang et al. (29) showed the activation of PI 3-kinase by insulin to be impaired in adipose tissue microvessels of insulin-resistant rats, whereas the activation of MAPK by insulin was unaffected. Cusi et al. (30) extended these findings to human muscle cells, and Madonna et al. (31) confirmed vascular cell adhesion molecule-1 amplification in human endothelial cells in vitro by insulin levels, reached in insulin-resistant subjects, by selectively blocking the PI 3-kinase pathway. These data indicate that “vascular insulin resistance” is characterized by an imbalance between PI 3-kinase and MAPK pathways. In contrast, “glucometabolic insulin resistance,” which represents the classical clinical phenotype of insulin resistance, is mediated exclusively via the PI 3-kinase pathway (32). In the vasculature, TZDs restore

the balance between the two insulin signaling pathways by inhibiting MAPK and enhancing the PI 3-kinase pathway (27); metabolically, TZDs only act via PI 3-kinase (32,33). As a consequence, an improvement of vascular insulin resistance does not need to be mirrored by an improvement of glucometabolic insulin resistance to the same extent, and this could explain our finding of improved endothelial dysfunction independent of glucometabolic insulin resistance.

At present, one can only speculate by what exact molecular mechanisms TZDs improve vascular insulin resistance. It has been established, however, that oxidative stress and local inflammatory activity play key roles in the pathogenesis of endothelial dysfunction in diabetes (34). TZDs were shown to antagonize proinflammatory nuclear factor- κ B (NF- κ B) activity as well as reduce inflammation markers, such as monocyte chemoattractant protein-1, plasminogen-activator inhibitor-1, or C-reactive protein (35–37), and to exert antioxidative effects, which can be explained at least in part by NF- κ B downregulation (35). This results in subsequent reduction of inducible NO synthase overexpression (38) as well as NADPH oxidase downregulation (38). The NF- κ B pathway and NADPH oxidase, in turn, play key roles in the inactivation of NO and generation of peroxynitrite in hyperglycemic conditions (39). Such effects of TZDs might, of course, contribute to the improved endothelial dysfunction observed in our study.

Metabolic effects and predictors of improved endothelial function

The HOMA index, which represents predominantly hepatic insulin sensitivity in the fasting state, as well as S_i , which describes a dynamic mapping of insulin sensitivity during an OGTT, improved through pioglitazone treatment. In contrast to the significant difference in the HOMA index at study end, only the change of S_i from baseline to study end reached statistical significance, probably because of the larger variability of the latter.

β -Cell function ($\Delta I_{30}/\Delta G_{30}$) improved significantly, indicating an insulin-sparing effect of treatment. This was confirmed by reduced first- and second-phase insulin secretion as a result of reduced insulin demands. Interestingly, no change in mean blood glucose during the OGTT occurred after the 12 weeks of treatment. A possible explanation for this finding could be the relatively short treat-

ment period because other studies showed a delayed effect of pioglitazone on glyemic control in manifest diabetic patients, with a maximum after 16–24 weeks of treatment (40,41).

The effects on lipids observed were in agreement with data already published (40,41). A significant increase of HDL cholesterol by pioglitazone was observed. Triglycerides at study entry were higher in the placebo group but without statistical significance ($P = 0.59$). At study end, the difference was more pronounced and reached statistical significance, whereas the change in triglyceride levels from baseline to study end showed a trend in favor of pioglitazone.

Regression analysis and modeling indicated pioglitazone therapy, endothelial function at baseline, and the change of triglycerides from baseline to study end as significant predictors of improved endothelial function. Our study therefore confirms and extends previous publications that indicated improvements of endothelial function after glitazone were independent from metabolic effects (13,21) in a secondary prevention population.

Study limitations

A limitation of our study is the lack of information on inflammatory markers in relation to endothelial function. Because past studies found improvement of these markers by glitazone therapy in diabetic and nondiabetic patients (15,21), we speculated that this could also be observed in our patients. We cannot exclude, therefore, that improved inflammatory response could also represent a predictor of improved endothelial function.

Another limitation of our study might be the rather short treatment duration of 12 weeks, which resulted in a failure to detect any effects of pioglitazone on postprandial hyperglycemia. This, however, does not corroborate our results because improvements of endothelial function were clearly independent from metabolic effects of pioglitazone.

Clinical aspects

Our study clearly established improvement of endothelial function by pioglitazone in patients with already manifest CAD and diabetes. We believe this observation to be of particular importance because previous investigations established that better endothelial function is associated with improved cardiovascular outcome in patients with manifest CAD (42). In addition, TZDs have been shown to

reduce progression of carotid intima-media thickness, a surrogate marker of atherogenesis (21,43). The full implication of all the above-mentioned findings are provided by the recently published PROactive results, which showed that pioglitazone reduces incident vascular events in type 2 diabetic patients with manifest atherosclerosis (16). Therefore, clinical evidence in favor of glitazone treatment is available, from improved endothelial function via reduced progression of carotid atherosclerosis to reduced incidence of vascular events. Furthermore, we suggest that the assessment of changes in endothelial function through pioglitazone treatment could possibly identify potential responders of a glitazone therapy with regard to cardiovascular outcomes.

In conclusion, our study demonstrates that in patients with manifest CAD and new-onset diabetes, pioglitazone improves endothelial dysfunction above that of preexisting statin therapy independently of changes in insulin resistance or glycemic control. We suggest that our findings justify further research into possible direct effects of glitazones in the vascular wall, as well as into the possible role of endothelial function to predict treatment-induced vascular benefit in glitazone-treated patients.

Acknowledgments—The study was supported in part by an unrestricted research grant from Takeda Austria.

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