In Type 2 Diabetes, Rosiglitazone Therapy for Insulin Resistance Ameliorates Endothelial Dysfunction Independent of Glucose Control

Frank Pistrosch, MD
Jens Passauer, MD
Sabine Fischer, MD
Katja Fuecker
Markolf Hanefeld, MD
Peter Gross, MD

OBJECTIVE — Insulin resistance is an independent risk factor for arteriosclerosis and cardiovascular mortality. However, the mechanism by which insulin resistance contributes to arteriosclerosis is unknown. Conceivably, endothelial dysfunction could be involved. Therefore, we asked whether therapy for insulin resistance ameliorates any endothelial dysfunction.

RESEARCH DESIGN AND METHODS — We performed a double-blind cross-over trial of 12 patients with recently diagnosed type 2 diabetes. They received rosiglitazone 4 mg b.i.d. for 12 weeks and nateglinide 60 mg b.i.d. for the same number of weeks in random order. To assess the degree of endothelial dysfunction, we used venous occlusion plethysmography. We studied vasodilation in response to acetylcholine (ACh) with and without exogenous insulin. The agents were infused into the brachial artery. Furthermore, we determined insulin resistance by euglycemic clamp.

RESULTS — Glycemic control was comparable under rosiglitazone and nateglinide. Rosiglitazone ameliorated insulin resistance by 60% compared with nateglinide. ACh response was significantly increased after rosiglitazone treatment (maximum forearm blood flow 12.8 ± 1.3 vs. 8.1 ± 1.3 ml/100 ml after rosiglitazone and nateglinide, respectively; P < 0.05) but did not attain the level of healthy control subjects (14.0 ± 0.7 ml/100 ml). Coinfusion of exogenous insulin increased ACh response further in the rosiglitazone group. N-monomethyl-L-arginine-acetate (l-NMMA), an antagonist of nitric oxide synthase, largely prevented the increased vasodilation after rosiglitazone, regardless of the presence or absence of insulin. Insulin sensitivity and blood flow response were found to be correlated (P < 0.01).

CONCLUSIONS — Insulin resistance is a major contributor toward endothelial dysfunction in type 2 diabetes. Both endothelial dysfunction and insulin resistance are amenable to treatment by rosiglitazone.

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Type 2 diabetes is an important risk factor for arteriosclerosis. According to recent literature, insulin resistance is a major aspect of this relationship (1–3). However, the mechanism by which insulin resistance contributes to arteriosclerosis is not known. An intact vascular endothelium is paramount to protection from arteriosclerosis. Endothelial dysfunction is a hallmark of arteriosclerosis. Endothelial dysfunction is most likely involved in both initiation and propagation of arteriosclerosis (4–6). In type 2 diabetes, impaired endothelial function, both impaired nitric oxide (NO)-mediated vasodilation and vasodilation mediated independent of NO or prostacyclin (PGI2), has been demonstrated (7–9). Therefore, we asked the question whether in type 2 diabetes endothelial dysfunction might be related to insulin resistance and whether insulin sensitization is capable of restoring this dysfunction. To provide an answer, we used rosiglitazone, a peroxisome proliferator-activated receptor-γ agonist, to bring about insulin sensitization, and we observed its effects on endothelial function in type 2 diabetes. These effects were further studied in detail to differentiate between NO-mediated effects and NO/PGI2-independent vasodilation. We expected a significant decrease in plasma glucose levels after rosiglitazone treatment. Because hyperglycemia itself may cause endothelial dysfunction (10), we designed a cross-over study with two treatment arms to control for the confounding effect of hyperglycemia. We enrolled only patients with recently diagnosed diabetes to focus on early functional changes rather than late structural abnormalities of the vasculature. We compared the rosiglitazone-treated group with the same patients while receiving nateglinide, an antidiabetic agent without direct effects on insulin sensitivity, providing comparable glucose control (11).
between diagnosis and enrollment in the study was 4.3 ± 0.5 weeks. Patients were encouraged to keep their lifestyle habits unchanged throughout the study. At entry, patients had HbA1c <7.5%, and there was no history or evidence of any of the following: cardiovascular events, diabetic microvascular complications, microalbuminuria, or abnormal result of retinal screening. None of the patients had previously taken antidiabetic medication. Concomitant disorders included hypertension (n = 10) and hypercholesterolemia (n = 6). Any medications for these disorders were maintained unchanged throughout our study. All patients were nonsmokers. The control group consisted of nine healthy volunteers (four women, five men; mean age 59.7 ± 1.2 years, range 45–70 years) with a normal glucose tolerance test and not taking any medication.

The study protocol was approved by the ethics committee of our institution. All participants gave written informed consent before inclusion into the study.

Patients were assigned in random order to rosiglitazone 60 mg b.i.d. each for 12 weeks. Thereafter, they maintained a washout period of 4 weeks. This was followed by a second treatment period of 12 weeks in which medication was given in reverse order. Forearm blood flow (FBF) studies for determination of endothelial function were performed at baseline and at the end of each treatment. Euglycemic-hyperinsulinemic clamp was performed to measure the degree of insulin resistance. Furthermore, body weight, body composition (by electrical impedance analysis), and levels of fasting plasma glucose (FPG), HbA1c, HDL, LDL, plasma insulin, and free fatty acids (FFAs) were determined. Patients were seen every 4 weeks for control of compliance and assessment of side effects. All measurements were performed once in healthy volunteers. They did not receive any medication.

FBF studies

Studies were performed in a quiet, temperature-controlled room (23–25°C) under fasting conditions with the patients resting supine. At least 10 h were maintained between FBF measurement and the preceding dose of the study medication. Patients took 1,200 mg ibuprofen orally 1 h before beginning FBF studies to block the effect of prostaglandin products on endothelial function. An arterial cannula (27 gauge; Cooper Needleworks, Birmingham, U.K.) was inserted into the brachial artery of the nondonor arm for delivery of test agents.

FBF was measured in both arms simultaneously using strain-gauge venous occlusion plethysmography (Gutmann Medizinelektronik, Eursburg, Germany) as published (14). This technique showed good short-term and long-term reproducibility of FBF during intra-arterial infusion of vasodilators (r = 0.9–0.97). Before the start of each measurement, a wrist cuff was inflated to 50 mmHg above systolic blood pressure to exclude the hand circulation from the measurement. The upper arm cuff for venous congestion was inflated to 40 mmHg during each FBF measurement. Determination of baseline FBF started 20 min after insertion of the arterial catheter. Each dose of the test agent was given intra-arterially for 4 min at a constant rate of 1 ml/min before FBF measurements were performed.

Baseline protocol

Healthy control subjects and diabetic patients, before intake of study medication, underwent baseline determination of FBF. Six doses of acetylcholine (ACh) (Micioh-E, Nürnberg, Germany), 1, 5, 10, 50, 100, and 300 nmol/min, were infused into the brachial artery, and FBF was determined for each dose. After 30 min of equilibration, sodium nitroprusside ([SNP] Schwarz Pharma, Monheim, Germany), an endothelium-independent vasodilator, was given at 2.5, 5, and 10 μg/min, and FBF was determined for each dose.

In addition, the following specific tests were performed in diabetic patients after each treatment period.

Protocol 1: Assessment of endothelial function both under baseline conditions and local hyperinsulinemia

After establishment of a baseline ACh dose-response curve, we kept a washout period of 30 min. Thereafter, insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) was infused into the brachial artery at 0.1 mU · kg⁻¹ · min⁻¹, together with glucose (75 μmol/min). This infusion leaves systemic insulin and glucose concentrations unaltered (15). To test any effects of local hyperinsulinemia on endothelium-dependent vasodilation, insulin and glucose infusion were maintained, stepwise ACh infusion was added, and serial determinations of FBF were made. Thereafter, we kept another 30-min washout period followed by SNP infusion, as described in the baseline protocol.

Protocol 2: Role of NO in the observed responses

To differentiate between NO and NO/PGI₂-independent factors contributing to the ACh-mediated vasodilation, we repeated the tests of endothelial function under conditions of a clamped NO system as described (16). Accordingly, NO generation in the forearm was blocked by infusion of N-monomethyl-L-arginine-acetate (L-NMMA) (Clinalfa, Laüfeningen, Switzerland) at 16 μmol/min. This specific rate was chosen because Dawes et al. (17) reported a maximal NO suppression at this infusion rate. SNP was added as necessary to restore FBF to its previous baseline before L-NMMA. ACh infusions were given as in the baseline protocol. Because PGI₂ generation was inhibited by ibuprofen, the resulting increase in FBF was attributable to NO/PGI₂-independent factors (16).

Euglycemic-hyperinsulinemic clamp

The euglycemic-hyperinsulinemic clamp technique was applied as described (18). After an overnight fast, blood samples for measurements of baseline plasma glucose and baseline plasma insulin levels were collected. Thereafter, intravenous insulin infusion was administered in descending dosage, calculated on the basis of body surface area for 10 min. From the 11th minute onward, insulin was administered at a constant infusion rate of 60 mU · m⁻² · min⁻¹). Plasma glucose concentration was measured every 5 min. A 20% glucose solution was infused to keep the plasma glucose concentration steady at 5.5 mmol/l (5.3–5.7) over the duration of the clamp procedure. The steady state was maintained for at least 60 min. Insulin sensitivity was expressed as M. It was calculated as the quantity of glucose metabolized divided by the measured plasma insulin concentration, normalized for body weight.

Biochemical analyses

Cholesterol and triglyceride measurements were performed using the CHOD-
HbA1c, or FPG between rosiglitazone and nateglinide treatments, although, as compared with baseline, both rosiglitazone and nateglinide lowered HbA1c significantly. Healthy control subjects, however, had the lowest levels of HbA1c and FPG. BMI and body composition were not significantly different among all groups.

**Forearm blood flow studies**

FBF before the start of each dose-response study was not different, neither between groups nor between the parts of the protocol within one group (baseline protocol: 2.9 ± 0.3 and 2.6 ± 0.5 ml/100 ml for diabetic and healthy control subjects, respectively; protocol 1: 2.5 ± 0.3, 2.8 ± 0.3, and 2.8 ± 0.4 ml/100 ml for rosiglitazone and 3.0 ± 0.4, 3.2 ± 0.5, and 2.7 ± 0.5 ml/100 ml for nateglinide; protocol 2: 2.4 ± 0.3 and 2.6 ± 0.4 ml/100 ml for rosiglitazone and 2.6 ± 0.3 and 2.6 ± 0.3 ml/100 ml for nateglinide). Blood flow in the control arm showed no differences between groups, and there were no significant changes of blood flow in the control arm throughout the course of protocols (data not shown).

As demonstrated in Fig. 1A, the ACh response was significantly increased after rosiglitazone compared with nateglinide or untreated patients at baseline (P = 0.021), although healthy control subjects had the best response. Maximal ΔFBF reached 14.0 ± 0.7 ml/100 ml in healthy control subjects, 12.8 ± 1.3 ml/100 ml after rosiglitazone, 8.8 ± 1.3 ml/100 ml after nateglinide, and 8.0 ± 1.3 ml/100 ml in patients at baseline. When the area under the curve (AUC) was considered, total ΔFBF after rosiglitazone was ~40% larger than total ΔFBF after nateglinide (2.459 ± 328 vs. 1.788 ± 273 ml·mmol·100 ml−1·min−1 for rosiglitazone and nateglinide, respectively; P = 0.018). The corresponding AUC for control subjects and patients at baseline were 2,906 ± 216 and 1,702 ± 314 ml·mmol·100 ml−1·min−1, respectively.

Addition of L-NMMA to the previous protocol (Fig. 1C) resulted in a significant decrease of the response to ACh in both groups, nateglinide and rosiglitazone. This decrease was relatively more pronounced for the lower four doses than for the upper two doses of ACh. At the two highest infusion rates of ACh, ΔFBF approximated values observed previously in the absence of L-NMMA in both groups. ANOVA for repeated measurements indicated an absence of a significant difference between rosiglitazone and nateglinide in the presence of L-NMMA.

During coinfusion of insulin, the ACh dose-response curve again showed a significant difference between the two treatment groups (Fig. 1B). However, the increase in the AUC for ΔFBF in the presence of exogenous insulin (Fig. 1B) compared with the AUC in the absence of exogenous insulin (Fig. 1A) was significantly larger after rosiglitazone than after nateglinide (565 ± 31 vs. 287 ± 25 ml·mmol·100 ml−1·min−1, for rosiglitazone and nateglinide, respectively; P < 0.05).

When the responses to ACh during L-NMMA infusion in the presence of insulin were compared in the two groups,
no significant differences were found (Fig. 1D). In addition, comparison of respective groups in Figs. 1C and D (i.e., nateglinide with nateglinide, rosiglitazone with rosiglitazone) also failed to show significant differences.

There were no significant differences in $\Delta$FBF between any groups during the infusions of three doses of SNP: 6.6 ± 1.4, 10.3 ± 1.0, and 12.6 ± 1.1 ml/100 ml for healthy control subjects; 6.6 ± 1.4, 10.1 ± 1.0, and 12.4 ± 1.1 ml/100 ml after rosiglitazone; 6.8 ± 1.0, 10.0 ± 1.5, and 11.6 ± 1.2 ml/100 ml after nateglinide; and 6.9 ± 0.9, 9.0 ± 1.0, and 10.0 ± 1.0 ml/100 ml for patients at baseline.

**Euglycemic-hyperinsulinemic clamp**
Insulin sensitivity (expressed as $M_e$) was significantly larger after rosiglitazone treatment than after nateglinide (3.7 ± 0.3 vs. 2.3 ± 0.3 mg·kg$^{-1}$·min$^{-1}$ per 100 µU/ml; $P < 0.001$). $M_e$ of healthy control subjects was 5.7 ± 0.5 mg·kg$^{-1}$·min$^{-1}$ per 100 µU/ml, i.e., significantly larger than nateglinide and rosiglitazone ($P < 0.001$). $M_e$ of patients at baseline was 2.0 ± 0.4 mg·kg$^{-1}$·min$^{-1}$ per 100 µU/ml. There was no significant difference in $M_e$ between the nateglinide-treated group and patients at baseline ($P = 0.51$).

We compared the differences in maximal $\Delta$FBF in response to ACh between both treatment groups (rosiglitazone minus nateglinide) with the corresponding differences in insulin sensitivity. There was a significant positive correlation (Fig. 2). We did not find a demonstrable significant correlation between the differences of FBF and corresponding differences of any of the following: blood pressure, LDL, FFA, FPG, and insulin.

**CONCLUSIONS**—In the literature, it is well known that insulin resistance in type 2 diabetes is an important risk factor for arteriosclerosis (2), but it has remained unclear how these effects come about. Endothelial dysfunction is involved in initiation and propagation of ar-
teriosclerosis (4,5). The present studies were undertaken to clarify whether insulin resistance per se might be related to endothelial dysfunction and whether insulin sensitization might improve it. We used the same group of diabetic patients as its own control by a double-blind, cross-over design of two antihyperglycemic treatments, of which rosiglitazone is known to improve insulin sensitivity. In this way, we sought to keep other risk factors for endothelial dysfunction unaltered, i.e., glycemic control, blood lipids, or blood pressure. We used the forearm perfusion technique, and for our tests, we relied primarily on the vasodilator response to ACh, both being established procedures by the literature to assess endothelial function (4).

In the present work, we found significant endothelial dysfunction in type 2 diabetic patients. This result is in accordance with previous reports in the literature (9). In addition, our findings with rosiglitazone as opposed to those with nateglinide strongly suggest that insulin resistance per se is related to endothelial dysfunction, independent of glycemic control, and that rosiglitazone had therapeutic effects on this endothelial dysfunction. These findings were also reproducible under conditions of local hyperinsulinemia. It was possible to show a direct and significant correlation between the gain of M<sub>1</sub> in response to rosiglitazone (compared with nateglinide) and the corresponding gain of maximal AFBF (Fig. 2). It could be argued that treatment with nateglinide might have worsened endothelial function or insulin sensitivity. However, as shown for baseline studies and those after nateglinide, this was not the case. Taken together, our observations show that in type 2 diabetes, insulin sensitization ameliorates endothelial dysfunction and that endothelial function is restored to nearly normal levels by rosiglitazone (Fig. 1A). These results imply that endothelial insulin resistance may be an aspect of insulin resistance in general (20).

Our observations are in agreement with previous literature. Animal studies in the rat also showed an improvement of ACh-dependent vasodilation in response to pioglitazone or rosiglitazone (21,22). A comparable observation involving troglitazone has also been published in diabetic patients (23). In the latter study, the investigators used flow-mediated vasodilation to assess endothelial dysfunction, which is a less precise and less specific test than venous occlusion plethysmography. On the other hand, a study in obese, insulin-resistant but nondiabetic humans (24) failed to show an improvement in ACh-dependent vasodilation in response to troglitazone. That study reported a troglitazone-induced improvement in insulin sensitivity of only 25%, as opposed to a 60% improvement in the present work. It is conceivable that a 25% improvement (24) is ineffective with respect to significant changes in endothelial function.

Our study was not designed to evaluate the biochemical mechanisms by which insulin sensitization might improve endothelial function. In principle, thiazolidinediones improve general metabolic control. This improvement could have beneficial effects on endothelium (25). For instance, hyperglycemia or high concentrations of FFA may contribute to endothelial dysfunction by production of reactive oxygen species or by interaction with NO synthase (10,26). However, in the present study, glucose control and FFA levels were comparable during rosiglitazone and nateglinide treatments. Therefore, other aspects of metabolic control or different effects of rosiglitazone may have been responsible for the improved endothelial function. Hypertension is known to cause endothelial dysfunction. However, in our study, blood pressure control was maintained unchanged and at normal levels throughout (Table 1), as were any antihypertensive regimens. It is, therefore, very unlikely that blood pressure differences or antihypertensive medications contributed to the improvement of endothelial function in this study.

Endothelial dysfunction has been demonstrated even in healthy nondiabetic first-degree relatives of type 2 diabetic patients in the absence of insulin resistance (27). This indicates that additional factors contribute to the genesis of endothelial dysfunction of type 2 diabetic patients.

The tests of NO-blockade (“NO-clamp”) during ACh-induced endothelial vasodilation (Figs. 1C and D) yielded two observations: 1) the effects of rosiglitazone to improve endothelial function were associated with an improvement in NO generation, a finding that is supported by a recent study of Vinik et al. (28). They measured increased NO in the human skin during local skin hyperemia after rosiglitazone treatment of type 2 diabetic patients; 2) NO blockade did not abolish the response to ACh completely nor did it abrogate fully the improved response to ACh after rosiglitazone. Because of administration of ibuprofen, these residual vasodilator responses may therefore be related to other NO/PGL<sub>2</sub>-independent factors (29). There are few studies that have focused on the role of such factors in endothelial dysfunction in diabetes. Most have described a profound impairment (9). Even in the absence of significance, the data in Fig. 1C suggest a larger splay of both curves in the area of higher doses of ACh. Accepting from the literature that the lower doses of ACh pri-
marily stimulate endothelial NO generation, these data for higher doses of ACh (>100 nmol/min) indicate that rosiglitazone also tends to improve non–NO/PGI2-related endothelium-dependent vasodilation. Our study design did not allow further differentiation of these factors.

During coinfusion of insulin, the improved vasodilator response to ACh seemed to be primarily NO mediated. This is in agreement with others (30). It might be explained by improvement of an insulin-signaling pathway, which seems to regulate both insulin-stimulated glucose uptake and insulin-stimulated endothelial function (31).

The vasodilator response to SNP was comparable in all groups, demonstrating an intact vascular smooth muscle function in all groups tested. Therefore, the observed effects of rosiglitazone to improve endothelial dysfunction are best explained by effects of rosiglitazone on arterial vascular endothelium.

In conclusion, our study showed that insulin resistance is a major contributor to endothelial dysfunction in type 2 diabetes; both insulin resistance and endothelial dysfunction are amenable to treatment by rosiglitazone. In the future, additional studies may be performed to take the present issues further: i.e., what are the biochemical mechanisms by which insulin sensitization improves endothelial function and what are the long-term effects of rosiglitazone and the improvement of endothelial function in type 2 diabetic patients on arteriosclerotic end points?

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