Fluid Retention and Vascular Effects of Rosiglitazone in Obese, Insulin-Resistant, Nondiabetic Subjects

ALEXANDER J.M. RENNINGS, MD[1,2]
PAUL SMITS, MD, PHD[2]
MURRAY W. STEWART, DM, FRCP[3]
CEES J. TACK, MD, PHD[1]

OBJECTIVE — The use of thiazolidinedione (TZD) derivatives is associated with fluid retention, especially when combined with insulin. Because TZDs improve the metabolic effect of insulin, they may also reverse the blunted vascular response to insulin. We hypothesize that improvement of the action of insulin on vascular tone or permeability is the key mechanism of TZD-related fluid retention.

RESEARCH DESIGN AND METHODS — In a randomized, double-blind, placebo-controlled, cross-over study in 18 obese, nondiabetic subjects with features of the metabolic syndrome, we investigated the effects of a 12-week treatment with 4 mg rosiglitazone twice a day on glucose disposal, hemodynamics (including forearm vasoconstrictor response to nitric oxide [NO]), synthase inhibition by *N*-monomethyl-L-arginine-acetate (L-NMMA), vascular permeability (transcapillary escape rate of albumin), and plasma volume during a hyperinsulinemic-euglycemic clamp (120 min, 120 mU/m^2 per min).

RESULTS — As expected, rosiglitazone increased the glucose infusion rate during clamping. However, neither vascular permeability nor forearm blood flow response to hyperinsulinemia or L-NMMA was affected by rosiglitazone. Compared with placebo, rosiglitazone decreased diastolic blood pressure by 5 mmHg (95% CI 2.35–6.87, $P = 0.0005$) and increased plasma volume by 255 ml/1.73 m^2 (80–430, $P = 0.007$). Interestingly, the positive effect of rosiglitazone on glucose disposal correlated with change in foot volume ($R^2 = 0.53$, $P = 0.001$).

CONCLUSIONS — Rosiglitazone improved insulin sensitivity but had no effect on NO-dependent vasodilatation in the forearm or vascular permeability in obese, insulin-resistant, nondiabetic subjects. As such, TZD-related fluid retention was not caused by improvement of the vascular actions of insulin. Nonetheless, rosiglitazone-induced improvement in insulin sensitivity appears to be correlated to edema formation.

Diabetes Care 29:581–587, 2006

Thiazolidinedione (TZD) derivatives improve insulin sensitivity and hence are valuable in the treatment of type 2 diabetes (1). Important adverse effects are fluid retention and peripheral edema formation. The precise mechanism(s) of these adverse effects are unclear and probably are multifactorial (2). Although multiple factors are involved, the existence of an initial trigger or main mechanism could be of clinical importance. In theory, the initial trigger of fluid retention may originate either from kidney, heart, or peripheral circulation. As TZD treatment is associated with a reduction in blood pressure (3–6), a primary renal mechanism seems unlikely. A primary cardiac origin also seems improbable, because long-term studies with rosiglitazone have not revealed any negative effect on myocardial structure or function (7).

The combination of blood pressure reduction, fluid retention, and edema formation is compatible with changes in the peripheral circulation resulting in capillary leakage. This may be induced by certain actions of TZDs, such as improved insulin-mediated vasodilatation, direct vasoactive effects (8), or increased endothelial permeability (9). Interestingly, the incidence of edema increases substantially when rosiglitazone (10) or pioglitazone (11) is used in combination with insulin. A number of findings suggest that the tendency for fluid retention is coupled to the effect of TZDs on the metabolic actions of insulin. For example, both glycemic efficacy and edema formation are dose-dependent features of TZD therapy (10). Furthermore, peroxisome proliferator–activated receptor (PPAR)γ agonists with more potent glucose-lowering effects seem to be associated with a higher incidence of edema formation (12). Besides a metabolic effect, insulin also has important vascular properties at several sites of the vascular tree. For instance, insulin increases vascular permeability (13) and induces vasodilatation in resistance arteries (14), venules (15), and precapillary arteriolae (16), thereby inducing capillary recruitment (17) and resulting in a decrease in capillary resistance. Acute hyperinsulinemia has been reported to increase the transcapillary escape rate of albumin (13), consistent with a direct effect of insulin on arteries and capillaries, promoting vascular leakage and therefore edema formation. If TZDs augment both the metabolic and vascular effects of insulin, the effect on glycemic control and fluid retention would indeed be coupled.

In the present study, we investigated whether rosiglitazone treatment, besides improving the metabolic action of insulin, can also reverse the blunted vasodilator response to insulin (18) and/or change vascular permeability in insulin-resistant subjects. To avoid confounding by im-

From the [1]Department of Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands; the [2]Department of Pharmacology-Toxicology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands; and [3]GlaxoSmithKline, Harlow, U.K.

Address correspondence and reprint requests to Alexander J.M. Rennings, MD, Department of Pharmacology-Toxicology 149, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB Nijmegen, Netherlands. E-mail: a.rennings@aig.umcn.nl.

Received for publication 5 August 2005 and accepted in revised form 28 November 2005.

Abbreviations: ANP, atrial natriuretic peptide; DBP, diastolic blood pressure; FBF, forearm blood flow; GIR, glucose infusion rate; L-NMMA, N^G-monomethyl-L-arginine; PPAR, peroxisome proliferator–activated receptor; SBP, systolic blood pressure; TERalb, transcapillary escape rate of labeled albumin; TZD, thiazolidinedione.

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

proved glycemic control, we studied nondiabetic subjects with characteristics of the metabolic syndrome.

RESEARCH DESIGN AND METHODS

— The study population consisted of 18 healthy, obese volunteers (BMI between 27 and 36 kg/m^2, aged 30–65 years) with either two or more features of the metabolic syndrome as defined by the National Cholesterol Education Program (19) or one of these features in combination with a first-degree relative having type 2 diabetes. Subjects were not eligible for inclusion if they had fasting plasma glucose >7.0 mmol/l or HbA_{1c} (A1C) >6.5%, if they used nonsteroidal anti-inflammatory drugs, fibrates, anticoagulants, antihypertensives, any investigational drug, or a PPARγ agonist, or if they had just started lipid-lowering therapy. Additional exclusion criteria were blood pressure exceeding 160/100 mmHg, unstable or severe angina or congestive heart failure, the presence of clinically significant hepatic or renal disease or anemia, pregnancy, lactation, lack of appropriate contraception for women with child-bearing potential, and alcohol or drug abuse. Study participants were selected by advertisement, received a payment, and gave written informed consent. This study was approved by the hospital ethics committee and was performed according to good clinical practice guidelines.

Within 6 weeks after screening, participants were randomly assigned to receive either rosiglitazone (4 mg twice daily) or placebo for 12 weeks in a double-blind, cross-over design. The primary end points of the study were measured at the end of each 12-week treatment period, and we considered this long enough to avoid a carryover effect. Therefore, we decided to not include an extra washout period between both treatment periods. At weeks 2 and 6 of each treatment period, adverse events and pill compliance were recorded. Physical examination was performed, foot volume was measured, and safety chemical, hematological, and glycemic profiles were determined. At the end of each 12-week treatment period the hemodynamic and metabolic effects of insulin were quantified during a hyperinsulinemic-euglycemic clamp procedure. During this test, vascular permeability was assessed by measurement of the transcapillary escape rate of labeled albumin (TERalb). Two weeks after the final treatment period, there was a follow-up visit. Participants were strictly advised to maintain their diet and not to change their lifestyle.

Protocol experimental day

After an overnight fast of at least 10 h the subject entered a quiet temperature-controlled room (23–24°C) at 8:00 A.M. A 20-gauge catheter (Angiocath; Becton Dickinson, Sandy, UT) was inserted into the left brachial artery under local anesthesia (0.3–0.4 ml of lidocaine HCl; 20 mg/ml), connected via an arterial pressure monitoring line to a Hewlett Packard 78353B monitor and kept patent with saline and heparin (0.9% NaCl and 2 units/ml heparin; NaCl, 3 ml/h). This catheter was used for both intra-arterial drug infusion (automatic syringe infusion pump, type STC-521; Terumo, Tokyo, Japan) and for blood sampling. One venous catheter (Venflon, 20 G, 32 mm) was inserted antegrade into a deep arm vein for the infusion of insulin and glucose.

After a 30-min equilibration period, the intra-arterial pressure wave signal was recorded for 5 min to calculate cardiac output and systemic vascular resistance using "model flow analysis" (20). Subsequently, forearm blood flow (FBF) (21,22) was measured simultaneously in the experimental and control arm using mercury-in-Silastic strain-gauge venous occlusion plethysmography. The FBF of the contralateral arm was used as a time-control value to observe systemic effects. After these baseline measurements, the hyperinsulinemic-euglycemic clamp (23,24) was started. Insulin (Actrapid; Novo-Nordisk, Bagsvaerd, Denmark) was infused intravenously at a dose of 720 pmol/m^2 per min (120 mU/m^2 per min). Insulin (50 units/ml) was diluted in 47.5 ml of 0.9% NaCl with the addition of 2 ml of the subject's blood to a concentration of 1 unit/ml. Euglycemia was maintained at 5.0 mmol/l by a variable infusion of 20% glucose solution, adjusted at 5-min intervals according to arterial glucose measurements. Glucose infusion rate (GIR) was defined as the GIR during the last 30 min of the clamp expressed in micromoles per kilogram per minute (25). Potassium chloride (1 mmol/ml) was infused to prevent hypokalemia.

Throughout the clamp procedure, FBF measurements were performed, intra-arterial pulse wave was recorded, and ^{125}I-albumin was injected for calculation of TERalb and plasma volume. Moreover, blood samples for insulin measurement were drawn. After 2 h of hyperinsulinemic-euglycemic clamping, the specific nitric oxide (NO) synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA) was infused into the brachial artery at a rate of 0.4 mg · min^{-1} · dl^{-1}, and the subsequent vasoconstrictor response was measured. L-NMMA (100 mg, Clinalpha, Läufelfingen, Switzerland) solution was freshly made with 25 ml of 0.9% NaCl immediately before use. After the experiment was finished, glucose infusion was continued, and the participants were served a carbohydrate-rich meal to avoid hypoglycemic events after the test.

TERalb

At 60 min, an additional venous needle (BD Valu-set, 0.6 × 20 mm) was inserted and 2–4 μCi of ^{125}I-albumin (Shering Nederland, Weesp, the Netherlands) was given as an intravenous bolus injection. During the next 60 min, seven plasma samples were collected from the arterial line for radioactivity measurements. Plasma volume and TERalb were calculated using the following formulas (26,27):

Plasma volume (PV) (milliliters)/1.73 m^2 = [counts per minute injected/counts per minute t = 0/milliliters]/surface (square meters)/1.73 m^2.

TERalb = fraction of the intravascular mass of albumin leaving the vascular system per hour.

$$\text{TERalb} = [1 - e^{3{,}600 \times \text{slope}}] \times 100\% \text{ (\%/h)}.$$

Analytical methods

Arterial plasma glucose was measured in duplicate with the glucose oxidation method (Beckman Glucose Analyzer 2; Beckman, Fullerton, CA). Atrial natriuretic peptide (ANP) concentrations were analyzed by radioimmunoassay after cartridge extraction. Insulin levels were measured using the Perkin-Elmer AutoDELFIA Insulin kit with an automatic immunoassay system. C-peptide was analyzed with C-peptide double-antibody (^{125}I) radioimmunoassay kit.

Control visits

During all control visits (0, 2, 6, 14, and 18 weeks), blood pressure and heart rate were assessed after the subject had been sitting quietly for at least 5 min. Blood pressure was measured by auscultation method with the nondominant arm supported at heart level. Moreover, foot volume was assessed using the water

displacement method, which measures volume displacement in an indirect way with an electronic balance (coefficient of variation is 0.30%) (28). The balance recorded the force necessary for a standardized immersion of the foot, which depends solely on the volume of the foot (Archimedes principle). The mean temperature of the water was 22.9°C and did not differ >1°C between visits of one subject.

Statistical analysis

The study was powered (90%) to detect a 50% increase in percentage change in FBF between the treatment groups with 16 evaluable subjects. All significance tests and CIs were two sided and the overall type I error was 5%. Descriptive statistics of population characteristics are presented as means ± SD. The comparison between rosiglitazone and placebo was conducted within each subject. The response was measured at the end of each treatment period, assuming that any carryover from the first treatment period should be washed out. All data were analyzed using ANOVA, with adjustment for period if applicable. We used a paired Student's *t* test or Wilcoxon rank test, if appropriate, and ANOVA repeated measures for sequential data to derive *P* values. Treatment effects are presented as means ± SE or, for relative changes, as mean percentage change derived from the geometric mean with CIs. Correlations were calculated using Pearson's or Spearman's correlation tests if appropriate. All statistical analyses were performed using the SPSS personal computer software package.

RESULTS — Included subjects represented an overweight (98 ± 12 kg; BMI 32 ± 3 kg/m^2), middle-aged (46 ± 9 years) population of 11 men and 7 women. Obvious features of the metabolic syndrome present in our population were increased waist circumference (109 ± 7 cm), diastolic blood pressure (DBP) (93 ± 5 mmHg), and plasma triglyceride levels (1.9 ± 0.9 mmol/l). Other characteristics were systolic blood pressure (SBP) (134 ± 10 mmHg), plasma total cholesterol levels (5.7 ± 1.0 mmol/l), plasma HDL levels (1.2 ± 0.3 mmol/l), plasma fasting plasma glucose levels (5.5 ± 0.4 mmol/l), and AIC (5.50 ± 0.33%). Ten subjects were randomly assigned to receive placebo first, and the remaining eight subjects received rosiglitazone first. All subjects completed both treatment regimens. Drug compliance, measured by tablet counting, was excellent. Subjects reported only mild side effects, equally distributed between both treatments. One subject developed moderate edema during rosiglitazone treatment.

Effect of rosiglitazone on the metabolic actions of insulin

During rosiglitazone, the fasting values of plasma glucose (0.28 mmol/l [95% CI 0.05–0.50], $P = 0.02$), insulin, and C-peptide concentrations (14 pmol/l [2–26], $P < 0.05$ and 0.13 nmol/l [0.01–0.25], $P < 0.05$, respectively) were significantly decreased as compared with placebo. During the final 30 min of the clamp procedure, blood glucose values were equal during rosiglitazone and placebo treatment (4.96 ± 0.12 and 4.96 ± 0.15 mmol/l, respectively) and stable (coefficients of variation 4.36 ± 2.08 and 4.15 ± 1.96%, respectively). Also, steady-state plasma insulin concentrations were similar (1,664 ± 533 pmol/l vs. 1,795 ± 688 pmol/l, $P = 0.29$). Insulin sensitivity, measured by GIR, significantly improved during rosiglitazone (39.6 ± 9.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) treatment compared with placebo (33.7 ± 11.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), resulting in a period-adjusted treatment effect of 5.26 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (95% CI 1.68–8.83, $P = 0.007$).

Effect of rosiglitazone on the vascular actions of insulin

Hyperinsulinemia (~1,700 pmol/l) did not change FBF during either treatment, consistent with persistent vascular insulin resistance (treatment effect for rosiglitazone −8.2% [95% CI −27.2 to 8.0], $P = 0.318$) (Fig. 1*A*). During L-NMMA infusion, blood flow decreased, but the reductions were similar during rosiglitazone and placebo treatment (−22.9% [−13.5 to −31.3] vs. −25.7% [−18.8 to −31.8], NS) (Fig. 1*A*). Rosiglitazone had no effect on vascular permeability measured with TERalb (+0.27%/h [−1.21 to 1.75], $P = 0.71$) (Fig. 1*B*).

Insulin infusion reduced systemic vascular resistance during placebo treatment (−6.2% [95% CI −9.1 to −3.2], $P < 0.001$) and not during rosiglitazone treatment (−4.5% [−10.2 to 1.6], $P = 0.14$), but these changes did not differ significantly between treatments (0.4% [−5.5 to 6.7], $P = 0.68$). Similarly, insulin increased cardiac output, but again these changes were not different between both treatments.

Effect of rosiglitazone on blood pressure

DBP was reduced during rosiglitazone treatment whether measured via auscultatory or intra-arterial methods (auscultatory −5 mmHg [95% CI −6.87 to −2.35], $P = 0.0005$; intra-arterially −2 mmHg [−3.6 to −1.6], $P = 0.03$) (Fig. 1*C*). Rosiglitazone seemed to reduce the calculated systemic vascular resistance, but the difference in this measure failed to reach statistical significance (−3.2% [−9.6 to 3.7], $P = 0.28$).

Effect of rosiglitazone on fluid compartments

During rosiglitazone, plasma volume increased by 255 ml/1.73 m^2 (95% CI 80–430) ($P = 0.007$) compared with placebo (Fig. 1*D*). Hematocrit decreased accordingly (−0.019 l/l [−0.03 to −0.01], $P = 0.002$). We observed an increase in plasma ANP with rosiglitazone (12.1 pg/ml [0.7–23.4], $P = 0.039$; rosiglitazone vs. placebo). Rosiglitazone did not induce an increase in foot volume over placebo (0.37% [−0.80 to 1.50], NS). However, a period effect was detected, with greater relative differences from baseline during the second period, probably related to a seasonal increase in outside temperature throughout the study. Post hoc analyses revealed a significant correlation between changes in foot volume and GIR (Fig. 2) ($R^2 = 0.53$, $P = 0.001$) and trends between changes in GIR and TERalb and between changes in GIR and DBP ($R^2 = 0.23$, $P = 0.07$ and $R^2 = 0.15$, $P = 0.11$, respectively).

Characterization of subject with TZD-induced edema

One subject developed moderate edema and showed an increase in body weight of 3.7 kg, in plasma volume of 544 ml/1.73 m^2, and in foot volume of 4.6% during rosiglitazone treatment. Compared with the whole study population, this subject had an equivalent treatment response with regard to insulin-mediated vasodilatation (−9 vs. −7.6% [95% CI −21.4 to +8.7] but a more pronounced response in insulin sensitivity (15.8 vs. 5.3% [1.7–8.8].

CONCLUSIONS — The first principal observation of the present study is that rosiglitazone, although improving the metabolic action of insulin, affected neither vascular permeability nor the NO-dependent vascular responses to insulin. The second is that rosiglitazone signifi-

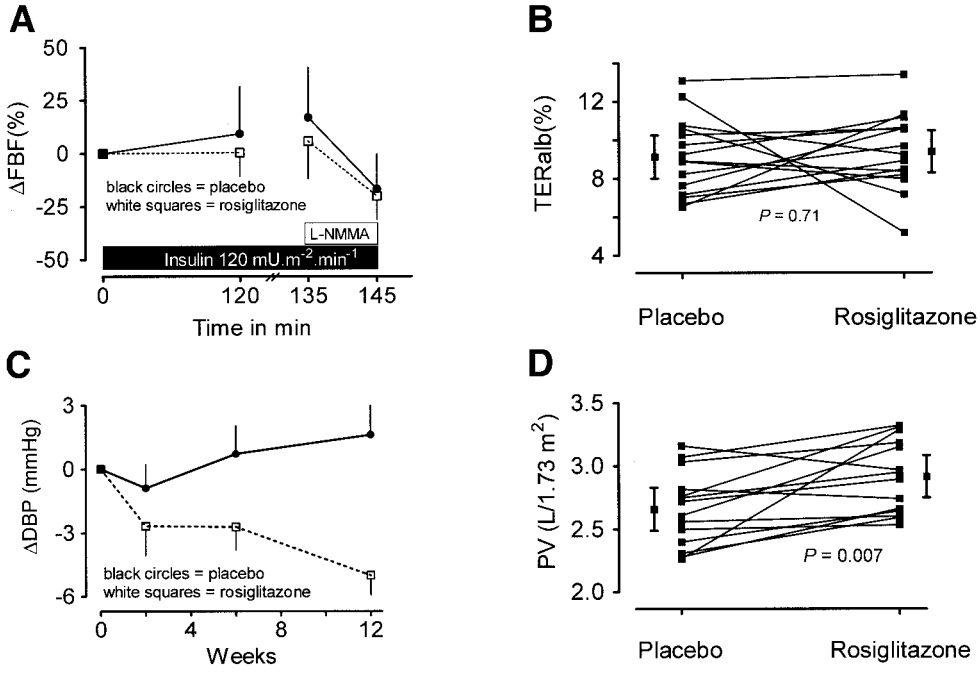

Figure 1—A: *Mean percentage change in FBF (experimental arm [mean and CI]) during hyperinsulinemic clamp and during subsequent infusion of* L-NMMA *into the brachial artery. There was no difference in response between placebo and rosiglitazone.* B: *Intrasubject changes in transcapillary escape rate of albumin (means ± SE). There was no difference in vascular permeability between the two treatments (9.14 ± 0.52 vs. 9.41 ± 0.51%).* C: *Absolute change in diastolic blood pressure (means ± SE) from the start of each treatment period. Rosiglitazone clearly reduced diastolic blood pressure.* D: *Intrasubject changes in plasma volume adjusted for body surface [(means ± SE). During rosiglitazone treatment the mean increase was 255 ml/1.73 m^2 compared with placebo. [*n *= 14: 4 subjects were excluded for this analysis. In one patient, no* ^{125}I*-albumin was available; in two patients, the correlation between (ln)plasma radioactivity and time did not exceed 0.85; and in one patient, we derived a nonphysiologic high plasma volume.]*

cantly increased plasma volume and lowered DBP. Taken together, these findings do not support the hypothesis that potentiation of the vascular effects of insulin, being either vasodilatation or increased vascular permeability, are the specific mechanism of TZD-induced fluid retention. Nevertheless, because the change in insulin-induced glucose uptake appeared to be related to the change in foot volume, our study does support some relationship between the effects of rosiglitazone on glucose uptake and interstitial fluid content.

In this study, rosiglitazone did not affect the vascular actions of insulin. In contrast, Paradisi et al. (29) found that troglitazone was able to reverse the blunted insulin-mediated vasodilatation in subjects with polycystic ovary syndrome. There are two important differences between the study of Paradisi et al. and ours: *1*) the population investigated and *2*) measurement of leg blood flow, whereas we measured FBF. Because previous studies have shown that the vasodilator response to acute hyperinsulinemia did not differ between the leg and the forearm vascular bed, our data may be extrapolated to the leg (30). Someone might still argue that rosiglitazone could exert a different effect on the response to insulin in forearm versus leg. However, in agreement with our forearm observations, we did not find any treatment effect of rosiglitazone on calculated total peripheral vascular resistance during hyperinsulinemia.

Two other studies are in complete agreement with our present findings. In a previous study, we did not find an effect of troglitazone on insulin-induced changes in FBF in obese subjects (23) and neither did Natali et al. (31) in patients with type 2 diabetes. In both studies a lower insulin dose (60 and 40 mU · m^{-2} · min^{-1}) was used. As such, the results of the present study confirm previous reports in obese or diabetic subjects using forearm measurements and extend it to high insulin infusion rates. Because our data are contrast with observations in the polycystic ovary syndrome, the vascular mechanism of action of rosiglitazone may be different in this particular form of insulin resistance.

Our observation that rosiglitazone did not reverse insulin-mediated vasodilatation seems to conflict with published reports showing a beneficial effect of rosiglitazone on NO-dependent vasodilatation (and hence on endothelial function) measured with acetylcholine infusion. For example, Pistrosch et al. (32) reported an increased vasodilator response to either acetylcholine alone or acetylcholine combined with locally infused insulin in rosiglitazone-treated patients compared with nateglinide-treated patients. Likewise, Natali et al. (31) found that rosiglitazone improved the vasodilator responses to acetylcholine but not to insulin in patients with type 2 diabetes. Of note, Natali et al. did not find any effect of rosiglitazone on the response to L-NMMA infusion, which is perfectly in line with our observations. It appears that insulin activation of the NO pathway is not strong enough to disclose the favorable effects of

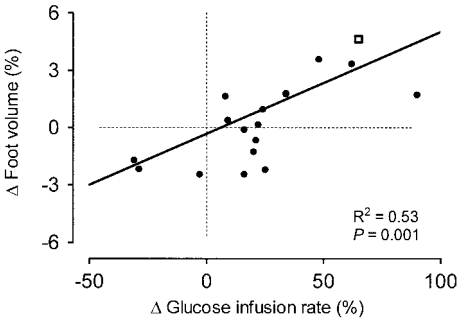

Figure 2—*Plot of correlation between differences in foot volume and glucose infusion rate between rosiglitazone and placebo treatment. This correlation is not driven only by the subject with edema (□);* n *= 18.*

rosiglitazone on endothelium and on insulin-mediated vasodilatation and that either a large improvement in insulin sensitivity (85% in Pistrosch et al.) or additional infusion of acetylcholine is needed.

This was the first human in vivo study investigating the influence of rosiglitazone treatment on TERalb. The finding that rosiglitazone did not change TERalb seems contradictory to an in vitro study with human pulmonary artery endothelial cells (9), but the discrepancy can be explained by clear differences in design and methodology. The absolute rate of TERalb in our population appeared to be rather high (33), although Pedrinelli et al. (34) reported a similar rate (9.6%) in subjects with essential hypertension, and Hilsted et al. (13) found a TERalb rate of 9.9% in normal individuals during a hyperinsulinemic-euglycemic clamp. Therefore, the observed high TERalb could either be the result of features of the metabolic syndrome such as hypertension or be due to the hyperinsulinemic state. Please note that TERalb is a measure of total body protein permeability. As such, we cannot exclude from these data the fact that rosiglitazone affects total body fluid filtration.

In the present study, rosiglitazone resulted in a decrease in DBP (but not SBP) when measured intra-arterially or auscultatory, which is in agreement with another study (31). As DBP is primarily determined by peripheral resistance, the reduction in blood pressure during rosiglitazone treatment could be caused by systemic vasodilatation. In support of this notion is our finding that the systemic vascular resistance was lower during rosiglitazone treatment before the start of the clamp. Interestingly, Shargorodsky et al. (35) did report that rosiglitazone lowers systemic vascular resistance. Apart from a potentiation of insulin effect, rosiglitazone may induce vasodilatation by inhibition of calcium currents (36,37), reduction of endothelin-1 secretion (38), or downregulation of the sympathetic nervous system (39).

Several studies have reported a decrease in hematocrit in response to TZD treatment, which has been interpreted as the result of an increase in plasma volume (40), but so far only one other study combined hematocrit with directly derived plasma volume measurements (41). Indeed, hematocrit decreased and plasma volume increased in our study, but, interestingly, we did not find a correlation between changes in hematocrit and changes in plasma volume. Also the observed elevation of plasma ANP levels during rosiglitazone treatment is consistent with plasma volume expansion. In healthy subjects, rosiglitazone increased plasma volume by only 1.8 ml/kg after 8 weeks of treatment (42). Apparently, the fluid-retaining effect of rosiglitazone is more pronounced in insulin-resistant subjects.

As there was no association between changes in the metabolic and vascular actions of insulin, our results do not support the view that insulin-induced glucose disposal is the consequence of enhanced total muscle blood flow (18). However, it should be acknowledged that opposing views exist in the literature as to whether the vasodilator effects of (physiological levels of) insulin contribute to the effect of insulin on tissue glucose uptake (17). The emerging view is that insulin may increase capillary recruitment and increase tissue perfusion, without necessarily increasing total blood flow (43). This view could be the explanation for the correlation between the change in foot volume and the metabolic but not vascular action of insulin, as found by post hoc analysis. Capillary recruitment will reduce systemic vascular resistance and increase glucose transport and fluid filtration. Therefore, capillary recruitment couples edema formation, reduced blood pressure, and insulin sensitization. In line with this reasoning, Bakris et al. (44) reported a correlation between the reduction of diastolic blood pressure and the improvement in insulin sensitivity during rosiglitazone treatment.

Altogether, our findings do not support the hypothesis that changes in the vascular effects of insulin, being either vasodilatation measured in the forearm or increased vascular permeability, are the specific mechanism of TZD-induced fluid retention. Although this conclusion is valid at the level of the whole study population, it also appears to be true for the single case with edema.

This study included an insulin-resistant nondiabetic population, which enabled us to investigate whether rosiglitazone can reverse the blunted vascular response of insulin, without any interference from changes in glycemic control. For example, hyperglycemia in itself could additionally impair endothelial function (45). The main outcome of the present study being no correlation between fluid retention (plasma volume) and changes in the vascular action of insulin probably holds true for a diabetic population as well. The incidence of edema may be expected to be higher in a diabetic population, for example, because of autonomic neuropathy (sympathetic nervous system dysfunction) or because of heart failure. As such, in a diabetic population the correlation between improved insulin sensitivity and edema formation could be less strong because of potential confounders.

The hypothetical framework of the present study leans heavily on capillary recruitment being the primary cause of edema formation, but the pathogenesis of fluid retention is probably multifactorial (2). At the moment, there are controversial reports about the potential of PPARγ agonists to stimulate the epithelial sodium channel, which could play an important role in TZD-related fluid retention (46–48).

In summary, this study provides no support for the view that TZDs increase transcapillary leakage of fluid as a result of either the augmentation of the NO-mediated vasodilator response to insulin or an increase of capillary permeability. The correlation between metabolic insulin sensitivity and edema formation may point to an alternative mechanism of TZD-related edema formation, possibly increased capillary recruitment.

Acknowledgments— This study was supported by GlaxoSmithKline. C.J.T. is a recipient of a clinical research fellowship of the Dutch Diabetes Foundation.

We thank Aarnout Janssen van Rozendaal for technical assistance during the clamp studies.

Parts of this study were published in abstract form in *Diabetologia* and *Diabetes*.

References

1. Yki-Jarvinen H: Thiazolidinediones. *N Engl J Med* 351:1106–1118, 2004
2. Nesto RW, Bell D, Bonow RO, Fonseca V, Grundy SM, Horton ES, Le Winter M, Porte D, Semenkovich CF, Smith S, Young LH, Kahn R: Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association: October 7, 2003. *Circulation* 108:2941–2948, 2003
3. Wang TD, Chen WJ, Lin JW, Chen MF, Lee YT: Effects of rosiglitazone on endothelial function, C-reactive protein, and components of the metabolic syndrome in nondiabetic patients with the metabolic syndrome. *Am J Cardiol* 93:362–365, 2004
4. Raskin P, Rendell M, Riddle MC, Dole JF,

Freed MI, Rosenstock J: A randomized trial of rosiglitazone therapy in patients with inadequately controlled insulin-treated type 2 diabetes. *Diabetes Care* 24: 1226–1232, 2001
5. Honisett SY, Stojanovska L, Sudhir K, Kingwell BA, Dawood T, Komesaroff PA: Rosiglitazone lowers blood pressure and increases arterial compliance in postmenopausal women with type 2 diabetes. *Diabetes Care* 26:3194–3195, 2003
6. Fullert S, Schneider F, Haak E, Rau H, Badenhoop K, Lubben G, Usadel KH, Konrad T: Effects of pioglitazone in non-diabetic patients with arterial hypertension: a double-blind, placebo-controlled study. *J Clin Endocrinol Metab* 87:5503–5506, 2002
7. St. John SM, Rendell M, Dandona P, Dole JF, Murphy K, Patwardhan R, Patel J, Freed M: A comparison of the effects of rosiglitazone and glyburide on cardiovascular function and glycemic control in patients with type 2 diabetes. *Diabetes Care* 25:2058–2064, 2002
8. Walker AB, Naderali EK, Chattington PD, Buckingham RE, Williams G: Differential vasoactive effects of the insulin sensitizers rosiglitazone (BRL 49653) and troglitazone on human small arteries in vitro. *Diabetes* 47:810–814, 1998
9. Idris I, Gray S, Donnelly R: Rosiglitazone and pulmonary oedema: an acute dose-dependent effect on human endothelial cell permeability. *Diabetologia* 46:288–290, 2003
10. Phillips LS, Grunberger G, Miller E, Patwardhan R, Rappaport EB, Salzman A: Once- and twice-daily dosing with rosiglitazone improves glycemic control in patients with type 2 diabetes. *Diabetes Care* 24:308–315, 2001
11. Rubin C, Egan J, Schneider R: Combination therapy with pioglitazone and insulin in patients with type 2 diabetes (Abstract). *Diabetes* 48 (Suppl. 1):A110, 1999
12. Saad MF, Greco S, Osei K, Lewin AJ, Edwards C, Nunez M, Reinhardt RR: Ragaglitazar improves glycemic control and lipid profile in type 2 diabetic subjects: a 12-week, double-blind, placebo-controlled dose-ranging study with an open pioglitazone arm. *Diabetes Care* 27:1324–1329, 2004
13. Hilsted J, Christensen NJ, Larsen S: Effect of catecholamines and insulin on plasma volume and intravascular mass of albumin in man. *Clin Sci (Lond)* 77:149–155, 1989
14. Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod P: Nitric oxide release accounts for insulin's vascular effects in humans. *J Clin Invest* 94:2511–2515, 1994
15. Bouskela E, Cyrino FZ, Wiernsperger N: Effects of insulin and the combination of insulin plus metformin (glucophage) on microvascular reactivity in control and diabetic hamsters. *Angiology* 48:503–514, 1997
16. Zhang L, Vincent MA, Richards SM, Clerk LH, Rattigan S, Clark MG, Barrett EJ: Insulin sensitivity of muscle capillary recruitment in vivo. *Diabetes* 53:447–453, 2004
17. Coggins M, Lindner J, Rattigan S, Jahn L, Fasy E, Kaul S, Barrett E: Physiologic hyperinsulinemia enhances human skeletal muscle perfusion by capillary recruitment. *Diabetes* 50:2682–2690, 2001
18. Laakso M, Edelman SV, Brechtel G, Baron AD: Impaired insulin-mediated skeletal muscle blood flow in patients with NIDDM. *Diabetes* 41:1076–1083, 1992
19. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults: Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 285: 2486–2497, 2001
20. Timmers HJ, Wieling W, Soetekouw PM, Bleijenberg G, Van Der Meer JW, Lenders JW: Hemodynamic and neurohumoral responses to head-up tilt in patients with chronic fatigue syndrome. *Clin Auton Res* 12:273–280, 2002
21. Tack CJ, Smits P, Willemsen JJ, Lenders JW, Thien T, Lutterman JA: Effects of insulin on vascular tone and sympathetic nervous system in NIDDM. *Diabetes* 45: 15–22, 1996
22. Whitney RJ: The measurement of volume changes in human limbs. *J Physiol* 121:1–27, 1953
23. Tack CJ, Ong MK, Lutterman JA, Smits P: Insulin-induced vasodilatation and endothelial function in obesity/insulin resistance: effects of troglitazone. *Diabetologia* 41:569–576, 1998
24. Defronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
25. Bergman RN, Finegood DT, Ader M: Assessment of insulin sensitivity in vivo. *Endocr Rev* 6:45–86, 1985
26. Parving HH, Gyntelberg F: Albumin transcapillary escape rate and plasma volume during long-term beta-adrenergic blockade in essential hypertension. *Scand J Clin Lab Invest* 32:105–110, 1973
27. Vervoort G, Lutterman JA, Smits P, Berden JH, Wetzels JF: Transcapillary escape rate of albumin is increased and related to haemodynamic changes in normo-albuminuric type 1 diabetic patients. *J Hypertens* 17:1911–1916, 1999
28. Van Hamersvelt HW, Kloke HJ, de Jong DJ, Koene RA, Huysmans FT: Oedema formation with the vasodilators nifedipine and diazoxide: direct local effect or sodium retention? *J Hypertens* 14:1041–1045, 1996
29. Paradisi G, Steinberg HO, Shepard MK, Hook G, Baron AD: Troglitazone therapy improves endothelial function to near normal levels in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 88:576–580, 2003
30. Yki-Jarvinen H, Utriainen T: Insulin-induced vasodilatation: physiology or pharmacology? *Diabetologia* 41:369–379, 1998
31. Natali A, Baldeweg S, Toschi E, Capaldo B, Barbaro D, Gastaldelli A, Yudkin JS, Ferrannini E: Vascular effects of improving metabolic control with metformin or rosiglitazone in type 2 diabetes. *Diabetes Care* 27:1349–1357, 2004
32. Pistrosch F, Passauer J, Fischer S, Fuecker K, Hanefeld M, Gross P: In type 2 diabetes, rosiglitazone therapy for insulin resistance ameliorates endothelial dysfunction independent of glucose control. *Diabetes Care* 27:484–490, 2004
33. Nielsen S, Hermansen K, Rasmussen OW, Thomsen C, Mogensen CE: Urinary albumin excretion rate and 24-h ambulatory blood pressure in NIDDM with microalbuminuria: effects of a monounsaturated-enriched diet. *Diabetologia* 38:1069–1075, 1995
34. Pedrinelli R, Penno G, Dell'Omo G, Bandinelli S, Giorgi D, Di Bello V, Navalesi R, Mariani M: Microalbuminuria and transcapillary albumin leakage in essential hypertension. *Hypertension* 34:491–495, 1999
35. Shargorodsky M, Wainstein J, Gavish D, Leibovitz E, Matas Z, Zimlichman R: Treatment with rosiglitazone reduces hyperinsulinemia and improves arterial elasticity in patients with type 2 diabetes mellitus. *Am J Hypertens* 16:617–622, 2003
36. Song J, Walsh MF, Igwe R, Ram JL, Barazi M, Dominguez LJ, Sowers JR: Troglitazone reduces contraction by inhibition of vascular smooth muscle cell Ca^{2+} currents and not endothelial nitric oxide production. *Diabetes* 46:659–664, 1997
37. Buchanan TA, Meehan WP, Jeng YY, Yang D, Chan TM, Nadler JL, Scott S, Rude RK, Hsueh WA: Blood pressure lowering by pioglitazone: evidence for a direct vascular effect. *J Clin Invest* 96: 354–360, 1995
38. Martin-Nizard F, Furman C, Delerive P, Kandoussi A, Fruchart JC, Staels B, Duriez P: Peroxisome proliferator-activated receptor activators inhibit oxidized low-density lipoprotein-induced endothelin-1 secretion in endothelial cells. *J Cardiovasc Pharmacol* 40:822–831, 2002
39. Yosefy C, Magen E, Kiselevich A, Priluk R, London D, Volchek L, Viskoper RJ Jr: Rosiglitazone improves, while glibenclamide worsens blood pressure control in treated hypertensive diabetic and dyslipidemic subjects via modulation of insulin resistance and sympathetic activity.

J Cardiovasc Pharmacol 44:215–222, 2004
40. Lebovitz HE: Differentiating members of the thiazolidinedione class: a focus on safety. *Diabetes Metab Res Rev* 18 (Suppl. 2):S23–S29, 2002
41. Young MM, Squassante L, Wemer J, van Marle SP, Dogterom P, Jonkman JH: Troglitazone has no effect on red cell mass or other erythropoietic parameters. *Eur J Clin Pharmacol* 55:101–104, 1999
42. Avandia (rosiglitazone maleate) [package insert]. Philadelphia, GlaxoSmithKline, 2000
43. Clerk LH, Vincent MA, Lindner JR, Clark MG, Rattigan S, Barrett EJ: The vasodilatory actions of insulin on resistance and terminal arterioles and their impact on muscle glucose uptake. *Diabetes Metab Res Rev* 20:3–12, 2004
44. Bakris G, Viberti G, Weston WM, Heise M, Porter LE, Freed MI: Rosiglitazone reduces urinary albumin excretion in type II diabetes. *J Hum Hypertens* 17:7–12, 2003
45. Makimattila S, Virkamaki A, Groop PH, Cockcroft J, Utriainen T, Fagerudd J, Yki-Jarvinen H: Chronic hyperglycemia impairs endothelial function and insulin sensitivity via different mechanisms in insulin-dependent diabetes mellitus. *Circulation* 94:1276–1282, 1996
46. Hong G, Lockhart A, Davis B, Rahmoune H, Baker S, Ye L, Thompson P, Shou Y, O'Shaughnessy K, Ronco P, Brown J: PPARγ activation enhances cell surface ENaCα via up-regulation of SGK1 in human collecting duct cells. *FASEB J* 17:1966–1968, 2003
47. Guan Y, Hao C, Cha DR, Rao R, Lu W, Kohan DE, Magnuson MA, Redha R, Zhang Y, Breyer MD: Thiazolidinediones expand body fluid volume through PPARγ stimulation of ENaC-mediated renal salt absorption. *Nat Med* 11:861–866, 2005
48. Nofziger C, Chen L, Shane MA, Smith CD, Brown KK, Blazer-Yost BL: PPARγ agonists do not directly enhance basal or insulin-stimulated Na^+ transport via the epithelial Na^+ channel. *Pflugers Arch* 451: 445–453, 2005