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

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 nitrate 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/m2 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 forearm blood flow change in foot volume (120 min, 120 mU/m2 per min).

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.

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

Address correspondence and reprint requests to Alexander J. M. Rennings, MD, Department of Pharmacology-Toxicology, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB Nijmegen, Netherlands. E-mail: a.renning@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\(^{\text{\textendash}1}\)-monomethyl-L-arginine; PPAR, peroxisome proliferator–activated receptor; SBP, systolic blood pressure; T\(_{\text{ERa}}\), transcapillary escape rate of labeled albumin; TZD, thiazolidinedione.

© 2006 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
proved glycemic control, we studied non-diabetic 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/m², 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 HbA1c (A1C) >6.5%, if they used nonsteroidal anti-inflammatory drugs, fibrates, anticoagulants, antihyper-tensives, any investigational drug, or a PPAR 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 anes-thesia (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 antecubital 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 (FFB) (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/m²/min (120 mU/m²/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 micro-moles 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 125I-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 NG-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, Laufelfingen, 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 carbo-hydrate-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 125I-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 m² = [counts per minute injected/cents per minute t = 0/milliliters/surface (square meters)/1.73 m².

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

TERalb = [1 − e⁻³.₆₀₀×slope] × 100% (/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 (123I) 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 CI 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/m²), 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 mmol/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 μmol · kg⁻¹ · min⁻¹) treatment compared with placebo (33.7 ± 11.7 μmol · kg⁻¹ · min⁻¹), resulting in a period-adjusted treatment effect of 5.26 μmol · kg⁻¹ · 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. 1A). During 1-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. 1A). Rosiglitazone had no effect on vascular permeability measured with TERab (+0.27% [−1.21 to 1.75], P = 0.71) (Fig. 1B).

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. 1C). 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 m² (95% CI 80–430) (P = 0.007) compared with placebo (Fig. 1D). 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) (R² = 0.53, P = 0.001) and trends between changes in GIR and TERRab and between changes in GIR and DBP (R² = 0.23, P = 0.07 and R² = 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 m², 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-
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. 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 lower insulin dose (60 and 40 mU \cdot m^{-2} \cdot 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.
Interestingly, we did not find a correlation between fluid retention (plasma volume) and 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
4. Raskin P, Rendell M, Riddle MC, Dole JF,
Fluid retention and rosiglitazone


42. Avandia (rosiglitazone maleate) [package insert]. Philadelphia, GlaxoSmithKline, 2000


