

## Effect of rosiglitazone on factors related to endothelial dysfunction in patients with type 2 diabetes mellitus

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Received 29 August 2006; received in revised form 19 December 2006; accepted 2 January 2007

Available online 5 February 2007

### Abstract

The effect of the insulin sensitizer rosiglitazone (RSG) on biological markers of endothelial dysfunction in subjects with type 2 diabetes mellitus (T2DM) was investigated in a 12-week, multi-center, randomized, double-blind study. One hundred and thirty-six subjects aged 40–70 years, with FPG  $\geq 7.0$  and  $\leq 15.0$  mmol/l, previously treated with a single oral anti-diabetic agent or diet/exercise, were randomized to RSG 8 mg/day ( $n=65$ ) or placebo (PBO,  $n=71$ ). Results revealed that RSG significantly reduced soluble (s)E-selectin by  $-10.9\%$  ( $P=0.004$ ) compared with PBO, but did not significantly alter soluble vascular cell adhesion molecule-1 ( $+0.6\%$ ,  $P=NS$ ). Compared with PBO, RSG also significantly reduced plasminogen activator inhibitor-1 ( $-36.9\%$ ,  $P<0.001$ ), tissue plasminogen activator antigen ( $-22.7\%$ ,  $P<0.001$ ), FPG ( $-2.8$  mmol/l,  $P<0.001$ ), fasting fructosamine ( $-42.0$  mg/dl,  $P<0.001$ ). Post-prandial AUC<sub>(0–4h)</sub> for free fatty acids (FFAs) reduced by  $-6.5$  mg/dl\*h from baseline ( $P=0.03$ ), a change that positively and significantly correlated with changes in sE-selectin ( $r=0.22$ ,  $P=0.05$ ). The incidence of adverse events was similar in the two groups (RSG: 35.4%; PBO: 40.8%); the majority mild or moderate. These data support the hypothesis that, in patients with T2DM, rosiglitazone has beneficial effects on biological markers of endothelial dysfunction. Improvements in insulin sensitivity and decreases in FFAs may play a role in these effects.

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**Keywords:** Randomized controlled trial; Type 2 diabetes mellitus; Rosiglitazone; Vascular endothelium; E-selectin; Vascular cell adhesion molecule-1; Free fatty acids

### 1. Introduction

Cardiovascular disease (CVD) is the leading cause of death in individuals with type 2 diabetes mellitus (T2DM), accounting for up to 75% of mortality [1]. Atherosclerotic CVD has been proposed to be an inflammatory disease that originates in endothelial dysfunction. Disturbances that

affect the balanced release of substances from the endothelium can create a prothrombotic vessel surface that is susceptible to atherosclerosis. For example, in patients with T2DM, both insulin resistance and its associated elevations in free fatty acids (FFAs) may impair endothelium-dependent vasodilation [2,3]. Other factors such as hypertension and dyslipidemia are also associated with endothelial injury, resulting in alterations in endothelial homeostasis, including adhesiveness and permeability [4].

Adhesion and migration of leukocytes from the circulation into the vessel wall, a process critical for repair of tissue injury, is tightly regulated by distinct families of adhe-

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sion molecules secreted by endothelial cells. Of these, the selectins, lectin-like glycoproteins, mediate the initial step of the process by weakly binding and rolling leukocytes, while members of the immunoglobulin supergene family, such as vascular cell adhesion molecule-1 (VCAM-1), contribute to firm adhesion and migration of leukocytes from the blood to the intima [5]. Increased leukocyte–endothelial cell adhesion has been implicated in atherosclerosis, and elevated levels of adhesion molecules have been reported in subjects with T2DM [6] and in prediabetic subjects [7].

The adhesion molecules E-selectin and VCAM-1 are both expressed on endothelial cells; however, while E-selectin is specific to activated endothelium, VCAM-1 is also expressed on various circulating blood cells [8,9]. Therefore, elevated levels of serum soluble (s)E-selectin may be a particularly strong indicator of endothelial dysfunction and one of the most important adhesion molecules involved in the atherosclerotic process [10]. In subjects with T2DM, levels of sE-selectin and sVCAM-1 have been shown to be higher than in non-T2DM subjects [6], to correlate with the degree of insulin resistance [11], and to decrease after improvement of glycemic control [6]. Endothelial cells also secrete a number of factors, including plasminogen activator inhibitor (PAI)-1, tissue plasminogen activator (tPA) and von Willebrand Factor (vWF), that are involved in clot formation and when present at raised levels, contribute to the pro-atherogenic state in T2DM [12–14]. Elevated circulating levels of PAI-1 have been found in poorly controlled subjects with T2DM, and are reversed after short-term improvements in glycemic control [15]. Elevated levels of PAI-1 are recognized as an early marker of endothelial dysfunction [16], while levels of vWF have long been thought to represent an index of endothelial damage [17].

Thiazolidinediones (TZDs) like rosiglitazone are insulin-sensitizing drugs as they are highly selective and potent agonists for the peroxisome proliferator activated receptor- $\gamma$  (PPAR $\gamma$ ). PPAR $\gamma$  is expressed in target tissues for insulin action, such as liver, skeletal muscle and most abundantly in adipose tissue. The TZDs are believed to exert their insulin-sensitizing action in muscle and liver through “fatty acid steal”, defined as a partitioning of circulating lipids away from muscle and liver and into adipose tissue [18]. TZDs consistently lower fasting and post-prandial glucose concentrations as well as FFA concentration in clinical studies [19]. It is thought that FFAs induce insulin resistance in human muscle at the level of insulin-stimulated glucose transport through a defective phosphorylation that impairs the insulin-signaling pathway [20]. Furthermore, activation of PPARs interferes with the earliest processes in atherogenesis to modulate production of chemokines, and thereby the expression of proinflammatory adhesion molecules in endothelial cells [21].

The purpose of this study was to test the effects of the insulin sensitizer rosiglitazone (RSG) on biological markers of endothelial dysfunction in subjects with T2DM.

## 2. Methods

### 2.1. Study population

This was a 12-week, multi-center, phase IIb, randomized, double-blind, placebo-controlled study to evaluate the effects of RSG 8 mg/day on sE-selectin, sVCAM-1, PAI-1, tPA-antigen and vWF-antigen levels in subjects with T2DM. Male and female subjects aged 40–70 years inclusive, with a diagnosis of T2DM defined by World Health Organization criteria [22], were eligible for inclusion. Subjects had to have been treated with diet or exercise alone or a single oral anti-diabetic agent within 3 months prior to screening and have fasting plasma glucose (FPG)  $\geq 7.0$  and  $\leq 15.0$  mmol/L. Subjects with systolic blood pressure  $>180$  mmHg or diastolic blood pressure  $>114$  mmHg were excluded, as were those with congestive heart failure grades II–IV according to NYHA classification, unstable angina, or severe angina requiring continual nitrate treatment.

### 2.2. Study design

Subjects were recruited from 22 centers in Austria, France, Germany, Ireland and the UK. Within 2 weeks of screening and discontinuation of previous anti-diabetic medication, subjects entered a 4-week, single-blind, placebo run-in period. Subjects were maintained on diet and exercise during run-in. Eligible subjects were randomized to receive either RSG 8 mg or placebo once daily during the 12-week study period, with all subjects also receiving diet and exercise advice at each study visit.

According to clinical and biological criteria, 100 healthy volunteer blood donors of the Blood Transfusion Center of Seine Saint-Denis, France, were selected and studied as control subjects. They met the following exclusion criteria: inflammatory disorder, dyslipoproteinemia, CVD, known diabetes or other endocrine disorder, hepatic or renal failure, overweight, smoking, alcohol consumption, hypertension, and/or therapy known to cause changes in lipoprotein profile (e.g. estrogen treatment). Some exclusion criteria were extended to the ascendants: CVD, known diabetes or other endocrine disorders, and dyslipoproteinemia.

The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki (1996). The protocol and informed consent of the subjects were approved by an ethics committee prior to each center's initiation.

### 2.3. Study evaluations

Patients were assessed at weeks 4, 8 and 12. Endpoints included changes from randomization (baseline/week 0) to week 12 in fasting levels of sE-selectin, sVCAM-1, PAI-1, tPA-antigen and vWF-antigen. Additional parameters measured included changes in FPG, fasting C-peptide, fasting insulin, sitting diastolic and systolic blood pressure, sitting

heart rate, and changes in  $AUC_{(0-4h)}$  for FFAs after an average 500 ml liquid meal (BOOST HP) drunk in 5 min. Safety and tolerability assessments included frequency and severity (mild, moderate or severe) of adverse events (AEs) and changes in body weight.

Assays were performed at Quest Diagnostics, Heston, UK, with the exception of PAI-1 (Scripps Reference Laboratory, San Diego, CA, USA) and VCAM-1 and E-selectin (Hôpital Avicenne, Bobigny, France). Serum sE-selectin and sVCAM-1 were assayed by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Abingdon, Oxfordshire, UK) using monoclonal antibodies specific for each adhesion molecule. PAI-1, tPA-antigen and vWF-antigen were measured on citrated plasma using ELISA. Plasma glucose was determined by automated glucose oxidase methodology. Serum FFAs were measured by enzymatic colorimetry (WAKO Chemicals, VA, USA).

Compliance during the treatment phase was assessed by recording the number of tablets dispensed at each clinic visit and the number returned unused at the next visit. A subject was considered compliant if they took between 80% and 120% of the study medication.

#### 2.4. Data analysis

The evaluable population consisted of all randomized subjects, with the exception of FPG, for which analysis was performed on the ITT population with LOCF applied for withdrawn patients or missing values, and FFAs, for

which analysis was performed on the ITT population without LOCF.

To assess differences between treatment groups with regard to continuous efficacy variables, an analysis of covariance (ANCOVA) procedure, which accounts for variability due to center, treatments and baseline, was employed. Treatment comparisons between the RSG group and the PBO group were performed using a significance level of 0.05. AE data were analyzed descriptively. Differences in change in body weight between treatment groups were analyzed using a one-way ANCOVA model with significance level of 0.05.

### 3. Results

#### 3.1. Study population

A total of 199 subjects with T2DM were screened, with 136 randomized into the study (PBO: 71; RSG: 65). The ITT population consisted of 135 subjects: 71 subjects from the PBO group and 64 from the RSG group. Of these, 124 completed the study (PBO: 62; RSG: 62). The two groups forming the ITT population were well matched for baseline characteristics, including FPG and mean duration of diabetes (Table 1). The majority of subjects in each treatment group (64% in the PBO group; 59% in the RSG group) had been treated previously with a single anti-diabetic agent; the remainder with diet and exercise alone. Two subjects in each group had received combination oral anti-diabetic agents in

Table 1  
Demographics and baseline characteristics (ITT population)

Parameter	Placebo ( <i>n</i> = 71)	Rosiglitazone 8 mg/day ( <i>n</i> = 64)
Male sex, <i>n</i> (%)	45 (63.4%)	42 (65.6%)
Age (years)	56.4 ± 6.9	55.5 ± 8.0
Race, <i>n</i> (%)		
White	68 (95.8%)	60 (93.8%)
Black	1 (1.4%)	3 (4.7%)
Oriental	2 (2.8%)	1 (1.6%)
Duration of diabetes (years)	4.1 ± 4.8	4.3 ± 5.2
Body weight (kg)	87.6 ± 17.7	90.2 ± 19.3
BMI (kg/m <sup>2</sup> ), median (interquartile range)	29.8 (26.9–32.9)	31.3 (27.1–36.3)
Waist/hip ratio	0.95 ± 0.07	0.95 ± 0.07
Fasting plasma glucose (mmol/l)	10.03 ± 2.67	9.81 ± 2.64
Fasting fructosamine (mg/dl)	312.0 ± 61.3	299.7 ± 48.3
Fasting C-peptide (nmol/l)	0.99 ± 0.39	1.09 ± 0.46
Fasting insulin (pmol/l)	97.5 ± 61.6	111.1 ± 67.5
Free fatty acids $AUC_{(0-4h)}$ (mg/dl*h)	−44.44 ± 43.96	−36.57 ± 19.98
Soluble E-selectin (ng/ml), geometric mean (CV)	58.46 (44.93)	66.26 (53.68)
Soluble vascular cell adhesion molecule-1 (ng/ml), geometric mean (CV)	437 (23.7)	461 (29.5)
Plasminogen activator inhibitor-1 (U/ml), geometric mean (−S.E., +S.E.)	1.82 (1.61, 2.07)	2.54 (2.26, 2.85)
Tissue plasminogen activator antigen (ng/ml), geometric mean (−S.E., +S.E.)	7.41 (7.06, 7.77)	7.86 (7.48, 8.27)
von Willebrand Factor antigen (%), geometric mean (−S.E., +S.E.)	132.4 (123.3, 142.2)	130.4 (122.0, 139.4)
Fibrinogen (g/l), geometric mean (−S.E., +S.E.)	3.06 (2.97, 3.16)	3.26 (3.18, 3.34)
Systolic blood pressure (mmHg)	138.1 ± 16.6	137.7 ± 21.2
Diastolic blood pressure (mmHg)	81.3 ± 11.2	81.6 ± 12.3
Heart rate (bpm)	72.7 ± 10.1	73.5 ± 9.4

Results are expressed as mean ± S.D. unless specified. Data are for ITT population, with the exception of plasminogen activator inhibitor-1, von Willebrand Factor, fibrinogen, E-selectin, vascular cell adhesion molecule-1, blood pressure and heart rate (all randomized patients).

the 3 months prior to study start. sE-selectin and sVCAM-1 levels at baseline were similar in the two groups of study subjects with diabetes (Table 1), although higher ( $P < 0.001$ ) compared with the blood-donor control group without known diabetes (sE-selectin:  $48.8 \pm 1.8$  ng/ml; sVCAM-1:  $388.6 \pm 6.3$ ; mean  $\pm$  S.E.). PAI-1, tPA-antigen, vWF-antigen and fibrinogen were similar at baseline in the two groups of T2DM patients (Table 1). Significant correlations were found at baseline between sE-selectin levels and fasting glycemia ( $r = 0.39$ ,  $P = 0.01$ ), fructosamine ( $r = 0.40$ ,  $P = 0.01$ ), triglycerides ( $r = 0.27$ ,  $P = 0.003$ ), C-peptide ( $r = 0.34$ ,  $P = 0.03$ ), ALAT ( $r = 0.90$ ,  $P < 0.001$ ), ASAT ( $r = 0.37$ ,  $P = 0.01$ ), PAI-1 ( $r = 0.52$ ,  $P < 0.001$ ), tPA-antigen ( $r = 0.55$ ,  $P < 0.001$ ), and between sVCAM-1 levels and fructosamine ( $r = 0.40$ ,  $P = 0.006$ ), ALAT ( $r = 0.47$ ,  $P = 0.001$ ), ASAT ( $r = 0.55$ ,  $P < 0.001$ ), vWF-antigen ( $r = 0.54$ ,  $P < 0.001$ ). At the study end, the proportion of randomized subjects who were deemed compliant with medication was 97.2% in the RSG group and 98.5% in the PBO group.

### 3.2. Effects of RSG on sE-selectin and sVCAM-1

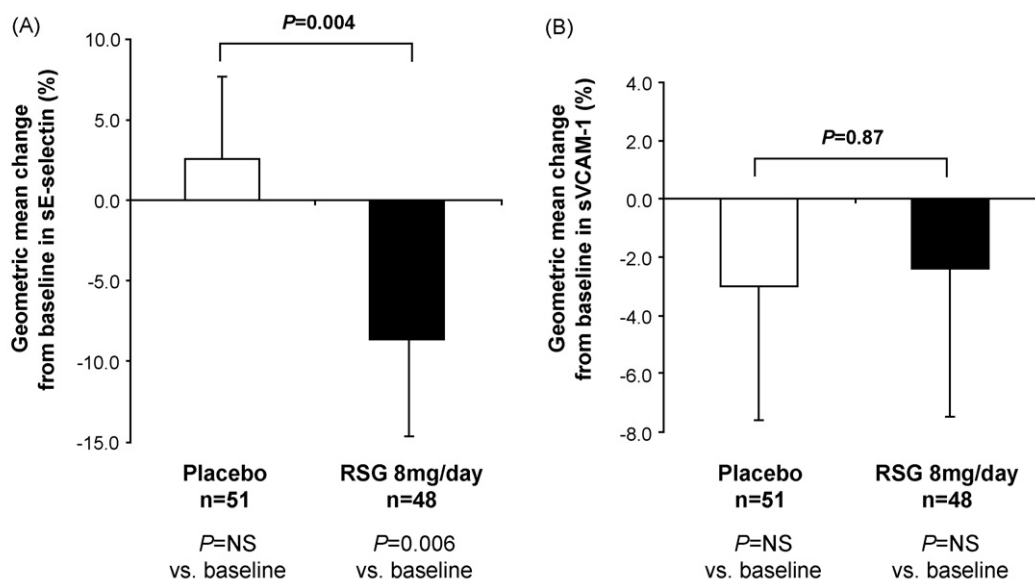
In subjects who received RSG, there was a statistically significant reduction in serum sE-selectin of  $-6.5$  ng/ml ( $-8.6\%$ ;  $P = 0.006$ ) at week 12 compared with baseline. Conversely, there was a non-significant increase in sE-selectin of  $+1.6$  ng/ml ( $+2.5\%$ ) in the PBO group, resulting in a statistically significant treatment effect of  $-8.1$  ng/ml ( $-10.9\%$ ,  $P = 0.004$ ) (Fig. 1A). Levels of sVCAM-1 were non-significantly reduced in the RSG and PBO treatment groups by  $-2.3$  ng/ml ( $-2.4\%$ ,  $P = \text{NS}$ ) and  $-13.2$  ng/ml ( $-3.0\%$ ,  $P = \text{NS}$ ), respectively, leading to a non-significant treatment effect of  $+0.6\%$  ( $P = \text{NS}$ ) (Fig. 1B).

### 3.3. Effects of RSG on PAI-1, tPA-antigen and vWF-antigen

In subjects randomized to RSG, plasma levels of PAI-1 and tPA-antigen were significantly reduced after 12 weeks compared with PBO, while there was a trend of reduction in vWF-antigen compared with PBO (Fig. 2). In the RSG group, PAI-1 decreased by  $-1.4$  U/ml ( $-36.1\%$ ,  $P < 0.001$ ) from baseline, compared with a non-significant increase of  $+0.1$  U/ml ( $+1.2\%$ ,  $P = \text{NS}$ ) with PBO, resulting in a statistically significant treatment effect of  $-1.5$  U/ml ( $-36.9\%$ ,  $P < 0.001$ ). Similarly, tPA-antigen decreased by  $-2.6$  ng/ml ( $-30.6\%$ ,  $P < 0.001$ ) from baseline with RSG. This change was statistically significant compared with the decrease of  $-0.8$  ng/ml ( $-7.2\%$ ,  $P = \text{NS}$ ) with PBO: the treatment effect was  $-1.5$  ng/ml ( $-22.7\%$ ,  $P < 0.001$ ). Levels of vWF-antigen decreased by  $-10.8\%$  (percentage change  $-5.6\%$ ,  $P = \text{NS}$ ) with RSG compared with an increase of  $+6.1\%$  (percentage change  $+11.8\%$ ,  $P = \text{NS}$ ) with PBO, giving a treatment effect of  $-16.8\%$  (percentage change  $-15.6\%$ ,  $P = 0.087$ ).

### 3.4. Metabolic effects

Following 12 weeks' treatment, RSG significantly reduced mean  $\pm$  S.D. FPG from baseline by  $-1.9 \pm 2.1$  mmol/l ( $P < 0.001$ ), compared with a significant rise of  $+0.8 \pm 2.0$  mmol/l ( $P < 0.001$ ) with PBO, producing a treatment effect of  $-2.8$  mmol/l ( $P < 0.001$ ). Similarly, mean fasting fructosamine significantly decreased from baseline in subjects treated with RSG ( $-29.1 \pm 42.2$  mg/dl,  $P < 0.001$ ) but significantly increased with PBO ( $+11.0 \pm 43.1$  mg/dl,  $P = 0.048$ ). The change with RSG was statistically significant compared with PBO (treatment effect  $-42.0$ ,



Data presented are mean  $\pm$  95% CI

Fig. 1. Effect of rosiglitazone on serum levels of (A) sE-selectin and (B) sVCAM-1. RSG, rosiglitazone; sVCAM-1, soluble vascular cell adhesion molecule-1.

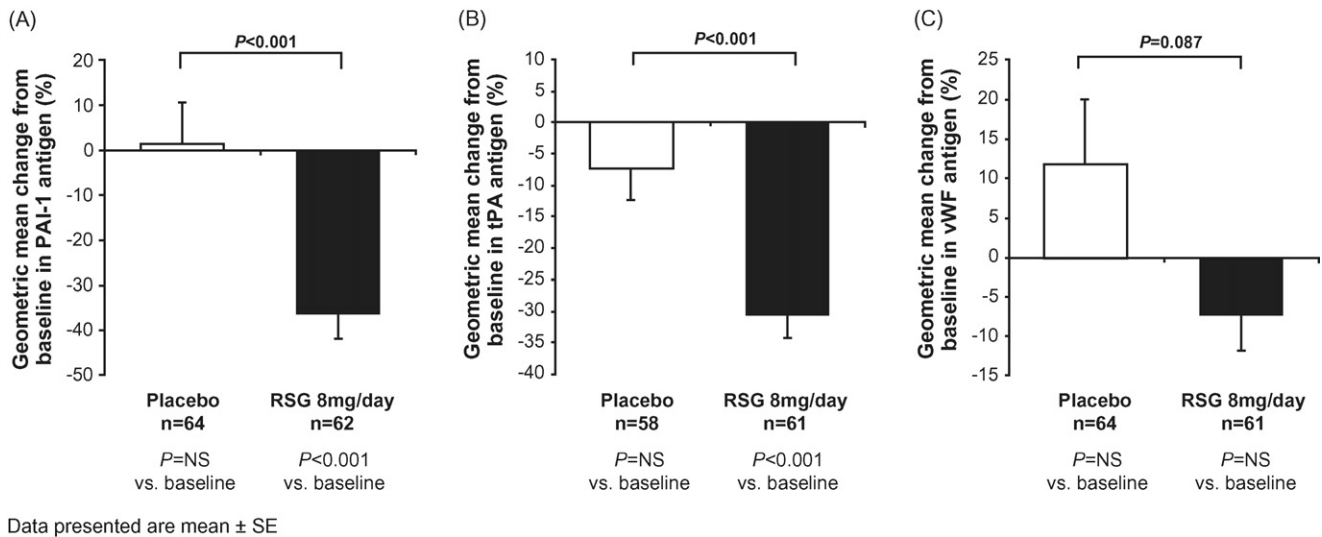


Fig. 2. Effect of rosiglitazone on (A) PAI-1; (B) tPA; (C) vWF levels. RSG, rosiglitazone; PAI-1, plasminogen activator inhibitor-1; tPA, tissue plasminogen activator; vWF, von Willebrand Factor.

$P < 0.001$ ). There were also significant decreases in both fasting C-peptide ( $-0.18 \pm 0.35$  nmol/l,  $P < 0.001$ ) and fasting insulin ( $-25.2 \pm 60.0$  pmol/l,  $P = 0.002$ ) with RSG, but no significant changes with PBO ( $-0.06 \pm 0.23$  nmol/l and  $-6.7 \pm 55.8$  pmol/l, respectively, both  $P = \text{NS}$ ), and the differences between treatment groups were not significant.

After 12 weeks, RSG significantly reduced FFAs as indicated by  $\text{AUC}_{(0-4\text{h})}$ , while there was no significant change with PBO. In subjects treated with RSG, mean  $\pm$  S.D. post-prandial FFA  $\text{AUC}_{(0-4\text{h})}$  was reduced by  $-6.5 \pm 22.5$  mg/dl\*h ( $P = 0.03$ ) (Fig. 3A). There was a decrease of  $-2.6 \pm 40.4$  mg/dl\*h in the PBO group ( $P = \text{NS}$ ), and as a result, the treatment effect between groups did not quite reach statistical significance ( $P = 0.07$ ). Assessment of the relationship between changes in sE-selectin and FFA  $\text{AUC}_{(0-4\text{h})}$  in the two groups taken together revealed a posi-

tive and significant correlation ( $r = 0.22$ ,  $P = 0.05$ ) (Fig. 3B). Changes in sE-selectin levels did not correlate with changes in FPG or C-peptide  $\text{AUC}_{(0-4\text{h})}$ .

### 3.5. Vital signs

There were small decreases from baseline in blood pressure and heart rate in both treatment groups after 12 weeks, but none was statistically significant. Systolic blood pressure decreased by  $-2.2 \pm 16.2$  mmHg with RSG and  $-2.9 \pm 14.6$  mmHg with PBO (both  $P = \text{NS}$ ), giving a treatment difference of  $+0.6$  mmHg ( $P = \text{NS}$ ), while the changes in diastolic blood pressure were  $-1.6 \pm 11.1$  mmHg ( $P = \text{NS}$ ) and  $-0.7 \pm 12.9$  mmHg ( $P = \text{NS}$ ), respectively, with a treatment difference of  $+0.9$  mmHg ( $P = \text{NS}$ ). Heart rate decreased by  $-0.3 \pm 6.7$  bpm ( $P = \text{NS}$ ) with RSG after 12 weeks' treatment and by  $-1.3 \pm 9.4$  bpm ( $P = \text{NS}$ ) with PBO.

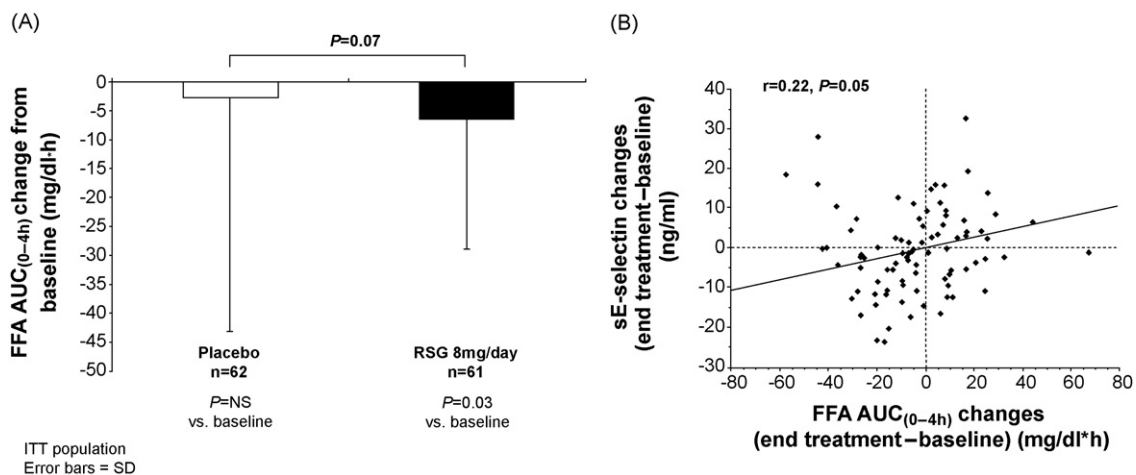


Fig. 3. Effect of rosiglitazone on (A) FFA  $\text{AUC}_{(0-4\text{h})}$  after the meal test and (B) correlation of changes in sE-selectin and FFA  $\text{AUC}_{(0-4\text{h})}$ . RSG, rosiglitazone; FFA, free fatty acids.



Comparison between groups showed that the treatment effect of +1.0 bpm was not statistically significant.

### 3.6. Safety and tolerability

Overall, treatment with RSG was well tolerated, with a similar incidence of AEs in the RSG and PBO groups. On-therapy AEs were recorded in 23 subjects (35.4%) in the RSG group and 29 (40.8%) in the PBO group; the majority were assessed by the investigator as mild or moderate in severity. Hyperglycemia was the only AE considered to be suspected or probably related to study medication that occurred in more than one patient, and was reported in 1.5% of patients in the RSG group and 8.5% in the PBO group. One subject in the RSG group experienced mild edema but this did not necessitate withdrawal. Six subjects withdrew from the study as a result of AEs—two in the RSG group and four in the PBO group. All were due to hyperglycemia with the exception of one case of atrial fibrillation in the RSG group. This subject had a history of atrial fibrillation and the worsening of the condition was described as possibly related to study medication. Body weight increased by  $+1.1 \pm 2.2$  kg ( $\pm$ S.D.) in the RSG group compared with baseline ( $P < 0.001$ ), but did not change significantly from baseline ( $-0.2 \pm 1.9$  kg) with PBO.

## 4. Discussion

Elevations in circulating adhesion molecules are an indication of early endothelial damage. Indeed, elevated levels of E-selectin have been shown to be a marker for development of atherosclerosis and coronary heart disease in non-T2DM subjects [23], and increased levels of sVCAM-1 are associated with risk of CV mortality in T2DM [24].

In this study population largely comprised of Caucasian subjects with T2DM, RSG significantly reduced sE-selectin levels compared with both baseline and PBO, an effect that showed a significant, positive correlation with concomitant changes in levels of FFA. The reduction in E-selectin is consistent with studies with troglitazone *in vitro* [25] and with RSG *in vivo* in non-T2DM patients [26]. However, it is unclear whether these reductions are associated with changes in glycemia. In this study, there were significant reductions in FPG and fructosamine with RSG compared with PBO, although glycated hemoglobin was not measured due to the duration of the study, and previous studies have been inconclusive. For example, a correlation between FPG and sE-selectin was reported in patients with poorly controlled T2DM after 14 days' intensive insulin treatment [6]. Conversely, a study investigating the influence of insulin and sulfonylureas in a small sample of patients with T2DM reported that changes were independent of glycemic control [27].

Previous clinical studies, including one with RSG [28] and our previously published study of a short-term improvement in glycemic control with insulin [6], support the lack of signif-

icant change in sVCAM-1 with RSG seen in this study. These data contrast with an *in vitro* study with troglitazone [25]. Several reasons could explain the lack of significant change *in vivo*. For example, VCAM-1 expression has been reported to be regulated by PPAR- $\alpha$  [29]. In addition, sVCAM-1 is not specific to the endothelium and baseline values were not as elevated as sE-selectin levels. The short duration of treatment (12 weeks) may also be a contributory factor.

Shear stress has been shown to affect expression of VCAM-1 and E-selectin [30]; therefore, it is possible that the previously reported effects of TZDs in lowering blood pressure [31] may be involved in changes in adhesion molecules. However, in this study decreases in blood pressure were small and similar in both treatment groups.

Raised levels of PAI-1, tPA and vWF are associated with increased CVD risk [32,33] and are frequently observed in subjects with T2DM [34]. In this study, RSG significantly reduced levels of PAI-1 and tPA, as observed previously and consistent with improvements in insulin resistance and endothelial function [35]. There was a marked, but non-significant, reduction in vWF ( $-16.8\%$ ) with RSG. Greater reductions in vWF levels have been reported previously following RSG treatment in non-T2DM subjects with CVD [26,28], perhaps related to higher circulating levels at baseline.

The effect of thiazolidinediones on a number of other important factors related to endothelial function has been studied previously. For example, rosiglitazone has been shown to reduce plasma monocyte chemoattractant protein-1 and C-reactive protein in obese subjects with and without diabetes [36], and to reduce levels of asymmetric dimethylarginine in subjects without diabetes [2,37]. Troglitazone has been reported to reduce the generation of reactive oxygen species *in vitro* [38]. Studies of other factors have failed to show conclusively positive effects. For example, rosiglitazone has shown no effect on intercellular adhesion molecule-1 in subjects with or without diabetes [28,36,39], while troglitazone has significantly reduced this factor in subjects without diabetes [40].

This was an *in vitro* study and we did not investigate direct effects of rosiglitazone on endothelial function. However, the indirect effects that we report reflect previous clinical observations of direct improvements in endothelial function, including endothelium-dependent flow-mediated vasodilatation and endothelium-independent nitroglycerin-induced vasodilatation, following RSG therapy [37,41,42]. Further assessments in larger numbers of subjects with T2DM, using direct measures of endothelium-dependent flow-mediated vasodilatation and *in vitro* assays of biological markers are required to support these conclusions.

Several metabolic disturbances associated with T2DM may account for the benefits seen with an insulin sensitizer. Interestingly, in the present study we found significant correlations at baseline between sE-selectin and a number of factors that attest to insulin resistance. In addition, in insulin-resistant subjects there is a reduction in

endothelium-dependent vasodilation [43], the severity of which correlates inversely with insulin-mediated glucose disposal [44], while insulin increases nitric oxide-mediated endothelium-dependent vasodilation [45]. Elevated FFA levels, associated with insulin resistance, have been shown to impair endothelium-dependent vasodilation in insulin resistant subjects [3] and also to have a proinflammatory effect, providing a potential link between inflammation and insulin resistance [46,47]. The correlation analyses performed in the present population suggest that the effects of changes in FFA levels on sE-selectin levels, and therefore on endothelium function, were of a greater magnitude than those related to blood glucose changes.

These results provide further support for the hypothesis that RSG has the potential to improve endothelial function. However, whether this translates into a reduction in CV events and improved patient outcomes is not yet known and is currently being examined in various long-term outcome studies [48,49].

## 5. Conclusions

Treatment with RSG was generally well-tolerated in subjects with T2DM and had beneficial effects on several factors related to endothelial dysfunction that are likely to be mediated through improvements in insulin sensitivity and decreases in serum FFA levels. These results provide further support for the hypothesis that RSG has the potential to improve endothelial function. However, further assessments in larger numbers of subjects with T2DM, using both direct measures of endothelium-dependent flow-mediated vasodilatation and *in vitro* assays of biological markers, are required to support our conclusions.

## Acknowledgements

This study was funded by GlaxoSmithKline Ltd. Data from this study were presented orally at the European Society of Cardiology Congress, Munich, Germany, 28 August–1 September 2004 and the European Association for the Study of Diabetes Annual Meeting, Munich, Germany, 5–9 September 2004, and in poster form at the American Heart Association Annual Scientific Sessions, New Orleans, United States, 7–10 November 2004. The authors wish to acknowledge the editorial assistance of Carol Mason in the development of this manuscript.

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