

## Effects of Rosiglitazone on Endothelial Function in Non-Diabetic Subjects with Metabolic Syndrome

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### OBJECTIVE

To evaluate the effects of rosiglitazone (ROSI), an insulin-sensitizer, on endothelial function and endothelial activation markers in a group of non-diabetic subjects with metabolic syndrome.

### METHODS

A group of eighteen subjects (12 women, 6 men), mean age  $41.2 \pm 9.7$  and BMI  $37.8 \pm 6.1$  Kg/m<sup>2</sup>, was treated with rosiglitazone 8 mg/day for 12 weeks. Another group of nine healthy subjects, mean age  $26.1 \pm 4.4$  and BMI  $21.7 \pm 1.7$  Kg/m<sup>2</sup>, was studied at baseline to compare vasodilator response. Endothelial function was evaluated by venous occlusion plethysmography after intra-arterial infusions of acetylcholine (Ach) and sodium nitroprusside (SNP). The following were measured: glucose, insulin, lipids, fibrinogen, and high-sensitivity C-reactive protein (CRP). HOMA and QUICKI indexes were calculated to quantify insulin resistance (IR).

### RESULTS

There was an improvement in insulin resistance, as evidenced by lower HOMA-R and higher QUICKI index, as well as a decrease in CRP and fibrinogen levels. Endothelium-dependent vasodilation also improved, as evidenced by greater increment in blood flow after Ach and greater decrement in vascular resistance. No difference in endothelium-independent vasodilation was noted.

### CONCLUSION

Rosiglitazone treatment reduced insulin resistance, fibrinogen, and CRP levels and improved endothelial function in non-diabetic subjects with metabolic syndrome. These data suggest that rosiglitazone plays a role in the regulation of endothelial function in patients at high cardiovascular risk.

### KEY WORDS

Endothelium, metabolic syndrome, rosiglitazone.

Although atherosclerosis has been recognized as a disease over the past 150 years, the mechanisms involved in its development have been evolving dramatically. The current view of atherosclerosis pathophysiology involves a complex interaction of several factors, quite different from the old view of a disease caused by abnormal lipid deposition in the vascular wall. Among these factors, inflammation and endothelial dysfunction are of particular importance<sup>1,2</sup>. The term endothelial dysfunction refers to impaired endothelium-dependent vasodilation and abnormal interplay between endothelial and blood cells, producing local inflammation and, later, vascular lesions and thrombosis. Endothelial dysfunction occurs when vasoconstrictive effects superimpose vasodilator effects, usually as a result of reduced nitric oxide (NO) bioavailability and consequent loss of its vascular protective action. Endothelial dysfunction is regarded as an early step in the atherosclerotic process, being found in all subsequent stages of the disease<sup>3,4</sup>.

Metabolic syndrome (MS) is defined by the presence of impaired glucose tolerance and/or type-2 diabetes mellitus (DM2), arterial hypertension, dyslipidemia, and abdominal obesity. Insulin resistance is the common denominator of this condition, preceding the onset of the aforesaid changes. Metabolic syndrome is currently recognized as a major risk factor for atherosclerotic disease and cardiovascular mortality<sup>5-7</sup>. It is widely known that DM2 patients have varying degrees of endothelial dysfunction. There is evidence, however, of early endothelial dysfunction even before any level of impaired glucose tolerance occurs<sup>8,9</sup>. Furthermore, patients with insulin resistance (IR) often have endothelial dysfunction and vascular inflammation<sup>10,11</sup>. Elevated levels of circulating free fatty acids (FFAs), oxidative stress, arterial hypertension, and a higher number of small, dense LDL particles are some of the potential mechanisms involved in reduced NO production secondary to insulin resistance<sup>11-13</sup>.

Peroxisome proliferator-activated receptors-gamma (PPAR $\gamma$ ) are members of a nuclear receptor family that regulates the expression of various genes. These receptors are expressed in several tissues, especially in the adipose, but also in vascular endothelial cells, macrophages, and pancreatic beta-cells<sup>14,15</sup>. Thiazolidinediones (TZDs) are their exogenous agonists and also potent insulin sensitizers (IS). Consequently, these agents have been used in the treatment of type-2 diabetes to improve IR both directly by acting on the adipose tissue (improve fatty acids and glucose uptake)<sup>16</sup> and indirectly by altering adipocytokines secretion (decrease in TNF $\alpha$  and increase in adiponectin)<sup>17-19</sup>, thus enhancing insulin sensitivity in other tissues.

Rosiglitazone, one of the TZDs currently available, not only affects glucose metabolism, but also has potent anti-inflammatory action<sup>17</sup>. Moreover, it has significantly improved endothelial function in diabetic patients<sup>20</sup>.

Therefore, early intervention with this agent in non-diabetics MS patients may contribute to enhanced endothelial function, and thanks to its anti-inflammatory action, reduce cardiovascular risks.

This study aims at evaluating insulin-sensitizer rosiglitazone (ROSI) effects on endothelial function and endothelial activation markers in a group of non-diabetic subjects with metabolic syndrome.

## METHODS

Eighteen subjects (6 men and 12 women, mean age  $41.2 \pm 9.7$ ) diagnosed with MS according to NCEP-ATPIII criteria<sup>21</sup> were studied (Table 1) before and after treatment with ROSI 8 mg/day during twelve weeks. Nine healthy subjects (5 men and 4 women, mean age  $26.1 \pm 4.4$ ) were studied solely to compare vasodilator response at baseline (Table 2).

This study was approved by the Ethics Committee of Hospital Universitário Pedro Ernesto, the teaching hospital of Universidade do Estado do Rio de Janeiro, and all patients signed an informed consent before entering the trial.

Patients were asked to maintain an isocaloric diet and the usual level of physical activity throughout the study. Those with hypertension were instructed not to stop taking their anti-hypertensive medication. Body mass index (BMI) was calculated by dividing weight (in kilograms) by squared height (in meters). Waist circumference was obtained by measuring the narrowest point midway between the iliac crest and the lower costal margin, and hip circumference was calculated by measuring the largest diameter of the gluteal region<sup>22</sup>. Waist-to-hip ratio (WHR) was determined by dividing the waist circumference by the hip circumference. Supine blood pressure was measured twice after a 15-minute rest using an automatic sphygmomanometer (Multiparameter patient monitor - Lifewindow LW6000, USA), and patients were classified as hypertensive according to JNC-7 criteria<sup>23</sup>.

Blood samples were collected, after a 12-hour fast, at baseline and after 12 weeks of rosiglitazone therapy. The following parameters were analyzed: glucose, total cholesterol, triglycerides, LDL and HDL cholesterol (all concentrations were determined by the automated enzymatic method), C-reactive protein (automated nephelometry), fibrinogen (coagulometric method),

**Table 1 – Diagnostic criteria for the metabolic syndrome according to NCEP-ATPIII.<sup>21</sup>**

Diagnosis based on at least three criteria:

Blood glucose  $\geq 110$  mg/dl

Triglycerides  $> 150$  (mg/dl)

HDL cholesterol  $< 50$  (Women)  $< 40$  (Men)

Blood pressure  $\geq 130 \times 85$  mm Hg

Waist circumference  $> 88$  cm (Women)  $> 102$  (Men)

**Table 2 – Clinical and laboratory parameters for the control group and the MS group at baseline**

	Control n = 9	MS n = 18	p value
Age (years)	26.1 ± 4.4	41.2 ± 9.7	< 0.001
Body weight (kg)	64.6 ± 13.7	105.3 ± 16.4	< 0.001
BMI (kg/height <sup>2</sup> )	21.7 ± 1.7	37.8 ± 6.1	< 0.001
Waist circumference (cm)	♂ 81.6 ± 2.8 ♀ 64.5 ± 5.2	♂ 108.6 ± 9.4 ♀ 106.8 ± 9.9	< 0.001 < 0.001
Hip circumference (cm)	♂ 97.4 ± 5.5 ♀ 90 ± 4	♂ 113.8 ± 5.5 ♀ 1119.9 ± 12.3	< 0.001 0.01
Waist-to-hip ratio	♂ 0.83 ± 0.09 ♀ 0.71 ± 0.04	♂ 0.97 ± 0.08 ♀ 0.91 ± 0.06	< 0.001 0.03
Glucose (mg/dl)	86 ± 8.1	98.4 ± 13.8	0.01
Insulin (μU/ml)	4.6 ± 1.65	14.5 ± 4.7	< 0.001
HOMA-R index	0.88 ± 0.48	3.5 ± 1.2	< 0.001
QUICKI index	0.421 ± 0.09	0.319 ± 0.013	< 0.001
CRP (mg/dl)	0.3 [0.1-1.0]	1.05 [0.3-9.6]	0.05
Fibrinogen (mg/dl)	244.9 ± 31.8	310.6 ± 73.3	0.01
Total cholesterol (mg/dl)	188.5 ± 30.1	206.6 ± 36.4	0.26
HDL cholesterol (mg/dl)	♂ 49 ± 5.6 ♀ 67.2 ± 14.3	♂ 44.8 ± 7.6 ♀ 44.7 ± 15.1	0.04 0.01
LDL cholesterol (mg/dl)	109.7 ± 19.3	128.8 ± 34.3	0.12
Triglycerides (mg/dl)	61.5 ± 17.7	168.3 ± 76.5	< 0.001

and insulin (chemiluminescence method). All patients underwent an oral glucose tolerance test (OGTT) using 75 g anhydrous glucose. The 2-hour blood glucose test was used to classify glucose tolerance<sup>24</sup>. Patients with DM2 were excluded. HOMA-R (insulin in μU/ml x glucose in mmol/l / 22.5)<sup>25</sup> and QUICKI (1/log insulin μU/ml + log glucose in mg/dl)<sup>26</sup> indexes were calculated to quantify insulin resistance.

Endothelial function was assessed by forearm venous occlusion plethysmography performed under standardized conditions. After an overnight fast, all patients rested for at least 30 minutes in a temperature-controlled room (22 ± 1 °C) prior to examination. They were requested to refrain from smoking and drinking alcohol and caffeine for 12 hours before the test. A 27-gauge needle was inserted into the brachial artery of the non-dominant arm under local anesthesia (1% Xylocaine) for drug infusion. Blood pressure was measured non-invasively throughout the study on the contralateral arm. Change in limb volume was measured by a sensor placed around the widest part of the forearm and attached to a plethysmograph (Hokanson E06, Washington, USA).

During flow analysis, venous return was occluded by inflating a cuff around the upper arm to 40 mm Hg using a rapid cuff inflator (Hokanson AG101, USA). Circulation to the hand was interrupted by inflating another cuff around the wrist to 40 mm Hg above the systolic blood pressure a minute before measurement. Measurements were taken in rest conditions and after infusion of acetylcholine (ACh) (Harvard Apparatus infusion pump, model 22, USA) at sequential doses of 7, 5, 15, and 30 μg/min every 5 minutes and sodium nitroprusside (SNP) at sequential doses of 2, 4, and 8 μg/min. A 30-minute interval between both infused drugs

was observed. Forearm blood flow (FBF) was measured during the last 2 minutes of each infusion. Four curves were determined at each study time point, and the mean value was used in the statistical analysis. PowerLab system (8SP-AD Instruments, Australia) was used to analyze plethysmograph signals.

Statistical analysis was performed using SPSS 8.0 for Windows. Normally distributed data were expressed as mean ± standard deviation and non-normally distributed data were expressed as median [minimum-maximum]. Normally distributed paired variables were compared by the Student's t-test, and non-normally distributed variables were compared by the Wilcoxon test. Independent variables (controls x patients) were compared by the Mann-Whitney test. The statistical significance level was set at 5%.

## RESULTS

Table 2 shows the clinical and metabolic characteristics of the control group and of the MS group. Of the eighteen patients with MS, nine had impaired glucose tolerance (IGT) and eight, systemic hypertension. Of the nine control subjects, not one had impaired glucose tolerance, arterial hypertension or dyslipidemia.

Table 3 shows the clinical and metabolic characteristics before and after ROSI therapy. After 12 weeks of rosiglitazone therapy, fasting glucose, CRP, and fibrinogen decreased and insulin resistance indexes improved (HOMA-R and QUICKI).

All patients experienced weight gain; however, when they were separated by sex, women had fat deposited peripherally, with increased hip circumference and

decreased waist-to-hip ratio (WHR) (Table 3).

With regard to lipid profile, there was an increase in total cholesterol, LDL cholesterol and, only in the female group, HDL cholesterol. No significant changes were observed in triglycerides levels.

No statistically significant changes were found in systolic ( $151.1 \pm 16.3 \times 144.9 \pm 14.5$  mmHg;  $p = 0.86$ ), diastolic ( $87 \pm 9.8 \times 84.4 \pm 9.7$  mmHg;  $p = 0.26$ ) and mean blood pressure ( $104 \pm 13.7 \times 103.3 \pm 15.4$  mmHg;  $p = 0.06$ ).

Figures 1 and 2 (A and B) illustrate blood flow and vascular resistance responses before and after Ach and SNP. Comparison of the MS group with the control group at baseline, demonstrated significant differences in blood flow increase after Ach ( $85\%[-26/551] \times 367\%[257/561]$ ) at the highest dose;  $p = 0.005$ ) and after SNP ( $120\%[-12/621] \times 399\%[135/1041]$ ) at the highest dose;  $p = 0.005$ ).

The MS group showed enhanced endothelium-dependent vasodilation following 12 weeks of rosiglitazone therapy, with increased blood flow after Ach ( $85\%[-26/551] \times 285.5\%[83/546]$ ) at the highest dose;  $p = 0.02$ ) and decreased vascular resistance ( $-47.5\%[-84/33] \times -74\%[-85/-46]$ ) at the highest dose;  $p = 0.003$ ) (fig.1). No change was observed in vasodilator response after SNP.

After treatment, the difference in blood flow increase after Ach between both groups disappeared ( $285.5\%[83/546] \times 367\%[257/561]$ ) at the highest dose;  $p = 0.23$ ), and the difference in endothelium-independent vasodilation was maintained ( $219.5\%[52/466] \times 399\%[135/1041]$ ) at the highest dosage;  $p = 0.007$ ) (fig.1).

## DISCUSSION

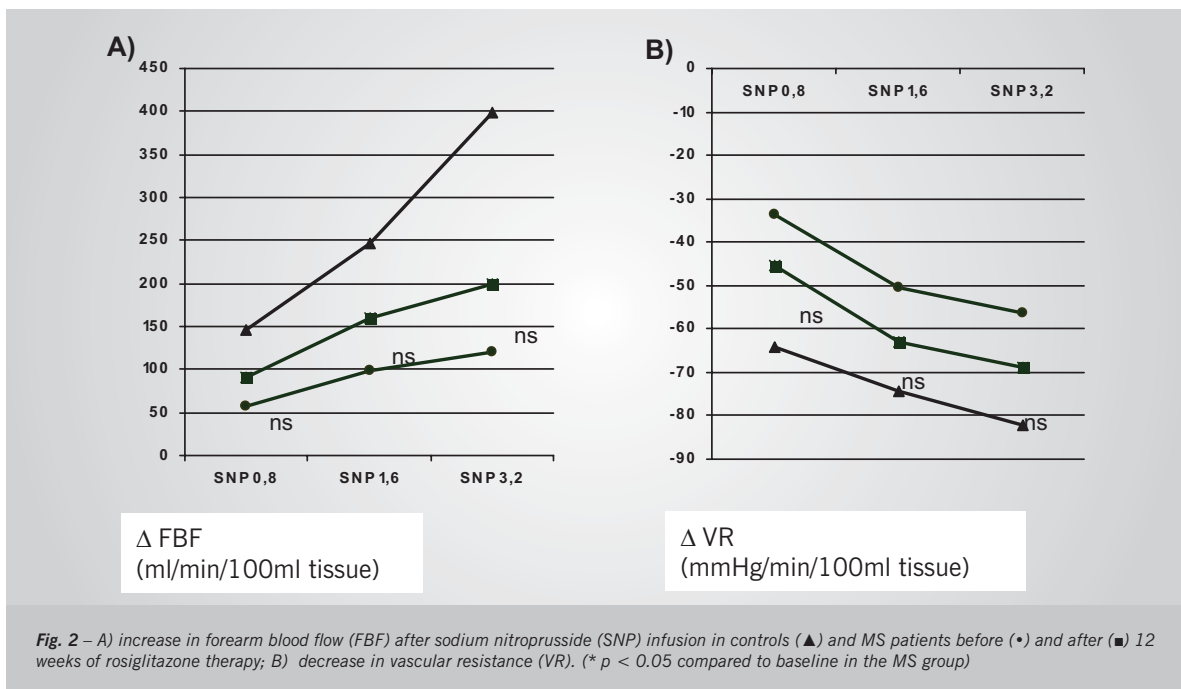
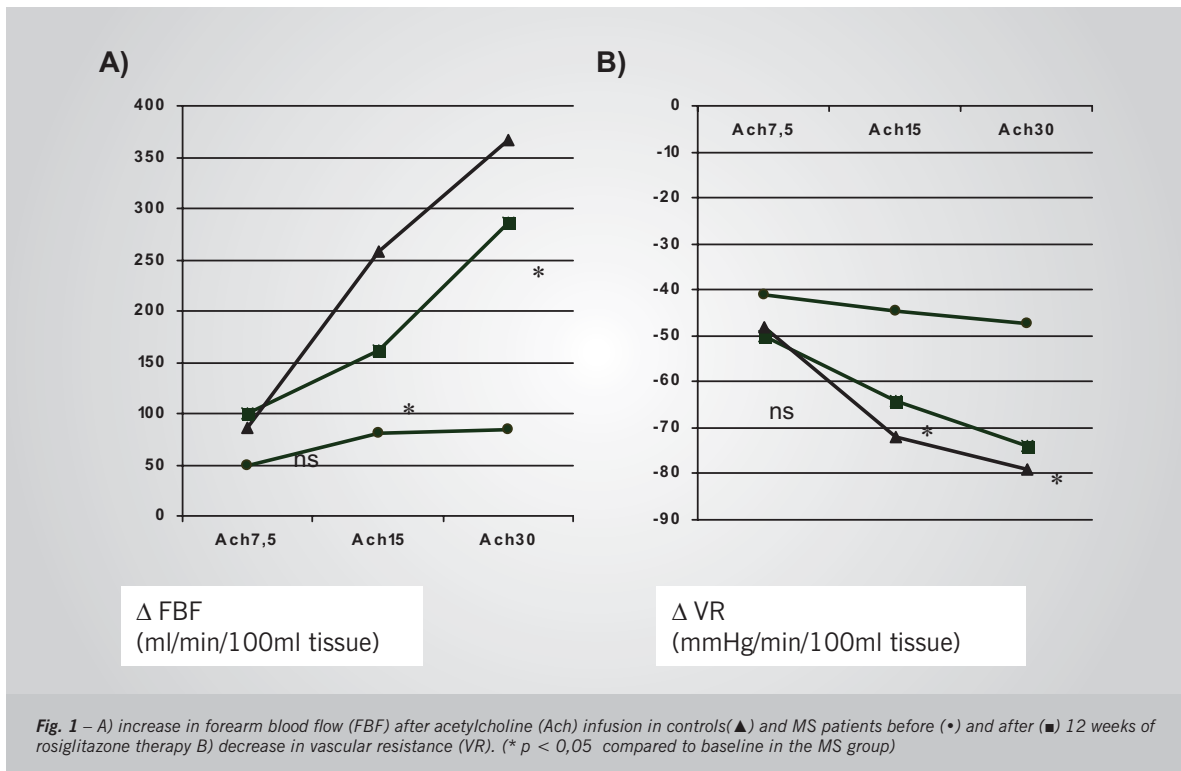
Metabolic syndrome is a well established risk factor for cardiovascular disease<sup>5,6,7</sup>, even in the absence of diabetes mellitus. In addition to being a common finding in MS patients, IR precedes the onset of metabolic changes<sup>27</sup>. More importantly, IR, evaluated by HOMA-R, is an independent risk factor for cardiovascular disease<sup>28</sup>, and it is associated with endothelial dysfunction and inflammation<sup>10,11</sup>. Therefore, a group of non-diabetic patients with MS, and thus at risk of developing cardiovascular disease, was studied to evaluate whether rosiglitazone would be able to improve endothelial function and inflammation. For this purpose, venous occlusion plethysmography was used with acetylcholine and sodium nitroprusside infusion, the method regarded as the gold standard in *in-vivo* assessment of endothelial function<sup>3</sup>.

Vasodilator response to Ach in patients with MS was significantly lower than that of the control group at baseline (Fig. 1). After rosiglitazone therapy, however, this significant difference between both groups disappeared. Furthermore, only endothelium-dependent vasodilator capacity was restored. As expected, endothelium-independent vasodilation remained unchanged. After 12 weeks of treatment with rosiglitazone 8 mg/day, HOMA-R significantly decreased, whereas QUICKI increased, characterizing IR improvement.

Inflammation markers, such as CRP and fibrinogen, declined by 52.4% and 18%, respectively. Interestingly, total cholesterol, LDL cholesterol and body weight increased, factors well known to be associated with endothelial dysfunction. These findings suggest that the primary mechanism involved in improved endothelial vasodilator response might have been the decrease

**Table 3 – Clinical and laboratory parameters for the MS group at baseline and after 12 weeks of rosiglitazone therapy**

	Basal	Twelve weeks	p value
Body weight (kg)	105.3 ± 16.4	107.5 ± 16.7	0.003
BMI (kg/height <sup>2</sup> )	37.8 ± 6.1	38.6 ± 6.4	0.004
Waist circumference (cm)	♂ 108.6 ± 9.4	♂ 108.4 ± 11.1	0.87
	♀ 106.8 ± 9.	♀ 107.7 ± 11.8	0.39
Hip circumference (cm)	♂ 113.8 ± 5.5	♂ 114.4 ± 5.4	0.59
	♀ 119.9 ± 12	♀ 123.3 ± 11.2	0.001
Waist-to-hip ratio	♂ 0.97 ± 0.08	♂ 0.94 ± 0.07	0.33
	♀ 0.91 ± 0.06	♀ 0.87 ± 0.07	0.02
Glucose (mg/dl)	98.4 ± 13.8	86.4 ± 10.3	0.002
Insulin (μU/mL)	14.5 ± 4.7	12.1 ± 6.2	0.07
HOMA-R index	3.5 ± 1.2	2.5 ± 1.5	0.02
QUICKI index	0.319 ± 0.013	0.336 ± 0.022	0.002
CRP (mg/dl)	1.05 [0.3-9.6]	0.5 [0.1-3.0]	< 0.001
Fibrinogen (mg/dl)	310.6 ± 73.3	255.5 ± 75.1	0.01
Total cholesterol (mg/dl)	206.6 ± 36.4	221.5 ± 33.8	< 0.001
HDL cholesterol (mg/dl)	♂ 44.8 ± 7.6	♂ 44.8 ± 8.1	0.99
	♀ 44.7 ± 15.1	♀ 49.5 ± 17.35	0.04
LDL cholesterol (mg/dl)	128.8 ± 34.3	141.0 ± 37.1	< 0.001
Triglycerides (mg/dl)	168.3 ± 76.5	160.8 ± 61.1	0.53



in insulin resistance and improvement in vascular inflammation. Other authors have already demonstrated the CRP reduction observed in this study after the use of TZDs in either diabetic or non-diabetic patients<sup>29,30,31</sup>, confirming the anti-inflammatory effect of this therapeutic drug class.

Wang et al<sup>31</sup> reported similar results in 50 patients with MS treated with ROSI during 8 weeks. Using brachial

artery Doppler, they observed flow-mediated vasodilation after ischemia, coupled with increased LDL cholesterol and apolipoprotein B levels. Nevertheless, nitroglycerin-induced vasodilation improvement (endothelium-independent) was also observed. Pistrosch et al<sup>20</sup> demonstrated improved endothelial function in insulin-resistant type-2 diabetics after 12 weeks of ROSI therapy. In this study, endothelial function improvement occurred

regardless of glycemic control, as demonstrated when compared with a group who received nateglinide. The authors, therefore, suggest that improvement in insulin resistance may be more important for improvement in endothelial function than blood glucose reduction. Caballero et al<sup>32</sup> also reported improvement in endothelial function in patients with recently diagnosed type-2 diabetes after troglitazone treatment. This beneficial effect, however, was not demonstrated in diabetic patients with established macrovascular disease. Sidhu et al<sup>30</sup> studied non-diabetic subjects with coronary heart disease and noted improvement in endothelial activation markers (CRP, circulating adhesion molecules, and von Willebrand factor). Yet, no improvement in flow-mediated vasodilation was observed. Natali et al<sup>33</sup> compared the effects of metformin and rosiglitazone in 74 type-2 diabetic patients and concluded that only the group who received rosiglitazone improved endothelium-dependent vasodilation and insulin sensitivity, although both groups have attained similar metabolic control.

Several mechanisms have been described to explain the improvement in endothelial function following treatment with PPAR $\gamma$  agonists. The decline in insulin resistance may increase NO delivery, since, physiologically, insulin increases endothelial nitric oxide synthase (eNOS) expression<sup>34</sup>. Recently, Goya et al<sup>35</sup> showed that these agonists directly stimulate eNOS expression *in vitro*. The reduction of circulating free fatty acid afforded by TZDs also improves endothelial function<sup>33</sup>. The anti-inflammatory effects of TZDs have already been well demonstrated in animals and humans alike<sup>37</sup>, evidenced by decreased NF $\kappa$ B<sup>38</sup> activation as well as cytokine and vascular adhesion molecules release<sup>38</sup>. Another possible mechanism involved in improvement of endothelial function is the antioxidant effect, thus decreasing free radical production by monocytes and polymorphonuclear cells<sup>39</sup>, peroxynitrite formation and induced endothelial NO synthase expression<sup>40</sup>.

The administration of rosiglitazone to non-diabetic patients with IR improved insulin sensitivity, an effect already demonstrated in other groups of patients with IR, such as women with polycystic ovaries<sup>41</sup>, AIDS patients on antiretroviral therapy, which causes lipodystrophy and insulin resistance<sup>42</sup>, and patients with impaired glucose tolerance<sup>43</sup>.

Thiazolidinediones usually cause weight gain, due to their effects on several genes associated with adipogenesis. There is an increase in adipocytes differentiation (pre-adipocytes  $\rightarrow$  mature adipocytes), particularly those located subcutaneously, which are

metabolically more effective; that is to say, they can enhance fatty acids and glucose uptake<sup>44</sup>. In this study, the mean body weight increase of 2.2 kg in 12 weeks was not followed by an increase in waist circumference, suggesting a more peripheral fat distribution (decreased WHR) in the female group). In some studies, TZDs have been shown to redistribute body fat, reducing visceral, hepatic, and intramyocellular fat<sup>45-47</sup>, sites of abnormal fat deposition associated with greater insulin resistance. A more accurate method to determine body fat was not used in the study, thus limiting further conclusions.

The effects of thiazolidinediones on lipid profile are controversial. Some studies show higher levels in total cholesterol and LDL cholesterol<sup>31</sup>, as observed in this group. PPAR receptors activation in macrophages seems to cause different effects. Stimulation occurs to induce the expression of CD36 receptors, facilitating the uptake of oxidized LDL molecules<sup>48</sup> whereas increasing ABCA1-induced reverse cholesterol transport<sup>49</sup>. Analysis of LDL particles shows that the TZDs change their morphologic pattern, reducing the number of small, dense LDL molecules and, thus, endothelial uptake<sup>50</sup>. There is no consensus in the literature regarding TZD effect on triglycerides, in that both reduced effects and no effect at all were reported<sup>51,52</sup>. In this study, no significant change in triglycerides level was found.

Matching the control group and the MS group by age was not possible, but this difference was useful to demonstrate that ROSI improved vasodilator response on level equivalent to that of a healthy younger group. The lack of an MS group treated with placebo somewhat limits study conclusions.

We concluded that ROSI induced IR, fibrinogen and CRP reduction and improved endothelial function in MS subjects. These data suggest a possible role of this substance in regulating endothelial function. The prophylactic use of TZDs in subjects at high risk for developing diabetes and cardiovascular disease (patients with metabolic syndrome and impaired glucose tolerance) is yet to be established. Controlled and prospective clinical trials are under way, and data are awaited to determine whether this approach may reduce long-term ischemic cardiovascular events.

#### Potencial Conflict of Interest

Amélio F. Godoy-Matos

- Conference speaker sponsored by GSK industry

The other co-authors did not declare any potential conflict of interest.

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