Original Article

Effects of Rosiglitazone (a Peroxysome Proliferator–Activated Receptor γ Agonist) on the Blood Pressure and Aortic Structure in Metabolically Programmed (Perinatal Low Protein) Rats

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This study investigated the effects of rosiglitazone on nutritionally programmed chronic disease, with a focus on blood pressure (BP) and aortic wall structural remodeling. Wistar pregnant rats were fed one of two diets: a normal protein diet (19% protein; NP rats) or low-protein diet (5% protein; LP rats). Male offspring at 3 months of age were randomly divided into four groups: NP offspring treated with rosiglitazone (NPR); untreated NP offspring (NP); LP offspring treated with rosiglitazone (LPR); untreated LP offspring (LP). Rosiglitazone was administered at a dose of 5 mg/kg/d until 6 months of age. BP was elevated in LP offspring. Rosiglitazone reduced BP beginning in the first week of treatment in the LPR offspring. The insulin sensitivity was increased in LP offspring, and was not altered by rosiglitazone. LP offspring exhibited a 40% reduction in the amount of elastic fibers in the aorta wall compared with NP offspring ($p<0.01$), and the quantity of elastic fibers was not altered by rosiglitazone. The smooth muscle cells, elastic lamellae, circumferential wall tension (CWT) and tensile stress (TS) were increased in LP offspring, indicating increased blood flow in the aorta. Rosiglitazone reduced both CWT and TS by 30% compared to the levels in untreated LP offspring ($p<0.01$ for both). Rosiglitazone restored the expressions of angiotensin II type 1 receptor and endothelial nitric oxide synthase nearly to the levels in the NP offspring. ANOVA disclosed a significant two-factor interaction between protein content in the diet and rosiglitazone treatment ($p<0.001$ for CWT and $p<0.00001$ for TS, two-way ANOVA). We conclude that rosiglitazone has beneficial effects in reducing the BP and the aortic tunica media hypertrophy with consequent balance of the wall stress in metabolically programmed offspring. (*Hypertens Res* 2008; 31: 965–975)

***Key Words*: chronic diseases, peroxysome proliferator–activated receptor γ agonist, arterial remodeling, hypertension, insulin**

Introduction

Human epidemiological studies and appropriately designed dietary interventions in animal models have provided considerable evidence to suggest that maternal nutritional imbalance and metabolic disturbances, when occurring during critical time windows of development, may have a persistent effect

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on the health of the offspring (*1*). According to this concept of "fetal programming," intrauterine conditions that impair fetal growth can program the fetus to express hypertension (*2*) in addition to having irreversible effects on glucose homeostatic mechanisms in the offspring that may predispose the offspring to diabetes in later life (*3*), and of course both hypertension and diabetes are risk factors for cardiovascular disease (*4*).

Hypertension leads to adverse remodeling in the arterial wall, particularly in the elastic (large) arteries (*5*). These arteries should no longer be considered passive conduits, but rather should be considered in terms of their active behavioral response to the mechanical forces to which they are subjected, reflecting changing patterns in the structural remodeling of the vessels (*6*, *7*).

Rosiglitazone is a potent thiazolidinedione insulin sensitizer with a high-affinity ligand for peroxysome proliferator–activated receptor γ (PPARγ), a nuclear hormone receptor that is expressed in adipocytes and that regulates terminal adipocyte differentiation (*8*). Rosiglitazone is indicated for the treatment of type 2 diabetes (*9*) as well as for the treatment of severely obese non-diabetic subjects (*10*). Furthermore, rosiglitazone reduces blood pressure (BP) in association with the amelioration of insulin sensitivity (*11*) that improves vessel function (*12*).

The present study was undertaken to investigate the effect of rosiglitazone on the nutritional programming of chronic disease. The focus of the analyses was on the carbohydrate metabolism, body fat mass and aortic wall structural remodeling in adult offspring from undernourished dams.

Methods

Sample and Procedures

All procedures were carried out in accordance with the US National Institutes of Health guidelines for the care and use of laboratory animals (US NIH 85-23, revised 1996). The experimental protocols were approved by the local committee for the use and care of experimental animals of the State University of Rio de Janeiro, Rio de Janeiro, Brazil (protocol number CEA/104/2005).

Wistar rats bred in our laboratory were maintained under controlled conditions (21±2°C, humidity 60±10%, 12:12 h dark-light and air replacement cycles) with free access to food and water. Virgin females were caged with males overnight and mating was confirmed by analyzing the vaginal plug or spermatozoa in the vaginal smear. Dams were housed individually and fed one of two diets throughout gestation and the first 10 d of lactation: a normal protein diet (190 g protein/kg diet), or a low-protein diet (50 g protein/kg diet). Both diets were isoenergetic (16,503.0 kJ/kg diet; the low-protein diet was compensated by the addition of carbohydrates). The mineral and vitamin contents in the two diets were identical and in accordance with the recommendations of the American Institute of Nutrition (AIN-93G) (*13*). Diets were produced by Rhoster (Sao Paulo, Brazil) and their compositions are described in Table 1.

Table 1. Composition of Perinatal Diets (Both Diets Are Isoenergetic)

Nutrients (g/kg)	Perinatal diet	
	NP	LP
Corn starch	539.486	682.336
Sucrose	100.0	100.0
Casein	190.0	50.0
Cystine	3.0	1.5
Coline	2.5	2.5
Fat (soybean oil)	70.0	70.0
Fiber (cellulose)	50.0	50.0
Mineral mix (AIN-93G)	35.0	35.0
Vitamin mix (AIN-93G)	10.0	10.0
tert-Butylhydroquinone	0.014	0.014
Energy (kJ/kg of diet)	16,503.0	16,503.0
Protein (%)	19.0	5.0
Carbohydrate (%)	65.0	79.0
Lipid (%)	16.0	16.0

Groups: LP, low protein; NP, normal protein. Vitamins and minerals mix formulated to meet the American Institute of Nutrition AIN-93G recommendation for rodents.

Immediately after delivery the litters were adjusted to 6 animals each to assure adequate and standardized nutrition until weaning (*14*). Beginning at postnatal day 10 and continuing for the remainder of the lactation period, dams were fed the standard rat chow (normal protein diet). Therefore, the protein restriction covered the whole period of organogenesis in rodents (*15*). At weaning (21 d of age), the offspring from the dams fed a normal-protein diet were designated NP offspring and those from the dams fed a low-protein diet were termed LP offspring. Male offspring was randomly selected from each mother to complete the sample size. At 3 months of age, the animals were randomly divided into four groups: NP offspring treated with rosiglitazone (NPR); untreated NP offspring (NP); LP offspring treated with rosiglitazone (LPR); untreated LP offspring (LP). Rosiglitazone was administered at a dose of 5 mg/kg/d by gavage every morning (8 AM) until 6 months of age. Each offspring was labeled, weighed and measured weekly. They had free access to chow during the study.

At 6 months of age offspring were deeply anesthetized by intraperitoneal administration of 50 mg/kg sodium pentobarbital, and blood samples were rapidly obtained by cardiac puncture and then centrifuged (120× *g* for 15 min) at room temperature to obtain plasma for insulin and leptin radioimmunoassays (plasma was stored −80°C until assay). The thoracic aorta were isolated and put in the fixative for 48 h at room temperature (freshly prepared formaldehyde 1.27 mol/L in 0.1 mol/L phosphate-buffered, pH 7.2). Aorta rings of 5

mm in length were embedded in Paraplast Plus (Sigma-Aldrich Co., St. Louis, USA), sectioned (3 μm), and stained by Masson's trichrome and orcinol-new fuchsin (*16*) in order to identify elastic fibers and smooth muscle cell (SMC) nuclei. Moreover, fat deposits (retroperitoneal fat and epididymal fat masses) were completely removed on both sides of the animal and weighed.

Body Mass and Blood Pressure

Body mass (BM) was measured every week throughout the experiment from 3 to 6 months of age (Thursday, 8 AM). Systolic BP was measured weekly in conscious rats through the non-invasive method of tail-cuff plethysmography (Letica LE 5100; Panlab, Barcelona, Spain) from the age of 3 to 6 months. Animals went through a 2-week period of adaptation before the beginning of the measurement of BP. Animals were manipulated by one person and were kept in a calm and silent room. Consequently, no restraint was applied to them and stress was minimized as no significant increment in heart rate was observed during the procedure. They were also slightly warmed to dilate the caudal artery and make the tail-pulse easier to recognize. The BP value was taken as the average of three recordings for each animal at each measurement period.

Blood Glucose

An oral glucose tolerance test (OGTT) was performed monthly from the age of 3 (before treatment) to 6 months. After a 6-h fast (from 7:00 AM to 1:00 PM), blood samples were collected from the cut tip of the tail of the animals immediately before and 30 min after glucose overload (0.5 g glucose/kg BM, by gavage). The fasting glucose (FG) measurement made during euthanasia was used to determine the insulin/glucose (I/G) ratio. The blood samples were collected using a glucometer according to the manufacturer's recommendations (Roche, Sao Paulo, Brazil).

Radioimmunoassay for Leptin and Insulin

Plasma leptin concentrations were measured by radioimmunoassay using a rat leptin RIA kit (Cat. RL-83K; Linco Research, St. Charles, USA) and plasma insulin concentrations were measured using a rat insulin RIA kit (Cat. RI-13K; Linco Research). All samples were analyzed in a double assay, for which the intraassay coefficient of variation was 4.2% for leptin and 1.4% for insulin. The I/G ratio was also analyzed in order to investigate insulin resistance.

Morphometry

Non-consecutive cross-sections perpendicular to the artery axis and stained with Masson's trichrome were used. Five random digital images per animal were acquired (TIFF format, 36-bit color, 1,280 × 1,024 pixels) with an LC Evolution camera and Olympus BX51 microscope, and analyzed with the Image-Pro Plus software package, version 5.0 (Media Cybernetics; Silver Springs, USA). To estimate the tunica intima and tunica media thickness (IMT), four measures per image were obtained at 0, 90, 180 and 270°. The lumen area (a) was estimated by drawing a line over the circle delimited by the inner face of the intima layer. The lumen diameter (d) was calculated as $d = 2\sqrt{a/\pi}$, a expressed in mm^2. The mean cross-sectional area of the intima plus media (intima-media area: IMA) was estimated as $[\pi (d/2 + \text{IMT})^2] - [\pi (d/2)^2]$. All measurements were corrected for tissue shrinkage due to fixation and further processing by multiplying by 1.28 (the value previously determined in a pilot study).

Elastic Fibers

Digital images of the stained aortic slices were obtained using the same equipment described previously. The density threshold tool was used to select the area of the aortic wall having positively stained elastic fibers (orcinol new-fuchsin), which was then expressed as a percentage of the total wall area. These measures were obtained from five aortic non-consecutive sections per animal. The total amount of elastic fibers in an aortic cross-section was calculated as the product of the IMA and the percentage area stained with orcinol, expressed in mm^2.

Circumferential Wall Tension and Tensile Stress

Circumferential wall tension (CWT) was calculated by Laplace's law as MSBP × (d/2) expressed in dyn/cm (where MSBP is the mean systolic BP in dyn/cm^2 and d is the lumen diameter in cm). Tensile stress (TS) was computed as CWT/IMT and expressed in dyn/cm^2 (*17*).

Stereology and Data Analysis

The numerical density per area (Q_A) of smooth muscle cell nuclei (SMCN) was estimated as the ratio between the number of SMCN profiles counted in the test frame and the test frame area (A_T).

$$Q_A[\text{smcn}] = \sum \text{SMCN profiles}/A_T$$

We used the vertical section methodology and the cycloid arcs test-system for data acquisition of tunica media elastic lamellae. The density of the surface of elastic lamellae (Sv[lamellae]) was estimated as the ratio between the intersections of the lamellae with the cycloid arcs (I) and the total length of the test line (L_T) (*18*).

$$\text{Sv[lamellae]} = 2I/L_T$$

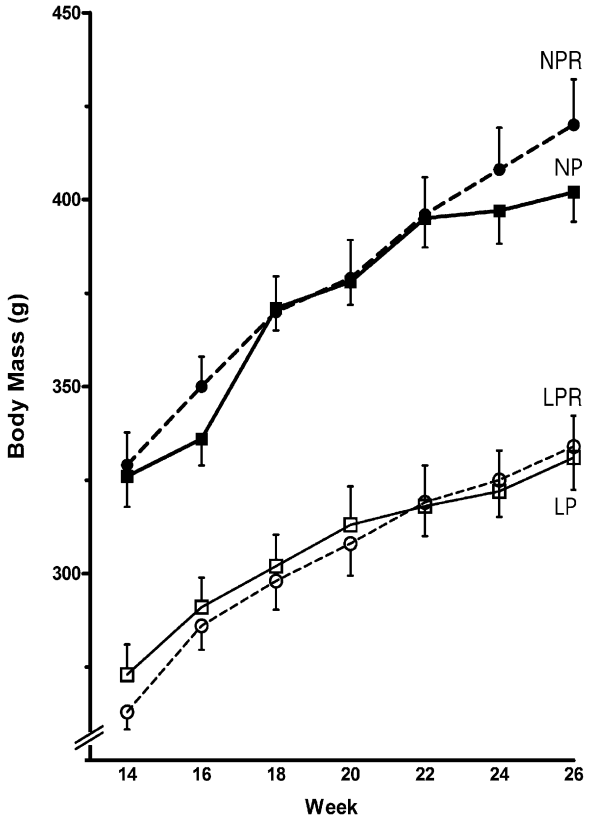

Fig. 1. *Body mass evolution during rosiglitazone administration (LP, low protein; NP, normal protein; R, treated with rosiglitazone). Both the LP groups (treated and untreated) showed significant reduction of body mass compared to the NP groups (treated and untreated). Rosiglitazone administration did not change this evolution of body mass.*

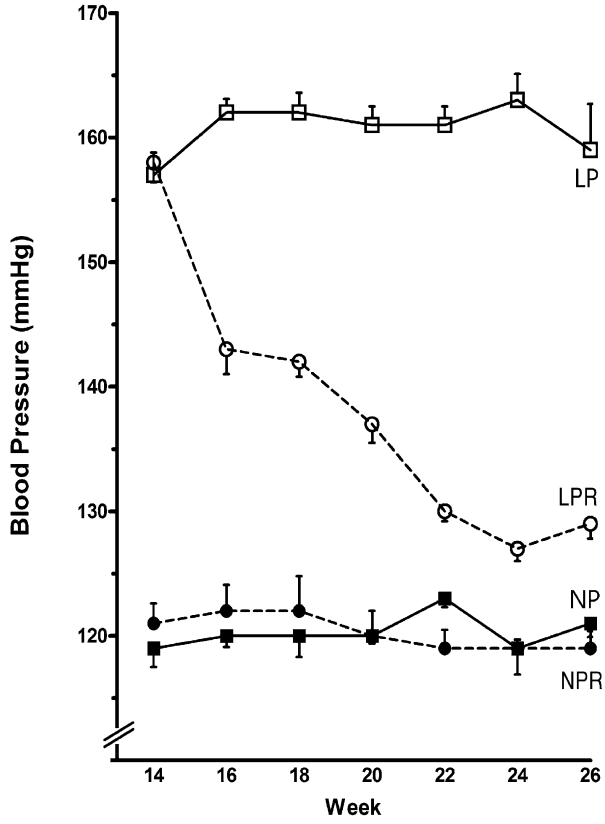

Fig. 2. *Systolic blood pressure evolution during rosiglitazone administration (LP, low protein; NP, normal protein; R, treated with rosiglitazone). The 14-week-old LP offspring showed elevated blood pressure compared to the NP group of the same age. After the beginning of rosiglitazone administration, blood pressure decreased rapidly, and at the end of the experimental period the BP value was the same as in the NP groups (treated and untreated).*

Immunohistochemistry

Sections (5 μm) of the aortic rings were incubated with antisera against angiotensin II (Ang II) receptor type 1 (AT1R, code sc-579; Santa Cruz Biotechnology, Santa Cruz, USA) and endothelial nitric oxide synthase (eNOS, code N9532; Sigma, St. Louis, USA) at 4°C overnight. The sections were rinsed with phosphate-buffered saline. A biotinylated antibody (K0679, Universal DakoCytomation LSAB + Peroxidase Kit; DakoCytomation, Glostrup, Denmark) was used as a secondary antibody detected by reaction with horseradish peroxidase–streptavidin–biotin complex. Positive immunoreaction was identified after incubation with 3,3′-diaminobenzidine tetrachloride (K3466, DAB; DakoCytomation) and counterstaining with Mayer hematoxylin. AT1R and eNOS expression were classified by an investigator using a semiquantitative scoring system (+, weak expression; ++, moderate expression; +++, strong expression).

After the confirmation of the normality and homocedasticity of variances, the differences among the groups were tested by one-way ANOVA and Tukey's post hoc test (pairwise design, honest significant difference for unequal "*n*"). In addition, the interaction between maternal protein restriction and rosiglitazone treatment was analyzed by a two-way ANOVA (Statistica version 7; Statsoft, Tulsa, USA). A *p*-value of 0.05 was considered statistically significant.

Results

Results are shown as the mean and standard error of the mean.

Body Mass

Animals from the maternal low protein protocol showed a significant reduction of BM (Fig. 1). Before rosiglitazone treatment (at 3 months of age) they showed BM 20% smaller than NP animals ($p<0.01$, one-way ANOVA), a difference that continued until the end of the experiment (6 months of

Table 2. Serum Concentrations of Last Fasting Glucose, Oral Glucose Tolerance Test (OGTT), Fasting Insulin, Insulin/Glucose (I/G) Ratio, and Leptin/Body Mass Index (BMI) Ratio

Measurements	Group			
	NP	LP	NPR	LPR
Fasting glucose (mmol/L)	4.8±0.1	4.7±0.1	4.9±0.1	4.8±0.2
OGTT (mmol/L)	5.6±0.2	6.2±0.1	5.8±0.3	5.7±0.3
Fasting insulin (pmol/L)	180.8±42.1	116.7±38.3†	192.5±43.5	116.7±25.4‡
I/G ratio (mol/mol)	45.7±5.2	27.6±4.7†	39.3±5.6	23.0±3.1‡
Leptin/BMI ratio (ng/mL)	99.0±20.4	100.5±5.2	91.6±5.3	79.9±7.5

Groups: LP, low protein; NP, normal protein; R, treated with rosiglitazone. Values are given as mean±SEM. †$p<0.05$ *vs*. NP; ‡$p<0.05$ *vs*. NPR.

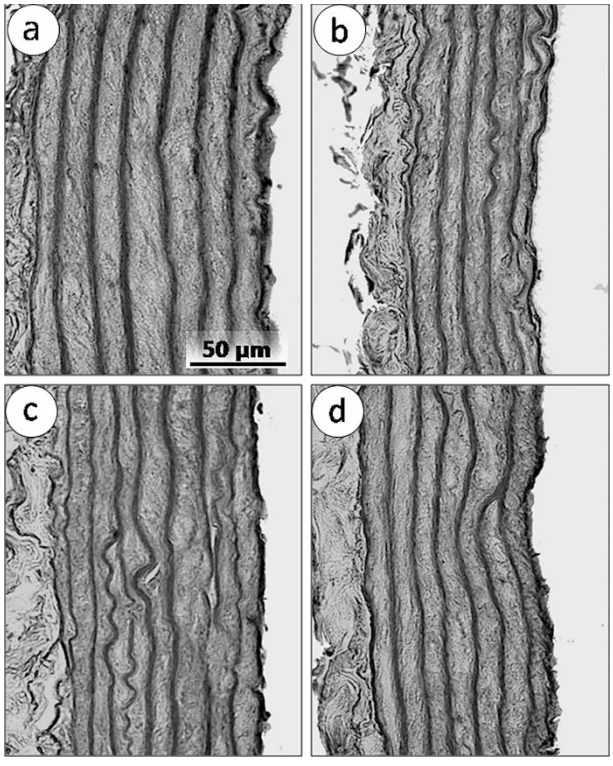

Fig. 3. *Photomicrographs of the thoracic aortic wall stained to identify elastic fibers by orcinol new-fuchsin (the same magnification is used in all photomicrographs): a: NP group, b: NPR group, c: LP group, d: LPR group. The aortic tunica media in LP offspring clearly showed reduced content of elastic fibers. Rosiglitazone administration did not change the level of elastic fibers (see quantification in Fig. 4).*

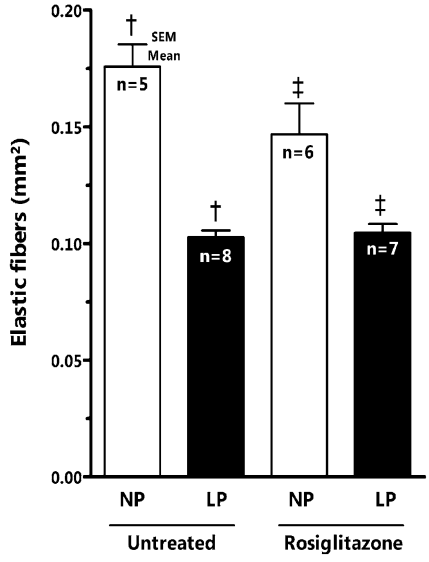

Fig. 4. *Aortic tunica media elastic fiber content (LP, low protein; NP, normal protein). Same symbol above the bars indicates significant difference between the groups ($p < 0.05$, one way ANOVA).*

age). The administration of rosiglitazone did not alter the BM evolution in either LP or NP offspring.

Blood Pressure

LP offspring showed significantly higher BP levels than NP offspring (Fig. 2). During all periods of the BP measurement, from 3 to 6 months of age, the BP levels were more than 30% higher in LP offspring than in NP offspring ($p<0.01$, one-way ANOVA). Rosiglitazone administration to NP offspring did not cause any change in BP. However, when rosiglitazone was administered to LP offspring, BP was immediately reduced in the first week of treatment, and this reduction continued until the end of the treatment ($p<0.01$, one-way ANOVA). From 3 to 6 months of age, rosiglitazone reduced BP in LP offspring by 20% (from 158.0±1.6 mmHg to 126.0±1.2 mmHg, $p<0.01$, paired *t*-test). At 6 months of age, LPR offspring did not show any difference compared to NP and NPR offspring. The protein content in the diet and the treatment interacted on BP levels ($p<0.0001$, two-way ANOVA).

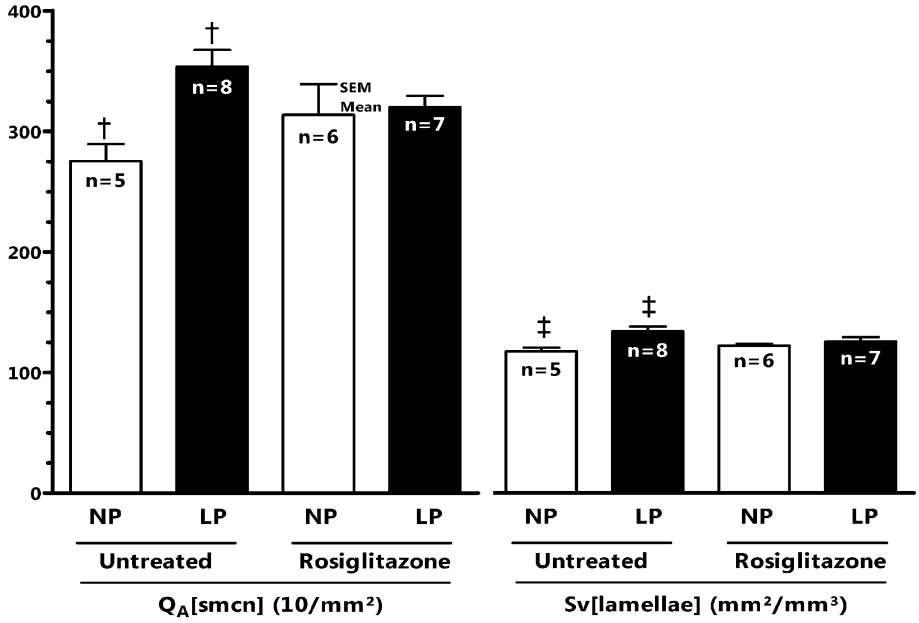

Fig. 5. *Aortic tunica media stereology. Q_A[smcn] is the numerical density per area of the smooth muscle cell nuclei, Sv[lamellae] is the surface density of the elastic lamellae. LP, low protein; NP, normal protein. Same symbol above the bars indicates significant difference between the groups ($p<0.05$, one way ANOVA).*

Carbohydrate and Fat Metabolism

NP and LP offspring did not show a significant alteration in fasting glucose or OGTT between before (data not shown) and after treatment with rosiglitazone (Table 2); the values were within the normal range for Wistar rats. An increased insulin sensitivity was observed in LP offspring. There was a 40% difference in I/G ratio between the groups, or 45.7±5.2 mol/mol in NP offspring and 27.6±4.7 mol/mol in LP offspring ($p<0.01$, one-way ANOVA). The rosiglitazone administration did not alter this finding: there was still a 40% difference between groups, or 39.3±5.6 mol/mol in NPR offspring and 23.0±3.1 mol/mol in LPR offspring ($p<0.01$, one-way ANOVA). No significant difference was found between NP and NPR offspring or between LP and LPR offspring.

The ratio between serum leptin and BM index (Lep/BM index ratio) was used to evaluate the availability of leptin, which regulates energy stores in the peripheral adipose tissue. The Lep/BM index ratio was not significantly different among the groups (Table 2), indicating that rosiglitazone was not responsible for changes in the food intake or fat deposits of the animals.

Arterial Wall Remodeling

Administration of a low protein diet to dams altered the elastogenesis of LP and LPR offspring, as evidenced by the much smaller amounts of elastic fibers in the aortic walls of those animals compared to NP and NPR offspring (−40%, $p<0.01$, one-way ANOVA). Rosiglitazone did not alter the structure of the elastic fibers in the LPR and NPR offspring (Figs. 3 and 4). The density of SMCN and the surface density of elastic lamellae were both greater in LP offspring than in NP offspring (Q_A[smcn] +20%, $p<0.01$, one-way ANOVA; Sv[lamellae] +15%, $p=0.02$, one-way ANOVA). There were no significant differences between LPR and NPR offspring (Fig. 5).

Circumferential Wall Tension and Tensile Stress

LP offspring showed the highest CWT and TS, indicating an increase of blood flow load in the aorta of those animals. Rosiglitazone administration reduced both the CWT and the TS in LPR offspring (CWT was 30% lower in LPR than in LP offspring, $p<0.01$, and was 15% lower in LPR than NPR offspring, $p<0.05$; TS was 35% lower in LPR than in LP offspring, $p<0.01$; one-way ANOVA) (Fig. 6). ANOVA disclosed a significant two-factor interaction between protein content in the diet and rosiglitazone treatment ($p<0.001$ for CWT and $p<0.00001$ for TS, two-way ANOVA), indicating that administration of a low protein diet to dams worsened the hemodynamic conditions in offspring, and this effect was minimized by rosiglitazone treatment.

AT1R and eNOS Immunostaining

In the aortic wall, the immunostaining of both AT1R (Fig. 7) and eNOS (Fig. 8) was positive. The AT1R was positive in

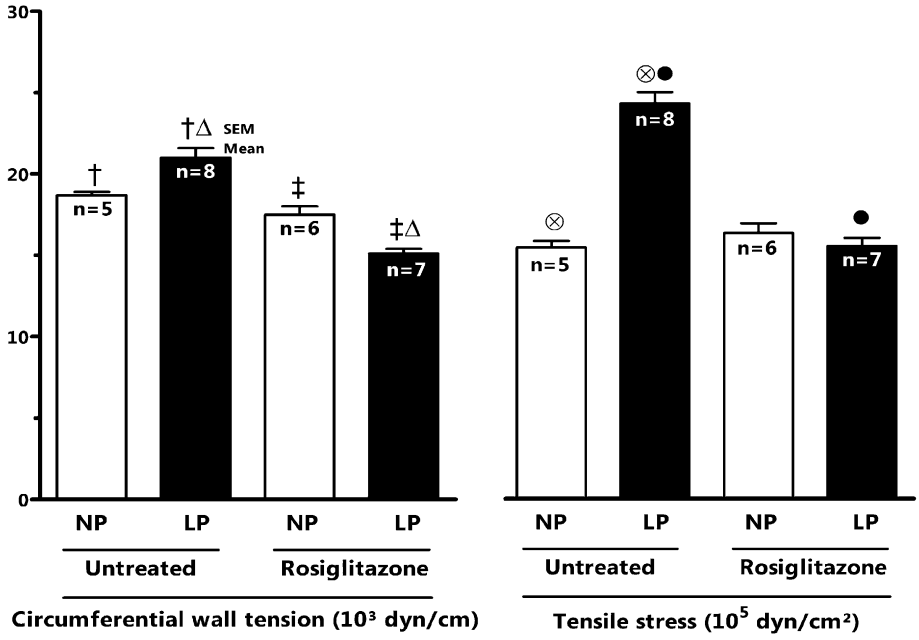

Fig. 6. *Aortic wall tension stress parameters. LP, low protein; NP, normal protein. Same symbol above the bars indicates significant difference between the groups ($p < 0.05$, one way ANOVA).*

the smooth muscle cell layer, while the eNOS was positive in the endothelial layer of all groups. The semiquantitative estimates of both AT1R and eNOS immunostaining are shown in Table 3.

Discussion

In this study, we programmed rat offspring to express chronic diseases in adult life by severe maternal protein restriction, a model that has been well documented in the literature (*19–21*). Consequently, 6-month-old offspring from undernourished dams showed high BP and structural alteration of the aortic wall, which agreed with previous reports (*22*, *23*). Moreover, they also showed increased insulin sensitivity with no alteration of either the fat mass or the blood leptin levels. Rosiglitazone administration did not alter the insulin sensitivity, the fat mass or the blood leptin of the programmed offspring, but reduced their BP and ameliorated their aortic wall structural remodeling. In spite of previous studies indicating rosiglitazone-induced body weight gain, the present results are in accordance with a recent report in which fat mass and fat mass reduction were unaltered in rosiglitazone-treated subjects (*10*).

Rosiglitazone is currently at the center of a debate, since a recent report announced a major risk of death from cardiovascular causes due to rosiglitazone administration (*24*). However, the analyzed data were considered insufficient to determine whether the drug was associated with an increase in the risk of myocardial infarction (*25*, *26*). Furthermore, rosiglitazone has been reported to show cardioprotective effects against myocardial ischemia-reperfusion injury due to an improvement in cardiac insulin sensitivity (*27*), or independently of its insulin-sensitizing properties, because is associated with significant over-expression of Ang II type 2 receptors (*28*), and with the synthesis of nitric oxide (*29*).

We know that the elastin network plays a major role in the mechanical adaptation of the arterial wall in hypertensive rats, not through variations of its total amount but through variations of the extent of anchorage to the muscle cells (*30*). This agrees with the present study, because while rosiglitazone ameliorated the aortic adaptation to tension stress, it did not change the elastic fiber content in the aortic tunica media. Moreover, the present findings showed a significant reduction of the elastic fiber content in the aortic tunica media of 6-month-old programmed offspring. Fetal under-nutrition in rats induced *via* a maternal low-protein diet caused a decrease in aortic wall thickness and elastin content without altering aortic dilator function. These changes in vascular structure may amplify aging-related changes to the vasculature and contribute to the pathophysiology of the putative link between impaired fetal growth and adult cardiovascular disease (*31*).

Changes in large-artery structure associated with hypertension and aging take considerable time to regress after blood pressure is lowered, and this may explain why, despite treatment, wall stress remains elevated (*32*). Elevated blood pressure induces a simultaneous growth adaptation in every component structure of the media of the thoracic aorta, leading to a disproportionate accumulation of scleroproteins (neosynthesized fiber proteins) that markedly exceeds that of the contractile component of the vessel wall. At a cellular level,

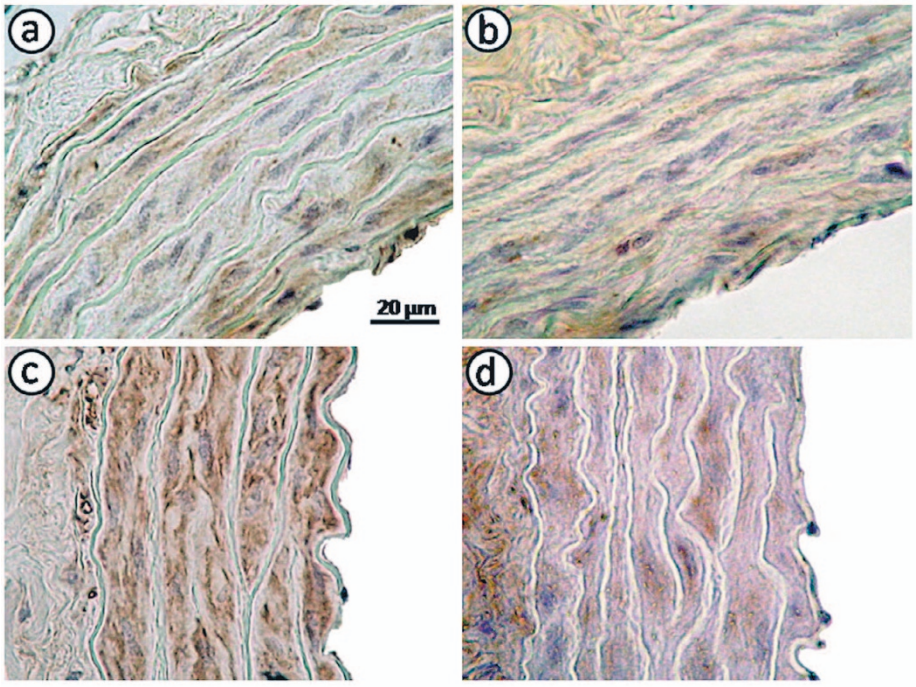

Fig. 7. *The angiotensin II type 1 receptor was positively expressed in the aortic wall (smooth muscle in tunica media). a: NP group, b: NPR group, c: LP group, d: LPR group. The intensity of expression was in the order of (from most to least intense) LP group > LPR group > NP = NPR groups (see Table 3).*

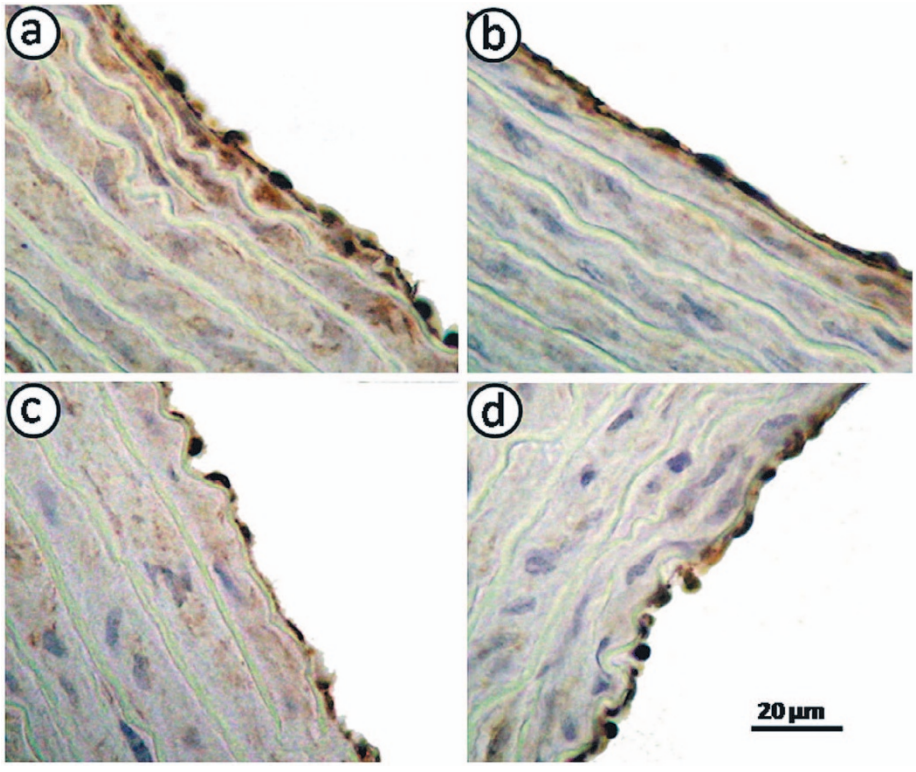

Fig. 8. *The eNOS was positively expressed in the aortic wall (endothelial layer in tunica intima). a: NP group, b: NPR group, c: LP group, d: LPR group. The intensity of expression was in the order of (from most to least intense) NP group = NPR group > LPR group > LP group (see Table 3).*

Table 3. Semiquantitative Estimate Scores of the Aortic Wall Immunostaining

Group	AT1R (tunica media)	eNOS (tunica intima)
NP	+	+++
NPR	+	+++
LP	+++	+
LPR	++	++

+, weak expression; ++, moderate expression; +++, strong expression). Groups: LP, low protein; NP, normal protein; R, treated with rosiglitazone. AT1R, angiotensin II receptor type 1; eNOS, endothelial nitric oxide synthase.

smooth muscle cell hypertrophy is the prevailing process that underlies the tissue response of the aorta in early hypertension (*5*). We investigated the association of tunica media smooth muscle cells and elastic lamellae with stereology, which is a less time-consuming and highly reproducible method to quantify vascular structures in all models of wall thickening (*33*). Rosiglitazone had beneficial effects on the relative growth of the smooth muscle and the elastic lamellae in the aortic tunica media of 6-month-old programmed offspring.

The renin-angiotensin system (RAS) is involved in the mechanism of BP elevation in this experimental animal model (*34*) and AT1R is highly expressed in offspring from protein-restricted fed dams with consequent vasoconstriction in these offspring (developmental model programming for hypertension) (*35*). Our findings in the aortic wall also demonstrated high expression of AT1R in LP offspring. The administration of rosiglitazone to LP offspring (LPR group) had the beneficial effect of diminishing AT1R expression to a level similar to that in NP offspring. Therefore, we can speculate that rosiglitazone contributed to the observed reduction in vasoconstriction in the LP offspring and that this result was mediated by RAS.

Multiple growth promoting signaling pathways, as well as vascular remodeling, are activated by Ang II through AT1R, such as phosphatidylinositol 3-kinase (PI3K/p85α) and extracellular signal–regulated kinase (ERK) 1/2. The PPARγ activator rosiglitazone negatively modulates theses pathways and restores AT1R expression to basal levels in the aorta (*36*, *37*). Some studies have concluded that these effects are independent of the insulin-sensitizing properties of rosiglitazone (*38*, *39*). We know that subcutaneous adipose tissue is a significant source of Ang II and that rosiglitazone downregulates the RAS in subcutaneous adipose tissue, contributing to the long-term effect of rosiglitazone on BP (*40*).

NO is an endogenous vasodilator, an inhibitor of vascular smooth muscle growth (*41*), and an anti-apoptotic agent (*42*). Intrauterine under-nutrition induces hypertension and alters endothelium-dependent responses in the aorta of the resulting offspring, thereby leading to endothelial dysfunction associated with a decrease in eNOS activity and expression (*43*, *44*). Rosiglitazone stimulates endothelial cell NO release (*45*) *via* an effect that may be dependent on phosphorylation of eNOS (*29*). The present findings showed lower expression of eNOS in offspring from protein-restricted dams (LP group). The administration of rosiglitazone to these offspring (LPR group) was effective for increasing the eNOS expression and reducing BP.

Modulations in Ang II–induced growth (*37*), endothelial function, oxidative stress and vascular inflammation (*46*, *47*) are involved in the regression of vascular remodeling in hypertensive animals treated by PPARγ activators (*48*). PPAR activators also play a role in decreasing endothelin-1 production (*49*). In Ang II–stimulated vascular smooth muscle cells, rosiglitazone causes an antiproliferative–anti-apoptotic effect and reduces extracellular matrix production, suggesting a role of PPARγ activators in preventing Ang II–induced vascular fibrosis (*50*). All these effects of rosiglitazone and other PPARγ activators likely played a role in reducing both the aortic circumferential wall tension and the tensile stress in treated offspring from protein-restricted dams in the present study.

In rat offspring, protein restriction during gestation and the first part of the lactation period led to hypertension and increased insulin sensitivity in adulthood. These effects in turn caused adverse aortic wall remodeling characterized by a reduction in the elastic fiber content and hypertrophy of the tunica media smooth muscle and elastic lamellae, and finally in increased wall tension and tensile stress. Rosiglitazone administration had beneficial effects on these changes, reducing the BP and the aortic tunica media hypertrophy, and ultimately balancing the wall stress in treated programmed offspring.

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