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Rosiglitazone increases extravasation of macromolecules and endothelial nitric oxide synthase in skeletal muscles of the fructose-fed rat model

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Abstract

Reduced extravasation of macromolecules in skeletal muscle has recently been documented in the fructose-fed rat model, corroborating a hypothesis that a functional obliteration of muscle regional microcirculation might lead to hypertension and restrict access of nutrients and hormones to their target cells. The goal of this study was to assess the impact of a treatment with rosiglitazone on the reduced muscle vasopermeability observed previously in the fructose-fed rat model. Fructose-fed Sprague–Dawley rats were gavaged with rosiglitazone (10 μmol kg^{-1} per day; $n = 21$) or the vehicle only ($n = 19$) for 3 consecutive weeks before assessing the extravasation of Evans Blue (EB) dye in vivo in distinct muscle groups. Relative to control group, rosiglitazone reduced mean arterial blood pressure ($\Delta = -16.7\%$, $P < 0.001$), plasma insulin ($\Delta = -39.1\%$, $P < 0.05$) and plasma triglyceride ($\Delta = -32.8\%$, $P < 0.01$) concentrations in a significant manner. Plasma VEGF concentrations were significantly lower in the rosiglitazone-treated animals compared to the control animals (32.7 ± 0.8 pg ml^{-1} versus 46.1 ± 1.2 pg ml^{-1}, $P < 0.001$). While no changes were observed in the lungs or the kidneys, fructose-fed rats treated with rosiglitazone had a 30–50% increase ($P < 0.005$) in the extravasation of EB regardless of the skeletal muscle group studied (rectus femoris, soleus, gastrocnemius lateralis, vastus lateralis and tibialis cranalis). In homogenates of skeletal muscles (vastus lateralis) of fructose-fed rats, rosiglitazone resulted in a significant increase in NO synthase (NOS) activity ($\Delta = +41.9\%$, $P < 0.003$) as well as endothelial NOS immunoreactive mass ($\Delta = +37.8\%$, $P < 0.01$) compared to the control animals. There was no change in the immunoreactive level of the *n*NOS isoform, the most abundant muscle isoform, or in the immunoreactive levels of VEGF.

In conclusion, rosiglitazone appears to restore a vascular dysfunction previously documented in the skeletal muscle microcirculation, as evidenced by improved skeletal muscle vasopermeability and upregulation of the muscle endothelium-NO system in the fructose-fed rat model. These effects on muscle per se might also result in a partial improvement of the insulin resistance phenomenon by improving the distribution of nutrients and insulin to skeletal muscle. This effect appears to be independent of circulating levels of VEGF since changes in plasma concentrations of this permeability factor were lower in the rosiglitazone-treated group.

Keywords: Thiazolidinediones; Rosiglitazone; Insulin resistance; Nitric oxide synthase; Fructose-fed rat model; Skeletal muscle; Vascular permeability; Microcirculation

1. Introduction

Insulin resistance is a complex entity associated with hypertension, Type 2 diabetes and dyslipidemia [1–3]. Both because of its close interconnections with these conditions but also its possible direct impact on the vasculature, insulin resistance is considered by many as an important cardiovascular risk factor [4–6]. Despite extensive clinical and experimental research, however, there remain unresolved questions about its real impact on vascular function, both as a factor leading to hypertension [7] or as a phenomenon having an impact on the access of insulin to its target tissues [8,9]. We have recently examined the microvascular permeability of skeletal muscles in the hypertensive and insulin resistant fructose-fed rat and found that there was a marked reduction in capillary permeability in this tissue [10] and that this change was

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associated with a downregulation of the *e*NOS isoform in skeletal muscle [11]. Given the key role of skeletal muscle in glucose uptake [12] and its very rich nutritive and non-nutritive vasculature [13], these data provided evidence, for the first time, that a reduction in capillary permeability could contribute to abnormal internal distribution of fluids in the body, reduced capillary delivery of insulin to target organs and perhaps hypertension.

Several classes of pharmacological agents are currently used to reduce insulin resistance in patients [14]. While these agents are predominantly used in patients for their glucose lowering effect in the fasting or the postprandial state, there is evidence that thiazolidenediones also have additive and singular impacts on systemic blood pressure [15] and urinary albumin excretion rate [16], suggesting a direct or indirect effect of PPARγ agonists on vascular tone and/or peripheral vascular resistance. If a defect of skeletal muscle capillary permeability has relevance to insulin resistance and/or Type 2 diabetes, anti-hyperglycemic agents such as thiazolidinediones could have a significant impact of the regulation of the skeletal muscle microvasculature.

The present study was undertaken to verify this hypothesis by investigating the effect of a 3-week oral treatment with rosiglitazone on the skeletal muscle capillary permeability in a fructose-fed rat model of insulin resistance. Since regulation of skeletal muscle microvasculature is dependent upon an adequate generation of nitric oxide (NO) by the endothelium [17,18], effects of rosiglitazone on the local skeletal muscle nitric oxide synthase (NOS) system were also examined. Plasma and tissue levels of vascular endothelial growth factor (VEGF) were also measured in these experiments because TZDs have been associated with an increase expression of VEGF possibly resulting in changes in microcirculation as well as fluid retention [19–23].

2. Material and methods

2.1. Materials

[^{3}H]-L-arginine was obtained from Amersham (Oakville, Ont., Canada). Primary antibodies for the Western blotting experiments were purchased from the following manufacturers: monoclonal anti-*e*NOS (BD Transduction Laboratories, Lexington, KY, USA), polyclonal anti-*n*NOS (BD Transduction Laboratories), polyclonal anti-*i*NOS (Oxford Biomedical Research, Oxford, MI), polyclonal anti-VEGF 147 (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Silica LK6D (0.25 mm × 20 cm × 20 cm) Whatman adsorption plates were purchased from Fisher Scientific (Nepean, Ont., Canada) as were all solvents used from chromatography. The immunoblotting membranes and the enhanced chemiluminescence (ECL) Western blotting detection system were obtained from Roche (Indianapolis, IN, USA). All other reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Animals and experimental protocol

Thirty male Sprague–Dawley rats (Charles River Breeding Laboratories, St-Constant, Que., Canada), initially weighing between 350 and 400 g, were used for these experiments. The animals were housed in temperature and humidity controlled conditions and were acclimated with a standard rat chow (Ralston Purina Canada, Woodstock, Ont., Canada), containing 53% vegetable starch, 4.5% fat, 22% protein, 0.36% sodium, and 1.08% potassium for 4 days prior to feeding them with a 60% fructose enriched diet (Harlan Teklad, Madison, WI, USA) for a total of 4 weeks. The fructose-fed animals were randomised to two experimental groups. The first group was fed with fructose for 4 weeks and was given rosiglitazone (10 μmol kg^{-1} per day) diluted in water once daily by gavage in the last 3 weeks of this dietary manipulation. The control group was similarly fed with fructose for 4 weeks but was gavaged with the water vehicle only in the last 3 weeks.

2.3. Blood pressure measurement

The tail cuff method was used to measure systolic blood pressure in the unanesthetised state. Ambient temperature was maintained at 30 °C. The equipment used included magnetic animal holders connected with a manual scanner, a pulse amplifier and a dual channel recorder. The mean of four consecutive readings was used as the measurement of blood pressure in each animal.

2.4. Measurement of capillary permeability

The Evans Blue (EB) dye technique was used to measure capillary permeability to albumin in various leg muscle groups from unanesthetised control and experimental rats. This technique is based on the principle that EB dye avidly binds to the negatively charged intravascular albumin and, thus, it is a reliable way of assessing transvascular fluxes of macromolecules. This technique has been described previously [10,11,24–26] and is a reliable estimate of the extravasation and interstitial accumulation of albumin [27]. Briefly, rats were injected in the caudal vein with EB (20 mg kg^{-1}). The EB dye was allowed to circulate for 10 min before sacrifing the experimental animals. The thorax was cut and the lungs perfused with 15 ml of Krebs solutions (10 ml min^{-1}) via the pulmonary artery to remove any intravascular dye that would interfere with the amount of EB that has extravasated in lungs. Several skeletal muscles, as well as lungs and kidneys were obtained to assess degree of extravasation of macromolecules in these various tissues. For skeletal muscle specifically, rectus femoris, soleus, gastrocnemius lateralis, vastus lateralis and tibialis cranalis were sampled in this

protocol because of their different content in type I or type II fibres; the rectus femoris having the highest proportion of type II (fast-twitch, glycolytic or white) fibres (96%), and the soleus the highest proportion of type I (slow-twitch, oxidative or red) fibres (84%) [28]. Tissues were weighed immediately. A third of each tissue sample was dried at 60 °C for 24 h, and a dry/wet weight ratio was calculated to avoid underestimation of EB concentration due to local edema. The other 2/3 was put in a formamide solution (4 ml g^{-1} wet tissue) for 24 h for dye extraction. The extracted amount of EB dye was determined by spectrophotometry at 620 nm using a μQuant (Bio-Tek Instruments Inc., Winooski, VT, USA) and 96-well microplates. Concentration of EB in each tissue was expressed in μg g^{-1} of dry tissue to avoid underestimation due to tissue edema. Complete and tight EB binding of serum albumin, extracellular equilibration of the marker, as well as entire extraction of the dye by formamide were all validated in our own [10,24], and other laboratories [29]. Results were calculated from a standard curve of EB (0.5–25 μg ml^{-1}) and expressed as EB μg g^{-1} of dry weight of tissue.

2.5. Preparation of muscle tissue extracts

Vastus lateralis from the contralateral leg of each animal were rapidly frozen in liquid nitrogen and stored at −70 °C until assayed. This particular muscle group was chosen because of its size, its facility of isolation and its general and common content in type I (2%) and type II (98%) fibres [28]. Muscles were homogenised on ice in a buffer containing 25 mM Tris–HCl, 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride and 5 μl ml^{-1} of a complementary anti-protease cocktail (Protease Inhibitor Cocktail P-8340, Sigma). The homogenate was centrifuged at 500 × *g* for 10 min at 4 °C. The infranatant of this homogenate was used for NOS activity as well as Western blots. Protein content was determined using the Pierce BCA Protein Assay (Pierce, Rockford, IL, USA).

2.6. Nitric oxide synthase activity assay

Nitric oxide synthase activity was quantified by the conversion of [^{3}H]-L-arginine to [^{3}H]-L-citrulline, with minor modifications [30]. Briefly, aliquots of the homogenates (200 μg proteins) were incubated in 50 mM HEPES (pH 7.4) with 20 nM [^{3}H]-L-arginine (0.1 μCi per tube), 24 μM cold L-arginine, 120 μM NADPH, 60 mM L-valine, 12 mM L-citrulline, 1.2 mM $MgCl_2$, 0.2 mM $CaCl_2$, 10 μg ml^{-1} calmodulin, 3 μM BH_4, 1 μM flavin adenine dinucleotide (FAD) and 1 μM flavin mononucleotide (FMN). The reaction was carried out for 1 h at 37 °C with or without L-NAME (2 mM) and terminated by adding 2 ml of ice-cold buffer (20 mM HEPES pH 5.5, 1 mM EGTA). [^{3}H]-L-citrulline was separated from [^{3}H]-L-arginine by TLC plates using a solvent system of chloroform–methanol–water–ammonium hydroxide (1:9:2:4 v/v/v/v) for 1 h. The plates were developed using ninhydrine and the citrulline band of each sample was scraped from the plate and counted by liquid scintillation. The NOS-dependent citrulline formation is expressed as pmol min^{-1} mg $protein^{-1}$ after having subtracted the L-NAME blank for each sample.

2.7. Western blots analyses in skeletal muscles (eNOS, nNOS, iNOS and VEGF)

For immunoblotting, samples (75 μg of protein per lane) were added to SDS buffer (250 mM Tris–HCl, pH 6.8, 2% glycerol, 5% mercaptoethanol), boiled for 5 min, and then separated on 8% SDS–polyacrylamide gel electrophoresis. Subsequently, proteins were transferred to a polyvinyldene difluoride (PVDF) filter membrane overnight (4 °C) and then blocked with a solution containing 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 0.1% Tween 20 and 5% non-fat milk for 1 h. The immunoblotted proteins were incubated then subsequently incubated overnight at 4 °C with primary antibodies. Antibodies were diluted 1:500 for *e*NOS, *i*NOS, VEGF and 1:250 for *n*NOS. The PVDF membranes were washed for 45 min, followed by 1 h incubation with either anti-mouse or anti-rabbit immunoglobulin G conjugated to horseradish peroxidase. After appropriate washing, the immunoreactive bands were visualised by enhanced chemiluminescence and autoradiography. Muscle standards were run on every gel for comparison of samples from different immunoblots. Values are expressed in arbitrary densitometric units relative to the muscle standards run on every gel.

2.8. Biochemical measurements

Blood was collected from the retroorbital sinus in a non-fasting state, under halothane anesthesia, 5 days prior to capillary permeability measurements and sacrifice of the animals. Blood samples were centrifuged, aliquoted, frozen and later assayed for glucose, insulin and triglyceride concentrations. Plasma glucose and triglyceride concentrations were measured enzymatically using colorimetric kits from VWR Canlab (Ville Mont-Royal, Que., Canada). Plasma insulin concentrations were assessed by RIA with a rat insulin specific kit from Linco (St. Charles, MO, USA), using rat insulin as standard. Plasma VEGF was measured using a commercial enzyme-linked immunosorbent assay that has also been validated for rat serum or plasma (Quantikine®, R&D Systems Inc., Minneapolis, MN, USA).

2.9. Statistical analyses

Data are reported as mean values ± S.E.M. Comparisons between the groups were performed using the non-paired Student's *t*-test. *P* values less than 0.05 were considered statistically significant.

Table 1
Animal characteristics and metabolic data at the end of each treatment period

	Control ($n = 19$)	Rosiglitazone ($n = 21$)	P value[a]
Weight (g)	447 ± 7	467 ± 8	<0.05
Weight gain[b] (g)	62 ± 5	84 ± 5	<0.004
Systolic blood pressure (mmHg)	147 ± 3	124 ± 2	<0.001
Diastolic blood pressure (mmHg)	98 ± 2	81 ± 2	<0.001
Plasma glucose (mM)	12.9 ± 0.3	12.1 ± 0.3	ns
Plasma insulin (pM)	780 ± 140	475 ± 46	<0.05
Plasma triglycerides (mM)	3.14 ± 0.33	2.11 ± 0.20	<0.01

Values are represented as means ± S.E.M.
[a] Non-paired Student's t-test.
[b] Weight gain over the entire study period (4 weeks).

3. Results

3.1. Weight and metabolic changes

Table 1 illustrates the impact of 3 weeks of rosiglitazone (versus the vehicle only) in fructose-fed animals on body weight, blood pressure, and plasma glucose, insulin and triglyceride concentrations. Mean weight gain in animals treated with rosiglitazone was significantly greater and more rapid than in the control group ($P < 0.05$). As expected, rosiglitazone reduced mean arterial blood pressure ($P < 0.001$), plasma insulin ($P < 0.05$) and triglyceride ($P < 0.01$) concentrations. Non-fasting plasma glucose concentrations were comparable between the two treatment groups ($P > 0.05$), supporting an impact of rosiglitazone on insulin resistance.

3.2. Plasma VEGF concentrations

A 3-week treatment with rosiglitazone was associated with significant reduction in plasma VEGF levels in this fructose-fed animal model with mean concentration of 46.1 ± 1.2 pg ml^{-1} in the vehicle-treated animals versus 32.7 ± 0.8 pg ml^{-1} in the rosiglitazone-treated group ($\Delta = -29.1\%$, $P < 0.001$).

3.3. Measurements of microvascular permeability

Fig. 1 compares, using the same Y-axis scale, the quantitative extravasation of Evans Blue in samples of rectus femoris and soleus muscles of the rosiglitazone-treated animals compared to the control group. Capillary permeability to Evans Blue in soleus, a muscle with a high content in oxidative or red fibres (type I), was approximately three-fold the one measured in rectus femoris samples; this difference was seen in both the control and the rosiglitazone-treated animal groups ($P < 0.005$). Treatment with rosiglitazone increased the extravasation of Evans Blue in a statistically significant manner in all muscle groups studied in this protocol: +34.7% for the rectus femoris, +34.1% for the soleus, +38.1% for the gastrocnemius lateralis, +49.9% for the tibialis cranialis, and +51.9% for the vastus lateralis (all with $P < 0.005$ compared to the vehicle-treated or control group). There were no statistically significant differences in the relative magnitude of this effect of rosiglitazone in all these distinct muscle groups.

In contrast to the significant increase seen for the extravasation of macromolecules in skeletal muscles, rosiglitazone did not have any impact on Evans Blue permeability in the lungs (110.7 ± 18.7 μg g^{-1} of dry tissue

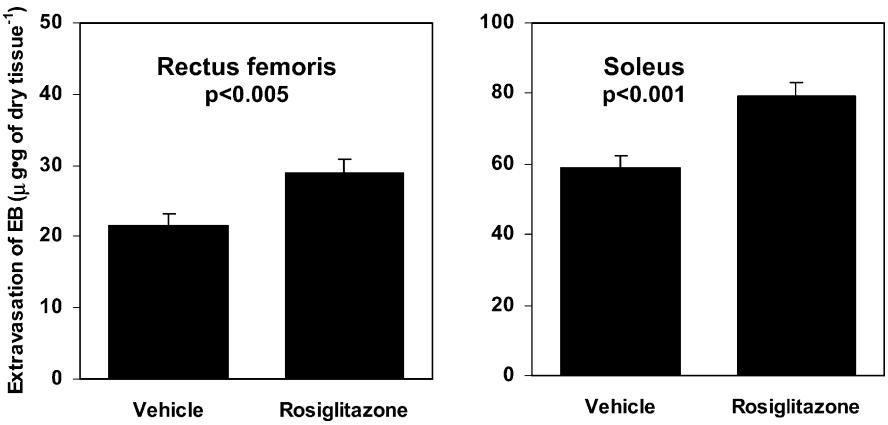

Fig. 1. Extravasation of Evans Blue dye in fructose-fed rats gavaged with the vehicle only or rosiglitazone for a total of 3 consecutive weeks. Data illustrated in this figure shows the effect of rosiglitazone in the rectus femoris (64% type II, 36% type I fibres) and the soleus (16% type II, 84% type I fibres) which are known to have different content in fast-oxidative glycolytic (type II) and fast-oxidative (type I) fibres. Each bar represents the means ± S.E.M. obtained from experiments conducted in groups of 19 and 21 animals, respectively.

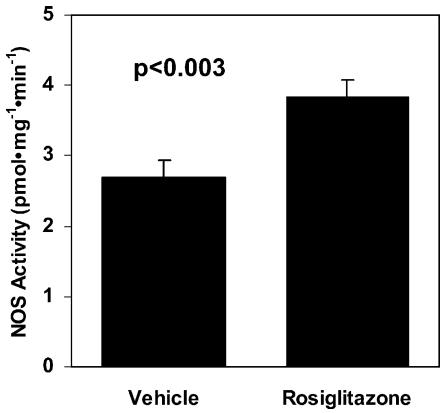

Fig. 2. Effects of fructose feeding on NOS activity in homogenates of vastus lateralis muscles obtained from control ($n = 19$) or rosiglitazone-treated animals ($n = 21$). Rats were fed for 3 weeks with rosiglitazone or the vehicle only. NOS activity was determined using equivalent muscle extracts (200 μg of total tissue proteins) from each animal (see Section 2). Results are means ± S.E.M.

weight in controls versus 117.2 ± 20.0 μg g^{-1} of dry tissue weight with rosiglitazone; P = ns) or in the kidneys (103.4 ± 7.4 μg g^{-1} of dry tissue weight in controls versus 107.8 ± 7.0 μg g^{-1} of dry tissue weight with rosiglitazone; P = ns).

3.4. Measures of nitric oxide synthase activity and immunoreactive mass

Fig. 2 illustrates the effect of rosiglitazone on nitric oxide activity in skeletal muscle homogenates (vastus lateralis) of these fructose-fed animals. Compared to the control group, rosiglitazone increased total tissue nitric oxide activity by 41.9% ($P < 0.003$). To try to ascertain which isoform was upregulated in response to rosiglitazone, we measured the immunoreactive protein mass of *e*NOS, *n*NOS and *i*NOS using specific antibodies. In these Western Blot experiments, there was a significant increase of *e*NOS immunoreactive mass in skeletal muscle homogenates from the rosiglitazone-treated animals compared to the controls ($\Delta = +37.8\%$, $P < 0.01$). These changes in *e*NOS were not paralleled by any significant changes in the *n*NOS immunoreactive mass, the most abundant NOS isoform in skeletal muscle (Fig. 3). The isoform *i*NOS was not detected in muscles sampled from this animal model in any of the treatment groups, even after a prefatory immunoprecipitation with the *i*NOS antibody (data not shown). Incidentally, VEGF immunoreactive mass levels, assessed with the same Western blotting technique in muscle homogenates, did not highlight any significant differences between the two treatment groups (vehicle-treated: 4.66 ± 1.18; rosiglitazone-treated: 5.33 ± 1.12 arbitrary densitometric units relative to a control sample run on each gel; P = ns).

4. Discussion

We have shown in the present study that treatment with rosiglitazone for 3 weeks improved the skeletal muscle vasopermeability abnormalities described previously by our group in the insulin resistant fructose-fed rat model [10,11]. This 30–50% increase in skeletal muscle vasopermeability was statistically significant when compared to control animals gavaged with the vehicle only. These

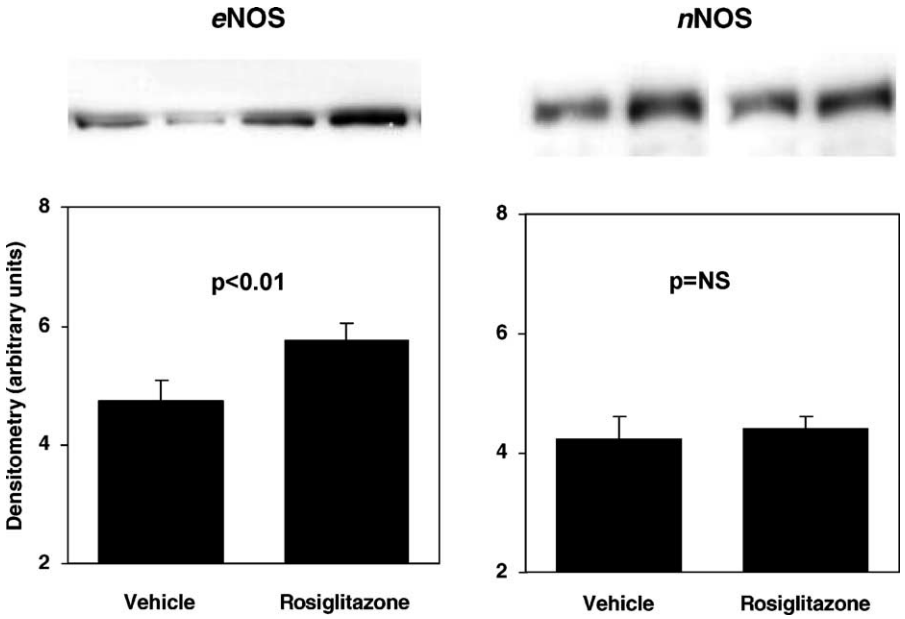

Fig. 3. Representative immunoblots of an experiment where equivalent amounts of muscle protein (75 μg) were resolved on SDS–PAGE and analysed by Western blotting using *e*NOS or *n*NOS specific antibodies. Both protein migrated as a single band of about 140,000 M_r for *e*NOS and about 155,000 M_r for *n*NOS. Results are means ± S.E.M. of muscle homogenates obtained from all the animals in which the NOS activity was assayed. Results are given in arbitrary densitometric units relative to a control sample run in duplicate on each individual gel.

changes were observed concomitantly with a significant reduction in mean arterial blood pressure, plasma insulin and plasma triglyceride concentrations, intimating a clear impact of rosiglitazone on the underlying insulin resistance syndrome of this animal model. Given the fact that thiazolidinediones have been linked to formation of pulmonary edema [31,32], we interestingly did not see any significant changes in the extravasation of Evans Blue in the lung tissue of this insulin resistance animal model.

The method used to assess tissue vasopermeability reflects predominantly the extravasation of macromolecules since it measures the quantity of Evans Blue dye affixed to albumin that is able to leave the muscle vascular compartment. Since skeletal muscle constitutes 80% of total body mass, takes up 20% of total cardiac output and is therefore an important component of the peripheral vascular resistance, a reduction in the functional obliteration of this tissue vascular bed could theoretically result in an improvement in arterial blood pressure and/or total body insulin resistance.

The improvement in skeletal muscle vasopermeability observed with rosiglitazone using the Evans Blue extravasation technique were interestingly accompanied by an increase in NOS activity in total skeletal muscle homogenates. Since this activity can result from an effect on either *e*NOS, *n*NOS or *i*NOS, we have examined the immunoreactive content of all isoforms in skeletal muscles of our experimental animals. *i*NOS could not be detected indicating that the expression of this isoform does not seem to play any role in this nonobese animal model of insulin resistance. In contrast, among *n*NOS and *e*NOS, only the latter isoform showed a significant improvement with rosiglitazone. Thus, the increase in skeletal muscle NOS activity was entirely attributable to change in the expression of *e*NOS, a finding concordant with recent work from our group in this insulin resistance animal model, showing unresponsiveness of the skeletal muscle capillary bed to bradykinin [11]. Because the *e*NOS isoform comes predominantly from the vascular component of skeletal muscle, these findings suggest that rosiglitazone has a clear impact on the skeletal muscle microcirculation. This effect could be mediated by an increase in the capillary surface area (through capillary recruitment) or by changes in the capillary permeability itself, as NO has been shown to impact both [33,34]. Our experiments were not designed to determine whether these changes in *e*NOS and EB extravasation in skeletal muscle were the result of a direct or indirect effect of rosiglitazone. Thus, for example, we cannot discount the possibility that the improvement in *e*NOS and BE extravasation resulted from a reduction in systemic blood pressure. There are, however, reasons to believe that rosiglitazone has a direct effect on skeletal muscle microcirculation. Firstly, PPARγ have been clearly shown to be expressed in endothelial cells [35,36]. Secondly, several groups have shown that thiazolidinediones are able to regulate secretion of vasoactive substances in endothelial [36] as well as in vascular smooth muscle cells [37]. Moreover, Uchida et al. have reported an acute hemodynamic effect of pioglitazone in isolated perfused rat hearts [38]. Finally, a direct vasodilatory effect of troglitazone has recently been demonstrated on the renal microcirculation by Arima et al. [39]. While these studies suggest a direct effect of TZDs on vasculature, our study is unique in that it was undertaken in vivo in whole animals and was focused on a tissue microcirculatory system which have not so far been the object of many experimental studies. Interestingly, no such effect of rosiglitazone was observed in the kidney, a richly vascularised *e*NOS-dependent tissue. Moreover, since the model used in our studies is not hyperglycemic and rosiglitazone did not have a significant impact on plasma glucose concentrations, it can be concluded that rosiglitazone ameliorates skeletal muscle vasopermeability and macromolecule trafficking independent of its glucose-lowering effect. Because plasma concentrations and muscle immunoreactive mass of VEGF were not increased by rosiglitazone, it appears unlikely that this cytokine is implicated in the phenomenon observed in this animal model.

Rosiglitazone improved skeletal muscle vasopermeability in all the individual muscle groups examined in this fructose-fed animal model suggesting that the effect of this medication is not a priori dependent on the content of type I or type II muscle fibres. The hypothesis to be tested was in relation to the possibility that muscles with a higher type I (slow-twitch, oxidative or red) fibre content would benefit more from an improvement in capillary permeability to meet their need for nutrients. While no such impact of rosiglitazone was observed, it is interesting to note that the Evans Blue extravasation in a muscle with a predominant content of type I fibres, such as the soleus, was three-fold that in muscles enriched in type II fibres, such as the rectus femoris. These observations support the view that muscles with a high content of type I fibres require a microcirculation that is more permeable and adaptative than muscles with a predominant content in type II (fast-twitch, glycolytic or white) fibres. It should also be considered that differences in EB extravasation could also be due to differences in microcirculatory density or capillary surface area.

The observation that plasma VEGF concentrations were lower in the rosiglitazone-treated animals compared to controls can a priori be felt as contrary to some data previously published on troglitazone [19–21]. It should however be pointed out that our experimental paradigm was different and that, to our knowledge, our paper is the first one to measure plasma VEGF concentrations in the fructose-fed rat model. However, our data are not completely inconsistent with the literature since thiazolidinediones have been shown to reduce VEGF production in tumour cells [40] and to suppress neovascularisation in the retina [41].

In conclusion, given the important metabolic role of skeletal muscle for insulin-mediated glucose disposal and

peripheral vascular resistance, our findings strengthen the view that skeletal muscle microcirculation is a novel potential target in the pathophysiology of insulin resistance. Our data point to a direct impact of thiazolidinediones on regulation of skeletal muscle vasculature and are compatible with a vasculoprotective effect of those agents on regional vascular bed such as the crucial one composing the skeletal muscle system. Further studies will need to investigate the relevance of these findings to the management of patients with insulin resistance.

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