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Reduction of endothelial NOS and bradykinin-induced extravasation of macromolecules in skeletal muscle of the fructose-fed rat model

Gérard E. Plante[a], Mylène Perreault[b], Alexis Lanthier[a], André Marette[b], Pierre Maheux[a,*]

[a]*Departments of Medicine, Pharmacology and Physiology, Division of Endocrinology and Metabolism, Faculté de Médecine, Université de Sherbrooke, 3001 12ème Avenue Nord, Sherbrooke, Québec, Canada J1H 5N4*

[b]*Department of Physiology and Lipid Research Unit, Université Laval, Laval, Québec, Canada G1V 4G2*

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Abstract

Objective: Reduced capillary permeability of the skeletal muscle vascular bed has been suggested to play a role in fructose-fed rats, corroborating a long held view that insulin resistance might partially be explained by the lack of access of insulin and glucose to its target organs, mainly skeletal muscles. The goal of this study was to explore mechanisms underlying this vascular abnormality, and more specifically the role of bradykinin and nitric oxide (NO) on skeletal muscle microcirculation and the extravasation of macromolecules. **Methods:** For that purpose, Sprague–Dawley rats were fed with either a fructose-enriched (F) or a normal chow (N) diet and extravasation of macromolecules was assessed at 4 weeks by measuring in vivo the extravasation of Evans Blue (EB) dye in the quadriceps muscles of both groups after the intravenous injection of the potent vasodilator bradykinin (150 μg/kg). **Results:** As expected, fructose-fed rats had less extravasation of EB in skeletal muscle in the basal state as compared to controls (F 17.6±4.4 vs. N 43.6±6.9 μg/g dry tissue; $P<0.01$). In response to bradykinin, the EB dye extravasation in skeletal muscle was 89.4% higher in rats fed the normal chow diet compared to the basal state ($P<0.03$). In contrast, no significant increase in vasopermeability was observed in fructose-fed animals acutely injected with BK (17.6±4.4 μg/g in the basal state versus 24.6±3.1 μg/g after the injection of BK; P=NS). To distinguish a functional from an anatomical/structural defect, hematoxylin–eosin sections as well as electron micrographs of skeletal muscle microvessels were examined in both groups of animals: no obvious abnormalities were found. However, in homogenates of skeletal muscles (quadriceps) of fructose-fed rats, there was a marked reduction of NO synthase (NOS) activity (−33.8%; $P<0.001$) as well as endothelial NOS immunoreactive mass (−23.4%; $P<0.04$) as compared to control animals. **Conclusion:** There is unresponsiveness of the skeletal muscle capillary bed to bradykinin in insulin-resistant animals most probably due to a reduction in endothelial NOS (activity and mass). Our results indicate a functional defect possibly involving responsiveness of the precapillary resistance and/or the endothelial barrier to bradykinin in skeletal muscles. Since insulin must cross the endothelial monolayer to reach its target cells on the abluminal side, it is suggested that reduced endothelial NOS and consequent reduced extravasation of macromolecules could exacerbate insulin resistance in skeletal muscles and hypertension in the fructose-fed rat.

Keywords: Capillaries; Diabetes; Endothelial function; Microcirculation; Regional blood flow

1. Introduction

Insulin resistance has long been recognized to be associated with hypertension, hyperinsulinemia and dyslipidemia, an association frequently referred as the metabolic syndrome [1,2]. The precise pathophysiological mechanism underlying the increase in blood pressure in this condition and, its precise relationship with the insulin resistance defect itself or the development of the subsequent diabetic syndrome have only been partially elucidated [3,4]. It is becoming clear that endothelial dysfunction and/or impaired vascular reactivity might play a pivotal role in generating the micro- and macrovascular complications characteristic of the insulin resistance

*Corresponding author. Tel.: +1-819-346-1110x14850; fax: +1-819-564-5292.

E-mail address: pierre.maheux@usherbrooke.ca (P. Maheux).

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syndrome and type 1 or type 2 diabetes [5–9]. However, this theory, while appealing, has been difficult to verify: methods used to examine this defect in vivo have often been limited by the fact that only large to medium size arteries can be studied [10,11]. Researchers have inferred that this defect also affects different vascular segments downstream from these large conduit arteries but neither the consequence nor the importance of this problem has been clearly established in conditions such as insulin resistance.

Using the fructose-fed insulin resistance rat model, our group has recently suggested that an endothelial dysfunction specifically located in the skeletal muscle vascular bed could affect extravasation of macromolecules, glucose or insulin, and possibly contribute to the increase in high blood pressure [12]. Because the vascular bed of skeletal muscle accounts for up to 25% of total cardiac output [13] and is a main target for the action of insulin, we hypothesized that an endothelial cell dysfunction at this level could be an important underlying mechanism for the chronic increase in systemic blood pressure, on one hand, and, at least partly, to the impaired insulin-mediated glucose uptake, on the other hand, well documented in the insulin resistance syndrome.

The purpose of the present study was to examine in more details the extend of this endothelial dysfunction in the fructose-fed rat model. To do this, the acute capillary response to the potent nitric oxide dependent vasodilator bradykinin was examined using an Evans Blue dye extravasation technique. This study shows that there is a markedly impaired response of skeletal muscle vasculature to the acute injection of bradykinin in this dietary-induced insulin resistance model. It also demonstrates that there are important defects in the local skeletal muscle nitric oxide system of this insulin resistance animal model.

2. Methods

2.1. Materials

[^{3}H]L-arginine was obtained from Amersham (Oakville, Ontario, Canada). Monoclonal (eNOS) and polyclonal (nNOS) antibodies were obtained from BD Biosciences (BD Transduction Laboratories, Lexington, KY, USA). Silica LK6D (0.25 mm×20 cm×20 cm) Whatman adsorption plates were purchased from Fisher Scientific (Nepean, Ontario, Canada). All the other reagents were purchased from Sigma (St. Louis, MO, USA). The immunoblotting membranes and the enhanced chemiluminescence (ECL) Western blotting detection system were obtained from Roche (Indianapolis, IN, USA). Finally, all solvents used from chromatography were supplied by Fisher Scientific (Nepean, Ontario, Canada).

2.2. Animals

Male Sprague–Dawley rats (Charles River Breeding Laboratories, St. Constant, Québec, Canada), initially weighing between 200 and 220 g, were used for these experiments. Because of the nature of the experiments to be described and the possible effects on muscle tissue variables of pharmacological agents administered intravenously, different groups of experimental animals had to be studied. More specifically, 34 animals were used for the Evans Blue experiments performed in vivo (16 controls and 18 fructose-fed animals), and 46 animals (21 controls and 25 fructose-fed animals) were used for the in vitro assessment of the various muscle tissue variables. In the latter group, blood pressure and plasma parameters were systematically measured. The animals were housed in temperature and humidity controlled conditions and were fed standard rat chow (Ralston Purina Canada, Woodstock, Ontario, Canada), containing 53% vegetable starch, 4.5% fat, 22% protein, 0.36% sodium, and 1.08% potassium prior to dietary manipulation. Rats were then randomly assigned to a standard chow diet or a 60% fructose enriched diet (Harlan Teklad, Madison, WI, USA) for a total of 4 weeks. This fructose-fed rat model has been studied and validated over the years for studying insulin resistance [14–16]. Blood was collected from the retro-orbital sinus, under halothane anesthesia, 2 days prior to sacrifice. This study was approved by our institutional ethics committee for animal experiments and conformed with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH Publication No. 85.23, revised 1996).

2.3. Blood pressure measurement

The tail cuff method was used to measure blood pressure. 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. Blood pressure was measured under unanesthetized conditions. The mean of four consecutive readings was used for the evaluation of blood pressure in each rat.

2.4. Measurement of vasopermeability in skeletal muscles

The Evans Blue (EB) dye technique was used to measure capillary permeability to albumin in the quadriceps muscle from unanesthetized control and experimental rats. This technique is based on the principle that Evans Blue dye avidly binds to the negatively charged intravascular albumin and is thus a reliable way of assessing transvascular fluxes of macromolecules. This technique has been described and validated previously [12,17–19] and

provides a reliable estimate of the extravasation and interstitial accumulation of albumin [20]. Briefly, rats were injected in the caudal vein with bradykinin (150 μg/kg; a generous gift from Professor Domenico Regoli, Université de Sherbrooke) followed 10 min later by an injection of EB (20 mg/kg; Sigma, St. Louis, MO, USA). This hypotensive and vasodilatory dose of bradykinin was chosen based on previous experiments from our group [21] as well as from others [22,23]. It takes into account that a large proportion of bradykinin injected intravenously undergoes degradation in the lungs. The EB dye was allowed to circulate for 10 min before sacrifice of the experimental animals. Only quadriceps muscles were sampled in this protocol. 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 Evans Blue concentration due to local edema. The other 2/3 was put in a formamide solution (4 ml/g wet tissue) for 24 h for dye extraction. The extracted amount of Evans blue dye was determined by spectrophotometry at 620 nm using a μQuant (Bio-Tek Instruments) and 96-well microplates. Concentration of EB in each tissue was expressed in μg/g of dry tissue. 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 recently validated in our own [12,17], and other laboratories [24]. We have previously published, using ^{51}Cr-labeled erythrocytes, that tissue contamination by residual blood in the vascular compartment is negligible representing exactly 1.4% in the particular case of skeletal muscle [12]. To avoid seasonal differences in EB extravasation in peripheral tissues such as skeletal muscles (unpublished observations), experiments in the fructose fed group and the controls were always conducted in pairs. Results were calculated from a standard curve of Evans Blue (0.5–25 μg/ml) and expressed as Evans Blue μg per g of dry weight of tissue.

2.5. Microscopy of skeletal muscle microcirculation

To study an eventual effect of fructose feeding on the general tissue and vascular architecture, quadriceps muscles were fixed in 10% buffered formalin and embedded in paraffin. Sections (4 μm thick) were stained with hematoxylin and eosin and mounted for light microscopy by standard procedures. Small pieces of skeletal muscle were also fixed in 2.5% glutaraldehyde, treated with 2% osmium, dehydrated, and embedded into Epon 812 for the examination of endothelial cell gaps by electron microscopy.

2.6. Preparation of muscle tissue extracts

Quadriceps from the contralateral leg of each animal were rapidly frozen in liquid nitrogen and stored at −70 °C until assayed. Muscles were homogenized on ice in a buffer containing 25 mM Tris–HCl, 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride and 5 μl/ml of a complementary antiprotease 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 determination of NOS activity as well as Western blots. Protein content was determined using the Pierce BCA protein assay (Pierce, Rockford, IL, USA).

2.7. Nitric oxide synthase activity assay

Nitric oxide synthase (NOS) activity was quantified by the conversion of [^{3}H]L-arginine to [^{3}H]L-citrulline, as described previously ([25]). Briefly, aliquots of the homogenates (200 μg of proteins) were incubated in 50 mM HEPES (pH 7.4) with 20 nM [^{3}H]L-arginine (0.1 μCi/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 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) for 1 h. The plates were developed using ninhydrin 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/mg protein after having subtracted the L-NAME blank for each sample.

2.8. Western blot analysis of level of eNOS and nNOS protein

For immunoblotting, samples of muscle homogenates (75 μg of protein/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 eNOS and 1:250 for nNOS. 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 visualized 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.

2.9. Biochemical measurements

Plasma glucose concentrations were measured enzymatically by the glucose oxidase, and plasma triglycerides with a GPO procedure using kits purchased from VWR Canlab (Ville Mont-Royal, Québec, 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.

2.10. 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. Correlations between continuous variables were measured using the Spearman test. *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. Weight and metabolic changes

Table 1 illustrates the impact of 4 weeks of fructose feeding versus a normal chow diet on body weight, blood pressure, and plasma glucose, insulin and triglyceride concentrations. These results demonstrate that weight was similar in both groups ($P>0.05$). As expected, fructose feeding was associated with a significant increase in tail blood pressure ($P<0.001$), plasma insulin ($P<0.04$) and triglyceride concentrations ($P<0.001$). Plasma glucose concentrations were comparable between the two groups confirming that fructose feeding is associated with an insulin resistance defect, a compensatory hyperinsulinemia, but not a full diabetic syndrome characterized by hyperglycemia.

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

	Normal ($n=21$)	Fructose ($n=25$)	*P* value[a]
Weight (g)	421±8	417±10	NS
Systolic blood pressure (mmHg)	123±5	153±6	<0.001
Diastolic blood pressure (mmHg)	77±6	85±5	<0.001
Plasma glucose (mM)	9.2±0.8	11.2±0.7	NS
Plasma insulin (pM)	327±43	485±58	<0.04
Plasma triglycerides (mM)	1.85±0.15	5.75±0.72	<0.001

Mean±S.E.M.
[a] Non-paired Student's *t*-test.

3.2. Measurements of microvascular permeability in response to bradykinin

Fig. 1 compares the quantitative extravasation of Evans Blue in quadriceps muscles of chow-fed as well as fructose-fed animals in the basal state and in response to an acute injection of bradykinin. It can be seen from these data that there was a marked reduction in skeletal muscle permeability at baseline in the fructose-fed animals compared to control animals ($P<0.02$) and that bradykinin failed to increase the extravasation of albumin-bound Evans Blue. More specifically, Evan Blue extravasation could be increased from 43.6±6.9 to 82.6±8.5 μg/g of dry tissue ($\Delta=+89.4\%$; $P<0.03$) with bradykinin in the chow-fed control animals as compared to an increase from 17.6±4.4 to 24.6±3.1 μg/g of dry tissue ($\Delta=+39.8\%$; $P>0.05$) in the fructose-fed animals. To test the hypothesis that an increase in skeletal vasopermeability in response to bradykinin was dependent on the generation of nitric oxide, we measured EB dye extravasation in skeletal muscles (quadriceps) in a series of experiments where chow-fed animals received either L-NAME (50 mg/kg; $n=8$) or normal saline (0.7 ml; $n=6$) intraperitoneally 15 min before the actual intravenous injection of bradykinin. As expected, Evans Blue extravasation measured after the injection of bradykinin was markedly reduced by inhibiting NOS with L-NAME (23.2±7.0 μg/g with L-NAME vs. 76.8±20.8 μg/g dry tissue with saline; $\Delta=-69.8\%$, $P<0.02$). It should be noted that the amount of Evans Blue measured in skeletal muscles of the chow-fed animals injected with L-NAME was actually less than the one measured in the basal state (23.2±7.0 μg/g with L-NAME vs. 43.6±6.9 μg/g in the basal state).

3.3. Microscopy of skeletal muscle

Hematoxylin and eosin staining of quadriceps muscles obtained from animals in each experimental groups were meticulously examined and did not reveal any gross structural abnormalities. Most specifically, capillaries and arterioles of skeletal muscles from each group had a normal appearance and were not surrounded by abnormal tissue reaction. More specifically, there was no evidence of inflammatory reaction nor matrix structural changes (Fig. 1). In electron microscopy, a careful and thorough examination of junctions between adjacent endothelial cells (intercellular clefts/gaps) did not unveil any significant difference between the control and the fructose-fed animals (data not shown).

3.4. Measures of nitric oxide synthase activity and immunoreactive mass

Fig. 2 depicts the reduction observed for total tissue nitric oxide activity in the fructose-fed animals compared to their controls (−33.8%; $P<0.001$). To establish which NOS isoform was more significantly affected, we mea-

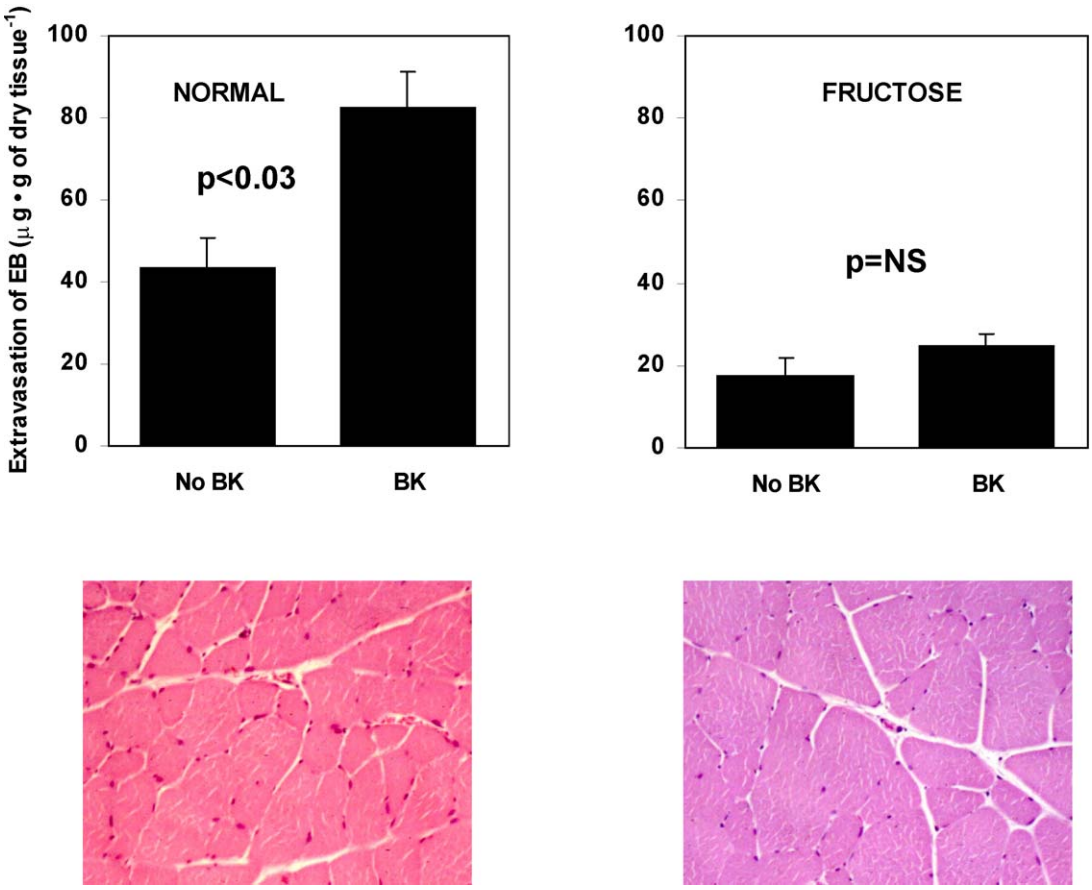

Fig. 1. Extravasation of Evans Blue dye in skeletal muscles (quadriceps) of Sprague–Dawley rats fed with a normal chow or a fructose-enriched diet for a total of 4 consecutive weeks. The microvascular permeability was assessed in the basal state (no BK) or after the acute injection of 150 μg/kg of bradykinin (BK). Each bar represent the mean±S.E.M. obtained from experiments conducted in groups of 8–9 animals each. Shown underneath are representative light micrographs (150X) of 4-μm thick section stained with H&E from skeletal muscle and demonstrating that capillaries in both dietary paradigms were comparable.

sured the immunoreactive protein mass of eNOS and nNOS using specific antibodies. In these Western blot experiments, there was a marked reduction only in eNOS immunoreactive mass in skeletal muscle homogenates of the fructose-fed animals compared to the chow-fed rats (−37.8%; $P<0.01$; Fig. 3). These changes in eNOS were not paralleled by any significant changes in the nNOS immunoreactive mass, the most abundant muscular NOS isoform. Of note, there was a moderate correlation between the eNOS immunoreactive mass and the tissue total NOS activity ($\rho=+0.53$; $P<0.001$). No such correlation was found between nNOS immunoreactive mass and NOS activity. Finally, there was no correlation between plasma triglyceride concentrations and either EB extravasation or NOS activity (data not shown).

4. Discussion

Our data add substantially to the evidence that insulin resistance is associated with an impaired permeability of the skeletal muscle vascular bed. This had previously been shown in the basal state at 4 and 8 weeks [12] but the present paper has explored the functionality of the skeletal muscle vasculature bed in response to an acute injection of a pharmacological dose of bradykinin in vivo. Bradykinin is a potent vasodilatator and its action is predominantly dependent on the generation of nitric oxide from the endothelial cells [26]. The resulting arteriolar dilation is accompanied by contraction of endothelial cells and widening of intercellular junctions, and by an increased venous pressure secondary to constriction of veins [26,27]. We were able to show that extravasation of macromolecules cannot be heightened as effectively, using a pharmacological dose of bradykinin, in the fructose-fed rat model as compared to controls. Given the particular mechanism of action of bradykinin but also the fact that its effect on macromolecule extravasation can be blocked by L-NAME, the results of our experiments really point to an endothelial cell dysfunction and a defect in skeletal muscle vascular permeability in this animal model of insulin resistance. In this last set of experiments, it should be noted that L-NAME suppressed the Evans Blue extravasation to a level less than the one measured in the basal conditions sug-

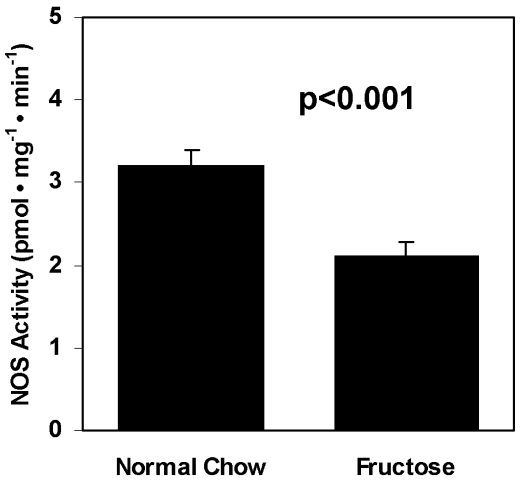

Fig. 2. Effects of fructose feeding on NOS activity in homogenates of quadriceps muscles obtained from control (n=21) or fructose-fed animals (n=25). Rats were fed for 4 weeks with a fructose-enriched diet. NOS activity was determined using equivalent muscle extracts (200 μg of total tissue proteins) from control or fructose-fed animals (see Section 2). Results are means±S.E.M.

gesting that NOS is also involved in setting up capillary permeability and/or precapillary resistance in the basal state. It is also important to pinpoint that this impaired response of skeletal muscle to bradykinin was seen despite the fact that bradykinin has a very short plasma half-life and that a 10-min period before the actual caudal vein injection of Evans Blue might have underestimated the magnitude of the difference observed between the two groups of animals. It is therefore possible that the vascular dysfunction observed in the skeletal muscle of the fructose-fed rat model might even be more important.

Coincident with these in vivo phenomena, we were able to demonstrate in vitro a significant reduction in total nitric oxide synthase activity as well as a reduction in the eNOS immunoreactive mass in skeletal muscle homogenates of the fructose-fed animals. The reduction in eNOS was very selective since no change in nNOS could be observed. In the absence of any change in nNOS, it is hypothesized that the reduction in total tissue NOS activity comes mostly, if not exclusively, from a reduced expression of eNOS, an assertion supported by the moderate correlation observed between eNOS immunoreactive mass and total tissue NOS activity. We infer that this reduction comes predominantly from the eNOS enzyme located in the skeletal muscle microvasculature (arterioles and small nutritive or non-nutritive arteries) as opposed to the myocytes themselves, although there are still controversies as to whether eNOS is expressed or not in myocytes [28–30]. The combination of these in vitro and in vivo observations, along with the fact

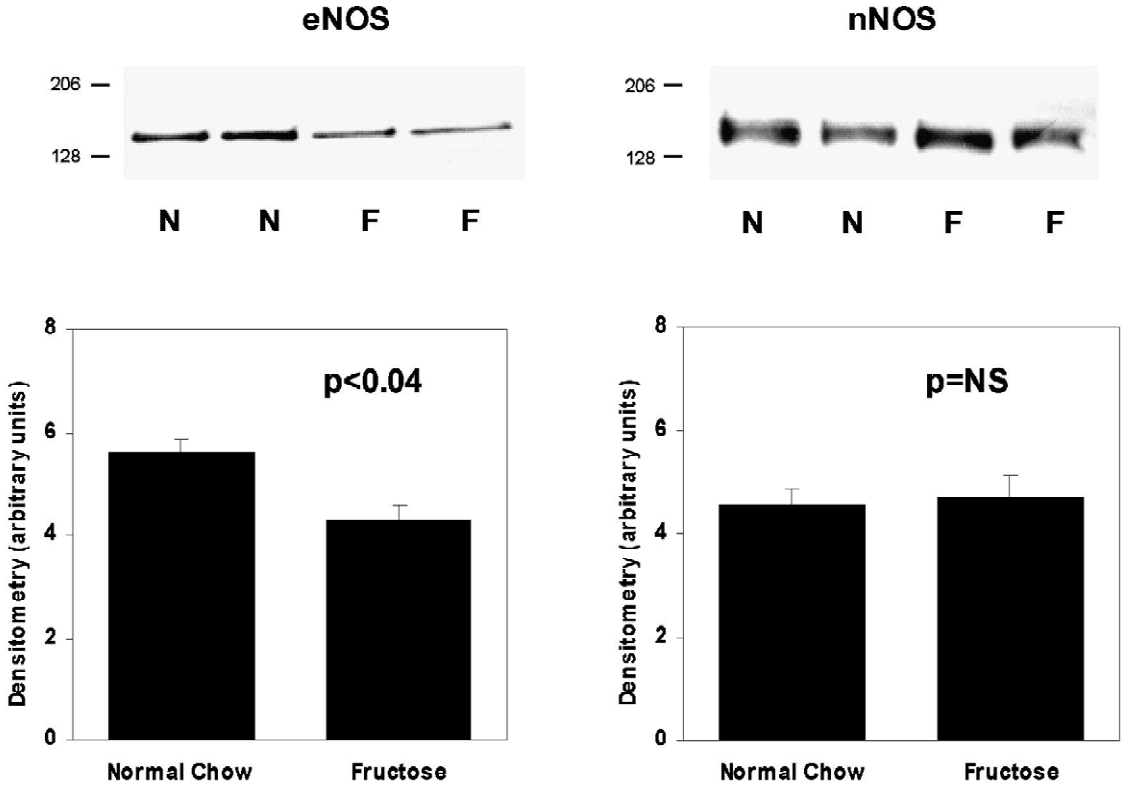

Fig. 3. Representative immunoblots of an experiment where equivalent amounts of muscle protein (75 μg) were resolved on SDS–PAGE and analyzed by Western blotting using eNOS or nNOS specific antibodies. Both protein migrated as a single band of about M_r 140 000 for eNOS and about M_r 155 000 for nNOS. 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.

that no abnormalities could be identified in light or electron microscopy on endothelial cells, suggests a functional problem with the vascular bed of skeletal muscle in this dietary-induced insulin resistance rat model. These data contrast with the observations previously reported in the streptozotocin-induced rat model [30]; while the reduction in total tissue NOS activity was of the same magnitude, both eNOS and nNOS were affected in this type 1 diabetes rat model characterized by marked hyperglycemia, hypoinsulinemia and high catabolic state. nNOS is indeed the most abundantly expressed isoform in skeletal muscle [31] but it possible that the metabolic profile induced by the fructose feeding paradigm does not in any way change nNOS expression in skeletal muscle. While we have not measured NO formation upon stimulation with bradykinin, all our experiments point to a dysregulation of the eNOS isoform in skeletal muscle of this insulin resistance rat model.

Endothelial dysfunction has been associated with reduced generation and action of NO, a finding consonant with the observations of the present study. Several studies have shown that endothelium-dependent relaxation is diminished in human and experimental diabetes [4–9], but the consequence of this phenomenon in organs using huge amount of energy, such as the skeletal muscle, is not well established and could very likely be tissue specific. While a decrease in bradykinin-mediated vasodilatory response in the hindlimb muscle bed of streptozotocin-induced rats has been documented previously [32], our study suggests that an abolished rise in EB uptake upon stimulation with bradykinin might indeed be also associated to an endothelial dysfunction in this insulin resistant but normoglycemic animal model. While the fructose-fed rat model has been associated with both a diminished ability of insulin to suppress hepatic glucose production [14] and a peripheral insulin resistance [15,16], we think that it is this latter defect that is responsible for the observations made in the present study.

Alterations of the skeletal muscle vasopermeability and a reduction in skeletal muscle eNOS activity may be important in the insulin resistance syndrome. Skeletal muscle plays an important role in postprandial uptake of glucose from the circulation [33] and modulation of the peripheral vascular resistance [13]. Hence, abnormal blood flow in skeletal muscle could well contribute or aggravate the insulin resistance defect and possibly increase the systemic blood pressure. Although the observed dysfunction could affect endothelial cells throughout the skeletal muscle vasculature, it might affect only strategic segments of the microcirculation, and, therefore, leads to an impaired EB leakage in the basal state as well as in response to acute injection of bradykinin. Terminal arterioles could logically be one such key site of regulation, because they control the precapillary resistance. If precapillary resistance is high, the capillary hydrostatic pressure drops and transfer of solutes to the adjacent interstitial fluid compartment could decrease. These terminal arterioles are also important in capillary recruitment and it has been reported recently that insulin resistance can be associated with less recruitment of capillaries in skeletal muscles [34,35]. Alternatively, fructose feeding and/or insulin resistance may impair the capillary membrane permeability itself by inhibiting the contraction of endothelial cells and narrowing intercellular junctions. Finally, it is possible that insulin resistance could decrease the postcapillary resistance, decreasing venous pressure and preventing water, solutes or the EB dye from passing to the interstitium. In any one of these scenarios, the transit time of blood through skeletal muscle would be reduced in this insulin resistance model and this could result in an impaired supply of blood and/or substrates to muscle cells. Interestingly, bradykinin is known to have an effect on the three vascular targets mentioned above and its effects were clearly blunted in the fructose-fed insulin resistance animal model [26,27]. It is difficult, with the in vivo methodology used on whole animal in the present study, to identify specific vascular sites as the locus of the effects seen. However, it is possible that a combination of all of these might play a role.

In conclusion, the fructose-induced insulin resistance model is associated with defects in skeletal muscle microcirculation, as assessed by measuring extravasation of macromolecules both in the basal state and after the acute injection of bradykinin. The in vivo experiments in conjunction with the marked reduction in NOS activity and immunoreactive mass are consistent with endothelial dysfunction in the skeletal muscle microvasculature system. Given the important role of skeletal muscle on peripheral vascular resistance and glucose uptake, we suggest that a reduction in skeletal muscle vasopermeability can, as alluded by others [36–38], contribute to abnormal internal distribution of fluids in body, reduced capillary delivery of insulin to target organs and perhaps hypertension. This phenomenon may take place in the absence of hyperglycemia.

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