ENDOTHELIAL DYSFUNCTION IN INSULIN RESISTANT RATS IS ASSOCIATED WITH OXIDATIVE STRESS AND COX PATHWAY DYSREGULATION

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Short title: Endothelial function in insulin-resistant rats

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SUMMARY

Because insulin resistance is inevitably associated with cardiovascular complications, there is a need to further investigate the potential involvement of oxidative stress and the cyclo-oxygenase (COX) pathway in the vascular modifications associated to this pathological context. Endothelial function was evaluated in control and fructose-fed rats (FFR) by i) in vitro study of endothelium-dependent and -independent relaxations of aortic rings, and ii) in vivo telemetric evaluation of pressor response to norepinephrine. After 9 weeks of diet, FFR displayed hypertriglyceridemia, hyperinsulinemia and exaggerated response to glucose overload. Aortic rings from control rats and FFR exhibited comparable endothelium-dependent relaxations to Ach. In the presence of indomethacin, relaxations were significantly reduced. FFR showed exaggerated pressor responses to norepinephrine that were abolished with indomethacin. Urinary nitrites/nitrates, 8-isoprostanes and thromboxane B_2 excretion levels were markedly enhanced in FFR, whereas the plasma levels of 6-keto prostaglandin $F_{1\alpha}$ were unchanged. In conclusion, fructose overload in rats induced hypertriglyceridemia and insulin resistance associated with an enhanced oxidative stress. This was associated with COX pathway dysregulation which could be one of the contributors to subsequent vascular dysfunction. Consequently, reduction of oxidative stress and regulation of the COX pathway could represent new potential therapeutic strategies to limit vascular dysfunction and subsequent cardiovascular complications associated with insulin resistance.

Keywords: endothelial dysfunction, insulin resistance, oxidative stress, cyclo-oxygenase

INTRODUCTION

Insulin resistance is typically defined by the reduced sensitivity to insulin actions that regulate glucose disposal, and results ultimately in type 2 diabetes mellitus. In patients with insulin resistance such as in the metabolic syndrome, cardiovascular risk is markedly increased (Grundy 2006). However, causes and consequences of insulin resistance on cardiovascular complications are yet to be explored in order to limit the cascade of sequelae and co-morbid disease (Haffner 1999).

Endothelium appears to play a key role in the vascular damages induced by insulin resistance associated with metabolic syndrome (Kim *et al.* 2006). Patients with metabolic syndrome or type 2 diabetes mellitus exhibit impaired endothelium-dependent vasodilation (Baron 1999). It is now recognized that these disturbances in endothelial function are principal players in the ischemic manifestations of coronary artery disease (Anderson *et al.* 1995, Meredith *et al.* 1993). In fact endothelial dysfunction has been suggested to precede the elevation of blood pressure (Katakam *et al.* 1998) and contribute to the development of cardiovascular diseases in insulin resistance (Shinozaki *et al.* 1995) and may therefore represent both a surrogate marker for cardiovascular risk as well as a relevant therapeutic target.

Oxidative stress has been suggested to both (i) contribute to insulin resistance (Carantoni *et al.* 1998, Gopaul *et al.* 2001), and (ii) play a crucial role in the pathogenesis of endothelial dysfunction (Esper *et al.* 2006, Sonnenberg *et al.* 2004). The most important consequence of increased oxidative stress on vascular endothelial function is the decrease in NO bioavailability resulting from both NO inactivation by superoxide anions and NO synthase uncoupling (Griendling *et al.* 1997). An increase in free radical production could also activate the cyclo-oxygenase (COX) pathway resulting in an imbalance between vasoconstrictor and vasodilator prostanoid synthesis. Indeed, it was suggested that both hyperglycemia (Cosentino *et al.* 2003)

and oxidative stress dysfunction (Bachschmid *et al.* 2005, Cosentino *et al.* 2003) were associated with an increase in vasoconstrictor thromboxane A_2 and a decrease in vasodilator prostacyclin (PGI_2) produced by COX. Thus, this modulation of the prostanoid production could result in endothelial dysfunction (Bachschmid *et al.* 2005, Cosentino *et al.* 2003).

We aimed to investigate new potential mechanisms linking disrupted glucose metabolism to subsequent cardiovascular complications by studying endothelial function and the potential involvement of oxidative stress and the COX pathway in the vascular modifications induced by insulin resistance. Since fructose consumption might be a contributing factor to the development of metabolic abnormalities observed in the metabolic syndrome (Bray *et al.* 2004, Elliott *et al.* 2002), we used the fructose-fed rat (FFR) as a model of insulin resistance. Endothelial function was evaluated both in vitro and in vivo by (1) the study of systemic endothelium-dependent relaxations by isometric tension studies on aortic rings, and (2) telemetric evaluation of arterial pressure and pressor responses to norepinephrine in conscious unrestrained rats. We also sought to determine the effects of fructose overload on biochemical indicators of the extent of oxidative stress, and COX pathway dysregulation in FFR.

METHODS

Experimental design

After a 1-week acclimatization period, male Wistar rats (Charles River, France, 180-220 g) were randomly placed on a purified control chow (Control: TD.03102) or on an isocaloric fructose-enriched diet (fructose-fed rats or FFR: TD.89247 containing 18.3 % protein, 60.3 % fructose and 5.2 % lard) (Teklad Labs, Madison , WI, USA) for the following 9 weeks. All procedures were

performed in accordance with the legislation on the use of laboratory animals (NIH publication N°85-23, revised 1996) and Animal care Regulations in force in France as of 1988.

After 9 weeks of diet, in vitro vascular reactivity was evaluated in a first set of animals (Control: n=12, FFR: n=12). In this set of rats, 24-hour urine and blood samples were collected for biochemical determinations.

In a second set of animals (Control: n=10, FFR: n=10), blood pressure and pressor responses to norepinephrine were investigated and oral glucose tolerance tests (OGTT) were performed in a subset of animals from this series (Control: n=8, FFR: n=8) after 9 weeks of diet.

A third set of animals (Control: n=8, FFR: n=8) was carried out to investigate the role of COX pathway in pressor responses to norepinephrine following indomethacin injection after 9 weeks of fructose-enriched diet.

In vitro vascular reactivity

Rats were deeply anesthetized with urethane (1.2 g/kg, i.p.). Aortic rings were obtained and placed in organ chambers (5 ml) filled with an oxygenated physiological salt solution (PSS: NaCl 118.0 ; KCl 4.6 ; $CaCl_2$ 2.5 ; KH_2PO_4 1.2 ; $MgSO_4$ 1.2 ; $NaHCO_3$ 25.0 and glucose 11.1 mM) at 37°C for isometric tension recording. After equilibration the preparations were precontracted by phenylephrine. Concentration-response curves to endothelium-dependent relaxant agonist (i.e. acetylcholine, ACh, 10^{-10} to 10^{-5} M) were performed in presence or not of indomethacin (10^{-5} M). Every 2 minutes, increasing doses of Ach were added to the organ bath. Since aortic relaxant responses to Ach were stable, relaxations were recorded during the last 20 seconds before adding a new dose. Indomethacin was added to the organ bath 30min before precontraction to phenylephrine preceding concentration-response curves.

To evaluate endothelium-independent relaxations, concentration-response curves to sodium nitroprusside (SNP, 10^{-10} to 10^{-6} M) were performed. For each concentration-response curve, a pD2 value (–log [EC_{50}] where EC_{50} was the concentration of drug that produced 50% of the maximum effect) and a maximal effect value (E_{max}, maximum response) were determined.

In vivo telemetric measurement of blood pressure

Before the end of the 8th week of treatment period, rats were anesthetized (2% inhaled isoflurane), and each animal was implanted with a radio-telemetry transmitter (model PA-C40, Data Sciences International, St. Paul, MN, USA). The catheter was introduced into the femoral artery and advanced to the abdominal aorta. The right jugular vein was catheterized to allow subsequent intravenous perfusion. After surgery, each rat was allowed to recover at least 7 days before blood pressure measurement. Telemetric measurements in conscious unrestrained rats were performed at the end of the treatment period (week 9). Briefly, after 30 min acclimatization blood pressure was recorded for 30 minutes (baseline parameters measured during the last 5 min). Subsequently, increasing doses of norepinephrine were infused i.v. for 5 min each (50, 100, 200, 400 ng/kg/min). Pressor responses were determined for each dose as an average of the recorded response during the final minute. In the third set of animals, to investigate the role of COX pathway in pressor responses to norepinephrine, indomethacin (7.5 mg/kg (Ruiz *et al.* 1994)) or its vehicle was intravenously injected 30 min before the beginning of the norepinephrine perfusion.

Evaluation of glucose metabolism

After telemetry BP measurements, rats were fasted overnight, then gavaged with a solution of glucose 1 g/kg and anesthetized with isoflurane. Blood samples were taken from the tail vein at 0,

10, 20, 30, 60 and 90 minutes after the gavage. Fasting levels of glycemia and insulinemia were determined at time 0. Blood glucose was determined immediately after collection (Accu-chek active, Roche diagnostics, France), insulin concentration was determined in plasma samples by enzyme immunoassay (Cayman Chemical, MI, USA). The insulin sensitivity index (ISI) was calculated using the formula of Matsuda and DeFronzo (Matsuda *et al.* 1999) as follows:

ISI = 10000 / √ [(FPG x FPI) x (mean OGTT glucose concentration x mean OGTT insulin concentration)], FPG being fasting plasma glucose (in mg/dL), FPI fasting plasma insulin (μU/mL) and mean OGTT (oral glucose tolerance test) glucose and insulin concentration being obtained from the area under the curve of glucose or insulin concentration evolution during the 90 min following oral gavage with 1 g/kg glucose solution.

Biochemical determinations

At the end of the 9th week of diet, rats to be included in *in vitro* vascular reactivity studies were fasted overnight and placed in metabolic cages to collect 24-hr urine samples, and plasma samples were also collected. Plasma and urinary creatinine was determined by spectrophotometry (Jaffe M. 1886). The urinary concentration of nitrates and nitrites, 8-isoprostanes and thromboxane B_2, and plasma 6-keto prostaglandin F1α were determined using commercially available assay kits (Cayman Chemical, MI, USA). Plasma triglycerides were measured using a colorimetric method (Sigma assay kit, St Louis, MO, USA). All urine concentrations were corrected by the clearance of creatinine to limit variability in the assays due to changes in renal excretory function (Behr-Roussel *et al.* 2000).

Statistical analysis

All data were expressed as mean ± SE. Most of the results were analyzed using Student's t-test. In vitro vascular relaxation responses curves and pressor responses to norepinephrine results were analyzed using a two-way ANOVA statistical analysis followed by Bonferroni's complementary analysis when relevant. For pD2 and Emax values, statistical analysis was performed according to the extra sum of squares F test principle with GraphPad Prism® 4.03 software. P values < 0.05 were considered significant.

RESULTS

Metabolic parameters

After 9 weeks of control or fructose-enriched diet, rat body weights were similar in both control and FFR groups (table 1).

Eventhough fasting glycemia was not significantly changed by 9 weeks of fructose overload, insulinemia was significantly increased in FFR compared to control rats (table 1, P=0.036). Moreover, oral glucose tolerance test revealed significant differences in terms of increase in plasma levels of glucose and insulin. Indeed, in response to oral administration of 1 g/kg glucose, FFR displayed a decreased insulin sensitivity index compared to control rats (table 1, P=0.014) indicating insulin resistance in these animals.

Mean arterial pressure (MAP) and heart rate, measured telemetrically in conscious animals after a 30 min acclimation period were unchanged after 9 weeks of fructose-enriched diet (table 1).

Finally, FFR were highly hypertriglyceridemic compared to the control rats (table 1).

In vitro vascular reactivity

Precontractions tensions elicited by 10^{-6} M Phenylephrine were similar in aortic rings from control and FFR, whatever the experimental condition (before ACh-dependent relaxation 572±66 mg/g wet weight in control vs 440±63 mg/g wet weight in FFR; P=0.17 Student's t-test - before ACh-dependent relaxation in the presence of indomethacin 402±47 mg/g wet weight in control vs 320±40 mg/g wet weight in FFR; P=0.20 Student's t-test - before SNP-dependent relaxation 675±99 mg/g wet weight in control vs 640±110 mg/g wet weight in FFR; P=0.82 Student's t-test). Aortic rings from control rats and FFR exhibited comparable endothelium-dependent relaxations to Ach (figure 1.A) with unchanged pD2 (7.65 ± 0.05 in control rats vs. 7.83 ± 0.08 in FFR, ns) and Emax (-99.1 ± 1.7 % in control rats vs. -95.2 ± 1.08 % in FFR, ns). However, when indomethacin was added to the organ bath, a significant reduction of endothelium-dependent relaxations to ACh was observed in aortas from FFR compared to control rats (P<0.001 2-way ANOVA) (figure 1.B), which is associated with a reduction of Emax (-104.0 ± 1.9 % in control rats vs. -90.7 ± 3.1 % in FFR, P<0.01)and unchanged pD2 (7.79 ± 0.07 in control rats vs. 7.70 ± 0.12 in FFR, ns). Conversely, aortic endothelium-independent relaxations to SNP were increased in the aortas from FFR compared to control rats (figure 1.C).

In vivo telemetric measurement of pressor response to norepinephrine

During the first 30 minutes of baseline recording, the pressure was stable in control rats and FFR. The perfusion of increasing concentrations of norepinephrine elicited a dose-dependent increase in arterial pressure (figure 2) with both an elevation of systolic and diastolic BP (data not shown). The response to norepinephrine was clearly enhanced in FFR compared to control rats (P<0.01 2-way ANOVA, figure 2.A).

The administration of indomethacin 30 min before the beginning of norepinephrine infusion significantly reduced the amplitude of the pressor response to norepinephrine in both controls (figure 2.B) and FFR (figure 2.C). However, the downward shift of the pressor response curve following indomethacin administration was more important in FFR than in control rats. Indeed, when indomethacin was intravenously injected, the pressor responses to norepinephrine in FFR (figure 2.A) were normalized and not significantly different from control rats ($P>0.05$ 2-way ANOVA, figure 2.D) except during the 400 ng/kg/min norepinephrine perfusion.

Biochemical evaluation of oxidative stress and cyclo-oxygenase products

Both plasma and urine creatinine levels were similar in control and FFR, resulting in a preserved creatinine clearance following 9 weeks of fructose-enriched diet (0.49±0.05 in control vs 0.39±0.05 in FFR mL/min; $P=0.16$ Student's t-test).

Both urinary nitrites/nitrates ($P<0.05$ Student's t-test) and 8-isoprostanes ($P<0.01$ Student's t-test) levels were markedly increased in FFR compared to control animals (figure 3.A). Whereas urinary thromboxane B_2 excretion was greatly enhanced in the FFR ($P<0.05$ Student's t-test) as a result of the fructose-enriched diet, the levels of the stable metabolite of prostacyclin (PGI_2), 6-keto prostaglandin $F_{1\alpha}$ were similar in control and FFR rats (figure 3.B).

DISCUSSION

In the present study, 9 weeks of fructose-enriched diet in rats induced hyperinsulinemia, impaired glucose tolerance and hypertriglyceridemia with no change in blood pressure. Many other studies using the FFR have also reported an increased fasting plasma insulin and/or exaggerated response

to glucose overload (Lee *et al.* 2006, Nagai *et al.* 2002, Nakagawa *et al.* 2006, Vasudevan *et al.* 2005) as well as a consistent hypertriglyceridemia in accordance with our results (Bartus *et al.* 2005, Nagai *et al.* 2002, Nakagawa *et al.* 2006, Nyby *et al.* 2005, Sanchez-Lozada *et al.* 2007, Shinozaki *et al.* 2000, Takagawa *et al.* 2002). Interestingly, insulin levels and high triglyceridemia are known to enhance free radical production (Bakker *et al.* 2000, Kim *et al.* 2006). Oxidative stress has been evaluated by the measure of the clinically validated biomarker: urinary 8-isoprostanes (Montuschi *et al.* 2004). We have confirmed the excessive non-enzymatic in vivo lipid peroxidation as a result of oxidative stress. In agreement with the present observation, oxidative stress has repeatedly been evidenced in FFR (Delbosc *et al.* 2005, Miatello *et al.* 2005, Nyby *et al.* 2005, Shinozaki *et al.* 2000). This may directly result from elevated glycemia and triglyceridemia present in these rats.

In contrast, a somewhat surprising finding in this study is the fact that 9 weeks of fructose-enriched diet did not modify baseline blood pressure and heart rate. These results are in contradiction with several studies reporting that FFR are hypertensive (Kamide *et al.* 2002, Miatello *et al.* 2005, Nagai *et al.* 2002, Nyby *et al.* 2005, Sanchez-Lozada *et al.* 2007, Takada *et al.* 2001). In these studies, BP has been measured by tail-cuff plethysmography. In contrast, using telemetry, D'Angelo *et al.* (D'Angelo *et al.* 2005) reported that 8 weeks of fructose feeding (66% fructose, 12% lard) produced no change in baseline MAP in agreement with our data. We believe that tail-cuff BP results must be cautiously interpreted since it may yield misleading results due to the restraint and thermal stress imposed to the animal. These challenging conditions may indeed provide BP measurements that may be better compared to a pressor response rather than a baseline BP (Pelaez *et al.* 2003).

Fructose-enriched diet associated with moderate amounts of fat (Reed *et al.* 1994) induced insulin resistance associated with hyperlipidemia in accordance with previously reported results

(Galipeau *et al.* 2001, Miatello *et al.* 2002, Song *et al.* 2004) with no change in BP. This depicts some of the abnormalities associated with an early stage of the metabolic syndrome development. Moreover, a direct consequence of these abnormalities (insulin resistance and hyperlipidemia) may be an enhanced oxidative stress which could constitute the starting point for cardiovascular complications associated with the metabolic syndrome.

In addition to its essential metabolic actions, insulin binding to its receptors has been demonstrated to stimulate the production of NO at the endothelial level (Baron *et al.* 1997). Since NO constitutes one of the major vasodilator mediator, the defect in insulin signaling pathway caused by insulin resistance appears to be closely associated with endothelial dysfunction. Interestingly, endothelium-dependent reactivity of isolated aortic rings from FFR did not seem to be affected. However, in the presence of indomethacin, marked endothelial dysfunction was revealed. These results suggest that, in FFR, the net balance between vasodilator and vasoconstrictor mechanisms does not appear to be modified. However, the contribution of each independent pathway seems to be modified, suggesting that compensatory relaxation mechanisms are still able to buffer specific dysfunctions of vasodilator or vasoconstrictor pathways already present at a very early stage of the metabolic syndrome. Such compensatory mechanisms have been previously demonstrated in SHR in which up-regulation of endothelium-independent vasodilation partly balanced endothelial dysfunction (Behr-Roussel *et al.* 2003). Interestingly, as we demonstrated in the present work, the same observation seems to hold true in FFR since endothelium-independent relaxing mechanisms were upregulated.

Next, we could closely examine the modulation of the endothelium-dependent vasodilation pathways in aortas from FFR (i.e. NO and COX products). The most likely event occurring in FFR to explain the alteration of endothelium-dependent relaxations is the impairment of the NO

pathway. In fact, insulin binding to its receptors has been demonstrated to stimulate the production of NO from the endothelium. Moreover, in FFR, insulin resistance seems to be mediated via a decreased insulin receptor expression (Catena *et al.* 2003). Although no direct measurement of impaired NO production was performed in the present study, several elements lead to suggest that NO bioavailability is disturbed in FFR. We have evidenced an increase in oxidative stress by elevated levels of IPT. The elevation of reactive oxygen species production observed in FFR could reduce NO bioavailability by inactivating NO to peroxynitrite. In this respect, the elevation of urinary nitrites/nitrates in FFR might indeed reflect the impaired NO bioavailability since peroxynitrite, as well as NO, is metabolized in nitrites and nitrates (Dedon *et al.* 2004). Several studies reported a decreased vascular eNOS expression and/or activity in FFR (Miatello *et al.* 2005, Nyby *et al.* 2005, Shinozaki *et al.* 1999, Shinozaki *et al.* 2000) while Shinozaki *et al.* showed eNOS uncoupling in FFR (Shinozaki *et al.* 1999). Taken together, all these elements are strongly in favor of a decreased NO bioavailability that could explain the impaired vascular endothelium-dependent relaxation in FFR.

COX products are also critical regulators of vascular tone (Davidge 2001). In the present study, since COX inhibition revealed endothelial dysfunction in FFR, it is suggested that an increased production of endothelium-dependent vasodilator COX products occurred in FFR (i.e. PGI_2 or PGE_2). Yet, basal circulating levels of the stable metabolite of PGI_2, 6-keto-prostaglandin$F_{1\alpha}$, were not modified in FFR which is in accordance with previous studies (Bartus *et al.* 2005). However, it must be kept in mind that our results were obtained in unstimulated conditions and this might not preclude a compensatory increased production of PGI_2 in response to a vasodilator stimulus. Vasodilator prostaglandin E_2 may also be upregulated in FFR but this possibility was not assessed and remains to be investigated.

We conclude that, in FFR, the increase in COX-derived vasodilators associated with the enhancement in endothelium-independent relaxation pathway may constitute compensatory mechanisms for a decreased production of other vasodilators among which NO is the most probable candidate.

In our experimental conditions, an exaggerated pressor response to norepinephrine was found in conscious FFR after 9 weeks of fructose-enriched diet, associated with increased thromboxane B_2 (TxB_2), the stable metabolite of thromboxane A_2, urinary levels.

A possible explanation to the heightened contractile response to norepinephrine could be a change in alpha-receptor expression in the vasculature. This specific issue was not addressed in the present work but seems improbable since in vitro pre-contraction of aortic rings to phenylephrine were similar in control and FFR.

Indomethacin infusion was able to correct the exaggerated response to norepinephrine in FFR. COX dysregulation, which could account for the in vitro vascular reactivity results, could thus constitute a relevant explanation for in vivo increased pressor response in FFR. Indeed, the enhanced COX-dependent vasoconstrictor TxB_2 production is in agreement with Galipeau *et al.* (Galipeau *et al.* 2001) showing that fructose overfeeding in rats leads to an increase in TxB_2 production. These observations support the concept that TxB_2 produced by COX is increased in FFR, therefore leading to an exaggerated pressor response to norepinephrine. This hypothesis was further reinforced by the fact that COX inhibition by indomethacin corrected this abnormal reactivity to stress in FFR. Therefore, despite the absence of elevated baseline blood pressure, FFR showed an exaggerated response to NE that could be associated with COX pathway dysregulation. This support the fact these FFR are in an early stage of the pathology, showing thus vascular dysfunction rather than a declared hypertensive state, which is already associated

with the dysregulation of the COX pathway, leading to production of endothelium-derived constricting factors as previously reported in several models of hypertension including spontaneously hypertensive rats (Luscher *et al.* 1986), NO-deficient hypertension (Paulis *et al.* 2006) or aged Wistar-Kyoto rats (Koga *et al.* 1989). Therefore, this suggests that COX pathway dysregulation may represent a common feature of endothelial-dysfunction.

Interestingly, oxidative stress could provide the missing link between insulin resistance / hypertriglyceridemia and COX dysregulation. Indeed, since NO exhibits an inhibitory effect on COX activity (Kanner *et al.* 1992), the likely defect of NO in FFR could contribute to COX dysregulation in these animals. Moreover, peroxynitrite was shown to promote preferential TxA_2 production by COX (Bachschmid *et al.* 2005). Thus, one of the consequences of increased oxidative stress in FFR might be the dysregulation of the COX pathway resulting in an increased vasoconstrictor TxA_2 production in response to stress, which could explain the exaggerated vasoconstrictor response to norepinephrine.

To conclude, fructose-enriched diet in rats leads to hypertriglyceridemia and insulin resistance. These metabolic abnormalities induced by fructose overload were associated with an enhanced oxidative stress which appears to dysregulate the COX pathway. As a result, in vitro endothelium-dependent relaxations are impaired in the FFR and the in vivo pressor responses to norepinephrine are enhanced. Consequently, oxidative stress markers such as 8-isoprostanes and biomarkers of COX activity such as TXB_2 may be good biomarkers of vascular dysfunction associated with the early stages of the metabolic syndrome. The reduction of oxidative stress and the normalization of the COX pathway could constitute new potential therapeutic strategies to limit vascular dysfunction and resulting cardiovascular complications associated with the

metabolic syndrome. To conclude the present work gives additional insights about the potential mechanisms linking insulin resistance and endothelial dysfunction.

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FIGURE LEGENDS

Figure 1. Comparison of endothelium-dependent (A, B) and –independent (C) relaxations obtained in *in vitro* experiments with aortic rings in absence (A, C) or in presence (B) of 10 µM indomethacin. 2-way ANOVA: NS: not significant, *P<0.05, ***P<0.001.

Figure 2. Concentration response curves to increasing doses of norepinephrine infusion on mean arterial pressure (MAP) measured in vivo in conscious animals (control and FFR) at the end of the treatment period (week 9) (A) or, 30 min after intravenous vehicle or indomethacin 7.5 mg/kg injection (B, C, D). 2-way ANOVA: NS: not significant, **P<0.01, ***P<0.001.

Figure 3. Levels of oxidative stress markers (nitrites/nitrates, 8-isoprostanes) (A), and COX products (thromboxane B_2 and prostaglandin $F_1\alpha$) (B) in control and FFR after 9 weeks of treatment. Student's t-test, NS: not significant, *P<0.05, **P<0.01.

Table 1. Metabolic parameters in control and FFR after 9 weeks of fructose-enriched diet.

	Control	**FFR**	**P=**
Obesity			
Body weight (g)	419 ± 9	408 ± 12	NS
Glucose metabolism			
Fasting glycemia (mg/dL)	113.4 ± 11.8	134.7 ± 5.8	NS
Fasting insulinemia (ng/mL)	1.26 ± 0.25	2.34 ± 0.38*	0.036
Insulin sensitivity index	2.17 ± 0.29	1.25 ± 0.13*	0.014
Baseline blood pressure			
Mean arterial pressure (mmHg)	106.5 ± 4.5	103.2 ± 5.6	NS
Heart rate (beats/min)	407± 19	380 ± 9	NS
Lipids			
Plasma triglycerides (mM)	1.28 ± 0.15	2.19 ± 0.28**	0.009

Values are expressed as means ± SE. Student's t-test, NS: not significant, *P<0.05, **P<0.01

FIGURE 1

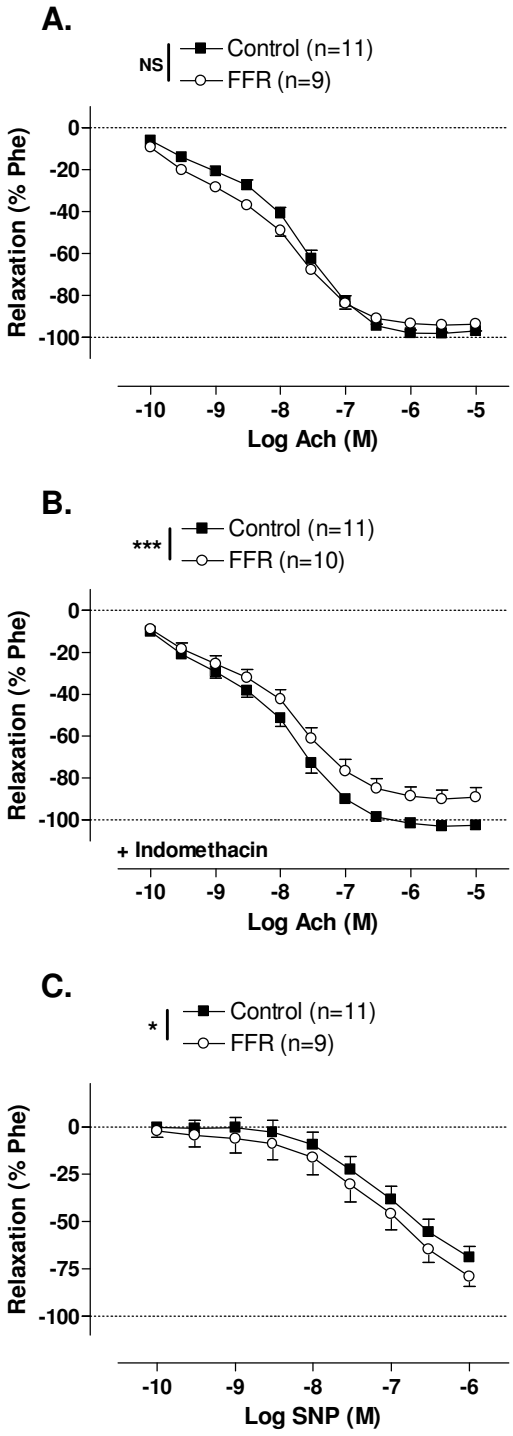

A.
NS
Control (n=11)
FFR (n=9)
Relaxation (% Phe)
0
-20
-40
-60
-80
-100
-10
-9
-8
-7
-6
-5
Log Ach (M)
B.
***
Control (n=11)
FFR (n=10)
Relaxation (% Phe)
0
-20
-40
-60
-80
-100
+ Indomethacin
-10
-9
-8
-7
-6
-5
Log Ach (M)
C.
*
Control (n=11)
FFR (n=9)
Relaxation (% Phe)
0
-25
-50
-75
-100
-10
-9
-8
-7
-6
Log SNP (M)

FIGURE 2

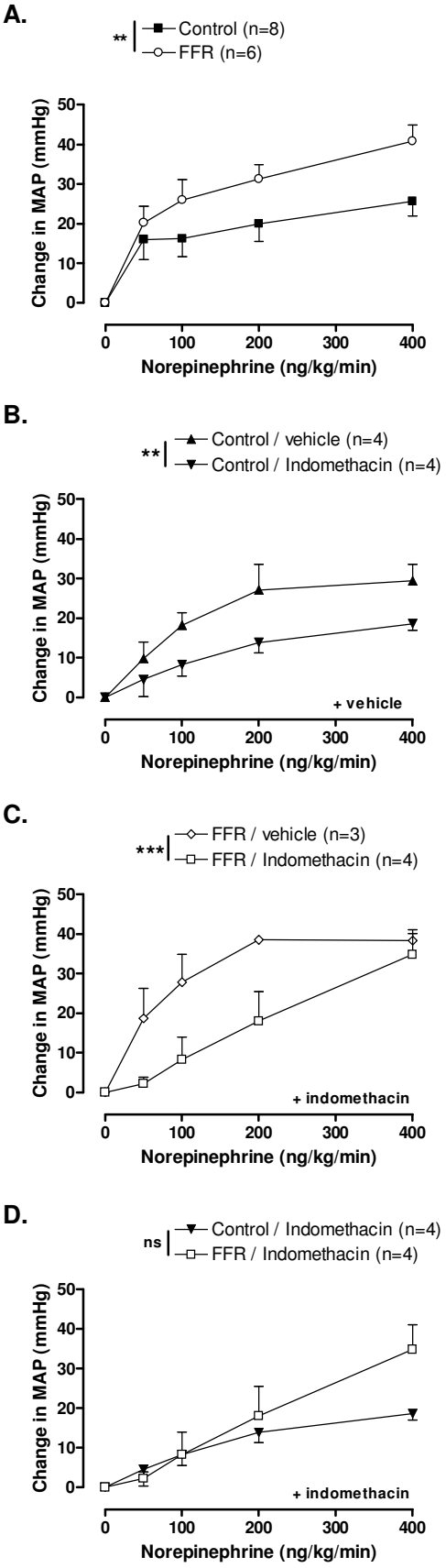
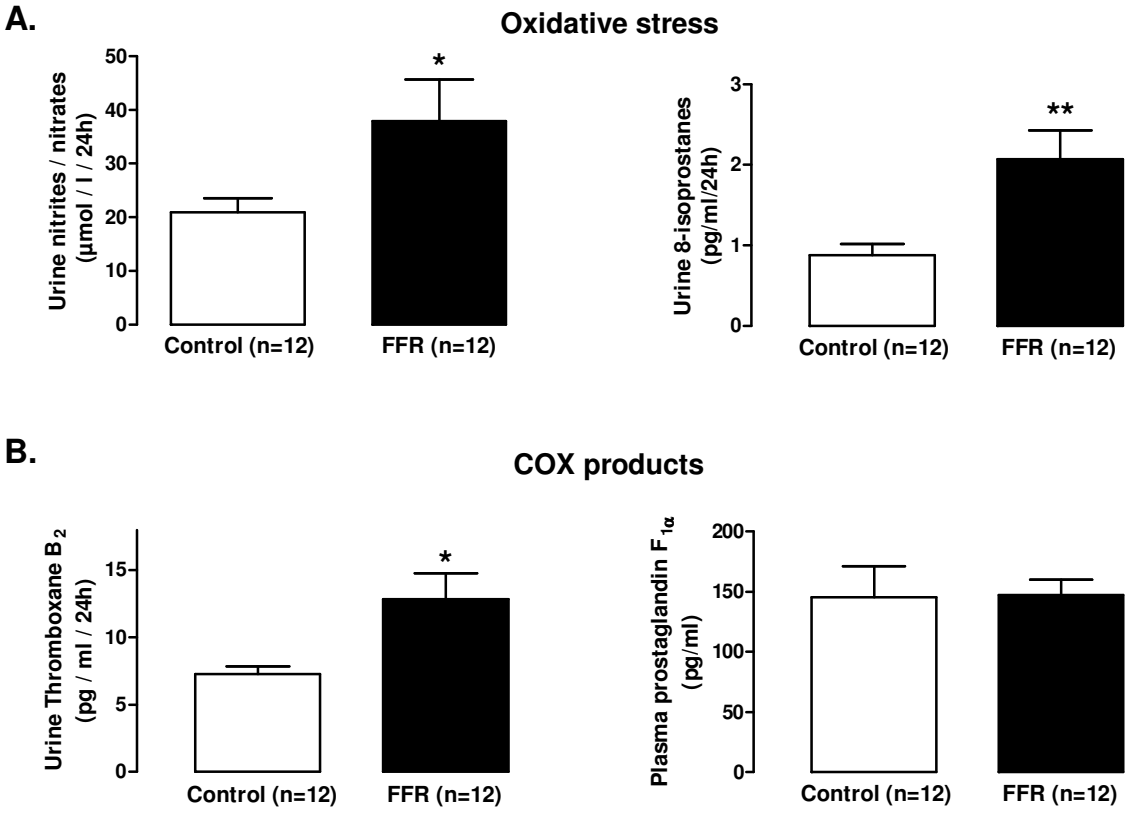

A.
**
Control (n=8)
FFR (n=6)
Change in MAP (mmHg)
Norepinephrine (ng/kg/min)
B.
**
Control / vehicle (n=4)
Control / Indomethacin (n=4)
+ vehicle
C.
***
FFR / vehicle (n=3)
FFR / Indomethacin (n=4)
+ indomethacin
D.
ns
Control / Indomethacin (n=4)
FFR / Indomethacin (n=4)
+ indomethacin

FIGURE 3


A.
Oxidative stress
Urine nitrites / nitrates (µmol / l / 24h)
50
40
30
20
10
0
*
Control (n=12)
FFR (n=12)
Urine 8-isoprostanes (pg/ml/24h)
3
2
1
0
**
Control (n=12)
FFR (n=12)
B.
COX products
Urine Thromboxane B2 (pg / ml / 24h)
15
10
5
0
*
Control (n=12)
FFR (n=12)
Plasma prostaglandin F1α (pg/ml)
200
150
100
50
0
Control (n=12)
FFR (n=12)