ORIGINAL ARTICLE

Losartan and Metformin Prevent Abnormalities in Perivascular Adipose Tissue and in Mesenteric Vascular Bed Prostanoid Release Induced by High-fat High-fructose Diet in Rats

Losartán y metformina previenen alteraciones en el tejido adiposo perivascular y en la liberación de prostanoides del lecho vascular mesentérico producidas por una dieta alta en grasa y sobrecarga de fructosa en ratas

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ABSTRACT

Objective: The aim of this study was to analyze the effects of losartan (30 mg/kg/day) and metformin (500 mg/kg/day) on the adiposity index and the mesenteric vascular bed prostanoid release, and their relationship with systolic blood pressure in a metabolic syndrome model induced by high-fat high fructose-diet in male Sprague-Dawley rats for 9 weeks..
Methods: Mesenteric vascular beds were extracted and incubated and prostanoids were measured by high-performance liquid chromatography. Systolic blood pressure was measured by an indirect method.
Results: High-fat high-fructose diet produced a significant increase in systolic blood pressure and mesenteric vascular bed adiposity index and in the release of vasoconstrictor prostanoids as thromboxane B_2 and prostaglandin $F_{2\alpha}$ and of prostaglandin E_2, a marker of inflammation. The PGI_2/TXA_2 ratio was significantly reduced. The administration of losartan and metformin prevented all these changes.
Conclusion: Both drugs have beneficial effects on mesenteric perivascular adipose tissue by improving endothelial dysfunction induced by an imbalance of vasoactive substances.

Keywords: Adipose Tissue - Metabolic Syndrome -Hypertensión - Mesentery/blood supply -Prostaglandins/metabolism

RESUMEN

Objetivo: El objetivo de este trabajo fue analizar los efectos del losartán (30 mg/kg/día) y de la metformina (500 mg/kg/día) sobre el índice de adiposidad y la liberación de prostanoides del lecho vascular mesentérico, así como su relación con la presión arterial sistólica en un modelo de síndrome metabólico inducido por una dieta alta en grasa y sobrecarga de fructosa en ratas Sprague-Dawley macho durante 9 semanas.
Material y métodos: Los lechos vasculares mesentéricos extraídos se incubaron y los prostanoides liberados se midieron por cromatografía líquida de alta eficiencia. La presión arterial sistólica fue medida por método indirecto.
Resultados: La dieta alta en grasa y la sobrecarga de fructosa produjo aumentos significativos en la presión arterial sistólica y del índice de adiposidad del lecho vascular mesentérico. Por su parte, la dieta alta en grasa y sobrecarga de fructosa incrementó la liberación de prostanoides vasoconstrictores tanto del tromboxano B_2 como de la prostaglandina F_{2alfa}; y del marcador de inflamación, la prostaglandina E_2. La relación PGI_2/TXA_2 se redujo significativamente. La administración de losartán como de metformina previnieron todas estas alteraciones.
Conclusión: Ambos fármacos ejercen efectos beneficiosos sobre el tejido adiposo perivascular del lecho mesentérico, lo que mejora la disfunción endotelial inducida por un desbalance de sustancias vasoactivas.

Palabras claves: Tejido Adiposo - Síndrome Metabólico - Hipertensión - Mesenterio/irrigación sanguínea - Prostaglandinas/suministro de sangre

Abbreviations

SBP	Systolic blood pressure	**PVAT**	Perivascular adipose tissue
PG	Prostaglandins	**TX**	Thromboxane
PR	Prostanoids		

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INTRODUCTION

The hypothesis that perivascular adipose tissue (PVAT) dysfunction is a common pathophysiological process that could link metabolic and cardiovascular diseases is becoming increasingly strong and is one of the new therapeutic targets to consider. (1-3)

Experimental models in rats with nutritionally unbalanced diets such as the high-fat high-fructose diet resemble the characteristic disorders of metabolic syndrome in humans (4-6) and are essential to understand the pathophysiological aspects of this syndrome and to be used in pharmacological studies. (7)

The mesenteric vascular bed was considered until now almost without clinical relevance, but it is important to take it into account since it is made up of resistance arteries, and the PVAT consists mainly of white visceral adipose tissue which has higher pathogenicity. In addition, it is a source of prostanoids including prostaglandins (PG) and thromboxanes (TX) which participate in the regulation of the vascular tone. (8, 9) We have previously found an abnormal pattern of prostanoid release in mesenteric vessels. (10)

Losartan and metformin are well-established drugs in daily clinical practice for the treatment of hypertension and metabolic syndrome, but their pleiotropic effects still generate great interest. (11, 12)

In this setting, the aim of this study was to analyze the effects of losartan and metformin on the adiposity index and the mesenteric vascular bed prostanoid release, and their relationship with systolic blood pressure (SBP) in a metabolic syndrome model induced by high-fat high fructose-diet in rats.

METHODS

Six-week-old male Sprague-Dawley rats weighing 180-210 g at the beginning of the study were randomly divided into six groups (n=6 in each group) and treated as follows for 9 weeks: C (controls) were fed standard rodent diet (Asociación Cooperativas Argentinas, with the following composition [w/w]: 20% proteins, 3% fat, 2% fiber, 6% minerals and 69% starch and vitamin supplements) and tap water to drink; HFHF (high-fat high fructose diet) had 50% (w/w) bovine fat added to standard rodent diet (elaborated in our laboratory) and a 10%(w/v) fructose solution to drink; CL and HFHFL were treated with losartan 30 mg/kg/day in the drinking water; CM and HFHFM were treated with metformin 500 mg/kg/day in the drinking water. All the animals were allowed to feed and drink ad libitum during the experiment. Diet and drug treatments started at the same time. The doses of losartan and metformin were chosen according to previous studies and references. (10, 13-17) Losartan and metformin of the highest commercial grade available were purchased from Droguería Saporiti SACIFIA (Buenos Aires, Argentina).

Blood pressure measurement

The animals were trained three times a week. Indirect systolic blood pressure (SBP) was determined at the beginning and at the end of the experimental period by means of an indirect plestimographic method using a sphygmomanometer consisting of an inflatable tail-cuff and a microphone connected to a Grass DC amplifier (model 7 DAC, Grass Instruments Co.) coupled to a polygraph chart recorder (Model 79, Grass Instruments Co.).

Body weight and mesenteric vascular bed adiposity index assessment

Body weight in rats was monitored throughout the study period. Body weight gain was calculated as the difference between body weight after and before treatment. Mesenteric vascular bed adiposity index was calculated as mesenteric vascular bed fat weight/body weight × 100.

Assessment of blood metabolic parameters

At the end of the treatment, all the animals were fasted for 5 h and immediately before being sacrificed blood samples were collected from the retro-orbital sinus under light anaesthesia (intraperitoneal xylazine 2 mg/kg and ketamine 60 mg/kg) and centrifuged at 2700 rpm during 20 minutes at 4°C. The following parameters were measured: plasma glucose (ACCU-Check®, Roche Diagnostics GmbH, Mannheim, Germany), plasma triglycerides with a commercially available kit (TG Color GPO/PAP AA, Wiener Labs, Rosario, Santa Fe, Argentina) by spectrophotometry and insulin by ELISA (Millipore Corporation, Billerica, MA, USA). The homeostasis of insulin resistance (HOMA-IR) assessment model was calculated after treatment using the following equation: HOMA = fasting plama glucose (mmol/L) × fasting insulin (mIU l−1)/22.5. (18)

Assessment of prostanoid release

The mesenteric vascular bed (which includes perivascular adipose tissue and blood vessels) was dissected from all the animals in the six groups and transferred to a Petri dish with Krebs solution (mmol/L): NaCl 118, KCl 4.7, MgSO4 1.2, NaH2PO4 1.0, CaCl2 2.6, NaHCO3 25.0, glucose 11.1, and incubated for 60 min at 37°C. Then, the mesenteric vascular beds were removed and weighed. At the end of the incubation period the medium was acidified to pH 3.5 with 1 mol/L formic acid and extracted three times with 2 volumes of chloroform to measure prostanoid release. The chloroformic fractions were pooled and evaporated to dryness. Reversed-phase high performance liquid chromatography (HPLC) was carried out on a column (BBS Hypersil C18, Thermo Electron Co., Bellefonte, PA, USA). The solvent system was 1.7 mmol/L H3PO4 67.2: acetonitrile 32.8 v/v. The flow rate was 1 mL/min, and UV absorption was measured at 218 nm. Dried samples were resuspended in 0.15 mL of the mobile phase and injected into the HPLC system. Authentic prostanoid standards of 6-keto prostaglandin (PG) F1α (stable metabolite of PGI2 or prostacyclin), PGE2, PGF2α and thromboxane (TX) B2 (stable metabolite of TXA2) (Sigma Chemical Co., Saint Louis, MO, USA) were run along with the samples, and a support assay was performed to determine the amount of prostanoids. All the values were corrected for loss of recovery as determined by parallel standards. The results were expressed as nanograms of prostanoid per milligram of wet tissue weight.

Statistical analysis

Statistical analysis was performed using InfoStat 2018 software package, FCA, Argentina. ANOVA was used for comparisons between groups, followed by Tukey's test. Pearson's correlation coefficients (r) of the data points from the experimental rats were calculated by linear regression. The results are expressed as mean ± SEM. A p value <0.05 was considered statistically significant.

Ethical considerations

The experiments were previously approved by the institutional Ethics Committee for the Care and Use of Laboratory Research (CICUAL, Comité Institucional para el Cuidado y Uso de Animales de Laboratorio, School of Pharmacy and Biochemistry, University of Buenos Aires, Resolution Nº 2259). All animals included in the experimental protocols were handled and housed following CICUAL guideline recommendations in accordance with the ethical standards established by international regulations and principles of care and use of experimental animals.

RESULTS

Effects of losartan and metformin on glucose, triglyceride and insulin levels and HOMA-IR

At the end of the treatment, rats fed a high-fat high-fructose diet showed significantly greater plasma levels of glucose, triglycerides and insulin and higher HOMA-IR index compared with the control group (HFHF vs. C, $p < 0.01$; Table 1). The administration of losartan and metformin prevented all these changes at 9 weeks of treatment (HFHFL vs. HFHF, HFHFM vs. HFHF $p < 0.01$ and $p < 0.05$, respectively; Table 1) in the group with high-fat high fructose diet.

Effects of losartan and metformin on systolic blood pressure, mesenteric vascular bed adiposity index and body weight gain

Systolic blood pressure, mesenteric vascular bed adiposity index and body weight gain were significantly higher in rats fed a high-fat high-fructose diet versus the control group (HFHF vs. C, $p < 0.01$; Table 1). Losartan reduced SBP in the control group (CL vs. C, $p < 0.01$; Table 1). In addition, we found that increased mesenteric vascular bed adiposity index had a positive correlation with SBP ($r = 0.82$, $p < 0.01$; Figure 1). Drug therapy not only prevented the increase in SBP but also the increase in the mesenteric vascular bed adiposity index and body weight gain in rats fed a high-fat high-fructose diet (HFHFL vs. HFHF, HFHFM vs. HFHF, $p < 0.01$; Table 1).

Effects of losartan and metformin on mesenteric vascular bed prostanoid release

Rats fed a high-fat high-fructose diet presented higher release of vasoconstrictor prostanoids (PR), thromboxane B2 and prostaglandin F2α (ng PR/mg of tissue, HFHF vs. C, $p < 0.01$, Figure 2 and Figure 3) compared with the control group. We also found that the increase of both PR had a positive correlation with the increase in SBP (TXB_2: $r = 0.89$, $p < 0.01$; $PGF_{2\alpha}$: $r = 0.80$, $p < 0.01$) and the mesenteric vascular bed adiposity index (TXB2: $r = 0.90$, $p < 0.01$ and $PGF_{2\alpha}$: $r = 0.84$, $p < 0.01$). Losartan and metformin prevented these abnormalities (ng PR/mg of tissue, HFHFL vs. HFHF, TXB2: $p < 0.01$, Figure 2; $PGF_{2\alpha}$: $p < 0.05$, Figure 3, and ng PR/mg of tissue, HFHFM vs HFHF, TXB2: $p < 0.05$, Figure 2; $PGF_{2\alpha}$: $p < 0.05$, Figure 3).

The high-fat high-fructose diet produced a significant increase in the release of PGE_2 (ng/mg, HFHF vs. C, $p < 0.01$, Figure 4), a marker of inflammation, compared with controls. We also found a positive and significant correlation between this increase and higher SBP ($r = 0.75$, $p < 0.01$, Figure 5) and higher mesenteric vascular bed adiposity index ($r = 0.82$, $p < 0.01$). Both drug treatments prevented this increase (ng PR/mg of tissue, HFHFL vs. HFHF, HFHFM vs. HFHF, $p < 0.05$, Figure 4).

The PGI_2/TXA_2 ratio (measured as their stable metabolites) was reduced by the high-fat high-fructose diet at 9 weeks of treatment compared with the control group (HFHF vs. C, $p < 0.01$, Figure 6). Losartan and metformin attenuated the reduction of this ratio (HFHFL vs. HFHF, HFHFM vs. HFHF, $p < 0.01$, Figure 6).

DISCUSSION

The present study reports, for the first time, the evidence of the preventive effect of losartan and metformin on the increase of mesenteric vascular bed adiposity and release of vasoconstrictor (TXB2 and $PGF_{2\alpha}$) and pro-inflammatory (PGE_2) prostanoids in an experimental model of metabolic syndrome induced by the combination of two diets which more adequately reflects the dietary patterns of the societies today. We also showed the positive effect of both drugs on the reduction of the PGI_2/TXA_2 ratio, a marker of endothelial dysfunction.

Regarding the results of the adiposity index, we found different criteria for the selection of visceral fat

Table 1. Systolic blood pressure, mesenteric vascular bed adiposity index, body weight gain, triglycerides, glucose, insulin and HOMA-IR index

	SBP (mmHg)	Adiposity index (%)	Weight gain (g)	Triglycerides (mg/dL)	Glucose levels (mg/dL)	Insulin (ng/mL)	HOMA-IR
C	119 ± 2	0.7 ± 0.1	182 ± 4	65 ± 9	116 ± 3	1.2 ± 0.1	0.1 ± 0.003
CL	108 ± 1†	0.7 ± 0.06	187 ± 10	66 ± 8	119 ± 4	0.9 ± 0.1	0.1 ± 0.01
CM	122 ± 1	0.6 ± 0.07	177 ± 4	56 ± 9	117 ± 3	1.3 ± 0.2	0.1 ± 0.02
HFHF	150 ± 3 *	1.8 ± 0.1 *	308 ± 21 *	188 ± 16 *	141 ± 3 *	3.4 ± 0.4 *	0.4 ± 0.05 *
HFHFL	112 ± 1 ‡	1.0 ± 0.05 ‡	169 ± 13 ‡	74 ± 8 ‡	131 ± 4	1.6 ± 0.2 ‡	0.1 ± 0.03 §
HFHFM	125 ± 1 ‡	1.3 ± 0.04 ‡	175 ± 9 ‡	64 ± 6 ‡	126 ± 2 ‡	1.3 ± 0.4 ‡	0.1 ± 0.05 §

Values are expressed as mean ± SEM. C: Control; CL: Control + losartan; CM: Control + metformin; HFHF: High-fat high-fructose diet; HFHFL: High-fat high-fructose diet + losartan; HFHFM: High-fat high-fructose diet + metformin; SBP: Systolic blood pressure. HOMA-IR: Homeostasis of insulin resistance. *$p < 0.01$ vs. C, CL, CM; † $p < 0,01$ vs. C; ‡ $p < 0,01$ vs. HFHF; § $p < 0.05$ vs. HFHF.

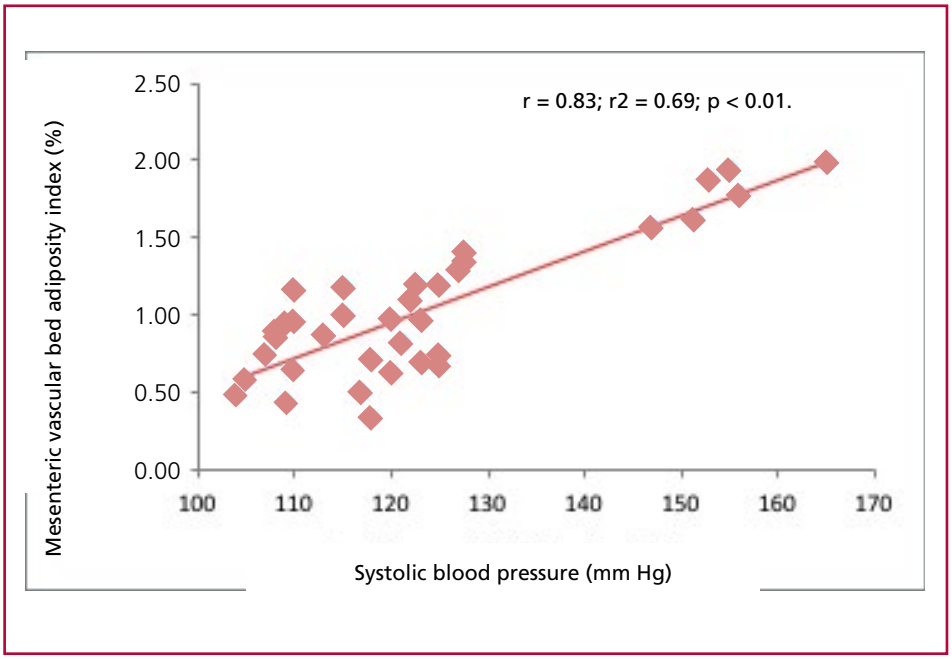

Fig. 1. Linear regression between mesenteric vascular bed adiposity index vs. systolic blood pressure.

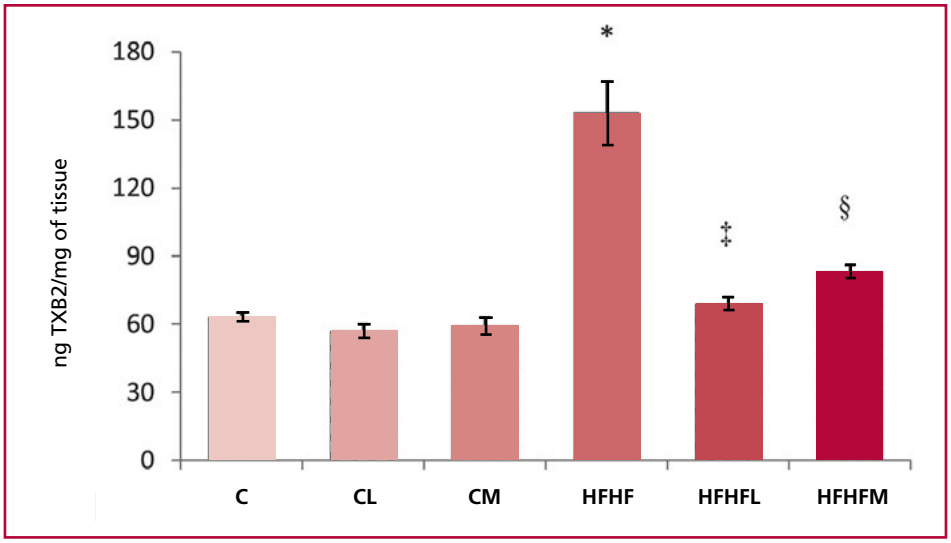

Fig. 2. Thromboxane B2 release from the mesenteric vascular bed.

Values are expressed as mean ± SEM. C: Control; CL: Control + losartan; CM: Control + metformin; HFHF: High-fat high-fructose diet; HFHFL: High-fat high-fructose diet + losartan; HFHFM: High-fat high-fructose diet + metformin, *p < 0.01 vs. C, CL, CM; ‡ p < 0.01 vs. HFHF; § p < 0.05 vs. HFHF.

to determine the abnormalities in the white adipose tissue mass. Mourand et al. (19) reported the effect of losartan in the visceral fat/gastrocnemius muscle ratio considered as an index of body composition. Tikko et al. (20) showed that the proportion of white adipose tissue/body weight increased in rats fed a high-fat diet and that treatment with metformin reduced this parameter. However, none of these authors specified in which organ white adipose tissue was measured. In an experimental model with SHR fed a high-fat diet plus losartan, Wang et al, (21) reported that these animals exhibited a significant reduction in the percent mesenteric fat pad weight/body weight ratio.

The accumulation of ectopic fat in the PVAT could be relevant for the pathogenesis of hypertension associated with insulin resistance. (22) One of the possible mechanisms involved could be due to PVAT dysfunction (according to the specific vascular bed) induced by a high-fat high-fructose diet that produces changes in the number and the expression pattern of vasoactive factors, contributing to the propensity of the vessels to develop vascular disease. Increased mass and lack of anti-contractile effect of the PVAT induced by a high-fat diet could be caused by an imbalance in the release of adipokines, inflammation, oxidative stress and endothelial dysfunction. (23-25)

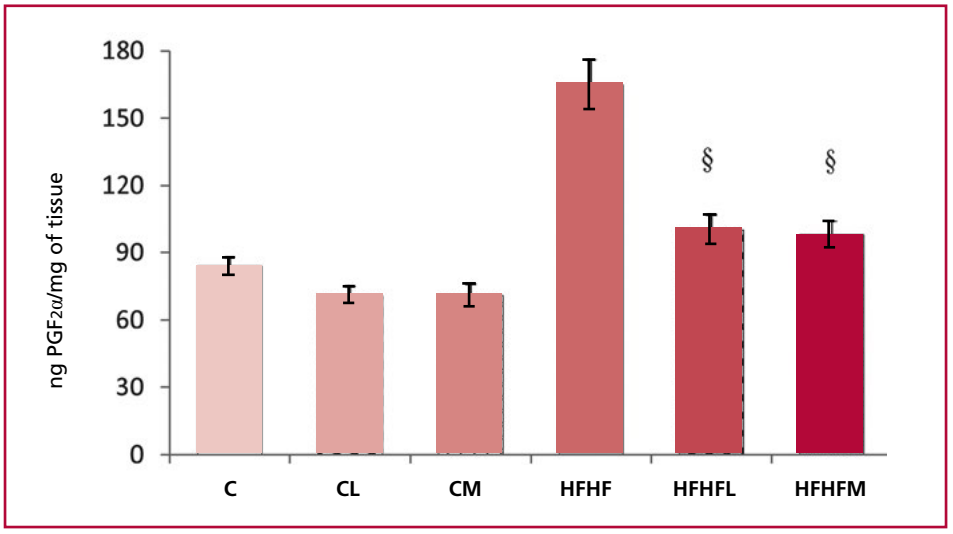

Fig. 3. Prostaglandin F2α release from the mesenteric vascular bed.

Values are expressed as mean ± SEM. C: Control; CL: Control + losartan; CM: Control + metformin; HFHF: High-fat high-fructose diet; HFHFL: High-fat high-fructose diet + losartan; HFHFM: High-fat high-fructose diet + metformin, *p < 0.01 vs. C, CL, CM; § p < 0.05 vs. HFHF.

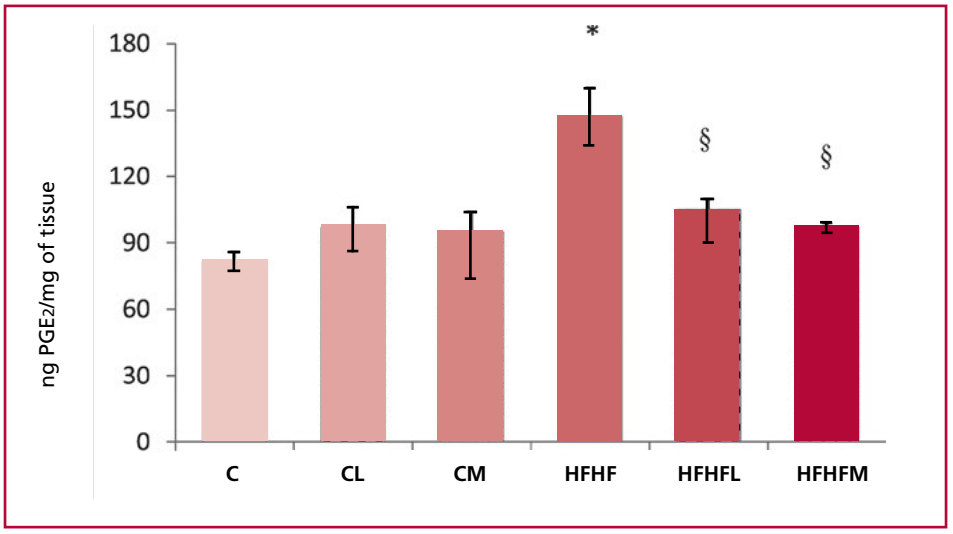

Fig. 4. Prostaglandin E2 release from the mesenteric vascular bed.

Values are expressed as mean ± SEM. C: Control; CL: Control + losartan; CM: Control + metformin; HFHF: High-fat high-fructose diet; HFHFL: High-fat high-fructose diet + losartan; HFHFM: High-fat high-fructose diet + metformin, *p < 0.01 vs. C, CL, CM; § p < 0,05 vs. HFHF.

Prostanoids are fundamental for endothelial physiology and there has been increasing interest in their possible actions on blood vessels. We now know that these substances can derive from both the endothelium and the PVAT, which is considered part of the vascular structure with paracrine function. The increase in vasoconstrictive prostanoids may be related to a higher production of reactive oxygen species, which seem to play an important role in the vascular impairment induced by PVAT. This would lead to a reduction in the expression of eNOS that affects the bioavailability of NO, thus contributing to endothelial dysfunction, which would correlate with AMPK reduction. (26-28)

In accordance with our results, Matsumoto et al. (29) had previously reported that metformin significantly reduced blood pressure and the release of TXB_2 and PGE_2 in the mesenteric arteries induced by acetylcholine in OLETF rats (a type 2 diabetes hypertensive model). In addition, the release of endothelium-derived prostanoids (6-keto-$PGF_{2\alpha}$, PGE_2, $PGF_{2\alpha}$ and TXB_2) in mesenteric artery rings was significantly suppressed in the losartan-treated OLETF group. (30)

Metformin activates AMPK which regulates adipocyte metabolism and vascular structure and function. (31-33) On the other hand, PVAT in the mesenteric arteries has a local renin-angiotensin system with a high density of angiotensin II type 1 receptors (AT1). (34-36.

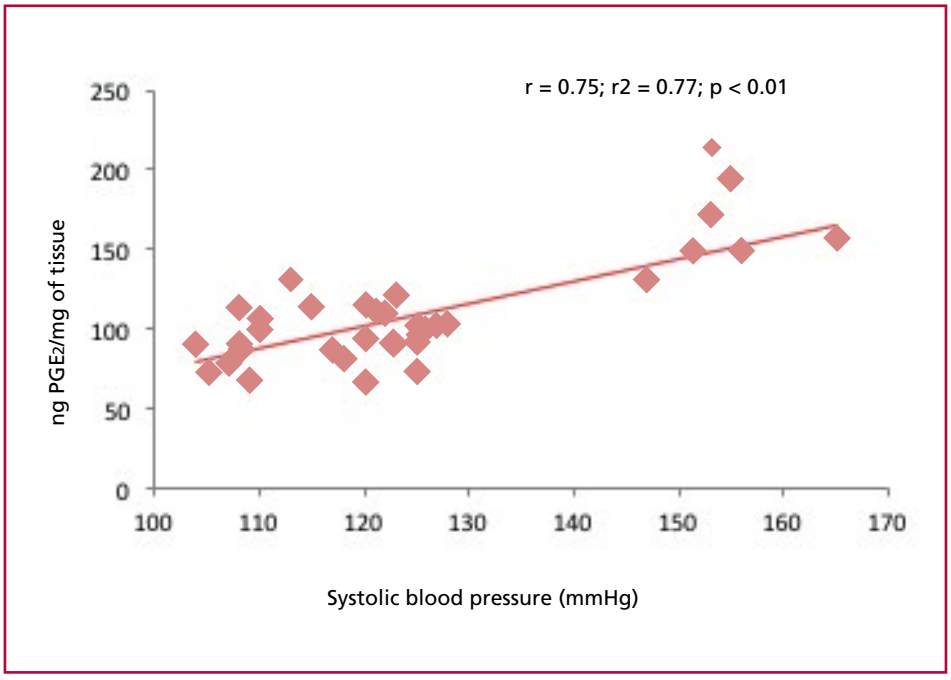

Fig. 5. Linear regression between prostaglandin E_2 vs. systolic blood pressure

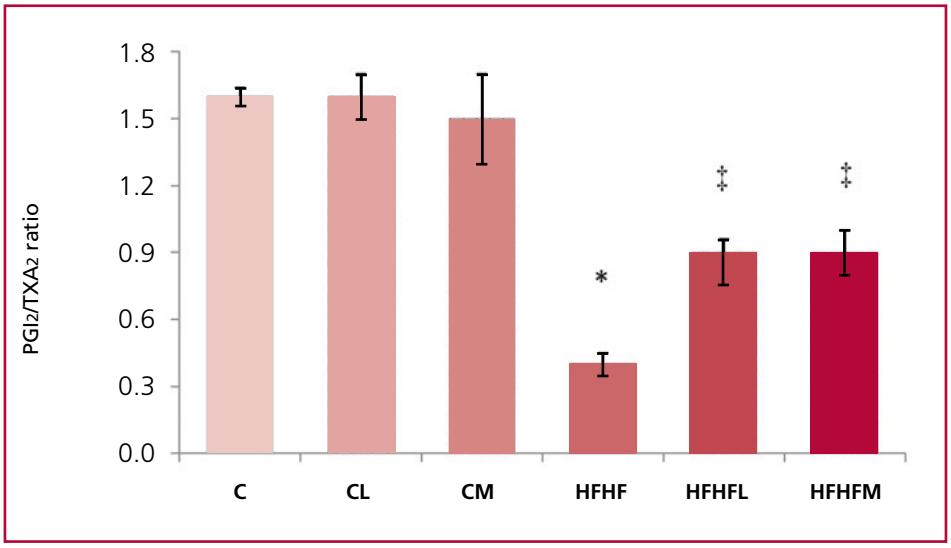

Fig. 6. Prostaglandin I_2/thromboxane A_2 ratio.

Values are expressed as mean ± SEM. C: Control; CL: Control + losartan; CM: Control + metformin; HFHF: High-fat high-fructose diet; HFHFL: High-fat high-fructose diet + losartan; HFHFM: High-fat high-fructose diet + metformin, *p < 0.01 vs. C, CL, CM; ‡ p < 0.01 vs. HFHF.

CONCLUSIONS

Losartan and metformin have beneficial effects on the mesenteric perivascular adipose tissue beyond their respective antihypertensive and insulin-sensitizing effects, by improving endothelial dysfunction induced by an imbalance of vasoactive substances in the mesenteric vascular bed in this experimental dietary model.

Conflicts of interest

None declared.

(See authors' conflicts of interest forms on the website/ Supplementary material)

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