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A high-fat plus fructose diet produces a vascular prostanoid alterations in the rat

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Summary

1. In the rat, a high-fat (HF) plus fructose (F) diet produces cardiovascular and metabolic alterations that resemble human metabolic syndrome. Prostanoids (PR), cyclo-oxygenase-derived arachidonic acid metabolites, have vasoactive properties and mediate inflammation.
2. The aim of this study was to analyse the effect of a HF+F diet on blood pressure (BP), metabolic parameters and mesenteric vascular bed PR production in male Sprague–Dawley rats.
3. Four groups were studied over 9 weeks (n = 6 each): control (C), standard diet (SD) and tap water to drink; F+SD and 10% w/v F solution to drink; HF 50% (w/w) bovine fat added to SD and tap water; and HFF, both treatments. PR were determined by HPLC.
4. Blood pressure was elevated in all experimental groups. Triglyceridaemia, insulinaemia and HOMA-IR were increased in the F and HF groups. HF+F animals showed elevated glycaemia, insulinaemia, HOMA-IR and triglyceridaemia. F decreased the vasodilator prostanoids PGI_2 and PGE_2 in the mesenteric vascular bed. Body weight was not significantly altered. In HFF, production of PGE_2, PGF_2alpha and TXB_2 was elevated.
5. The increased BP in HF and HFF could be partly attributed to the imbalance in vascular PR production towards vasoconstrictors. On the other hand, this dietary modification could induce inflammation, which would explain the elevation of PGE_2. In the F group, hypertension could be related to decreased vasodilator PRs.
6. The simultaneous administration of HF and F in the rat produces deleterious effects greater than observed when treatments are applied separately.

Keywords: high fat, fructose, hypertension, prostanoids, metabolic syndrome

Introduction

Changes in food habits, specially an increased intake of lipids and carbohydrates induce an elevation of plasma catecholamines, which produces sympathetic activation, an increase in plasma insulin and can cause insulin resistance (Arone *et al.*, 1995). These alterations can provoke a mild hypertensive state. Experimental models such as fructose-overload and/or high-fat diets in rats could mimic the dietary alterations observed in western populations over the last decades, resembling human metabolic syndrome (Valensi, 2005; Jenssen, 2008; Hariri & Thibault, 2010).

Agents derived from the vascular wall could also be involved in metabolic syndrome-induced hypertension. Eicosanoids, metabolites of arachidonic acid, are a large class of lipids with relevant biological activity and have a fundamental role in the maintenance of homoeostasis. Eicosanoids levels are frequently altered in pathophysiological states and can contribute to the evolution of disease processes. Among them are the prostanoids, metabolites of arachidonic acid through the cyclo-oxygenase pathway. These substances have been implicated in increased peripheral resistance, which has been postulated as one of the mechanisms involved in fructose-induced elevation of blood pressure. Moreover, an altered pattern of prostanoid release has been previously found in mesenteric vessels of experimental diabetic (Peredo *et al.*, 2006) and fructose-treated rats (Puyó *et al.*, 2004).

Therefore, the aim of this study was to analyse the effects of fructose overload, a high-fat diet and the combination of both treatments on metabolic parameters, blood pressure and prostanoid release in the rat mesenteric vascular bed.

Methods

Male 6-week-old Sprague–Dawley rats weighing 170–210 g at the beginning of the study were

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used. The experiments were approved in advance by the local ethics committee on animal research. Animals were maintained in a room at 22 ± 2 °C where the air was adequately recycled.

The rats were acclimatized to the procedure of blood pressure measurement at 10.00 AM, twice a week, for 2 weeks prior to sacrifice. Indirect systolic blood pressure was measured by means of a photoelectric tail-cuff connected to an amplifier (II TC model 47; Innovators in Instrumentation, Landing, NJ, USA) in series with an oscilloscope (type 532, Tektronic Inc., Portland, OR, USA). In addition, rats were weighed before dietary manipulation and at the end of the study.

Twenty-four rats were randomly divided in for four groups ($n = 6$ each): controls (C), tap water to drink and fed with standard rodent diet (SD, Asociación Cooperativas Argentinas) with the following composition (w/w): 20% proteins, 3% fat, 2% fiber, 6% minerals and 69% starch and vitamins supplements; fructose-overloaded (F), fructose solution (10% w/v) to drink, SD; high-fat diet (HF), tap water to drink, fed with 50% (w/w) bovine fat added to SD; and fructose plus high-fat diet (HFF), both treatments.

After 9 weeks, all groups were fasted for 5 h and weighed. Blood samples were collected from the retro-orbital sinus and centrifuged at 4 °C. Plasma triglyceride levels were immediately measured by means of commercial kits (TG Color GPO/PAP AA, Wiener Labs, Rosario, Santa Fé, Argentina) using spectrophotometric methods; plasma glucose by a blood glucose meter (Accu-Chek, Roche Diagnostics GmbH, Mannheim, Germany) and insulin by ELISA (Millipore Corporation, Billerica, MA, USA). After sample collection, the animals were killed and the liver, kidneys and heart dissected and weighed, to calculate the hypertrophy index as the ratio between mg of heart and weight/g of body weight. Homoeostasis model of assessment – insulin resistance index (HOMA-IR) was calculated using the following equation: HOMA = fasting glucose (mM) × fasting insulin (mIU l^{-1})/22.5 (Matthews *et al.*, 1985).

The mesenteric beds of animals of all groups were dissected and transferred to a Petri dish with Krebs′ solution (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, NaH_2PO_4 1.0, $CaCl_2$ 2.6, $NaHCO_3$ 25.0, glucose 11.1. The tissues were incubated in that solution for 60 min at 37 °C. To measure prostanoid release, at the end of the incubation period, the media was acidified to pH 3.5 with 1 M formic acid and extracted three times with 2 volumes of chloroform. The chloroform fractions were pooled and evaporated to dryness. Reversed-phase HPLC was carried out on a column (BBS Hypersil C18, Thermo Electron Co., Bellefonte, PA, USA). The solvent system was 1.7 mM H_3PO_4 67.2: acetonitrile 32.8 v/v. The flow rate was 1 ml min^{-1}, 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) F_1alpha (stable metabolite of PGI_2 or prostacyclin), PGE_2, PGF_2alpha and thromboxane (TX) B_2 (stable metabolite of TXA_2) (Sigma Chemical Co., Saint Louis, MO, USA) were run along with the samples, and a bracket assay was performed to determine the amount of prostanoid. All values were corrected for recovery loss as determined by parallel standards. Results were expressed as nanograms of prostanoid per milligram of wet tissue weight.

Statistical analysis

All data are expressed as mean ± SEM. Intergroup comparisons were made by one-way analysis of variance (ANOVA). When necessary, the Tukey *post test* was applied. Linear regression was performed of systolic blood pressure against body weight. A P value <0.05 was considered statistically significant.

Results

Systolic blood pressure, body and organ weight, hypertrophy index, homoeostasis model assessment: insulin resistance (HOMA-IR) and plasma data are shown in Table 1. Systolic blood pressure levels were elevated in all groups compared to controls (F, $P < 0.05$; HF, $P < 0.01$ and HFF, $P < 0.001$ *vs.* C). Triglyceridaemia ($P < 0.01$ *vs.* C) and insulinaemia ($P < 0.05$ *vs.* C) were increased in the fructose overload and high-fat diet groups. The animals with both treatments showed elevated glycaemia, insulinaemia ($P < 0.05$ *vs.* C) and triglyceridaemia ($P < 0.001$ *vs.* C). The HOMA-IR, an index of insulin resistance, was elevated in all treated groups ($P < 0.01$). Neither body, liver and kidney weights nor the hypertrophy index were different among groups. In spite of the fact that body weight was not significantly increased in treated animals, that parameter and systolic blood pressure showed a positive and significant correlation; on the contrary, it was not significant in control animals (Fig. 1).

Fructose overload decreased the production of the vasodilator prostanoids PGI_2 (Fig. 2) and PGE_2 (Fig. 3) in the mesenteric vascular bed ($P < 0.01$ *vs.* C). None of the vasoconstrictor prostanoids measured, $PGF_2\alpha$ (Fig. 4) and TX B_2 (Fig. 5) production was modified by fructose treatment. On the other hand, in the HF and HFF groups, PGI_2 (Fig. 2) release was not altered compared to controls. However, PGI_2 levels were lower in HFF rats to the high-fat diet alone. As

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Table 1 Systolic blood pressure, body and organs weight, hypertrophy index, homoeostasis model assessment: insulin resistance (HOMA-IR) and plasma data

Groups (n = 6)	Control	Fructose	High-fat diet	High-fat diet + Fructose
Body weight (g)	438 ± 15	437 ± 17	486 ± 14	495 ± 20
Liver weight (g)	14.6 ± 1.1	15.9 ± 1.3	16.2 ± 0.6	18.2 ± 1.0
Kidneys weight (g)	2.9 ± 0.2	2.9 ± 0.2	2.9 ± 0.1	3.0 ± 0.2
Hypertrophy index (mg g^{-1})	2.6 ± 0.1	2.6 ± 0.2	2.7 ± 0.1	2.8 ± 0.1
Systolic blood pressure (mm Hg)	121 ± 2	138 ± 3 *	141 ± 3 **	149 ± 5 #
Glycaemia (mg dl^{-1})	124 ± 6	148 ± 4	131 ± 6	159 ± 7*
Insulinaemia (ng ml^{-1})	1.7 ± 0.2	3.6 ± 0.7*	3.4 ± 0.4*	3.7 ± 0.3*
HOMA-IR	11 ± 2	22 ± 2**	26 ± 5**	33 ± 6**
Triglyceridaemia (mg dl^{-1})	89 ± 8	153 ± 12**	161 ± 8**	188 ± 16#

*P < 0.05 *vs.* C, **P < 0.01 *vs.* C, #P < 0.001 *vs.* C.

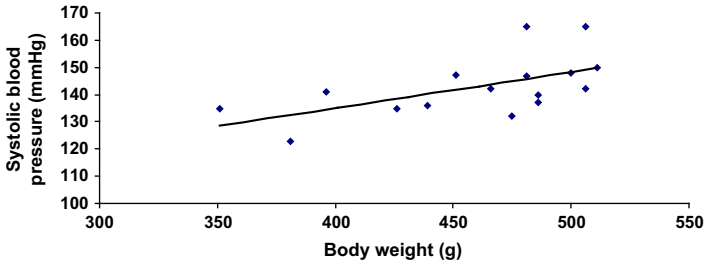

Figure 1 Linear regression of systolic blood pressure against body weight in treated animals: fructose-overloaded (n = 6), high-fat diet-fed (n = 6) and fructose plus high-fat diet (n = 6); r = 0.580, r^2 = 0.336, P < 0.01.

can be seen in Fig. 3, the combined treatment produced an elevation of PGE_2 (P < 0.05 *vs.* C). Furthermore, the high-fat diet alone showed a tendency to increase PGE_2 levels.

Both HF and HFF treatments increased $PGF_2\alpha$ release (Fig. 4, P < 0.01 *vs.* C). Meanwhile, TXA_2 production was augmented only by the combined treatment (Fig. 5, P < 0.05 *vs.* C). The prostacyclin/thromboxane release ratio (measured as their stable metabolites), an index of endothelial dysfunction (Fig. 6), was reduced in all groups compared (F and HFF P < 0.001 *vs.* C; HF P < 0.05 *vs.* C).

Discussion

The present study shows that a high-fat diet, fructose overload and the combination of both treatments produced an increase in triglyceridaemia, insulinaemia, insulin resistance and systolic blood pressure levels in the rat; meanwhile, glycaemia was increased only by combined treatment with a high-fat diet and fructose overload. Regarding mesenteric vascular bed prostanoid production, fructose reduced PGI_2 and PGE_2; high-fat diet alone increased PGF_2 alpha release and the combined treatment increases PGE_2, PGF_2 alpha and

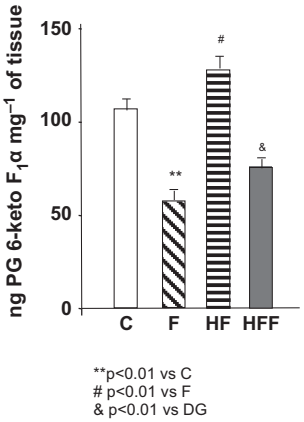

Figure 2 Release of 6-keto PGF_1alpha (ng mg $tissue^{-1}$) in control (C, n = 6), fructose-overloaded (F, n = 6), high-fat diet-fed (HF, n = 6) and fructose-high fat (HFF, n = 6). Results are expressed as mean ± SEM. **P < 0.01 *vs.* C; #P < 0.01 *vs.* F; &P < 0.01 *vs.* HF.

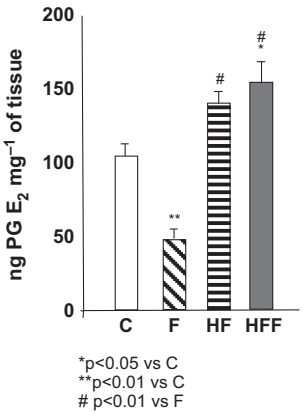

Figure 3 Release of PGE_2 (ng mg $tissue^{-1}$) in control (C, n = 6), fructose-overloaded (F, n = 6), high-fat diet-fed (HF, n = 6) and fructose-high fat (HFF, n = 6). Results are expressed as mean ± SEM. *P < 0.05 *vs.* C; **P < 0.01 *vs.* C; #P < 0.01 *vs.* F

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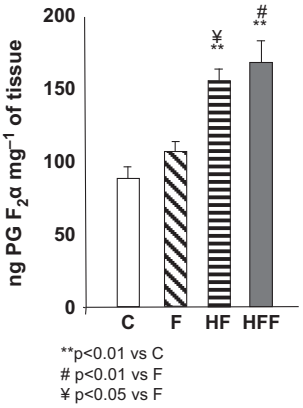

Figure 4 Release of PGF_2 alpha (ng mg $tissue^{-1}$) in control (C, $n = 6$), fructose-overloaded (F, $n = 6$), high-fat diet-fed (HF, $n = 6$) and fructose-high fat (HFF, $n = 6$). Results are expressed as mean ± SEM. $^{**}P < 0.01$ *vs.* C; $\#P < 0.01$ *vs.* F; $¥P < 0.05$ *vs.* F.

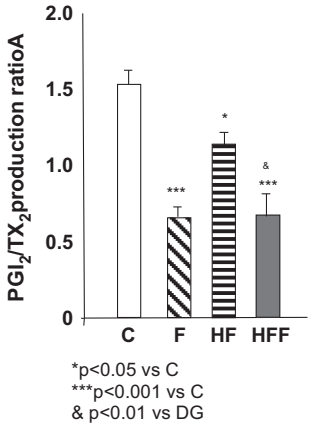

Figure 6 Ratio of PGI_2/TXA_2 in control (C, $n = 6$), fructose-overloaded (F, $n = 6$), high-fat diet-fed (HF, $n = 6$) and fructose-high fat (HFF, $n = 6$). Results are expressed as mean ± SEM. $^{*}P < 0.05$ *vs.* C; $^{***}P < 0.001$ *vs.* C; $\&P < 0.01$ *vs.* HF.

TXA_2. Moreover, the prostacyclin/thromboxane release ratio was reduced in all treated groups relative to control.

Elevated blood pressure levels were previously found in Wistar rats (Panchal *et al.*, 2011; Wong *et al.*, 2012; Alam *et al.*, 2013) with the combined high-fructose-high-fat diet. In spite of the fact that body weight was not significantly increased in either treated groups, we found a positive and significant correlation between systolic blood pressure and body weight in those animals. Epidemiologic studies clearly demonstrate the correlation between body weight and blood pressure in obese and lean populations (Wofford *et al.*, 2008). Moreover, Di Nardo *et al.* (2009) found that in fatty Zucker rats, there was a correlation between mean arterial pressure and body weight.

Regarding the metabolic parameters measured in this study, higher levels of plasma glucose, triglycerides and insulin were also reported in Wistar rats (Wada *et al.*, 2010) showing the characteristics of metabolic syndrome; and an elevation of plasma insulin was found in Sprague–Dawley rats after 10 weeks of administration (Elmarakby & Imig, 2010). Wang *et al.* (2012) reported that in Wistar rats a high-fat diet increased HOMA-IR.

In this study, we found an altered profile of prostanoids production. Previously, we have reported a decrease of prostacyclin and PGE_2 release from the mesenteric vascular bed of fructose-overloaded rats. Both prostanoids have vasodilator properties, and we suggested that this decrease in vasodilators could be involved in the blood pressure elevation in this model (Peredo *et al.*, 2008).

On the other hand, in the high-fat diet group, the production of the vasoconstrictor prostaglandin F_2 alpha was elevated; and in the high-fat plus fructose group of animals, both vasoconstrictor metabolites of arachidonic acid measured, PGF_2 alpha and TXA_2, were increased. In addition, vascular PGE_2, which is also a well-known mediator of inflammation, was elevated in the combined diet group and showed a strong tendency to augment in the high-fat group. Iyer *et al.* (2012) reported that a 16-week administration of high carbohydrate-high-fat diet induced an elevation in PGE_2 concentrations in adipose tissue and plasma of Wistar rats.

As previously reported, one of the mechanisms involved in the elevation of blood pressure in fructose-overloaded rats could be a decrease in the production of vasodilator prostanoids. On the contrary, in the animals with a high-fat diet added to the fructose, the main alteration observed in the vascular prostanoid levels was an increase in the vasoconstrictors PGF_2 alpha and TXA_2. The latter was found elevated in blood

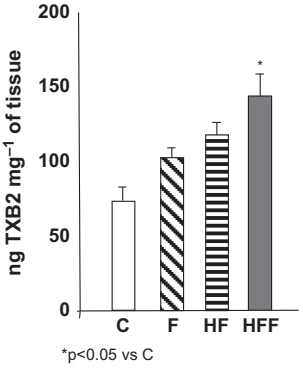

Figure 5 Release of TXB_2 (ng mg $tissue^{-1}$) in control (C, $n = 6$), fructose-overloaded (F, $n = 6$), high-fat diet-fed (HF, $n = 6$) and fructose-high fat (HFF, $n = 6$). Results are expressed as mean ± SEM. $^{*}P < 0.05$ *vs.* C.

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serum of patients with metabolic syndrome (Novgrodtseva *et al.*, 2011). It has been also shown that after 10 weeks of high-fat diet, prepubescent Sprague–Dawley rats developed hypertension and that arachidonic acid-induced vasoconstriction was increased in the aorta of those animals (Smith & Dorrance, 2006).

As far as we know our findings are the first reporting an alteration in vascular prostanoids release in animals under these diet modifications. In another model of insulin resistance and hypertension, the 12-month old offspring of diabetic rats, Ramos-Alves *et al.* (2013) also found elevated levels of PGs E_2 and F_2 alpha, and TXB_2 in stimulated mesenteric vessels.

It is a well-known fact that TXA_2 has an important role in cardiovascular diseases such as atherosclerosis and myocardial infarction. This prostanoid alters endothelial cells function by mean of stimulation of leucocyte adhesion to the vessel wall, an increase of vascular permeability and an enhancement of the expression of adhesion molecules implicated in the inflammatory response. In addition, TXA_2 induces proliferation of smooth muscle cell promoting neointima formation. Song *et al.* (2009) showed that the stimulation of TXA_2 receptors (TP) impairs insulin signalling in vascular endothelial cells by selectively activating the Rho/Rho-associated kinase/LKB1/PTEN pathway. As a result, there is a worsening of insulin resistance with subsequent hyperinsulinemia. In our study, TXA_2 levels were elevated in the group where both diet modifications were administered, coincidentally to high insulin and blood pressure levels.

PGF_2alpha, the other vasoconstrictor prostanoid measured in this study, has been recently proposed to be a new fibrotic hormone (Olman, 2009), and insulin resistance is related to fibrosis (Witteles & Fowler, 2008). Ding *et al.* (2014) showed that the PGF_2alpha-F-prostanoid receptor ($PGF_2\alpha$-FP) is also involved in this process as silencing it may exert a protective effect on diabetic cardiomyopathy. In addition, Tian *et al.* (2012) reported that the release of PGF_2alpha was increased in acetylcholine-stimulated renal arteries of Sprague–Dawley renovascular hypertensive rats, suggesting a role for this prostanoid in endothelial dysfunction.

We also found that the prostacyclin/thromboxane release ratio, a well-known index or endothelial dysfunction (Bunting *et al.*, 1983) was reduced in all treated groups. Accordingly to our results, Chu *et al.* (2011) reported that a high-fat diet shifted the prostanoid balance in favour of thromboxane production over prostacyclin in swine.

In conclusion, the administration of a high-fat diet plus a fructose overload produces metabolic and haemodynamic modifications that are more marked than those of each treatment alone. The elevation in blood pressure found in these experimental models of insulin resistance in the rat could be related, at least in part, to an altered vascular prostanoid release profile. This dietary combination could resemble the so-called junk food, common in many countries at present.

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