ORIGINAL ARTICLE

Effects of Renin-Angiotensin System Blockade on the Renal Dopaminergic System in an Experimental Model of Chronic High-Fat Diet

Efectos del bloqueo del sistema renina angiotensina sobre el sistema dopaminérgico renal en un modelo experimental de dieta crónica alta en grasa

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ABSTRACT

Background: The renal dopaminergic system (RDS) exerts natriuretic and diuretic effects through D1 receptors and anti-inflammatory actions through D2 receptors. In contrast, angiotensin II, via AT1 receptors, generates opposite responses. Chronic consumption of high-fat diets (HFD) is associated with increased blood pressure and renal inflammation.
Objective: This study aimed to evaluate the impact of treatment with losartan, an AT1 receptor antagonist, on the RDS, blood pressure, and renal damage induced by a HFD.
Methods: Male Sprague–Dawley rats were studied for 8 weeks and randomly assigned to four experimental groups (n=4–6): control (C), high-fat diet (HFD), control + losartan (CL), and high-fat diet + losartan (HFDL). Systolic blood pressure (SBP), body, and plasmatic and urinary biochemical and metabolic parameters were assessed. Renal function, urinary excretion of L-dopa and dopamine (L-dopa/dopamine index), expression of receptors, dopamine transporters, and markers of inflammation, as well as renal structure and ultrastructure were also evaluated. Statistical analysis was performed using Student's t-test, one-way ANOVA with Tukey's post hoc test, Pearson's correlation, and linear regression. Results are expressed as mean ± standard error and $p<0.05$ was the level of significance.
Results: Losartan prevented the increase in SBP and the L-dopa/dopamine index (HFD vs. C, $p<0.01$; HFDL vs. HFD, $p<0.01$); the reduction in fractional and urinary sodium excretion and diuresis (HFD vs. C, $p<0.01$; HFDL vs. HFD, $p<0.05$); and decreased expression of the membrane transporter protein OCTN-1,2,3 (HFD vs. C, $p<0.01$; HFDL vs. HFD, $p<0.05$). It avoided overexpression of the dopamine D1 receptor (D1R) and Na^+K^+ATPase (HFD vs. C, $p<0.01$; HFDL vs. HFD, D1R $p<0.01$ and Na^+K^+ATPase $p<0.05$) and reduced the activation of nuclear factor kappa B, and transforming growth factor beta 1 (HFDL vs. HFD, $p<0.01$). It also mitigated structural alterations in the proximal tubules, increased interstitial fibrosis (HFD, $p<0.01$) and ultrastructural changes in the podocyte pedicels observed in HFD.
Conclusions: Under conditions of chronic consumption of a HFD, early administration of losartan favored RDS activity, prevented an increase in SBP, and attenuated interstitial fibrosis and renal inflammation, contributing to protection against target organ damage.

Keywords: Renal dopamine – High-fat diet – Blood pressure – Inflammation – Fibrosis – Losartan

RESUMEN

Introducción: El sistema dopaminérgico renal (SDR) ejerce efectos natriuréticos y diuréticos mediante receptores D1 y acciones antiinflamatorias mediante receptores D2. En contraste, la angiotensina II, vía receptores AT1, genera respuestas opuestas. El consumo crónico de dietas ricas en grasas se asocia con incremento de la presión arterial e inflamación renal.
Objetivo: Evaluar el impacto del tratamiento con losartán, antagonista de receptores AT1, sobre el SDR, la presión arterial y el daño renal inducido por una dieta rica en grasas.
Material y métodos: Ratas macho Sprague–Dawley fueron estudiadas durante 8 semanas y asignadas aleatoriamente a cuatro grupos experimentales (n=4–6): control (C), dieta grasa (DG), control + losartán (CL) y dieta grasa + losartán (DGL). Se determinaron presión arterial sistólica (PAS), parámetros corporales, bioquímicos, metabólicos plasmáticos y urinarios, función renal, excreción

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urinaria de L-dopa y dopamina (índice L-dopa/dopamina), expresión de receptores, transportadores de dopamina y marcadores de inflamación, así como estructura y ultraestructura renal. Análisis estadístico: prueba t+ de Student, ANOVA de una vía con post hoc de Tukey, correlación de Pearson y regresión lineal. Los resultados se expresaron como media ± error estándar; significancia $p<0,05$.
Resultados: El losartán previno el aumento de la PAS y del índice L-dopa/dopamina (DG vs. C, $p<0,01$; DGL vs. DG, $p<0,01$); la reducción de la excreción fraccional y urinaria de sodio y de la diuresis (DG vs. C, $p<0,01$; DGL vs. DG, $p<0,05$); y la disminución en la expresión de la proteína trasportadora de membrana OCTN-1,2,3 (DG vs. C, $p<0,01$; DGL vs. DG, $p<0,05$). Evitó la sobreexpresión del receptor dopaminérgico D1R y la Na^+K^+ATPasa (DG vs. C: $p<0,01$; DGL vs. DG: D1R $p<0,01$; Na^+K^+ATPasa $p<0,05$) y redujo la activación del factor nuclear kappa B, NF-κB, y el factor de crecimiento transformante beta 1 TGF-beta1 (DGL vs. DG, $p<0,01$). Mitigó las alteraciones estructurales de los túbulos proximales, el incremento de fibrosis intersticial (DGL vs. DG, $p<0,01$) y los cambios ultraestructurales en los pedicelos podocitarios observados en DG.
Conclusiones: En condiciones de consumo crónico de dieta rica en grasas, la administración temprana de losartán favoreció la actividad del SDR, previno el aumento de la PAS y atenuó la fibrosis intersticial y la inflamación renal, contribuyendo a la protección frente al daño de órgano blanco.

Palabras clave: Dopamina renal – Dieta rica en grasas – Presión arterial – Inflamación – Fibrosis – Losartán

INTRODUCTION

The worldwide consumption of diets rich in carbohydrates and fats is a key factor in the development of metabolic syndrome (MS), defined by the concurrence of at least three metabolic disorders—hyperglycemia, hypertriglyceridemia, insulin resistance, hypertension (HTN), systemic inflammation, or increased waist circumference — that damage various organs, particularly the kidneys, which are essential for electrolyte homeostasis and blood pressure control. (1,2) High-caloric diets induce structural and functional renal dysfunction and increase the risk of HTN, a chronic and asymptomatic disease responsible for approximately 50% of global cardiovascular mortality. (3,4) According to a 2021 World Health Organization population study published in The Lancet, including 104 million participants, the prevalence of HTN was 59% in women (aged 55–62) and 49% in men (aged 46–52), with only 47% and 38%, respectively, receiving treatment. (5) More recently, in 2023, the WHO report "Global Report on Hypertension: The Race Against a Silent Killer" noted a global prevalence of 34% in men and 32% in women, a difference that tends to disappear with advancing age. (6). In Argentina, the RENATA 2 study (2017) reported an incidence of 36.3% in adults aged 26–60 years, higher in men. (7)

The renal dopaminergic system (RDS) and the renin-angiotensin system (RAS) play a key role in the renal regulation of blood pressure. The RDS, located in the proximal convoluted tubule, promotes natriuresis, diuresis, and anti-inflammatory effects by acting on D1 and D2 receptors, counteracting the action of insulin and angiotensin II (Ang II). (8) Conversely, overactivation of the RAS stimulates tubular reabsorption of sodium and water and increases inflammation and oxidative stress. Both systems maintain functional antagonism, as angiotensin II inhibits dopaminergic synthesis and activity. (9) In this context, the L-dopa/dopamine ratio has been proposed as an early biomarker of renal damage in animal models of fructose-induced HTN. (10)

Pharmacological treatment of HTN includes AT1 receptor (AT1R) antagonists, including losartan (L), a potent and selective competitive blocker that specifically inhibits the effects of Ang II. (11) Although its antihypertensive efficacy is well known, its ability to modulate the RDS–RAS balance in MS induced by high-fat diets (HFD), as part of its nephroprotective action, has not been fully clarified. In this context, we proposed to evaluate whether a HFD alters this balance as a mechanism of HTN and whether early treatment with L prevents these alterations in an experimental model of MS induced by HFD for 8 weeks. The usefulness of the L-dopa/dopamine index as an early biomarker of renal dysfunction was also analyzed.

METHODS

A) Animals, experimental design, and diet.

Male Sprague-Dawley rats (6 weeks old, 180–200 g; Central Animal Facility, FFyB-UBA) were kept under controlled conditions (22±2°C, 50–70% humidity, 12 h light/dark) and studied for 8 weeks. The animals were divided into four groups (n=4–6):

- C: standard diet (SD) and water ad libitum (Asociación Cooperativas Argentinas; 20% protein, 3% fat, 2% fiber, 6% minerals, 69% starch, vitamins; 3.3 kcal/g);
- HFD: SD + 50% w/w fat and water ad libitum (Faty, Quick-food S.A., Argentina; 9 kcal/g, 99% total fat, 77% saturated, 19% trans);
- CL: C + losartan (L), 30 mg/kg/day, water. (Losartan potassium, 100% dry basis, Droguería Saporiti S.A.C.I.F.I.A, Buenos Aires, Argentina);
- HFDL: HFD + CL.

B) Body weight, food consumption, and calories.

Body weight was recorded daily. Food and drink intake was measured every 48 hours. Calculation included: I) food (g/day) = offered – remaining; II) drink (mL/day) = offered – remaining; III) calories: C and CL = SD (g) × 3.3 kcal/g; HFD and HFDL = SD (g) × 3.3 kcal/g + fat (g) × 9 kcal/g.

C) Systolic blood pressure (SBP).

This was measured at the start and before sacrifice using tail plethysmography with a photoelectric sphygmomanometer (Grass D.C. 7DAC) and oscilloscope (Grass D.C. 79D, Grass Instruments Co., USA).

D) Urine and blood collection.

Three days before sacrifice, the animals were housed in metabolic cages for 48 hours. Urine collection: I) the urinary fraction from the first 6 hours was recovered in an aqueous solution of hydrochloric acid with a 6N concentration (HCl 6N), aliquoted in HCl 1N and stored at −80°C for L-dopa

and dopamine assessed by high-performance liquid chromatography (HPLC); II) The urinary fraction from the following 18 hours was stored at −20°C for sodium, creatinine, and albumin assessment (Spectrum CCX, Abbott Diagnostics, USA). Diuresis was measured by total volume urinated in 24 h. On the sacrifice day, after 6-h fasting, blood was obtained by retro-orbital puncture under anesthesia (ketamine 80 mg/kg + xylazine 12 mg/kg, PRO-SER SA).

E) Tissue collection.

The animals were sacrificed under anesthesia in a CO2 chamber. The kidneys were decapsulated, weighed, and randomly distributed for histology, transmission electron microscopy (TEM), and Western blot.

F) Assessments.

F-1) Plasma metabolism: Blood glucose was assessed with Accu-Chek Performa Nano (Roche Diagnostics, Germany), triglyceridemia with TG Color GPO/PAP AA kit (Wiener Lab., Argentina) and insulin by ELISA test (Mercodia Rat Insulin ELISA, Sweden). The triglyceride-glucose product (TyG) (12) and the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) index were calculated. (13)

F-2) Renal function: Sodium and creatinine were measured in plasma and urine (Spectrum CCX), glomerular filtration rate (GFR) was estimated by creatinine clearance and tubular function was assessed by urinary sodium excretion (UNa+V) and fractional excretion of sodium (FENa).

F-3) Urinary catecholamines: L-dopa and dopamine were measured by reverse-phase HPLC (Zorbax RxC18 column; DuPont, USA; amperometric detection, ESA, USA). Internal standard: 3,4-dihydroxybenzylamine (Sigma-Aldrich, Cat. No. 858781). External standards were: L-dopa (Cat. No. 13248) and dopamine (Cat. No. 21992), both from Cayman Chemical. The detection limit was 20 pg/sample.

F-4) Albuminuria: It was measured with Cobas equipment (ALBT2 Tina-quant Albumin Gen.2; Roche Diagnostics, Switzerland); The urinary albumin/creatinine ratio was determined, considering microalbuminuria between 30–300 mg/g. (14)

F-5) Renal protein expression of the amino acid and thyroid hormone transporter LAT2, membrane transport proteins OCT2 and OCTN1,2,3, dopaminergic receptors D1R and D2R, Ang II receptor, sodium-potassium ATPase (Na^+K^+ATPase), transforming growth factor beta 1 (TGF-beta1), and nuclear factor kappa B1 (NFkB1) were assessed by Western blot. Beta-tubulin was used for load control and optical densitometry (ImageJ) for analysis. Because several of the antibodies have similar molecular weights, the membrane stripping technique with 5% acetic acid was used after the primary antibody was developed in order to mark the expression of the load control. (Table 1)

F-6) Renal histology: 8-micron thick sections were stained with hematoxylin-eosin and Sirius Red (15). Cortical fibrosis was quantified in 20 fields/animal (ImageJ) using a Nikon Type 104c microscope to obtain the images. Cortical fibrosis is expressed as: fibrosis percentage (%) = [Σ cortical interstitial collagen / (Total area – Σ vessels)] × 100.

F-7) Renal ultrastructure; 1 mm^3 renal cortical fragments were used for TEM and observed with a Zeiss EM 109T TEM with Gatan ES1000W digital camera.

Statistical analysis

InfoStat statistical package was used for Student's t-test, one-way ANOVA with Tukey post-hoc test, and Pearson and linear regression correlations. Results were expressed as mean ± standard error of the mean (SEM) and significance was $p<0.05$. GraphPad Prism v10.2.3 was used for graphs.

Ethical considerations

Procedures were approved by CICUAL-UBA (Res. CD No. 1881/1999, director Dr. M. R. Choi), in accordance with international ethical standards in animal experimentation.

RESULTS

Table 2 shows that HFD induced a significant increase in body weight compared with C, which was prevented by L in HFDL. Food intake decreased in HFD versus C, an effect reversed by L, while calorie intake increased in HFD with no differences between HFD and HFDL. Water intake did not vary between groups. Plasma parameters showed significant increases in HFD compared with C, with reductions in triglycerides and insulin in HFDL; Triglyceride-glucose product and HOMA-IR did not differ between groups.

Table 3 shows how HFD increased SBP and the urinary L-dopa/dopamine index compared with C, effects attenuated by L in HFDL. The CL group showed an additional reduction in SBP compared with C. No changes were observed in the urinary albumin/creatinine ratio or GFR. Diuresis, $FENa^+$, and UNa^+V decreased in HFD and were prevented by L in HFDL.

Hematoxylin-eosin staining showed cytoplasmic vacuoles in cortical tubular cells in HFD versus C, with no alterations in CL compared with C. Fewer vacuoles were present in HFDL than in HFD (Figure 1 A-D). The podocyte ultrastructure revealed shortening and fusion of pedicels in HFD, which was prevented by L in HFDL, and with no difference between CL and C (Figure 1 E to H).

HFD overexpressed AT1R and reduced the membrane transport protein OCTN1,2,3, compared with C, while L prevented this decrease. L also prevented the overexpression of the D1R dopaminergic receptor in HFDL compared with HFD and D2R, which was decreased in HFD and was not modified by L. LAT2 and OCT2 did not vary between groups. Na^+K^+ATPase was increased in HFD, but L returned it to normal values in HFDL compared with HFD (Figure 2).

The L-dopa/dopamine index correlated positively with SBP, insulinemia, and D1R, and inversely with UNa^+V and OCTN1,2,3. Losartan prevented these alterations in HFDL compared with HFD (Figure 3).

HFD significantly increased the expression of NFkB1 and TGF-beta1, effects that were prevented by L in HFDL. Interstitial fibrosis increased in HFD compared with C, and L significantly prevented this in HFDL compared with HFD, with residual foci and no changes in CL compared with C. Semi-quantitative analysis confirmed these findings (Figure 4).

DISCUSSION

High fat diet consumption significantly increased body weight compared with C, an effect that was prevented by L. Although food intake was lower in HFD, the caloric density of the diet resulted in 33% more calories consumed, which explains the increase in body weight, in agreement with findings reported by Pinhal et al. (16) Our results coincide with Smith et

Table 1. Primary antibodies used for Western blot.

Protein	Antibody	Dilution	Molecular weight	Supplier and catalog number
D1R	Rabbit polyclonal anti-D1R	1:1000	50 kDa	Proteintech, catalog number 17934-1-AP
D2R	Rabbit polyclonal anti-D2R	1:600	51 kDa	Proteintech, catalog number 55084-1-AP
OCTN 1,2,3	Rabbit polyclonal anti-OCTN 1,2,3	1:2000	90 kDa	Santa Cruz Biotechnology, Inc., catalog number sc-33534
OCT2	Goat polyclonal anti-OCT2	1:800	50 kDa	Santa Cruz Biotechnology, Inc., catalog number sc-19814
LAT2	Goat polyclonal anti-LAT2	1:800	50 KDA	Santa Cruz Biotechnology, Inc., catalog number sc-27581
AT1R	Rabbit polyclonal anti-AT1R	1:100	50 KDA	Proteintech, catalog number 25343-1-AP
Na+K+ATPase	Rabbit polyclonal anti-alpha-1 Na+ K+ ATPase	1:10,000	105 KDA	Abcam, catalog number ab74945
NFkB1	Rabbit polyclonal anti-NFkB1	1:1000	50 KDA AND 105 KDA	Proteintech, catalog number 14220-1-AP
TGF-beta1	Rabbit polyclonal anti-TGF-beta1	1:500	44 KDA	Proteintech, catalog number 21898-1-AP
Beta-tubulin	Rabbit polyclonal anti-beta-tubulin	1:3000	50 KDA	Abcam, catalog number ab6046

Table 2. Body weight, food, water, and calorie intake and plasma metabolic parameters.

Parameter	C	HFD	CL	HFDL
Body weight (g)	415.7±8.7	473.8±14.1*	389.7±3.8	359.13±7.93§
Food intake (g/24 hours)	20.9±3.6	13.8±1.3†	28.4±3.5	18.6±1.7‡
Water intake (mL/24 hours)	12.5±3.9	12.4±0.7	20.5±2.7	19.1±1.3
Calorie intake (kcal/24 hours)	60.5±10.8	103.6±11.5*	72.4±11.4	119.3±11.1
Triglycerides (mg/dL)	59.68±6.13	119.25±4.17†	53.20±4.41	82.97±6.93‡
Blood glucose (mg/dL)	117.5±7.5	152.2±4.6†	100.3±29.6	173.7±14.0‡
TyG index	8.0±0.2	10.5±0.1†	8.1±0.3	10.2±0.2
Insulin (ng/mL)	1.20±0.10	4.20±0.50†	1.3±0.2	2.8±0.4‡
HOMA-IR	0.7±0.1	3.3±0.5†	1.5±0.7	3.2±0.5

Results are expressed as mean ± SEM. Significance is considered as *p<0.05 C vs. HFD; †p<0.01 C vs. HFD; ‡p<0.05 HFDL vs. HFD; §p<0.01 HFDL vs. HFD.
C: standard diet and water ad libitum (SD); CL: C + losartan (L); HFD: SD + 50% w/w fat and ad libitum water; HFDL: HFD + CL; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; SEM: standard error of the mean; TyG: triglyceride-glucose product

al., (17) who described a reducing effect of L on body weight, suggesting a regulating role of AT1 antagonism in HFD-induced obesity. Moreover, Hosseini et al. (18) suggested that L may improve leptin resistance and modulate body weight in conditions of diet-induced obesity. Water intake did not differ between groups, and the previously validated L administration in drinking water (19) avoided the bias associated with chronic gavage (force-feeding).

The metabolic profile showed insulin resistance in HFD versus C, with increased triglycerides, blood glucose, insulin, TyG, and HOMA-IR, consistent with previous reports in HFD-induced MS models. (18) Losartan partially attenuated triglycerides and insulin without modifying blood glucose or indices, while CL did not differ from C. These findings are consistent with the literature regarding metabolic alterations caused by HFD and the beneficial effects of L, which could be due to the reduction of oxidative stress via the activation of superoxide dismutase (SOD), although the precise mechanism remains unclear. (17,20)

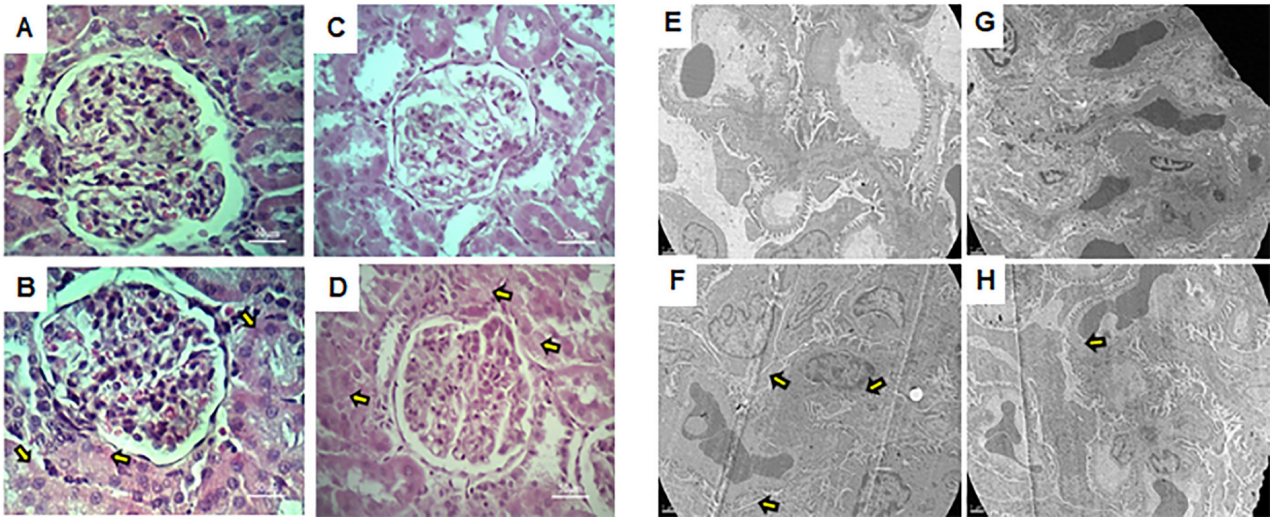
HFD significantly increased SBP compared with C, in agreement with findings of Jin et al. and Li et al. (21,22), who demonstrated that HFD induces obesity, insulin resistance, and hyperinsulinemia, accompanied by RAS activation, increased Ang II, aldosterone,

Table 3. Systolic blood pressure, urinary L-dopa/dopamine index and plasma and urinary parameters of renal function.

Parameter	C	DG	CL	DGL
SBP (mmHg)	120.7±2.3	134.8±2.3†	106.4±2.5 ¶	109.5±2.0§
L-dopa/dopamine index	1.1±0.1	2.9±0.2†	1.8±0.1	1.8±0.1§
Albumin/urinary creatinine (mg/g)	17.42±1.98	12.93±0.66	15.31±1.52	8.45±1.13
Diuresis (mL/24 hours)	16.4±2.2	3.5±0.3†	17.28±1.38	6.23±0.93‡
GFR (mL/min)	1.65±0.27	1.38±0.11	1.77±0.20	1.42±0.09
FENa (%)	0.50±0.06	0.16±0.02†	0.51±0.02	0.32±0.05‡
UNa^+V (mEq/24hours)	1.78±0.18	0.45±0.05†	1.95±0.01	0.78±0.10‡

Results are expressed as mean ± SEM (standard error of the mean). Significance is considered as: *p<0.05 C vs. HFD; †p<0.01 C vs. HFD; ‡p<0.05 HFDL vs. HFD; §p<0.01 HFDL vs. HFD; ¶p<0.01 CL vs. C; GFR: glomerular filtration rate; FENa: fractional excretion of sodium; SBP: systolic blood pressure; SEM: standard error of the mean; UNaV: urinary sodium excretion.

Figure 1. Structure and ultrastructure of the renal cortex



A, B, C, and D photos: Optical microscopy with hematoxylin and eosin staining, total magnification:400X E, F, G, and H photos: Transmission electron microscopy, total magnification: 7000X. Nomenclature: A-E: C group. B-F: HFD group. C-G: CL group. D-H: HFDL group. In A, B, C, and D, the yellow arrows point to the cytoplasmic vacuoles of the tubular cells. In E, F, G, and H, the yellow arrows point to the podocyte pedicels with altered morphology.

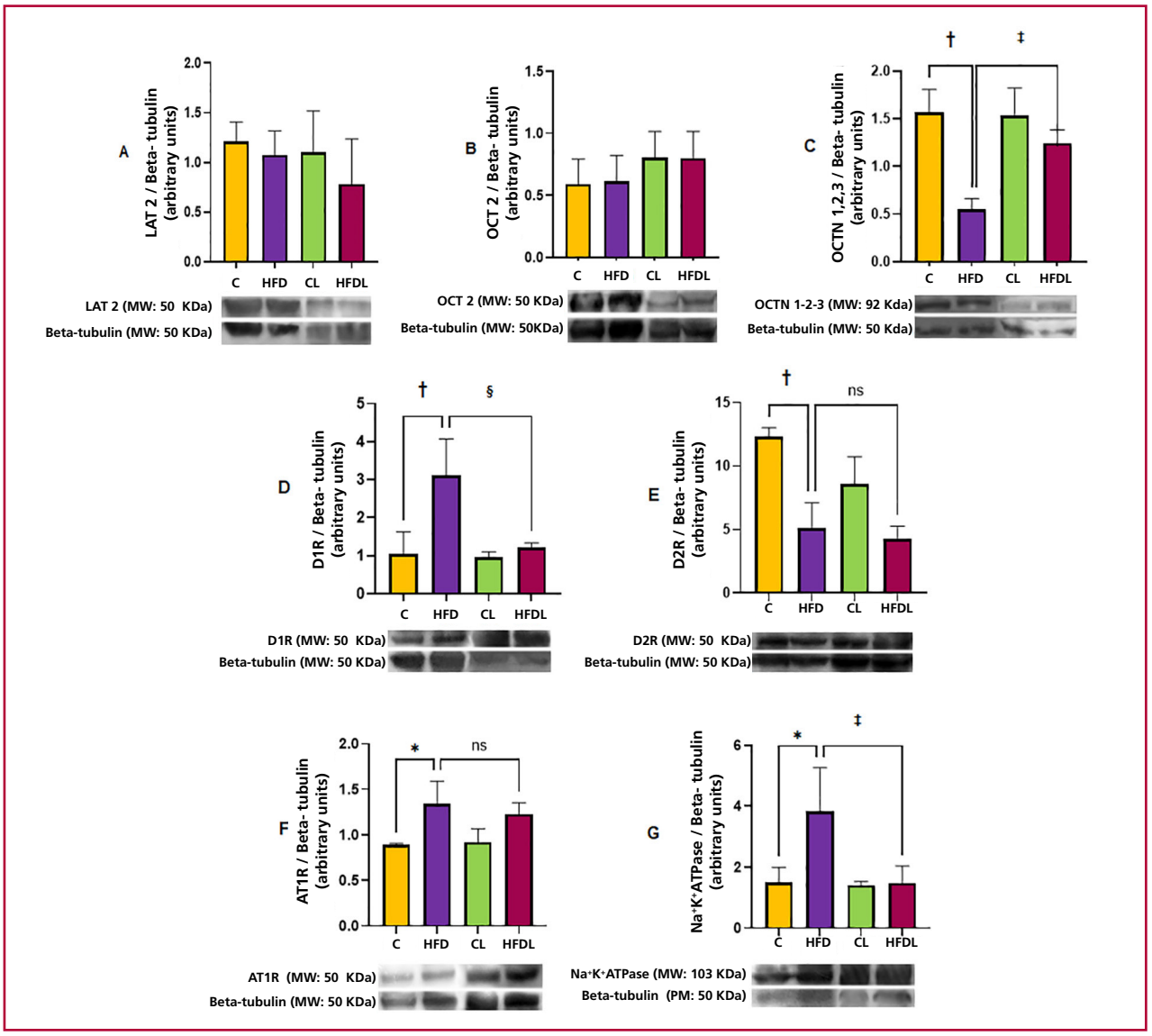
and AT1R expression, along with inflammation and renal fibrosis. In our model, concomitant administration of L prevented the increase in SBP in HFDL compared with HFD, an effect attributable to its antagonistic action on AT1R. (16) In addition, L significantly reduced SBP in normotensive controls (CL), in line with findings reported by Lee et al. (19) Li et al. (22) described an allosteric effect of L on AT1R, which potentiates D1R activity, contributing to its antihypertensive action.

Glomerular filtration rate showed no differences between groups. Similar results were reported by Roza et al. (23) in mice treated with HFD at 8 and 12 weeks. In addition, other groups reported increased levels associated with glomerular hyperfiltration in obese animals. (24,25) These findings suggest that the effect of HFD on GFR is variable. Treatment with L also did not modify this parameter between the groups evaluated.

The urinary albumin/creatinine ratio, which is more reliable than the absolute values of the metabolites separately, did not exceed 30 mg/g in any group, ruling out microalbuminuria. L did not modify this marker, suggesting that the alterations derive from the diet and not from the drug. In contrast, Sánchez-Navarro et al. (26) reported a significant increase of GFR under longer-term HFD, indicating that exposure time could be a determining factor.

HFD reduced diuresis versus C as reported by Sánchez-Navarro et al, (26) possibly due to lower RDS activity and higher RAS activity. (10) L prevented this reduction in HFDL, suggesting restitution of RDS function by AT1R blockade. (22) Similarly, $FENa^+$ and UNa^+V were reduced in HFD, partially prevented

Figure 2. RDS, RAS, and Na^+K^+ATPase protein expression in the renal cortex by Western blot



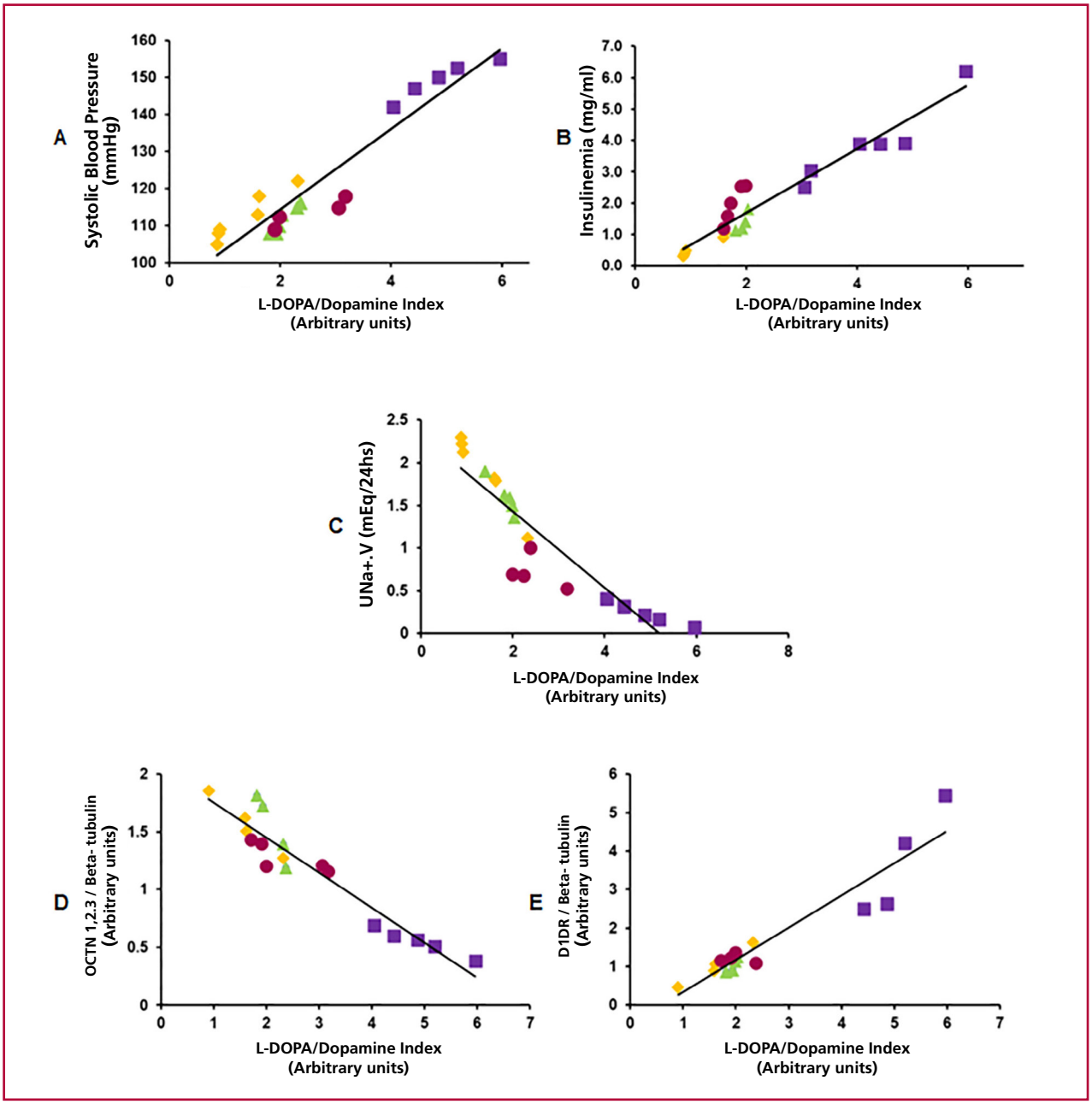
A: LAT2. B: OCT2. C: OCTN 1,2,3. D: D1R. E: D2R. F: AT1R. G: Na^+K^+ATPase. Results are expressed as mean ± SEM. Significance is considered as: *p<0.05 C vs. HFD; †p<0.01 C vs HFD; ‡p<0.05 HFDL vs HFD; §p<0.01 HFDL vs HFD.
C: standard diet and water ad libitum (SD); CL: C + losartan (L); HFD: SD + 50% w/w fat and ad libitum water; HFDL: HFD + CL; RAS: renin angiotensin system; RDS: renal dopaminergic system; SEM: standard error of the media. Remaining abbreviations in the text

by L in HFDL, although without reaching CL values, indicating the possible involvement of sodium reabsorption mechanisms independent of Na^+K^+ATPase, such as the Na^+/H^+ exchanger or the epithelial sodium channel (ENaC). (27,28) Previous results show disparity: Roza et al. (23) observed no changes, while Pinhal et al. (16) reported decreased FENav and UNa^+V in obesity or prolonged HFD. HFD is thus associated with the observed alterations, and L exerts a partial preventive effect.

Histologically, HFD induced cytoplasmic vacuoles in the proximal convoluted tubule (PCT), which was absent in C and partially prevented by L in HFDL, with no changes in CL. Although lipid stains were negative, the literature suggests that they could correspond to lipid vacuoles (4,29) or hydropic degeneration due to Na^+K^+ATPase dysfunction associated with lipotoxicity (30), and attenuated by L through reduction of inflammation and oxidative stress. (19) Ultrastructural analysis with TEM showed obliteration and interdigitation of pedicels and podocyte hypertrophy in HFD, which was partially prevented by L and is consistent with the nephroprotective role of dopamine (31) and the harmful effect of Ang II on AT1R. (32)

HFD-induced MS is associated with HTN through RAS activation, particularly through the action of Ang II on AT1R, which stimulates the Na^+K^+ATPase pump, promoting sodium retention and reducing its

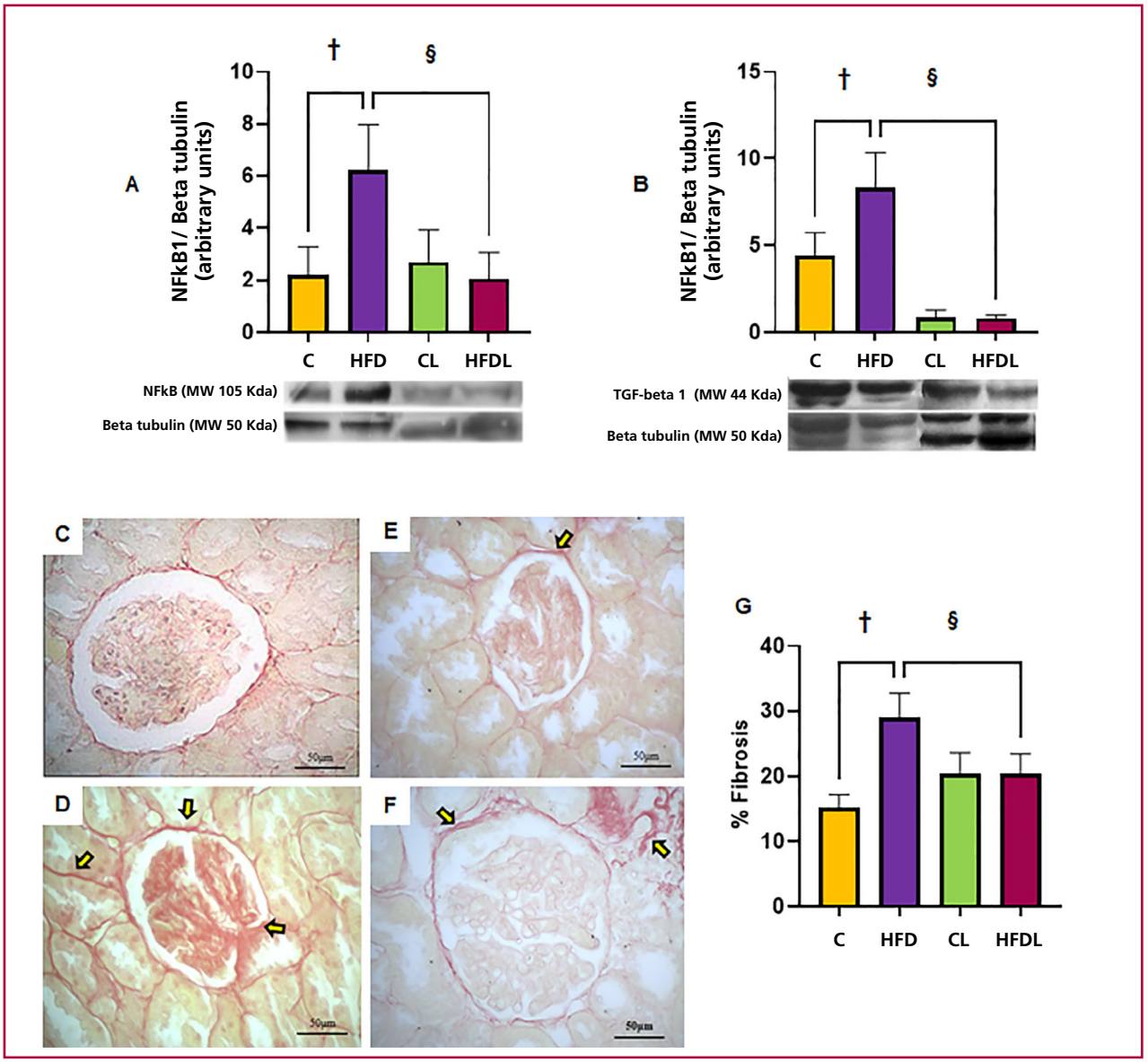
Figure 3. Functional correlations with the L-dopa/dopamine index



A: Linear regression SBP versus L-dopa/DA index; r = 0.95, R2 = 0.90, p<0.01; B: Linear regression of insulinemia versus L-dopa/DA index; r = 0.96, R2 = 0.91, p<0.01; C: Linear regression of UNa+V versus L-dopa/DA index; r = 0.91, R2 = 0.83, p<0.01; D: Linear regression of OCTN 1,2,3 versus L-dopa/dopamine index; r = 0.95, R2 = 0.91, *p<0.01 and E: Linear regression of D1R versus L-dopa/dopamine index; r = 0.95, R2= 0.89, *p<0.05. Group and color codes: C: Yellow. HFD: Violet. CL: Green. HFDL: Burgundy.

excretion. (1,22) Concomitantly, HFD inhibits RDS activity, enhancing the anti-natriuretic effect. There is negative reciprocity between AT1R and D1R in PCT cells, where activation of one induces internalization and abolition of signaling of the other, acting as a unit of opposites together with Na^+K^+ATPase as a common target. (33) In normotensive humans, a negative interaction between RDS and RAS has been demonstrated in the regulation of renal sodium transport, dependent on sodium intake. (34) HFD increased renal expression of AT1R compared with C, in accordance with models of MS and RAS activation. (21) L showed a tendency toward reduction in HFDL, suggesting that longer times may be necessary to demonstrate the effect. In contrast, L normalized the L-dopa/dopamine index, which was increased in HFD vs. C, (35) evidencing greater tubular dopamine availability and urinary excretion associated with res-

Figure 4. Evaluation of inflammation markers in the renal cortex by Western blot and fibrosis percentage by histology with Sirius red staining.



A: NFkB1. B: TGF-beta1. Photo C: C group. Photo D: HFD group. Photo E: CL group. Photo F: HFDL group. Graphic G: fibrosis percentage. The yellow arrows point to sites of increased interstitial fibrosis. Total magnification: 400X Results are expressed as mean ± SEM. Significance is expressed as: †p<0.01 C vs. HFD; §p<0.01 HFDL vs. HFD.

toration of OCTN1,2,3 transporters. LAT2 and OCT2 were not modified, in contrast to fructose overload. (35) HFD increased D1R, which was prevented by L, and reduced D2R, unchanged by L, reflecting renal dopaminergic alteration (36). HFD significantly increased cortical Na^+K^+ATPase compared with C, a finding consistent with Deji et al. (9,37). L prevented this increase in HFDL, without modifying baseline values (CL vs. C), an effect explained by its allosteric action on AT1R–D1R. (21)

The L-dopa/dopamine index correlated positively with SBP and insulin, and negatively with UNa^+V and OCTN1,2,3, which directly links RDS to blood pressure regulation and sodium balance, in interaction with insulin resistance. (38) L normalized these correlations by blocking AT1R and inhibiting Na^+K^+ATPase. Taken together, the results highlight the HFD-induced RDS-RAS imbalance and the potential of L to prevent associated renal and hemodynamic dysfunction.

In terms of inflammation, RDS exerts anti-inflammatory effects, while RAS potentiates them. (10,39) Renal dopamine, via D1R and D2R, attenuates the effects of Ang II by reducing inflammation and fibrosis (16). In contrast, HFD induced a proinflammatory environment with overexpression of NFkB1 and TGF-

beta1, increased interstitial fibrosis, and activation of oxidative pathways. (3,11,39) L prevented these changes by blocking Ang II and allowing the protective action of RDS to reduce fibrosis and oxidative stress. (40)

In conclusion, preventive and continuous treatment with L significantly attenuated RAS activity and reactivated RDS. AT1R inhibition by losartan reduced the action of Na^+K^+ATPase and normalized D1R expression, restoring OCTN1,2,3 transporter levels favoring dopamine access to the tubular lumen. Consequently, urinary dopamine excretion increased, the L-dopa/dopamine index normalized, and interaction with D1R was enhanced, promoting natriuresis and restoring RDS-RAS balance. In addition, L improved interstitial fibrosis, generated an anti-inflammatory renal environment, and preserved PCT structure and podocyte ultrastructure.

Limitations

One of the limitations of the present study was that the expression of the total Na^+K^+ATPase pump was assessed, without discriminating between the phosphorylated or active form and the non-phosphorylated or inactive one. Moreover, the activity of this transporter was not measured, based on the working group's own background showing in a fructose overload model with L that there were no variations between the different experimental groups.

Furthermore, it was not possible to perform immunohistochemistry on either the RDS transporters or receptors, which would have provided information about the cellular location of the different proteins, possibly directly related to the functionality of these proteins.

As a complement to TEM, it would have been suitable to determine the expression of nephrin and podocalyxine as structural markers of glomerular filtration barrier integrity, as well as to determine the expression of IL-6 as an acute marker of inflammation complementary to NFkB1.

These assessments are planned for the future in order to complete the corresponding profile..

Conflicts of interest

(See authors' conflict of interests forms on the web).

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