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CENTRO DE INVESTIGACIÓN EN CIENCIAS DE LA SALUD Y BIOMEDICINA (CICSaB)

LA DENERVACIÓN BILATERAL RENAL ES INSUFICIENTE PARA PREVENIR LA HIPERTENSIÓN Y LAS COMPLICACIONES RENALES EN UN MODELO MURINO DE DIETA ALTA EN GRASA.

TESIS QUE PRESENTA

M.C. OMAR FLORES SANDOVAL

PARA OBTENER EL GRADO DE

DOCTOR EN CIENCIAS BIOMÉDICAS BÁSICAS

DIRECTORA DE TESIS

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CRÉDITOS INSTITUCIONALES

Este trabajo se llevó a cabo bajo la tutoría de la Dra. Nadia Saderi en los laboratorios de Neuroanatomía Funcional y Ritmos Biológicos de la Facultad de Ciencias, de la Universidad Autónoma de San Luis Potosí, con la beca otorgada por el CONACYT con número (CVU) 554951. Para la realización de este trabajo se utilizaron los recursos de los proyectos: “Alteraciones circadianas: un factor de riesgo para la salud” con número 183078 y “Participación del Núcleo Arcuato del Hipotálamo en el desarrollo del Síndrome Metabólico e Hipertensión causados por una dieta alta en grasa número 243298” otorgados por el CONACYT.

Tesis que presenta:
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The Living State: With Observations on Cancer (p. 1).
Academic Press. New York, New York, USA. 1972.

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Bilateral renal denervation does not prevent hypertension and kidney injury in rats fed with a high-fat diet.

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ABSTRACT

One of the hallmarks of metabolic syndrome is the increased renal sympathetic nerve activity, which contributes to hypertension and leads to chronic kidney disease. Clinical evidence and animal models of this condition have shown that renal sympathetic denervation transiently attenuates hypertension while renal injury worsens. Leptin and insulin resistance and other alterations of energy homeostasis play a role in metabolic syndrome's cardiovascular and renal complications. To investigate this issue, we compared the metabolic, cardiovascular, and renal health of male rats that underwent bilateral renal denervation and were fed either with a standard or a high-fat diet for 8 and 12 weeks. The results indicate that the animals fed with hypercaloric diet does not affect the amount of noradrenaline in the kidneys but display increased blood pressure and plasma levels of angiotensin II, proteinuria, decreased glomerular filtration rate, urinary flow, and potassium excretion independently from the surgery. Nevertheless, bilateral renal denervation attenuates the ketonuria observed in intact, high-fat diet-fed rats. The comparison of intracellular proteins in the kidney, downstream of angiotensin II, leptin, and insulin signaling (AKT, PI3K, and ERK1/2) in the kidney showed a combined effect of the renal sympathetic input, the energy content of the diet and time, especially in the long term. Based on of these results, we suggest that renal sympathetic nerve activity plays a role in the early-intermediate stages of the metabolic syndrome. At the same time, the effects of chronic, long-term exposure to the hypercaloric diet prevails on the autonomic regulation of kidney homeostasis.

INTRODUCTION

In 2021, the World Health Organization reported that 1.28 billion people in the world suffer from systemic arterial hypertension (HT) or high blood pressure (HBP) (*NCD Risk Factor Collaboration, 2021*). Although nowadays the clinical practice avails itself of several antihypertensive agents, the prevalence of HT exceeds 50%, and a significant gap exists in the diagnosis and therapy between high- and low-income countries. Regardless of the underlying etiology, the clinical progression of HT results in organ failure, principally the kidneys and heart, leading to premature death (*Al Ghorani et al., 2021*).

A large body of literature links the pathophysiology of HT with the obesity epidemic because a nearly linear relationship exists between the increase in the body mass index (BMI) and HBP, where the former shifts the frequency distribution towards higher levels of the latter (*Hall et al., 2019*). The comorbidity of HT and obesity is characterized by several interrelated disorders, put together under the denomination of Metabolic Syndrome (MetS) (*Lin et al., 2022*). The clinical evidence, as well as the data obtained from experimental animal models, indicate that HT, visceral fat accumulation (abdominal obesity), dyslipidemia, glucose intolerance, and insulin resistance are characterized by the upregulation of the renal sympathetic nerve activity (RSNA) and the increased risk of chronic kidney disease (CKD) (*Amann and Benz, 2013*; *Oparil et al., 2018*; *DeLalio et al., 2020*), leading to the hypothesis that the interruption of the brain-kidney communication might prevent HT and renal failure. Radiofrequency ablation of sympathetic nerves has been suggested and recurrently tried as a therapeutical remedy in patients with resistant hypertension and obese subjects, with beneficial results over blood pressure and metabolic homeostasis (*Mahfoud et al., 2011*; *Witkowski et al., 2011*; *Kampmann et al.,*

2017; *Lee DP, 2020*). Nevertheless, several issues emerged from the clinical studies, and the safety and efficacy of renal denervation in the long-term control of HBP are still uncertain (*Miroslawska et al., 2016*; *Veerlop et al., 2015*; *Lohmeier and Hall, 2019*; *Grassi G., 2021*; *Miroslawska et al., 2021*). Besides, increased noradrenaline renal spillover also occurs in obese, normotensive subjects (*Grassi et a., 1995*; *Vaz et al., 1997*), suggesting that obesity promotes SRNA and may impair renal physiology, independently from HT.

Several authors found evidence that MetS-related HT depends on the sustained stimulation of the brain by the angiotensin II (A-II) systemic and local signaling, as well as on adiposity signals, such as insulin and leptin, which all promote sympathoexcitation (*Rahmouni et al., 2003*; *Hilzendeger et al., 2012*; *Campos et al., 2015*). The renin-angiotensin system (RAS) is a physiological mechanism that increases blood pressure by promoting thirst, vascular constriction, Na^+ and H_2O reabsorption, and sympathetic activation (Oparil et al., 2003). There is plenty of evidence that the RAS is overactivated in obesity and insulin resistance (*Kumari et al., 2019*). For instance, a positive-feedback loop has been described for the type 1 angiotensin-II receptor (AT1-R) and dyslipidemia, where A-II, the active product of the RAS-peptide cascade, stimulates LDL uptake and oxidation. At the same time, oxidized LDL promotes AT1-R expression (*Nickenig et al., 1997*). Angiotensin-II (A-II) triggers several changes that predispose to renal injury, such as efferent arterioles constriction and Na^+ retention, by increasing glomerular filtration, regulating the transport proteins turnover in the tubules, upregulating the sympathetic tone and vasopressin, and aldosterone release (*Johnson and Malvin., 1977*; *Barton et al., 1997*; *Neuhofer and Pittrow, 2006*; *Harrison-Bernard LM, 2009*). The systemic and local increase of angiotensin-II (A-II) levels is also involved in the complex relationship

between oxidative stress, endothelial dysfunction, chronic inflammation, and insulin resistance. The upregulation of A-II signaling, oxidative stress, and kidney endothelial dysfunction are directly proportional to insulinemia (*Sarafidis and Ruilope, 2006*). In addition, insulin promotes the proliferation of renal cells and the extracellular matrix, and the production of other growth factors, leading to the fibrotic changes in renal CKD (*Abrass et al., 1994*; *Abrass et al., 1995*). The importance of leptin in mediating HT and CKD is MetS needs to be clarified. However, plasma leptin concentration is correlated with sympathetic activity, including in the kidneys, and may increase renal Na^+ retention (*Haynes et al., 1997*; *Shek et al., 1998*; *Carlyle et al., 2002*; *Kim et al., 2013*). Interestingly, obese humans with genetic leptin deficiency and sympathetic dysfunction also display decreased RAS response to upright posture (*Ozata et al., 1999*).

Given this premise, the present study aims to investigate the influence of RSNA on the onset of CKD in an animal model of MetS induced by a high-fat diet (HFD), characterized by HT, obesity, dyslipidemia and increased levels of A-II, insulin, and leptin. For that, we compared blood pressure, metabolic and renal function parameters, and hormone signaling proteins (Akt, PI3K, and ERK1/2) in rats subjected to bilateral renal denervation (BRDx) and then exposed to either a standard diet or HFD for 8 and 12 weeks.

MATERIALS AND METHODS

Animals

Young-adult male Wistar rats weighing 100-120 g, were donated by the Laboratory of Biological Rhythms and Metabolism of Universidad Nacional Autónoma de Mexico. The animals were housed individually in acrylic cages, under 12-12 light-dark cycle, at 23±1ªC, with free access to food (Laboratory Rodent Chow 5001, LabDiet, USA) and

filtered water, until the day of the surgery. The experimental procedures were approved by the Ethical Committee of the Facultad de Ciencias Químicas of Universidad Autónoma de San Luis Potosí (CEID-FCQ, CEID2014030), in strict accordance with the Mexican Guidelines for the Care and Use of Experimental Animals (NOM-062-ZOO-1999) and the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe No 123, Strasbourg 1985).

Experimental protocol

Series 1, 8 weeks

The animals were randomly assigned into four groups: the rats that underwent sham surgery and then fed with a standard diet (SHAM-SD) or with the HFD (SHAM-HFD), and those that underwent bilateral renal denervation and then fed with a standard diet (BRDx-SD) or with the HFD (BRDx-HFD).

The standard lab-diet (vide supra) contained 4.5% fat and provided 3.36 kcal/g. The HFD was obtained by adding 15% pork fat (HEB, Texas, USA; 886Kcal/g) and 15% margarine (Primavera, Upfield, Mexico City, Mexico; 545Kcal/g;) to the standard diet. According to the nutritional facts indicated by the manufacturers, the HFD diet contained ~28.60% of fat and provided ~217 kcal/g.

Animals stayed under general conditions before the surgery (SHAM or BRDx) until they reached or exceeded their pre-surgery weight; only the rats meeting this requirement were subjected to the feeding protocol until the sacrifice. The rats were anesthetized with an overdose of sodium pentobarbital (Pisabental PiSA Agropecuaria, Hidalgo, México). The renal hili were occluded, both kidneys were quickly removed and immediately frozen at -80 °C for further analysis. Then, animals were transcardially perfused with 0.9% saline,

followed by 4% paraformaldehyde (PFA; Sigma-Aldrich Corp., St. Louis, MO, USA) diluted in phosphate buffer (PB 0.1 M, pH 7.4). The retroperitoneal, epididymal, and visceral adipose depots were removed for weighing.

Series 2, 12 weeks

The animals of series 2 were submitted to the same procedure as series 1, except rats were given access to their respective diets for 12 weeks instead of 8 weeks.

Bilateral Renal Denervation

Briefly, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p., Pisabental PiSA Agropecuaria, Hidalgo, México) and placed on a thermostatic operating table at 37 ºC, with general asepsis and under stereoscope visualization (X25). Following the abdominal midline incision, both kidneys were exposed, and the renal vessels were isolated from connective tissue and perirenal fat. After mechanically doffing the visible nerves from the kidney up to the renal aortic ganglion (also removed), the vessels were coated for 2 min with 10% phenol in absolute ethanol. The muscle layers of the abdominal wall were stitched with absorbable suture (Chromic Gut 4-0, COVIDIEN, Dublin, Ireland), and the skin was closed using a nonabsorptive suture (SEDA-SILK, 4-0, ETHICON, Johnson & Johnson, USA). Ceftriaxone (1g/kg, PiSA Farmacéutica, Jalisco, México) and Ketoprofen (0.5g/kg Profenid, Farmar Health Care Services, Madrid, España) were administered after the surgery. Rats were placed in warmed cages until complete recovery from the anesthesia (30–50 min) and then returned to their home cages.

In the sham surgery, the renal nerves and ganglia were isolated from the connective tissue but preserved.

ANALYTICAL TECHNIQUES

Animals from series 1 and 2 and the respective samples obtained before and after the sacrifice were analyzed as follows.

Bodyweight, food and water consumption, and blood pressure recording

Bodyweight (BW) and food and water intake (FI and WI, respectively) were measured every 48 hours. Systolic, diastolic, and mean blood pressure (SBP, DBP, and MBP, respectively) were assessed weekly, utilizing a non-invasive tail-cuff CODA device (Kent Scientific, Torrington, CT, USA). For that, animals were maintained in acrylic restrainers while an occlusion-cuff (O-cuff) was placed at the base of the tail, and a sensor (VPR-cuff) was placed 1 cm from the O-cuff. 15 correct BP measures were obtained from each animal. The efficiency of this system provides about 99% correlation with telemetry and direct blood pressure measure (*Feng et al., 2008*; *Fraser et al., 2001*).

Plasma metabolic and hormonal parameters

Two days after the metabolic cage assays, rats fasted for 24 hours; the tail was cleaned with 70% ethanol and cut 1 mm from the tip using a sharp, sterile scalpel. Fasting blood glucose (GLU) was measured with a glucometer (Accu-chek, Roche, USA), then 1 g/kg of D-glucose solution (50%, PiSA, Jalisco, México) was administered intraperitoneally. Blood samples collected before (time 0) and 15, 30, 60, and 120 minutes after glucose administration were stored in Eppendorf tubes (~200 µL) containing a clot-activator gel and centrifuged at 4000 rpm for 10 min. These samples were used to determine glucose concentration and stored at -80ºC for later insulin (INS) quantification. This was performed using a sandwich ELISA kit (APLCO Immunoassays, Salem, NH, USA) following the manufacturer's instructions and using 30 µL of serum per sample.

Blood samples (~2-3 mL) collected during the sacrifice and centrifuged as previously mentioned, were used to determine triglycerides (TG) and HDL-Cholesterol (HDL) concentration by colorimetric enzymatic kits (Spinereact, Girona, España; Colestat, Weiner Lab, Rosario, Argentina), as well as leptin (LEP) and angiotensin-II (ANG-II) by sandwich ELISA kit (BioVendor, Brno Czech Republic; MyBioSource, USA), following the manufacturer's instructions and using 2.5-50 µL of serum per sample. Results were quantified at 450-500 nm by means of a spectrophotometer (Novaspec II Visible, Amersham Pharmacia Biotech, Cambridge, UK).

Renal noradrenaline quantification

To assess the efficiency of the BRDx, the content of norepinephrine (NE) in a kidney from each animal was quantified by ELISA. Successful denervation was defined when the tissue content of NE was less than 10% of the mean value in the sham group (*Muhlbauer et al., 1997*).

Renal parameters

On the same day of blood pressure measurement, the urine proteins, ketone bodies (KET) and urobilinogen content, and pH were quantified by mean of a dry chemical method kit (Urine strips, URIN-10, Spinereact, Girona, España).

To determine renal excretory function, four days before the end of each protocol, 5 rats were randomly selected from each group and placed in metabolic cages with their respective diets to collect 24 h urine and plasma. Urine flow rate (UFR) was estimated as V/T x BW, where V was the urine volume in mL, and T was the time in minutes. Blood samples (0.4-0.5 mL) were collected from the tail vein, put in clot-activator gel tubes (BD Vacutainer 367983, BD, Mexico City, Mexico), and centrifuged at 4000 rpm for 10 min.

Absolute Na^+ and K^+ excretion ($U_{Na}V$, U_KV) was taken as U_{Na} x UFR and U_K x UFR, being U_{Na} and U_K measured by flame photometry (Flame photometer model 943; Instrumentation Laboratory SpA, Milano, Italy). Creatinine clearance (C_{cr}) was used to estimate the glomerular filtration rate (GFR), as follows: GFR= C_{cr} = U_{cr} x UFR/P_{cr}, with U_{cr} and P_{cr}, being urine and plasma creatinine concentrations determined by colorimetric enzymatic kit (Creatinine-J, Spinereact, Girona, España) at 500 nm with a spectrophotometer (vide supra).

Kidney protein extraction and immunoblotting

Whole kidney tissue was placed in an ice-cold lysis buffer solution containing 1 mM HEPES, 150 mM NaCl, 1 mM EGTA, 0.1 mM $MgCl_2$, 0.5% Triton X-100 and supplemented with a protease and phosphatase inhibitor cocktail (1×, Halt, Thermo Scientific, Germany). The samples were immediately homogenized (Tissue Tearor Homogenizer, Biospec Products, USA) at 4°C for ~15 s., and then centrifuged (Thermo MicroCL 21R, ThermoFisher Scientific, Germany) at 14,800 rpm for 15 min at 4°C. Protein concentration was determined by the acid bicinchoninic method (Sigma-Aldrich, St. Louis, MO, USA).

Kidney protein samples (10-50 µg) were mixed with 2× Laemmli buffer and heated at 95 °C for 5 min, then electrophoretically separated on 8–12% sodiumdodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels at 120 V/50 mA for 90 min. Separated proteins were transferred by semi-dry transfer apparatus (Trans-Blot SD Cell, Bio-Rad Laboratories, USA) to polyvinylidene difluoride (PVDF) membranes (0.45 µm, Immobilon-P, Millipore, USA). Molecular weight markers (~10–250 kDa; Precision Plus Protein All Blue Standars, Bio-Rad Laboratories, USA) were used to estimate molecular

mass. Blots were blocked with 5% bovine serum albumin or 5 % nonfat dry milk in Tris-buffered saline (pH 7.6)/0.1% Tween-20 (TBST). Blots were incubated with primary antibodies to Akt (1:1000, #9272; Cell Signaling, USA), phospho-Akt (pAkt) (Ser473, 1:500, #4060, Cell Signaling, USA), PI3K p85 (1:1000, #4292, Cell Signaling, USA), phospho-PI3K p85 (pPI3K) (Tyr458; 1:1000, #4228, Cell Signaling, USA), p44/42 ERK1/2 (ERK) (1:1000, #9102, Cell Signaling, USA) and phospho-p44/42 ERK1/2 (pERK) (Thr202/Tyr204, 1:1000, #4370, Cell Signaling, USA), and loading control protein GAPDH (1:4000, sc-25778; Santa Cruz Biotechnology Inc., Santa Cruz, CA) at 4°C, overnight. Blots were washed with TBST and then exposed to the second antibody conjugated to the horseradish peroxidase (1:5000, sc-2004; Santa Cruz Biotechnology Inc., Santa Cruz, CA) diluted in TBST/5% bovine serum albumin or 5 % nonfat dry milk at 25°C for 90 min. Detection of specific proteins was accomplished using an enhanced chemiluminescence kit (Immobilon Western; Millipore, USA); according to the manufacturer's instructions, blots were exposed to X-ray film (The Number 1 Dental Fil, Carestream Dental, Atlanta, GA).

Experimental design

The animals were randomly assigned to their respective group using the R software (V3.4) when they reached a BW between 225 and 255 g. Following the elimination criteria, the excluded rats were replaced with others, which were assigned sequentially as the others were eliminated (a random process) through a spiral tracking strategy until we completed the groups were completed. Because the study's main response variable was the BP value in both series, it was a three-way, completely randomized, longitudinal study (*Cook & Ware, 1983*). The three ways were denervation, a diet with two levels each, and

time with repeated measurements. The BP, BW, FI, WI, and urine parameters (PROT, KET, URI, and pH) were measured weekly, on the same day, while the amount of fat depots, blood metabolic parameters (LEP, ANG-II, INS, GLU, TRY, and HDL), renal hemodynamic parameters (UFR, GFR,$U_{Na}V$, U_KV) and signaling proteins (Akt, pAkt, PI3K, pPI3K, ERK, pERK) were cross-sectional measured at final of each protocol. Densitometric results were reported as integrated values (area density of band) and expressed as a ratio of interest protein to the loading control (GAPDH) using the ImageJ Software V1.52 (NIH, USA).

Statistical analysis

The overall time course of each continuous variable (BP, BW, FI, WI, GGT, Insulin, PROT, KET, URI, and pH) was analyzed (95% CI) using three-way repeated-measures of multivariate ANOVA (3WRM-MANOVA) (*Weinfurt KP, 2000*), one-way being the diet, another the denervation and the other time, with repeated measurements, looking for time, group, and/or time*group interaction effects. To assess the effect of time on each group, an RM-MANOVA by the group was performed. The response variables were modeled by linear fixed models (regression analysis, one-way, two-way, and three-way ANOVA), their interactions, and by mixed effects with Geisser-Greenhouse correction; after testing the residuals for normality (Shapiro-Wilk test) and homoscedasticity (Brown-Forsythe test) (Heiberger & Holland, 2015), the best models were chosen based on the highest r^2 (determination coefficient = explained variation), the lowest Akaike Information Criterion (AIC)(Akaike, 1974), and the parsimony principle. Post-hoc multiple comparisons were performed through a Tukey test. The alpha level imposed was 0.05. Statistical analysis was performed using JMP V10 (SAS Institute, Cary, NC, USA) and

GraphPad Prism V9 (GraphPad Software, La Jolla, CA) software, and all values are represented as mean ± standard error of the mean (SEM).

RESULTS

Renal denervation

Successful denervation was confirmed by assessing that renal NE content was ≈ 72% - 80% lower in BRDx animals compared with SHAM in both series of rats ($P < 0.0001$) (Figure 1), independently from the diet regimen.

Effects of BRDx and HFD on the body weight, food, and water intake

The overall analysis of the body weight (BW) (Figure 2A), as well as the one by group, indicated an effect of time ($P < 0.0001$) since day 24. HFD intake promoted a significant BW increase with respect to SD-fed groups, independently from BRDx (group*time interaction, $P = 0.001$) from day 68 onwards (P from 0.0457 to 0.0005). No changes were observed in in the average amount of food consumed per week (Figure 2B). While there was no effect of time on the water intake (Figure 2C), this was decreased by the HFD when compared to SD-fed groups from week 4 ($P = 0.001$). At weeks 10 and 11, denervation seemed to restore water intake in BRDx-HFD; however, it was not so at the end of the 12 weeks.

The results obtained from the 8 weeks series are represented in Supplementary Figure 1 and Supplementary Figure 2.

Effect of HFD and BRDx on fat depots and leptin, triglycerides, and HDL-cholesterol serum concentrations

The Figure 3A shows the amount of visceral, epididymal, and retroperitoneal adipose tissue extracted from animals of the 8 weeks series, where HFD produces a significant

increase ($P < 0.0001$) compared to the SD, and it is not affected by BRDx. This trend repeated in the 12 weeks series ($P < 0.0001$), where HFD increases by ~ 95% of the values observed at 8 weeks. Consistently, blood leptin (Figure 3B), TG (Figure 3C), and HDL-cholesterol (Figure 3D) concentrations were upregulated by HFD ($P < 0.0001$ in all cases) compared to the SD-fed groups. Renal denervation did not affect fat mass, leptin, and HDL-cholesterol in both BRDx-SD and BRDx-HFD. Interestingly, TG concentration was significantly lower in BRDx-HFD compared to SHAM-HFD at 8 weeks, but not at 12 weeks.

Effect of HFD and BRDx on blood glucose and insulin.

No difference in fasting glucose was found in the 8 weeks series (Figure 4A); however, it was significantly increased in both HDF-fed groups at 12 weeks (Figure 4C) ($P < 0.0011$). In the GGT, the overall MANOVA indicated an effect of time ($P < 0.0001$), group ($P = 0.0056$), and group*time interaction ($P = 0.0394$) in both the 8 weeks and 12 weeks series. The analysis by time point showed that glycaemia was significantly higher at 60, 90 and 120 min after glucose infusion in the SHAM-HFD animals of the 8 weeks series compared to SD-fed animals. BRDx-HFD animals showed the same pattern, except on 90, when glycemia was significantly lower than BRDx-HFD. In the SHAM-HFD of the 12 weeks series, glycemia was significantly increased at 15, 90, and 120 minutes after glucose infusion in comparison with SD groups; the response was similar in the BRDx-HFD, again with the exception that the 90 minutes, when glucose level was significantly lower than in the SHAM-HFD.

Concerning to insulin levels (Figure 4B), the global MANOVA showed an effect of group ($P = 0.0057$), time ($P < 0. 0001$), and group*time interaction ($P = 0.0057$). Analysis by

time point indicated that insulin levels were upregulated by HFD (P = 0.0072) at 60, 90, and 120 min in the 8 weeks series and were not affected by BRDx. In the 12 weeks series (Figure 4D), the overall MANOVA only showed an effect of time (P = 0.0019) but not of group nor group*time interaction, while analysis by time point indicated that the insulin level of the BRDx-HFD was significantly higher (P = 0.0234) concerning the SD-fed groups only 120 minutes after glucose infusion.

Effect of BRDx and HFD on blood pressure and serum Ang-II

For the weekly SBP values (Figure 5A), the overall MANOVA indicated time effect (P < 0.0001) from week 3 onwards, group effect (P < 0.0001), and group*time interaction (P < 0. 0001). The HFD significantly increased SBP compared to the SD groups (P = 0.0089) since week 5. The SBP of BRDx-HFD was significantly lower than SHAM-HFD (P = 0.0197) during weeks 11 and 12, although the BRDx-HFD rats still were hypertensive. For DBP (Figure 5B), the overall MANOVA showed an effect of time (P < 0.0001), group (P < 0.0001), and group*time interaction (P = 0.0058). The DBP was significantly higher in SHAM-HFD and BRDx-HFD compared to the SD-fed groups (P = 0.0025), and no difference compared to BRDx was found. MBP (Figure 5C) showed the same pattern as DBP from week 5, with the overall MANOVA indicating the same effects (P from 0.0011 to < 0.0001).

The quantification of plasma Ang-II at the end of each series (Figure 5D) showed that BRDx-attenuated HFD-induced increase at 8 weeks (P = 0.003) but not at 12 weeks.

Effect of BRDx and HFD on renal function

In the weekly quantification of urine protein concentration (Figure 6A), the overall MANOVA showed time (P < 0.0001), group (P < 0.0001), and group*time interaction (P

< 0.0001) effects. 0001). HFD promoted a sharp increase in protein excretion after the first week of the experiment, followed by an equally blunt decrease. No differences were observed between the groups until week 9 when SHAM-HFD and BRDx-HFD displayed a progressive increase in urine protein content. Concerning the SD-fed groups, from week 9 and week 10, respectively (P = 0.0001 and P < 0.0001). However, BRDx attenuated this increase, and protein excretion was significantly lower in BRDx-HFD than in HFD-SHAM (P = 0.0004).

For urine KETs (Figure 6B), the overall MANOVA indicated an effect of time (P < 0.0001), group (P < 0.0001), and group*time interaction (P < 0.0001). HFD caused a significant increase in ketones excretion with respect to SD-fed groups (P = 0. 0005) at week 2, independently from BRDx. Nevertheless, a long-term increase in urine KETs was detected in SHAM-HFD (P = 0.0059), which BRDx-HFD severely attenuated.

HFD reduces the UFR significantly independently from BRDx (P < 0.0001) (Figure 7A) concerning SD-rats, in both 8-weeks and 12-weeks series, while GFR decreased significantly only after 12 weeks of HFD (P < 0.0001) (Figure 7B). The UNaV (Figure 7C) significantly decreased in the SHAM-HFD (P = 0.0019) with respect to the other groups at week 8, while no differences were found in the 12 weeks series. There is a statistical increase in Na^+ excretion in the BRDx-HFD animals of the 12 weeks series compared to those sacrificed at 8 weeks (P = 0.0003). The HFD significantly decreased UKV (P < 0.0001) in both the 8- and 12-weeks series (Figure 7D) for SD-fed groups, BRDx elicited no effect. No changes were found in urobilinogen excretion and urine pH (Supplementary Figure 3).

Effect of BRDx and HFD on signaling proteins in the kidney

The renal content of Akt (Figure 8A) and the pAkt/Akt (Figure 8C) ratio did not change in the kidney the 8 weeks series, although a decrease was observed in pAkt (Figure 8B) of the BRDx-SD compared to SHAM-HFD group ($p = 0.016$). In the 12 weeks series, Akt significantly decreases in SHAM-SD, BRDx-SD, and SHAM-HFD compared to the 8 weeks results (p from 0.0020 to 0.0005). Similarly, pAkt decreased in BRDx-SD ($p = 0.0003$) and increased in SHAM-HFD ($p = 0.0062$) concerning 8 weeks series. In addition, pAkt was downregulated in BRDx-SD and SHAM-HFD compared to BRDx-HFD ($p < 0.0001$ in both cases). All these changes did not result in significant changes in pAkt/Akt, except that the ratio was higher in SHAM-SD of the 8 weeks series compared to the 12 weeks series ($p = 0.0069$).

BRDx-HFD displayed a significant increase in the renal content of PI3K (Figure 8D) concerning BRDx-SD and SHAM-HFD ($p = 0.0005$), and PI3K (Figure 8E) compared to the other groups (p from 0.0176 to < 0.0001), resulting in no changes in the pPI3K/PI3K ratio (Figure 8F). In SHAM-SD, SHAM-HFD, and BRDx-SD of the 12 weeks series, the PI3K content was lower than in the 8 weeks series (p from 0.0029 to < 0.0001), whereas pPI3K increased in BRDx-HFD ($p < 0.0001$). As a result, no significant changes were found in pPI3K/PI3K from the 8 weeks series, whereas a significant upregulation was detected by comparing BRDx-SD ($p < 0.0001$) and SHAM-HFD ($p = 0.0264$) of the 12 weeks series. In addition, the increase of pPI3K/PI3K observed in BRDx-SD was significantly different when compared with SHAM-SD ($p < 0.0001$), SHAM-HFD ($p < 0.0001$), and BRDx-HFD group ($p = 0.0012$).

In the 8 weeks series, the renal content of ERK (Figure 8G) and pERK (Figure 8H) did

not show any change between groups, although the ratio resulted in a significant decrease in SHAM-HFD concerning BRDx-SD and BRDx-HFD ($p = 0.0012$ and 0.0048, respectively) (Figure 8I). An upsurge of ERK was observed in BRDx-SD of the 12 weeks series compared to the values obtained from the 8 weeks series ($p = 0.0244$). The ERK content in BRDx-HFD of the 12 weeks series was significantly lower concerning BRDx-SD ($p = 0.0336$) and SHAM-HFD ($p = 0.0493$). The level of pERK raised in the SHAM-SD when compared to 8 weeks ($p < 0.0001$) and was also significantly higher with respect to BRDx-SD, SHAM-HAD and BRDx-HFD of the same analysis (p from 0.0002 to < 0.0001). As a result, pERK/ERK increased in the SHAM-SD from the 8 weeks to 12 weeks series ($p = 0.0069$), and it was also significantly elevated concerning BRDx-SD ($p < 0.0001$), SHAM-HFD ($p < 0.0001$) and BRDx-HFD ($p = 0.0072$) of the 12 weeks series. Finally, pERK/ERK was significantly lower in BRDx-SD than in BRDx-HFD ($p = 0.0038$). Representative pictures of the Western Blots are in Supplementary Figure 4.

DISCUSSION

In the present work, we explored the contribution of sympathetic nerve activity in the onset of renal impairment in a rat model of metabolic syndrome induced by a high-fat diet. As expected, the hypercaloric diet promoted a progressive and significant rise in body weight (Figure 2A), fat accumulation (Figure 2A-B), and a series of other metabolic, cardiovascular, and renal symptoms. Surprisingly only in a few cases, these changes are prevented by bilateral sympathetic denervation of the kidneys. HFD-fed rats, with or without kidney denervation, display almost uniformly all the signs of the metabolic syndrome: increased levels of circulating leptin (Figure 3B) and HDL-cholesterol (Figure

3D), glucose intolerance (Figure 4), and hypertension (Figures 5A-B-C). Nevertheless, the results of BRDx provide several interesting issues that require further discussion.

High-fat diet does not affect the amount of noradrenaline in the kidneys.

The noradrenaline content within the kidney was evaluated to assess the efficiency of the bilateral renal denervation and, as it is illustrated in Figure 1, the surgical procedure was performed successfully. Rodionova and colleagues (*2016*) demonstrated that renal noradrenaline increases ~38% 12 weeks after denervation. In the present study, we cannot discard that reinnervation occurs; however, the renal noradrenaline is significantly lower in all denervated groups compared to the intact ones.

Despite the putative role of RSNA in obesity-associated HT and MetS, the animals fed with the HFD are hypertensive, and blood pressure is slightly, non-significantly different between obese intact and denervated animals (Figure 5). It is also worth observing that, in SHAM rats, HFD-induced obesity does not affect renal noradrenaline concerning the normocaloric, standard diet. All this is consistent with what was reported for obese-hypertensive humans, who do not significantly increase noradrenaline spillover compared to normotensive subjects (Rumantir et al., 1999; Esler et al., 2006). Thus, RSNA does not account for the increased blood pressure associated with obesity and metabolic syndrome.

Long-term high-fat diet promotes kidney injury, despite the bilateral renal denervation.

The kidneys' health is usually evaluated by analyzing the urine and blood composition and estimating the glomerular filtration rate (GFR). The presents work found that urine pH and urobilinogen content do not change between groups (Supplementary Figure 1).

In contrast, while urine protein and ketones concentration increase (Figures 6A and 6B), and the GFR decreases (Figure 7B). These data indicate that, although the kidneys of the HFD-fed rats can still compensate for the metabolic and cardiovascular challenge, there is a renal injury in progress.

The RSNA appears to exert specific effects on renal functioning. For instance, the urine protein is significantly lower in BRDx-HFD than in SHAM-HFD but considerably higher than in the STD-fed groups. Proteinuria is a sign of increased glomerular permeability and, together with the decreased GFR, indicates the degeneration of the glomerular structure. This picture suggests that in the HFD-related factors (such as hypertension and the metabolic alterations) promote renal injury, and the RSNA exacerbates the damage produced by the HFD, despite noradrenaline content being similar in SHAM-HFD and BRDx-HFD.

Bilateral renal denervation prevents late ketonuria in rats fed with an HFD.

The ketone bodies (acetone, acetoacetate, and β-hydroxybutyrate) are water-soluble molecules produced mainly by the hepatic fatty acid catabolism and used as an energy source by extrahepatic tissues in conditions of negative metabolic balance. The rate of ketogenesis increases in several disorders, including diabetes mellitus, insulin resistance, and non-alcoholic liver steatosis (*Puchalska & Crawford, 2017*), while ketonuria is commonly associated with poorly compensated diabetes and other situations of reduced glucose availability (*Comstock & Garber, 1990*). HFD rats display several risk factors for hyperketonemia and ketonuria [overweight, abnormal fat accumulation, hepatic steatosis (data not shown), glucose intolerance]. Still, the considerable increase in renal ketones excretion detected in SHAM-HFD is prevented by BRDx (Figure 6B). This pattern

resembles the plasma TG-concentration, which is significantly higher in SHAM-HFD when compared to STD-fed rats and BRDx-HFD in the 8 weeks series (Figure 6D).

At physiological concentrations, acetoacetate and β-hydroxybutyrate are freely filtered by the glomeruli and then shuttled back from the ultrafiltrate by the type-2 Na^+-coupled monocarboxylate symporter (SMCT2) at the apical surface and by the type-1 H^+-monocarboxylate antiporter (MCT1) at the basolateral side of the tubular epithelium (*Rojas-Morales et al., 2021*). When ketogenesis increases, the reabsorption of filtered ketone bodies rises as an adaptive response aimed at preventing the loss of metabolic fuels and NH_4^+, Na^+, and K^+ (*Palmer & Clegg, 2021*). In both groups fed with the HFD, we observed a transient ketonuria within the first two weeks of hypercaloric food intake. This suggests that the rat metabolism quickly adapts to use ketone bodies as an energy source. Later in the feeding protocol, the excretion of ketone bodies increases in HFD, suggesting that ketonuria could depend on the saturation of the tubular transporters. Very little is known about the molecular, biochemical, and pharmacological regulation of these proteins in rat kidneys. Nevertheless, it has been reported that metabolic acidosis decreases SMCT2 mRNA expression in the mouse nephron (*Becker et al., 2010*). This indicates that the downregulation of ketones reabsorption in HFD rats might prevent ketoacidosis by increasing excretion. Thus, the drastic reduction of ketonuria in BRDx-HFD suggests that the RSNA prevents renal ketone elimination. This evidence conflicts with the report of Pierre and colleagues (*2003*), who observed that noradrenaline does not affect MCT1 immunoreactivity in cortical culture but upregulates MCT2. However, De Oliveira and colleagues (*2021*) reported that renal bilateral denervation reduces the Na^+-glucose cotransporter (SGLT2) expression in the tubular epithelium in a rat model of type-

1 diabetes. If it is the case, the increased Na^+ concentration in the ultrafiltrate of BRDx-HFD with respect to SHAM-BRDx (Figure 7C) might drive ketone bodies reabsorption. Regardless of the mechanism by which noradrenaline inhibits renal ketone recycling, it is unclear whether sympathetic bilateral denervation might have beneficial or detrimental consequences on the body. On one side, the overproduction of ketones bodies has a cytoprotective effect because it steals substrates to the oxidative metabolism (*Puchalska and Crawford, 2017*) and prevents kidney damage and HT (*Chakraborty et al., 2018; Ishimwe et al., 2020*). Conversely, the pharmacological inhibition of Na^+-glucose reabsorption is strongly associated with ketoacidosis, which is likely to precipitate in patients affected by metabolic syndrome (*Somagutta et al., 2021*). Hence, the consequences of decreased ketonuria in BRDx-HFD must be explored carefully.

High-fat diet decreases water intake and urine K^+ but does not affect Na^+ excretion. The decrease in water consumption is a behavior extensively reported for human-obese subjects (*Yamada et al., 2022*) but less studied in animals. Volcko and colleagues (*2020*) also observed that HFD-fed rats consume less water than the normocaloric controls, consistent with what has been detected in the SHAM-HFD and BRDx-HFD. Both in obese humans and animals, the reduced water intake has been associated with changes in body fluids distribution and the interplay between diet composition and thirst sensation (*Marken Lichtenbelt & Fogelholm, 1985*; *Waki et al., 1991*; *Dos-Santos et al., 2022*). Independently of how the rat body deals with water distribution in HFD and RSNA, the kidneys respond, reducing the urinary flow rate (Figure 7A) and urine K^+ (Figure 7D). This result conflicts with the hypothesis that RSNA is the primordial input for water and solutes traffic in the kidney rather than a participant among other players.

Conversely, Na^+ excretion decreases in SHAM-HFD animals with respect to the BRDx-HFD after 8 weeks of HFD (Figure 7C), while no change has been found between SD-fed animals, suggesting that RSNA promotes the Na^+ re-uptake during the early stages of obesity. Moreover, renal denervation prevents A-II upsurge in HFD-fed animals (Figure 5D), which likely depends on the dampened catecholamine-stimulated renin release (*Hackental et al., 1990*) and contributes to Na^+ excretion.

The difference in urine Na^+ between SHAM-HFD and the other groups gets lost in the 12 weeks series, which indicates that, in these animals, other factors different from arterial pressure (because the SD-fed rats are normotensive), RSNA (because there is no difference between SHAM and denervated rats) and A-II (because plasma levels are similar in SHAM-HFD and BRDx-HFD), may promote natriuresis. This pattern is consistent whit what was concluded by Bie (*2018*), who stated that "*the regulation of renal sodium excretion is a parallel operation of several relatively independent mediators based on different feedback pathways*"

The reduction of K^+ excretion observed in the SHAM-HFD rats after 8 weeks of the hypercaloric protocol (Figure 7D) may depend on the rise of plasma A-II (Figure 5D) and drop of the urine flow (Figure 7A), which counterweight the decrease of water consumption (Figure 2C). However, the amount of excreted K^+ and water are similar in BRDx-HFD, although these animals do not display any increase in plasma A-II. The concentration gradient might drive the K^+ recycling and transepithelial potential (Palmer & Clegg, 2008) resulting from the boosted water reabsorption in the distal portions of the renal tubules promoted by the antidiuretic hormone (ADH) (*Boone & Deen, 2008*). The release of ADH is primarily regulated by body fluid volume and osmolarity ("osmotic

release of ADH") and not only in response to RAS activation or under autonomic control ("non osmotic release of ADH") (*Schrier & Goldberg, 1980*). In the SHAM-HFD, combining A-II and ADH signaling in the kidney might contribute to decreased urine Na^+ and override aldosterone-promoted K^+ secretion.

After 12 weeks, the chronic up-regulation of plasma A-II in both SHAM-HFD and BRDx-HFD might also inhibit the renal outer medullary K^+ (ROMK) channels of intercalated cells in the connecting duct, which in turn dampens the electrogenic K^+ secretion. Furthermore, in HFD-fed rats, the reduced GFR (Figure 7B) decrease the K^+ filtration. Since luminal K^+ is essential for Na^+ re-uptake and K^+ is only one-third of the filtered Na^+ load, Na+ reabsorption likely occurs at the expense of K^+ excretion.

Bilateral renal denervation does not prevent the adiposity-related alterations of the metabolic syndrome.

As already reported by several authors, abnormal plasma levels of A-II and leptin, dyslipidemia, glucose intolerance, and insulin resistance are intermingled with renal (and non-renal) sympathetic overactivation via the brain-periphery communication loop (*Thorp & Schlaich, 2015*; *DeLalio et al., 2020*). The renal denervation attenuates the HFD-dependent A-II uprise in the 8 weeks series. However, this adjustment disappears in the 12 weeks series (Figure 5D), likely because, in obesity and metabolic syndrome, the increase of the RAS activity depends not only on the canonic, systemic RAS peptide cascade. For example, the expansion of the adipose tissue contributes to about 30% of the circulating levels of angiotensinogen, and in addition, the local, intrarenal RAS results hyperactivated (*Phillips et al., 1993*; *Giani et al., 2015*).

AT1-R is expressed throughout the kidney; its activation and transactivation account for most of the A-II physiological and pathological effects on renal function (*Forrester et al., 2018*). A glance at AT-1R downstream signaling proteins shows that the increase of A-II input in the 8 weeks series does not affect pPI3K/PI3K and pAkt/Akt ratio, while renal denervation prevents pERK/ERK decrease in HFD-fed animals. This result conflicts with the hypothesis that SHAM-HFD rats should be the most exposed to the harmful effect of the diet since A-II-dependent ERK signaling promotes renal inflammation, epithelial-mesenchymal transition, and fibrosis (*Mondorf et al., 2000*). Nevertheless, the pleiotropic A-II intracellular transduction comprises the recruitment of the AT1-R by β-arrestin, paradoxically enhancing ERK signaling and promoting tissular injury (Turu et al., 2019). Supporting this model of "biased signaling," there is that the pathological response to A-II renal infusion, as well as hypertension, are boosted by selective AT1-R deficiency in the immune system, suggesting that renal A-II signaling may have anti-hypertensive and anti-inflammatory effects (*Schellings et al., 2006*; *Chen et al., 2010*; *Kendall et a., 2014*; *Karnik et al., 2015*), which, according to the present results, might require RSNA.

A-II intracellular signaling cross-talks with the pathways regulated by leptin and insulin. Leptin displays a significant rise in the plasma of both SHAM and BRDx HFD-fed animals (Figure 3B) that mirrors fat accumulation (Figures 3A and 3B). Although chronic hyperleptinemia and leptin resistance stimulate the RSNA via hypothalamic and brainstem relays (*Haynes et al., 1997*; *Kuo et al., 2001*; *Mark et al., 2009*; *Dubinion et al., 2011*; *do Carmo et al., 2009*), they also directly disturb kidney homeostasis and harm virtually the whole renal tissue (*Korczynska et al., 2021*). Leptin is filtered by the glomerulus and then up-taken by receptor-mediated endocytosis in the proximal

convoluted tubule in proportion to plasma concentration, and since the low GFR in CDK results in reduced leptin excretion, a vicious circle might contribute to an increase in the circulating levels of this hormone in obesity and MetS. In the kidney, leptin signaling promotes the proliferation of glomerular endothelial and mesangial cells, and fibrogenesis. Hyperphosphorylation of Akt is a marker of renal leptin resistance (*Beltowski et al., 2010*) and endothelial dysfunction (*Ding et al., 2016*). Leptin also promotes mesangial cell hypertrophy via the PI3K and ERK pathways (Lee et al., 2005). However, the results show that both the RSNA and diet affect the internal signaling and do not help to dissect the contribution of these factors, at least at this point of the experimental procedure. For instance, there is no difference in pPI3K/PI3K between SHAM-SD and SHAM-HFD (Figure 8F) despite leptin being significantly higher in the blood of the latter (Figure 3B), suggesting that hyperleptinemia could not affect this pathway.

Interestingly, the denervation triggers a huge pPI3K/ PI3K increase in BRDx-SD with respect to SHAM-SD, but a decrease in BRDx-HFD in comparison with BRDx-SD, which indicates that the RSNA regulates the PI3K pathway according to the metabolic state. Analogous observations about insulin and the IP3K-Akt cascade in the 12 weeks series can be expressed. Both RSNA and HFD, alone or in combination, decrease the pERK/ERK ratio (Figure 8F). Hence, noradrenaline release in the kidney changes the interplay of the RSNA with leptin, insulin, or another metabolic parameter that respond to energy availability and regulate the IP3K pathway and the downstream proteins.

In the 8 weeks series, the systemic insulin resistance observed in HFD-fed animals does not parallel any change in the IP3K-Akt cascade (Figure 8C and 8F). On the other hand, SHAM-HFD displays a decrease in pERK/ERK, which is reverted in BRDx-HFD. Given

the role of the ERK pathway in cell proliferation and growth, renal denervation in obesity and MetS might have detrimental effects on kidney structure and function.

Finally, in the 12-week series, the PI3K, Akt, and ERK phosphorylated forms abound in SHAM-STD concerning the analog group sacrificed after 8 weeks (Figure 8C, 8F, and 8I), despite there is no change in plasma A-II, which suggests the influence of age on the internal renal signaling. It has been suggested that chronic kidney disease shares some features with aging, which may include the dampened response to oxidative insults and shortening of the cell life span promoted by decreased PI3K/Akt and increased ERK signaling, respectively (*Terada et al., 2001*; *Shimamura et al., 2003*; *Downward J., 2004*; *Slack et al., 2015*). Thus, since pAkt/Akt and pERK/ERK (but not pPI3K/PI3K) are significantly higher in SHAM-STD than BRDx-STD, the consequence of renal denervation results is ambiguous.

The framing of the problem described in the introduction of the present work refers to bilateral renal denervation as a clinical tool to control the cardiovascular and renal complications of overweight and obesity in human subjects already diagnosed with metabolic syndrome and drug-resistant hypertension. Nevertheless, the present work explores the influence of the autonomic innervation of the kidney in the onset and progress of the metabolic syndrome; for that, the bilateral renal denervation was performed before the rats were shifted to the HFD and, hence, previously the observation of the overt metabolic and cardiovascular imbalance. The results of the experiments do not contribute to solving the problem of the safety and efficiency of the surgery in humans. Still, they merely try to dissect the effects of the neural input to the kidney from the changes in hormonal signaling promoted by the hypercaloric diet. The autonomic renal

innervation does not play a pivotal role because obese animals show hypertension and the early signs of CKD, independently from the surgery, which blames A-II, leptin, insulin, and all the other possible players that link the alteration of the metabolic homeostasis with the cardiorenovascular sequelae. On the other hand, the results obtained from the 12-week series underscored that renal denervation might also have risky effects, such it is suggested by glucose handling and ketonuria in BRDx-HFD rats. This observation opens the way to additional questions about the neural and hormonal interplay in the pathophysiology of metabolic syndrome, especially in the kidney.

CONCLUSION

The present work explored the contribution of the RSNA on cardiovascular and renal performance in a rat model of metabolic syndrome induced by a high-fat diet. As a result, the interruption of RSNA in HFD-fed animals does not significantly improve blood pressure in most of the parameters analyzed to diagnose renal impairment, especially in the long term. For example, bilateral renal denervation only delays the increased protein renal excretion in HFD rats. Likewise, the surgical procedure normalizes Na^+ excretion and prevents the upsurge of plasma A-II promoted by HFD in the 8-week but not in the 12-week series. Furthermore, the changes in plasma TG and urine ketone bodies concentration observed in BRDx-HFD when compared with SHAM-HFD suggest that the RSNA plays a specific, peculiar role in lipid oxidation when the energy balance is positive. Similar conclusions can be obtained by observing how both the RSNA and A-II, leptin, and insulin signaling interplay according to the feeding state and, possibly, the age of the animals. For instance, RSNA seems essential to prevent renal tissular injury triggered by

A-II in HFD-fed rats. Nevertheless, the procedure increases the ratio of pERK/ERK in these animals, which could be more susceptible to the mitogenic, proliferative effect of leptin and insulin. Hence, further experiments will be necessary to dissect the neural and hormonal control of kidney physiology and understand how this organ responds and adapt to metabolic challenges.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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AUTHOR'S CONTRIBUTION

N.S. directed the study. O.F.S., S.C.R., R.C.S.D. and A.B.R development of methodology, acquisition, and data analysis. C.E.B provided animals. O.F.S. and N.S. interpretation of data, writing, review of the manuscript. All authors contributed to the discussion of the data and edition of the manuscript.

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FIGURES

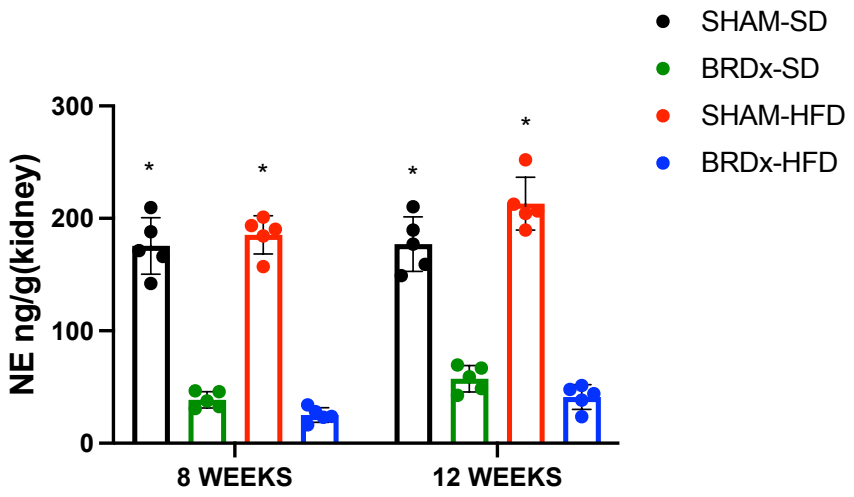


FIGURE 1. The bilateral renal denervation decreases the kidney content of Noradrenaline. Quantification of NE content in the kidney of the rats sacrificed 8 weeks or 12 weeks after the surgery (n = 5 per group). The three-way ANOVA + Tukey test: * P from to < 0.0001 denervated groups vs sham groups. NE: noradrenaline.

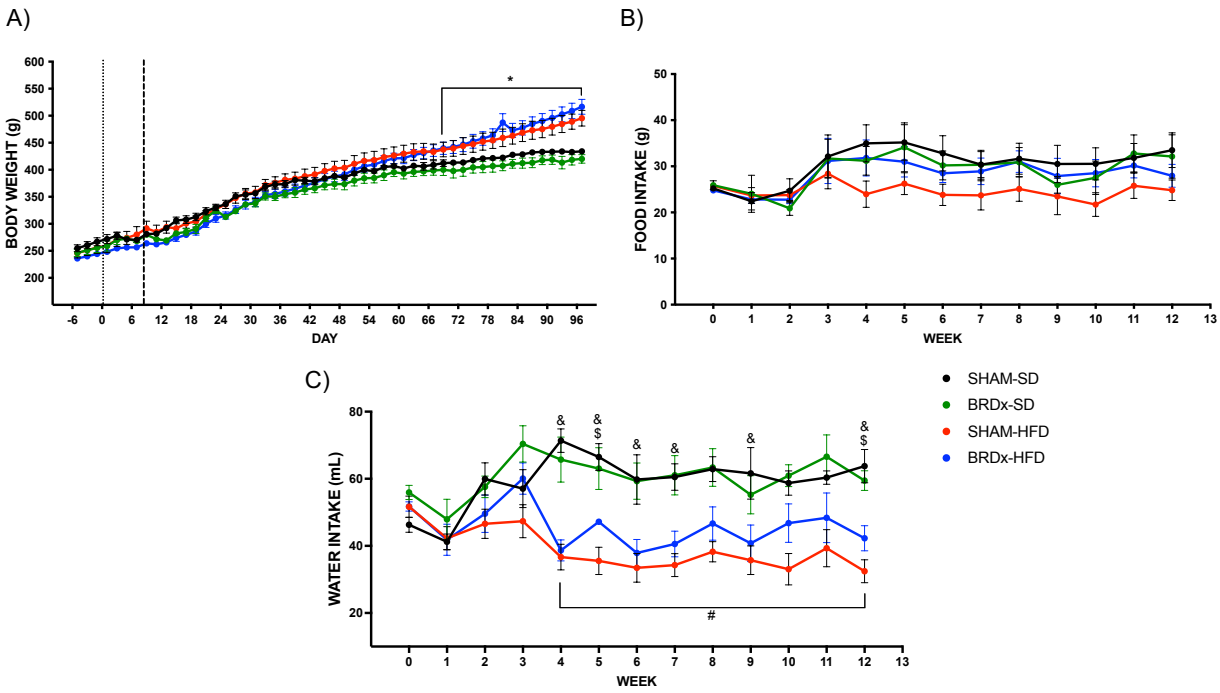


FIGURE 2. The high-fat diet increases body weight and decreases water intake in both intact and denervated rats. Bodyweight (A), food (B), and water (C) intake for each group for the entire protocol. The dotted line represents the surgery day, while the discontinuous one is the starting diet day (n=6 for all groups). The overall RM-MANOVA indicated time effects (P<0.0001) from day 68 in body weight and from week 4 in the water intake to the end, and time*group interaction effects (p<0.001) in the same points. RM-MANOVA by group: (P from 0.013 to 0.004) day 0 versus day 24 onwards all groups in body weight, (P from 0.005 to 0.001) week 0 versus week 4 onwards all groups except for BRDx-HFD in water intake. Two-way ANOVA + Tukey test: * P from 0.0457 to 0.0005 SHAM-HFD versus all groups; # P from 0.001 to 0.0007 SHAM-HFD versus SHAM-SD and BRDx-SD; & P from 0.015 to 0.0014 BRDx-HFD versus SHAM-SD and BRDx-SD; $ P from 0.0411 to 0.0402 BRDx-HFD versus SHAM-SD, BRDx-SD and BRDx-HFD.

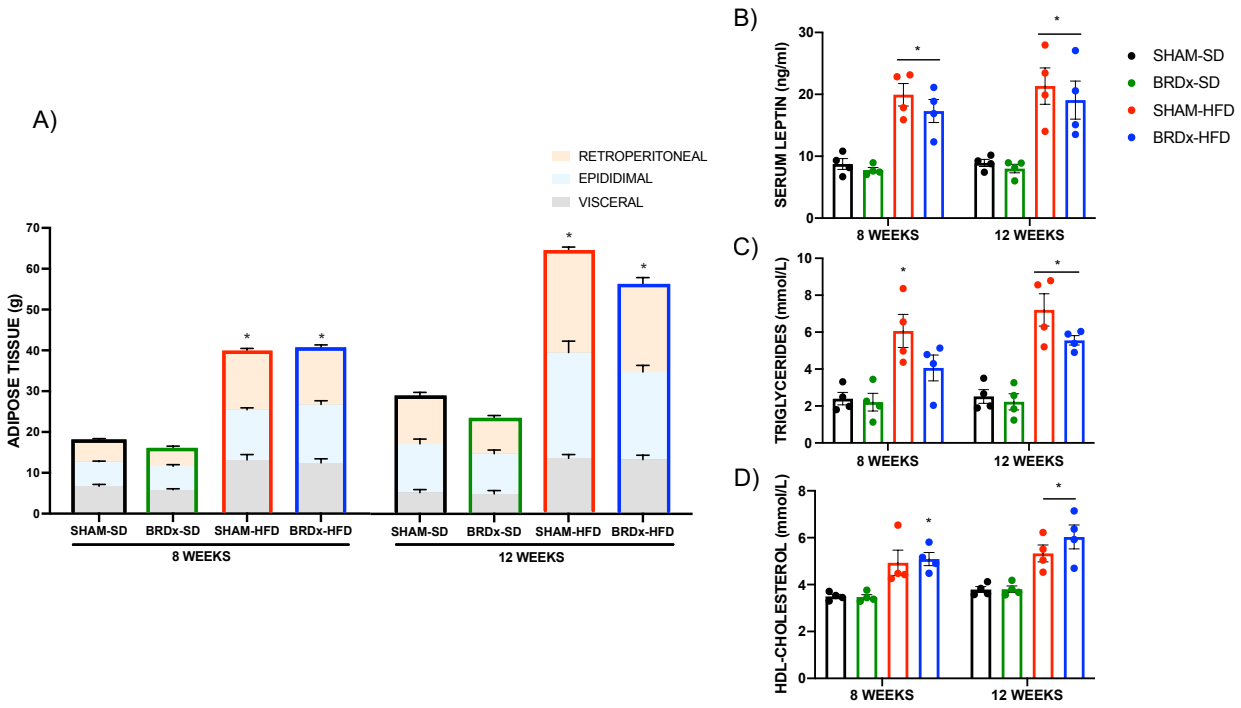


FIGURE 3. Bilateral renal denervation does not prevent fat accumulation, hyperleptinemia and dyslipidemia promoted by the high-fat diet. Adipose tissue at 8-week and 12-week (A), serum leptin (B), serum triglycerides (C), and HDL-Cholesterol (D). Two and Three-way ANOVA + Tukey test (adipose tissue and serum parameters respectively): * P from 0.0492 to < 0.0001 SHAM-HFD and BRDx-HFD versus SHAM-SD and BRDx-SD in the same week.

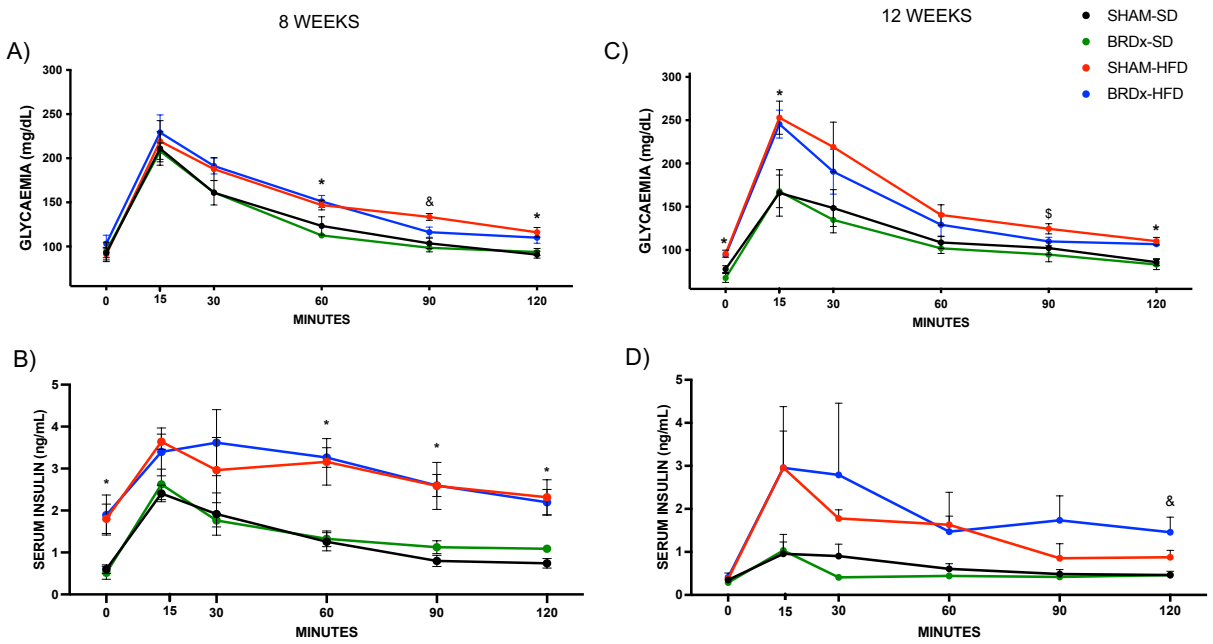


FIGURE 4. Bilateral renal denervation does not prevent glucose intolerance and insulin resistance promoted by the high-fat diet. Glycaemia at 8 (A) and 12 (C) weeks, and serum insulin at 8 (B) and 12 (D) weeks. The overall RM-MANOVA indicated time effects (P from 0.033 to < 0.0001) in all groups and no time*group interaction effects. RM-MANOVA by group: (P from 0.0303 to 0.01) minute 0 versus minute 15 onwards all groups. Two-way ANOVA + Tukey test: * P from 0.0492 to 0.005 SHAM-HFD and BRDx-HFD versus SHAM-SD and BRDx-SD; & P from 0.020 to 0.003 SHA-HFD versus all groups; $ P from 0.02 to 0.003 SHAM-HFD versus SHAM-SD and BRDx-SD; & P = 0.0402 BRDx-HFD versus SHAM-SD and BRDx-SD.

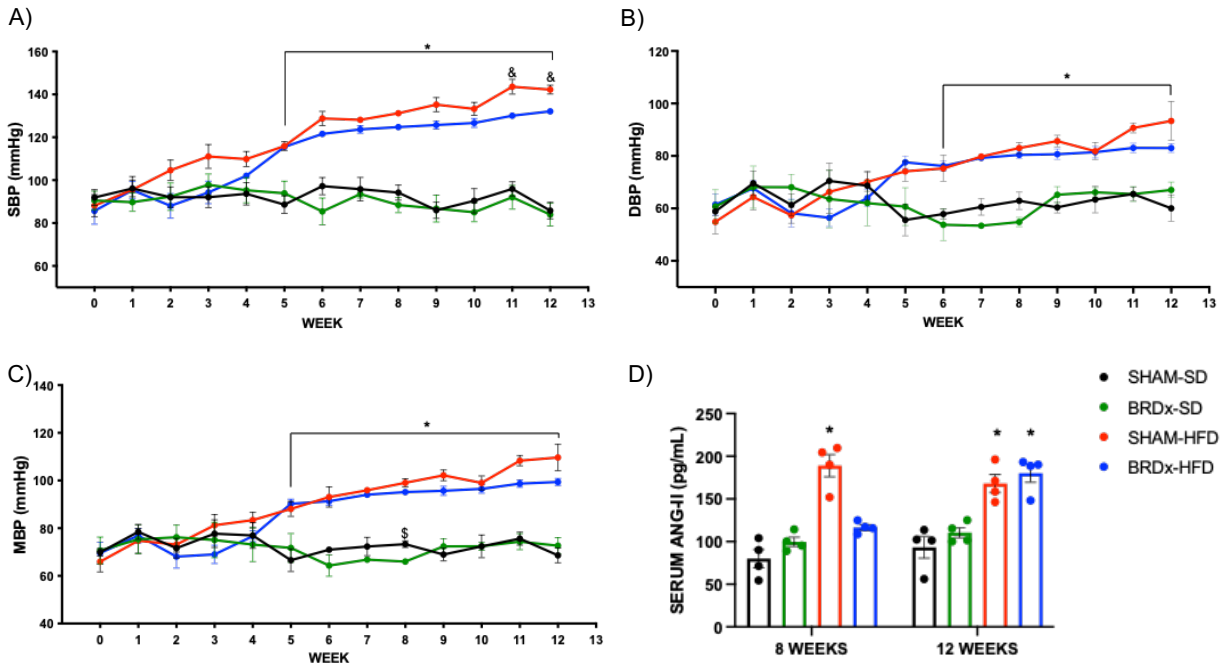


FIGURE 5. Bilateral renal denervation does not prevent blood pressure and serum Ang-II increase promoted by the high-fat diet. Systolic Blood Pressure (A), Diastolic Blood Pressure (B), and Mean Blood Pressure (C) were measured for 12 weeks (n=6 in all groups), and serum Ang-II (D). The overall RM-MANOVA indicated time effects (P<0.0001) from week 5 to the end in all groups in SBP and MBP and from week 6 in DBP; and time*group interaction effects (p<0.0001) in the same points. RM-MANOVA by group: in SBP (P from 0.003 to 0.0002) from week 3 onwards in SHAM-HFD and from week 5 in BRDx-HFD; in DBP and MBP (P from 0.004 to 0.0001) from week onwards in SHAM-HFD and BRDx-HFD. Two-way ANOVA + Tukey test: * P from 0.0057 to 0.0001 SHAM-HFD and BRDx-HFD versus SHAM-SD and BRDx-SD; & P from 0.0165 to 0.001 SHAM-HFD versus BRDx-HFD; $ P = 0.0206 SHAM-SD versus BRDx-SD.

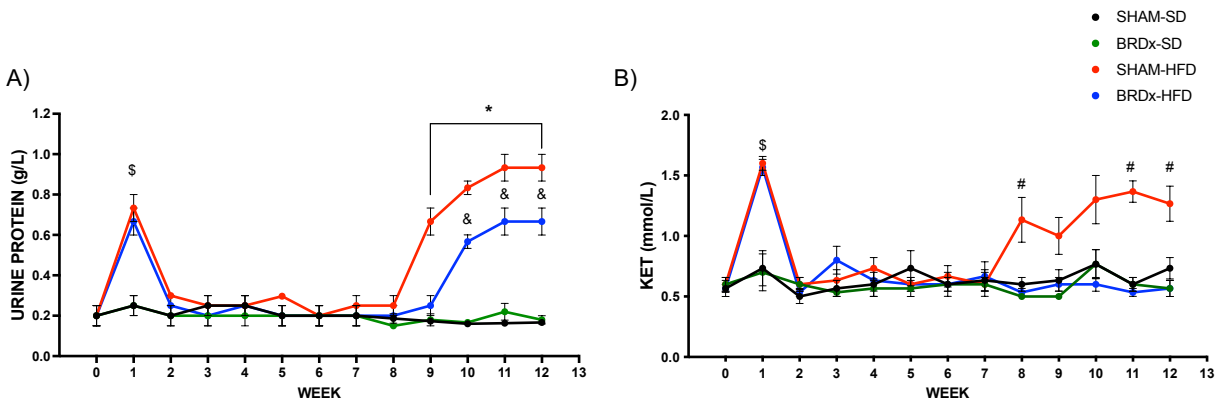


FIGURE 6. Bilateral renal denervation decreases protein ketones excretion in rats fed with the HFD. Urinary protein (A) and Urine Ketone Bodies (B) weremeasured weekly for 12 weeks (n=6 in all groups). The overall RM-MANOVA indicated time effects (P<0.0001) from week 1 to the end of all groups and in both variables; and time*group interaction effects (p<0.0001) in the same points. RM-MANOVA by group: in SBP (P from 0.003 to 0.0002) from week 3 onwards in SHAM-HFD and from week 5 in BRDx-HFD; in DBP and MBP (P from 0.004 to 0.0001) from week onwards in SHAM-HFD and BRDx-HFD. Two-way ANOVA + Tukey test: * P from 0.0057 to 0.0001 SHAM-HFD and BRDx-HFD versus SHAM-SD and BRDx-SD; & P from 0.0165 to 0.001 SHAM-HFD versus BRDx-HFD; $ P = 0.0206 SHAM-SD versus BRDx-SD.

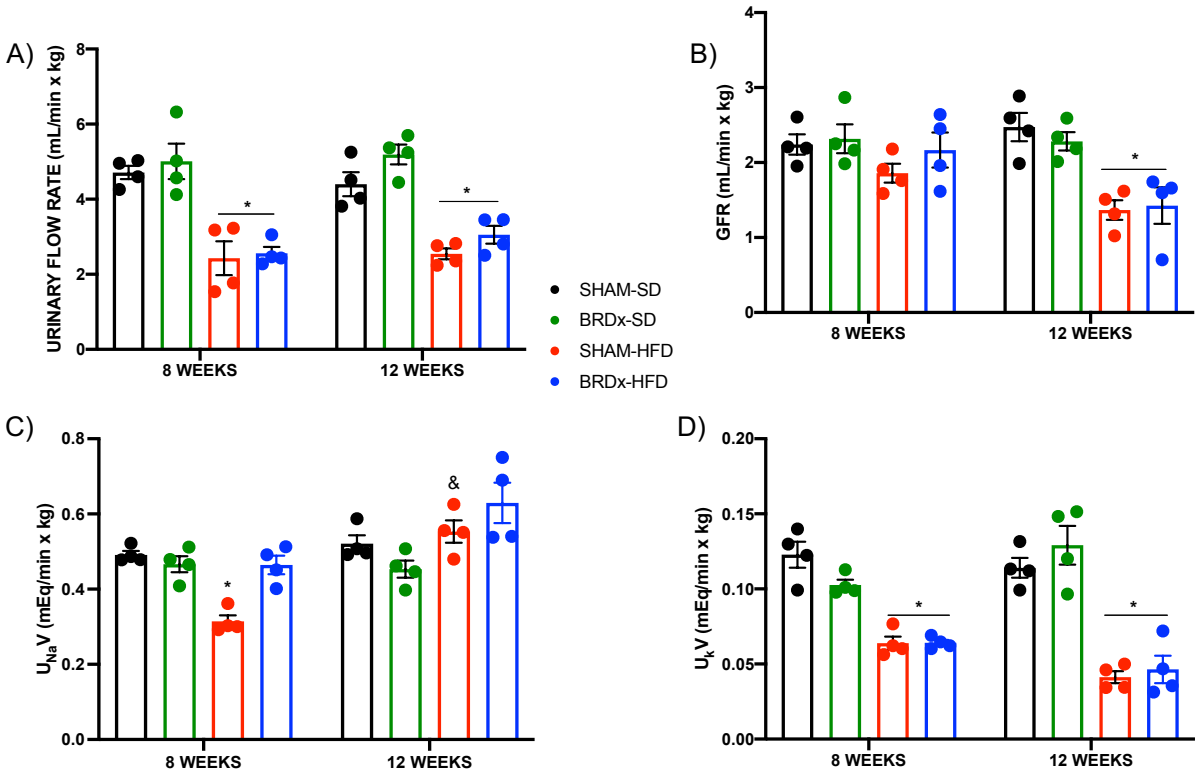


FIGURE 7. Bilateral renal denervation does not prevent the decrease in urinary flow and glomerular filtration rate promoted by the HFD. Urinary flow rate (A), Glomerular filtration rate (B), Absolute Na+ renal excretion (C), and Absolute K+ renal excretion (D). Three-way ANOVA + Tukey test: * P from 0.0235 to < 0.0001 SHAM-HFD and BRDx-HFD versus SHAM-SD and BRDx-SD in the same week; & P = 0.0008 SHAM-HFD 8 week versus SHAM-HFD 12 week.

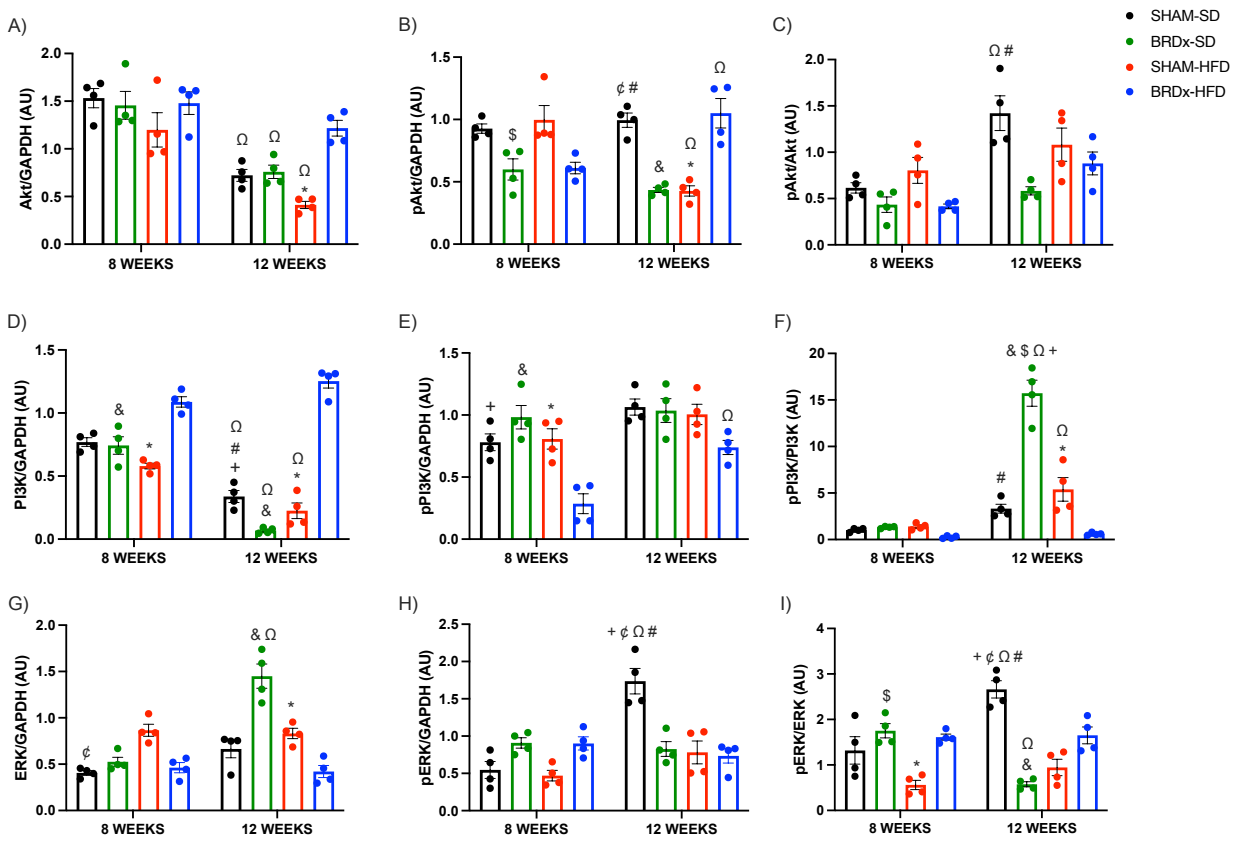
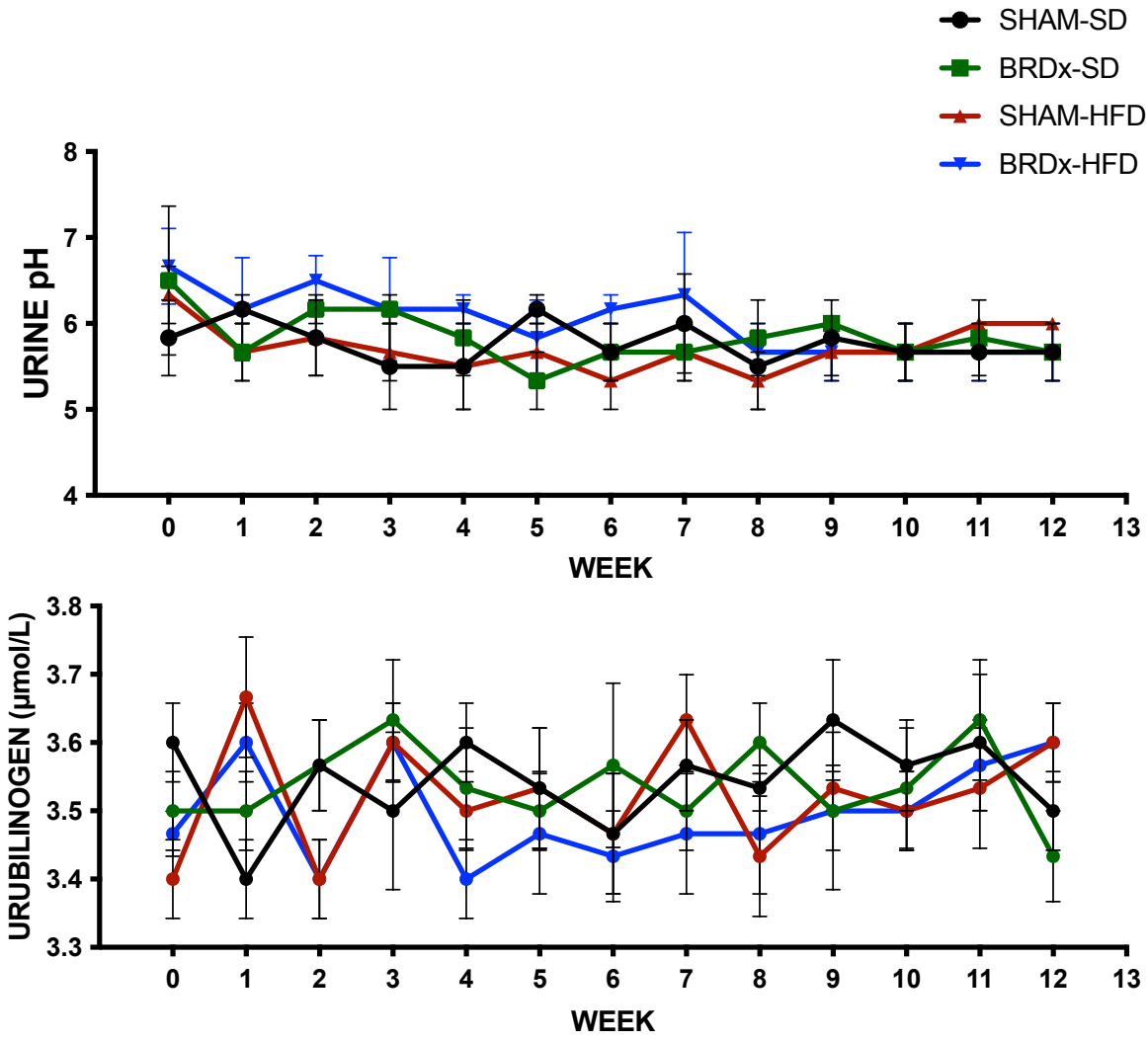
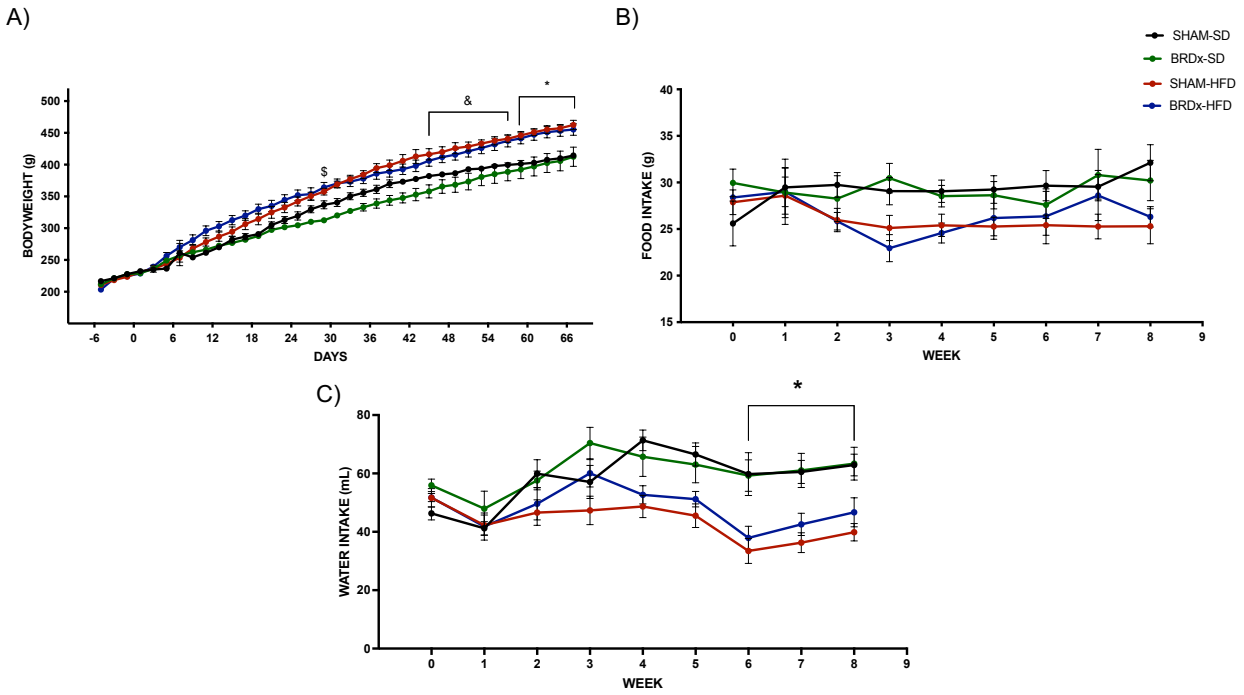


FIGURE 8. Quantification of the unphosphorylated and phosphorylated AKT, PI3K, and ERK signaling proteins in the kidney. Akt (A), pAkt (B), pAkt/Akt ratio (C), PI3K (D), pPI3K (E), pPI3K/PI3K ratio (F), ERK (G), pERK (H), pERK/ERK ratio (I) relative expression to GAPDH at final of 8 and 12 weeks. # SD-SHAM vs. SD-BRDx in the same week, P from 0.0084 to < 0.0001; ¢ SD-SHAM vs. HFD-SHAM in the same week, P < 0.0001; + SD-SHAM vs. HFD-BRDx in the same week, P from 0.0072 to < 0.0001; $ SD-BRDx vs. HFD-SHAM in the same week, P from 0.0012 to < 0.0001; & SD-BRDx vs. HFD-BRDx in the same week, P from 0.0336 to < 0.0001; * HFD-SHAM vs. HFD-BRDx in the same week, P from 0.0493 to < 0.0001; Ω the same condition at 8 weeks vs 12 weeks, P from 0.0193 to < 0.0001.

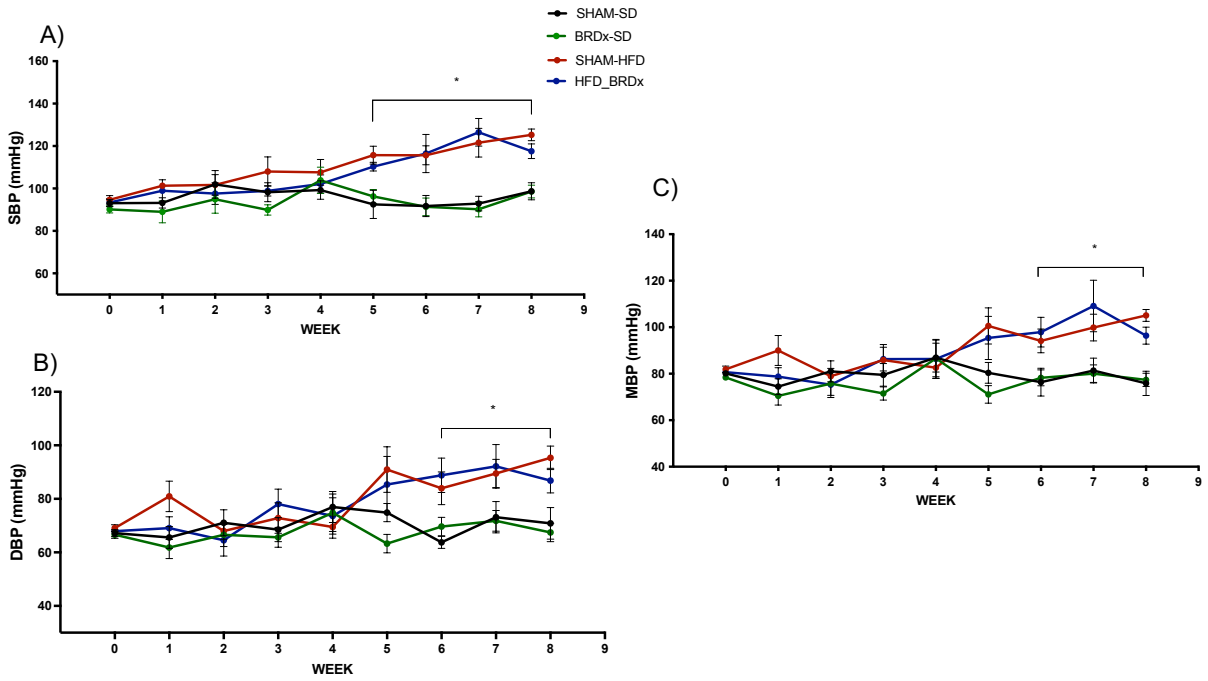
SUPLEMENTARY FIGURES



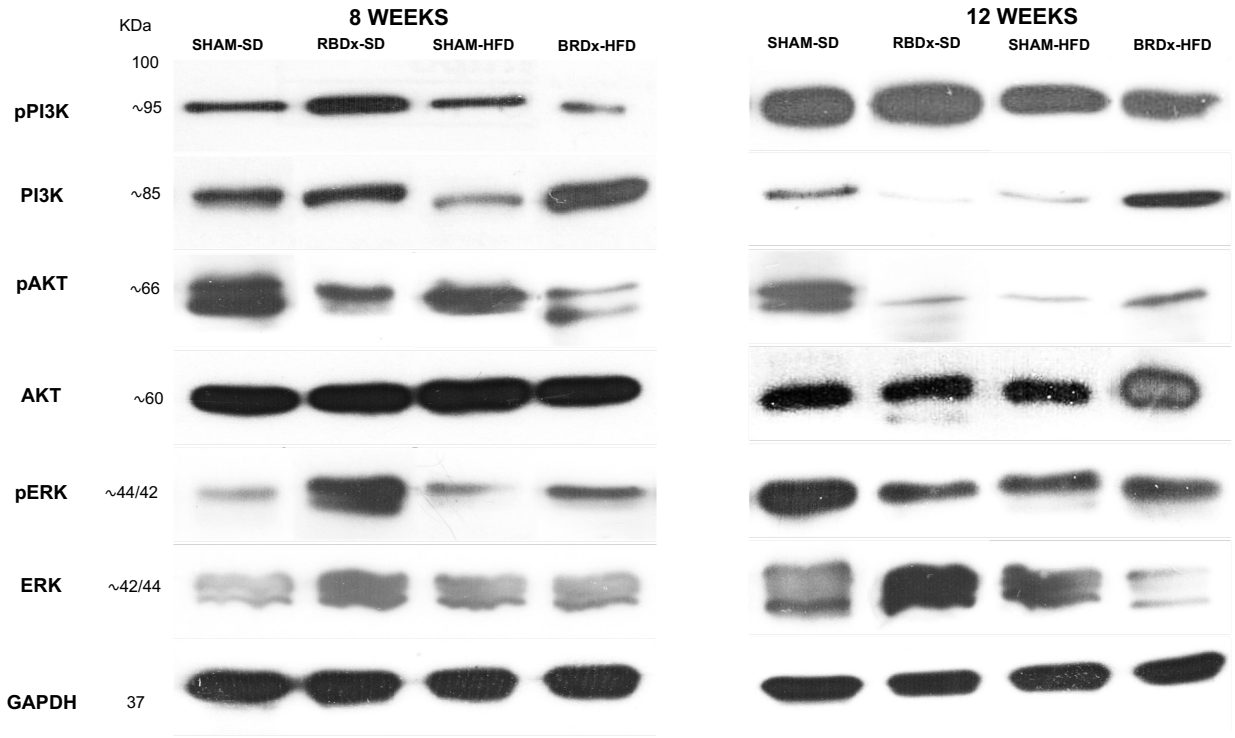
SUPLEMENTARY FIGURE 1. Neither bilateral denervation nor HFD affects urine pH (A) and urine urobilinogen concentration (B).



SUPLEMENTARY FIGURE 2. Bodyweight (A), food (B), and water (C) intake for each group for the 8-week series. The overall RM-MANOVA indicated time effects (P<0.0001) from day 36 in body weight and from week 6 in the water intake to the end, and time*group interaction effects (p<0.001) in the same points. RM-MANOVA by group: (P from 0.019 to 0.005) day 0 versus day 18 onwards all groups in body weight, (P from 0.003 to 0.001) week 0 versus week 6 onwards all groups in water intake. Two-way ANOVA + Tukey test: * P from 0.04 to 0.0001 SHAM-HFD and BRDx-HFD versus SHAM-SD and BRDx-SD; & P from 0.001 to 0.0007 SHAM-HFD versus BRDx-SD; $ P = 0.002 BRDx-SD versus all groups.



SUPLEMENTARY FIGURE 3. Blood pressure in 8 weeks series. Systolic Blood Pressure (A), Diastolic Blood Pressure (B), and Mean Blood Pressure (C) were measured for 8 weeks (n=6 in all groups). The overall RM-MANOVA indicated time effects (P<0.0001) from week 5 to the end of all groups in SBP and DBP and from week 6 in MBP; and time*group interaction effects (p<0.0001) in the same points. Two-way ANOVA + Tukey test: * P from 0.01 to 0.0001 SHAM-HFD and BRDx-HFD versus SHAM-SD and BRDx-SD.



SUPLEMENTARY FIGURE 4. Representative pictures of the Western Blot results unphosphorylated and phosphorylated Akt, PI3K, and ERK signaling proteins in the kidney.

The FASEB Journal

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Direct Evidence that in Rat the Renal Interstitium Contracts *in vivo*

Manuel Rodríguez-Martínez, Juan Francisco López-Rodríguez, Omar Flores-Sandoval, Miriam Zarahí Calvo-Turrubiartes, Lilia LLamazares-Azuara

First published: 03 October 2018 | https://doi.org/10.1096/fasebj.31.1_supplement.701.4

TOOLS SHARE

Abstract

Dibutyryl-cAMP abolishes the RIHP increase in response to renal medullary direct interstitial volume expansion (DIVE = 100 µl in 30 s) with 0.9% saline solution (SS) in hydropenic rats (FASEB J 30:739.9: 2016), suggesting that renal interstitium contracts *in vivo*. To assess more directly if such an event happens *in vivo*, we decided to evaluate in hydropenic rats the time course of RIHP before and after either an acute sham (CTR group, n = 9) or an acute (1s) real renal interstitial medullary application (RIMA) of a 5µ bolus of either vehicle (0.9% SS, SS group, n = 9) or 10 µM alpha-trinositol (anti-inflammatory drug that increases β1 integrin-mediated collagen fiber network contraction, A-T group, n = 9). Left subcapsular RIHP (subcRIHP) and % change of renal outer medullary blood flow (%ΔRoMBF by L-D) were continuously measured in anesthetized rats with right nephrectomy, acute left renal denervation, hormonal clamp and renal perfusion pressure control (100 mmHg), before (baseline = BL = 30 min) and after sham or real RIMA of 5 µl bolus of either SS or A-T. There were no differences among groups in body weight, hematocrit nor plasma protein concentration at any time. The table below shows both BL RIHP and ΔRIHP (RIHPafter – RIHPbefore) values (mean ± SEM) obtained at different times points after sham or real RIMA. Three were no differences among groups in BL RIHP. Overall RMMANOVA of ΔRIHP data showed no time effect, but if group (P<0.0004) and time * group interaction effects (P<0.009); 1WRMMANOVA:* P = 0.05, ** P <0.001 vs. Before; 1WANOVA + Tukey test:*P<0.04 vs. SS group, **P<0.001 vs. CTR group. The %ΔRoMBF showed a similar decrease over time in the three groups.

CONCLUSIONS

The results show that acute RIMA of alfa-trinositol is able to increase the RIHP in hydropenic rats by a mechanism independent of increased renal medullary blood flow, which reinforces the idea of that, at least in hydropenia, renal interstitium contracts *in vivo*.

Support or Funding Information

C15-FAI-04-96.66/UASLP

Group	subcRIHP (mmHg)Before RIMA	subcΔRIHP (mmHg)After RIMA			
	BL	15 min	30 min	45 min	60 min
CTR	3.0 ± 0.1	−0.85 ± 0.22**	−1.11 ± 0.16**	−1.17 ± 0.19**	−1.16 ± 0.17**
SS	3.1 ± 0.03	−0.30 ± 0.20	−0.15 ± 0.36	−0.38 ± 0.56	−0.51 ± 0.55
A-T	3.1 ± 0.06	0.54 ± 0.25*♦	1.26 ± 0.51*,♦	0.76 ± 0.37♦	0.68 ± 0.38♦

Volume 31, Issue S1
Special Issue: Experimental Biology 2017 Meeting Abstracts
April 2017
Pages 701.4-701.4

Related Information

The FASEB Journal

Physiology | Free Access

Bilateral renal denervation does not prevent the kidney damage caused by a high fat diet in rats

Omar Flores-Sandoval, Skarleth Cárdenas-Romero, Roberto Salgado-Delgado, Nadia Saderi

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TOOLS SHARE

Abstract

One of the hallmarks of the metabolic syndrome (MS) is the increase of renal sympathetic nerve activity (RSNA), which leads to the upregulation of the renin-angiotensin system (RAS). Interestingly, the sympathetic hyperactivity in the kidney of obese humans often occurs in absence of overt systemic hypertension. The stimulation of RSNA contributes to the glomerular lesions and the progressive loss of nephron functionality, resulting in acute renal injury (AKI) and, in the long term, chronic kidney disease (CKD), which represent the most common complication of MS. Clinical evidence and animal models of MS have shown that renal sympathetic denervation (RSDN) induces a transient attenuation of the hypertensive symptoms, while the markers of kidney damage continue worsening. Therefore, it is likely that the renal derangement in MS might also depend on the metabolic impairment, such as the upregulation of plasma leptin and insulin, independently from the RSNA. To investigate this issue, we explored the effect of MS over blood pressure and kidney functionality, in intact and renal denervated rats. For that, animals were either fed with a standard diet (SD) or a high-fat diet for 12 weeks (HFD), with or without bilateral renal denervation performed at the beginning of protocol (n = 6). Results indicate that, as expected, HFD in intact animals promotes fat accumulation, glucose intolerance and hypertension, but also decreases water consumption and induces marked proteinuria from week 7, slight hematuria from week 9, with respect to SD rats. Remarkably, the renal denervation prevents the changes that HFD triggered in blood pressure and renal parameters only in the short and medium term, but it is ineffective in the long term. On the bases of these results, we suggest that the hypertension associated to MS is not only due to hyperactivation of RSNA, but it could involve hormonal/hemodynamic factors, typical of metabolic impairment, which could contribute to renal injury.

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This abstract is from the Experimental Biology 2019 Meeting. There is no full text article associated with this abstract published in *The FASEB Journal*.

Volume 33, Issue S1
Experimental Biology 2019 Meeting Abstracts
April 2019
Pages 569.5-569.5

Related Information

Recommended

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Patric J. D. Delhanty, Martin Huisman, Lucy Y. Baldeon-Rojas, Iris van den Berge, Aldo Grefhorst, Thierry Abribat, Pieter J. M. Leenen, Axel P. N. Themmen, Aart-Jan van der Lely

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PLOS ONE

RESEARCH ARTICLE

Additional evidence that the rat renal interstitium contracts *in vivo*

Manuel Rodríguez-Martínez*☯, Juan Francisco López-Rodríguez☯, Omar Flores-Sandoval☯, Miriam Zarahí Calvo-Turrubiartes, María Eugenia Sánchez-Briones, Ana Sonia Silva-Ramírez, Vianney Guerreo-Ojeda

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Abstract

We recently provided highly suggestive preliminary evidence that the renal interstitium contracts reactively *in vivo*. We demonstrated that renal medullary direct interstitial volume expansion (rmDIVE = 100 μl bolus infusion of 0.9% saline (SS)/30 s) brought about a biphasic renal interstitial hydrostatic pressure (RIHP) response which was abolished when dibutyryl-cAMP was concomitant and interstitially infused. To assess more deeply the feasibility of the concept that the renal interstitium contracts *in vivo*, two experimental series (S1, S2) were performed in hydropenic rats subjected to acute left renal-denervation, hormonal clamping, and control of renal arterial pressure. In S1, RIHP and renal outer medullary blood flow (RoMBF) were continuously measured before and after a sudden micro-bolus (5μl) injection, into the renal medullary interstitium, of SS containing α-trinositol (α-TNS, anti-inflammatory drug) to either two doses 2 or 4 mM (SS + 2 α-TNS and SS + 4 α-TNS groups). No overall differences between groups in either ΔRIHP or %ΔRoMBF time courses were found; however, in the SS + 2 α-TNS group the data were less scattered and the ΔRIHP time course tended to peak faster and then persisted there, so that, this α-TNS dose was selected for S2. In S2, RIHP and RoMBF were similarly measured in rats randomly assigned to three groups: the CTR group (sham time-control), SS group (SS alone), and SS + α-TNS group. The micro-bolus injection of SS alone (SS group) was unable to increase ΔRIHP. The group with no micro-bolus injection (CTR group) experienced a decrease in ΔRIHP. The micro-bolus injection of SS + 2 α-TNS was accompanied by a differential increase in ΔRIHP (vs. CTR and SS groups). These responses were not associated with differential changes among groups in %ΔRoMBF or hemodilution parameters. These results provide additional evidence that the renal interstitium contracts *in vivo*.

Introduction

Given the *sui-generis* architecture of the renal interstitium [1] and supported both in the well-known phenomenon of cellular subcutaneous tissue contraction [2] and in the fact that renal

The FASEB Journal

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Renal Interstitial Injection of a-Trinositol Increases Renal Interstitial Hydrostatic Pressure (RIHP) but Does Not Modify its Low Frequency Oscillatory Pattern

Manuel Rodríguez-Martínez, Juan Francisco López-Rodriguez, Omar Flores-Sandoval, Ana Sonia Silva-Ramírez, Vianney Guerrero-Ojeda

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TOOLS SHARE

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Pages 1-1

Related Information

Abstract

RIHP increases after renal interstitial medullary injection (RIMI) of a 5μ bolus of saline solution (SS) + 2 μM α-Trinositol (α –T, drug that increases collagen fiber network contraction, FASEB J 31:701.4: 2017). To assess whether this increase is induced by changes in the frequency and/or amplitude of oscillatory pattern of RIHP we evaluate in hydropenic rats the time course of RIHP before and after either an acute sham (CTR group, n = 9) or an acute (1s) real RIMI of a 5μ bolus of either vehicle (0.9% SS, SS group, n = 9) or SS + 2 μM α - T (α – T group, n = 9). Left subcapsular RIHP (RIHP) was continuously measured (1 Hz) in anesthetized rats with right nephrectomy, acute left renal denervation, hormonal clamp and renal perfusion pressure control (100 mmHg), before (baseline phase = BL = 30 min) and 30 min after (experimental phase = EXP) sham or real RIMI of 5 μl bolus of either SS or SS + α-T. The table 1 shows the 30 min average BL RIHP (1800 s, s = samples or seconds), ΔRIHP (EXP RIHP – BL RIHP) values (mean ± SEM) obtained 30 min after sham or real RIMI during 300 s. It also shows the RIHP frequency (F) of major oscillations (cycles/min = cpm) and the standard deviation (SD, an oscillatory amplitude index) of global RIHP oscillation before and 30 min after RIMI. Three were no differences among groups in BL RIHP, F or SD. For ΔRIHP data there were time * group interaction effect ($P < 0.009$); * $P = 0.05$, ** $P < 0.001$ vs. BL; ˚ $P < 0.04$ vs. SS group, ¨ $P < 0.001$ vs. CTR group. For SD data ° $P < 0.001$ between phases.

CONCLUSIONS

The results show that RIHP oscillates at an equally low frequency (≈ 0.03 Hz) before and after sham or real RIMI but with lower amplitude after sham or real RIMI in any of the groups. This suggests that α-T increases RIHP but not through modify the frequency or amplitude of its low frequency oscillatory pattern.

Support or Funding Information

Own financial resources of Integrative Physiology Lab.

	RIHP (mmHg)		RIHP Frequency cpm		RIHP SD (mmHg)	
		Delta				
	BL 30 min	EXP-BL at min 30	BL last 5 min	EXP at min 30	BL 30 min	EXP at min 30
Group	1800 s	300 s	300 s	300 s	1800 s	300 s
CTR	3.0 ± 0.10	−1.1 ± 0.16	2.2 ± 0.26*^	2.1 ± 0.26	0.54 ± 0.07	0.20 ± 0.04*
SS	3.1 ± 0.03	−0.15 ± 0.36	2.1 ± 0.44	2.2 ± 0.46	0.50 ± 0.06	0.18 ± 0.03*
a-T	3.1 ± 0.06	1.26 ± 0.51	2.2 ± 0.32*˚♦	2.2 ± 0.25	0.35 ± 0.04	0.19 ± 0.04*

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ORIGINAL ARTICLE

Temporal dysregulation of hypothalamic integrative and metabolic nuclei in rats fed during the rest phase

Oscar D. Ramirez-Plascencia[a,b], Nadia Saderi[a], Skarleth Cárdenas Romero[a], Omar Flores Sandoval[a], Adrián Báez-Ruiz[a], Herick Martínez Barajas[a], and Roberto Salgado-Delgado[a]

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ABSTRACT

Temporal coordination of organisms according to the daytime allows a better performance of physiological processes. However, modern lifestyle habits, such as food intake during the rest phase, promote internal desynchronization and compromise homeostasis and health. The hypothalamic suprachiasmatic nucleus (SCN) synchronizes body physiology and behavior with the environmental light–dark cycle by transmitting time information to several integrative hypothalamic nuclei, such as the paraventricular nucleus (PVN), dorsomedial hypothalamic nucleus (DMH) and median preoptic area (MnPO). The SCN receives metabolic information mainly via Neuropeptide Y (NPY) inputs from the intergeniculate nucleus of the thalamus (IGL). Nowadays, there is no evidence of the response of the PVN, DMH and MnPO when the animals are subjected to internal desynchronization by restricting food access to the rest phase of the day. To explore this issue, we compared the circadian activity of the SCN, PVN, DMH and MnPO. In addition, we analyzed the daily activity of the satiety centers of the brainstem, the nucleus of the tractus solitarius (NTS) and area postrema (AP), which send metabolic information to the SCN, directly or via the thalamic intergeniculate leaflet (IGL). For that, male Wistar rats were assigned to three meal protocols: fed during the rest phase (Day Fed); fed during the active phase (Night Fed); free access to food (*ad libitum*). After 21 d, the daily activity patterns of these nuclei were analyzed by c-Fos immunohistochemistry, as well as NPY immunohistochemistry, in the SCN. The results show that eating during the rest period produces a phase advance in the activity of the SCN, changes the daily activity pattern in the MnPO, NTS and AP and flattens the c-Fos rhythm in the PVN and DMH. Altogether, these results validate previous observations of circadian dysregulation that occurs within the central nervous system when meals are consumed during the rest phase, a behavior that is involved in the metabolic alterations described in the literature.

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KEYWORDS
Suprachiasmatic nucleus; dorsomedial hypothalamic nucleus; paraventricular nucleus; median preoptic area; circadian asynchrony

Introduction

Body homeostasis and health are tightly dependent on the synchrony between the daily light–dark alternation and the preservation of physiological and behavioral rhythms. The coordination between environmental cues and internal homeostasis is mainly accomplished by the hypothalamic Suprachiasmatic nucleus (SCN) hierarchically. The SCN receives light signals via glutamatergic projections from retinal ganglion cells and then transmits time-related information to several nuclei which, in turn, control the neuroendocrine axis and the autonomic nervous system, among other outputs directed inside and outside of the hypothalamus (Buijs et al. 2020). The second-order relays allow organisms to anticipate and adapt to the incoming challenges according to the day phase. In addition to the light–dark cycle, the synchronizing properties of the SCN depend also on a wide range of peripheral inputs, which makes it possible to coordinate physiology and behavior with other environmental cues, such as food availability or social interaction (Espitia-Bautista et al. 2017; Zerón-Rugerio et al. 2020).

The brain areas which mainly exchange information with the SCN and function as effectors for their circadian rhythmicity are the hypothalamic paraventricular nucleus (PVN), the dorsomedial hypothalamic nucleus (DMH) and median preoptic area (MnPO). These nuclei integrate the temporal information with internal cues to regulate body temperature, stress response and endocrine secretion, metabolism and sleep, among other homeostatic and behavioral functions (Buijs et al. 2003; Chou et al. 2003; Gooley et al. 2006; Guzmán-Ruiz et al. 2015; Neumann et al. 2019; Oster 2020; Saper and Machado 2020).

An extensive amount of evidence shows that conflicts between the rhythms ruled by the SCN and alterations of physiology and behavior could be harmful to any

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Leptin and adiponectin regulate the activity of nuclei involved in sleep-wake cycle in male rats

Oscar Daniel Ramírez-Plascencia[1,2], Nadia Saderi[1], Skarleth Cárdenas-Romero[1], Fabio García-García[3], Carolina Peña-Escudero[3], Omar Flores-Sandoval[1], Lucia Azuara-Álvarez[1], Adrián Báez-Ruiz[1] and Roberto Salgado-Delgado[1]*

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Epidemiological and experimental evidence recognize a relationship between sleep-wake cycles and adiposity levels, but the mechanisms that link both are not entirely understood. Adipose tissue secretes adiponectin and leptin hormones, mainly involved as indicators of adiposity levels and recently associated to sleep. To understand how two of the main adipose tissue hormones could influence sleep-wake regulation, we evaluated in male rats, the effect of direct administration of adiponectin or leptin in the ventrolateral preoptic nuclei (VLPO), a major area for sleep promotion. The presence of adiponectin (AdipoR1 and AdipoR2) and leptin receptors in VLPO were confirmed by immunohistochemistry. Adiponectin administration increased wakefulness during the rest phase, reduced delta power, and activated wake-promoting neurons, such as the locus coeruleus (LC), tuberomammillary nucleus (TMN) and hypocretin/orexin neurons (OX) within the lateral hypothalamus (LH) and perifornical area (PeF). Conversely, leptin promoted REM and NREM sleep, including increase of delta power during NREM sleep, and induced c-Fos expression in VLPO and melanin concentrating hormone expressing neurons (MCH). In addition, a reduction in wake-promoting neurons activity was found in the TMN, lateral hypothalamus (LH) and perifornical area (PeF), including in the OX neurons. Moreover, leptin administration reduced tyrosine hydroxylase (TH) immunoreactivity in the LC. Our data suggest that adiponectin and leptin act as hormonal mediators between the status of body energy and the regulation of the sleep-wake cycle.

KEYWORDS

VLPO, sleep-wake, metabolism, obesity, circadian misalignment, timing of food intake, hypothalamus

EFECTO DE LA LITERATURA SOBRE EL CEREBRO Y SUS BENEFICIOS EN LA SALUD

SKARLETH CÁRDENAS-ROMERO*, OMAR FLORES-SANDOVAL*, ÓSCAR DANIEL RAMÍREZ-PLASCENCIA**

La narración es parte central de nuestra esencia como seres humanos. Todas las culturas conocidas tienen historias que se divulgan de generación en generación, ya sea de forma oral o escrita, y son tan importantes que se ha propuesto que el origen del hombre moderno se encuentra en la transmisión de esas historias (Smith *et al.*, 2017). La escritura facilitó la difusión de historias que se daban de forma oral, convirtiendo al libro en el objeto que permitió una de las revoluciones culturales más importantes de la humanidad.

La literatura nace de estas narraciones y su desarrollo ha acompañado al ser humano desde el inicio. Los libros son más que información en papel, a través de ellos compartimos conocimiento, contamos historias, conocemos personajes reales y ficticios, recreamos sucesos históricos, inventamos mundos mágicos y futuristas. Gran parte de las personas pueden identificar algún libro que disfrutaron, que cambió su forma de pensar o incluso que marcó su vida de forma significativa. Si un libro puede causar este tipo de impresiones ¿es posible que tenga la fuerza suficiente para generar cambios en nuestro cerebro, e incluso en nuestra salud?

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SLIDES PRESENTATION

UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ

CBB Ciencias Biomédicas Básicas POSGRADO

La denervación simpática bilateral renal no previene la hipertensión ni las complicaciones renales del síndrome metabólico

Defensa de tesis de doctorado
Presenta:
MC. Omar Flores Sandoval

DIRECTORA DE TESIS
Dra. Nadia Saderi

ASESORES INTERNOS:
Dr. Roberto C. Salgado Delgado
Dr. Ricardo Espinosa Tanguma
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SINODAL SUPLENTE:
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1

INTRODUCCIÓN

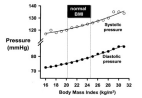
Más de mil millones de personas (2 de cada 7) en el mundo padecen hipertensión.

Cada año su prevalencia aumenta, especialmente con la edad.

HIPERTENSIÓN

- Esencial ≠ Secundaria
- Es el principal factor de riesgo para enfermedades cardio-renales.
- El número de personas con hipertensión incontrolada sigue aumentando, a pesar de los avances en farmacológicos y clínicos.
- La hipertensión engendra más hipertensión, lo que produce daño a órganos diana (**riñón**), insuficiencia orgánica y muerte prematura.

RELACIÓN ENTRE PRESIÓN ARTERIAL & INDICE DE MASA CORPORAL



Montani JP. et al., Int J Obes Relat Metab Disord., 2002.

PAM= Gasto cardíaco X Resistencia Periférica Total

BLOOD PRESSURE CATEGORY	SYSTOLIC mm Hg (upper number)		DIASTOLIC mm Hg (lower number)
NORMAL	LESS THAN 120	and	LESS THAN 80
ELEVATED	120 – 129	and	LESS THAN 80
HIGH BLOOD PRESSURE (HYPERTENSION) STAGE 1	130 – 139	or	80 – 89
HIGH BLOOD PRESSURE (HYPERTENSION) STAGE 2	140 OR HIGHER	or	90 OR HIGHER
HYPERTENSIVE CRISIS (consult your doctor immediately)	HIGHER THAN 180	and/or	HIGHER THAN 120

(Whelton PK et al., Hypertension, 2017)

2

INTRODUCCIÓN

Más de mil millones de personas (2 de cada 7) en el mundo padecen hipertensión.

Cada año su prevalencia aumenta, especialmente con la edad.

3

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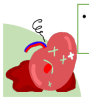

4

PAM= Gasto cardíaco X Resistencia Periférica Total

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NORMAL	LESS THAN 120	and	LESS THAN 80
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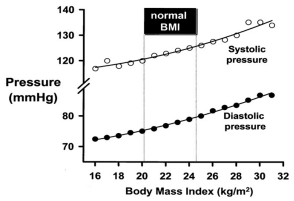
HYPERTENSIVE CRISIS (consult your doctor immediately)	HIGHER THAN 180	and/or	HIGHER THAN 120

(Whelton PK et al., Hypertension, 2017)

5

RELACIÓN ENTRE PRESIÓN ARTERIAL & ÍNDICE DE MASA CORPORAL



Montani JP. et al., Int J Obes Relat Metab Disord., 2002.

6

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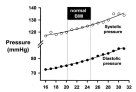
RELACIÓN ENTRE PRESIÓN ARTERIAL & INDICE DE MASA CORPORAL



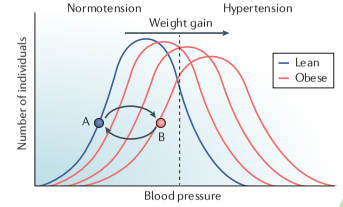
Montani JP. et al., Int J Obes Relat Metab Disord., 2002.

PAM= Gasto cardíaco X Resistencia Periférica Total

(Whelton PK et al., Hypertension, 2017)

7

El aumento de peso afecta la distribución de frecuencia de la presión arterial hacia la hipertensión.



Hall J. et al., Hypertension 2019.

8

Comorbilidad de condiciones metabólicas y cardiovasculares

Table 1 Metabolic syndrome definitions provided by various authorities

Authority, yr	WHO 1998	EGIR 1999	ATP III (2001/4)	IDF 2005
Insulin resistance	IGT, IFG, T2D; insulin resistance + 2 below	Plasma insulin >75th percentile + 2 below	None; any 3 of the 5 below	None
BMI or body fat distribution	Men WHR >0.90 Women WHR >0.85 or BMI >30	WC ≥94 cm men or ≥80 cm women	WC >102 cm men or >88 cm women	Increased WC (pop. specific) + 2 below
Lipids	TG ≥150 mg/dL HDL <35 men or <39 women	TG ≥ 150 mg/dL HDL <39 in men or women	TG ≥150 mg/dL HDL <40 men or <50 women	TG ≥150 mg/dL or Rx, HDL <40 men or <50 women or Rx
Glucose	IGT, IFG, T2D	IGT or IFG	≥110 mg/dL	≥100 mg/dL or diabetes
Blood pressure	≥140/≥90	≥140/≥90 or Htn Rx	≥130/≥85	≥130/≥85 or Htn Rx
Other	Urine alb >20 mg /min; ACR >30 mg/g			

WHO World Health Organization, *EGIR* European Group for the study of Insulin resistance, *ATP* adult treatment panel, *IDF* international diabetes federation, *IGT* impaired glucose tolerance, *IFG* impaired fasting glucose, *T2D* type 2 diabetes, *BMI* body mass index, *WHR* waist:hip ratio, *Htn* hypertension, *Rx* pharmacologic treatment, *alb* albumin, *ACR* albumin:creatinine ratio

Lopes H et al, Diabetol Metab Syndr, 2016

9

Cambios neurohumorales, hemodinámicos y renales en humanos y en modelos animales de obesidad causados por una dieta alta en grasa

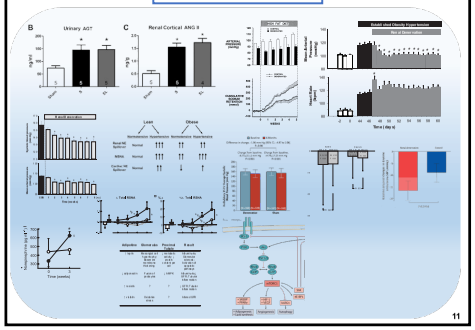
Table 1 | **Haemodynamic, renal and neurohormonal changes in obesity**

Parameter	Humans	Dogs	Rabbits	Rats	Mice
Kidney function					
GFR[a]	↑	↑	↑	↑ or ↔	↑
Renal blood flow[a]	↑	↑	↑	↑ or ↔	NA
Renal Na^+ reabsorption[a]	↑	↑	↑	↑ or ↔	↑ or ↔
Kidney compression	↑	↑	↑	↔	↔
Hormonal changes					
Fasting plasma insulin	↑	↑	↑	↑	↑
Insulin sensitivity	↓	↓	↓	↓	↓
Fasting plasma leptin	↑	↑	↑	↑	↑
Plasma renin:angiotensin II	↑	↑	↑ or ↔	↑ or ↔	↑
Plasma aldosterone	↑	↑	↑	↑	↑ or ↔
Neural control					
Renal sympathetic activity	↑	↑	↑	↑	↑
Cardiac sympathetic activity	↓ or ↔	NA	NA	NA	NA
Muscle sympathetic activity	↑	NA	NA	NA	NA
Baroreflex sensitivity	↓	↓	↓	↓	↓

Hall et al., Hypertension 2019.

10

ANTECEDENTES



11

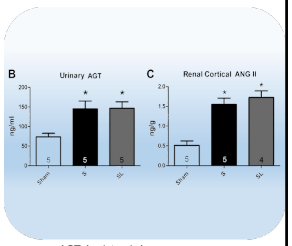
ANTECEDENTES

La activación simpática (RSNA) renal estimula el RAS.

(Pontes R.B et. al. Am J Physiol Renal Physiol . 2015)

La activación del RAS contribuye a la lesión glomerular y la pérdida de nefronas.

(Engeli S. & Sharman A., J Mol Med., 2001)

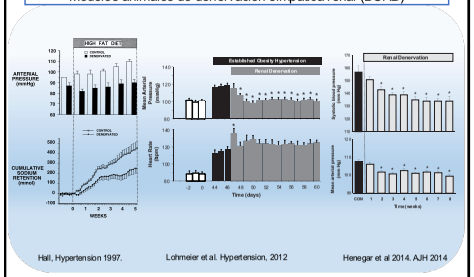


AGT: Angiotensinógeno
ANG II: Angiotensina II
RAS: "*Renin - Angiotensisn System*"
RSNA: "*Renal Sympathetic Nerve Activity*"

12

ANTECEDENTES

Modelos animales de denervación simpática renal (BSRD)



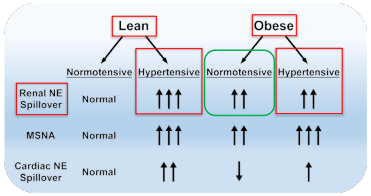
Hall, Hypertension 1997. Lohmeier et al. Hypertension, 2012 Henegar et al 2014, AJH 2014

BSRD: "*Bilateral Sympathetic Renal Denervation*"

13

ANTECEDENTES

El aumento de la RSNA no es una explicación suficiente para la hipertensión y el daño renal asociados a la obesidad.



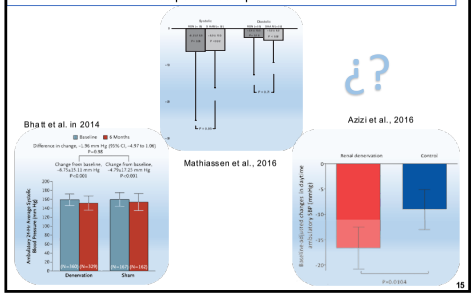
MSNA: "*Muscular Sympathetic Nerve Activity*"
NE: Noradrenalina
RSNA: "*Renal Sympathetic Nerve Activity*"

Grassi G et al. Hypertension, 2015

14

ANTECEDENTES

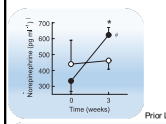
La denervación simpática renal no disminuye los valores de presión arterial de pacientes hipertensos.



15

ANTECEDENTES

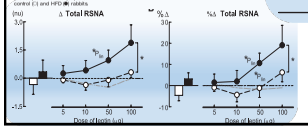
La actividad simpática y el daño renal son directamente proporcionales a la concentración plasmática de Leptina.



Prior L. et al. Hypertension, 2009

Adipokine	Glomerulus	Proximal Tubule	Result
↑ leptin	Mesangial cell hypertrophy; Basement membrane thickening	↓ metabolic activity; ↓ protein content per cell	Albuminuria; Glomerular sclerosis; Activation of apoptotic pathways
↓ adiponectin	Fusion of podocytes	↓ AMPK	Albuminuria; ↓ GFR; Tubular inflammation
↑ resistin	?	?	↓ GFR; Tubular inflammation
↑ visfatin	Oxidative stress	?	Altered GFR

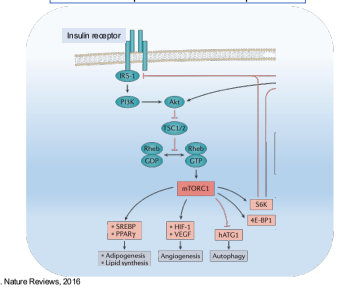
Brifa et al. Am J. Physiol Renal Physiol, 2004.



16

ANTECEDENTES

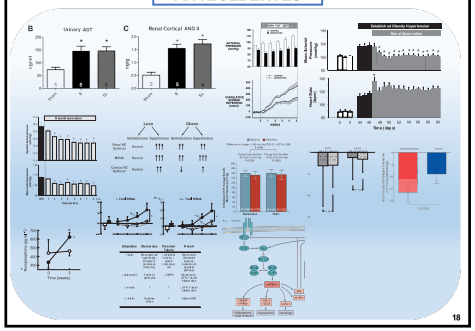
Señalización por insulina en los podocitos



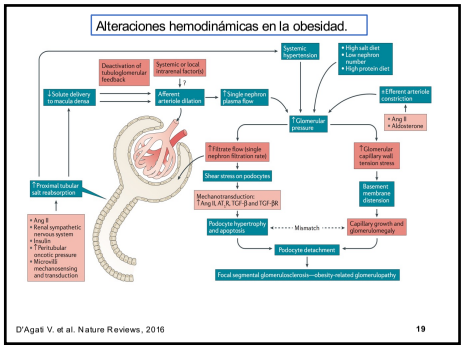
D'Agati V. et al. Nature Reviews, 2016

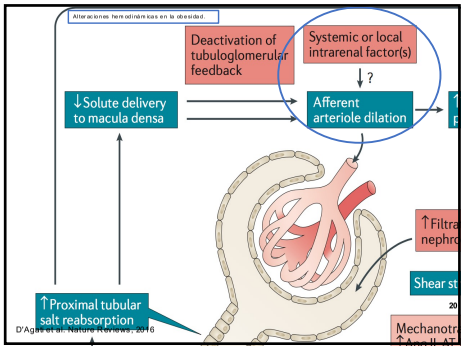
17

ANTECEDENTES



18


Alteraciones hemodinámicas en la obesidad.
Systemic hypertension
• High salt diet
• Low nephron number
• High protein diet
Deactivation of tubuloglomerular feedback
Systemic or local intrarenal factor(s)
↓Solute delivery to macula densa
Afferent arteriole dilation
± Efferent arteriole constriction
↑Single nephron plasma flow
↑Glomerular pressure
• Ang II
• Aldosterone
↑Filtrate flow (single nephron filtration rate)
↑Glomerular capillary wall tension stress
Shear stress on podocytes
↑Proximal tubular salt reabsorption
Basement membrane distension
Mechanotransduction: ↑Ang II, AT1R, TGF-β and TGF-βR
Podocyte hypertrophy and apoptosis
Mismatch
Capillary growth and glomerulomegaly
Podocyte detachment
Focal segmental glomerulosclerosis—obesity-related glomerulopathy
• Ang II
• Renal sympathetic nervous system
• Insulin
• ↑Peritubular oncotic pressure
• Microvilli mechanosensing and transduction
D'Agati V. et al. Nature Reviews, 2016
19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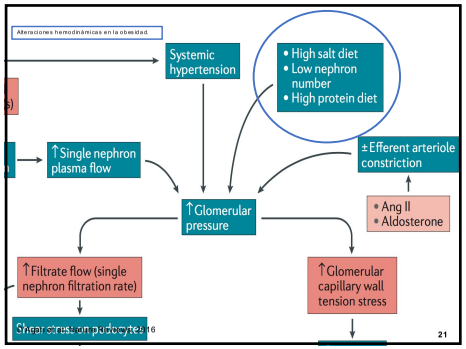
Shear st
Mechanotra
D'Agati et al. Nature Reviews, 2016
20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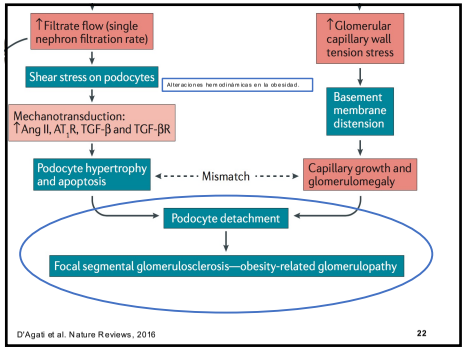
Shear stress on podocytes
21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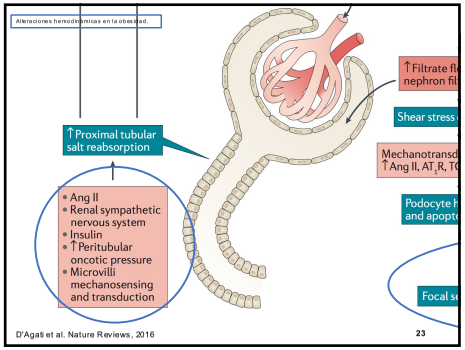
22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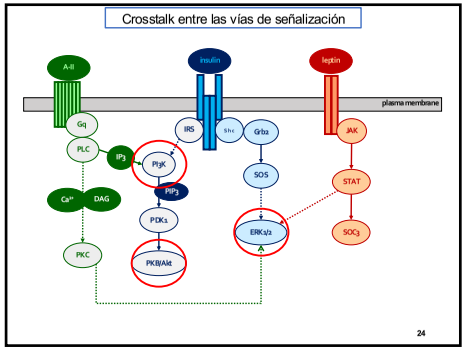
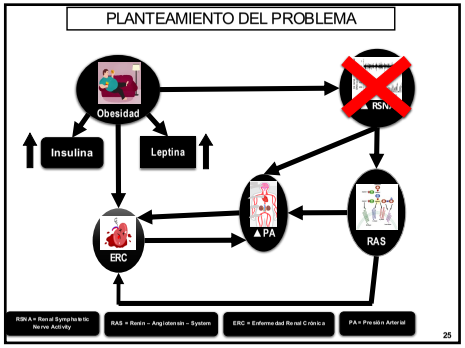
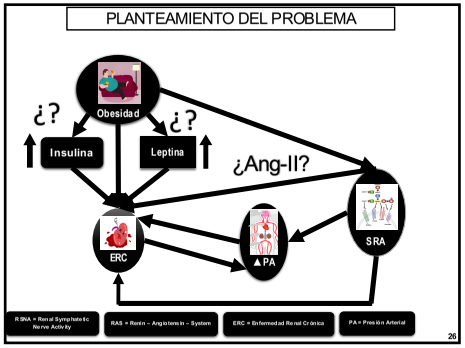
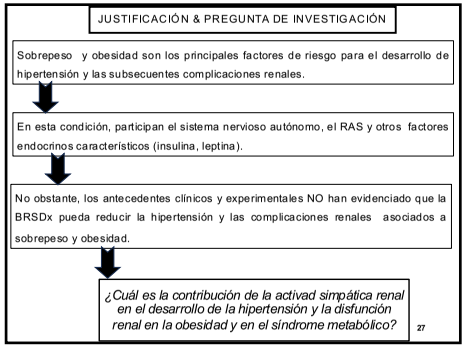
• Renal sympathetic nervous system
• Insulin
• ↑Peritubular oncotic pressure
• Microvilli mechanosensing and transduction
Shear stress
Podocyte H
and apopt
Focal s
D'Agati et al. Nature Reviews, 2016
23


Crosstalk entre las vías de señalización
A-II
Insulin
leptin
plasma membrane
Gq
IRS
Shc
Grb2
JAK
PLC
IP3
PI3K
SOS
STAT
PIP3
Ca++
DAG
PDK1
ERK1/2
SOCS
PKC
PKB/Akt
24


PLANTEAMIENTO DEL PROBLEMA
Obesidad
Insulina
Leptina
RSNA
PA
RAS
ERC
RSNA = Renal Sympathetic Nerve Activity
RAS = Renin – Angiotensin – System
ERC = Enfermedad Renal Crónica
PA = Presión Arterial
25


PLANTEAMIENTO DEL PROBLEMA
Obesidad
¿?
¿?
Insulina
Leptina
¿Ang-II?
PA
ERC
SRA
RSNA = Renal Sympathetic Nerve Activity
RAS = Renin – Angiotensin – System
ERC = Enfermedad Renal Crónica
PA = Presión Arterial
26


JUSTIFICACIÓN & PREGUNTA DE INVESTIGACIÓN
Sobrepeso y obesidad son los principales factores de riesgo para el desarrollo de hipertensión y las subsecuentes complicaciones renales.
En esta condición, participan el sistema nervioso autónomo, el RAS y otros factores endocrinos característicos (insulina, leptina).
No obstante, los antecedentes clínicos y experimentales NO han evidenciado que la BRSDx pueda reducir la hipertensión y las complicaciones renales asociados a sobrepeso y obesidad.
¿Cuál es la contribución de la activad simpática renal en el desarrollo de la hipertensión y la disfunción renal en la obesidad y en el síndrome metabólico?
27

HIPÓTESIS

La innervación simpática renal no es una condición necesaria y suficiente para la hipertensión y el daño renal asociados a la obesidad en un modelo de síndrome metabólico en rata inducido por dieta alta en grasa.

- Las ratas obesas y sometidas a denervación simpática renal bilateral presentaran hipertensión y síntomas de daño renal.
- Los riñones de las ratas afectadas por el síndrome metabólico presentaran niveles alterados de las proteínas de señalización intracelular de A-II, leptina e insulina.

28

OBJETIVO GENERAL

Evaluar la *contribución de la activad simpática renal en el desarrollo de la hipertensión y la disfunción renal en un modelo murino de dieta alta en grasa*

29

OBJETIVOS ESPECÍFICOS

1. Evaluar el efecto de la ablación de los nervios renales en dos series de animales con seguimiento a las 8 y 12 semanas.

2. Evaluar los marcadores de síndrome metabólico en ratas expuestas a 8 y 12 semanas de dieta alta en grasa (HFD).

3. En cada grupo de animales experimentales y en sus respectivos controles, evaluar el desarrollo de las complicaciones renales mediante:
   - marcadores indirectos de daño renal: proteinuria y/o KET
   - antes del sacrificio: flujo urinario, TFG, UNaV,y UKV;
   - después del sacrificio: niveles renales de NE, niveles plasmáticos de Lep, Ang-II e Insulina

4. Evaluar a las 8 y 12 semanas los niveles renales de las proteínas: pPI3K, pERK1/2, y pAkt, y sus respectivas proteínas totales.

30

Materiales and Métodos

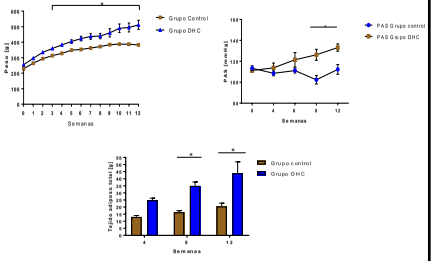
Animales
Ratas Wistar macho destetadas
peso: 100-120 g

Condiciones de bioterio
- alojamiento individual
- temperatura 21 ± 1 ° C
- 12 horas de ciclo de luz / oscuridad
- agua filtrada *ad libitum*

Dieta estándar con 0.4% de Na⁺ y 5 % de grasa

31

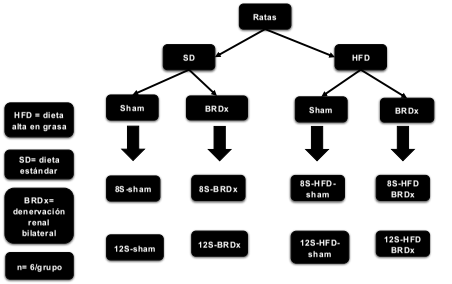
Modelo de síndrome metabólico estandarizado en el laboratorio



Donjuan G., Tesis de maestría 2017-18

32

Grupos

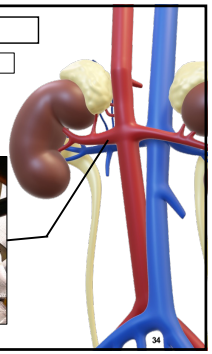


33

Denervación renal bilateral

BRD = Bilateral Renal Denervation

Anestesia general con pentobarbital (50 mg/kg i.p.).

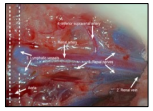


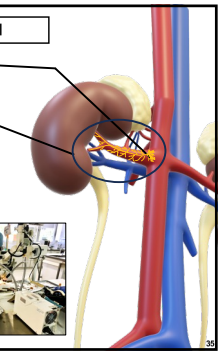
34

Denervación renal bilateral

Ganglio aórtico renal.

Los vasos se recubrieron durante 2 min con fenol al 10% en etanol absoluto.

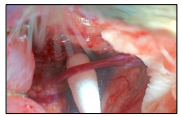


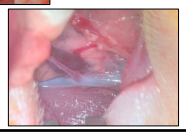


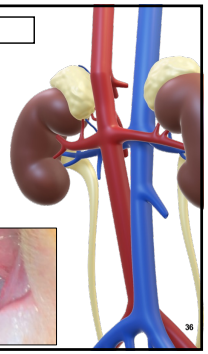
35

Denervación renal bilateral

Se les administró ceftiaxona (1 g/Kg) y ketoprofeno (0.5 g/kg).







36

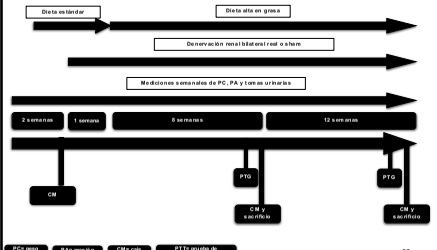
Dieta alta en grasa (HFD)

La dieta estándar contenía un 4.5% de grasa y aportaba 3.36 kcal/g. La HFD se obtuvo añadiendo a la dieta estándar un 15% de grasa de cerdo (886Kcal/g) y un 15% de margarina (545Kcal/g).

Según los datos nutricionales indicados por los fabricantes, nuestra dieta HFD contenía ~ 28.60% de grasa y aportaba ~ 217 kcal/g.

37

PROTOCOLO EXPERIMENTAL

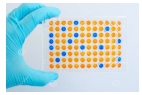


38

Medición de las variables

- Valores de presión arterial→ CODA

- Norepinefrina Renal→ ELISA



- Parámetros plasmáticos:
  - Leptina, insulina y Ang-II→ ELISA
  - Glucosa → Glucómetro

- Parámetros Renales:
  - Creatinina, proteínas, KET, glucosa, pH, urobilinógeno→ métodos enzimáticos colorimétricos
  - TFG, $U_{Na}V$ y U_KV → tomas urinarias de 24 hrs en la jaula metabólica y las concentraciones medidas por fotometría de flama.
  - Akt, pAkt, ERK1/2, pERK1/2, PI3K y pPI3K → Western blot

39

Diseño experimental

- Experimental, prospectivo, longitudinal, comparativo, con la aplicación transversal sub-protocolos longitudinales diagnósticos.
- **UE**: rata
- **Estructura del tratamiento**: trifactorial cruzado 2 x 2 x 2, un factor siendo dieta con dos niveles, otro factor siendo denervación con dos niveles y el otro factor siendo el punto temporal con 2 niveles.
- **Estructura del diseño**: completamente aleatorizado
- **Procedimiento diagnóstico**: medición de las variables

40

Tamaño de la muestra

- Dieta = 2
- Denervación = 2
- Interacción = 1
- Punto temporal = 2
- Grados de libertad → 6
- n Teórica→ 10-20 repeticiones x GL = 60 - 120 repeticiones
- n planeada → 6 animales x 4 grupos x 3 puntos temporales = 72 repeticiones
- Nivel de alfa impuesto: 0.05

- Modelo: **SBP**~ Dieta + DRB + Dieta*Denervación
  prot de señalización~ Dieta + DRB + Dieta*Denervación

Fainstein, 1996

41

Criterios de inclusión y eliminación

- **Criterios de inclusión**
  - Ratas macho Wistar, con pesos al día del experimento entre 180 y 200 g
  - Curva de peso normal desde 10 días antes del protocolo.
  - Sin síntomas de enfermedad respiratoria
- **Criterios de eliminación**
  - Ratas que no alcancen el peso pre-denervación
  - Ratas que presenten complicación post-quirúrgica
  - Imposibilidad de haber medido correctamente todas las variables de respuesta
  - Ratas que no resulten adecuadamente denervadas (< 15 % basal)
  - Ratas que a la semana 8 no alcancen valores hipertensivos.

42

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  - Ratas que a la semana 8 no alcancen valores hipertensivos.

43

Definición operacional de las variables

Código	Nombre	Significado	Escala de medición	Valor
PAM, PS, PD	Presión arterial media, sistólica y diastólica	Valores de presión sanguínea	Continua	mmHg
NERe	Norepinefrina renal	ANSER	Continua	ng/g de tejido
Ang-II, IN, Lep	Angiotensina II, insulina y leptina	Parámetros plasmáticos	Continua	pg/ml o ng/ml
GLU	Glucosa	Parámetros plasmáticos	Continua	mg/dL
TFG, UNaV, UKV,	Filtración glomerular, excreción de sodio, excreción de potasio	Parámetros renales	Continua	mL/min, %, mg albúmina/mg creatinina
PROT, KET, URU, pH	Proteínas, cuerpos cetónicos, urubilinógeno y pH urinarios	Parámetros renales	Continua	g/L y mmol/L
pAkt, pERK1/2, pPI3K	Proteínas/segundos mensajeros	Vías de señalización	Continua	Unidades arbitrarias

44

ANÁLISIS DE DATOS

- Preliminarmente, se realizó un análisis exploratorio para apoyar la aleatorización y evaluar la distribución de las variables experimentales de interés.
- El curso temporal PAS, GLU, BW, consumo de alimento y agua se analizó a través del análisis de varianza multivariable (MANOVA) con medición repetida en el factor tiempo para detectar efectos de componentes principales y/o interacción grupo * tiempo.
- Las variables de respuesta se modelaron mediante modelos lineales fijos (análisis de regresión, ANOVA de una, dos y tres vías), sus interacciones y mediante efectos mixtos con corrección de Geisser-Greenhouse.

45

ANÁLISIS DE DATOS

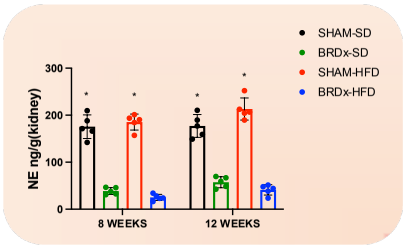
- Se probaron los criterios paramétricos por Brown-Forsyte (homo/heterocedasticidad) y Shapiro Wilk (ajuste a la distribución normal) en los residuales de los modelos y no fue necesario ninguna transformación de los datos.
- El nivel de alfa impuesto fue de 0.05.
- El análisis estadístico fue realizado con el programa JMP V5.01 (SAS Institute) y GraphPad Prisma V9.1 como los datos se ajustaron a una distribución normal se expresaron como la media ± EE.

46

RESULTADOS

47

El protocolo de BRDx fue exitoso, la cantidad de NE decae entre un 72 y 80 % de sus valores basales.

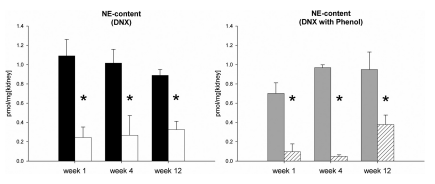


Datos no publicados

* 8W DS, HS Y 12W DDS Y HS vs 8W DD, HD Y 12W DD, HD, p< 0.0057

48

El contenido de NE no se recuperó completamente.

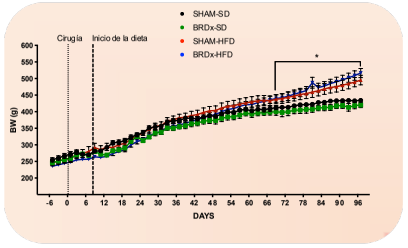


Estos resultados sugieren que un excedente neto a largo plazo de la influencia aferente sobre el riñón puede estar contribuyendo a varios procesos renales.

Rodionova K. et al. Am J Physiol Regul Integr Comp Physiol. 2016

49

HFD produjo un aumento de peso que no fue atenuado o modificado por la BRD

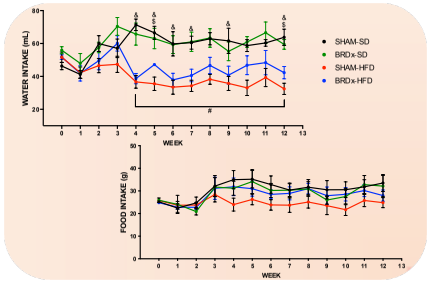


Two-way ANOVA + Tukey test: * P from 0.0457 to 0.0005 HFD-SHAM versus all groups; #P from 0.001 to 0.0007 HFD-SHAM versus DST-SHAM and DST-RBDx; & P from 0.015 to 0.0014 HFD-RBDx versus DST-SHAM and DST-RBDx; $ P from 0.0411 to 0.0402 HFD-RBDx versus DST-SHAM, DST-RBDx and HFD-RBDx.

Datos no publicados

50

Los protocolos no modificaron el consumo de alimento mientras que HFD disminuyó el consumo de agua



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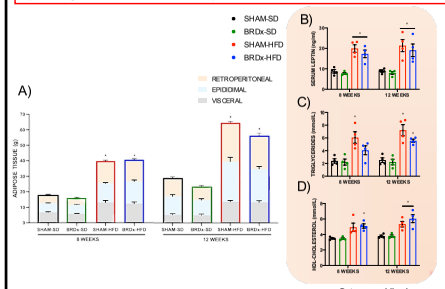
Datos no publicados

51

La BRD no modifica el aumento de la masa adiposa ni de los niveles plasmáticos de leptina, triglicéridos ni HDL inducidos por la dieta.

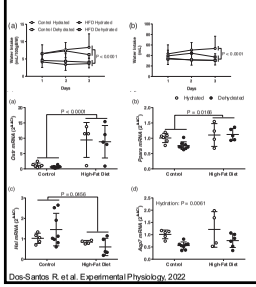


ANOVA de dos vías + Tukey test: * P < 0.003 HFD-SHAM y HFD-RBDx vs DST-SHAM y DST-RBDx

Datos no publicados

52

El aumento del tejido adiposo causado por la HFD puede mitigar los efectos de la deshidratación.

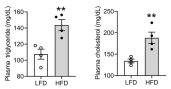


La oxitocina posiblemente media la respuesta lipolítica.
La lipólisis, como la acuaporina-7 y el PPRα, podrían contribuir a los diferentes efectos observados en las ratas control y con HFD.

Dos-Santos R. et al. Experimental Physiology. 2022

53

El riñón puede amortiguar los incrementos en $[TG]_p$ sin la presencia del estímulo nervioso.



Sun Y. et al. Cell Death & Disease, 2020.

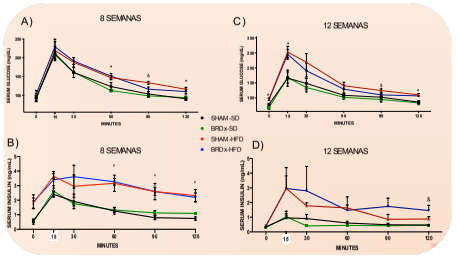
Changes in parameters.

	Pre-RSD (n = 20)		Post-RSD (n = 20)	
	Mean	SD	Mean	SD
(C) Metabolic indices				
BMI, kg/m²	37.2	5.4	36.9	5.9
Waist/hip ratio	0.94	0.04	0.93	0.04
Neck circumference, inches	16.1	1.0	17.9	0.9
Total cholesterol, mmol/L	4.61	0.88	4.61	0.96
HDL, mmol/L	1.15	0.40	1.24	0.30
LDL, mmol/L	2.60	0.94	2.35	0.80
TG, mmol/L	2.39	1.77	1.95	0.94

Daniels F. et al. Sleep Medicine, 2017.

54

A las 12S la HFD indujo un aumento en la glucosa y en insulina independiente de la BRD mientras que a las 8S solo lo retarda.

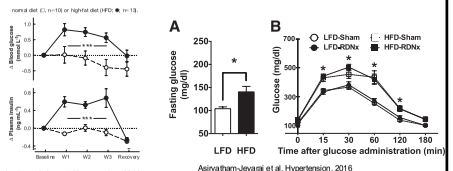


MANOVA : * HS y HD vs DS y DD p= 0.0309; & HS vs DS y DD p= 0.0295

Datos no publicados

55

La BRD puede tener efectos sobre el metabolismo de la glucosa y la insulina, pero los resultados no son consistentes.



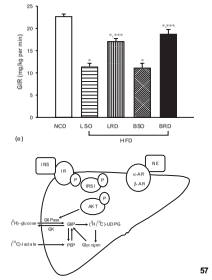
Armitage J. A. et al. Hypertension, 2016.

Asirvatham-Jeyaraj et al. Hypertension, 2016

56

La BRD puede mejorar el metabolismo de la glucosa y la sensibilidad a la insulina.

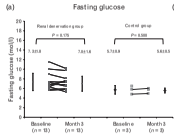
A través de la supresión de la producción hepática de glucosa que se debe a la disminución de la glucogenólisis y gluconeogénesis vía flujo de Glucosa 6-fosfatasa y aumentando la sensibilidad hepática a la insulina.



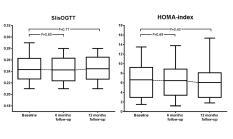
Chen W et al. Journal of Hypertension, 2016

57

La BRD no modifica los niveles de insulina y glucosa.



Tsioufis C et al. Journal of Hypertension, 2016

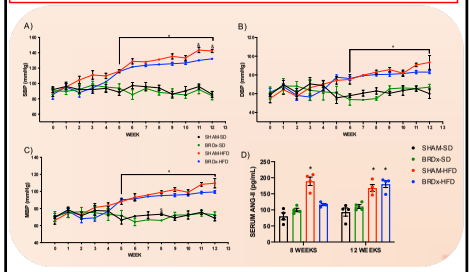


Verloop W. et al. Hypertension, 2015

La presencia de HFD mantiene un efecto desfavorable sobre la recuperación de las células de los islotes, lo que a su vez provoca un aumento de los niveles de insulina

58

La BRD sólo atenúa el amento de presión inducido por la HFD mientras que a las 8S atenúa el aumento de [ANG-II]$_p$ que no se ve modificado a las 12S



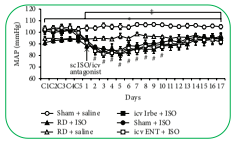
RM-ANOVA indicated time effects ($P<0.0001$) and time* group interaction effects ($p<0.0001$).
Tukey-test ANOVA + Tukey test: † P from 0.0057 to 0.0001 SHAM-HFD and BRDx-HFD versus SHAM-SD and BRDx-SD; & P From 0.0183 to 0.001 SHAM-HFD versus BRDx-HFD; $ P = 0.0038 SHAM-SD versus BRDx-SD.

Datos no publicados

59

La BRD sólo atenúa el amento de presión inducido por la HFD mientras que a las 8S atenúa el aumento de [ANG-II]$_p$ que no se ve modificado a las 12S

BRD puede disminuir la presión arterial en modelos de HFD reduciendo la retención de sodio, la sobreactivación simpática y la inflamación renal.

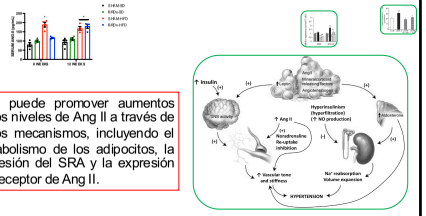


Li J-D. et al. Oxidative Medicine and Cellular Longevity, 2016

60

La BRD sólo atenúa el amento de presión inducido por la HFD mientras que a las 8S atenúa el aumento de [ANG-II]$_p$ que no se ve modificado a las 12S

HFD puede promover aumentos en los niveles de Ang II a través de varios mecanismos, incluyendo el metabolismo de los adipocitos, la expresión del SRA y la expresión del receptor de Ang II.

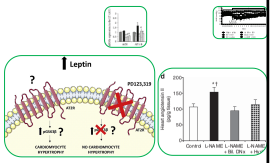


Cabandugama P. K. et al. Med Clin N Am, 2017

61

La BRD sólo atenúa el amento de presión inducido por la HFD mientras que a las 8S atenúa el aumento de [ANG-II]$_p$ que no se ve modificado a las 12S

La BRD puede influir en "normalizar" los receptores de Ang II (en especial el 2), en la presión arterial y en la inflamación renal, pero sus mecanismos no se conocen con detalle.



Lima V. M. et al. Molecular and Cellular Endocrinology, 2019

Etiguchi M. et. al. Kidney International, 2015

62

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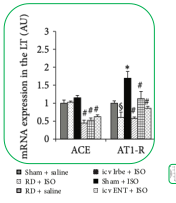
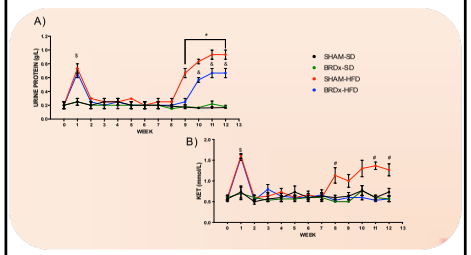


Li J-D. et al. Oxidative Medicine and Cellular Longevity, 2016

63

La BRD atenúa, pero no elimina la excreción de proteínas causada por la HFD mientras que sí previene la excreción de KET.



Datos no publicados

RM-MANOVA indicated time effects (P<0.0001) and time*group interaction effects (p<0.0001) in the same points. Two-way ANOVA + Tukey test: * P from 0.0057 to 0.0001 SHAM-HFD and BRDx-HFD versus SHAM-SD and BRDx-SD; & P from 0.0165 to 0.001 SHAM-HFD versus BRDx-HFD; $ P = 0.0206 SHAM-SD versus BRDx-SD.

64

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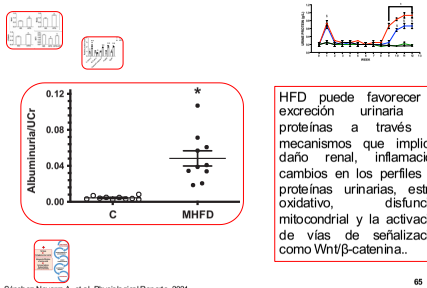

HFD puede favorecer la excreción urinaria de proteínas a través de mecanismos que implican daño renal, inflamación, cambios en los perfiles de proteínas urinarias, estrés oxidativo, disfunción mitocondrial y la activación de vías de señalización como Wnt/β-catenina..

Sánchez-Navarro A. et al. Physiological Reports, 2021

65

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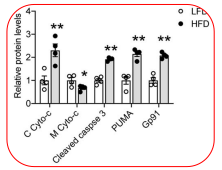

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Sun Y. et al. Cell Death & Disease, 2020

66

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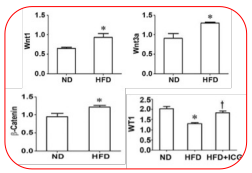

HFD puede favorecer la excreción urinaria de proteínas a través de mecanismos que implican daño renal, inflamación, cambios en los perfiles de proteínas urinarias, estrés oxidativo, disfunción mitocondrial y la activación de vías de señalización como Wnt/β-catenina..

Yu Y. et al. Frontiers in Medicine, 2022

67

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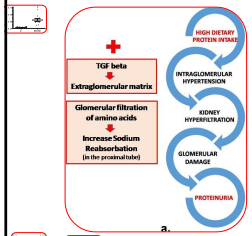

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Ria P. et al. Int. J. Mol. Sci. 2022

68

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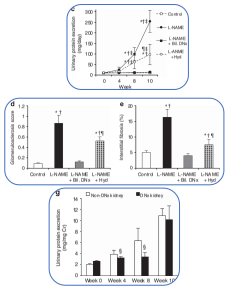
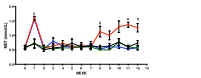


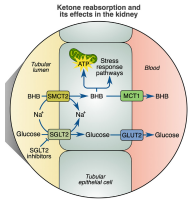
BRD reduce la proteinuria a través de la disminución de la presión arterial, la mejora de la hemodinámica renal, la reducción de la inflamación y la fibrosis renales, y la modulación de las vías de señalización.

Enjuchi M. et al. Kidney International, 2015

69

La BRD previene la excreción de KET





ketogénesis → KET → filtración → reabsorción → SMT2 MCT1

Puchalska P & Crafford P.A. Cell Metab, 2016
Palmer B.F. & Clegg D. J. Adv Physiol Educ, 2016
Rojas-Morales P. et. al. Adv Redox Research, 2021
de Oliveira T.L. et. al. Life Sciences, 2021

70

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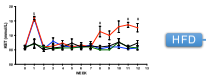

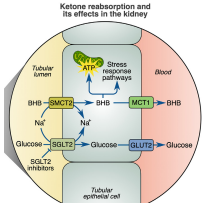

HFD → ↑Ketogénesis → Cetonuria
↑Ketogénesis → KET → Filtración → Reabsorción → SMT2 MCT1 → Pérdida de NH4, Na+, K+
Cetonuria → Diabetes descompensada

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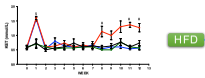

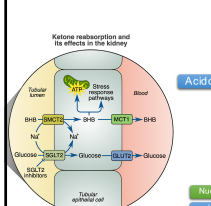

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Reabsorción → Acidosis metabólica → Tiempo

Nuestro grupo
Literatura

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72

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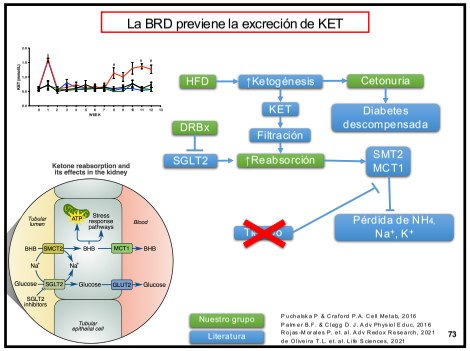

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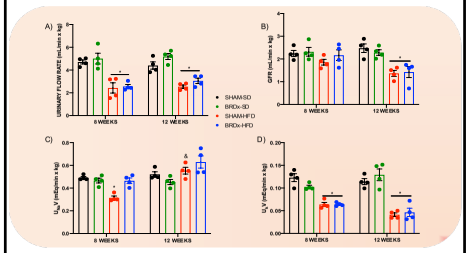
73

La HFD promueve una disminución del flujo urinario, de la TFG y de la U_KV que no se ve modificado por la BRD, mientras que la $U_{Na}V$ no sufre cambios.



Datos no publicados

Three-way ANOVA + Tukey test. * P from 0.0235 to < 0.0001 SHAM-HFD and BRDx-HFD versus SHAM-SD and BRDx-SD in the same week; & P = 0.0008 SHAM-HFD 8 week versus SHAM-HFD 12 week.

74

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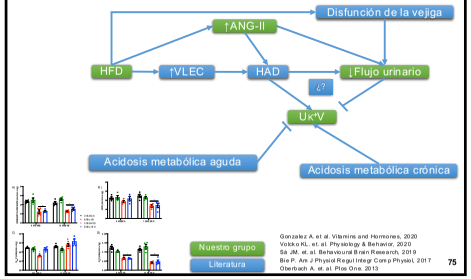

Gonzalez A. et al. Vitamins and Hormones. 2020
Volkis KL. et. al. Physiology & Behavior. 2020
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Bie P. Am J Physiol Regul Integr Comp Physiol. 2017
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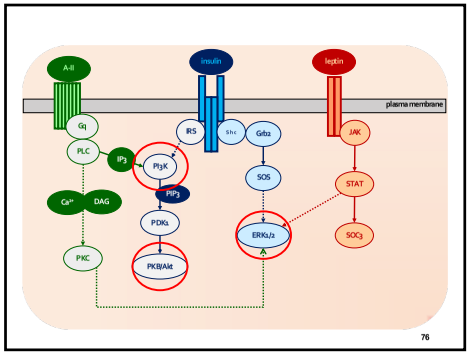
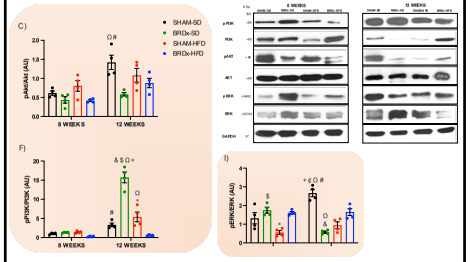
75



76

La activación intracelular de PI3K, AKT y ERK, muestran una combinación de efecto de tiempo y dieta que no es completamente mediado por el SNS

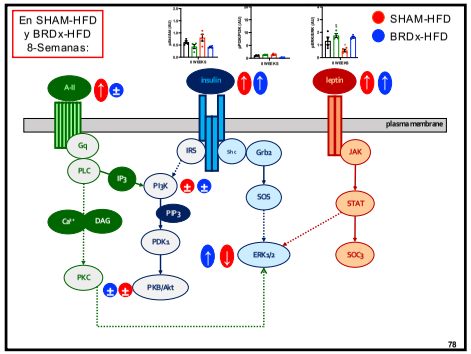


ANOVA 3W. # SD-SHAM vs SD- BRDx in the same week, P from 0.0084 to < 0.0001; # SD- SHAM vs HFD- SHAM in the same week, P < 0.0001; + SD- SHAM vs HFD-BRDx in the same week, P from 0.0072 to < 0.0001; $ SD- BRDx vs HFD- BRDx in the same week, P from 0.0107 to < 0.0001; ^ HFD- BRDx in the same week, P from 0.0493 to < 0.0001; Q the same variable at 8 week vs 12 week.

Datos no publicados

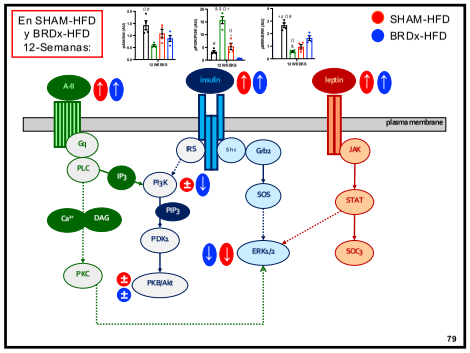
77

En SHAM-HFD y BRDx-HFD 8-Semanas:



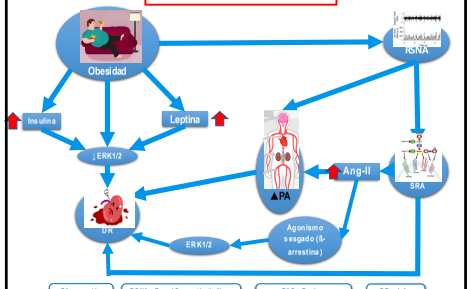
78

En SHAM-HFD y BRDx-HFD 12-Semanas:



79

Conclusiones



80

Limitaciones del estudio & Perspectivas

El presente trabajo explora la influencia de la inervación autonómica del riñón en la aparición y progresión del síndrome metabólico.

81

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A este punto temporal, la inervación autonómica renal parece no desempeñar un papel fundamental, ya que los animales obesos presentan hipertensión y los primeros signos de ER.

85

Limitaciones del estudio & Perspectivas

Evaluación de la histología renal

86

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Realizar un protocolo similar pero con 16 semanas de HFD

87

Limitaciones del estudio & Perspectivas

Evaluación de la histología renal

Realizar un protocolo similar pero con 16 semanas de HFD

Llevar a cabo un protocolo de cambio a las 8 semanas de HFD a una dieta normocalórica

88

GRACIAS

89

Gracias

- CONACYT-CB-243298
- Beca CONACYT 556514

- Laboratorio de Neuroanatomía Funcional
- Laboratorio de Ritmos Biológicos.

Facultad de Ciencias, Universidad Autónoma de San Luis Potosí.

90