Dissociation of antihypertensive and metabolic response to losartan and spironolactone in experimental rats with metabolic sindrome

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Submitted on: 29/03/2012.
Approved on: 11/08/2012.

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DOI: 10.5935/0101-2800.20120021

Abstract

Introduction: The treatment of arterial hypertension (AH) in patients with metabolic syndrome (MS) is a challenge, since non drug therapies are difficult to implement and optimal pharmacological treatment is not fully established. **Objective:** To assess the blockade of the rennin angiotensin aldosterone system (RAAS) in blood pressure (BP) in renal function and morphology in an experimental model of MS induced by high fat diet. **Methods:** Wistar rats were fed on high fat diet from the fourth week of life, for 20 weeks. The groups received Losartan or Spironolactone from the eighth week of life. We weekly evaluated the body weight and BP by tail plethysmography. At the end of the experiment oral glucose tolerance, lipid profile, creatinine clearance tests, and the direct measurement of BP were performed. A morphometric kidney analysis was performed. **Results:** The administration of high-fat diet was associated with the development of MS, characterized by central fat accumulation, hypertension, hyperglycemia and hypertriglyceridemia. In this model there were no changes in renal histomorphometry. The blockade of angiotensin II (Ang II) receptor AT1 prevented the development of hypertension. The mineralocorticoid blockage did not have antihypertensive efficacy but was associated with reduction of abdominal fat. **Conclusion:** The dissociation of the antihypertensive response to the blockades of Ang II receptors and mineralocorticoid indicates the involvement of Ang II in the pathogenesis of hypertension associated with obesity. Reduction of central obesity with Spironolactone suggests the presence of mineralocorticoid adipogenic effect.

Keywords: animals, hypertension, metabolic syndrome x, obesity, renin-angiotensin system.

Introduction

Obesity is a worldwide epidemic that occurs due to economic, social, and demographic changes in different populations. The World Health Organization estimates that there are, at present, approximately 400 million obese individuals in the world and that the prevalence of obesity has increased substantially in recent decades.[1,2]

Obesity is a multifactorial disease, related to hereditary factors and primarily to poor eating habits. This changing profile of nutritional patterns culminates in a positive energy balance, compounded by an association with a sedentary lifestyle, which together contribute to the development of metabolic syndrome (MS).[3]

Accumulated adipose tissue is a metabolically active organ that secretes several substances called adipokines that exert inflammatory, hormonal, and hemodynamic effects. These adipokines are mostly related, directly or indirectly, to processes that contribute to the development of atherosclerosis, hypertension (HTN), insulin resistance (IR), diabetes mellitus (DM), and dyslipidemia; in other words, they are the link between adiposity and MS.[4,5] The latter is characterized by the association of 3 or more comorbidities, including central obesity, HTN, glucose intolerance, increased levels of triglycerides, and reduced high-density lipoprotein cholesterol levels.[6,7] Together, these factors result in the high risk of the development of cardiovascular disease and renal injury, and the severity of this risk is proportional to the number of MS components.[8,9]

To date, there is no specific treatment for MS; however, it is believed that the treatment of its components could reduce cardiovascular risk. Ideally, this treatment should be based on weight loss, since this is the most effective measure for preventing diabetes, lowering cholesterol and triglyceride levels, and reducing IR and blood pressure.[10] However, the success rate of this therapeutic measure is low, which leads to the need for specific treatment of dyslipidemia, insulin resistance, and HTN.[11]

One of the biggest challenges for the clinician relates to the treatment of HTN in individuals with MS. As previously mentioned, nonpharmacological measures are often difficult to implement and drug-based treatment is not yet fully established.[11]

Some authors recommend the use of diuretics, which can interfere with metabolic parameters, while others suggest the importance of drugs that do not interfere with those parameters, such as calcium channel blockers and renin-angiotensin-aldosterone system (RAAS) inhibitors.

In recent years, the involvement of aldosterone in the genesis of HTN-associated obesity has been suggested. Studies in dogs[15] and Sprague-Dawley rats[16] demonstrated that blocking this system prevented increases in blood pressure as well as aldosterone-induced vascular injury. However, in both studies, the animals showed a positive sodium balance, a factor that has been described recently as a mediator of the vasculotoxic action of aldosterone.[17]

Blockade of the RAAS seems effective in the control of HA as well as hypertriglyceridemia and IR. However, the available studies are scarce and involve only a small number of patients, and therefore do not allow the generalization of this strategy for the treatment of HTN associated with MS.[11,20]

The use of experimental models of MS is an alternative to the study of causal mechanisms and the evaluation of treatment with antihypertensive medication. Among these models, those that are induced by dietary modifications best reproduce the metabolic changes of human obesity, including dyslipidemia, glucose intolerance, and hyperinsulinemia.[21-23]

In the present study, the effects of angiotensin and aldosterone blockade on renal blood pressure, function, and morphology were assessed in an experimental model of MS induced by a hyperlipidic diet.

Methods

Experimental model

Male Wistar rats aged 4 weeks were provided by the Center for Reproductive Biology Federal University of Juiz de Fora and were randomly divided into 4 groups of 12 animals. In the control group (C), animals were fed a commercial Nuvital® (Nuvilab, Colombo, PR, Brazil) normocaloric diet (68% carbohydrates, 19% protein, 3.5% lipids, 4.5% fiber, 5% vitamins and minerals, and 370 kcal/100 g) for 20 weeks. In the hyperlipidic group (H), the rats were fed a prefab high-fat diet (PragSoluções Comércio e Serviços Ltda., Jaú, SP, Brazil) containing: 35% carbohydrates, 19% protein, 36.5% lipids, 4.5% fiber, 5% vitamins and minerals, and 524 kcal/100 g for 20 weeks (Table 1).

Table 1 Nutritional composition of the hyperlipidic diet (g/kg)

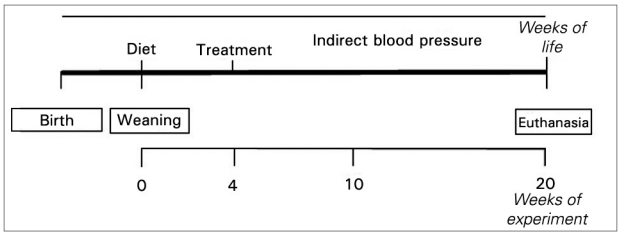
Ingredients	Grams/Kg
Corn starch	200
Soybean meal	300
Fat	190
Saturated fatty acids	158.5
Unsaturated fatty acids	70
Fiber	30
L cystine	3.88
Choline chloride	2.58
BHT (antioxidant)	0.014
Mineral Mix*	35
Vitamin Mix#	10
Total	999.974
Total Kcal	5240
Lipidic Kcal	65%

* Mineral Mix (g/kg of mix) contains: 30.5 g MgSO4.7H2O, 65.2 g NaCl, 105.7 g KCl, 200.2 g KH2PO4, 38.8 g MgCO3. Mg(OH)2.3H2O, 40 g FeC6H5O7.5H2O, 516.4 g CaCO3, 0.8 g KI, 0.9 g NaF, 1.4 g CuSO4.5H2O, 0.4 g MnSO4, and 0.05 g CoNO3.
# Vitamin Mix (g/kg of mix) contains: 3 g thiamine mononitrate, 3 g riboflavin, 3.5 g pyridoxine HCl, 15 g nicotinamide, 8 g calcium pantothenate, 1 g folic acid, 0.1 g biotin, 0.005 g cyanocobalamin, 0.013 g acetomenaphtone, 0.6 g vitamin A acetate, 25 g RRR-D-tocopherol acetate and 10 g choline chloride.

The hyperlipidic/losartan (H+L) and hyperlipidic/spironolactone (H+E) groups received the same diet as group (H). From the age of 8 weeks, group H+L received losartan at 10 mg/kg/day (Merck SA, Rio de Janeiro, RJ, Brazil) and group H+E received spironolactone at

40 mg/kg/day (Pfizer SRL, Buenos Aires, Argentina) by once-daily gavage for 16 weeks (Figure 1).

Figure 1. Experimental design.



All animals were monitored for 20 weeks. Twenty-four hours prior to euthanasia, urine was collected in metabolic cages and the animals were implanted with catheters to measure direct blood pressure and evaluate oral glucose tolerance. Euthanasia was performed by exsanguination under anesthesia with ketamine (90 mg/kg ip.) and xylazine (10 mg/kg ip.) (König SA®, Avellaneda, Argentina). Kidney and blood samples were collected at the time of euthanasia (Figure 1).

All experimental procedures were approved by the Ethics Committee on Animal Experimentation of the Federal University of Juiz de Fora (No. 001/2009).

Obesity

We considered animals obese when the weight difference between the groups with hyperlipidic diets (H, H+L, and H+A) was significant compared to that of group C.[24,25] Bilateral abdominal lipectomy was performed at 20 weeks of the experiment after euthanasia with ketamine and xylazine and exsanguination. Subsequently, retroperitoneal (RET) and right and left epididymal (EP) adipose tissue weight (g) was obtained. These parameters were used as criteria for visceral obesity.[24]

For the calculation of food (g) and energy (kcal/day) consumption, the animals were weighed and individually assigned to metabolic cages, with fixed quantities of dietary consumption measured once a week for a period of 24 hours.

Blood pressure

Indirect method

Systolic blood pressure (SBP) was measured in mmHg, weekly, using the noninvasive tail plethysmography method (plethysmograph LE5001; Panlab®, Barcelona, Spain). After preconditioning in the containment chamber, the animals were preheated to 35 ± 2°C for 5 minutes and 10 consecutive readings were obtained. The individual blood pressure measurement was calculated by averaging the readings obtained.[26]

Direct method

Direct measurements of SBP and diastolic blood pressure (DBP) were obtained in mmHg, at 20 weeks after the start of the experiment, by cannulation of the right femoral artery under anesthesia with ketamine (90 mg/kg i.p.) and xylazine (10 mg/kg i.p.) (König SA®). Twenty-four hours after surgery, blood pressure measurement was performed using the pulsatile blood pressure method with the ML865-25T pressure transducer (ADInstruments®, Sydney, Australia).[27]

Lipids

Serum triglyceride and total cholesterol levels were evaluated 20 weeks after starting the diets, at the time of euthanasia, and after 8 hours of fasting, using commercially available kits (Labtest®, Lagoa Santa, Brazil).

Oral Glucose Tolerance Test (OGTT)

Nineteen weeks after starting the diet, blood samples were collected from the cannula used to measure blood pressure (after an 8-hour fast) to evaluate the glucose level at time zero (T_0) in all groups. A solution of 50% glucose was administered by gavage at a dose of 2 g/kg of body weight. Subsequently, blood samples were collected at time T_1 (15 min), T_2 (30 min), T_3 (60 min), and T_4 (120 min) and all samples were analyzed in a glucometer (MediSense, Abbott®, Chicago, IL, USA). The area under the curve was calculated using Origin 3.5 software (Microcal Software, Northampton, MA, USA).[28]

Serum glucose levels were also measured at the time of euthanasia using a commercially available testing kit (Labtest®, Lagoa Santa, Brazil).

Renal function

At the end of the experiment, blood samples were collected from all groups at the time of euthanasia (after an 8-hour fast) to measure serum creatinine (Cr) levels. Urinary Cr was measured in 24-hour urine samples harvested in the metabolic cage at this same point. The assays were performed using commercially

available kits (Labtest®, Lagoa Santa, Brazil) in a Labmax progress automatic analyzer (Labtest®, Lagoa Santa, Brazil).

After collection of individual urine (24-hour) samples, creatinine clearance (Ccr) was calculated from the urinary Cr, serum Cr, 24-hour urine volume, and body weight, using the following equation: Ccr (mL/min/kg) = [Urine Cr (mg/dL) × urine volume (mL)/serum Cr (mg/dL)] [1000/body weight (g)] [1/1440 (min)].[29]

Renal morphology

The right kidney was sectioned transversely and fixed in Baker's formaldehyde-calcium, subsequently embedded in paraffin and sectioned at 5-µm thickness, and stained with hematoxylin and eosin to assess glomerular morphology. Glomerular area and volume were assessed by morphometry. The glomerular area was quantified at 400× magnification. Only glomeruli in which the vascular pole was identified in the plane under study were selected for measurements in the cutting area, to ensure that the measured glomeruli were sectioned in similar planes. The glomerular images were scanned using an HBO50 Zeiss Axiophot microscope equipped with an Axicam ICc3 camera (Carl Zeiss®, Jena, Germany). After scanning, the glomerulus was identified and the area was calculated in μm^2 using analysis software (Optimas 5.1; Optimas Corporation, Seattle, WA, USA). Twenty cortical glomeruli were measured in each animal, with 6 animals from each group. The glomerular volume (GV) was calculated from the cross-sectional area of the glomerulus (CG), using the formula: $GV = \beta/\kappa\ (CG)^{3/2}$, where $\beta = 1.38$ is the shape coefficient for a sphere and $\kappa = 1.1$ is the size distribution coefficient.[30]

Statistical analysis

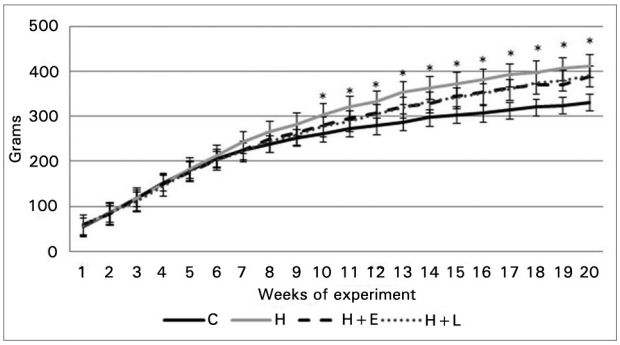
Results are presented as the mean and standard deviation. The distribution of the sample was evaluated by the Kolmogorov-Smirnoff test. The control and hyperlipidemic groups were compared by *Student's t-tests*. The hyperlipidic groups were compared by two-tailed analysis of variance (ANOVA) and post hoc Dunnett's tests. The level of significance was set at $p < 0.05$. The Statistical Package for the Social Sciences (SPSS) 15.0 software was used for all analyses (SPSS Inc., Chicago, IL, USA).

Results

Obesity and metabolic syndrome

Despite the difference in dietary composition characterized by a higher percentage of lipids in group H, energy intake was similar between groups (Table 1). Over the 20-week study period, all animals gained weight. In the 10th week of the experiment, group H showed a 17.5% higher weight gain than group C ($p < 0.001$). From this point, groups H, H+E, and H+L maintained a higher weight than group C, with no difference between these hyperlipidic groups. At the end of the experiment, the weight gain of the 3 experimental groups was 22.8% higher than group C ($p < 0.001$, Figure 2).

Figure 2. Body weight measurements of the study groups. Data are presented as mean ± standard error. Body weight measurements were performed weekly at different time points. The control and hyperlipidic groups were compared by *Student's t-tests* and the hyperlipidic groups were compared with each other by ANOVA and Dunnett's tests. * $p \leq 0.05$, control group vs. group H (*Student's t-test*).



Besides the increase in body weight, groups H, H+E, and H+L developed visceral and EP fat accumulation at the end of the experiment (Table 2). On comparison, only the H+E group had lower RET fat accumulation than group H (11.0 ± 2.3 vs. 14.8 ± 3.1 g, $p < 0.01$).

From the 17th experimental week, group H showed elevated SBP (Figure 3). This increase was confirmed at week 20 after direct measurement of blood pressure, in which group H showed a significant increase compared to group C (159.0 ± 10.1 vs. 118.7 ± 8.4 mmHg, $p < 0.001$). The same behavior was observed for DBP (94.3 ± 8.5 vs. 83.2 ± 6.2 mmHg, $p < 0.001$) (Figure 4).

Regarding the metabolic profile, we observed increased triglyceride levels in animals that consumed a hyperlipidic diet (53.8 ± 12.4 vs. 28.0 ± 4.8 mg/dL) in

Table 2 Energy consumption and retroperitoneal and epididymal adipose tissue weight of the study groups

	(C)	(H)	(H+E)	(H+L)
	N = 12	N = 12	N = 12	N = 12
Daily intake (g/day)	17.0 ± 2.4	13.1 ± 2.3*	13.0 ± 2.2	13.4 ± 2.2
Energy intake (kcal/day)	63.1 ± 8.9	68.3 ± 12.1	67.6 ± 11.3	69.8 ± 11.6
Retroperitoneal fat (g)	5.4 ± 1.8	14.9 ± 3.8*	11.0 ± 2.3#	14.4 ± 4.2
Epididymal fat (g)	2.5 ± 0.6	6.6 ± 1.4*	5.4 ± 1.0	6.9 ± 1.9

Data are presented as mean ± standard deviation. Daily and energy intake are presented as average weekly evaluations from the first week of the experiment. Epididymal and retroperitoneal fat were weighed at the time of euthanasia by the end of the study. The control and hyperlipidic groups were compared by Student's t-tests and the hyperlipidic groups were compared with each other using ANOVA and Dunnett's tests. * $p \leq 0.05$ vs. control group (*Student's t-test*). # $p \leq 0.05$ vs. hyperlipidic group (Dunnett's test).

Figure 3. Blood pressure levels evaluated by indirect measurement in the study groups. Data are presented as mean ± standard deviation. Systolic blood pressure measurements presented as the mean, with an average of 10 individual readings. The control and hyperlipidic groups were compared by *Student's t-tests* and the hyperlipidic groups were compared with each other by ANOVA and Dunnett's tests. * $p \leq 0.05$, group H vs. control group (*Student's t-test*). # $p \leq 0.05$, group H+L vs. hyperlipidic group (Dunnett's test).

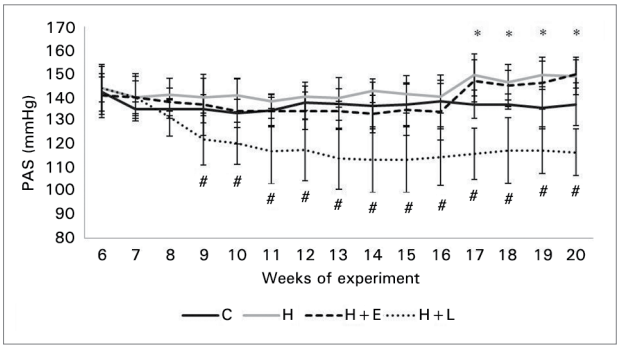
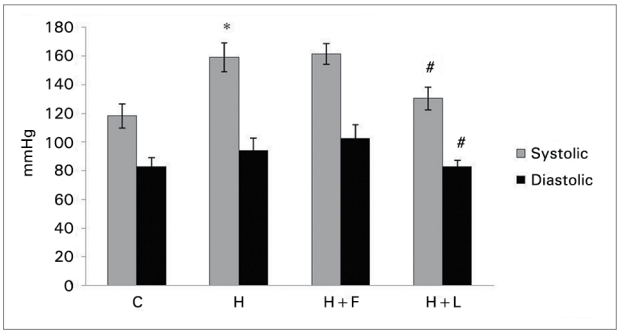


Figure 4. Blood pressure levels evaluated by direct measurements in the study groups at week 20. Data are presented as mean ± standard deviation. The control and hyperlipidic groups were compared by *Student's t-tests* and the hyperlipidic groups were compared by ANOVA and Dunnett's tests. * $p \leq 0.05$, group H vs. control group (*Student's t-test*). # $p \leq 0.05$, group H+L vs. hyperlipidic group (Dunnett's test).



groups H and C ($p \leq 0.001$). Cholesterol levels did not change (67.6 ± 4.69 vs. 63.2 ± 9.2 mg/dL) (Table 2). Fasting glucose levels were not different in groups C and H; however, a significant increase in blood glucose was observed in group H 120 minutes after glucose overdose (Table 2). Furthermore, the area under the glucose curve in group H was significantly greater than that of group C in the OGTT (15.885 ± 1.837 vs. 21.449 ± 3.692).

Treatments

Blood pressure

SBP remained high throughout the experiment in groups H and H+E. However, in the losartan-treated group (group H+L), a significant reduction occurred from the beginning of treatment (Figure 3).

Metabolic Profile

Triglyceride levels were not altered by the treatments, although increased cholesterol levels were observed in group H+L compared to group H ($p < 0.001$), as shown in Table 3.

There was also no change in the glycemic profile (fasting glucose level and area under the glucose curve) of animals undergoing antihypertensive treatments (Table 3 and Figure 5).

Renal function and morphology

At the end of the study, the glomerular filtration status as assessed by Ccr did not change significantly between the groups. The same finding was observed in relation to GV and glomerular area (Table 4).

Discussion

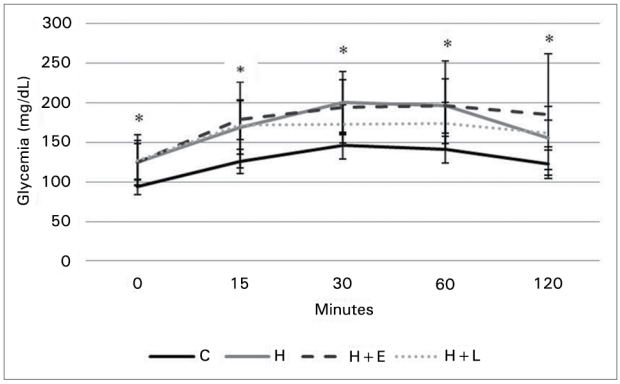
The obesity epidemic observed in the past decades has reached alarming proportions. It is estimated that 48% of the Brazilian population is overweight and about 15% is classified as obese.[31] Obesity progresses with several metabolic, inflammatory, and humoral changes that are associated with the development of malignant neoplasms, DM, dyslipidemia, and HTN. Most HTN patients are overweight or obese, and the results of epidemiological studies suggest that

Table 3 Total cholesterol, triglycerides, area under the curve, glucose/fasting, and glucose/120 min in the study groups at week 20

	(C)	(H)	(H+E)	(H+L)
	N = 11	N = 9	N = 11	N = 9
Total cholesterol (mg/dL)	67.6 ± 4.6	63.2 ± 9.2	73.0 ± 21.1	84.3 ± 10.2#
Triglycerides (mg/dL)	28.0 ± 4.8	53.8 ± 12.4*	59.7 ± 14.8	57.3 ± 12.8
Area under the curve (gluc. vs t)	15885 ± 1837	21449 ± 3692*	22329 ± 5597	20097 ± 2786
Glucose/fasting (mg/dL)	142.9 ± 13.3	143.5 ± 20.2	141.6 ± 23.2	137.3 ± 31.0

Gluc: glucose; t: time. Data are presented as mean ± standard deviation. The control and hyperlipidic groups were compared by *Student's t-tests* and the hyperlipidic groups were compared with each other by ANOVA and Dunnett's tests. * $p \leq 0.05$ vs. control group (*Student's t-test*). # $p \leq 0.05$ vs. hyperlipidic group (Dunnett's test).

Figure 5. Glycemic profiles of both groups after glucose load. Data are presented as mean ± standard deviation. The control and hyperlipidic groups were compared by *Student's t-tests* and the hyperlipidic groups were compared with each other by ANOVA and Dunnett's tests. * $p \leq 0.05$, group H vs control group (*Student's t-test*).



65–75% of the risk of developing essential HTN can be directly attributed to excess body weight.[32]

In this population, drug treatment of HTN is intended to reduce cardiovascular and renal morbidity and mortality and prevent metabolic worsening.[11] Some clinical and experimental evidence following RAAS blockade suggests the effective participation of this system in the pathogenesis of HTN and kidney lesions.[12,15] This strategy appears promising, as studies have shown attenuation of other components of MS, such as hypertriglyceridemia, after RAAS blockade.[12,18] However, the available studies do not provide sufficient clinical evidence for the generalization of this strategy for the treatment of HTN associated with MS through the administration of RAAS blockers. Thus, the use of experimental models constitutes an alternative approach for gaining knowledge of the efficacy of various antihypertensive treatment protocols in MS.[11,20]

Numerous experimental models of obesity have been described. These range from genetic models such as Zucker rats and knockout mice, to hypothalamic lesion-induced models related to growth and high-fat diet-induced models in dogs, rabbits, mice, and Sprague-Dawley rats.[33]

On the other hand, there are few reports of obesity induction in Wistar rats in order to study MS.[23,34] In the present study, administration of a lipid-rich diet for a period of 20 weeks induced obesity, hypertriglyceridemia, glycemic disorders, and HTN in rats; these findings are consistent with a diagnosis of MS. Despite the similar energy consumption, the difference in composition of the lipid-rich diet was responsible for the emergence of MS.[25]

The importance of using experimental models that resemble the profile of MS observed in humans is unquestionable, given the scarcity of recommendations for the treatment of this syndrome in humans. Currently, the therapeutic approach is based on the goal of reducing body weight and/or pharmacological

Table 4 Creatinine clearance, glomerular area, and glomerular volume of the study groups at week 20

	(C)	(H)	(H+E)	(H+L)
	N = 11	N = 9	N = 11	N = 9
Creatinine clearance (mL/min/kg)	3.2 ± 0.5	3.0 ± 0.3	3.5 ± 1.0	3.2 ± 1.2
	N = 6	N = 6	N = 6	N = 6
Area of glomerulus ($\times 10^2$ μm^2)	63 ± 9	59 ± 5	63 ± 11	66 ± 5
Glomerular volume ($\times 10^4$ μm^3)	64 ± 13	58 ± 13	64 ± 16	68 ± 8

Data are presented as mean ± standard deviation. The control and hyperlipidic groups were compared by *Student's t-tests* and the hyperlipidic groups were compared with each other by ANOVA and Dunnett's tests.

treatment of comorbidities comprising the syndrome. Specifically with regard to obesity-related HTN, despite the relative knowledge of its causal mechanisms, the optimal treatment is not yet established and there are no specific guidelines for the HTN treatment in this population. In general, drugs that do not interfere with the metabolic profile and encompass pathophysiological mechanisms such as sympathetic overactivity, sodium retention, and RAAS blockade have been recommended.[11,35]

In the present study, administration of a high-fat and normosodic diet to Wistar rats was associated with the development of HTN (Figure 3). In these animals, treatment with losartan prevented HTN development, a finding indicative of the importance of the RAAS in the pathogenesis of HTN in obese rats. These data are consistent with the literature and confirm the importance of this system in the pathogenesis and progression of HTN.[36]

On the other hand, mineralocorticoid antagonist treatment did not prevent the increase of blood pressure associated with MS in this study. This finding is in disagreement with a previous study by our group in obese dogs, in which we demonstrated the importance of aldosterone in the pathogenesis of obesity-related HTN.[15] In humans with MS, administration of spironolactone monotherapy significantly reduced blood pressure, suggesting the clinical importance of this hormone system in HTN in this context. Besides a reduction in blood pressure, this treatment led to improvement in glucose and lipid profiles, which is a clinically relevant finding given the characteristics of the subjects assessed.[18]

The discrepancy between the present study and data from previous studies by our group could be attributed to differences between species, diets, or the period of obesity exposure of the animals. Another aspect that could have interfered with this dissociation is the dose of spironolactone used, which eventually could have been low. However, this possibility does not seem likely, since in a previous study we observed a significant reduction in blood pressure in MS subjects treated with only 25-50 mg of spironolactone. In this study, the hypotensive action has been attributed to possible non-genetic effects.18 This finding is similar to that observed in the RALES study, which showed that treatment with spironolactone at a low dose (25 mg/day) was associated with a decrease in hospitalization and mortality and improvement in symptoms of serious heart failure patients.[37]

Furthermore, recent studies suggest that aldosterone induces vascular injury and HTN only in the presence of increased sodium intake. Sodium acts synergistically in the activation of the mineralocorticoid receptor (MR) by aldosterone by increasing the expression of cytokines and proinflammatory mediators, with cyclooxygenase-2, monocyte chemoattractant protein-1, and osteopontin being responsible for the development of microalbuminuria, vasculopathy, and HTN.[17] Rocha *et al*. showed that MR activation by coadministration of aldosterone and sodium exacerbated the severity of coronary lesions and HTN in Sprague-Dawley rats; however, in the absence of one of the agonists or presence of a mineralocorticoid antagonist, lower vasculotoxic and hypertensive actions were observed.[16] Since the diet used in this study was normosodic, this fact could explain the lack of a spironolactone-mediated hypotensive effect, and thus the discrepancy between our data and those from other studies in which MR blockade with eplerenone dramatically improved HTN and proteinuria and reversed podocyte damage in SHR rats fed with high-sodium diets.[17] The clinical offset to this observation is demonstrated in Yanomami Indian populations, where high plasma concentrations of aldosterone are unable to raise the blood pressure of these individuals due to a low dietary sodium intake.[38]

In the present study, administration of losartan or spironolactone did not significantly alter the metabolic and glycidic profiles of the animals. Moreover, the spironolactone-treated group showed a significant 25% reduction in RET fat. Similar effects have been described in C57BL/6 mice with MS.[39] In that study, obese mice treated with spironolactone showed a significant reduction of EP fat. This finding has been attributed to the inhibition of clonal expansion, differentiation, and accumulation of triglycerides in the adipocytes and by decreased expression of the peroxisome proliferator-activated receptor- due to MR blockade.[20,39]

We did not observe changes in renal function, as assessed by Cr levels, Ccr, and renal histology. These data are in disagreement with those presented by other authors who described kidney injury in experimental models of obesity, which are usually characterized by increased glomerular filtration rate, proteinuria, and

increased GV.[15,40] Although it has been evaluated previously, proteinuria was not included in the present study due to technical and interpretation difficulties, which made it impossible to use the results. However, it is worth speculating that no observable glomerular histological changes might have been secondary to the short period of obesity exposure of the animals and only slightly elevated blood pressure levels. This possibility seems likely considering that the renal damage from obesity in Wistar rats occurs only in senile animals.[40] Another important consideration is that we cannot dismiss entirely a more incipient nephropathy, since we did not evaluate indicators of injury at an earlier stage, such as the expression of inflammatory mediators, growth factors, and protein markers of epithelial-mesenchymal transition.

In conclusion, RAAS blockade in Wistar rats with MS was shown to be an effective measure for reducing blood pressure when inhibition of the angiotensin II receptor occurs. However, the same hypotensive effect was not observed following MR blockade, which inhibited adipogenic activity and prevented the accumulation of abdominal fat.

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