Increased diuresis, renal vascular reactivity and blood pressure levels in young rats feeding high sodium, moderately high fructose, or their association: a comparative evaluation.
Increased diuresis, renal vascular reactivity and blood pressure levels in young rats feeding high sodium, moderately high fructose, or their association: a comparative evaluation.

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Abstract

Background and aim: Excessive intakes of sodium or fructose have been described as risk factors for hypertension. We hypothesized that even a moderately high fructose diet (6% fructose), either alone or in combination with high sodium (4% NaCl), may impair diuresis, renal and systemic vascular reactivity, contributing to the onset of high blood pressure in rats.

Methods and results: Male Wistar rats were fed chow containing 4% NaCl (HS), 6% fructose (MHF), or both 4% NaCl and 6% fructose (HSMHF) for six weeks and had their diuresis, plasma creatinine, vascular reactivity of perfused kidneys and systemic arterial pressure evaluated. We found no differences in augmented diuresis among animals given HS, MHF or HSMHF diets. After six weeks both the HS and HSMHF groups had increased weight in their left kidneys, but only the HSMHF group showed augmented plasma creatinine. The effects of phenylephrine on renal vascular perfusion pressure were similarly enhanced in kidneys from the HS, MHF, and HSMHF groups, but not on the systemic arterial pressure. Although when evaluated in anesthetized rats only the HSMHF group presented augmented blood pressure, evaluation in conscious animals revealed that both the MHF and HSMHF diets, but not the HS alone, were able to induce tachycardia and hypertension.

Conclusion: A MHF diet containing 6% fructose was enough to render the renal vascular bed hyperreactive to phenylephrine, and to induce both hypertension and tachycardia. The combination of 6% fructose with 4% NaCl led to plasma accumulation of creatinine and accelerated the development of tachycardia.

Keywords: hypertension, tachycardia, renal function, dietary fructose
Introduction

Hypertension is a well known risk factor for more severe cardiovascular diseases, such as myocardial infarction, heart failure, and kidney disease (Chobanian et al. 2003). More than 95% of individuals with hypertension are diagnosed with primary hypertension, which is putatively associated with a combination of genetic and environmental factors, such as dietetic habits (Carretero and Oparil 2000). Overconsumption of sodium is among the main dietary risk factors known for hypertension (Cook et al. 2007). Besides elevated amounts of salt, processed foods such as soft drinks, candies, crackers, baked goods and others contain high levels of fructose, which has been increasingly produced and used worldwide as a sweetener over the past 45 years (Vuilleumier 1993; Guthrie and Morton 2000). Notably, excessive consumption of fructose has been linked with metabolic syndrome and obesity in both clinical and experimental analyses (Basciano et al. 2005; Tran et al. 2009), and these two conditions are associated with hypertension. Indeed, a number of studies have shown that overconsumption of fructose leads to hypertension in laboratory animals (Hwang et al. 1987; Tran et al. 2009), and is an independent risk factor for the development of hypertension (Jalal et al. 2010). Although the mechanisms involved in the influence of fructose on blood pressure remain poorly understood, the continuous intake of a high fructose diet has been associated with kidney disease and renal micro vascular damage (Gersch et al. 2007; Sanchez-Lozada et al. 2007; Nakayama et al. 2010). Notably, the kidneys exert a range of effects on the systemic arterial pressure through regulation of water and sodium balance (Knepper et al. 2015). In addition, renal vasoconstriction plays a major role in the development of essential hypertension (Gomez 1951).

In spite of the previously mentioned studies, the role of fructose in the genesis of hypertension is still controversial, mainly because the majority of the experimental studies in this
field were conducted with chow containing 60-70% fructose (Hwang et al. 1987; Gersch et al. 2007). Even in studies describing the use of moderate amounts of fructose (10-20%) added to drinking water (Dai and McNeill 1995; Sanchez-Lozada et al. 2007), the final consumption of fructose remains equivalent to a diet containing around 40% fructose in the chow.

Recent studies have suggested that increased dietary fructose intake stimulates salt absorption in the small intestine and kidney tubules, contributing to salt-induced hypertension (Singh et al. 2008). Interestingly, the exposure of normotensive and healthy rats to high salt ingestion (up to 8%) does not render these animals hypertensive, despite a range of changes in vascular biology (Crestani et al. 2014). Thus, this study aimed to investigate the hypothesis that even a moderately high fructose diet (6% fructose), either alone or in combination with high sodium (4% NaCl), may impair diuresis, renal and systemic vascular reactivity, contributing to the onset of high blood pressure in rats.

Methods

Animals and experimental groups

This study was performed using male Wistar rats provided by the Universidade Federal do Paraná (UFPR, Curitiba, PR, Brazil). All procedures adopted in this study were approved by the Institutional Ethics Committee for Animal Use from UFPR (601/2012). The animals were kept under standard laboratory conditions (12 hour light/dark cycle, 21 ± 2 °C, water and food ad libitum). The animals were taken on the day of weaning (50-60 g) and were separated in four distinct groups (5-6 animals per cage). The first group, used as a control, received standard chow (gross energy: 3.91 kcal/g) containing regular amounts of NaCl (0.27%), calcium (1–1.4 %), protein (minimal: 22%), fat (minimal: 4%, ether extract), crude fiber (maximal: 7%), and
carbohydrates (55%) (Nuvital®, Curitiba, PR, Brazil). The second group, the high-salt (HS) group, received standard chow with 4% NaCl added. The third group received a moderately high fructose diet (MHF group), which was the standard chow containing 6% fructose. Finally, the fourth group received standard chow with both 4% NaCl and 6% fructose added (HSMHF group). These diets were given for six weeks, when the experiments were ended. The high sodium and fructose diets used for HS, MHF, and HSMHF groups were prepared in-house using powdered regular chow supplied by the same manufacturer. The mineral and vitamin amounts presented in the chow used for all experimental groups met the recommendation specified in AIN-93 (Reeves et al. 1993).

**Evaluation of body weight, consumption of water and chow, and hematological and blood biochemistry parameters**

The body weight of all the experimental groups was measured weekly. During the six weeks, the amounts of water and chow provided and consumed for each animal cage were also measured, and used to assess the average of food and water intake. For hematologic and biochemical analysis, blood samples were collected at the sixth week from the same animals used for isolation of the kidney. The animals were subjected to general anesthesia induced by ketamine/xilazine (100/20 mg/kg, i.p), the abdomen was opened and a polyethylene catheter was inserted into the posterior vena cava, allowing the draw of 1 mL of blood, which was immediately placed in tubes containing EDTA (40 µL, 6.8 mM). To determine leukocytes, hemoglobin, platelets, and hematocrit, 30 µL of blood was immediately analyzed using an automatized blood counter (HORIBA ABX®, Micros 6; HORIBA, Montpellier, France). The plasmatic concentration of creatinine and uric acid were measured in blood plasma using
commercial kits according to the manufacturer’s instructions (K016 and K052, respectively; Quibasa/Bioclin, MG, Brazil). Blood glucose was measured by tail-vein sampling with a blood glucose meter (Accu-Chek Active, Roche, Mannheim, Germany). Fasting glucose was measured in animals maintained without access to chow during an entire day (7:00 AM to 6:00 PM).

**Assessment of diuresis**

Urinary volume was determined once a week, as previously described (Kau et al. 1984), with minor modifications. Briefly, the animals were accommodated individually in metabolic cages with free access to water for 8 h. The urine was collected after 1, 2, 4, 6, and 8 hours and the total volume was measured using automatic pipettes.

**Ex vivo analyses of the renal vascular reactivity**

The isolation of the kidneys and the setup of the perfusion apparatus were conducted as previously detailed (Sant'Helena et al. 2015). In brief, the left kidney was removed from animals under general anesthesia (induced by ketamine/xilazine, 100/20 mg/kg, i.p.), and maintained under constant perfusion (4 mL/min) with physiological saline solution (PSS, composition in mM: NaCl 131.3, KCl 4.7, KH₂PO₄ 1.18, MgSO₄7H₂O 1.17, NaHCO₃ 14.9, D-glucose 5.5, CaCl₂2H₂O 1.6, EDTA 0.08; pH 7.4) at 37 ºC and continuously aerated with 5% CO₂/95%O₂, allowing the assessment of the renal vascular perfusion pressure (RVPP). After stabilization for 30 min the basal RVPP was recorded and the effects of noncumulative doses of phenylephrine (0.1, 0.3, 1, 3, 10, 30, 100, and 300 nmol), given at 10 min intervals, were measured and compared among the groups.
Direct measurement of blood pressure in anesthetized rats

The animals were subjected to anesthesia induced by intramuscular injection of ketamine/xilazine (100/20 mg/kg), supplemented at 45-50 min intervals. The right femoral vein and the left carotid artery were surgically isolated and cannulated for drug administration and for recording of mean arterial pressure (MAP), as previously described (Crestani et al. 2009). The blood pressure was allowed to stabilize for 15 min and the basal MAP recorded at this point was used for comparisons among the groups. In addition, the animals received intravenous bolus injections of phenylephrine and acetylcholine (6, 20, and 60 nmol/kg for both). The doses of the vasoactive agents were administered at 10 min intervals and their effects on MAP compared among the groups. The animals were euthanized with an overdose of anesthetic agents at the end of the experiments. The heart, thoracic aorta, and left kidney from these animals were removed, cleaned of adjacent tissues, and weighed to determine their relative weights.

Measurement of systolic blood pressure in conscious rats

The animals were subjected to the tail-cuff method at 4 and 6 weeks after the beginning of the diets. We used a non-invasive blood pressure system (model IN125/R, AD Instruments, Australia) coupled to a pulse transducer/pressure cuff (LE5160R, Panlab, Spain), which allowed the detection of both the systolic arterial pressure (in mm Hg) and heart rate (in beats per minute). To reduce stress-induced variations in blood pressure, the animals were subjected to restrain, tail-cuff inflation, and room temperature (maintained at 29 °C) daily for 1 week before the first assessment of blood pressure. To ensure the accuracy of our results the data were obtained from average of three to five measurements made in each session per animal.
Drugs and reagents

Phenylephrine and acetylcholine were purchased from Sigma (St Louis, MO, USA). Fructose was obtained from Won Nutrition (Caçapava, SP, Brazil) and NaCl was purchased from Vetec Química (Duque de Caxias, RJ, Brazil). Ketamine and xilazina were from Syntec (São Paulo, SP, Brazil). All the salts used to prepare the physiological nutritive solution were from Merck (Darmstadt, Germany).

Statistical analysis

The results are expressed as mean ± SEM. The data were analyzed by ordinary one-way analysis of variance (ANOVA) followed by Dunnett’s post hoc test, two-way ANOVA followed by Bonferroni’s multiple comparison, or multiple t test, when applicable. A value of p < 0.05 was accepted as statistically significant. For multiple comparisons, the threshold of statistical significance was set following the Bonferroni correction (0.05/3). Graphs were drawn and statistical analyses were performed using GraphPad Prism version 6.0g for Mac (GraphPad Software, La Jolla, CA, USA).

Results

Despite the increased platelet counts seen in the HSMHF group, we found no major effects of the consumption of 4% NaCl or 6% fructose, or their association, on hematological parameters (Table 1). In addition, blood glucose levels, including fasting glycemia, remained unaltered in all groups (Table 1). During the six weeks of evaluation, animals exposed to the 6% fructose diet did not gain more weight than animals subjected to regular chow. However, the body weight of animals from both the HS and HSMHF groups were reduced by 10% in the sixth
week compared with the control group (Fig. 1A). The low weight gain in the HSMHF group, but not in the HS group, was accompanied by diminished food intake (Fig. 1B). Sodium overconsumption was associated with increased water intake (measured weakly directly from animal cages) and augmented diuresis (individually assessed once a week in metabolic cages), as found at 1, 3, and 6 weeks in the HS group (Fig. 1C and D, hachured bars). Animals from the MHF group showed unchanged water intake, but also presented increased diuresis when subjected to metabolic cages after 6 weeks fed chow containing 6% fructose (Fig. 1C and D, gray bars). In addition, NaCl and fructose overload in the chow also resulted in increased water consumption and augmented urine output, as found in the HSMHF group (Fig. 1A and B, black bars).

Analyses of the serum uric acid and creatinine levels determined in the sixth week after the beginning of the diets showed that creatinine, but not uric acid, was significantly increased only in animals from the HSMHF group, compared with samples from the control group (Table 1). We did not find any differences in the weight of the heart and the thoracic aorta among the control, HS, MHF, and HSMHF groups (data not shown), but the relative weight of the left kidney was significantly increased in both the HS and HSMHF groups (Fig. 2A). Importantly, phenylephrine-increased renal vascular perfusion pressure was significantly enhanced in the kidneys obtained from the HS, MHF, and HSMHF groups (Fig. 2C), but only kidneys from the HS and HSMHF groups displayed increased basal perfusion pressure (Figure 2B).

In experiments with anesthetized rats, the basal MAP recorded in animals treated with the chow containing 4% NaCl and 6% fructose was 126 ± 6 mm Hg, significantly higher than the MAP found in the control, HS, and MHF groups (Fig. 3A). Evaluation of the vascular responsiveness showed no differences in the vasodilatory effects of acetylcholine among the
groups (Fig. 3B). Compared with the control group, only animals subjected to the moderately high fructose diet showed impaired responses to phenylephrine, which had its vasopressor effect reduced (Fig. 3C, gray bars). Despite this hyporeactivity to phenylephrine and the unaltered MAP when blood pressure was recorded under anesthesia, the measurement of blood pressure in conscious animals revealed that after six weeks of receiving the diets animals from both the HF (Figs. 4C and 4D) and HSMHF (Figs. 4E and 4F) groups showed systolic arterial pressure and heart rates significantly higher than animals from the control group. Interestingly, in the HSMHF group heart rate was also increased in the fourth week of evaluation (Fig. 4F).

**Discussion**

In this study, we aimed to investigate the effects of sodium and fructose overconsumption on the cardiovascular function of rats. As previously mentioned, the proportion of fructose in the chow used in our experiments was limited to 6%, giving us a moderately high fructose diet, significantly below the amounts used by other studies (Hwang et al. 1987; Dai and Mcneill 1995; Gersch et al. 2007; Sanchez-Lozada et al. 2007). Indeed, unlike data from studies using 60% fructose in the diet, the animals from our MHF and HSMHF groups were not overweight or hyperglycemic, suggesting that the findings described in our study, i.e. augmented plasma creatinine, enhanced reactivity of the renal vascular bed, and increased blood pressure, are not likely related to obesity, diabetes or metabolic syndrome, conditions tightly associated with both cardiovascular disorders and fructose overload (Basciano et al. 2005; Tran et al. 2009). Nevertheless, whether the moderate intake of 6% fructose in chow may or not result in impaired insulin sensitivity, mainly after longer periods of intake, deserves further investigation.
Augmented urine excretion and histological evidence of damage in nephrons has been previously shown in the kidneys of animals given high levels of fructose in water over long period (16 months), although changes in weight may not occur (Kizhner and Werman 2002). We did not explore the morphological effects of our MHF diet on the kidneys, but our results show that exposure to a diet containing 6% fructose for six weeks also results in increased diuresis, as individually assessed using metabolic cages. Importantly, the combination of 4% NaCl and 6% fructose in the chow (HSMHF group) did not exacerbate fructose-induced diuresis, despite of the well-known diuretic effects of high-salt diets. Interestingly, the relative weight of kidneys was increased in animals subjected to HS and HSMHF diets, but only the HSMHF group showed plasma accumulation of creatinine. Taking into account that both metabolic and kidney damage generated by a 60% fructose diet in rats may be prevented by dietary sodium depletion (Oudot et al. 2013), our data reinforces the relevance of the deleterious interaction between elevated sodium and fructose for renal function, even when the dietary fructose is maintained at moderately high levels.

Our results did not allow us to establish any association between the effects of HS and MHF diets on renal vascular reactivity, as the kidneys obtained from the HS, MHF, and HSMHF groups displayed a similar pattern of hyperreactivity to the selective $\alpha_{1A}$-adrenergic receptor agonist phenylephrine. Notably, both salt and fructose are putative risk factors for hypertension and, although the hyperreactivity of the renal vascular bed has been previously described after high salt intake (dos Santos et al. 2006), this is the first study demonstrating enhanced vascular reactivity to phenylephrine in kidneys from fructose fed rats. We also investigated if the 6% fructose intake could also increase the pressor effects of phenylephrine, but our data revealed that animals from the MHF group were hyporeactive to phenylephrine, suggesting that the
enhanced vascular reactivity to phenylephrine found in the fructose fed rats is not a systemic event but depends on the vascular system evaluated. Indeed, the effects of phenylephrine, endothelin, and angiotensin II remained unchanged in the mesenteric vascular bed of rats given 60% fructose chow (Navarrocid et al. 1995). The mechanisms involved in the impaired vascular reactivity found in kidneys, and its contribution to the development of renal dysfunction and hypertension induced by fructose deserves further investigation.

Although the assessment of blood pressure under anesthesia revealed increased mean arterial pressure only in animals receiving the HSMHF diet, we found that when measured by the tail-cuff method animals subjected to the MHF diet also presented high blood pressure levels, suggesting that fructose, even in amounts significantly lower than in previous studies, was the main factor responsible for the elevation of blood pressure. Although we cannot explain the different levels of blood pressure found when anesthetized and awake animals were used, the sympathoexcitatory event associated with stress induced by the tail-cuff method may have been a determinant stimulus to unmask hypertension, as previously described (Verma et al. 1999). In addition, despite the poor evidence of fructose-induced changes in heart rate in clinical and experimental studies (Le et al. 2012; Zemancikova and Torok 2014), we found that the MHF diet used in our experiments resulted in tachycardia, which appeared earlier in those animals receiving the combined HSMHF diet.

We conclude that even a moderate intake of fructose (6% added in chow) may be enough to raise blood pressure in normotensive and healthy rats, at least when overconsumption starts from weaning. Compared with human stages, it can be considered that in our experiments the animals were maintained under high dietary levels of fructose and sodium throughout infancy until reaching early adulthood. Importantly, although epidemiological studies did not reveal a
clear correlation between high fructose feeding and cardiovascular disturbances in children (for review see Rizkalla 2010), our results suggest that the high consumption of fructose and sodium at early life stages may contribute for early cardiovascular diseases in this population. The continuous use of this moderately high fructose diet for six weeks also rendered the renal vascular bed hyper reactive to vasoconstriction and increased the heart rate of the animals. Importantly, a combination of 6% fructose with 4% NaCl was not enough to potentiate the fructose-induced hypertension, but it did lead to plasma accumulation of creatinine and accelerated the development of tachycardia. Despite the descriptive nature of our study, the findings presented here may improve our current knowledge regarding the cardiovascular risks of excessive consumption of fructose, including when combined with increased dietary levels of sodium.

Conflict of interest statement

The authors declare no conflict of interest

Acknowledgements

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References


Table 1 Effects of high-salt, moderately high-fructose, and a combination of both diets on hematologic and biochemistry parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>HS</th>
<th>MHF</th>
<th>HSMHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes (x 10^3/µL)</td>
<td>9.9 ± 1.44</td>
<td>9.5 ± 0.86</td>
<td>9.2 ± 1.2</td>
<td>6.9 ± 0.77</td>
</tr>
<tr>
<td>Hemoglobin (x 10^3/µL)</td>
<td>12.6 ± 0.52</td>
<td>12.9 ± 0.28</td>
<td>12.1 ± 0.45</td>
<td>11.8 ± 0.31</td>
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<tr>
<td>Hematocrit (%)</td>
<td>46.6 ± 2.39</td>
<td>46.1 ± 0.85</td>
<td>45.4 ± 2.19</td>
<td>41.8 ± 1.02</td>
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<tr>
<td>Platelets (x 10^3/µL)</td>
<td>1062 ± 67.6</td>
<td>1197 ± 10.0</td>
<td>1017 ± 91.6</td>
<td>1282 ± 58.7*</td>
</tr>
<tr>
<td>Lymphocytes (x 10^3/µL)</td>
<td>7.54 ± 1.39</td>
<td>6.98 ± 0.66</td>
<td>7.36 ± 1.02</td>
<td>5.14 ± 0.57</td>
</tr>
<tr>
<td>Monocytes (x 10^3/µL)</td>
<td>1.02 ± 0.12</td>
<td>1.18 ± 0.13</td>
<td>0.92 ± 0.13</td>
<td>0.86 ± 0.11</td>
</tr>
<tr>
<td>Granulocytes (x 10^3/µL)</td>
<td>1.00 ± 0.13</td>
<td>1.36 ± 0.19</td>
<td>0.94 ± 0.15</td>
<td>0.92 ± 0.18</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>2.70 ± 0.25</td>
<td>2.83 ± 0.17</td>
<td>2.71 ± 0.26</td>
<td>3.17 ± 0.35</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.88 ± 0.07</td>
<td>0.90 ± 0.09</td>
<td>1.11 ± 0.24</td>
<td>1.49 ± 0.10*</td>
</tr>
<tr>
<td>Glycemia (mg/dL)</td>
<td>127.2 ± 3.94</td>
<td>121.3 ± 2.72</td>
<td>124.3 ± 2.35</td>
<td>131.2 ± 2.39</td>
</tr>
<tr>
<td>Fasting glycemia (mg/dL)</td>
<td>100.7 ± 0.66</td>
<td>114.7 ± 3.52</td>
<td>98.5 ± 5.90</td>
<td>113.8 ± 2.13</td>
</tr>
</tbody>
</table>

Mean values with their standard errors, n=5 rats per group. HS (high-salt intake group, 4% NaCl added in the chow), MHF (moderately high-fructose intake group, 6% fructose added in the chow), HSMHF (high-salt plus moderately high-fructose intake, 4% NaCl and 6% fructose added in the chow). The control group received regular chow only. The values show the mean ± SEM of blood samples collected after six weeks under the diets. Statistical analyses were performed by means of one way-analyses of variance (ANOVA) followed by Dunnett’s multiple comparison test. * P < 0.05 versus control group.
Figure Legends

**Fig. 1.** Body weight (A), chow (B) and water (C) intake, and diuresis in rats receiving the regular (control), 4% NaCl (HS), 6% fructose (MHF), or the combined 4% NaCl /6% fructose chow for six weeks. The values shown the mean ± SEM of 5 animals per group; *p* < 0.05 compared with the respective control.

**Fig. 2.** Relative weight of left kidney (A), basal (B) and phenylephrine-increased (C) renal vascular perfusion pressure of kidneys obtained from rats subjected to regular (control), 4% NaCl (HS), 6% fructose (MHF), or the combined 4% NaCl/6% fructose chow for six weeks. The values shown the mean ± SEM of kidneys from 8 animals per group; *p* < 0.05 compared with the respective control.

**Fig. 3.** Basal mean arterial pressure (A), and responses to acetylcholine (B) and phenylephrine in anesthetised rats subjected to regular (control), 4% NaCl (HS), 6% fructose (MHF), or the combined 4% NaCl/6% fructose chow for six weeks. The values shown the mean ± SEM of 6-8 animals per group; *p* < 0.05 compared with the respective control.

**Fig. 4.** Systolic arterial pressure (A, B and C) and heart rate (D, E and F) measured in conscious rats subjected to regular (control), 4% NaCl (HS), 6% fructose (MHF), or the combined 4% NaCl/6% fructose chow for six weeks. The values shown the mean ± SEM of 5 animals per group; *p* < 0.05 compared with the respective control.
Figure 1
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159x140mm (300 x 300 DPI)
Figure 3
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Figure 4
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