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Kidney Function in Rats Treated With a High-Fructose Diet and Streptozotocin

Review Article

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Introduction

High fructose intake has been associated with metabolic syndrome in the United States [1]. Fructose is mainly present in breakfast cereals, high-fructose corn syrup, and soft drinks, which are consumed in large amounts by the American population. Metabolic syndrome is characterized by specific signs as abdominal obesity, elevated triglycerides, reduced HDL-C, elevated blood pressure, and hyperglycemia [2, 3]. These signs are risk factors for cardiovascular disease and chronic kidney disease; they are designated as cardiorenal metabolic syndrome.

The link between obesity, insulin resistance, elevated plasma uric acid levels, non-alcoholic fatty liver, and cardiovascular or chronic kidney disease may be oxidative damage [4, 5].Obesity and elevated plasma uric acid levels are associated with systemic oxidative stress, inflammation, endothelial dysfunction, and pancreatic β-cell injury [5].

Fructose depletes ATP, increases nucleotide turnover and increases plasma uric acid levels, impairing the renin-angiotensin-system and inducing oxidative stress [6]. Fructose-induced uric acid synthesis in hepatocytes has lipogenic effects such as liver fatty acid accumulation and an increase in plasma triglycerides [7]. A high-fructose diet also induces inflammation in hepatocytes and impairment of insulin signaling in the liver [8].

Adherence to a high fructose intake diet (HFID) for long periods is being used to mimic metabolic syndrome in rats. Studies have successfully induced metabolic syndrome in rats or mice using a 60% fructose diet [9], usually during a long period of treatment such as 8 months [10]. Nevertheless, other strategies potentiate or accelerate the development metabolic syndrome signs, such as cardiovascular or kidney diseases associated with this syndrome.

A study suggested that a high-fructose and high-fat diet could mimic some of the signs of metabolic syndrome [10]. In addition, another study suggested that fiber-free white flour together with fructose is more effective at triggering metabolic syndrome than only fructose [11].

The goal of the present study was to analyze the association between high fructose intake in rats treated with low doses of streptozotocin, focusing on kidney function and histological alterations. In our model, the high-fructose diet induced some of the metabolic syndrome signs such as obesity, elevated triglycerides, and increased plasma uric acid levels. In the rat kidney, an increased glomerular filtration area, urine volume, and urinary protein excretion, possibly mediated by an increase in kidney oxidative stress, were observed. In animals treated with low doses of streptozotocin and fructose, the same effects were observed except oxidative stress. Our work suggests that fructose together with low doses of streptozotocin can potentiate, at least partially, the effects of high fructose intake in the induction of metabolic syndrome and provide new insights into the effect of fructose on metabolic syndrome.

Materials and Methods

Animal treatment

The experimental protocol was approved by the ethics committee of the Universidade Federal de São Paulo (process number 0590/10).

Male Wistar rats weighing 200–250 g were divided into three groups. One group was treated with tail injection of streptozotocin at low concentration (STZ, Sigma, St. Louis, Mo, USA; 30 mg/kg dissolved in sodium citrate buffer, pH 4.5) and fed with 20% HFID+STZ in drinking water for 90 days. The second group was fed only with a 20% HFID in drinking water for 90 days, and

the third group received tap water during the entire period and was used as the control (CTL).

In some experiments, as indicated in the text, the animals were fed during the experimental protocol with a 2% fructose diet. All animals had free access to standard rat chow and tap water during experimental protocol.

At 0 (baseline), 30, 60, and 90 days after the beginning of the experimental protocols, blood samples were collected from the retinal or the tail vein, and the animals were maintained in metabolic cages for 24 h for urine collection.

The animals were killed after 90 days and both the right and left kidneys were removed for histological analyses. Biochemical parameters were determined by analyzing plasma and urine samples.

Biochemical analysis

Plasma glucose concentration was determined for all rats by analyzing tail blood samples (Accuchek, Boehringer Mannheim, Indianapolis, Ind., USA). The levels of blood creatinine, urea, and urinary protein were assayed spectrophotometrically according to standard procedures using commercially available diagnostic kits (Labtest Diagnostica, Minas Gerais, Brazil). Creatinine was determined by using the Jafé reaction [12], and urea was determined by measuring urease activity [13]. Serum uric acid was determined by the uricase enzyme reaction based on the formation of allantoin and hydrogen peroxide [14].

Plasma triglycerides were assessed by measuring hydrolyzation by lipase, which results in the formation of glycerol and fatty acids; glycerol reacts with glycerol kinase followed by oxidase in a system that forms hydrogen peroxide [15]. HDL cholesterol was assessed by precipitation of lipoproteins followed by quantification of HDL in the supernatant. The samples were hydrolyzed with cholesterol esterase and cholesterol oxidase, forming hydrogen peroxide [16]. The hydrogen peroxide formed in the reactions described above was analyzed by performing the horseradish peroxidase reaction in a chromogenic system.

Urinary protein was analyzed by using the pyrogallol red-molybdate method [17]. The results were expressed in mg/dL for creatinine, urea, uric acid, triglycerides, HDL cholesterol, and mg/24 h for urinary protein. Urine volume was expressed in mL, rat weight was expressed in grams, and urinary sodium was analyzed with flame photometry (Micronal B462, São Paulo, Brazil) and expressed in mEq/24 h.

Lipid peroxidation

Lipid peroxidation was determined in urine samples by quantifying TBARS (thiobarbituric acid reactive substances). The reactive substances combine with thiobarbituric acid, forming a red compound whose concentration can be assessed spectrophotometrically at an absorbance of 535nm. The thiobarbituric acid assay was performed as described previously [18]. Malondialdehyde (MDA) was used to generate a standard curve, and the results were expressed as nM of MDA/mg creatinine. Urine samples were added to a solution of 0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25 N HCl (Sigma-Aldrich, St. Louis, USA), after which they were continually agitated while heated to 95°C for 20 min. Subsequently, the samples were allowed to cool to room temperature. Assessment of the creatinine level in urine samples was performed as previously described and used to normalize the results of MDA.

Urinary Hydroperoxide

Urinary levels of hydroperoxides were measured using the ferrous oxidation method with Xylenol Orange called FOX2 assay for lipid hydroperoxide [19]. Briefly, 100-µL aliquots of urine were transferred into microcentrifuge vials followed by the addition of 900 µL FOX2 reagent.

After incubation at room temperature for 30 min, the vials were centrifuged at 12,000 rpm at 25°C for 10 min. The absorbance of the supernatant was then determined at 560 nm. Lipid hydroperoxide content in urine samples was determined according to the molar coefficient (4.3 104 M-1cm-1). Assessment of the creatinine level in urine samples was performed as previously described and used to normalize the results of urinary hydroperoxide measurements.

Histological analysis

The kidneys were dissected along the nonhilar axis and fixed in 10% phosphate-buffered formalin (Erviegas, São Paulo, Brazil). Kidney sections were fixed with 4% buffered paraformaldehyde and embedded in paraffin (Erviegas, São Paulo, Brazil). Four-micrometer thick sections were cut for Masson's Trichrome (TM; Erviegas, São Paulo, Brazil) and Periodic Acid Schiff (PAS; Erviegas, São Paulo, Brazil) staining, and then examined by performing light microscopy in a blinded manner.

The morphometric study was performed in kidney sections stained with PAS and TM in a Leica image analyzer computer system (Hessen, Germany). The glomerular analyzes is represented by the renal corpuscle area, glomerular tuft area, and the Bowman space, as shown by the difference between them in PAS-stained sections. The fraction of PAS-positive staining areas within the glomerular tuft area was measured in 10 randomly selected glomeruli to indicate the mesangial matrix expansion.

The kidney sections stained with TM were used to evaluate fibrosis in the glomeruli. The percentage of the area stained blue was measured per unit tuft area in 15 randomly selected glomeruli per animal.

Statistical Analysis

The results are expressed as a mean ± SE. The data were analyzed by one-way ANOVA followed by Tukey's test. A value of $p < 0.05$ was considered statistically significant.

Results and Discussion

Studies have suggested that a fructose diet combined with other stimuli such as a high-fat diet [10, 20] or even a fiber-free white flour diet [11] could mimic metabolic syndrome in animal models. The hypothesis of the present study was that low doses of streptozotocin could accelerate or potentiate the symptoms of metabolic syndrome.

Tables 1 and 2 show the biochemical parameters measured in the CTL, HFID, and HFID+STZ groups. The STZ+HFID animals gained significantly more weight than control or HFID animals after 30 days of treatment (Table 1).

Table 1: Physiological Parameters

	Days	Weight(g)	Blood Glucosis (mg/dL)	Urine Volume (mL)	Creatinine serum (mg/dL)	Urea (mg/dL)
Control	**0**	375±11	83.2±4.8	13.8±1.9	0.50±0.13	34.4±2.3
	30	395±10	79.8±2.8	11.8±2.0	0.61±0.07	28.5±4.7
	60	437±8	87.0±2.3	9.9±1.8	0.52±0.14	29.2±1.3
	90	458±9	95.8±5.3	12.0±2.0	0.63±0.07	28.0±1.3
HFID	**0**	396±6	82.8±5.1	10.7±0.9	0.36±0.05	35.5±7.2*
	30	420±3	90.2±5.3	11.1±3.3	0.35±0.02	46.8±1.8
	60	441±3*	103.0±6.6	31.0±3.4*	0.42±0.04	33.6±1.5*
	90	442±2	106.6±5.3	36.2±2.8*	0.65±0.09	36.8±0.8
STZ+HFID	**0**	411±6	93.2±3.0*	9.1±1.3	0.40±0.08	48.5±5.8
	30	444±7*	108.0±6.2*	14.6±3.6	0.03±0.06	40.8±0.7
	60	468±8*	108.0±4.8	42.8±1.7*	0.40±0.06	28.9±2.4
	90	469±9*	106.7±4.8	34.6±2.3*	0.48±0.04	30.1±2.4

Table 2

	Days	Urinary Protein (mg/24h)	Uric Acid Serum (mg/dL)	Tryglicerydes (mg/dL)	C-HDL (mg/dL)	Sodium Urinary (mEq/24h)
Control	**0**	6.6 ± 0.5	1.60 ± 0.75	64.6 ± 4.7	52.3 ± 5.3	0.57 ± 0.02
	30	4.8 ± 0.4	1.21 ± 0.27	45.5 ± 1.3	46.3 ± 1.5	ND
	60	4.1 ± 0.5	0.88 ± 0.01	60.3 ± 2.4	49.2 ± 3.4	ND
	90	4.8 ± 0.6	0.83 ± 0.38	55.0 ± 4.5	52.5 ± 4.3	0.63 ± 0.09
HFID	**0**	5.5 ± 0.9	1.58 ± 0.49	59.6 ± 18.8	55.1 ± 8.1	0.48 ± 0.08
	30	3.5 ± 1.0	3.75 ± 0.23*	75.9 ± 26.7	58.4 ± 2.2*	ND
	60	16.6 ± 2.4*	1.45 ± 0.07*	137.9 ± 2.0*	57.9 ± 4.7	ND
	90	10.7 ± 0.5*	1.38 ± 0.31	115.7 ± 5.7*	56.1 ± 0.6	0.39 ± 0.07
STZ+HFID	**0**	6.5 ± 0.5	1.33 ± 0.07	59.6 ± 18.8	62.6 ± 4.5	0.48 ± 0.07
	30	3.7 ± 1.0	1.65 ± 0.32	70.9 ± 6.4*	68.9 ± 4.7*	ND
	60	25.1 ± 4.8*	1.25 ± 0.13	192.0 ± 18.8*	59.5 ± 6.3	ND
	90	12.8 ± 2.8*	2.38 ± 0.19*	149.7 ± 38.1*	64.8 ± 2.7	0.35 ± 0.03*

(*)p< 0.05 vs. CTL

Data were expressed as the mean ± SEM. The parameters were analyzed in the CTL (control), HFID (high fructose intake diet, 20%), STZ+HFID (streptozotocin, 30 mg/mL and high fructose intake diet, 20%) groups at 0, 30, 60, and 90 days of diet.

No persistent increase in plasma glucose levels was detected in any of the experimental groups in comparison to the control group, and a significant increase was observed in the STZ+HFID animals only at the beginning of the experiment (Table 1).

The fructose diet induced a significant increase in uric acid after 30 days in the HFID group and after 90 days in the STZ+HFID group, and a persistent increase in plasma triglycerides was observed in both groups in comparison to the control group after 30 days of diet. The percentage increase was 210% and 251% in plasma triglycerides after 90 days of diet in the HFID and STZ+HFID groups, respectively (Table 2). Nevertheless, a small increase in HDL cholesterol was detected after 30 days of diet in the HFID and STZ+HFID groups.

The same observations were reported in other studies. Animals fed with fructose developed hypertension, hyperuricemia, and hypertriglyceridemia, and these effects increased progressively with an increase in fructose intake from 10 to 60% of fructose [9].

The correlation between these signs may be an increase in uric acid or alterations of the oxidant/antioxidant status. A high-fructose diet induced hypertension, liver steatosis, hyperinsulinemia, and hypertriglyceridemia, and these effects were prevented by allopurinol treatment. Uric acid also increased the oxidant status of the animals treated with fructose [21, 22].

The streptozotocin treatment together with the high-fructose diet (20%) potentiated some of the signs of metabolic syndrome in our experimental model, such as body weight, plasma glucose, and triglyceride levels. These results suggest that low doses of streptozotocin can accelerate the development of metabolic syndrome.

The kidney function analyzes showed that in both groups analyzed, HFID and STZ+HFID, the diuresis increased after 60 days

of diet (Table 1). In HFID and STZ+HFID, the increase was 301% and 288%, respectively. Although, no differences in creatinine or urea were observed in the HFID and STZ+HFID groups in comparison to the control group (Table 1).

Table 2 shows the increase in urinary protein in the HFID and STZ+HFID groups after 30 days of diet and a decrease in urinary sodium only in the STZ+HFID group (45%) after 90 days of diet in comparison to the control group. Interesting in STZ+HFID group the diuresis and the urinary protein were higher than fructose fed animals, suggesting that the effects of fructose in the kidney function were potentiated by streptozotocin treatment.

Figure 1 shows the urinary lipid peroxidation (A) and urinary hydroperoxides (B) in the HFID and STZ+HFID groups in comparison to the values in the control group. No difference in urinary hydroperoxides was detected between the groups at baseline (0 time); however, there was a significant increase in urinary hydroperoxides in the HFID group (107.9 ± 31.9 versus 23.8 ±10.0 nM/mg of creatinine compared with the control) after 90 days ($p < 0.05$). This increase was related to the high-fructose diet, as indicated by the fact that when animals were fed a 2% fructose diet for 90 days (13.6 ± 1.6 nM/mg of creatinine), there was no increase in lipid peroxidation. No significant increase was observed in the STZ+HFID, although a tendency towards an increase was observed in lipid peroxidation (63.0 ± 21.7 nM/mg of creatinine) (Figure 1A).

This result was confirmed by quantification of urinary peroxides (Figure 1B). We observed a significant increase in urinary hydroperoxides in the HFID group (752 ± 190 versus 87.7 ± 29.8 nM/mg of creatinine compared with control group) after 90 days of

Figure 1. (A) Urinary hydroperoxides (nM/mg creatinine)(B) TBARS levels (nM/mg creatinine) in the control group (CTL) , HFID (high fructose intake diet) and rats treated with streptozotocin and high fructose induced diet (STZ+HFID) at 0 and 90 days.

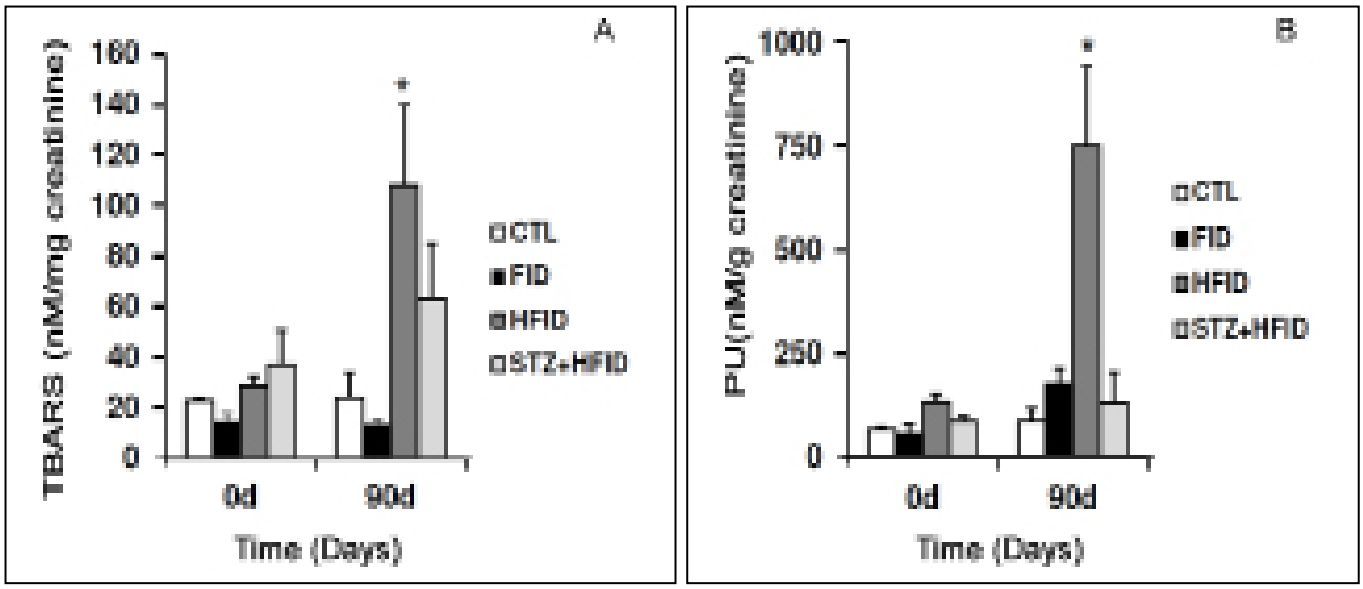
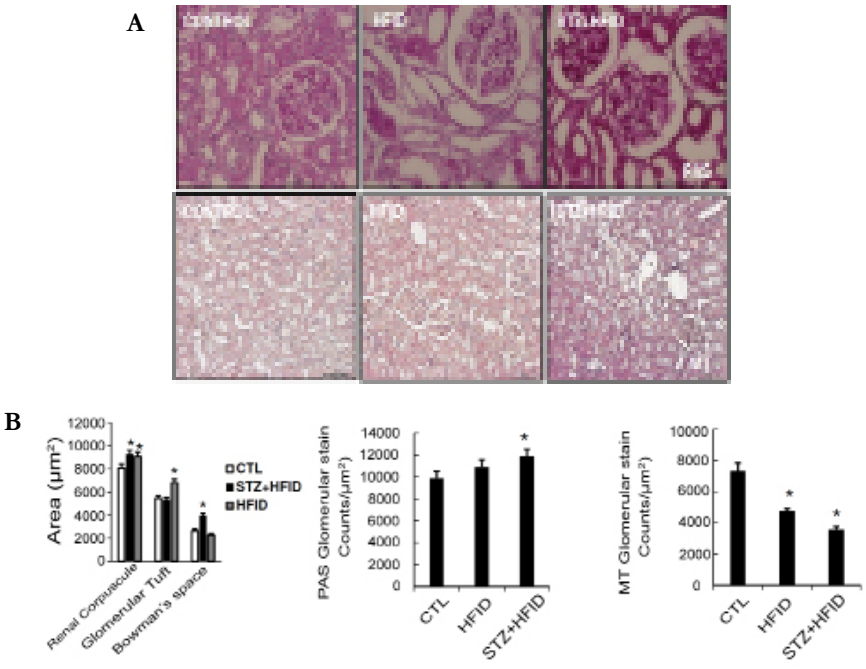


Figure 2. (A) Light microscopy of kidney sections stained with Periodic Acid Schiff (PAS) at 400× and Masson Trichrome stain (TM) at 200× in control rats (CTL), HFID (high fructose intake diet) and rats treated with streptozotocin and high fructose induced diet (STZ+HFID). (B) Quantification of glomerular tuft, glomerular area, and Bowman's space expressed in μm², PAS glomerular stain in counts/μm² and TM glomerular stain in counts/μm².



diet, but not in animals fed a 2% fructose diet (175.2 ± 39.1 nM/mg of creatinine) or in the STZ+HFID group (130.7 ± 73.7 nM/mg of creatinine).

Taken together these results suggest that a high-fructose diet significantly increased lipid peroxidation as a consequence of oxidative stress and the inhibition of antioxidant defenses, as reported previously [23, 24]. Nevertheless, low doses of streptozotocin together with a high fructose diet attenuates the increase in lipid peroxidation, although the consequences of this effect remain to be determined. One hypothesis is that fructose consumption results in ATP depletion, and the AMP generated enters into the purine degradation pathway and increases uric acid levels, which together with the fructose can result in oxidative stress [25]. In our experimental model, the increase in uric acid plasma concentration was delayed in animals treated with streptozotocin and fed with fructose in comparison to the group that only received the carbohydrate.

Figure 2 shows the results of morphometric analyses of kidney sections from HFID and STZ+HFID animals compared to those of the control group after 90 days of high-fructose diet. The renal corpuscle area was increased in both groups fed with fructose (HFID: 9062 ± 373 μm^2; STZ+HFID: 9248 ± 368 μm^2) in comparison to that in the control group (CTL: 8076 ± 328 μm^2) (Figure 2A).

Interestingly, in the HFID group, the glomerular tuft area (6802 ± 298 μm^2) was increased in comparison to that in the STZ+HFID group (5321 ± 209 μm^2) and the control group (5446 ± 230 μm^2). Nevertheless, Bowman's space was increased in the HFID+STZ (3927 ± 244 μm^2) in comparison to the control (2630 ± 163 μm^2) and HFID (2260 ± 158 μm^2) groups (Figure 2 A and B). This result suggests that in both experimental situations, the renal corpuscle was increased, although this increase was related to an increase in Bowman's space in the STZ+HFID group and to an increase in glomerular filtration area in the HFID group.

It is also important to point out that in the STZ+HFID group, there was a significant increase in PAS stain (11,912 ± 611 count/μm^2) in comparison to the control (9,889 ± 636 count/μm^2) and HFID (10,893 ± 669) groups, suggesting that the protein glucose residue increased in the glomerular filtration area (Figure 2 A and B). No increase in TM positive staining was observed in the experimental groups when compared to the control group; however, a significant decrease was observed in both experimental groups.

Taken together these results showed that animals treated with streptozotocin and fed a high fructose diet showed an increase in renal corpuscle area, basement membrane thickness, urine volume, and protein excretion, and a decrease in urinary sodium excretion, suggesting impairment in kidney function in animals treated with streptozotocin and fed with fructose. Although in animals fed with high fructose, the kidney analyses showed an increase in glomerular tuft area, a concomitant increase in protein excretion and urine volume also suggested an increase in glomerular filtration rate.

Fructose and glucose can glycate the glomerular basement membrane and increase its permeability [26]. Additionally, fructose itself can increase tubular sodium reabsorption [27], and this effect appeared to be potentiated in streptozotocin-treated and fructose-fed animals in comparison to the other groups analyzed.

Taken together, the results of the present study suggest that the association of a high fructose diet with low or mild pancreatic lesions promoted by low doses of streptozotocin accelerate, at least partially, the signs of metabolic syndrome. Additionally, our results provide new insights about the effects of prolonged high fructose intake combined or not with low doses of streptozotocin on the kidney.

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