High-fructose Diet Induces Earlier and More Severe Kidney Damage than High-fat Diet on Rats

Original Article

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ABSTRACT

Background: Several previous studies explored the toxic effect of high-fat diet (HFD) and high-fructose diet (HFrD), however little is known regarding their differential detrimental effects on the kidney.

Aim: This study was conducted to compare the biochemical, histological, and molecular changes in the kidney induced by consumption of HFD and HFrD for 4 and 8 weeks.

Materials and Methods: Thirty-five rats were randomly divided into five groups (n = 7/group): control, HFD1 (fed HFD for 4w), HFrD1 (for 4w), HFD2 (for 8w), and HFrD2 (for 8w). The studied parameters involved kidney function markers [urea, creatinine, and retinol-binding protein (RBP)] in the serum, histological examination using H&E stains, immunohistochemical examination of alpha-smooth muscle actin (αSMA), and expression of inflammation and glomerulosclerosis-related *genes* [tumor necrosis alpha (*TNFα*), interleukin 1β (*IL1β*), and nuclear factor kappa B (*NFκB*)], and necrosis-related *genes* [poly(ADP-ribose) polymerase-1 (*PARP1*) and receptor-interacting protein (*RIP1*)] in the kidney using real-time PCR.

Results: Earlier and more severe renal damage were noticed in rats fed HFrD than HFD-fed rats as evidenced by 1) significantly (p<0.05) higher levels of kidney function parameters (urea, creatinine, and RBP), 2) a higher kidney histopathological score (glomerulosclerosis, glomerular necrosis, Bowman's space dilation, coagulative necrosis, cloudy swelling, and fat droplets deposition in renal tubules, congestion and mononuclear cells infiltration in interstitium), 3) a significantly (p<0.01) higher positive αSMA staining in renal capillaries and some tubular cells, 4) a significantly (p<0.05) upregulated expression of (*TNFα, IL1β,* and *NFκB*), 5) a significantly (p<0.05) increased expression of (*PARP1* and *RIP1*). The highest damage order was noticed in HFrD2, followed by HFrD1 and then HFD2. No notable renal damage was observed in HFD1 as compared to the control group.

Conclusion: The findings of this study highlight the ability of HFrD to induce earlier and more severe renal damage than HFD.

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Key Words: Glomerulosclerosis; high-fat diet; high-fructose diet; kidney histopathological score; necrosis.

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INTRODUCTION

Obesity is a major health issue, affecting up to 30% of adults in the developed world and is related to increased dietary consumption of fat and sedentary lifestyles[1]. High obesity prevalence has severe global health and economic burden[2]. Obesity is related to metabolic syndrome (MS) that includes chronic conditions such as high blood pressure, heart and blood vessel diseases, and metabolic disorders including non-alcoholic fatty liver disease[3,4]. MS is also accompanied by diabetes type 2 (due to insulin insensitivity), hypercholesterolemia, and dyslipidemia[5]. Consumption of high-fat diets (HFD) is the main cause of both MS and obesity[6].

Fructose is the main element in several commonly used food products. Table sugar, soft and fruit drinks, and jams are the main dietary sources of fructose[7]. Consumption of high fructose diets (HFrD) can cause liver damage due to rapid fructose metabolism in the liver, which subsequently increases fat synthesis and deposition in this organ[8]. Similar to HFD, the consumption of HFrD also increases the prevalence of obesity and MS[5]. Furthermore, consumption of high carbohydrates, as in the case of HFrD, even with or without HFD, can lead to diabetes type 2[9]. The rise in MS patients worldwide has attracted the interest of investigators in the implementation of a lab animal model closely similar to humans. Animal models fed 40–60 % HFD and/or HFrD could induce MS model closely similar to that of humans[4,10].

Obesity and MS can also induce renal damage[11,12]. Abdominal obesity initiates kidney disease, even when there are no other obesity-related clinical signs such as high blood pressure and cholesterol, or pre-existing kidney disorders[13,14]. Thus, MS may promote kidney damage before the advent of hypertension and diabetes. Indeed, Amaral, *et al*.[13] and Tang, *et al*.[12] found that HFT induced very early histological changes (wider Bowman's space, elevated glomerular cell proliferation, and infiltration of mononuclear

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cells) and functional alterations (glomerular hyperfiltration and sodium retention) in rat kidney. Many other studies reported similar histological and functional disruption following administration of HFD to animals[13,15-20]. Consumption of HFrD also caused similar histological changes and dysfunction in rat kidney[21-27].

Although the actual mechanism by which HFD and HFrD could induce renal damage is not fully known, it was reported that inflammation may be the main determinant of this damage[25]. Consumption of these diets induced mononuclear cells, mostly macrophage, infiltration in renal interstitium which further produced inflammatory cytokines, such as TNFα, IL1β, and IL6[24,25,28]. Among these cytokines, TNF was shown to play a crucial role in the induction and progression of glomerulosclerosis in humans[29]. A recent microarray-based study by Otalora, *et al*.[30] screened for glomerulosclerosis specific markers in biopsied specimens of patients and found 15 podocyte-specific markers. Among these markers, TNFα and IL1β cytokines and the inflammation and immunity related *gene* NFkB showed higher expression. For this reason, these three *genes* were chosen as inflammation and glomerulosclerosis related *genes* in our study. Moreover, previous studies reported induction of necrosis in both glomeruli and tubular cells following consumption of HFD and HFrD[13,15-27]. However, this conclusion based only on the histological examination, and none of these previous studies confirm the occurrence of necrosis at a molecular level. This prompts us to detect the expression of two important necrosis-related *genes PARP1* and its downstream target *RIP1*[31] in kidneys of rats fed HFD and HFrD.

As previously mentioned, many studies investigated the nephrotoxic effect of HFD and HFrD, however little is known regarding the differential detrimental effects of both diets on the kidney. It is important to know which of these two diets can induce earlier and/or more severe renal damage to choose the most appropriate MS model for studying renal damage that could mimic human MS. Therefore, herein, we compared the detrimental effects of consumption of HFD and HFrD for 4 and 8 weeks on the kidney through investigation of kidney function parameters (creatine, urea, and RBP), histopathological damage score, αSMA positive immunostaining, and expression of some inflammatory and necrotic *genes*.

MATERIAL AND METHODS

The experimental protocol was approved by the Animal Ethics Committee of Kafrelsheikh University and following NIH guidelines on animal care.

Animals and experimental design

Adult male Wistar rats (n = 35, 205–235 g body weight) purchased from the National Research Center (Cairo, Egypt) were housed in polypropylene cages under standard environmental conditions (22-23 °C, 12-h light/dark cycle, and 62-70% humidity) with free access to food and water. After 1 week of acclimatization, the animals were randomly divided into five groups (n = 7/group); control, HFD1, HFrD1, HFD2, and HFrD2 group. Rats in the control group fed normal pellet diet (containing 26% protein, 60% carbohydrate, 5% fat, 8% fibers, and 1% vitamins and minerals with a total estimated energy of 3.15 kcal/g food). In HFD1 and HFD2 groups, rats fed HFD for 4w and 8w, respectively, while in HFrD1 and HFrD2 groups rats fed HFrD for 4w and 8w, respectively. HFD contained 15% fat [13% sheep tallow (obtained from a commercial source) and 2% cholesterol powder and Tween 80% (El- Gomhouria Co. for Trading Chemicals and Medical Appliances, Cairo, Egypt)], 21% protein, 60% carbohydrate, 3% fibers, and 1% vitamins and minerals with a total calory of 5.3 kcal/ g][32]. HFrD was the same as the normal pellet diet but with 25% fructose (Sigma, USA) in drinking water[33].

Sampling

At the end of the experiment, the rats were anesthetized by 500 µL of Ketamine-Xylazine intraperitoneal injection (100 and 20 mg/kg body weight, respectively). Blood samples were collected from venous plexus at the medial canthus of the eye in plain tubes to separate serum as previously described[34]. After euthanization, both kidneys were dissected immediately and rinsed with saline to get rid of any blood spots. Specimens from each kidney were either fixed in 10% neutral buffered formalin (for histology) or stored in -80°C freezer (for real-time PCR).

Biochemical assays

Kidney function markers including creatinine and urea were determined in serum using commercially available kits and following manufacturer's instruction (Diamond Diagnostics Company, Egypt). Retinol-binding protein (RBP) levels were also determined in sera using an Elisa kit following the manufacturer's protocol (LSBio, Inc., USA, # LS-F28065).

Histological examination

Following fixation in 10% neutral buffered formalin, tissue specimens were dehydrated through alcohols, cleared in xylene, and then embedded in paraffin wax. Sections (5µm) were stained with hematoxylin and eosin[35]. Five slides from each kidney of five animals per group were analyzed.

Renal histopathological score

Renal histopathological score was determined in 10 randomly chosen, non-overlapping fields (at a magnification of X400). This score involved some histopathological changes in the renal corpuscle (glomerulosclerosis, glomerular necrosis, Bowman's space dilation), in renal tubules (dilation, coagulative necrosis, cloudy swelling, and fat droplets deposition), and in the interstitium (congestion and mononuclear cells infiltration). The score grades were - (no lesion), + (mild damage), ++ (moderate damage), and +++ (high damage)[36].

Immunohistochemistry

Immunostaining of tissue sections was carried out as previously detailed[37,38]. Briefly, deparaffinized kidney sections were first treated with 2% H_2O_2 in humidified boxes (to block endogenous peroxidase activity), then with 5% fetal calf serum (to block nonspecific binding). Sections were then incubated with anti-alpha smooth muscle actin (αSMA) primary mouse monoclonal antibody (1:200 dilution, DakoCytomation, Glostrup, Denmark) overnight at 4 °C. Afterward, sections were incubated with secondary biotinylated mouse antibodies (1:1000, Dako, CA, # K4003) for 1 h at room temperature. The color was developed by incubating tissue sections with streptavidin-biotin peroxidase conjugate (1:100, Vector Laboratories, Burlingame, CA) for 1h followed by diaminobenzidine chromogen. Before examination with a Leica BM5000 microscope, all slides were counterstained with Harris 's modified hematoxylin. The positive area percentage of αSMA was determined using an image analyzer (Leica Q 500 DMLB, Leica, UK). For each kidney specimen, a total of 30 different non-overlapping was examined at magnifications of X400. Ten specimens were examined in each group.

Real-time PCR

The relative expression of inflammation and glomerulosclerosis related *genes* (*TNFα*, *IL1β*, and *NFκB*) and necrosis-related *genes* (*PARP1* and *RIP1*) in the kidneys was determined using real-time PCR (qPCR). β actin was utilized as an internal reference (housekeeping) *gene*. RNA was extracted from kidney tissue using the RNeasy Mini kit (Qiagen, #74104) following the manufacturer's protocol. RNA samples were quantified by Nanodrop (Quawell 5000, USA) and reverse transcribed into cDNA by Quantiscript reverse transcriptase (Qiagen, #205310) as previously described[39]. The primers were designed by the Primer 3 web-based tool based on the published rat sequences (Table 1). The qPCR was done using QuantiTect SYBR Green Master Mix with reaction setup, and melting curve condition as previously described[40]. Fold change of *gene* expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Table 1: Primers used for real-time PCR

Gene	Forward primer (5'……3')	Reverse primer (5'……3')
TNFα	GCATGATCCGCGACGTGGAA	AGATCCATGCCGTTGGCCAG
IL1β	CACCTCTCAAGCAGAGCACAG	GGGTTCCATGGTGAAGTCAAC
NFκB	CCTAGCTTTCTCTGAACTGCAAA	GGGTCAGAGGCCAATAGAGA
PARP1	ACGCACAATGCCTATGAC	CCAGCGGAACCTCTACAC
RIP1	AGGTACAGGAGTTTGGTATGGGC	GGTGGTGCCAAGGAGATGTATG
β actin	TACAACCTCCTTGCAGCTCC	GGATCTTCATGAGGTAGTCAGTC

Statistical analysis

One-way Analysis of Variance followed by Duncan's multiple range test was used to compare between effect of different treatments on analyzed parameters using Graph Pad Prism 5 software (San Diego, CA, USA). Data were presented as mean ± standard error of mean (SEM) and significance was set at $p<0.05$.

RESULT

H & E results

Kidneys of rats in the control group had normal histology of glomeruli (G), Bowman's capsule (arrow), the proximal (P), and distal (D) convoluted tubules (Figure 1A). The proximal tubule (P) had a small lumen and a single layer of cuboidal cells with eosinophilic, granular cytoplasm. The distal convoluted tubules (D) were lined with cuboidal cells with rounded large nuclei (Figure 1A).

The kidney of HFD-fed rats for 4 weeks (HFD1) showed slight histological changes (Figure1B). Bowman's space was slightly enlarged (double arrow), lumens of some proximal convoluted tubules had detached cells (arrows), and lumen of some distal tubule was enlarged (arrowheads). However, in the HFrD1 group the histological alterations were more pronounced (Figure 1C1-4) as compared to the HFD1 group. As shown in Fig.1C1, there were numerous dilated renal tubules (red arrows), multiple foci of mononuclear cells aggregation in the interstitial tissue (black arrows) around congested blood capillaries (stars). (Figure 1C2) shows tubular hyaline casts, early coagulative necrosis of some renal tubules (arrows), and numerous dilated distal tubules (stars). As revealed from (Figure 1C3), a large amount of white adipose tissues was subcapsularly accumulated (stars). (Figure 1C4) shows glomerular necrosis and enlarged Bowman's space with notable glomerulosclerosis in some glomeruli (arrows).

Kidneys of rats in the HFD2 group showed cloudy swelling in some renal tubules with numerous tubular dilatation (stars), and mononuclear cells infiltration in the interstitium (double arrows, Figure 1D1). Glomerular sclerosis with enlarged bowman`s capsule was clearly noticed in some renal corpuscles (arrows, Figure 1D1, 2). Some lipid droplets were found in the distal tubules (arrowheads, Figure 1D2). However, Kidneys of HFrD2 showed severe glomerular sclerosis as revealed by highly collapsed glomeruli with very wide bowman`s capsule (arrows, Figure 1E1, 2). Also, numerous renal tubules suffered from cloudy swelling and coagulative necrosis with mononuclear cell infiltration in the interstitium (stars, Figure 1E1, 2). Many lipid droplets appeared in some renal tubules (black arrows, Figure 1E1).

The effect of HFD and HFrD on the kidney damage score was summarized in (Table 3). Based on data presented in this table, HFrD-fed rats showed earlier and more severe renal histological damage than HFD-fed rats and this was compatible with results of renal damage parameters.

Immunohistochemical results

The positive αSMA immunostaining area was only recognized in the nuclei of smooth muscle of interstitial blood capillaries in control and HFD1 groups (black arrowheads, Figure 2A, B). However, in other groups (HFD2, HFrD1, HFrD2) this positive area was also extended to involve tubular cells (red arrowheads) along with different size blood vessels (black arrowheads, Figure 2C-E). Additionally, the % of αSMA positive area was significantly ($p < 0.05$) higher in HFrD2 group (1.93 ± 0.10), followed by HFrD1 (1.25 ± 0.08), and HFD2 (1.00 ± 0.07) group than in HFD1 (0.62 ± 0.02) and control group (0.51 ± 0.01). However, no significant ($p > 0.05$) difference was observed between HFD1 and the control group.

Biochemical results

Kidney function parameters (urea, creatinine, and retinol-binding protein) levels were significantly ($P< 0.05$) higher in sera of all treated groups, except HFD1 which showed an insignificant increase as compared to the control group (Table 2).

Gene expression results

Real-time PCR (qPCR) was applied to determine changes in the relative expression of inflammation and glomerulosclerosis related *genes* (*TNFα*, *IL1β,* and *NFκB*) and necrosis-related *genes* (*PARP1* and *RIP1*) in the kidneys following different treatments. The obtained qPCR data reveled a significant increase in the expression of these five *genes* in the kidneys of rats in HFrD1, HFD2, and HFrD2 groups as compared to HFD1 and control groups (Figure 3). The highest significant expression was noticed in HFrD2, followed by HFrD1 and then HFD2 group. However, no significant difference was found between HFD1 and control groups.

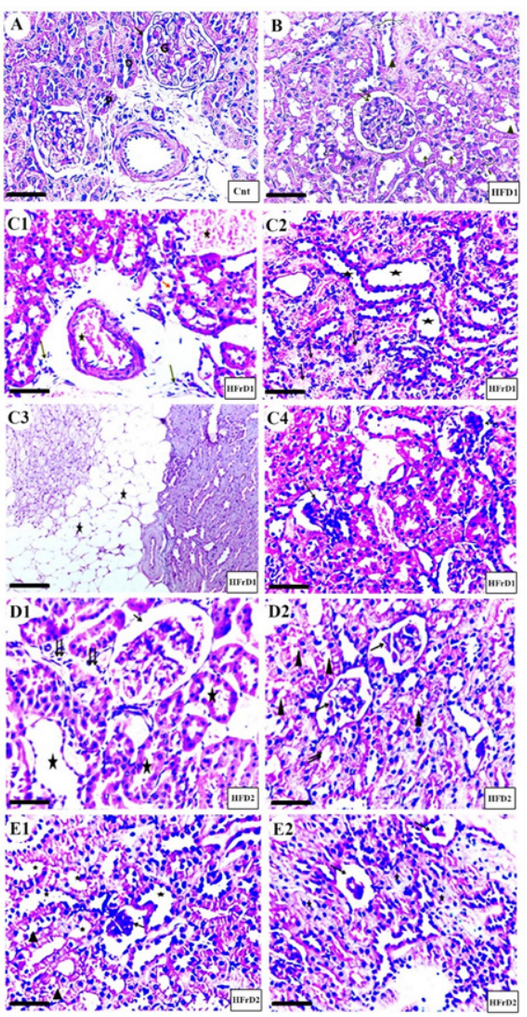

Fig. 1: Photomicrographs of rat renal cortex sections stained with H&E in different experimental groups. Scale bars = 40 µm (in A-C2, C4, D2-E2), 30 40 µm (in C3), and 45 µm (in D1).

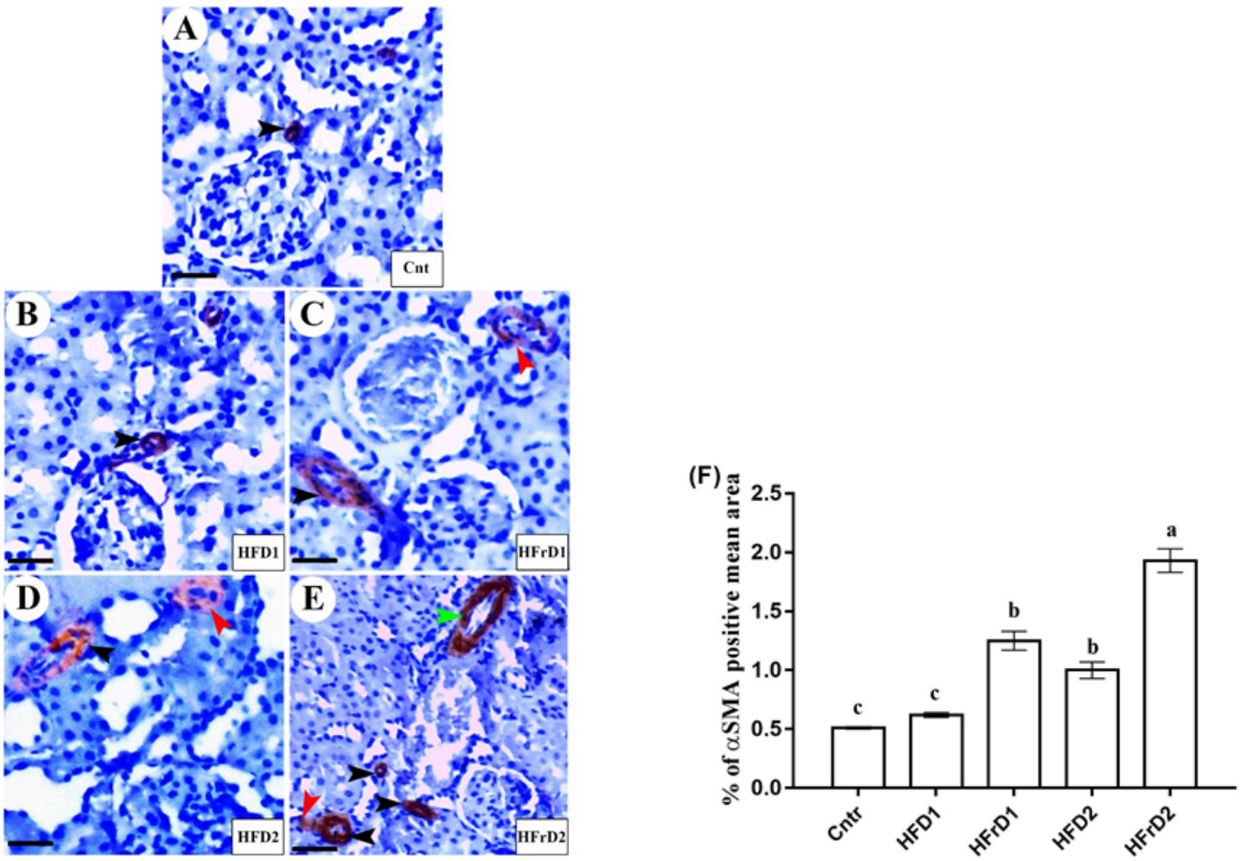

Fig. 2: (A-E) Photomicrographs of rat renal cortex sections immune-stained with anti-αSMA primary antibody in different experimental groups. Scale bars = 50 µm in all images except (E) which is 40 µm. (F) Quantification of αSMA positive immune-stained area percentage. Data were presented as mean ± standard error of mean (n = 5/group). Columns with different lower-case letters [a (highest values) – c (lowest value)] are significantly different at $p < 0.01$.

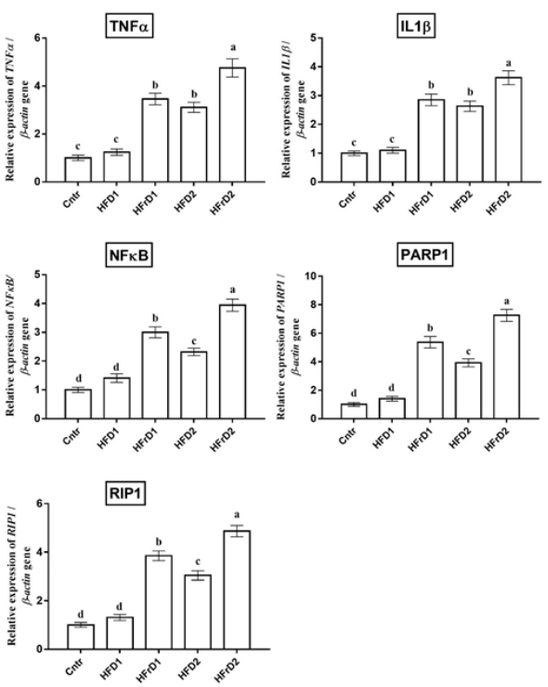

Fig. 3: Relative expression of *TNFα, IL1β, NFκB, PARP1*, and *RIP1* genes in kidneys as detected by qPCR. Gene expression was presented as fold change mean ± standard error of mean (n = 5/group). Values (columns) with different superscript letters [a (highest value) – d (lowest value)] are significantly different ($p \leq 0.05$).

Table 2: Effect of HFD and HFrD on kidney function markers in rats

Animal groups	Urea (mg/dl)	Creatinine (mg/dl)	Retinol binding protein (ng/ml)
Control	18.46±1.05[d]	1.21±0.03[d]	36.19±1.84[d]
HFD1	20.37±1.25[d]	1.34±0.03[d]	38.60±1.71[d]
HFrD1	41.08±2.63[b]	1.95±0.06[b]	90.28±5.36[b]
HFD2	29.82±1.49[c]	1.62±0.05[c]	74.36±4.02[c]
HFrD2	48.11±2.90[a]	2.23±0.07[a]	112.30±6.20[a]

Data are presented as mean ± SEM (n = 7/group). Mean values with different superscript letters [a (the highest values) – d (the lowest value)] in the same column are significantly different at ($p \leq 0.05$).

Table 3: Effect of HFD and HFrD on kidney histopathological score

Site	Lesion	Cnt	HFD1	HFrD1	HFD2	HFrD2
Renal corpuscle	Glomerulosclerosis	-	-	++	++	+++
	Glomerular necrosis	-	-	++	++	+++
	Bowman's space dilation	-	+	++	++	+++
Renal tubules	Dilated tubules	-	+	++	++	++
	Coagulative necrosis	-	-	+	++	+++
	Cloudy swelling	-	-	+	++	+++
	Fat droplets	-	-	-	++	++
Interstitium	Congestion	-	-	++	++	++
	Mononuclear cells infiltration	-	-	++	++	+++

Score: - no lesion, + mild, ++ moderate, and +++ high

DISCUSSION

A large body of published data studied the detrimental effects of HFD and/or HFrD on many organs including liver and kidney. However, little is known regarding their differential detrimental effects on the kidney.

HFD1 group of the present study showed no clear kidney damage relative to the control group. They only showed slight histological changes in form dilated Bowman's space and convoluted tubules. It is possible that these mild initial histological changes do not induce pronounced alterations in renal functionality as revealed by non-significant changes in urea and creatinine serum levels, αSMA positive immune-stained area, and mRNA levels of TNFα, IL1β, NFκB, PARP1, and RIP1 as compared to the control group. In contrast to our findings, Salim, *et al*.[41] reported earlier histological changes (including glomerulosclerosis, tubular necrosis, and mononuclear cell infiltration) in kidneys of rats fed HFD for only 2w. These contradictory results may be attributed to the type of fat used, total calories received, and method of administration. Indeed, Salim, *et al*.[41] fed rats 2ml cow fat emulsion by oral gavage but no information was provided regarding the total calories received by this diet.

Animals fed HFD for longer time (for 8w, HDF2 group) exhibited clear renal damage as revealed by significant elevated urea and creatinine levels, increased renal histopathological score, αSMA positive immune-staining area, and upregulated expression of *TNFα, IL1β, NFκB, PARP1*, and *RIP1* genes. The structural changes in the HFD2 group included glomerular sclerosis with enlarged bowman`s capsule, tubular cloudy swelling, lipid droplets deposition in tubular epithelial cells, and pro-inflammatory mononuclear cells infiltration in the interstitium. Similar histological changes in kidneys were also reported by several other studies following feeding rodents on HFD for ≥ 8w[13,15-20]. Interestingly, Karam, *et al*.[42] only found lipid infiltration in the renal tubules without any other structural changes in kidney of adult male rat fed HFD, similar in composition to that used in our study, for 7w. In support, Crinigan, *et al*.[43] also did not find any biochemical (kidney function parameters and inflammatory markers), or structural changes in kidneys of young male rats fed HFD (60% fat/kcal) for 6w, though these animals had higher body mass with higher deposition of fat in their viscera. Based on Crinigan, *et al*.[43] findings, using a higher fat content diet above that used in our study did not exert any notable structural changes. Searching the literature, we did not find any other studies investigating the effect of HFD on kidney following feeding schedule from 4w to 8w in male rats.

The result of HFrD group in the present study should sever renal damage that was confirmed by biochemical (urea, creatinine, and RBP), structural (histology and immunohistochemistry), and molecular (*genes*) levels. Elevated serum levels of urea and creatinine after HFrD feeding for 4-5w were also reported in other studies[24,27,44-50]. The structural alterations induced by feeding HFrD for 4w were similar to those induced by HFD for 8w (glomerulosclerosis, glomerular necrosis, dilated Bowman's space, aggregation of inflammatory mononuclear cells in the interstitium, congested blood capillaries, coagulative necrosis and dilation of renal tubules) but with less pronounced cloudy swelling and clear hyaline cast within the lumen of tubules and in the interstitium. Although a large amount of fat was accumulated underneath the renal capsule, no fat droplets

deposited in tubular cells during this stage. However, feeding HFrD for 8w resulted in more remarkable structural changes with notable renal function disruption as compared to all other groups. In general, these structural changes could decrease kidney function leading to lower glomerular filtration rate and higher urea and creatinine levels in the blood[51]. This could illuminate the higher urea and creatinine serum levels in HFrD groups which also showed prominent kidney damage. Similar histological changes were also observed by other studies following feeding rats HFrD for 4w[26,27], 5w[24,25], 6w[23], and 8w[21,22]. However, unlike Saleh, *et al*.[27] and Yang, *et al*.[25], who found interstitial fibrosis, we did not find interstitial renal fibrosis in all examined sections stained with H&E. Therefore, Masson's Trichrome, as a specific stain for connective tissue, is recommended to precisely detect renal fibrosis. On the other hand, de Castro, *et al*.[46]found similar histological changes, but with non-significant changes in urea and creatinine in the kidneys of adult rats fed HFrD for 13w.

The deposition of fat droplets in epithelial tubular cells, as seen in kidneys of HFD2 and HFrD2 groups, can alter the metabolism of these cells and trigger release of free radicals and pro-inflammatory cytokines, thereby leading to inflammatory-oxidative stress-induced renal damage, which also called lipotoxicity[52-54]. The most two cellular structures affected by lipotoxicity are the cell membrane and mitochondria which their damage induces apoptosis and necrosis in renal cells[13].

Kidneys of rats in HFD2, HFrD1, and HFrD2 groups of the present study showed a significantly increased αSMA, a marker for myofibroblasts, positive staining area as compared to other groups. In agreement, other studies also reported higher αSMA positive areas in the kidneys of rats fed HFrD[23,24,55]. Abdel-Kawi, *et al*.[24] found localization of αSMA positive staining in the wall of blood capillaries and tubular cells but failed to detect the expression in the interstitium as detected by Nakayama, *et al*.[23]. Higher αSMA expression, but less than that in HFrD groups, was also noticed in kidneys of rats fed HFD for 8w (HFD2) as compared to HFD1 and control groups. Localization of αSMA in the HFD2 group was similar to that observed in HFrD groups. However, unlike the results obtained by Wei, *et al*.[56], we did not find any positive staining in the glomeruli.

Renal inflammation is crucial for the induction and progression of kidney damage[25]. In the present study, kidneys of rats in HFD2, HFrD1, and HFrD2 group exhibited significantly increased mRNA levels of inflammation and glomerulosclerosis related *genes* (*TNFα, IL1β,* and *NFκB*), with a higher expression in HFrD2, followed by HFrD1 and HFD2, as compared to control and HFD1 groups. These results agreed with the histological results which showed a clear lesion of inflammation, such as infiltration of mononuclear cells, mostly macrophages, in the interstitium around congested blood vessels. Consistent with these results, other studies also reported that consumption of HFrD triggered renal inflammation through induction of mononuclear cells aggregation and subsequently overproduction of inflammatory cytokines[24,25,28]. Fructose can trigger the expression of macrophage-activator MCP-1 in tubular cells, which further recruits many macrophages to the kidney[57]. These macrophages produce many cytokines, particularly TNFα and IL16 which initiate inflammatory cascade[58]. A recent study by Otalora, *et al*.[30] specified TNFα, IL1β, and NFkB as three important markers for the diagnosis and prognosis of glomerulosclerosis in patients.

Among the four treated groups, rats fed HFrD exhibited significantly ($P< 0.05$) higher kidney function parameters than HFD-fed animals at the two timepoints, 4w and 8w. Comparing the two timepoints, all kidney function parameters were significantly ($P< 0.05$) higher at 8w (HFD2, HFrD2) than 4w (HFD1, HFrD1). These results indicate earlier renal damage induced by the consumption of HFrD as compared to HFD.

CONCLUSIONS

The present study concluded that consumption of a diet rich in fructose caused earlier and more severe kidney damage (as indicated by higher urea, creatinine, and RBP levels, histological damage score, αSMA positive staining, and inflammatory and necrotic *gene* expression) than consumption of fat-rich diet. Therefore, a high fructose diet could be effectively used as a short-lasting metabolic syndrome model in adult male rats to study renal damage better than using high-fat diet.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Kramer H, Luke A. Obesity and kidney disease: A big dilemma. Curr Opin Nephrol Hypertens 2007;16:237-241.
2. Withrow D, Alter DA. The economic burden of obesity worldwide: A systematic review of the direct costs of obesity. Obesity reviews 2011;12:131-141.
3. Grundy SM. Obesity, metabolic syndrome, and cardiovascular disease. The Journal of Clinical Endocrinology & Metabolism 2004;89:2595-2600.
4. Zaki SM, Fattah SA, Hassan DS. The differential effects of high-fat and high- -fructose diets on the liver of male albino rat and the proposed underlying mechanisms. Folia morphologica 2019;78:124-136.
5. Bocarsly ME, Powell ES, Avena NM, Hoebel BG. High-fructose corn syrup causes characteristics of obesity in rats: Increased body weight, body fat and triglyceride levels. Pharmacology, biochemistry, and behavior 2010;97:101-106.
6. Auberval N, Dal S, Bietiger W, *et al*. Metabolic and oxidative stress markers in wistar rats after 2 months on a high-fat diet. Diabetology & metabolic syndrome 2014;6:130.

7. Bantle JP. Is fructose the optimal low glycemic index sweetener? In: Nutritional management of diabetes mellitus and dysmetabolic syndrome, Karger Publishers, 2006, pp 83-95.
8. Ouyang X, Cirillo P, Sautin Y, *et al*. Fructose consumption as a risk factor for non-alcoholic fatty liver disease. Journal of hepatology 2008;48:993-999.
9. Pereira-Lancha LO, Campos-Ferraz PL, Lancha AH, Jr. Obesity: Considerations about etiology, metabolism, and the use of experimental models. Diabetes, metabolic syndrome and obesity : targets and therapy 2012;5:75-87.
10. Collison KS, Zaidi MZ, Saleh SM, *et al*. Effect of trans-fat, fructose and monosodium glutamate feeding on feline weight gain, adiposity, insulin sensitivity, adipokine and lipid profile. The British journal of nutrition 2011;106:218-226.
11. Abrass CK. Overview: Obesity: What does it have to do with kidney disease? Journal of the American Society of Nephrology 2004;15:2768-2772.
12. Tang J, Yan H, Zhuang S. Inflammation and oxidative stress in obesity-related glomerulopathy. International journal of nephrology 2012;2012.
13. Amaral LS, Silva JA, Trindade TM, *et al*. Renal changes in the early stages of diet-induced obesity in ovariectomized rats. Physiological research 2014;63.
14. Fujita T. Aldosterone in salt-sensitive hypertension and metabolic syndrome. Journal of Molecular Medicine 2008;86:729.
15. Handa K, Kreiger N. Diet patterns and the risk of renal cell carcinoma. Public health nutrition 2002;5:757-767.
16. Jiang T, Wang Z, Proctor G, *et al*. Diet-induced obesity in c57bl/6j mice causes increased renal lipid accumulation and glomerulosclerosis via a sterol regulatory element-binding protein-1c-dependent pathway. Journal of biological chemistry 2005;280:32317-32325.
17. Altunkaynak ME, Özbek E, Altunkaynak BZ, Can İ, Unal D, Unal B. The effects of high-fat diet on the renal structure and morphometric parametric of kidneys in rats. Journal of anatomy 2008;212:845-852.
18. Danilewicz M, Wagrowska-Danielwicz M. Morphometric and immunohistochemical insight into focal segmental glomerulosclerosis in obese and non-obese patients. Nefrologia 2009;29:35-41.
19. Jackson CM, Alexander BT, Roach L, *et al*. Exposure to maternal overnutrition and a high-fat diet during early postnatal development increases susceptibility to renal and metabolic injury later in life. American Journal of Physiology-Renal Physiology 2012;302:F774-F783.
20. Esquinas P, Rios R, Raya AI, *et al*. Structural and ultrastructural renal lesions in rats fed high-fat and high-phosphorus diets. Clinical Kidney Journal, sfaa009, https://doiorg/101093/ckj/sfaa009 2020.
21. Li X, Zhang L, Yang Y, *et al*. Histopathology and urinary metabonomics reveal the role of dietary salt on the pathogenesis of fructose-induced kidney injury. Int J Clin Exp Med 2018;11:6662-6673.
22. Sivakumar AS, Viswanathan P, Anuradha CV. Dose-dependent effect of galangin on fructose-mediated insulin resistance and oxidative events in rat kidney. Redox Report 2010;15:224-232.
23. Nakayama T, Kosugi T, Gersch M, *et al*. Dietary fructose causes tubulointerstitial injury in the normal rat kidney. American Journal of Physiology-Renal Physiology 2010;298:F712-F720.
24. Abdel-Kawi SH, Hassanin KM, Hashem KS. The effect of high dietary fructose on the kidney of adult albino rats and the role of curcumin supplementation: A biochemical and histological study. Beni-suef university journal of basic and applied sciences 2016;5:52-60.
25. Yang M, Liu C, Jiang J, *et al*. Ginger extract diminishes chronic fructose consumption-induced kidney injury through suppression of renal overexpression of proinflammatory cytokines in rats. BMC complementary and alternative medicine 2014;14:174.
26. Okasha EF. Effect of high-fructose diet on the renal cortex of adult male albino rats: Histological and immunohistochemical study. Egyptian Journal of Histology 2011;34:639-649.
27. Saleh R, Merghani BH, Awadin W. Effect of high fructose administration on histopathology of kidney, heart and aorta of rats. Journal of Advanced Veterinary and Animal Research 2017;4:71-79.
28. Glushakova O, Kosugi T, Roncal C, *et al*. Fructose induces the inflammatory molecule icam-1 in endothelial cells. Journal of the American Society of Nephrology 2008;19:1712-1720.
29. Pedigo CE, Ducasa GM, Leclercq F, *et al*. Local tnf causes nfatc1-dependent cholesterol-mediated podocyte injury. The Journal of clinical investigation 2016;126:3336-3350.
30. Otalora L, Chavez E, Watford D, *et al*. Identification of glomerular and podocyte-specific *genes* and pathways activated by sera of patients with focal segmental glomerulosclerosis. PLoS One 2019;14:e0222948.
31. Elkeiy M, Khamis A, El-Gamal M, Abo Gazia M, Zalat Z, El-Magd M. Chitosan nanoparticles from artemia salina inhibit progression of hepatocellular carcinoma in vitro and in vivo. Environ Sci Pollut Res Int 2018;27:19016-19028.
32. Attia RT, Abdel-Mottaleb Y, Abdallah DM, El-Abhar HS, El-Maraghy NN. Raspberry ketone and garcinia cambogia rebalanced disrupted insulin resistance and leptin signaling in rats fed high fat fructose diet. Biomedicine & Pharmacotherapy 2019;110:500-509.

33. Lozano I, Van der Werf R, Bietiger W, *et al*. High-fructose and high-fat diet-induced disorders in rats: Impact on diabetes risk, hepatic and vascular complications. Nutrition & metabolism 2016;13:15.
34. Abdel-Aleem GA, Shafik NM, El-Magd MA, Mohamed DA. Soya bean rich diet is associated with adult male rat aggressive behavior: Relation to rf amide-related peptide 3-aromatase-neuroestrogen pathway in the brain. Metabolic Brain Disease 2019;34:1103-1115.
35. Abu Gazia M, El-Magd MA. Ameliorative effect of cardamom aqueous extract on doxorubicin-induced cardiotoxicity in rats. Cells, tissues, organs 2018;206:62-72.
36. Chang CL, Sung PH, Chen KH, *et al*. Adipose-derived mesenchymal stem cell-derived exosomes alleviate overwhelming systemic inflammatory reaction and organ damage and improve outcome in rat sepsis syndrome. American journal of translational research 2018;10:1053-1070.
37. Abu Gazia M, El-Magd MA. Effect of pristine and functionalized multiwalled carbon nanotubes on rat renal cortex. Acta Histochemica 2018;121(2)::207-217.
38. Helal MB, Labah DA, El-Magd MA, Sarhan NH, Nagy NB. Thyroidectomy induces thyroglobulin formation by parotid salivary glands in rats. Acta Histochemica 2020;122:151568.
39. Abdelhady D, El-Abasy M, Abou-Asa S, *et al*. The ameliorative effect of aspergillus awamori on aflatoxin b1-induced hepatic damage in rabbits. World Mycotoxin Journal 2017;10:363-373.
40. El-Magd MA, Khamis A, Nasr Eldeen SK, Ibrahim WM, Salama AF. Trehalose enhances the antitumor potential of methotrexate against mice bearing ehrlich ascites carcinoma. Biomedicine & Pharmacotherapy 2017;92:870-878.
41. Salim HM, Kurnia LF, Bintarti TW, Handayani H. The effects of high-fat diet on histological changes of kidneys in rats. Biomolecular and Health Science Journal 2018;1:109-112.
42. Karam I, Ma N, Yang Y, Li J. Induce hyperlipidemia in rats using high fat diet investigating blood lipid and histopathology. J Hematol Blood Disord 2018;4:104.
43. Crinigan C, Calhoun M, Sweazea KL. Short-term high fat intake does not significantly alter markers of renal function or inflammation in young male sprague-dawley rats. Journal of nutrition and metabolism 2015;2015.
44. Amin KA, Sana'a RG, Wala'a GH, Shima'a MR. Effects of orlistat and herbal mixture extract on renal function and oxidative stress biomarkers in a rat model of high fat diet. International Journal of Biochemistry Research & Review 2014;4:173.
45. Yurt KK, Kayhan E, Altunkaynak BZ, Tümentemur G, Kaplan S. Effects of the melatonin on the kidney of high fat diet fed obese rats: A stereological and histological approach. J Exp Clin Med 2013;30:153-158.
46. de Castro UGM, Silva ME, de Lima WG, Campagnole-Santos MJ, Alzamora AC. Age-dependent effect of high-fructose and high-fat diets on lipid metabolism and lipid accumulation in liver and kidney of rats. Lipids in health and disease 2013;12:136.
47. Cha Y, Huang X, Liu J, *et al*. Protective effects of berberine on high fat-induced kidney damage by increasing serum adiponectin and promoting insulin sensitivity. International journal of clinical and experimental pathology 2015;8:14486.
48. Roza NA, Possignolo LF, Palanch AC, Gontijo JA. Effect of long-term high-fat diet intake on peripheral insulin sensibility, blood pressure, and renal function in female rats. Food & nutrition research 2016;60:28536.
49. Estrany ME, Proenza AM, Gianotti M, Lladó I. High-fat diet feeding induces sex-dependent changes in inflammatory and insulin sensitivity profiles of rat adipose tissue. Cell biochemistry and function 2013;31:504-510.
50. Ali M, Abd El-Megeid A, Mostafa R. Effect of some levels from ginseng, barley and carob on lipid profile and kidney functions of rats fed on high fructose diets. Journal of American Science 2012;8:152-162.
51. Kim S, Choi JE, Choi J, *et al*. Oxidative stress-dependent toxicity of silver nanoparticles in human hepatoma cells. Toxicology in vitro : an international journal published in association with BIBRA 2009;23:1076-1084.
52. Sasson AN, Cherney DZ. Renal hyperfiltration related to diabetes mellitus and obesity in human disease. World journal of diabetes 2012;3:1.
53. Cherney DZ, Scholey JW, Miller J. Insights into the regulation of renal hemodynamic function in diabetic mellitus. Current diabetes reviews 2008;4:280-290.
54. Balarini CM, Oliveira MZ, Pereira TM, *et al*. Hypercholesterolemia promotes early renal dysfunction in apolipoprotein e-deficient mice. Lipids Health Dis 2011;10:220.
55. Marotta F, Kumari A, Catanzaro R, *et al*. A phytochemical approach to experimental metabolic syndrome-associated renal damage and oxidative stress. Rejuvenation Research 2012;15:153-156.
56. Wei P, Lane P, Lane J, Padanilam BJ, Sansom SC. Glomerular structural and functional changes in a high-fat diet mouse model of early-stage type 2 diabetes. Diabetologia 2004;47:1541-1549.
57. Cirillo P, Gersch MS, Mu W, *et al*. Ketohexokinase-dependent metabolism of fructose induces proinflammatory mediators in proximal tubular cells. Journal of the American Society of Nephrology : JASN 2009;20:545-553.
58. Galkina E, Ley K. Leukocyte recruitment and vascular injury in diabetic nephropathy. Journal of the American Society of Nephrology : JASN 2006;17:368-377.

الملخص العربى

النظام الغذائي عالي الفركتوز يحدث اضرار مبكرة واكثر شدة من النظام الغذائي عالي الدهون فى كلي الجرذان

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الخلفية: اوضحت العديد من الدراسات السابقة التأثير السام للنظام الغذائي عالي الدهون والنظام الغذائي عالي الفركتوز ولكن لا يعرف الا القليل عن مقارنة آثارهما الضارة على الكلى.

الهدف: هدفت هذه الدراسة الى مقارنة التغيرات الكيميائية الحيوية والهستولوجية والجزيئية في الكلى والناجمة عن اتباع نظام غذائي عالي الدهون واخر عالي بالفركتوز لمدة أربع وثمانية أسابيع.

المواد والطرق: استخدم خمسة وثلاثون جرذا فى هذه الدراسه وتم تقسيمهم الى خمس مجموعات كل مجموعة تحتوي على سبع فئران وهم المجموعة الأولى الضابطة والمجموعة الثانية تم تغذيتها على وجبات عالية الدهون لمدة اربع أسابيع والمجموعة الثالثة تم تغذيتها على وجبات بها نسبة عالية من الفركتوز لمدة اربع أسابيع بينما المجموعة الرابعة تم تغذيتها على وجبات عالية الدهون لمدة ثمانية أسابيع والمجموعة الخامسة تم تغذيتها على وجبات بها نسبة عالية من الفركتوز لمدة ثمانية أسابيع وتم قياس تركيز اليوريا والكرياتينين والبروتين المرتبط بالريتينول في الدم وفحص تركيب الكلي النسيجى والتعبير النسيجى الكيميائى المناعى لاكتين العضلات الملساء-الفا وتعبير جينات الالتهابات والتصلب والتنخر الكلوى عن طريق تفاعل البلمرة المتسلسل حقيقى الوقت.

النتائج: لوحظ تلف كلوي مبكر وأكثر شده في الفئران التي تغذت على وجبات بها نسبة عالية من الفركتوزمقارنة بالفئران التي تغذت على وجبات عالية الدهون واتضح ذلك من خلال ارتفاع دلالات وظائف الكلى (اليوريا والكرياتينين والبروتين المرتبط بالريتينول) في الدم بها وظهور نسبة عالية من التغيرات النسيجية الخلوية والنسيجيه الكيميائيه المناعيه في الكلي وزيادة التعبير للجنيات المتعلقة بالالتهابات والتصلب والتنخر الكلوى. وتم ترتيب المجموعات من حيث الأعلى في نسبة التلف كالتالي: الجرذان التي تم تغذيتها على وجبات عالية الفركتوز لمدة ثمانية أسابيع كانت اعلى نسبة يليها الجرذان التي تم تغذيتها على وجبات عالية الفركتوز لمدة اربع أسابيع ثم التي تم تغذيتها على وجبات عالية الدهون لمدة ثمانية أسابيع ولم يلاحظ أي تلف في الكلية في الجرذان التى تم تغذيتها على وجبات عالية الدهون لمدة اربع أسابيع مقارنة بالمجموعة الضابطة.

الخلاصة: أوضحت هذه الدراسة قدرة النظام الغذائي عالي الفركتوز على احداث أضرار مبكرة وأكثر شدة على الكلية مقارنة بالنظام الغذائي عالي الدهون.