Long-Term Fructose Intake: Biochemical Consequences and Altered Renal Histology in the Male Rat

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The use of fructose as a pure sugar has considerably increased in the last 3 decades, especially as a sweetener in carbonated beverages. Our previous studies showed that long-term fructose intake adversely affected several age-related metabolic parameters. The purpose of the present study was to compare the consequences of long-term fructose intake with those of glucose or sucrose on renal morphology and on several biochemical parameters used to estimate renal function. Male rats were fed a commercial diet for 16 months, and had free access either to water (control) or to 250 g/L solutions of fructose, glucose, or sucrose. Fructose-drinking rats exhibited higher liver weights compare to the other dietary groups. Control rats excreted significantly less urinary output than all sugar groups, which did not differ from each other. No differences were observed in fasting plasma fructose, glucose, and creatinine levels, or in urinary glucose levels. Fructose consumption resulted in elevated urinary fructose levels, higher creatinine clearance, and marked proteinuria. The tested sugars had influence on the molecular weight distribution of urinary proteins in the ranges of 10 to 16, 25 to 35, and 75 to 85 kd. Histological examination revealed that fructose consumption led to the formation of foci of cortical tubular necrosis with chronic inflammatory infiltrate, accumulation of tubular hyaline casts, thickening of the Bowman's capsule, mesangial thickening due to collagen deposits, and the occurrence of hemosiderin in tubular cells. These data suggest that fructose has a negative impact on kidney function and morphology. Further research is required to elucidate the precise mechanisms by which long-term fructose consumption hampers renal metabolism.

THE MONOSACCHARIDE fructose has long been a component of the human diet. In addition to being sweeter than sucrose, fructose contributes both physical and functional properties to food and beverage applications such as flavor enhancement, humectancy, color and flavor development, freezing-point depression, and osmotic stability.[1] Fructose has been promoted as an appetite depressant, as a food component for non–insulin-dependent diabetics, for use in parenteral feeding, and as a food supplement for endurance athletes.[2]

Most of the metabolic effects of fructose are due to its rapid utilization by the liver, leading to far reaching consequences of carbohydrate and lipid metabolism. Fructose overload increases hepatic pyruvate and lactate production, activates pyruvate dehydrogenase, and causes a shift from oxidation to esterification of nonesterified fatty acids, resulting in increased secretion of very-low-density lipoprotein (VLDL).[2] These effects are augmented by long-term fructose consumption, which causes enzymatic adaptation that increase lipogenesis and VLDL secretion, leading to hyperlipidemia, decreased glucose tolerance, and hyperinsulinemia.[2] In addition, acute fructose loading of the liver causes sequestration of inorganic phosphate in fructose-1-phosphate and diminished adenosine triphosphate (ATP) synthesis. Consequently, the inhibition of the enzymes of adenine nucleotide degradation is removed and uric acid formation accelerates with consequent hyperuricemia.[2]

Furthermore, the reducing free carbonyl group of fructose may react with free amino groups of biological macromolecules in a process known as fructation, also known as the Maillard reaction.[3] The initial phase of this reaction results in the formation of an acid labile Schiff base, which undergoes rearrangements to form the more stable Amadori (or Heyns) products. These compounds are considered to be intermediates in the reaction, and can undergo a cascade of slow reactions to form irreversible advanced glycation end products (AGEs). AGEs are heterogeneous compounds found in plasma, cells, and tissues, which can accumulate in various tissues such as vessel walls and the kidney. The advanced reactions can generate reactive oxygen intermediates acting as signal mediators, and may be deleterious to molecules, cells, or tissues. AGEs and reactive oxygen intermediates may induce cellular dysfunctions, and interfere with gene expression of peptides and cytokines regulating cell proliferation and vascular functions.[4] The sugar involved determines the rate of the reaction. Glucose, the most abundant sugar in living tissue, has a very low reactivity.[5] In vitro studies have demonstrated that the rate of the reaction is 10 times faster with fructose compared to glucose, and it has been postulated that fructation may occur in vivo.[6] The Maillard reaction is one of the hypotheses that may explain both the aging process and the complications of diabetes.[5]

Representing half of the sucrose molecule, fructose has been recognized for many years as being largely responsible for the metabolic effects of high-sucrose diets.[2] Diets rich in sucrose or fructose have been reported to produce varying degrees of pathology in the rat kidney,[7,8] with differences between rat strains.[7] A diffuse glomerulosclerosis and marked proteinuria, similar to changes found in diabetic rats, have been detected following long-term ingestion of a sucrose-rich diet.[9,10]

During the last 3 decades, there has been a significant in-

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Table 1. Body and Relative Organ's Weights, Feed, Liquid, and Energy Intakes, and Urine Excretion in Male Rats Following the Consumption of Various Sugar Solutions (250 g/L) for 16 Months

Parameter	Dietary Treatment			
	Water	Glucose	Sucrose	Fructose
No. of rats	7	7	6	7
Body weight (g)	452 ± 32^{c}	503 ± 46^{bc}	610 ± 76^{a}	592 ± 31^{ab}
Liver (mg · g^{-1} BW)	2.73 ± 0.11^{b}	2.79 ± 0.07^{b}	2.82 ± 0.13^{b}	3.18 ± 0.10^{a}
Kidney (mg · g^{-1} BW)	0.72 ± 0.04	0.70 ± 0.06	0.69 ± 0.07	0.77 ± 0.03
Liquid intake (mL · d^{-1} · 100 g^{-1} BW)	6.8 ± 0.1^{c}	18.8 ± 0.4^{a}	14.4 ± 0.3^{b}	12.8 ± 0.1^{b}
Feed intake (g · d^{-1} · 100 g^{-1} BW)	6.2 ± 0.3^{a}	2.8 ± 0.1^{c}	3.9 ± 0.1^{b}	3.6 ± 0.1^{b}
Energy intake (kJ · d^{-1} · 100 g^{-1} BW)	112.5 ± 4.5^{a}	116.7 ± 3.0^{a}	113.2 ± 2.6^{a}	102.4 ± 1.8^{b}
Urine excretion (mL · 24 h^{-1})	11.75 ± 1.94^{c}	18.05 ± 2.41^{a}	18.40 ± 1.24^{a}	15.46 ± 1.98^{ab}

NOTE. Data are means ± SE. Within a row, values with different superscripts differ significantly ($P < 0.05$).

crease in the availability of dietary fructose, particularly with the introduction of crystalline fructose and high-fructose syrup derived from corn.[11,12] The food industry utilizes the majority of the high-fructose corn syrup to sweeten carbonated beverages. We have previously demonstrated that fructose intake (via drinking), compared to glucose or sucrose, accelerates the normal aging process.[13] Thus, the purpose of the present study was to investigate whether or not long-term intake of fructose, versus glucose, sucrose, or water, would inflict detrimental consequences on renal performance in healthy male rats.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals were purchased from Sigma-Aldrich and Bio-Labs, Tel Aviv, Israel.

Animals and Diets

Male Sprague-Dawley rats weighing 45 to 50 g each, were obtained from the animal colony of the Department of Food Engineering and Biotechnology, Technion, Haifa, Israel. The animals were randomly divided into 4 groups of 7 rats each, and housed individually in polycarbonate cages, bedded with wood shavings and fitted with stainless steel wire mesh tops. The animal room was maintained at 21°C with 12-hour light/dark periods. The animals were treated according to the Ethics Committee of the Technion for experimentation in animals. All rats were fed a balanced commercial diet (#19510, Koffolk, Tel Aviv, Israel). The composition of the diet was as follows (g/kg diet): carbohydrates, 500; protein, 210; moisture, 130; ash, 70; fat, 45; cellulose, 45. One group of animals received tap water while the rest were provided with one of the following sugar solutions: glucose, sucrose, or fructose at a concentration of 250 g/L, each. Rats were allowed free access to the diet and their respective drinking solutions. Every 2 weeks rats were weighed and during the last week of the study, both liquid and feed intakes were determined. Biochemical and histological examinations were conducted at the end of the 16-month feeding period.

Sample Collection

At the end of the feeding period, rats were deprived of food and placed in metabolic cages for a 24-hour urine collection. Animals were then weighed and killed by CO_2 asphyxiation. The abdominal cavity was opened, and blood was drawn from the aorta into syringes containing Na_2EDTA and kept on ice. Plasma was separated by centrifugation (2,500 × *g* for 30 minutes, at 4°C) and stored at -80°C for metabolite analysis. The liver and kidneys were removed, blotted, and weighed, and kidneys were cut into thin (1-mm) slices and fixed in 10% buffered formalin solution for light microscopic examination.

Biochemical Analysis

Plasma and urine fructose concentrations were measured colorimetrically according to Roe.[14] Commercial kits (Sigma-Aldrich) were used to measure plasma and urine glucose levels. Urine soluble protein levels were measured colorimetrically after precipitation with trichloroacetic acid as described by Pesce and Strande.[15] Plasma and urine creatinine concentrations were determined using a commercial kit (Sigma-Aldrich, kit #555). The electrophoretic pattern of excreted urinary proteins was performed according to Sambrook et al.[16] Gels

Table 2. Plasma and Urine Metabolites, and Creatinine Clearance in Male Rats Following the Consumption of Various Sugar Solutions (250 g/L) for 16 Months

Parameter	Dietary Treatment			
	Water	Glucose	Sucrose	Fructose
No. of rats	7	7	6	7
Plasma glucose (mmol · L^{-1})	4.39 ± 0.74	4.18 ± 0.15	4.49 ± 0.36	4.41 ± 0.31
Plasma fructose (mmol · L^{-1})	1.21 ± 0.12	1.21 ± 0.18	1.29 ± 0.17	1.30 ± 0.13
Plasma creatinine (mmol · L^{-1})	28.92 ± 3.35	26.69 ± 2.19	21.03 ± 3.54	28.52 ± 5.93
Urine glucose (μmol · 24 h^{-1})	10.58 ± 3.56	11.48 ± 3.56	18.38 ± 2.97	11.70 ± 1.81
Urine fructose (μmol · 24 h^{-1})	7.13 ± 1.51^{c}	9.87 ± 0.18^{c}	25.34 ± 3.25^{b}	34.01 ± 5.35^{a}
Urine creatinine (mmol · 24 h^{-1})	185.84 ± 15.45^{ab}	92.04 ± 9.36^{c}	138.05 ± 37.00^{bc}	217.31 ± 16.53^{a}
Urine protein (mg · 24 h^{-1})	23.62 ± 3.49^{b}	15.39 ± 0.78^{c}	22.74 ± 6.01^{b}	143.78 ± 32.52^{a}
Creatinine clearance* (mL · s^{-1})	0.08 ± 0.01^{b}	0.04 ± 0.01^{c}	0.08 ± 0.01^{b}	0.20 ± 0.08^{a}

NOTE. Data are means ± SE. Within a row, values with different superscripts differ significantly ($P < .05$).
*Creatinine clearance = Urine (mL/s) · urine creatinine (mmol/L)/plasma creatinine (mmol/L).

Table 3. Molecular Weight Distribution of Excreted Urinary Proteins in Male Rats Following the Consumption of Fructose, Glucose, or Sucrose Solutions (250 g/L) for 16 Months

Parameter	Dietary Treatment			
	Water	Glucose	Sucrose	Fructose
No. of rats	7	7	6	7
Molecular weight (kd)				
<10	0.6 ± 0.3	1.5 ± 1.1	0.5 ± 0.3	0.3 ± 0.1
10-16	10.9 ± 0.4^{ab}	12.5 ± 4.2^{a}	3.6 ± 0.8^{c}	6.0 ± 1.3^{bc}
16-20	ND	ND	ND	0.5 ± 0.3
20-25	2.3 ± 1.4	8.8 ± 4.7	9.4 ± 6.8	0.6 ± 0.5
25-45	0.6 ± 0.3^{c}	7.5 ± 3.0^{a}	5.1 ± 1.8^{ab}	4.3 ± 1.7^{ab}
45-55	11.6 ± 5.5	5.2 ± 1.7	14.3 ± 4.0	9.9 ± 4.2
55-67	67.9 ± 5.9	57.3 ± 11.2	62.3 ± 9.3	67.8 ± 4.6
67-75	4.3 ± 1.4	2.2 ± 0.9	1.5 ± 1.3	4.7 ± 2.9
75-85	0.5 ± 0.2^{b}	6.7 ± 2.7^{a}	2.0 ± 0.7^{b}	3.2 ± 0.8^{ab}
85-125	0.5 ± 0.3	0.6 ± 0.4	0.6 ± 0.6	1.5 ± 1.3
170-250	1.0 ± 0.5	0.7 ± 0.3	0.7 ± 0.3	1.4 ± 0.6

NOTE. Values (% of integrated density of the sample) are means ± SE. Within a row, values with different superscripts differ significantly ($P < .05$).

Abbreviation: ND, not detected.

were scanned, and the RFLP-SCAN software (Scanalytics, Billerica, MA) was used to quantitatively analyzed protein bands using rat serum albumin (Sigma-Aldrich) as a standard. Molecular mass reference standards (Bio-Labs) were used to analyze the molecular-weight distribution of the excreted urinary proteins.

Light Microscopic Examinations

Fixed kidney slices were dehydrated, embedded in paraffin, and evaluated on 7-μm sections stained with hematoxylin & eosin, Schiff periodic acid, Masson-trichrome, and Perls' stain. These procedures were performed at the Department of Pathology, Afula Medical Center, Israel. Randomly numbered kidney specimens were examined and evaluated by a pathologist who was blinded to the group assignment of the rats. The presence of necrotic inflammatory infiltrates, hyaline casts, mesangial and Bowman's capsule thickening, and hemosiderin were evaluated using a semiquantitative score.

Histomorphometry

Hematoxylin & eosin–stained tubular casts appeared as red granules and nonstained tissue appeared light pink by light microscopy. An image analyzer installed on a light microscope and attached to a personal computer equipped with a frame grabber was used to analyze the extension of positively stained hyaline casts. Images were captured, digitized, and displayed on a high-resolution colored monitor. Avoiding artifacts, the ten most intensely stained fields were analyzed at a power lens of 20 × 10. Images were then loaded on screen buffers having a resolution of 760 × 570 pixels, and measured in standardized frames (62,993 μm^2). Image Pro Plus 4 software (Media Cybernetics, Baltimore, MD) was used to assess the extent of positively stained areas after segmentation and thresholding. The percent of positively stained area per microscopic field was determined to assess the extent of cast involvement.

Statistical Analysis

Animal data were analyzed using SAS/STAT software, version 6.12, SAS Institute, Cary, NC. Data were analyzed by 1-way anlaysis of variance (ANOVA) followed by Duncan's multiple range tests. Histomorphometric data were analyzed using the SPSS 6.0. Comparison between groups used the nonparametric Kruskal-Wallis ANOVA followed by a corrected Mann-Whitney test for multiple comparisons. In both analyses a probability level of .05 was selected to indicate significance.

RESULTS

During the 16-month feeding period, one rat from the sucrose group died. Necropsy of the deceased animal did not identify the reason of death.

Table 4. Scores of Histologic Changes of Components in the Kidneys of Male Rats Following the Consumption of Fructose, Glucose, or Sucrose Solutions (250 g/L) for 16 Months

	Observations																			
	NI				HC				BCT				MT				HS			
Dietary Treatment	−	+	++	+++	−	+	++	+++	−	+	++	+++	−	+	++	+++	−	+	++	+++
Water (7)	7	0	0	0	6	1	0	0	7	0	0	0	7	0	0	0	7	0	0	0
Glucose (7)	7	0	0	0	7	0	0	0	7	0	0	0	7	0	0	0	7	0	0	0
Sucrose (6)	5	1	0	0	3	2	1	0	6	0	0	0	6	0	0	0	5	1	0	0
Fructose (7)	1	2	4	0	0	1	2	4	2	2	2	1	2	4	1	0	4	2	1	0

NOTE. −: denotes absence of observations; +: denotes the presence of observations with different degrees (+: slight; ++: moderate; +++: severe). Values are number of rats affected.

Abbreviations: NI, necrosis and inflammatory infiltrates; HC, hyaline casts; BCT, Bowman's capsule thickening; MT, mesangial thickening; HS, hemosiderin.

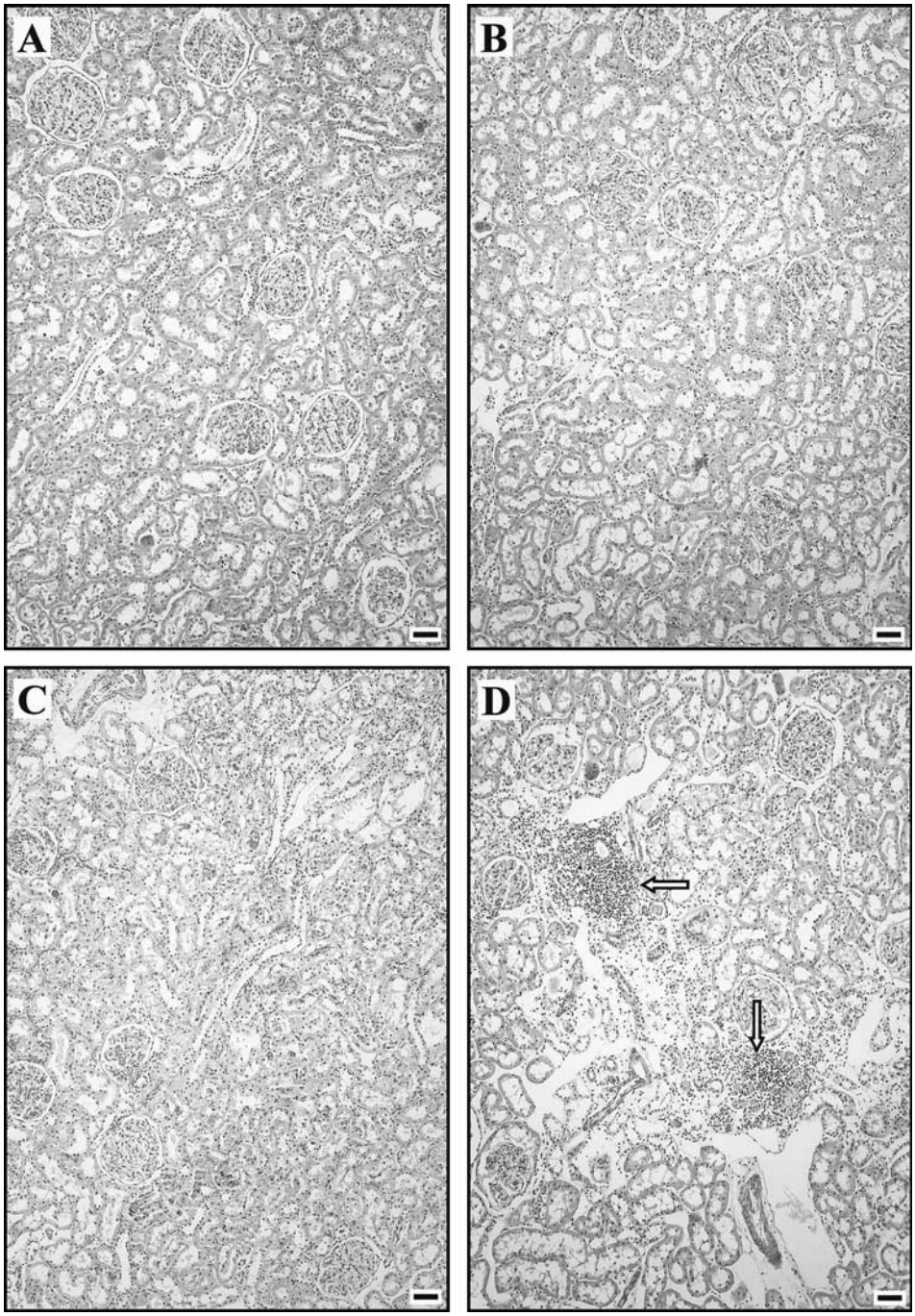

Fig 1. Light micrograph (hematoxylin-eosin staining) of renal cross-section from 16-month-old rats drinking various sugars solutions (250 g/L): (A) Control (water); (B) glucose; (C) sucrose; (D) fructose. In the fructose-drinking rat notice the occurrence of inflammatory infiltrates in the cortical stroma, and the accumulation of lymphocytes between renal tubules (arrows). Original magnification × 100; bar indicates 100 μm.

Analysis the abdominal cavity at the end of the feeding period revealed that both sucrose- and fructose-drinking rats contained extensive fat layers compared to glucose-drinking and control rats. The influence of the dietary sugars on body and relative organs weights, daily feed intakes, liquid consumption, total energy intake, and daily urinary excretion are presented in Table 1. Sucrose-drinking rats demonstrated the highest body weight, although it was not statistically different from the fructose group. The highest liver relative weights were observed in fructose-drinking animals, while no significant differences were observed in relative kidney weights. Rats in the glucose group consumed the highest amounts of liquid, while control (water-drinking) rats the lowest. In contrast, control rats consumed significantly more solid feed than the sugar-drinking animals, while among the sugar-drinking rats, glucose-drinking ones consumed the lowest amount of feed. Daily energy intakes were calculated based on the amount of energy-providing nutrients in the consumed feed and liquid. Control, glucose-, and sucrose-drinking rats consumed significantly more energy than the fructose groups. Control animals

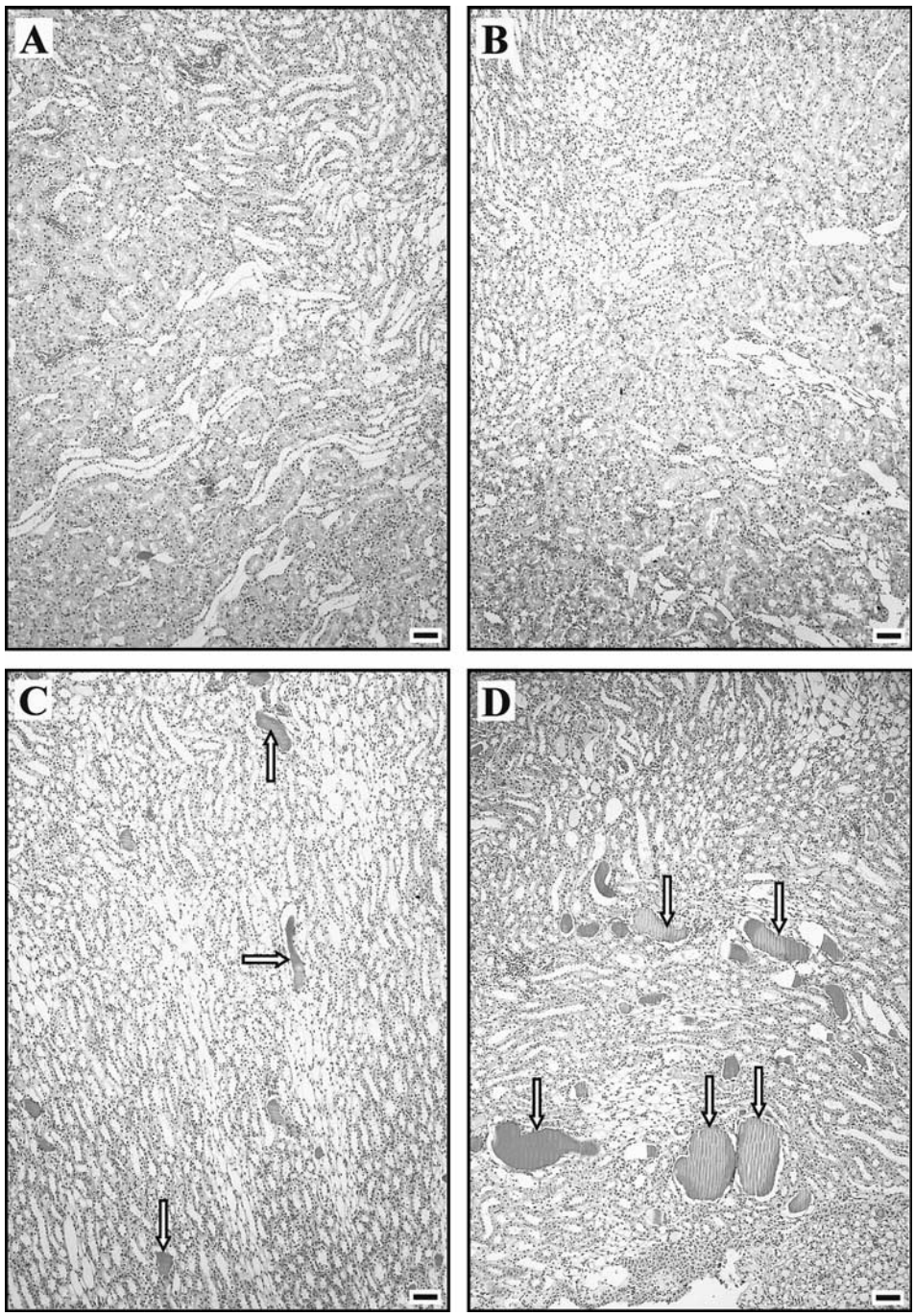

Fig 2. Light micrograph (hematoxylin-eosin staining) of renal cross-section from 16-month-old rats drinking various sugars solutions (250 g/L): (A) Control (water); (B) glucose; (C) sucrose; (D) fructose. In the fructose-drinking rat notice the occurrence of expanded dilated tubules filled with hyaline casts (arrows), mainly in the medullar area of the kidney. Original magnification × 100; bar indicates 100 μm.

excreted the lowest amounts of urine, while no significant differences were observed among the sugar-drinking animals.

The influence of the tested dietary sugars on plasma and urinary metabolites and creatinine clearance are presented in Table 2. Fasting plasma fructose, glucose, and creatinine as well as urine glucose levels were not affected by the tested dietary sugars. However, daily urinary excretion levels of fructose were the highest in fructose-drinking rats and the lowest in the glucose-drinking and control rats. While no significant differences in urinary creatinine levels were observed between the control and fructose-drinking rats, the latter animals demonstrated significantly higher levels than glucose- and sucrose-drinking animals. Fructose-drinking rats excreted the highest levels urinary protein. Creatinine clearance values were the highest in fructose-drinking as compared to control and other sugar-treated rats. Glucose-drinking rats showed the lowest creatinine clearance values.

The molecular weight distribution of excreted urinary proteins following the consumption of the tested dietary sugars is presented in Table 3. Molecular weights of the urinary proteins

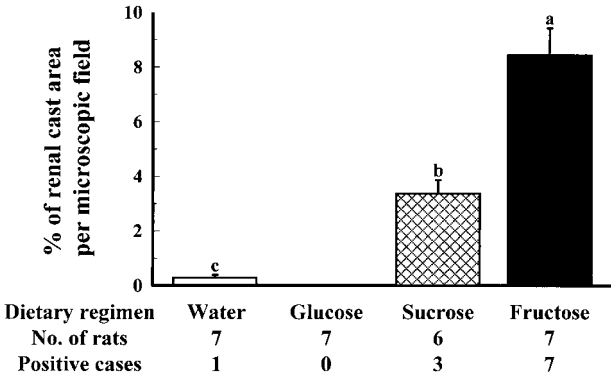

Fig 3. Histomorphometric measurements of renal casts extension in 16-month-old male rats drinking water or sugars solutions (250 g/L) of glucose, sucrose, or fructose (see Methods for details). Values are means ± SD, and bars with different letters differ significantly ($P < .05$).

were distributed over a wide range from 10 to 250 kd, but the main fractions are within the 10- to 16- and the 55- to 67-kd ranges, accounting for up to 70% of the total excreted proteins. Glucose-treated rats excreted 2- and 3.5 times more proteins of the 10- to 16-kd range then fructose- and sucrose-fed rats, respectively. At this molecular range, control rats excreted significantly more urinary proteins then sucrose-fed rats, but were not different than glucose- or fructose-treated rats. At the molecular range of 25- to 45-kd, sugar-treated rats excreted 7 to 12 times more urinary proteins then control rats, and no differences were observed among sugar-drinking rats. At the 75-to 85-kd range, glucose-drinking rats excreted 3- and 13 times more proteins then sucrose-drinking and control rats, respectively.

Histologic examination (Table 4) revealed that kidney tissue of the control and glucose-drinking rats appeared normal, and did not show any noticeable morphological change (except the presence of few sporadic hyaline casts in 1 rat of the control group). Histopathologic changes were more obvious in the fructose-drinking group with an increase in both the number of affected rats and in the severity of the morphologic changes. Six of 7 fructose-fed rats demonstrated foci of cortical tubular necrosis, with a mild to moderate amount of chronic inflammatory infiltrate, mostly small lymphocytes, in the cortical stroma between the renal tubules (Fig 1). All fructose-drinking rats showed expand dilated tubules filled with hyaline casts, some of which cystic, within the medullar area of the kidney (Fig 2). Hyaline casts were also found in 3 of 6 sucrose-drinking rats. Histomorphometric measurement of renal cast extension revealed that in positively stained renal sections, casts covered significantly more area in fructose-drinking rats compared to the other groups (Fig 3). Glomeruli regenerative changes such as thickening of the mesangium and Bowman's capsule (Fig 4), as well as mesangial collagen deposits (Fig 5) were observed in 5 of 7 fructose-drinking rats. The hemosiderin pigment was present in the cytoplasm of tubular cells (Fig 6) in 3 of 7 fructose-drinking rats and in 1 sucrose-drinking rat.

There were no observable histopathologic changes in the renal blood vessels of any of the tested groups.

DISCUSSION

Normal age-related changes in renal function are the most dramatic of any tissue or organ system.[17] Furthermore, it was noticed that the common reason for animal death during experiments employing long-term consumption of sugar-rich diets is the failure of renal function.[18] In a recent study conducted in our laboratory, Levi and Werman[13] concluded that consumption of a fructose solution, 250 (g/L) for 1 year adversely affects age-related parameters. We therefore hypothesized that long-term fructose drinking may also cause undesirable changes in renal structure, which may consequently impair renal function.

Age-related alterations in physiologic and biochemical parameters are drastic during the first months of life in the rat, and become fairly stable after reaching sexual maturity.[19] Indeed, body weights of control and glucose-drinking rats remained relatively constant following the fifth month, while those of sucrose- and fructose-drinking rats constantly rose throughout the study. This phenomenon is probably due to the effect of fructose on lipid metabolism in these rats. Dissection of the animals revealed that the abdominal cavity of both sucrose- and fructose-drinking rats contained extensive amounts of fat, which may have been responsible for their higher body weight. Elevated plasma triglycerides levels have been documented following the intake of sucrose or fructose-rich diets,[2,20] and accelerated lipogenesis was reported as the main reason for elevated liver weights after fructose consumption.[2] Hyperinsulinemia, which develops during the consumption of diets high in sucrose, may also contribute to the deposition of extensive fat layers.[21]

Increased kidney weight, either absolute or relative to body weight, is often, but not always, demonstrable after feeding male rats with sucrose- or fructose-rich diets for periods ranging from 10 weeks to 1 year or longer.[7] In the present study, however, no significant differences were observed in relative kidney weight between groups, although fructose-drinking rats showed somewhat higher kidney weights (Table 1).

Rats provided with sugar solutions consume significantly more liquid than the control group (Table 1). However, among the sugar-drinking rats, the glucose group consumed the highest volumes and the fructose group the lowest. The sensory effects of each of the tested sugars may have influenced liquid consumption. Until recently, it was thought that simple sugars produce only a single type of gustatory sensation in rodents.[22] However, Ackroff and Sclafani[23] suggested that fructose-conditioned preferences are based primarily on its sweet taste, while glucose can condition strong preference based both on taste and postingestive reinforcing actions, which can stimulate substantial increases in flavor acceptance as well as flavor preference. Regarding fructose verses sucrose, Ramirez[24] indicated that rodents could discriminate between these sugars, although they share some chemosensory properties. Therefore, we assume that the observed differences in liquid intakes, due to diverse sensory reactions to the provided sugars, may have affected urine volumes. The increase consumption of sugar solutions clearly explains the reduced intake of solid feed of sugar-drinking rats compared to the control group. Since sugar

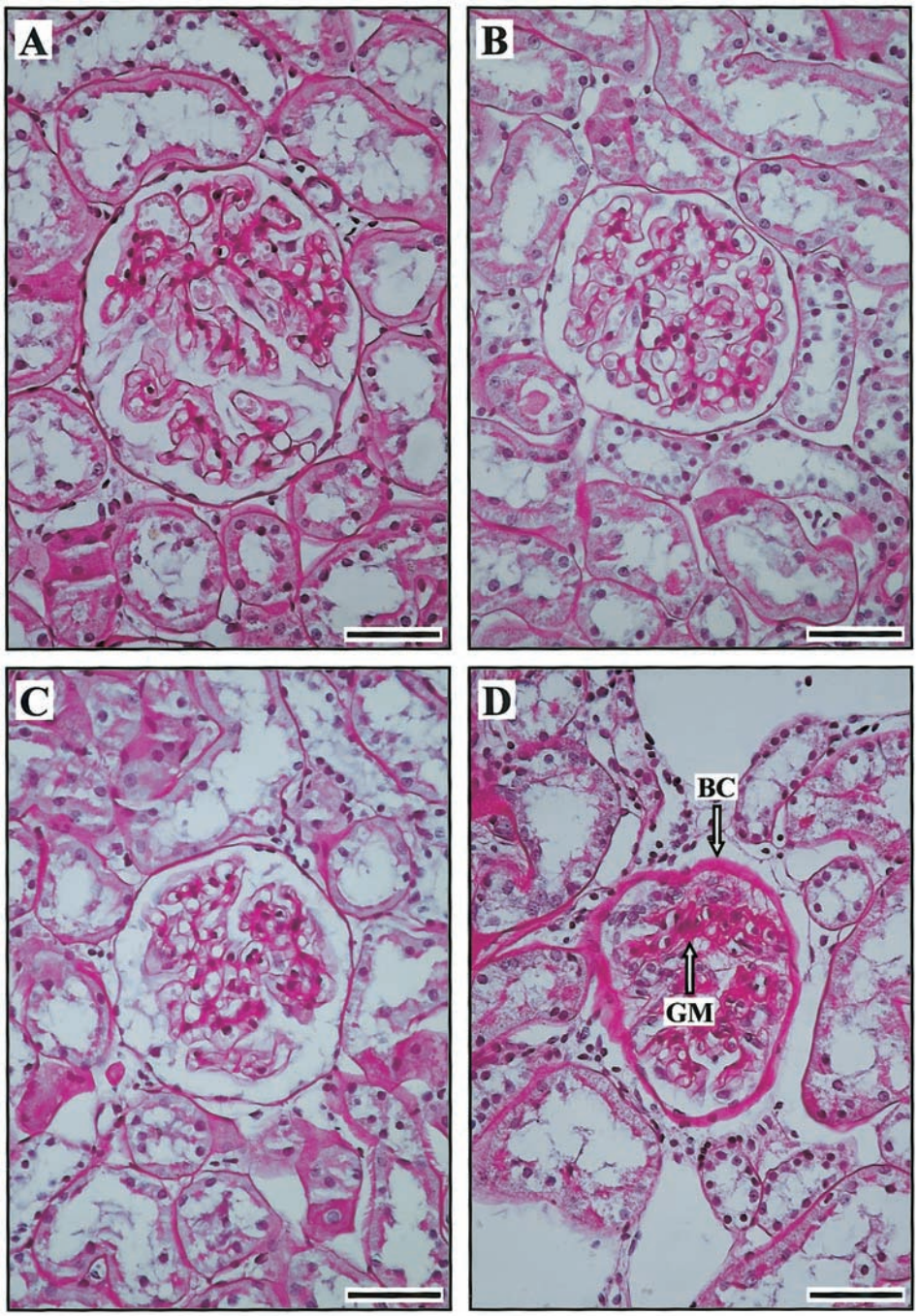

Fig 4. Light micrograph (periodic acid Schiff staining) of renal cross-section from 16-month-old rats drinking various sugars solutions (250 g/L): (A) Control (water); (B) glucose; (C) sucrose; (D) fructose. In the fructose-drinking rat notice the thickening of the Bowman's capsule (BC) and the glomerular mesangium (GM). Original magnification × 400, bar indicates 100 μm.

solutions provided considerable energy, sugar-drinking rats generally consumed less solid feed to meet their energy needs. However, the control, glucose, and sucrose groups consumed significantly more total energy than the fructose-drinking animals.

Histological examination of the kidney revealed considerable morphological changes in fructose-drinking rats compared to the other dietary groups. The appearance of hyaline casts (Fig 2) explains the observed tubular expansion in fructose-fed rats. The cast formation probably resulted from injury in the basal lamina of the glomerular filtration barrier, composed of collagen type IV, laminin, and heparan sulfate proteoglycans.[25] These long-live macromolecules may serve as targets to reducing sugars like glucose and fructose, and undergo glycation or fructation, respectively. Glycation of collagen type IV and laminin in the basement membrane modifies their chemical and physiological characteristics and may influence renal function.[26] The kidneys of fructose-drinking rats showed both tu-

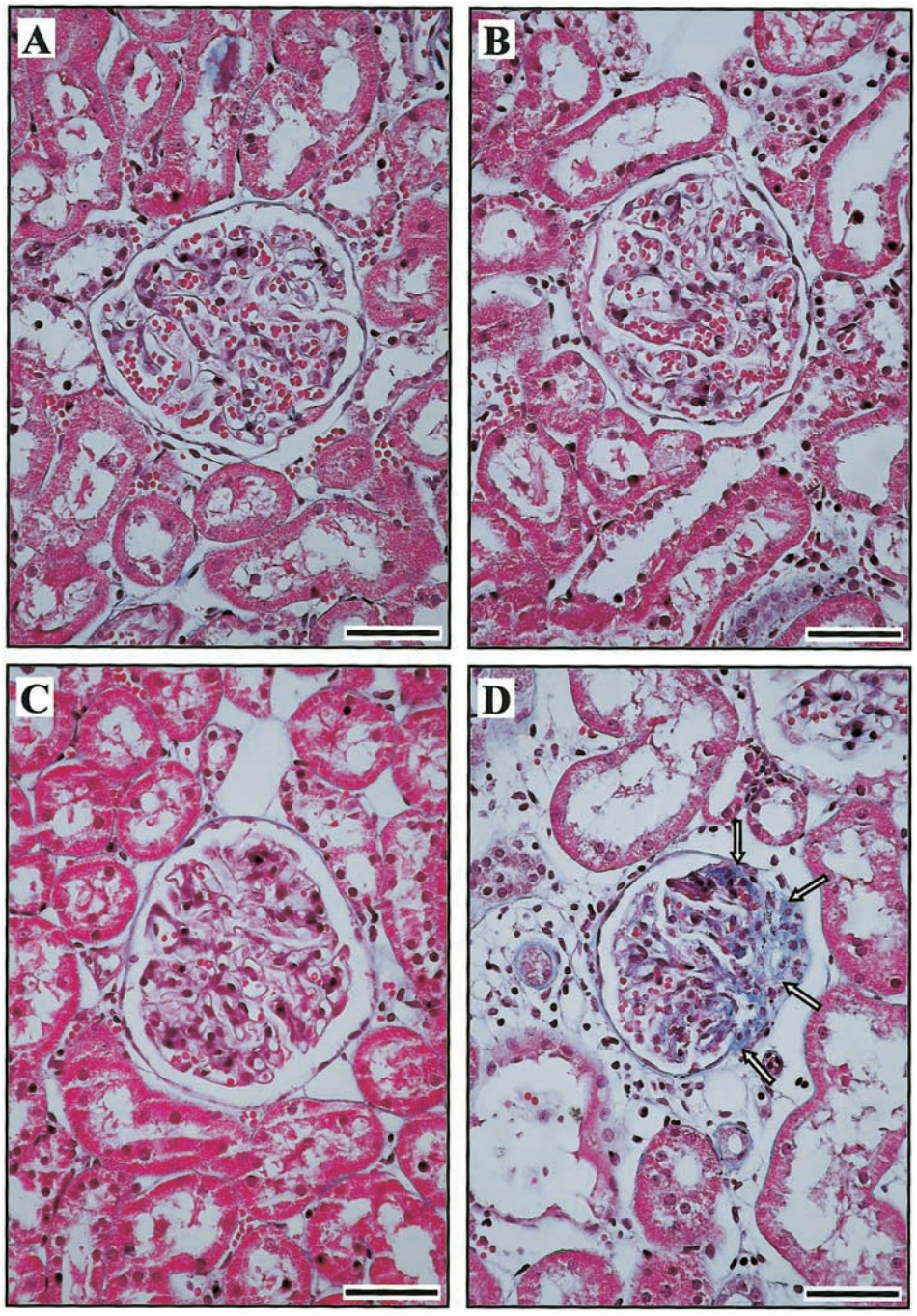

Fig 5. Light micrograph (Masson-Trichrome staining) of renal cross-section from 16-month-old rats drinking various sugars solutions (250 g/L): (A) Control (water); (B) glucose; (C) sucrose; (D) fructose. In the fructose-drinking rat notice the mesangial accumulation of collagen (arrow). Original magnification × 400; bar indicates 100 μm.

bular and glomerular changes (Figs 2, 4, and 5) even though these findings occurred in the presence of normal fasting blood glucose or fructose levels (Table 2). However, in a current ongoing study we observe significantly higher postprandial blood fructose levels in fructose-drinking rats as compared to sucrose- or glucose-drinking animals, with no apparent differences in post-prandial blood glucose levels. This may explain the higher levels of fructose in the urine of fructose-drinking rats (Table 2). Because fructose is a more potent glycating agent than glucose, one cannot rule out the involvement of fructose-originated alterations in the tubules and glomerulus of fructose-fed rats.

Lee et a[27] suggested that following intravascular hemolysis of red blood cells, hemosiderin may deposit in the renal cortex. Findings of the present study (Table 4) can neither support nor oppose the occurrence of intravascular hemolysis in fructose-drinking rats. Wixom et al[28] suggested that hemosiderin might also originate from ferritin breakdown. According to Williams

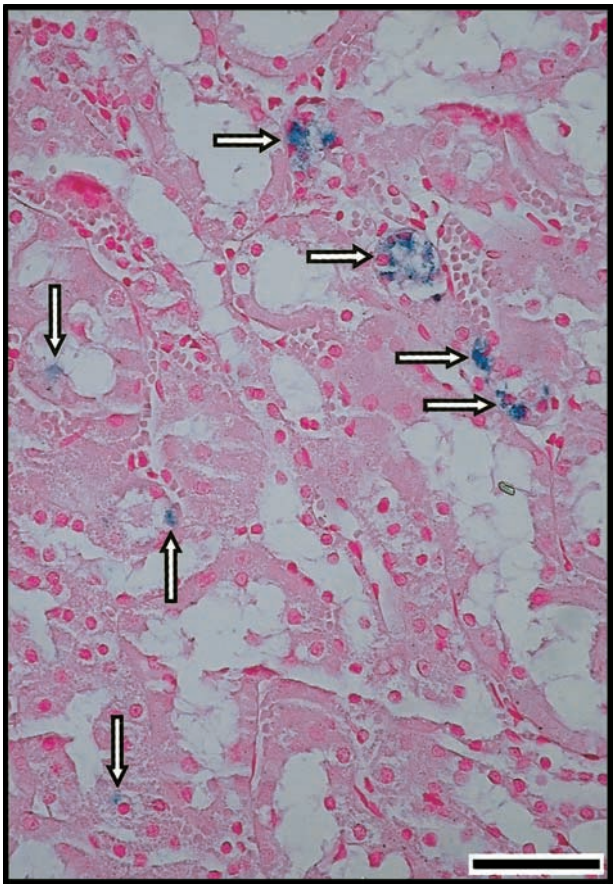

Fig 6. Light micrograph (Perls' staining) of renal cross-section from 16-month-old rats drinking fructose (250 g/L). Notice the accumulation of hemosiderin (arrows) in the tubular epithelial cells. Original magnification × 400, and the bar indicates 100 μm.

and Siegal,[29] infusion of glycated ferritin into rat kidneys results in its increased transglomerular passage and clearance in urine. Thus, we cannot rule out the possibility that ferritin may have reacted with fructose, permeated preferentially through the altered glomeruli, been reabsorbed in the proximal tubule, underwent proteolysis, and contributed iron as hemosiderin deposits. To the best of our knowledge, no reports concerning hemosiderin appearance in tubules, following a long intake of simple sugars in rats, are reported in the literature.

The most striking change in renal function of aging Sprague-Dawley rats is proteinuria.[30] The level of excreted urinary protein in healthy Sprague-Dawley rats has been reported to be 88 and 284 mg/d by 6 and 12 months of age, respectively.[31] In contrast, our rats, at 16 months of age, excreted considerably lower levels of proteins. Rats of the control, glucose and sucrose groups maintained relatively low urinary protein excretion (15 to 25 mg/d) while fructose-drinking rats reached levels as high as 144 mg/d (Table 2). Accordingly, rats in our breeding colony may be classified as low-protein excretors. Appearance of protein in the urine is controlled by both glomerular filtration and tubular reabsorption. Molecular size and configuration, net electrical charge, and hemodynamic forces that act at the surface of the glomerular endothelium affect trans-glomerular passage of proteins.[29] Proteins of molecular weight greater than 125,000 kd are believe to be restricted from transglomerular passage, while proteins with the size of serum albumin (69,000 kd) exhibit limited passage across the glomerular filtration barrier. Tubular reabsorption is an energy-dependent process and occurs by endocytosis of proteins attached to the brush border membranes.[32] Cationic proteins are more freely filtered than anionic proteins, while cationic proteins are reabsorbed more efficiently than anionic proteins. Tubular reabsorption of glomerular-filtered proteins is nearly complete, and the urinary level of low-molecular-weight proteins is less than 1% of the filtered load.[33] Identification of urinary excreted proteins can be used as a mean of distinguishing glomerular from tubular disorders. Thus, any tubular dysfunction will result in low-molecular-weight proteinuria.[30,32] The amount of excreted protein and level of cast formation in fructose-drinking rats differ considerably from that of the other groups. Therefore, fructose intake may cause alterations in the glomeruli and tubules, which result in abnormalities such as proteinuria. In diabetes, glycated albumin is known to increase glomerular permeability compare to nonglycated albumin and to induce hyperfiltration and proteinuria.[34]

Creatinine clearance is an additional biochemical parameter that distinguished rats consuming fructose from the other tested dietary sugars. Rehberg[35] first proposed this parameter as a marker for the rate of glomerular ultrafiltration in humans and experimental animals.[36] In addition, urinary creatinine is assumed to be a reliable index of muscle mass either in animals or in humans.[37] Creatinine is a degradation product of phosphocreatine and is excreted in the urine. If the kidney is unable to handle and excrete nitrogenous waste products, the concentration of creatinine will increase in blood. In our present study, plasma creatinine values in all rats (Table 2) were found to be within the normal range,[38] and not affected by the tested sugars. Thus, we assume that the kidneys of these rats are functionally able to dispose of nitrogenous waste products. However, fructose-consuming rats demonstrate elevated levels of urine creatinine, and consequently its clearance was significant higher compared to control, sucrose-, or glucose-drinking animals. Our observation reinforces that of Manitius et al,[39] who demonstrated elevated creatinine clearance after 2 weeks feeding of fructose compared to starch.

The present study presents evidence that long-term fructose consumption resulted in altered renal morphology and related biochemical parameters in rats. In view of the increased utilization of fructose by the food industry during the last 3 decades, advanced studies are needed to further clarify the exact mechanisms that mediate the harmful effects of fructose and whether these effects occur in humans. Based on our studies regarding the detrimental properties of fructose, we believe that it is time for the food industry to carefully reconsider the extensive use of this sugar.

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