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Sucrose induces fatty liver and pancreatic inflammation in male breeder rats independent of excess energy intake

Carlos A. Roncal-Jimenez[a,*], *Miguel A. Lanaspa*[a], *Christopher J. Rivard*[a], *Takahiko Nakagawa*[a,b], *L Gabriela Sanchez-Lozada*[a,c], *Diana Jalal*[a], *Ana Andres-Hernando*[a], *Katsuyuki Tanabe*[a], *Magdalena Madero*[c], *Nanxing Li*[a], *Christina Cicerchi*[a], *Kim Mc Fann*[a], *Yuri Y. Sautin*[b], *Richard J. Johnson*[a,b]

[a] *Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO 80016, USA*
[b] *Division of Nephrology, Hypertension and Transplantation, University of Florida, Gainesville FL, USA*
[c] *Instituto Nacional de Cardiología Ignacio Chávez, 14080 Mexico City, Mexico*

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ABSTRACT

Fructose induces metabolic syndrome in rats; but studies have been criticized for using high concentrations of fructose that are not physiologic, for using only pure fructose, and for not controlling for energy intake. We tested the hypothesis that a 40% sucrose diet (containing 20% fructose) might induce features of metabolic syndrome in male breeder rats independent of excess energy intake. Male Sprague-Dawley breeder rats were pair fed 40% sucrose or isocaloric starch diet for 4 months and evaluated for metabolic syndrome and diabetes. In vitro studies were performed in rat insulinoma cells (RIN-m5F) exposed to uric acid, and markers of inflammation were assessed. Rats fed a 40% sucrose diet developed accelerated features of metabolic syndrome with up-regulation of fructose-dependent transporter Glut5 and fructokinase. Fatty liver and low-grade pancreatic inflammation also occurred. Uric acid was found to stimulate inflammatory mediators and oxidative stress in islet cells in vitro. Sucrose, at concentrations ingested by a subset of Americans, can accelerate metabolic syndrome, fatty liver, and type 2 diabetes mellitus in male breeder rats; and the effects are independent of excess energy intake.

1. Introduction

Diets enriched in fructose are known to induce features of metabolic syndrome in rats [1,2]. However, these studies are frequently criticized for using excessive concentrations of fructose that are not physiological (eg, 60%) or for administering fructose alone (when most exposure to humans is as sucrose or high-fructose corn syrup) [3,4]. Furthermore, there remains debate whether the effects of fructose to induce metabolic syndrome are simply a consequence of excessive energy intake [5]. Finally, whereas there are some data that added sugars may increase the risk for type 2 diabetes mellitus both experimentally [6] and in humans [7,8], there is only minimal evidence that they have specific effects to induce islet cell dysfunction [9-11].

We therefore tested the hypothesis that a sucrose-based diet containing only 20% fructose might induce features of fatty liver and metabolic syndrome. To address whether the effects

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* *Corresponding author*. University of Colorado Health Sciences Center, Division of Renal Diseases and Hypertension, Box C281, Aurora CO 80045, USA. Tel.: +1 303 724 4898; fax: +1 303 724 4831.
E-mail address: carlos.roncal@ucdenver.edu (C.A. Roncal-Jimenez).

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observed were specific to fructose, we administered an identical diet to control rats in which the sucrose was replaced with starch. Furthermore, all rats were pair fed so that each animal ate the exact same number of calories. To further ensure that intake was not excessive, all rats were fed approximately 90% of normal intake. The slight restriction in caloric intake is to simulate a dieting individual who still ingests a diet high in added sugars. Studies were performed in male Sprague-Dawley breeder rats, which are known to spontaneously develop features of metabolic syndrome as they age [12,13]. Using this model system, we report that sucrose accelerated the development of metabolic syndrome and type 2 diabetes mellitus compared with starch-fed rats, in association with the development of mild inflammation in the pancreas.

2. Methods

2.1. Animals and diets

Eight-month-old male Sprague-Dawley breeder rats (Charles Rivers, Wilmington, MA) were housed in the animal facility at the University of Colorado. Rats were kept under temperature- and humidity-controlled specific pathogen-free conditions and maintained on a 12-hour light-dark cycle. The experimental protocols were approved by the University of Colorado Animal Care and Use Committee.

Rats were randomly divided into 2 groups, consisting of sucrose-fed (n = 10) or starch-fed (n = 10) rats. The sucrose group received a 40% sucrose diet (protein, 18.3% by weight and 20.2% kcal; carbohydrate, 60.5% by weight [consisting of 40% sucrose and 20.5% starch] and 66.9% kcal; fat, 5.2% by weight and 12.9% kcal; TD.09019 Harlan, Indianapolis, IN). The control group received an isocaloric (based on weight) diet in which the sucrose was replaced by starch (selected nutrient; protein, 18.3% by weight and 20.2% kcal; carbohydrate, 60.5% by weight and 66.9% kcal; fat, 5.2% by weight and 12.9% kcal; TD.06391 Harlan). The diets were identical in all nutrients other than replacing the starch for sucrose in the control diet (Table 1).

All rats were placed in individual cages and pair fed the same amount of total calories each evening for the 16 weeks. Food intake was measured daily and averaged approximately 21 g/d. This represented about 10% less than normal intake in these rats. Rats generally ate about 9 g in the first 2 hours and then the rest of their food over the following 18 to 20 hours. All food was ingested by 24 hours.

Table 1 – Composition of the diets

	Sucrose diet (g/kg)	Control diet (g/kg)
Casein	207.0	207
DL-Methionine	3.0	3.0
Corn starch (glucose polymer)	12.0	456
Maltodextrin	200.0	200.0
Sucrose	400.0	0
Lard	50.0	50.0
Cellulose	60.21	16.36
Mineral mix, Roger-Harper (170760)	50.0	50.0
Zinc carbonate	0.04	0.04
Vitamin mix, Teklad (40060)	10.0	10.0
Potassium chloride	7.6	7.6
Pink food color	0.15	

2.2. Blood sampling

Fasting (8-hour) blood samples were obtained from the tail vein from unanesthetized rats at baseline, 1 month, and 4 months. Serum was analyzed for glucose using the Glucose Assay Kit (Cayman Chemical Company, Ann Arbor, MI). Triglycerides and uric acid were analyzed using the Vetace autoanalyzer (Alfa Wassermann, West Caldwell, NJ). Serum insulin was measured by enzyme-linked immunosorbent assay (Crystal Chemical, Chicago, IL). Animal body weights were measured weekly.

2.3. Blood pressure

Blood pressure (BP) was measured by intraaortic telemetry in conscious, unrestrained rats using the DSI (St Paul, MN) telemetry system. Ten days before initiating the study, a BP sensor (model TA11PA-C40; DSI) was inserted into the aorta below the level of the renal arteries; and the radiofrequency transmitter was fixed to the peritoneum. For BP measurements, a plastic cage containing the rat was placed on top of a Lab Pro Data Acquisition System receiver (DSI); and systolic BP was continuously recorded at 5-minute intervals, with each BP reading representing the average of approximately 60 readings during a 10-second interval.

2.4. Liver oil red O stain

Liver tissue collected under isoflurane anesthesia was embedded in Optimal Cutting Temperature gel (OCT; Sakura Finetek, Torrance, CA) and frozen in liquid nitrogen. Air-dried cryostat tissue sections (8 μm) were dipped in formalin, washed with running tap water, rinsed with 60% isopropanol, and stained with oil red O counterstained with hematoxylin. *Macrovesicular fat deposition* was defined as the presence of lipid vacuoles that are larger than the nucleus and usually displaces it to the periphery of the cell.

2.5. Pancreatic histology and immunostaining

The pancreas was dissected under isoflurane anesthesia, fixed in methyl Carnoy solution, and embedded in paraffin; and sections (3 μm) were stained with periodic acid Schiff. Immunostaining was performed using our standard procedure [14] with the following antibodies: mouse anti-rat insulin (Cell Signaling Technology, Danvers, MA), ED-1 (mouse antibody to rat monocytes and macrophages; Serotec, Raleigh, NC), and rat URAT1 (murine anti-SLC22A12/Urat-1; ABNOVA, Walnut, CA). A horseradish peroxidase–conjugated secondary antibody was used followed by detection with diaminobenzidine-H_2O_2 (Vector, Burlingame, CA). For quantification, at least 5 islets were examined per biopsy. Images were captured using the ScanScope Scanner Console and Aperio Image Scope software (APERIO Technologies, Vista, CA); and by color saturation, the percentage of positive immunostaining area

was quantified. Infiltration of macrophages in pancreas interstitium was assessed by quantifying the percentage of positive area in 5 grids not containing islets for each biopsy. Mean scores for the rats from each group were compared. All studies were analyzed blinded.

2.6. Western blotting of liver and pancreas

Protein lysates were prepared from liver and pancreatic tissues as previously described [15]. Sample protein content was determined by the BCA protein assay (Pierce, Rockford, IL). Fifty micrograms of total protein was loaded per lane for sodium dodecyl sulfate polyacrylamide gel electrophoresis (10% wt/vol) analysis and transferred to polyvinylidene difluoride membranes. Membranes were incubated with the following primary antibodies: fatty acid synthase (FAS; Cell Signaling), fructokinase isoform C (KHK-C; Sigma, St Louis, MO), adenosine monophosphate deaminase 2 (AMPD2; Abcam, Cambridge, MA), enoyl CoA hydratase (ECoAH1; ProteinTech Group, Chicago, IL), URAT1 (murine anti-SLC22A12/Urat-1; ABNOVA), and β-actin (Cell Signaling). Blots were then incubated with a horseradish peroxidase secondary antibody and Immunstar HRP chemiluminescence kit (Bio-Rad, Hercules, CA) as described by the manufacturer. Chemiluminescence was recorded with an Image Station 440CF, and results were analyzed with the 1D Image Software (Kodak Digital Science, Rochester, NY).

2.7. RNA extraction, analysis, and message quantification

Total RNA was isolated from liver, pancreas, and RIN-m5F cells using the RNeasy kit (Qiagen, Valencia, CA); and integrity was assessed as the 28S to 18S ribosomal RNA ratio by capillary electrophoresis. RNA was converted to complementary DNA using the iScript complementary DNA synthesis kit

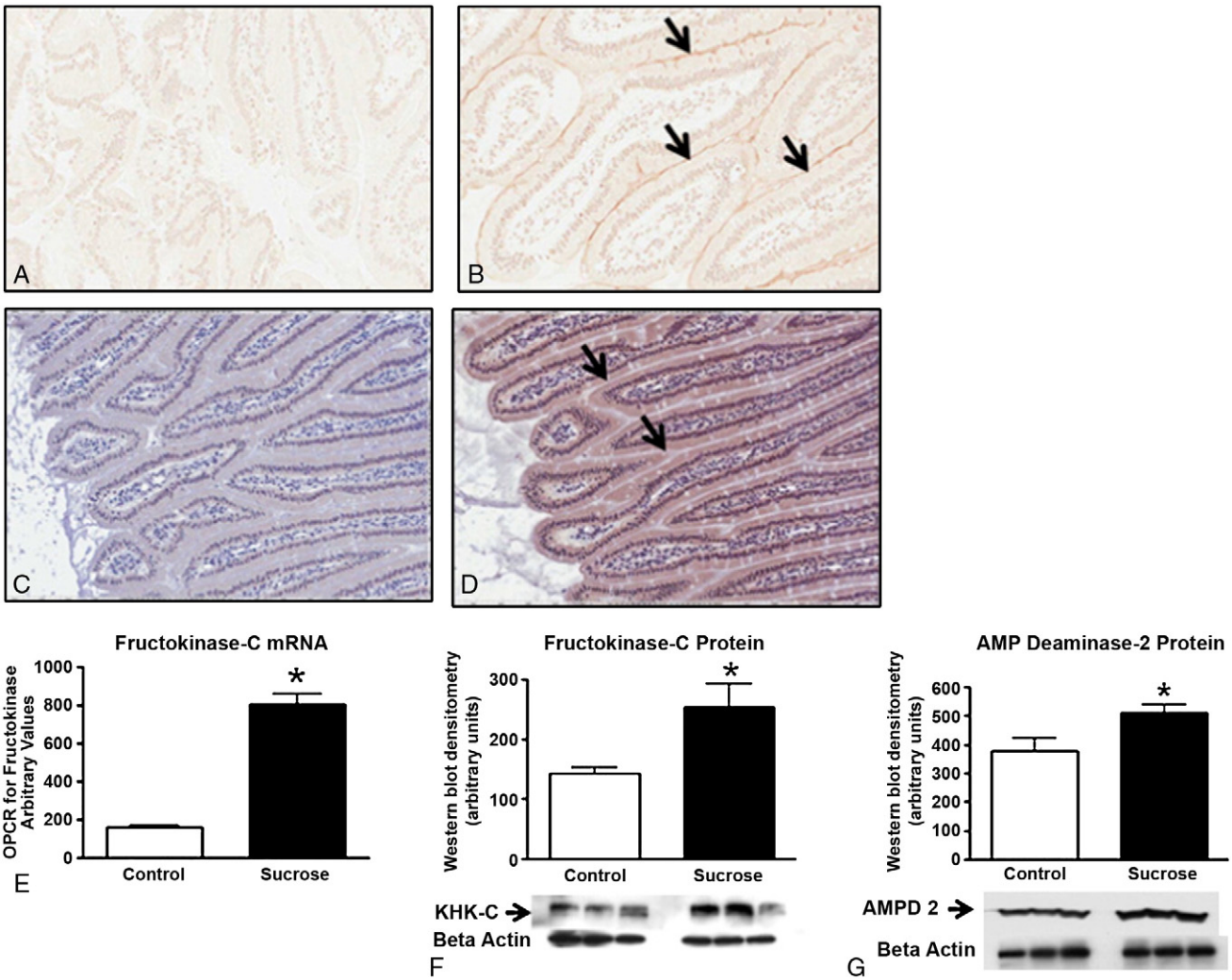


Fig. 1 – Expression of Glut5 and fructokinase. Jejunal tissues obtained from starch- and sucrose-fed rats were examined for the fructose transporter Glut5 and for fructokinase-C. In contrast to control rats (A), immunostaining showed the expression of Glut5 along the apical border (B, light brown line highlighted by arrows). Compared with control rats (C), fructokinase was also increased in the cytoplasm of the sucrose-fed rats (D, brown-colored cytoplasm, see arrows). Sucrose-fed rats showed an increased hepatic expression of fructokinase mRNA (E), fructokinase protein (F), and AMPD2 protein (G) compared with starch-fed rats. A and B, 40×; C and D, 20×. *P < .05.

(Bio-Rad). Quantitative polymerase chain reaction (PCR) primers specific to rat KHK-C (for GGTGTGGATGTGTCTCAAGTG, rev TGGCAGGTTCGTGTC), FAS (for GCGAGTCTATGCCACTATTC, rev AGCTGATACAGAGAACGGATG), adenosine triphosphate citrate lyase (ACL) (for CAGTGAACAACAGACCTATGAC, rev CAATGCTGCCCTCCAATGATG), chemokine (C-X-C motif) ligand 1 (KC or CXCL-1) (for GATGGCGTCTGTCTGGTG, rev AGGACCCTCAATAGAAATCGTAAA), monocyte chemoattractant protein–1 (MCP-1) (for GCATCAACCCTAAGGACTTCA, rev GCATCACATTCCAAATCACACT), interleukin-6 (IL-6) (for GGACCAAGACCATCCAACTC, rev CAACATTCATATTGCCAGTTCT), insulin (for ACAGCACCTTTGTGGTCC, rev GGACTCAGTTGCAGTAGT), and β-actin (for TATCGGCAATGAGCGGTTC, rev AGCACTGTGTTGGCATAGAG) were designed using Beacon Designer 5.0 software (Premier Biosoft International, Palo Alto, CA). Quantitative PCR was performed using the primers at 70 mol/L and the SYBR Green JumpStart Taq Readymix qPCR kit (Sigma) on a Bio-Rad I-Cycler. Quantitative PCR runs were analyzed by agarose gel electrophoresis and melt curve to verify if the correct amplicon was produced. β-Actin RNA was used as internal control, and the amount of RNA was calculated by the comparative C_T method as recommended by the manufacturer.

2.8. Cell culture studies

The established rat insulinoma cell line RIN-m5F (American Type Culture Collection, Manassas, VA) cells were grown in RPMI 1640 medium (Gibco, Carlsbad, CA) supplemented with 10% fetal bovine serum and 100 U/mL penicillin and 100 μg/mL streptomycin. To test the effects of uric acid, the media in culture dishes were supplemented with uric acid (0-6 mg/dL) for different time points. Water-soluble probenecid (Molecular Probes, Eugene, OR) was used at 2-mmol/L concentration. Experiments were initiated once cells reached confluence in 24-well flat-bottom tissue culture plates (35-3047; Falcon BD Labware, Franklin Lakes, NJ), with each experimental time point after exposure to uric acid performed in triplicate.

2.9. Determination of intracellular oxidative stress in RIN-m5F cells

Reactive oxygen species in live RIN-m5F cells plated in 96-well plates was determined using the Image-iT LIVE Green Reactive Oxygen Species Detection Kit I36007 (Molecular Probes) as per manufacturer's instructions. Briefly, cells were grown to 90% confluency in the presence or absence (control) of uric acid (3 or 6 mg/dL) alone or in combination with 2 mmol/L probenecid for 24 hours. After treatment, cells were washed with warm phosphate-buffered saline and incubated with 25 μmol/L of the cell-permeant fluorescent probe carboxy H2-dichlorodihydrofluorescein (DCF) for 30 minutes at 37°C protected from light. After incubation, cells were washed with warm buffer; and total fluorescence was determined using a multimode microplate reader (Biotek Synergy 2, Winooski, VT) with the following settings: excitation, 458 ± 20 nm; emission, 528 ± 20 nm. The DCF fluorescence signal was expressed as total fluorescence intensity normalized to milligram of soluble protein.

2.10. Statistical analysis

Data are presented as least square mean ± SE unless otherwise noted. The starch- and sugar-treated groups were compared using mixed-model repeated-measures analysis with preplanned contrasts to take into account correlated measurements for each rat. Within-group comparisons were also analyzed using mixed models in paired analyses. At 4 weeks, data were obtained on 5 rats in each group; but estimates were obtained using PROC MIXED in SAS (9.2; Cary, NC). Significance was defined as $P < .05$ for preplanned contrasts.

3. Results

Rats were pair fed isocaloric diets containing sucrose or starch for 4 months. Because all rats were pair fed, all rats received the identical energy intake.

3.1. Effect of sucrose diet on fructose transport and metabolism

Diets high in fructose (50%-60%) up-regulate Glut5 (fructose transporter in the gut) and fructokinase in the intestine and liver [16-19]. As shown in Fig. 1, rats fed 40% sucrose (which contains 20% fructose) showed increased Glut5 (jejunum) and KHK-C expression in their jejunum and liver. Adenosine monophosphate deaminase is stimulated during fructose metabolism in vitro, which eventually leads to the generation of uric acid [20]. The expression of AMPD2 (the major hepatic isoform) was significantly increased in

Table 2 – Metabolic parameters at various times following diet

	Control	Sucrose fed	P values
Baseline			
Body weight (g)	606 ± 11	590 ± 11	NS
Fasting blood glucose (mg/dL)	94 ± 10	95 ± 11	NS
Serum uric acid (mg/dL)	2.8 ± 0.2	3.0 ± 0.2	NS
Serum triglyceride (mg/dL)	111 ± 19	101 ± 19	NS
Serum insulin (pg/mL)	248 ± 93	234 ± 94	NS
4 wk			
Body weight (g)	606 ± 11	596 ± 11	NS
Fasting blood glucose (mg/dL)	82 ± 14	108 ± 14	NS
Serum uric acid (mg/dL)	2.6 ± 0.2	3.5 ± 0.2*	.007
Serum triglyceride (mg/dL)	121 ± 26	287 ± 26*	.0002
Serum insulin (pg/mL)	754 ± 119*	1151 ± 119*	.03
16 wk			
Body weight (% change)	-0.85 ± 1.3	2.69 ± 1.7	NS
Body weight (g)	601 ± 11	604 ± 11	NS
Fasting blood glucose (mg/dL)	124 ± 11	181 ± 14*	.001
Serum uric acid (mg/dL)	2.2 ± 0.2*	2.3 ± 0.2*	NS
Serum triglyceride (mg/dL)	165 ± 19	165 ± 21	NS
Serum insulin (pg/mL)	1198 ± 100*	801 ± 100*	.01

Data are presented as least square mean ± SE. NS indicates not significant.
* $P < .05$ within-group comparisons to baseline.

rats on the sucrose diet ($P < .05$, Fig. 1). Thus, a 40% sucrose diet up-regulates transporters and enzymes involved in fructose metabolism.

3.2. *Effect of sucrose diet on metabolic syndrome*

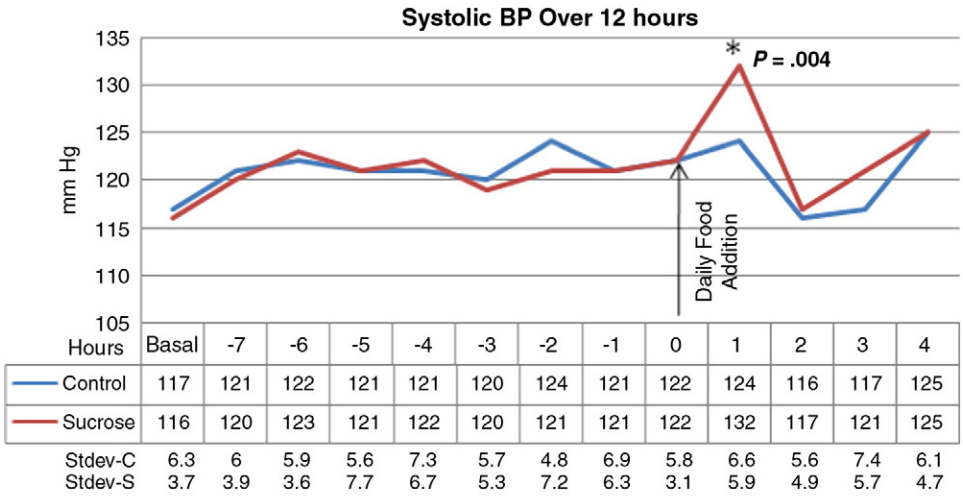
At baseline, mean serum glucose, fasting insulin levels, and serum triglycerides were in the reference range in both groups (Table 2). At 4 weeks, control rats developed fasting hyperinsulinemia, although serum glucose and triglycerides remained in the reference range (Table 2). However, sucrose-fed rats developed significantly higher levels of triglycerides, serum insulin, and uric acid values, consistent with an insulin-resistant state. At 16 weeks, control rats showed borderline elevated blood glucose levels with hyperinsulinemia and hypertriglyceridemia. Sucrose-fed rats had significantly higher blood glucose levels; however, the striking finding was that serum insulin levels had fallen to levels below that observed in the control rats (Table 2).

Mean body weights were not different between control and sucrose groups at any time during the study (Table 2).

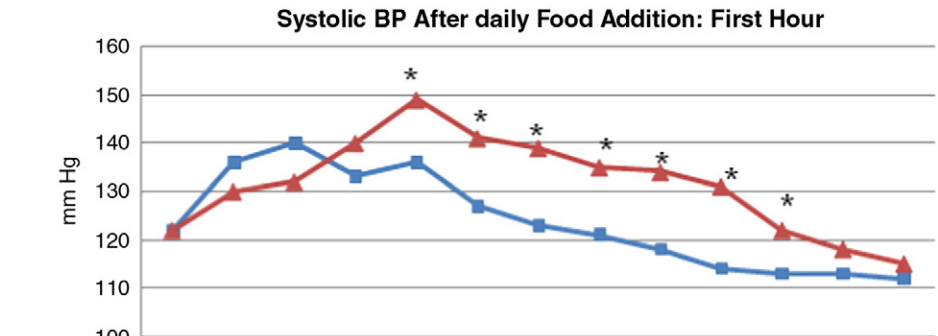
Blood pressure was measured in conscious unrestrained rats using an aortic telemetry system. Mean 24-hour BP, measured at monthly intervals, were not different between groups. However, when hourly BP measurements were obtained at each of these time points, a consistent increase in systolic BP was observed in the sucrose-fed rats during the first hour after food was provided (Fig. 2).

3.3. *Sucrose diet induces fatty liver*

Diets high in fructose (60%) induce fatty liver in male Sprague-Dawley rats, but the effects of lower concentrations of fructose on fatty liver are unknown. As shown in Fig. 3, sucrose-fed rats developed macro- and microvesicular steatosis as noted by oil



Hours	Basal	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4
Control	117	121	122	121	121	120	124	121	122	124	116	117	125
Sucrose	116	120	123	121	122	120	121	121	122	132	117	121	125
Stdev-C	6.3	6	5.9	5.6	7.3	5.7	4.8	6.9	5.8	6.6	5.6	7.4	6.1
Stdev-S	3.7	3.9	3.6	7.7	6.7	5.3	7.2	6.3	3.1	5.9	4.9	5.7	4.7



Minute	0	5	10	15	20	25	30	35	40	45	50	55	60
Control	122	136	140	133	136	127	123	121	118	114	113	113	112
Sucrose	122	130	132	140	149	141	139	135	134	131	122	118	115
Stdev-C	5.8	7	7.3	5.8	11.6	10.8	10.4	13.3	10	6.9	5.5	10.9	7.5
Stdev-S	3.1	9.5	7.3	12	5.1	8.7	8.4	10	12	13	10	9.2	6
P value		0.148	0.055	0.134	0.016	0.010	0.003	0.026	0.014	0.007	0.044	0.293	0.374

Fig. 2 – Systolic BP in control vs sucrose-fed rats. Upper panel: Systolic BPs as measured by telemetry over a 12-hour period. Mean BPs remain no different between control (starch fed, blue line, n = 8) and sucrose-fed (red line, n = 9) rats except for the first hour after introduction of food. Lower panel: Serial BPs during the first hour after exposure to food shows significant increases in BP in the sucrose-fed rats. *P = .004. Shown are measurements obtained at 2 weeks after initiating the diet.

red O stain. Previous studies have suggested that sucrose and fructose induce both an increase in fat synthesis and a decrease in fat oxidation [21,22]. Consistent with this observation, Western blotting livers of sucrose-fed rats showed increase in FAS and ACL (involved in fat synthesis) and decreases in ECoAH1 (involved in β fatty acid oxidation (Fig. 3).

3.4. *Sucrose effects on the pancreas*

Pancreatic tissues from sucrose-fed rats showed hyalinization with focal inflammatory infiltrates (Fig. 4). Macrophage infiltration was greater in the pancreatic interstitium of sucrose-fed rats compared with starch-fed rats (4.8% vs 1.6% interstitial area, $P < .0006$). In contrast, infiltration of macrophages within the islets was not different between groups, although they tended to be greater in sucrose-fed rats (5.2% vs 2.6% islet area, $P < .08$). These findings were accompanied by an increase in whole pancreas messenger RNA for chemokine (C-X-C motif) ligand 1 (KC or CXCL-1), MCP-1, and IL-6 (Fig. 4). There was also significantly less insulin staining in the islets, consistent with the lower insulin levels and higher glucose levels observed in the blood in the sucrose-fed rats (Fig. 5 and Table 2).

3.5. *Potential involvement of uric acid in islet inflammation*

Uric acid is generated during sucrose and fructose metabolism because of the breakdown of adenine nucleotides and the stimulation of AMP deaminase [20]. Uric acid has been shown to enter cells via specific transporters such as URAT1 where it can induce proinflammatory and prooxidative effects [23,24]. Interestingly, URAT1 expression was increased in the islets and pancreatic blood vessels of sucrose-fed rats by immunostaining (Fig. 6). Western blotting confirmed an increase in URAT1 protein in whole pancreas. In addition, incubation of RIN-m5F cells with uric acid resulted in a stimulation of several inflammatory mediators, including MCP-1, KC, and IL-6 messenger RNA (mRNA), as well as intracellular oxidative stress as determined by increased intensity of the fluorescence probe DCF. Uric acid–dependent effects could be inhibited by probenecid, an organic anion transport inhibitor that has been shown to block URAT1-mediated uptake of uric acid in proximal tubular cells [25] (Fig. 7).

4. Discussion

Although high concentrations of fructose are known to induce metabolic syndrome in male Sprague-Dawley rats, the studies have been criticized as being nonphysiologic. In addition, studies using pure fructose have been criticized because they do not mimic dietary habits in humans in which most fructose is from added sugars that also contain glucose either as a disaccharide (sucrose) or as a mixture of free monosaccharides (high-fructose corn syrup). Furthermore, there is significant debate over whether the effects of fructose (or of added sugars in general) induce features of metabolic syndrome

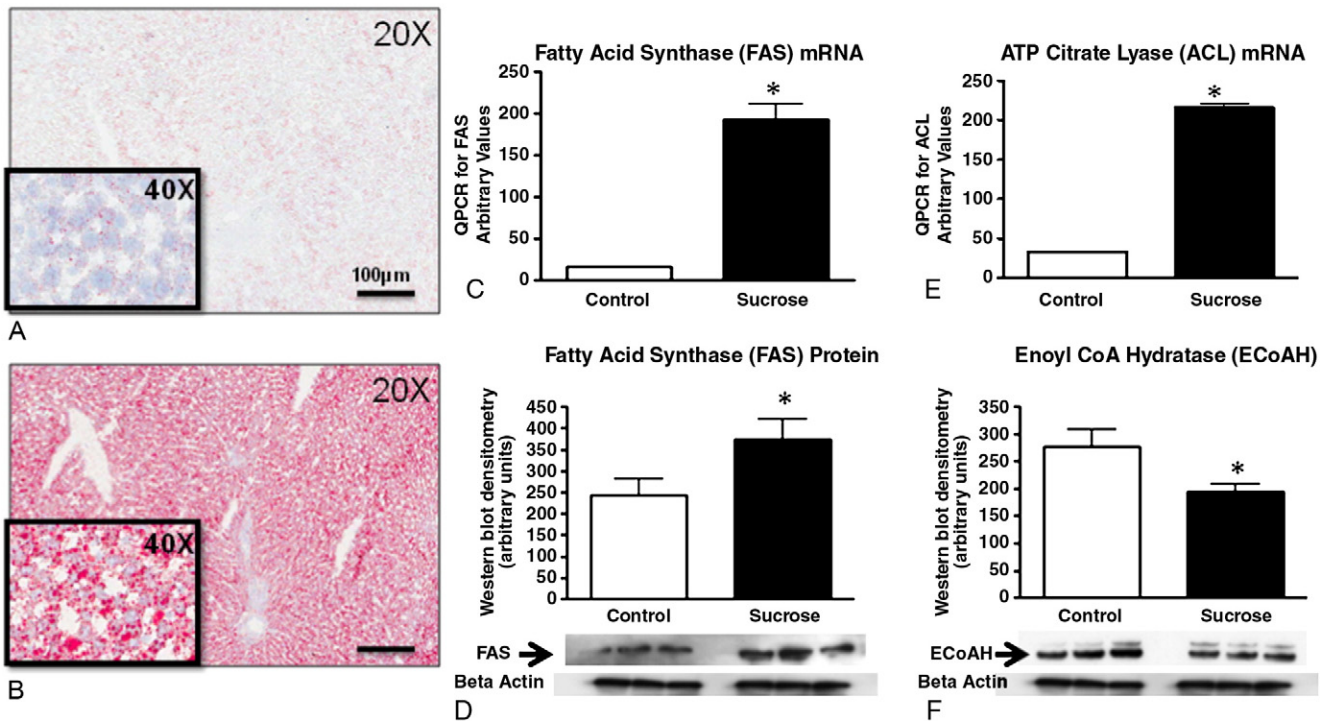


Fig. 3 – Effects of diet on fatty liver. Rat liver tissue obtained at 4 months shows negative staining for fat by oil red O stain in starch-fed, control rats (A), whereas diffuse micro- and macrovesicular fat deposits are present in sucrose-fed rats (B). Quantitative PCR analysis of whole liver tissues shows an increase in FAS (C) and ACL mRNA (D) in sucrose-fed rats. Western blots confirmed an increase in FAS protein (E) and a decrease in ECoAH1 protein in sucrose-fed rats (F). Key: A and B, oil red O stain, 20× magnification. *$P < .05$

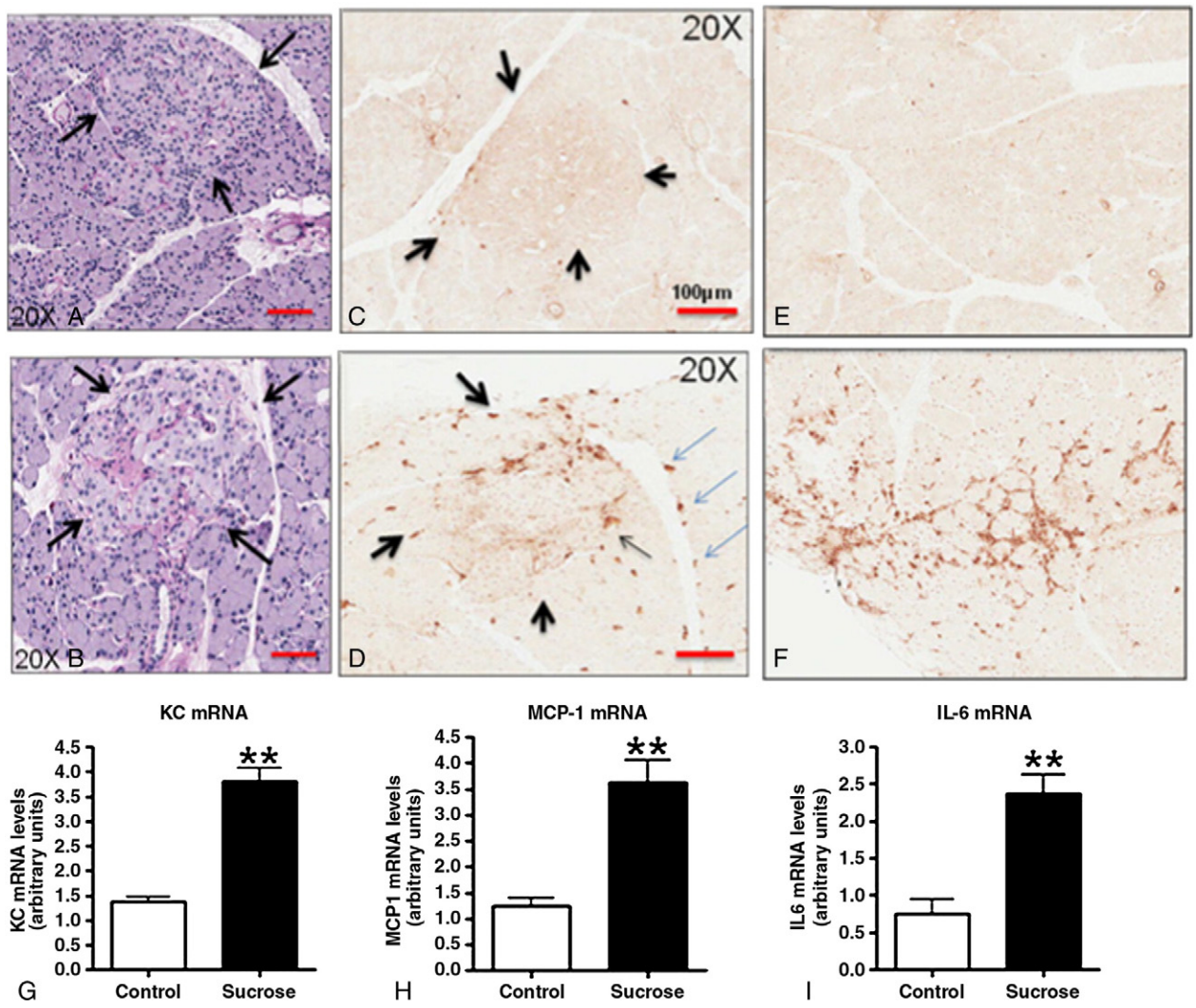


Fig. 4 – Islet injury and inflammation. Periodic acid Schiff–stained pancreas shows that islets from starch-fed control rats (identified by arrows) appear normal (A, 20×). In contrast, islets from sucrose-fed rats show focal hyalinization (B, 20×). Whereas macrophages (ED-1 staining) were infrequent in islets (C, 20×) and pancreatic interstitium (E, 10×) of starch-fed rats, macrophages were present in both the islets (D, arrows) in venous endothelium (D, light blue arrows) and the interstitium (F, 10×) in sucrose-fed rats. These changes were associated with an increase in KC mRNA, MCP-1 mRNA, and IL-6 mRNA in whole pancreas RNA extracts from sucrose-fed rats compared to starch-fed controls (G-I). *P < .05; **P < .01.

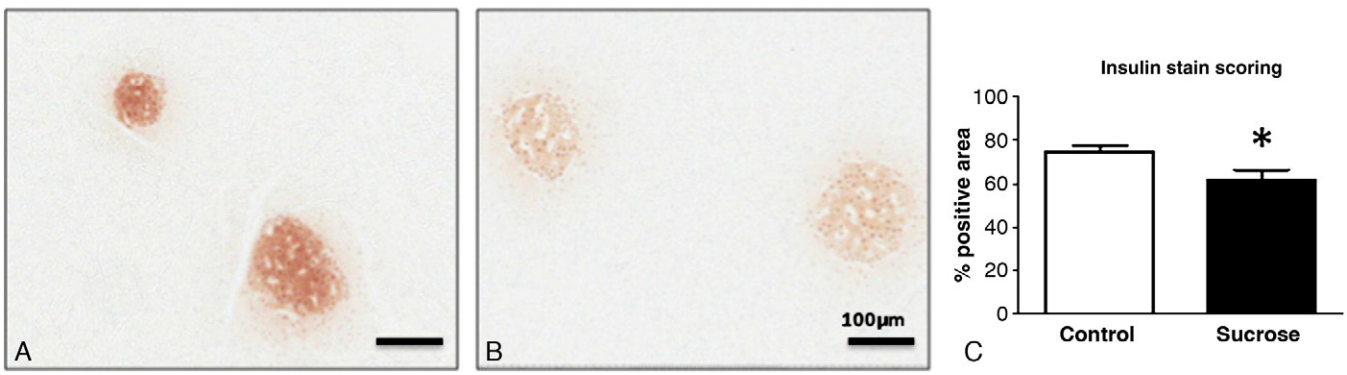


Fig. 5 – Decreased insulin staining and expression of inflammatory mediators in sucrose-fed rats. In contrast to starch-fed rats (A), sucrose-fed rats had decreased insulin staining of islets by both intensity and area (B) (A, B, magnification 20×). Quantification documented decreased insulin staining area (C). Insulin staining of islets quantified by computer image analysis. *P < .05; **P < .01. 10×.

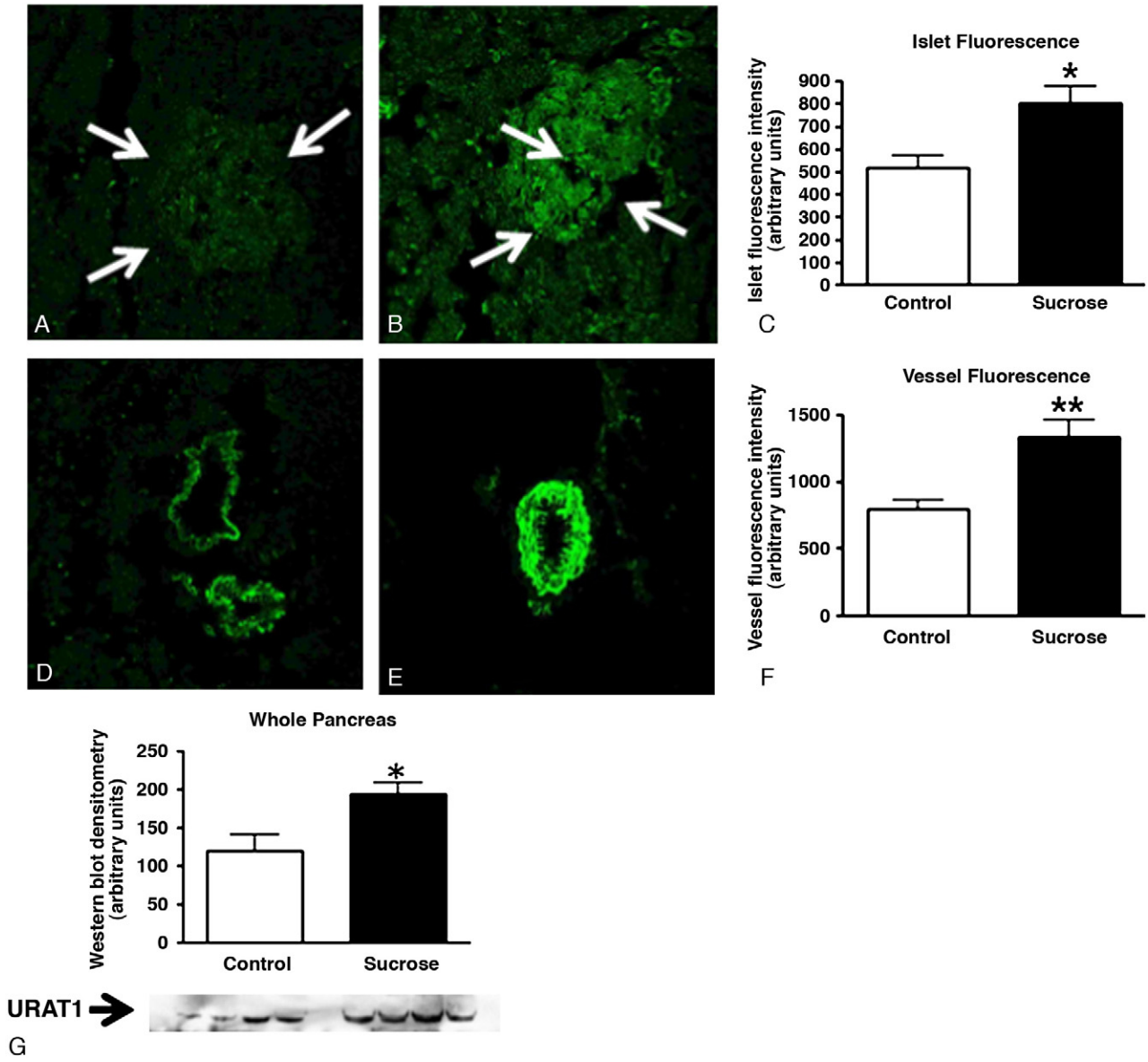


Fig. 6 – URAT1 expression in pancreatic islets. Minimal URAT1 was expressed by islets in starch-fed rats by immunofluorescence (highlighted by arrows, A) in contrast to sucrose-fed rats (B). Blood vessels also showed minimal URAT1 staining in starch-fed (D) in contrast to sucrose-fed rats (E). (A, B, D, E, magnification 40×.) Quantitative immunofluorescence for URAT1 staining in the islets and blood vessels is shown in C and F. Total URAT1 protein in whole pancreas by Western blotting showed an approximately 30% increase in URAT1 in sucrose-fed rats (G). The relatively small increase in URAT1 protein by Western blot despite the marked difference in immunostaining is likely because the former is of whole pancreatic extracts that include the acinar tissue. *P < .05. 20×.

independent of excessive energy intake. The purpose of this study was therefore to determine if metabolic syndrome can be induced in rats by administering a sucrose-based diet in which the fructose content is substantially less than classic models, and whether the effects of sucrose are independent of excessive energy intake. Studies were performed in older male Sprague-Dawley breeder rats, which are known to develop features of metabolic syndrome as they age [12,13]. Hence, these studies address whether sucrose diets can accelerate metabolic syndrome and islet abnormalities as opposed to causing these effects de novo.

Rats were administered 40% sucrose or starch diets for 4 months. The diets were identical in all nutrients except that sucrose was replaced with starch, and all rats were pair fed to ensure identical caloric intake. We also mildly diet restricted all rats to ensure that there would be no concern that intake was not excessive. Furthermore, this approach might simulate individuals who calorically restrict as an attempt to diet but who may not reduce their sugar intake. The first new finding was that the sucrose-based diet induced up-regulation of Glut5 and fructokinase in the jejunum and jejunum and liver, respectively. Fructose absorption is known to vary

dramatically in individuals, and this may be due to variable expression of Glut5 [26]. The observation that a sucrose-based diet containing 20% fructose can up-regulate the Glut5 transporter suggests that diets high in added sugars may increase the absorption of fructose and hence its metabolic effects. These data are consistent with our observation that fructokinase mRNA expression is increased in subjects with nonalcoholic fatty liver disease who are known to be ingesting high levels of fructose-containing beverages [27]. This could provide a potential explanation for why the effect of fructose on insulin resistance and serum triglycerides is greater in overweight or hyperinsulinemic individuals [28,29].

The second new finding was that a sucrose-based diet containing 20% fructose could accelerate the development of metabolic syndrome and cause fatty liver. Previously, most studies in rats have evaluated the effects of high concentrations of fructose on metabolic syndrome and/or have not pair fed rats given control diets to determine if the effects are independent of energy intake [30,31]. Our studies show that, even when diets are restricted to 90% of normal diet, features of metabolic syndrome can be accelerated in the male Sprague-Dawley rat. The most impressive finding related to the effects on the liver, where fatty liver developed only in the sucrose-fed rats.

A third finding was that sucrose intake could increase BP during the immediate postprandial period. Studies in rats have shown that 24-hour BP is not increased by telemetry with diets high in fructose [32]. However, a recent study by Brown et al [33] reported that the administration of fructose, but not glucose, will *acutely* raise BP in humans. Studies in mice have also confirmed that fructose raises systemic BP by intraaortic telemetry during feeding [34]. The mechanism may relate to the rapid rise in both intracellular and serum uric acid that occurs with fructose ingestion [35,36]. Increasing serum uric acid has been found to raise BP in animals [37]. Hyperuricemia is also associated with hypertension in humans; and in pilot studies, the lowering of uric acid can reduce BP [38,39]. Although short-term trials have generally not shown an effect of fructose on BP in humans, a significant increase in BP with large amounts of fructose was shown in the Menorca study [40]. Furthermore, in this study, the rise in BP was associated with an increase in fasting uric acid levels; and the rise in both uric acid and BP was prevented in the subjects randomized to allopurinol [40]. The chronic ingestion of fructose-containing drinks is also associated both with an elevation of serum uric acid [41] and with the development of hypertension independent of body mass index [36,42]. Furthermore, epidemiology studies suggest that reducing sugar-containing soft drinks results in a reduction in BP independent of weight change [43].

Finally, this study documented that sucrose could induce pancreatic inflammation. Specifically, sucrose-fed

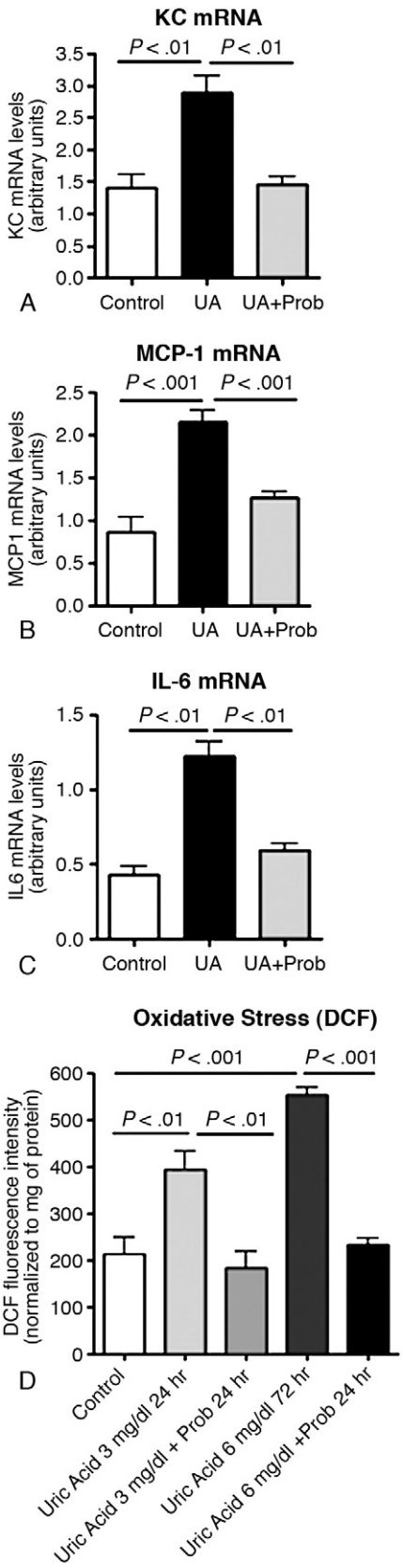


Fig. 7 – Effect of uric acid on rat pancreatic islet cells. Pancreatic RIN-m5F cells were exposed for 48 hours to varying concentrations of uric acid in the presence or absence of the URAT1 inhibitor probenecid (1 mmol/L). Uric acid (UA, 6 mg/dL) significantly increased the mRNA expression of the inflammatory markers KC (A), MCP-1 (B), and IL-6 (C). Uric acid (3 and 6 mg/dL) also stimulated oxidative stress, as assessed by oxidized DCF fluorescence (D). The effects of uric acid to induce cytokine expression and oxidative stress were blocked by probenecid (2 mmol/L) (A-D).

rats showed evidence for mild islet injury associated with a reduction in insulin by immunostaining and with the development of type 2 diabetes mellitus. Macrophage infiltration was increased in the interstitium of the pancreas and tended to be higher in the islets, and this was associated with increased mRNA expression in whole pancreatic RNA for a variety of inflammatory mediators including MCP-1. Although there are likely many mechanisms possible to account for these findings, we performed pilot studies to evaluate the role of uric acid. The rationale is based on the fact that the metabolism of fructose in the liver results in the stimulation of AMP deaminase with the production of uric acid intracellularly that then results in a rise in serum uric acid. Although serum uric acid is most elevated during fructose ingestion, we also observed higher levels during fasting at week 4 (Table 2). In turn, uric acid has been shown to enter a variety of cells via organic anion transporters such as URAT1 [23,44] where it can induce proinflammatory and prooxidative effects [45-50]. Uric acid has also been found to independently predict the development of insulin resistance and type 2 diabetes mellitus by meta-analysis [51]. Of particular interest are reports by Wexler [52] and Wexler and Greenberg [53] that chronic hyperuricemia induced by a uricase inhibitor can induce hypertension, hypertriglyceridemia, fatty liver, and diabetes in the male Sprague-Dawley rat. Of interest, the ability to induce these changes correlated with the serum uric acid levels, although proof that this was due to uric acid by studies using uric acid–lowering therapy was not found [52,53]. Furthermore, in one of these studies, a comparison of sex and breeder status was performed; and the male breeder rats showed the greatest elevations in uric acid and the most severe metabolic changes [53].

In turn, we found that URAT1 expression was induced in the islets of sucrose-fed rats and that uric acid could induce proinflammatory and prooxidative effects in islet cells that could be prevented by incubation with the organic anion transport inhibitor probenecid. Others have also reported that fructose-based diets can induce β-cell loss in a genetic model of insulin resistance [9-10] as well as in normal Wistar rats [11]. Recently, Cummings et al [6] also reported that a diet of 10% fructose could accelerate the development of type 2 diabetes mellitus in a specific diabetes-prone rat in association with a reduction in islet cells. There is also a report that soft drink ingestion correlates with evidence for β-cell dysfunction in Hispanic adolescents [54]. Collectively, these data support the possibility that fructose-containing sugars may increase the risk for diabetes not only by causing insulin resistance but by direct effects on the islets themselves and that one possible mechanism may involve the ability of fructose to raise serum uric acid levels.

This study has several limitations, including small sample size and potential individual variability in rate of food intake. We also used male Sprague-Dawley breeder rats, which are known to be prone to develop metabolic syndrome and pancreatic islet changes as they age [12-13]. Fructose is also known to be more likely to induce metabolic changes in older male rats [55-57] and in older male humans [58,59]. Hence, our studies may or may not be relevant to younger or female animals. We are currently studying potential mechanisms to account for the sex- and age-based differences in the metabolic responses to sucrose and/or fructose with a hypothesis that this may relate to the degree of intracellular and extracellular uric acid generation.

In conclusion, sucrose is not simply an energy source that may have a role in obesity, but rather has specific metabolic effects that favor the development of fat accumulation and insulin resistance independent of excessive energy intake. These observations are consistent with a recent meta-analysis that has found that the intake of sugar-sweetened beverages increases the risk for metabolic syndrome and diabetes in humans [8]. Further studies investigating the potential mechanisms by which sucrose may lead to these metabolic alterations are needed.

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Conflict of Interest

Dr R Johnson, Dr Nakagawa, and Dr Lanaspa have patent applications related to lowering uric acid or blocking fructose metabolism in the treatment of metabolic syndrome. Dr Johnson also has a book, *The Sugar Fix* (Rodale, 2008; and Simon and Schuster, 2009), which discusses the potential role of fructose in the obesity epidemic.

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