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Cardiovascular Interactions Between Losartan and Fructose in Mice

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Abstract

Aim—To determine whether pharmacological blockade of angiotensin (Ang) ATI receptors alters the cardiovascular, metabolic, and angiotensin-converting enzyme (ACE and ACE2) responses to a fructose diet in mice.

Methods—C57BL male mice were fed with a 60% fructose diet for 8 weeks in combination with losartan treatment on week 9 (30 mg/kg per day). Blood pressure (BP), heart rate (HR), and autonomic balance were monitored using radiotelemetry with spectral analysis. Renal ACE and ACE2 activity and protein levels as well as Ang II and Ang 1-7 were measured.

Results—Fructose impaired glucose tolerance and increased plasma cholesterol and insulin. These effects were not corrected by losartan treatment. Fructose increased BP and HR but only during the dark period. Short-term losartan treatment decreased BP by 16% in the fructose group but had no effect in controls. This was accompanied by a decrease in BP variance and its low-frequency component. Fructose increased Ang II (plasma and kidney) and ACE 2 (renal activity and protein expression). Losartan alone increased plasma Ang II in plasma and ACE2 in kidney. There were no changes in renal Ang 1-7 levels.

Conclusions—Losartan reversed the pressor effect of a high fructose diet, demonstrating that there are prominent interactions between a dietary regimen that produces glucose intolerance and an antihypertensive drug that antagonizes Ang signaling. The mechanism of change may be via renal Ang II rather than the ACE2/Ang 1-7 pathway because the fructose losartan combination resulted in lowered renal Ang II without changes in Ang 1-7.

Keywords

angiotensin; autonomic nervous system; blood pressure; fructose; losartan; metabolism; mice; renin angiotensin system; glucose tolerance

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Declaration of Conflicting Interest

The authors declared that they had no conflicts of interests with respect to their authorship or the publication of this article.

Introduction

Consumption of high levels of fructose in humans and animals leads to insulin resistance, obesity, cardiovascular and autonomic dysfunction, and lipid abnormalities.^{1,2} Insulin resistance plays an important role in the progression of type 2 diabetes, which is associated with activation of the renin angiotensin system (RAS) and cardiovascular and autonomic dysfunction.³⁻⁵ Angiotensin (Ang) receptor blockers (ARBs) are a method of choice for treatment of diabetics because they not only reduce blood pressure (BP) but also improve metabolic status.⁶

In humans, acute fructose ingestion increased BP and pressure variability, an indication of sympathetic activation.⁷ Likewise, we showed that a high fructose diet in mice altered BP and sympathetic balance.⁸ Blood pressure and its variance were increased but only during the dark period when the mice are active and sympathetic modulation is greatest. These findings raise concerns because fructose ingestion is associated with autonomic dysfunction, which in turn has been linked to end-organ damage and higher risk of mortality in diabetic patients.⁹

The rodent fructose model is a recognized experimental syndrome of insulin resistance and metabolic and cardiovascular dysregulation.^{8,10-12} Fructose consumption has been shown to activate the renin Ang and sympathetic nervous systems.^{8,13} Renin angiotensin system blockade using ACE inhibitors (ACEi) or ARBs ameliorates the cardiovascular and metabolic effects of a high fructose diet.^{13,14} A role for the RAS in the cardiovascular dysfunction produced by fructose was further documented using Ang AT1a gene deletion mice.¹⁵ In this genetic model, a fructose diet lowered BP rather than increasing it and reduced plasma Ang II. This provided further evidence for a role of Ang and Ang AT1 receptors in the pathological sequel to fructose.

Angiotensin-converting enzyme and ACE 2 are critical in Ang peptide metabolism and play counteracting physiological roles.¹⁶ With regard to the synthetic pathways, ACE2 cleaves Ang I and Ang II, generating Ang 1-9 and Ang 1-7, respectively, while ACE is important in formation of Ang II. Angiotensin-converting enzyme is present in the circulation and tissues, while ACE2 is most prominent in a variety of tissues.¹⁷ The fact that Ang 1-7 is a vasodilator and has growth effects opposite to that of Ang II has led to the idea that ACE 2 counterbalances ACE.

It has been shown previously that organ-specific ACE 2 is modified in experimental diabetes¹⁸⁻²⁰ as well as after ACE blockade.²¹ In *db/db* diabetic mice, renal ACE2 expression is increased prior to the development of nephropathy.^{19,22} With the progression of kidney disease, renal ACE2 expression and activity are decreased.²³ Further evidence for renoprotective effects of ACE2 was seen in animals lacking ACE2 or those treated with ACE2 inhibitors in which diabetes-induced kidney damage was extensive.²⁴ In the fructose model, there is activation of the RAS, seen as increases in plasma and aortic ACE, as well as higher levels of plasma Ang II.^{8,13} There is no information on ACE2 status in animals on a high fructose diet and questions as to its involvement in the autonomic and cardiovascular pathologies.

The objective was to determine whether short-term, pharmacological blockade of Ang AT1 receptors ameliorated the BP and autonomic responses to a high fructose diet and to determine whether the effects might be explained by changes in renal and circulating ACE, ACE2, and Ang peptides. Experiments combined integrative methods for study of changes in BP and autonomic balance with biochemical assays for Ang peptides and ACE and ACE2 activity and protein.

Methods

Animals and General Procedures

C57BL/6 male mice (Harlan Inc, Indianapolis, Indiana), body weight ~20 g, were randomly assigned to standard (58% carbohydrate, 30% protein, and 12% fat) or high fructose diet (67% carbohydrate—of which 98% is fructose, 20% protein, and 13% fat). Animals were housed at 22°C under a 12/12 light/dark cycle with ad libitum access to water and pellet chow (standard or high fructose). After 7 weeks of diet, radiotelemetric probes were inserted into the carotid artery. Losartan (30 mg/kg per day) was given in the drinking water for 1 week (week 9 of diet). Cardiovascular measurements were made at 8 (baseline control and baseline fructose) and 9 weeks (losartan control and losartan fructose). The time points were chosen because previous data from our laboratory showed that the nocturnal increase in BP was established after 7 weeks ingestion of a high fructose diet.

For metabolic measurements, animals were submitted to the same diet and drug regimen. Animals were decapitated at the end of week 9 for blood and tissue collection. There were four groups: control, fructose, control/losartan, and fructose/losartan. Plasma aliquots, with and without the protease inhibitor, bestatin, were stored at -80°C. Kidneys were homogenized using lysis buffer (Complete Lysis-M, EDTA-free, Roche Diagnostics, Mannheim, Germany), centrifuged, and supernatant collected for peptide and enzyme activity assays. All experimental protocols were approved by the WSU Animal Care and Use Committee.

Cardiovascular Parameters

Cardiovascular parameters were recorded by radiotelemetry, baseline, and after losartan treatment. For probe insertion, mice were anesthetized with ketamine/xylazine (120:20 mg/kg, intramuscular [IM]) and radiotelemetric catheters (model TA11PA-C10, Data Sciences International, St Paul, Minne-sota) were inserted into the left common carotid artery. The transmitter body was positioned subcutaneously on the right flank.^{15,25} After mice had recovered from the surgery (~7 days), 2 types of cardiovascular recordings were made: (1) continuous 24-hour BP recordings for determination of light/dark patterns and (2) 60-minute BP recordings (5 kHz) made during the light/dark phases for the purpose of spectral analysis.

Variability and Spectral Analysis

Peak interval (PI) and systolic arterial pressure (SAP) variabilities (heart rate variability [HRV] and blood pressure variability [BPV], respectively) were assessed in time and frequency domains using autoregressive spectral analysis.²⁶ Peak interval and SAP series were divided into 350 beats segments and overlapped by 50%. A spectrum was obtained for each of the segments via Levinson-Durbin recursion, with the model order chosen according to Akaike's criterion, ranging between 10 and 14. The oscillatory components were quantified in low (low frequency [LF]: 0.1 to 1 Hz) and high (high frequency [HF]: 1 to 5.0 Hz) frequency ranges.

Glucose Tolerance Test

Glucose tolerance test (GTT) was performed before and after losartan treatment. Mice were fasted for 6 hours and blood samples were taken from a tail cut at 0, 15, 30, 60, and 90 minutes after intraperitoneal (IP) glucose load (1.5 g/kg). Blood glucose was determined by Accu-Check Advantage Blood Glucose Monitor (Roche Diagnostic Corporation, Indianapolis, Indiana).

Plasma Endocrine Measurements

Plasma Ang II was measured by radioimmunoassay (ALPCO Diagnostics, Windham, New Hampshire). Plasma adiponectin was measured by enzyme-linked immunosorbent assay (ELISA; Adiponectin: United Chemi-Com, Inc, Rosemont, Illinois). Total plasma cholesterol and triglycerides were determined by colorimetric enzymatic assays (Thermo Fisher Scientific Inc, Waltham, Massachusetts). Plasma insulin and leptin levels were measured using a multianalytic profiling beads assay (Linco Research Inc, St Charles, Missouri).

Kidney Ang II and Ang 1-7 Measurements

Kidneys were homogenized in acid/ethanol (80% vol/vol 0.1 N HCl) containing a cocktail of protease inhibitors including 0.44 mmol/L 1,20 ortho-phenanthroline monohydrate (Sigma, St Louis, Missouri.), 0.12 mmol/L pepstatin (Peninsula Labs, Belmont, California), 1 mmol/L Na *p*-hydroxymercuribenzoate (Sigma, St Louis, Missouri), 15% EDTA, and 0.01 mmol/L WFML-1 (acetyl-His-Pro-Phe-Val-Statine-Leu-Phe), a rat renin inhibitor (AnaSpec Inc, San Jose, California) centrifuged at 12 000 rpm for 20 minutes at 4°C and stored overnight at 4°C. Samples were recentrifuged at 12 000 rpm for 20 minutes at 4°C and supernatant was removed and added to 1% *n*-heptafluorobutyric acid (HFBA) and refrigerated overnight at -20°C. The supernatant was extracted using Sep-Pak columns activated with 5 mL wash of a mixture of HFBA: methanol (0.1%:80%) and sequential washes of 0.1% HFBA. After the sample was applied to the column, it was washed with 0.1% HFBA and followed with a water wash. The sample was eluted with 3.3 mL washes of a mixture of acid methanol (0.1%:80%). A sample of the homogenate was taken to determine total protein content (Bio-Rad Protein Assay Reagent, Hercules, California). The sample was eluted, reconstituted, and split for the 2 RIAs (radioimmunoassay). For Ang II, samples were reconstituted in assay buffer and for Ang 1-7, a Tris buffer with 0.1% bovine serum albumin (BSA) was used. Samples were corrected for recoveries. Angiotensin II was measured using the Alpco (Windham, New Hampshire) and Ang 1-7 was measured using the antibody previously described.²⁷

Plasma ACE and Kidney ACE and ACE2 Activity Measurements

Plasma ACE activity was measured by a surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF)-mass spectroscopy assay.²⁸ 2-(*N*-morpholine)-ethane sulphonic acid (MES) buffer, containing phenylmethylsulfonyl fluoride (PMSF; 2 mmol/L) and bestatin (20 µmol/L), was added to plasma (0.5 µmol/L). Samples were spiked with Ang I (5 µmol/L) and incubated at 37°C. Reaction mixture (1 µmol/L) was spotted onto weak cation exchange (WCX2) ProteinChip. Generated Ang II was directly read in a ProteinChip reader system, PBS (protein biology systems) II (Biosystems, Palo Alto, California). Kidney ACE activity was measured using an assay kit (ALPCO Diagnostics, Windham, NH) with synthetic H³-hippuryl-glycyl-glycine as a substrate. Liberated H³-hippuric-glycyl-glycine acid produced by ACE was measured using a β scintillation counter (Packard 1800TR Liquid Scintillation Analyzer). Angiotensin-converting enzyme 2 activity was measured in kidney extracts using fluorogenic substrate, Mca-APK [Dnp] (BIOMOL International, L.P. Plymouth Meeting, Pennsylvania), as described by Vickers et al²⁹ with some modifications. Angiotensin-converting enzyme 2 fluorogenic activity assays were performed with final concentration of 100 µmol/L fluorogenic substrate in total volume of 100 µL of the ACE 2 assay buffer (75 mmol/L Tris buffer, 300 mmol/L NaCl, 10 µmol/L of zinc chloride, pH 7.4). The reaction product (fluorescence units) was quantified using a standard solution of Mca (7-methoxycoumarin-4-yl)acetyl.

Western Blot Protein Measurement of Kidney ACE and ACE2

Kidneys extracts were mixed with 25 μ L Laemli loading buffer (4% sodium dodecyl sulfate [SDS], 100 mmol Tris Cl, pH 6.8, 20% glycerol, 0.2% bromophenol blue, and 200 mmol/L dithiothreitol) and boiled for 10 minutes. A total of 50 μ g protein per lane) were loaded onto a 10% SDS–tris-glycine polyacrylamide gel and separated by electrophoresis. Proteins were electrotransferred onto a polyvinylidene fluoride (PVDF) membrane in transfer buffer (20 mmol/L Tris, 150 mmol/L glycine, 400 mL methanol). Polyvinylidene fluoride membranes were blocked for 1 hour in 10% nonfat dry milk solution in 2% Tween-PBS. Membranes were incubated with the primary antibody, monoclonal mouse anti-ACE (1:200; Chemicon International Ltd, Temecula, California) or polyclonal rabbit anti-ACE2 (1:200; Santa Cruz Biotechnology Ltd, Santa Cruz, California) at 4 °C. Membranes were washed in Tween-PBS and incubated with the secondary antibody, horseradish peroxidase (HRP)-conjugated donkey antimouse immunoglobulin G (IgG; 1:40 000; Jackson ImmunoResearch Laboratories Inc, West Grove, Pennsylvania) for 2 hours and washed (3 $\times$ 15 minutes in Tris-buffered saline Tween [TBST]). β -actin western blotting was used as an internal standard to demonstrate equal loading of the protein samples. Blots were detected using enhanced chemiluminescence and visualized using Fuji film image analyzer (LAS 3000 image Quant, Sunnyvale, California). Blots were analyzed using ImageJ (NIH [National Institutes of Health]), an image processing program that calculates area and pixel value statistics of user-defined selections. Angiotensin-converting enzyme, ACE2, and β -actin bands were visualized at apparent molecular weights of 170, 90, and 43 kD, respectively.

Statistics

Values were expressed as mean $\pm$ SEM. Data were analyzed using 3-way analysis of variance (ANOVA) followed by Newman-Keuls test. Differences were considered statistically significant at $P < .05$.

Results

Body weight was not affected by the fructose diet or the combination with losartan treatment (Table 1). Plasma glucose was not changed by diet or drug treatment, while plasma insulin was increased in fructose mice (Table 1; $P < .05$, control vs fructose). There were no additional effects of losartan. Figure 1A shows the time course of the glucose tolerance test and Figure 1B the composite data from the GTT (area under the curve [AUC]). Fructose consumption impaired glucose tolerance with no effect of losartan (Figure 1B, $P < .05$ control vs fructose).

Cholesterol levels were increased in fructose-fed mice, with no additional effects of losartan (Table 1; $P < .05$, control vs fructose). Triglycerides, leptin, or adiponectin levels were not affected by fructose or losartan treatment (Table 1).

Light/dark patterns of mean arterial pressure (MAP) and HR were determined using 24-hour telemetric recordings. There was a significant main effect of light/dark on MAP (Table 2; $P < .0001$) and HR (Table 2; $P < .0001$). There was a clear light/dark rhythm for MAP and HR under all conditions ($P < .03$; dark vs light).

For MAP, there were significant effects of diet (Table 2; $P < .0001$) and interaction between diet and losartan treatment (Table 2; $P < .003$). Fructose increased MAP ($P < .05$; control vs fructose) but only during the dark period. Losartan treatment lowered MAP in fructose-fed mice by 16% ($P < .05$, control vs fructose. $P < .05$, untreated vs losartan) during dark phase and by 9% ($P < .05$, control vs fructose. $P < .05$, untreated vs losartan) during the light phase. Losartan had no effect on MAP in mice on the standard diet (Table 2).

With regard to HR, there was a main effect of diet (Table 2; $P < .0005$). As with MAP, dark phase HR was higher in fructose-fed mice (Table 2; $P < .05$, control vs fructose). Losartan had no effect on HR in either control or fructose groups (Table 2).

Autoregressive spectral analysis of SAP and PI were used to evaluate autonomic modulation of the heart and vasculature. For both SAP variance and SAP-LF (Table 3), there were significant effects of light/dark (variance: $P < .00003$. LF: $P < .00005$) and interaction between diet and treatment (variance: $P < .05$. LF: $P < .03$) and light/dark and treatment (variance: $P < .003$. LF: $P < .005$). Control and fructose-fed mice showed a light/dark rhythm for SAP variance (Table 3; $P < .03$, dark vs light) and SAP-LF (Table 3; $P < .03$, dark vs light). The cardiovascular rhythms were abolished after losartan treatment. The fructose diet increased both SAP variance (Table 3; $P < .05$, control vs fructose) and SAP-LF (Table 3; $P < .05$, control vs fructose) during the dark phase. Systolic arterial pressure variance (Table 3; $P < .05$, untreated vs losartan) and SAP-LF (Table 3; $P < .05$, untreated vs losartan) were decreased after losartan treatment.

There were no changes in PI variance or PI-LF (Table 4). For PI-HF, there were significant effects of treatment (Table 4; $P < .001$), light/dark (Table 4; $P < .03$) and interaction between treatment and light/dark (Table 4; $P < .05$). Fructose-fed mice showed decreased PI-HF during the dark phase after losartan treatment ($P < .01$; light vs dark). However, during the light phase, losartan treatment increased PI-HF in fructose mice ($P < .05$, control vs fructose. $P < .005$, untreated vs losartan).

For plasma Ang II (Figure 2A), there were significant effects of diet ($P < .03$) and drug treatment ($P < .05$). Fructose increased plasma Ang II (Figure 2A; $P < .05$, control vs fructose) and plasma ACE activity (1.1 ± 0.6 vs 4.6 ± 1.1 ; Ang II formation, control vs. fructose). Losartan also increased plasma Ang II with no additional effect of the diet/drug combination (Figure 2A; $P < .05$, untreated vs treated). For renal Ang II levels (Figure 2B), there was a significant effect of diet ($P < .03$). Fructose increased renal peptide levels, an effect that was reversed by losartan treatment (Figure 2B). Renal Ang 1-7 levels were not affected by fructose diet and/or losartan treatment (Figure 2C).

Renal ACE activity or protein levels were not affected by the fructose diet and/or losartan treatment (Figures 3A and C). However, ACE protein levels were lower in fructose mice treated with losartan when compared to control mice given losartan (Figure 3C; $P < .03$, control losartan vs fructose losartan). Angiotensin-converting enzyme activity was increased by fructose (Figure 3B; $P < .003$) as was ACE 2 protein (Figure 3D; $P < .03$). These effects were not observed after losartan treatment (Figure 3B and 3). In control mice, losartan increased ACE 2 protein levels (Figure 3D; $P < .03$, untreated vs losartan) but had no effect on ACE 2 activity (Figure 3B).

Discussion

There is much interest in uncovering the means by which a high fructose diet alters metabolic and cardiovascular function. This is related to the public's concern that consumption of fructose-containing beverages and processed foods may have detrimental health effects. Our data demonstrate that consumption of a high fructose diet in mice produces a mild but consistent increase in BP. A role of the RAS was suggested by the marked BP reduction produced by the commonly used antihypertensive, losartan, and the modulation of renal ACE2 and Ang peptide levels.

A role of the RAS in diabetes-induced pathologies in humans is supported by the depressor and renoprotective effects of ARBs⁴ as well as the drug-associated decrease in the incidence of new-onset diabetes.³⁰ Results from animal studies show that ARBs reduce the

cardiovascular complications in models of both type 1 and type 2 diabetes.^{23,31,32} In the dietary fructose model, characterized by glucose intolerance and a rise in BP, there is activation of both the peripheral and central Ang systems.^{8,13,32} Studies using the Ang AT1a receptor gene deletion model showed that fructose exerted a depressor action.¹⁵ In addition, studies in a type 1 diabetic model showed that hypertension was absent in the Ang AT1 receptor knockout model.²⁵ Our findings extend these results to show that the fructose diet sensitized animals to Ang receptor blockade. In fructose-fed mice, a 1-week treatment with a low dose of losartan markedly reduced BP, providing evidence for diet/drug interactions.

Studies of long-term changes in BP in mice require the use of telemetry to provide accurate data on time and treatment-related changes. This was noted in the study by Farah et al,⁸ which showed in telemetric-monitored mice that fructose feeding increased BP but only during the behaviorally active period (dark phase). Because the circadian oscillation in activity was the same in both groups, it could be excluded as a factor responsible for the rise in BP observed in fructose mice. The light/dark difference is actually accentuated in fructose-fed mice and an evidence of a disturbance in the cardiovascular circadian pattern. Because abnormal circadian BP rhythms are improved with losartan treatment,³³ we also evaluated the effect of losartan on the light/dark BP pattern. Losartan decreased BP in fructose-fed mice with a greater effect noted during the dark period. Thus, the enhanced light/dark difference observed in fructose mice was corrected by losartan treatment. Control mice treated with losartan showed no BP changes as reported in other studies.³⁴

Consumption of the high fructose diet produced glucose intolerance, increased insulin, and hypercholesterolemia, consistent with previous studies in mice and rats.^{35,36} This supports the usefulness of the fructose diet to produce a model for the experimental syndrome of insulin resistance, a precursor condition to type 2 diabetes. Clinical and basic studies have shown that Ang AT1 blockade improves β -cell function, glucose tolerance, and delays the onset of type 2 diabetes.^{30,37} This was supported by studies in mice lacking Ang AT1a receptors.¹⁵ The gene deletion strain metabolized glucose better than wild-type mice. In the fructose model, there are numerous reports that ARBs improve glucose handling.^{32,34} Our results showed that short-term pharmacological blockade of Ang AT1 receptors did not improve glucose tolerance, estimated as AUC. This lack of improvement is likely related to the short treatment time and the low dose of losartan. Nevertheless, the cardiovascular results illustrate that the drug is functioning as an ARB.

Cardiovascular autonomic neuropathy is associated with a higher risk of mortality in diabetic patients.^{5,9} The pathological consequences are even greater when hypertension coexists with autonomic dysfunction.³⁸ Autonomic pathologies are often evaluated using statistical methods for measuring sympathetic and parasympathetic modulation of the cardiovascular system. In particular, analysis of HR and BP variability in time (variance) and frequency domains (spectral analysis) provides a sensitive and easily applied method.³⁹ The importance of BPV in clinical pathologies was established with reports that increased BPV is associated with end-organ damage²⁶ and early stages of autonomic neuropathy.⁴⁰

The fructose diet increased SAP variance and LF, only during the dark period, consistent with our previous studies.^{8,15} Because the LF oscillations are related to sympathetic modulation of the circulation,⁴¹ this suggests that fructose consumption is tied with sympathetic activation and cardiovascular risks. A role of the RAS in the autonomic changes of the fructose model was suggested by studies in Ang AT1a gene deletion mice.¹⁵ In AT1a knockout mice, fructose failed to increase BPV and its LF component. A test of pharmacological blockade of the RAS showed that losartan not only decreased SAP variance and LF in fructose-fed mice but also abolished the light/dark pattern. This agrees with previous studies that showed that losartan decreased both BPV and LF in hypertensive

rats.⁴² It also fits with our studies in the Ang AT1a gene deletion model in which BPV and LF were markedly lower.¹⁵

For HRV, the HF range reflects parasympathetic activity, whereas the interpretation of the LF range is more controversial.^{39,41} A reduction in HRV is an early marker for diabetic neuropathy in humans.⁴³ However, in the dietary-induced insulin-resistant model, there were no changes in HRV in wild type⁸ or AT1a knockout mice.¹⁵ The results of the current study are consistent with this finding, a lack of fructose-induced changes in HR or HRV.

Activation of the RAS is a key mechanism involved in the increased BP produced by a high fructose diet.^{8,15,44} Fructose increased ACE (plasma), Ang II (kidney and plasma), and brain expression of AT1a receptor messenger RNA (mRNA).⁸ To clarify the role of the renal RAS in the integrative responses to fructose, we focused on the study of ACE and ACE2 with measurements of activity, protein, and respective generated peptide levels. The kidney has a complete functional local RAS, including ACE and ACE2.²⁰ Angiotensin-converting enzyme (ACE 2) plays a role in degrading Ang II to Ang 1-7, providing a rationale for further exploration of its role in pathophysiological states, including hypertension and diabetic complications.¹⁶ This is the first report to show changes in ACE2 in the fructose model. There were increases in renal ACE2 activity and protein with no change in renal ACE. There were also increases in plasma ACE and Ang II, coincident with the increase in BP. Studies have described a renoprotective role for ACE2.^{18,19,22,45} The enzyme downregulation^{18,45} or pharmacological blockade²⁴ has been associated with renal lesion progression in experimental diabetes. In addition, studies in early stages of experimental diabetes show increased renal ACE2^{19,22,46} that precede its decrease as nephropathy develops.^{18,23} Our results showed a shift toward ACE 2 in kidney that could be renoprotective as suggested in other studies.

When fructose-fed mice were challenged with losartan, there was a dramatic drop in BP. The depressor response could be related to attenuation of Ang signaling. Fructose is known to activate the Ang receptor system, perhaps via Ang II.⁸ It has been shown that Ang II infusion produced a profound upregulation of AT1 receptors in the brain.⁴⁷ The decrease in BP could also be due to alterations in central sympathetic outflow. This is supported by the data that show a correction in SAP variability after combined treatment with fructose and losartan. However, these changes could be due to losartan's hypotensive effect per se. Therefore, the use of hypotensive pharmacological agents that have no direct influence on the RAS could be useful to elucidate the cardiovascular autonomic modulation in the fructose model. The decrease in BP was also associated with a reduction in renal Ang II without changes in plasma Ang II levels or renal Ang 1-7, ACE, and ACE2 activity. These findings suggest that circulating and renal RAS could be differentially modulated by the diet/drug interaction. Components of the RAS, ACE2, Ang 1-7,⁴⁸ and ACE⁴⁹ show different distribution patterns within the kidney. Therefore, specific studies focusing on the different areas of the kidney might disclose region-specific changes in the RAS.

Angiotensin II generation is regulated not only by ACE but also by other peptidases such as chymase.⁵⁰ It is possible that other enzymes involved in Ang II synthesis might be downregulated by the diet/drug treatment. With regard to Ang II degradation, ACE 2 plays a biological role in generating Ang 1-7 from Ang II.¹⁶ Studies in rats show that AT1 receptor blockade with losartan increases cardiac ACE2 mRNA and ACE2 activity in kidney and heart.^{21,51} Our data in mice showed that losartan treatment alone increased renal ACE2 protein without changing activity or Ang 1-7 levels. There are many instances in which protein content is not correlated with enzyme activity or peptide content. Although fructose and losartan individually increased kidney ACE 2, losartan treatment had no additional

effect on kidney ACE2 activity or protein expression. In addition, kidney Ang 1-7 levels were not affected by diet/drug interaction.

In conclusion, our results show, for the first time, that a high fructose diet activates renal ACE2 in mice, possibly as a reno-protective mechanism. In addition, we showed a prominent interaction between a fructose rich diet and the antihypertensive action of an Ang AT1 antagonist. The diet produced a mild, yet consistent, BP rise that sensitized the animals to the RAS pharmacological therapy.

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Figure 1.

A, Blood glucose levels during a glucose tolerance test in control and fructose-fed mice, untreated and treated with losartan. Glucose levels were measured after an intraperitoneal (IP) glucose load (1.5 g/kg). There were significant effects of diet ($F_{1,90} = 32.5$, $P < .0001$), treatment ($F_{1,90} = 12.5$, $P < .001$), and time ($F_{4,90} = 38.8$, $P < .0001$). B, Glucose tolerance test (GTT) estimated by area under the time curve (AUC) in control and fructose-fed mice, untreated and treated with losartan. There was a significant effect of diet ($F_{1,18} = 9.9$, $P < .005$). $^{\dagger}P < .05$ control versus fructose, $n = 8$ per group.

Figure 2.

Plasma angiotensin II (Ang II) (A), kidney Ang II (B), and kidney Ang 1-7 (C) in control and fructose-fed mice, untreated and treated with losartan. For plasma Ang II, analysis of variance (ANOVA) showed main effect of diet ($F_{1,16} = 6.3$, $P < .03$) and treatment ($F_{1,16} = 10.6$, $P < .05$). For kidney Ang II, there was a significant effect of interaction between diet and treatment ($F_{1,19} = 17.5$, $P < .0005$). There were no changes in kidney Ang 1-7 levels. [†] $P < .05$ control versus fructose. [#] $P < .05$ untreated versus losartan, $n = 6$.

Figure 3.

Renal angiotensin-converting enzyme (ACE) (A) and ACE 2 (B) activity and renal ACE (C) and ACE 2 (D) protein levels (Western blot, normalized by β-actin) in control and fructose mice, treated and untreated with losartan. A, There were no changes in ACE activity. B, ACE 2 activity was increased in fructose mice. There was a significant effect of diet ($F_{1,19} = 12.3$, $P < .003$). C, Angiotensin-converting enzyme protein levels were decreased in fructose mice treated with losartan. There was a significant effect of treatment ($F_{1,19} = 3.4$, $P < .005$). D, Angiotensin-converting enzyme 2 protein levels were increased in fructose mice and control mice treated with losartan. There were significant effects of diet ($F_{1,19} = 4.4$, $P < .003$) and treatment ($F_{1,19} = 5.7$, $P < .003$). [†] $P < .05$ control versus fructose. [#] $P < .05$ untreated versus losartan, $n = 6$.

Table 1Body Weight, Glucose, Insulin, Cholesterol, Triglycerides, Leptin, and Adiponectin Plasma Levels^a

Treatment Diet	Untreated		Losartan	
	Control	Fructose	Control	Fructose
Initial BW (g)	23.7 ± 0.3	23.6 ± 0.3	22.6 ± 0.2	23.5 ± 0.2
Final BW (g)	29.3 ± 0.5 ^b	27.8 ± 0.5 ^b	26.6 ± 0.4 ^b	27.4 ± 0.2 ^b
Glucose (mg/dL)	160 ± 4.8	166 ± 25.2	137 ± 7.8	170 ± 12.5
Insulin (ng/dL)	0.5 ± 0.1	1.1 ± 0.3 ^c	1.0 ± 0.2	1.5 ± 0.2
Cholesterol (mg/dL)	89 ± 9	161 ± 18 ^c	91 ± 8	169 ± 8 ^c
TGL (mg/dL)	753	75 ± 4	72 ± 4	71 ± 4
Leptin (ng/dL)	1.5 ± 0.3	2.4 ± 0.8	0.7 ± 0.3	1.8 ± 0.6
Adiponectin (ng/dL)	9.0 ± 0.2	8.5 ± 0.6	8.0 ± 0.5	9.5 ± 0.2

^aBody weight (BW) was measured at the 1st and 9th week of protocol. Glucose, insulin, cholesterol, triglycerides (TGL), leptin, and adiponectin were measured in nonfasted mice at the 10th week. For BW, there was a significant effect of time ($F_{1,38} = 66.5$, $P < .00001$). There were no changes in blood glucose. There was a significant effect of diet for insulin ($F_{1,21} = 6.87$, $P < 0.03$) and for cholesterol ($F_{1,24} = 40.8$, $P < .0001$). There were no changes in TGL, leptin, or adiponectin.

^b $P < .05$ initial versus final.

^c $P < .05$ control versus fructose, $n = 8$.

Table 2

Mean Arterial Pressure (MAP) and Heart Rate (HR)^a

Diet	Phase	Control		Fructose	
		Untreated	Losartan	Untreated	Losartan
MAP (mm Hg)	Light	101 ± 2.3	98 ± 2.3	98 ± 1.9	89 ± 1.7 ^{c,d}
	Dark	110 ± 2.1 ^b	108 ± 2.3 ^b	117 ± 1.3 ^{b,c}	98 ± 2.7 ^{b,c,d}
HR (bpm)	Light	507 ± 10	503 ± 12	531 ± 14	532 ± 20
	Dark	574 ± 13 ^b	588 ± 13 ^b	625 ± 7 ^{b,c}	594 ± 11 ^b

^a Effect of losartan treatment on mean arterial pressure (MAP) and heart rate (HR) in control and fructose-fed mice, with and without losartan. Mean arterial pressure and HR were recorded for 24 hours and analyzed during light and dark phases. For MAP, there were significant effects of treatment ($F_{1,69} = 24.6$, $P < .0001$) and light/dark ($F_{1,69} = 47.8$, $P < .0001$) and interaction between diet and treatment [$F_{1,69} = 10.46$, $P < .003$]. For HR, there were significant effects of diet ($F_{1,69} = 14.1$, $P < .0005$) and light/dark ($F_{1,69} = 75.6$, $P < .0001$).

^b $P < .03$ light versus dark.

^c $P < .05$ control versus fructose.

^d $P < .05$ untreated versus losartan, $n = 10$.

Table 3

Systolic Arterial Pressure (SAP) Variability in Time and Frequency Domains^a

Diet	Phase	Control		Fructose	
		Untreated	Losartan	Untreated	Losartan
SAP variance (mm Hg ²)	Light	11 ± 0.7	18 ± 2.4	14 ± 0.7	17 ± 3.2
	Dark	21 ± 1.8 ^b	22 ± 2.3	28 ± 1.3 ^{b,c}	17 ± 1.9 ^d
SAP-LF (mm Hg ²)	Light	8 ± 0.5	11 ± 2.6	11 ± 0.5	13 ± 2.8
	Dark	17 ± 1.4 ^b	16 ± 1.9	21 ± 1.3 ^{b,c}	11 ± 1.6 ^d

^aBlood pressure (BP) signals (5 kHz) were submitted to spectral analysis and systolic arterial pressure (SAP) variability was analyzed under time (variance) and frequency domains (low frequency [LF]). For SAP variance, there were significant effects of light/dark ($F_{1,63} = 25.9, P < .00003$) and interactions between diet and treatment ($F_{1,63} = 7.8, P < .05$) and treatment and light/dark ($F_{1,63} = 10.3, P < .003$). For SAP-LF, there were significant effects of light/dark ($F_{1,63} = 19.4, P < .00005$) and interactions between diet and treatment ($F_{1,63} = 6.3, P < .03$) and treatment and light/dark ($F_{1,63} = 9.3, P < .005$).

^b $P < .03$ light versus dark.

^c $P < .05$ control versus fructose.

^d $P < .05$ untreated versus losartan, $n = 8$.

Table 4

Pulse Interval (PI) Variability in Time and Frequency Domains^a

Diet	Phase	Control		Fructose	
		Untreated	Losartan	Untreated	Losartan
PI variance (ms ²)	Light	42 ± 8	63 ± 6	47 ± 11	68 ± 8
	Dark	39 ± 6	45 ± 5	37 ± 6	40 ± 6
PI-LF (ms ²)	Light	23 ± 5	28 ± 4	25 ± 6	20 ± 4
	Dark	19 ± 4	18 ± 3	13 ± 4	12 ± 3
PI-HF (ms ²)	Light	15 ± 3	25 ± 3	18 ± 5	35 ± 7 ^{b c}
	Dark	16 ± 3	19 ± 3	16 ± 2	19 ± 2 ^d

^a Heart rate (HR) signals (5 kHz) were submitted to spectral analysis and peak interval (PI) variability was analyzed under time (variance) and frequency domains (low frequency [LF] and high frequency [HF]). There were no changes in PI variance or LF. For HF, there were significant effects of treatment ($F_{1,72} = 13.2$, $P < .001$), light/dark ($F_{1,72} = 5.6$, $P < .03$), and interaction between treatment and light/dark ($F_{1,72} = 4.0$, $P < .05$).

^b $P < .05$ control versus fructose.

^c $P < .005$ untreated versus losartan, $n = 8$.

^d $P < .01$ light versus dark.