Fructose Feeding in Rats Is Not Associated With Sodium Retention

Shridhar N. Iyer and Michael J. Katovich

Chronic fructose treatment in rats repeatedly has been shown to elevate blood pressure associated with insulin resistance and hyperinsulinemia. Activation of the sympathetic nervous system and the renin-angiotensin system, vascular hypertrophy, and sodium retention by the kidney tubules have been proposed to be some of the mechanisms by which insulin elevates blood pressure. The precise mechanism by which hypertension develops in fructose-fed rats is still not known. The purpose of the current study was twofold. The first objective was to assess the effect of a fructose-enriched diet on urinary sodium excretion. The second objective was to investigate any changes in plasma volume and extracellular volume in fructose-fed rats. In both experiments, male Sprague-Dawley rats were divided into equal groups. Rats in the control group were fed Purina Laboratory Chow, whereas those in the experimental group were fed a 60% fructose diet. There was a significant elevation in the blood pressure of fructose-fed rats at the end of the second week of treatment, and it remained elevated for the remainder of the dietary intervention. In the first experiment, there was no significant difference in sodium, potassium or urine excretion throughout the 6 weeks of dietary treatment. At the end of this study, the serum insulin levels of fructose-fed rats were significantly greater than the levels in the control group. In the second experiment, which was a 4-week study, there was no significant difference in hematocrit, plasma volume, or extracellular fluid volume between control and fructose-fed animals at 2 or 4 weeks of dietary treatment. The results of these two in vivo studies are the first to document that elevation of blood pressure in fructose-fed rats does not occur directly via sodium retention or an increase in fluid volume. Am J Hypertens 1996;9:1018-1023

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tionship. Brands et al directly tested the relationship between insulin, salt, and blood pressure, and concluded that insulin-induced hypertension in Sprague-Dawley rats is not salt sensitive.

Acute insulin infusion also has been reported to have antinatriuretic effects; thus, sodium retention may play a role in the elevation of blood pressure in fructose-fed rats that are hyperinsulinemic. Furthermore, the elevation of plasma ANF observed in fructose-fed rats could increase blood pressure by directly or indirectly increasing sodium reabsorption. In turn, sodium retention could be associated with an increase in blood volume, which also could contribute to the elevated blood pressure observed in the fructose-fed rat. Hwang et al speculated that volume retention occurs in the fructose-fed rat because they observed an increase in atrial natriuretic factor (ANF) in rats after 4 weeks of fructose treatment. Thus, one purpose of this study was to directly assess the effect of a fructose-enriched diet on urinary excretion of sodium associated with chronic fructose feeding in rats. In an additional study, we also determined the effects of chronic fructose treatment on changes in hematocrit, plasma volume (PV), and extracellular fluid volume (ECF).

METHODS

Male Sprague Dawley rats (Charles River, Raleigh, NC) weighing 200 to 225 g were used for all experiments. All animals were housed in individual cages in a room maintained at 25 ± 2°C with a 12-h light/dark cycle. Rats were allowed to adapt to their new environment for a week and were provided laboratory Purina Laboratory Chow (Ralston Purina, St. Louis, MO) and tap water ad libitum.

Experiment 1 The purpose of this study was to determine whether sodium retention occurs in the fructose-fed rat. Rats were divided randomly into two equal groups (n = 7/group). The control group was fed normal rodent chow (Purina Laboratory Chow), whereas the experimental group was fed a 60% fructose diet (Teklad Labs, Madison, WI) for 6 weeks. The sodium and potassium concentrations in the Purina Laboratory Chow and fructose diet were the same. Prior to dietary intervention, baseline body weight and indirect systolic blood pressures were determined. Thereafter, body weight, food intake, water intake, and urine volume were determined each day for the first 10 days after initiation of fructose treatment, and subsequently on days 16, 22, 29, 30, 37 and 42. Rats were housed in individual Nalgene metabolic cages (Nalge Company, Rochester, NY) throughout the study. Urine sodium and potassium were determined using NOVA sodium/potassium analyzers (NOVA Biomedical, Waltham, MA).

Blood pressure was determined at the end of each week by indirect tail cuff measurements. At the conclusion of the study, direct blood pressure measurements were determined with indwelling catheters, as described previously, to validate the indirect measurements. Twenty-four hours following the insertion of the carotid catheter, blood pressure was recorded from the carotid artery in the free-moving, nonrestrained animal by connecting the PE-50 catheter to a pressure transducer that was coupled to a Digi-Med blood pressure analyzer (Micro-Med, Inc., Louisville, KY). Blood pressure was allowed to stabilize for a period of 30 min, and then the blood pressure was recorded. Animals were maintained in their home cages during the measurements of blood pressure.

Twenty-four hours after determining direct blood pressures, rats were decapitated after an overnight fast, and trunk blood was collected for determination of serum insulin concentrations. Serum insulin concentrations were determined by a suitable radioimmunoassay procedure using a rat insulin standard (Linco Research, Inc., St Louis, MO).

Experiment 2 The purpose of this study was to determine the hematocrit, plasma volume, and extracellular volume of fructose-fed rats, and to compare these variables to control animals. Male Sprague-Dawley rats were used in this study, and were equally divided into control and fructose-fed groups as described in Experiment 1. In this study rats (n = 14/group) were fed the two diets for 4 weeks. At the end of the second and fourth week, seven rats from each group were used for determination of plasma volume and extracellular volume. Plasma volume and extracellular volume were determined by previously described methods of Gregersen and Rawson and Crandal and Anderson, respectively.

Rats were anesthetized with a mixture of ketamine (30 mg/kg), xylazine (6 mg/kg), and acepromazine (1 mg/kg) administered intramuscularly (0.7 mL/kg). A PE-50 catheter was implanted into the carotid artery to collect blood samples, and silastic tubing was implanted into the jugular vein for injecting Evans’s blue and sodium thiocyanate solutions. Prior to injecting the dye solutions, a blood sample was collected for hematocrit measurements. A bolus intravenous injection (0.4 mL) of Evan’s blue solution in saline (3.5 mg/mL) was injected, followed by 0.4 mL of sodium thiocyanate (5% solution) in saline. The catheter was flushed with 0.2 mL heparinized saline (10 U/mL). Arterial blood samples (0.25 mL) were collected 10, 20, 30, 40, 50, and 60 min after injection of the dye. Plasma and extracellular fluid volume were determined spectrophotometrically by means of a calibration curve at 620 and 480 nm, respectively. Twenty-four hours after these determinations, mean blood pressure and heart rate were monitored.
Results are expressed as mean ± SEM. Body weight and indirect blood pressure measurements were analyzed by repeated measures ANOVA. Differences between individual groups were determined by Scheffe's test. Direct blood pressure measurements and plasma insulin were analyzed by unpaired Student's t-test. P-values ≥ .05 were considered statistically significant.

**RESULTS**

*Experiment 1* Prior to dietary intervention, animals from both groups had comparable body weights. Similarly, at the end of the study, the body weights of the control and fructose-fed rats were similar (460 ± 10 g and 466 ± 6 g, respectively).

Chronic fructose feeding resulted in an elevation of systolic blood pressure (Figure 1, panel A). Indirect measurement of blood pressure demonstrated that fructose treatment significantly elevated blood pressure, compared to that of the control group, by the end of the second week. Systolic blood pressure remained elevated throughout the remainder of the study. At the end of the study, mean blood pressure, determined through indwelling catheters, was significantly elevated \((P < .01)\) in fructose-fed groups (127 ± 4 mm Hg) compared to that of the control group (108 ± 3 mm Hg).

Throughout the study there was no significant difference in food and water intake between the two groups. The 24-h excretion of urine, sodium, and potassium for the two groups is summarized in Figure 1, panels B, C, and D, respectively. Urine output of fructose-fed rats appeared lower compared to that of the control group during the first 10 days, but did not attain statistical significance. By day 16, the volume of urine excretion tended to increase and was comparable to that of the control group throughout the remainder of the study. Throughout the study there was no significant differ-

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**FIGURE 1.** Effect of chronic fructose feeding on systolic blood pressure determined by indirect tail plethysmography (panel A), urine excretion (panel B), sodium excretion (panel C), and potassium excretion (panel D). Data are expressed as mean ± SEM. *Indicates significantly different \((P < .01)\) from the control group.
ence in urine sodium or potassium excretion between the two groups. Sodium balance during the first 10 days of fructose treatment were the same for the two groups, and ranged from 0.04 to 0.08 mEq/24 h and 0.04 to 0.09 mEq/24 h for the control and fructose-treated rats, respectively. At the end of the study, fasting serum insulin level in the fructose-fed rats (90 ± 8 μU/mL) were significantly greater than in the controls (44 ± 3 μU/mL).

Experiment 2  The second experiment was designed to determine whether feeding a fructose-enriched diet significantly altered plasma or extracellular fluid volume. The results are summarized in Table 1. They demonstrate that there were no significant changes in hematocrit, plasma volume, and extracellular fluid volume, in both groups of rats, at 2 and 4 weeks of fructose treatment. The mean blood pressure and heart rate were significantly elevated in fructose-fed rats compared to those of the controls. These results also are summarized in Table 1.

**DISCUSSION**

The results confirm the previous observations by other investigators that chronic fructose feeding is associated with increases in serum insulin and blood pressure. However, a few studies have failed to demonstrate hyperinsulinemia and an elevation of blood pressure in rats fed carbohydrate-enriched diets. The reason for this discrepancy may be due to a difference in the age or the strain of rat used. Many different mechanisms have been proposed regarding the role of insulin in mediating an elevation in blood pressure. One of these mechanisms is the sodium retaining effect of insulin. However, the results of the first experiment demonstrate that in fructose-treated rats there appears to be no significant sodium retention, and sodium balance was not altered in either of the two groups. Furthermore, the urine output of the two groups was comparable. The results of the second experiment also indicate that fructose feeding does not appear to result in an elevation in plasma or extracellular fluid volume. Collectively, the results of these studies suggest that neither sodium retention nor volume loading are the mechanisms by which blood pressure is increased in the fructose-fed rat model of hypertension.

Acute hyperinsulinemia has been reported to enhance sodium and water retention. Thus, sodium retention has been speculated to play a role in the elevation of blood pressure associated with the hyperinsulinemic state. The exact mechanism of insulin-induced antinatriuresis is not known, although it appears to be a direct effect on renal tubular sodium reabsorption. Theoretically, sustained hyperinsulinemia should result in sodium retention and chronic hypertension. However, investigators have been unable to confirm the increased proximal sodium reabsorption and whole body exchangeable sodium usually has been found to be decreased or unchanged in essential hypertensive patients. Brands et al also have reported that chronic insulin infusion in rats causes a modest increase in blood pressure without significant changes in sodium retention. Thus, although sodium reabsorption has been reported after acute insulin infusion, which could lead to volume expansion, there is little evidence of sodium retention in essential hypertensive patients with insulin resistance and in animals with chronic hyperinsulinemia. The absence of sodium retention may be partially due to pressure natriuresis, or it may occur due to a resistance to the effect of insulin on membrane cation transport. Feraille et al recently reported resistance to Na-K-ATPase in the nephrons of rats fed a fructose-enriched diet for 2 weeks. These animals were hyperinsulinemic and hypertensive. Thus, during insulin resistance in fructose-treated rats, the sodium-retaining effect of insulin is not manifested. The overall maintenance of sodium balance in these fructose-fed rats may be related to an interplay of several other endocrine systems, such as the elevation AII and atrial natriuretic factor, which have been reported previously.

Hall et al demonstrated an initial sodium retention in dogs during insulin infusion. However, there was a rapid escape from the antinatriuretic effect of insulin.

| TABLE 1. EFFECT OF 2 AND 4 WEEKS OF FRUCTOSE TREATMENT ON MEAN BLOOD PRESSURE (MBP), HEART RATE (HR), HEMATOCRIT, PLASMA VOLUME, AND EXTRACELLULAR FLUID VOLUME (ECF) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Group           | MBP (mm Hg)     | HR (beats/min)  | Hematocrit      | Plasma Volume   |
|                 |                 |                 |                 | (mL/kg body weight) | ECF (mL/kg body weight) |
| Week Two        |                 |                 |                 |                 |
| Control         | 105 ± 5         | 328 ± 26        | 43 ± 2          | 47 ± 1          | 308 ± 21         |
| Fructose        | 124 ± 4*        | 491 ± 32*       | 43 ± 2          | 49 ± 2          | 297 ± 15         |
| Week Four       |                 |                 |                 |                 |
| Control         | 112 ± 2         | 355 ± 16        | 43 ± 2          | 42 ± 4          | 314 ± 11         |
| Fructose        | 124 ± 3*        | 422 ± 05*       | 39 ± 1          | 46 ± 3          | 337 ± 22         |

Data are expressed as mean ± SEM.

* Significantly different (P < .05) from the control group.
after 2 days of infusion. Similar results were described in fructose-fed dogs by Martinez et al, who reported sodium retention between days 5 and 7, followed by pressure natriuresis and a return to normal levels by day 16. However, in a subsequent study from the same laboratory done under identical conditions, these investigators did not observe sodium retention or fasting hyperinsulinemia as reported in their previous study, but they did observe a reduction in urinary potassium excretion. Unfortunately, the discrepancy between their two studies was not addressed. In our study, no sodium retention was observed throughout the 6 weeks of fructose treatment in rats. The lack of transient sodium retention in rats may be species specific or related to the degree of hyperinsulinemia achieved.

The results of the present studies are the first to directly demonstrate that neither plasma volume nor extracellular fluid volume is altered in fructose-fed animals. These findings suggest that fructose feeding is not associated with volume loading. This would support the suggestion of Brands et al that the increase in blood pressure following insulin infusion is not associated with volume retention. However, the results contradict previous reports by Hwang et al which suggested that volume expansion may be involved in fructose-induced hypertension, as plasma ANF levels were elevated. However, no volume measurements were made by these investigators.

Although our results suggest that neither volume loading or salt retention are causal to the hypertension induced by fructose feeding, the mechanism(s) responsible for the increase in pressure have not been clearly elucidated. We have suggested that activation of the renin-angiotensin system is responsible for the hypertension and insulin resistance associated with fructose feeding. Iimura et al also has reported similar findings. Balon et al have suggested that the insulin resistance and elevation of blood pressure in the fructose-fed animals is a result of a magnesium deficiency. Although we observed a hypertensive state in our fructose-enriched diet when compared to a starch diet with identical magnesium sulfate levels (unpublished observations) we did not determine whether a magnesium deficiency occurred in the present study. However, the concept of a magnesium deficiency and an enhanced renin-angiotensin system may help to explain the pathogenesis of this model of hypertension. Nadler et al previously observed decreased insulin sensitivity and increased angiotensin II actions after dietary magnesium deficiency in humans. Resnick et al also demonstrated that serum magnesium levels were lower in patient with high renin hypertension. Thus, there may be a strong association with magnesium deficiency, hypertension, insulin resistance, and the renin-angiotensin system. In fact, this association may extend to other conditions such as diabetes and obesity, as has been previously suggested by Resnick. Additional studies addressing this interaction warrant further investigation.

In conclusion, the results of this study suggest that chronic fructose feeding causes an elevation of blood pressure associated with hyperinsulinemia in the rat. The elevation of blood pressure in fructose-fed rats does not occur by sodium retention or by an increase in blood volume. Thus, the sodium-retaining effects of acute hyperinsulinemia may not be manifested during chronic hyperinsulinemia. The results of this in vivo study, coupled with the recent in vitro results of Feraille et al, strongly suggest that sodium retention is not the mechanism by which hypertension develops in fructose-fed rats.

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REFERENCES


