

Fructose-Induced Insulin Resistance and Hypertension in Rats

I-SHUN HWANG, HELEN HO, BRIAN B. HOFFMAN, AND GERALD M. REAVEN

SUMMARY To determine if hypertension could be produced in normal rats by feeding them a fructose-enriched diet, Sprague-Dawley rats were fed either normal chow or a diet containing 66% fructose as a percentage of total calories for approximately 2 weeks. At the end of this period systolic blood pressure had increased from 124 ± 2 to 145 ± 2 (SEM) mm Hg in the fructose-fed rats, whereas no change occurred in the control group. In addition, hyperinsulinemia and hypertriglyceridemia were associated with hypertension in fructose-fed rats. The addition of clonidine to the drinking water inhibited fructose-induced hypertension, but not the increase in plasma insulin or triglyceride concentration seen in fructose-fed rats. Thus, the metabolic changes associated with fructose-induced hypertension are unlikely to be secondary to an increase in sympathetic activity. Whether or not this is also true of the hypertension remains to be clarified. (Hypertension 10: 512-516, 1987)

KEY WORDS • hypertension • insulin resistance • hyperinsulinemia • fructose • clonidine

It has been apparent for some time that increases in dietary carbohydrate intake can raise blood pressure in experimental animals.^{1,2} In particular, the ability of sucrose feeding to accentuate the magnitude of the blood pressure elevation already present in spontaneously hypertensive rats has been documented.³⁻⁵ Since sucrose feeding stimulates sympathetic nervous system activity,⁶ it has been suggested that sucrose-induced increases in sympathetic activity may elevate blood pressure in susceptible animals.⁴ Indirect support for this hypothesis was provided by the observation that rats with sucrose-induced hypertension had faster heart rates and larger hypotensive responses to α -adrenergic blockade with phentolamine than did their normotensive controls.⁷ This point of view has recently received added support from the demonstration that hypertension produced by sucrose feeding is associated with evidence of increased catecholamine

secretion.⁵ However, the physiological effects of sucrose are not limited to an increase in sympathetic activity, and rats fed a high sucrose diet also become insulin-resistant and hyperinsulinemic.^{8,9} Since several recent observations have documented an association between hyperinsulinemia and hypertension in humans,¹⁰⁻¹² it seemed important to see if a similar phenomenon could be found in carbohydrate-induced hypertension in rats. Thus, the current study was initiated to address this issue, and we have taken a somewhat different approach than has been conventionally used in an effort to broaden the nature of inquiry. First, we used normal Sprague-Dawley rats, not animals with spontaneous hypertension, to see if high carbohydrate diets can induce hypertension in rats with no apparent genetic propensity to become hypertensive. Second, we used fructose, not sucrose, as the source of dietary carbohydrate. Fructose feeding can also cause insulin resistance and hyperinsulinemia in normal rats.^{13,14} If fructose also produces hypertension, it would suggest that the insulin resistance and hyperinsulinemia associated with either fructose or sucrose feeding are the common factors in the mechanism of carbohydrate-induced hypertension. Finally, we examined the ability of clonidine, a potent suppressor of the sympathetic nervous system activity, to modulate both the metabolic and the hemodynamic effects of fructose feeding. The results demonstrate that insulin resistance and hyperinsulinemia develop when normal rats are fed a

From the Department of Medicine, Stanford University School of Medicine, and the Geriatric Research, Education and Clinical Center, Veterans Administration Medical Center, Palo Alto, California.

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Address for reprints: Gerald M. Reaven, M.D., VA Medical Center, GRECC/182B, 3801 Miranda Ave., Palo Alto, CA 94304.

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high fructose diet and that this maneuver can induce hypertension in normal rats. Furthermore, clonidine blocks the hypertension, but not the metabolic effects of fructose.

Materials and Methods

General Protocol

Male Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA, USA), initially weighing 160 to 180 g, were used for all experiments. Prior to dietary manipulation, all rats were fed standard rat chow (Wayne Lab Blox; Allied Mills, Chicago, IL, USA), containing 60% vegetable starch, 11% fat, and 29% protein, and maintained on a 12-hour light/dark (0600/1800) cycle. In addition, rats were acclimated to the procedure of blood pressure measurement at 1300 daily for 1 week. Following the training period, control rats continued on a diet of standard chow, while the experimental animals were placed on a diet containing 66% fructose, 12% fat, and 22% protein (Teklad Labs, Madison, WI, USA). The electrolyte content of the two diets was reasonably comparable. The chow diet contained 3.6 g/kg sodium and 10.8 g/kg potassium; the sucrose diet contained 4.9 g/kg sodium and 4.9 g/kg potassium. Rats continued to eat standard rat chow or the fructose diet for 12 days. Food was removed at 0800 on the 13th day, rats were weighed, and blood was taken by tail-bleeding at 1300. The diets were continued for 2 more days, and insulin suppression tests then were performed as described in a subsequent section.

Similar experiments were performed with fructose-fed rats that also had clonidine (0.75 μ g/ml) added to their drinking water. These rats drank approximately 25 ml of water/24 hours. The general protocol was similar to that just described, with measurements made after 13 days. In half of these rats, clonidine was then discontinued and the fructose diet continued; measurements were repeated 3 days later.

Blood Pressure Measurement

Rats were removed from the animal room and taken to the laboratory at 0900; they were allowed free access to diet and water and kept in a quiet area before the blood pressure was measured at 1300. The tail-cuff method, without external preheating, was used to measure the systolic blood pressure.¹⁵ Ambient temperature was kept at 30°C. The equipment used included magnetic animal holders connected with manual scanner (Model 65-12; IITC, Woodland Hills, CA, USA), pulse amplifier (Model 59; IITC), and dual-channel recorder (Model 1202; Linear Instruments, Reno, NV, USA). The systolic blood pressure was measured in the conscious state and has been shown with this technique to be similar to that obtained by direct arterial cannulation.¹⁶ The mean of five consecutive readings was used as the measurement of the systolic blood pressure of each rat for that day, and the average blood pressure on the 2 days before starting the diet and on the last 2 days of each diet period were averaged, calculated, and used for statistical comparisons.

Measurement of In Vivo Insulin Action

In vivo insulin action was quantified by a modification of a method previously used in humans¹⁷ and in rats.¹⁸ Food was withdrawn at 0800 the morning of the study, and the procedure was started at 1200. Rats were anesthetized by an intraperitoneal injection of sodium thiamylal (7.5 mg/100 g body weight), and the right internal jugular was exposed and cannulated for administration of the infusate. Rats received a continuous infusion of glucose (8 mg/kg/min) and insulin (2.5 mU/kg/min) for 180 minutes. With this technique, comparable steady state plasma insulin (SSPI) levels are reached in all animals during the last hour of the study. By measuring the steady state plasma glucose (SSPG) concentration during the 3rd hour, it is possible to obtain a direct assessment of the ability of a fixed concentration of insulin to stimulate glucose uptake in the various groups. SSPG and SSPI values were calculated from the mean of tail blood samples taken at 10-minute intervals during the last 60 minutes of the infusion.

Biochemical Measurements

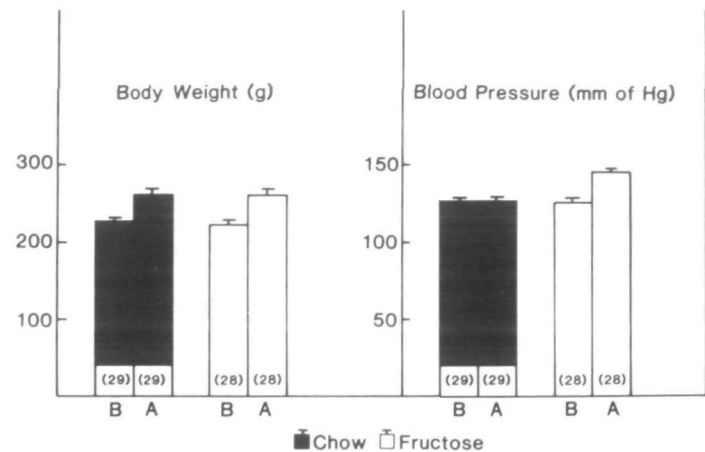
Tail blood samples were taken at the beginning of each experiment and 13 days later. They were centrifuged, aliquoted, frozen, and later assayed for glucose,¹⁹ insulin,²⁰ triglyceride (TG),²¹ and free fatty acid (FFA)²² concentrations. Not enough blood could be removed for measurement of glucose, insulin, TG, and FFA concentration in every animal before and after dietary intervention, and only data in which both of these values are available are presented. The means of assayed results of all groups of rats were compared using Student's *t* test.

Results

The effects of the fructose diet on body weight and blood pressure of normal rats are shown in Figure 1. These results indicate that the increment in body weight was similar after 13 days of eating either conventional rat chow or the 66% fructose diet. It can also be seen that the blood pressure was similar in the two groups before the dietary intervention. On the other hand, systolic blood pressure rose significantly ($p < 0.001$) in the fructose-fed rats. The actual values for systolic blood pressure before initiating the dietary intervention were 124 ± 2 and 125 ± 2 mm Hg in the rats continuing on chow, as compared with 123 ± 2 and 124 ± 2 mm Hg in the rats switched to the fructose diet. At the end of the period of dietary intervention, the blood pressure was 124 ± 3 and 123 ± 2 mm Hg in the chow-fed rats, as compared with 146 ± 2 and 144 ± 2 mm Hg in the rats consuming the fructose diet.

Figure 2 summarizes the effect of fructose feeding on plasma glucose, FFA, TG, and insulin concentrations. Plasma glucose and FFA concentrations of the two groups of rats were similar before and after dietary intervention. However, the plasma TG and insulin concentrations of the two groups were quite different; both TG and insulin concentrations increased signifi-

FIGURE 1. Mean (\pm SEM) body weight and blood pressure in rats fed either chow or the high fructose diet. Measurements were made before (B) and after (A) the introduction of the fructose diet. The number of animals in each group is shown in parentheses. The blood pressure increased in the rats fed the high fructose diet ($p < 0.001$).



cantly only in the rats eating the fructose diet ($p < 0.001$).

The results of the measurements of insulin action are illustrated in Figure 3 and indicate that SSPG concentrations were higher ($p < 0.001$) in the fructose-fed rats. This increase occurred even though SSPI concentrations were also higher in this group ($p < 0.001$). The finding that SSPG concentrations were higher in the rats fed fructose, in conjunction with coexisting higher insulin levels, indicates that the ability of insulin to stimulate disposal of a glucose load was impaired by feeding normal rats a high fructose diet. The higher SSPI in the fructose-fed animals suggests the possibility of an alteration in the clearance of insulin in these rats.

The ability of clonidine to modify the hypertensive and metabolic effects of the high fructose diet was evaluated in two separate experiments. Twenty fructose-fed rats were studied in each experiment, 10 with

clonidine added to their drinking water and 10 without. The first study was ended after 13 days as usual, whereas the second one was extended another 3 days after the clonidine had been withdrawn. The data from the two studies are combined and summarized in Figure 4. The clonidine treatment did not change body weight gain, plasma glucose concentrations, or FFA concentrations compared with values found in rats receiving the fructose diet alone (data not shown). In these separate experiments the ability of the fructose diet to produce hypertension in normal rats was again evident. However, clonidine prevented the fructose-induced increase in blood pressure. This suppression of the hypertensive response was reversible: 3 days after clonidine was withdrawn, the blood pressure in these rats was comparable to the hypertensive values found in rats fed the fructose diet alone. The remainder of the data in Figure 4 demonstrates that the ability of clonidine to modulate the effects of fructose feeding

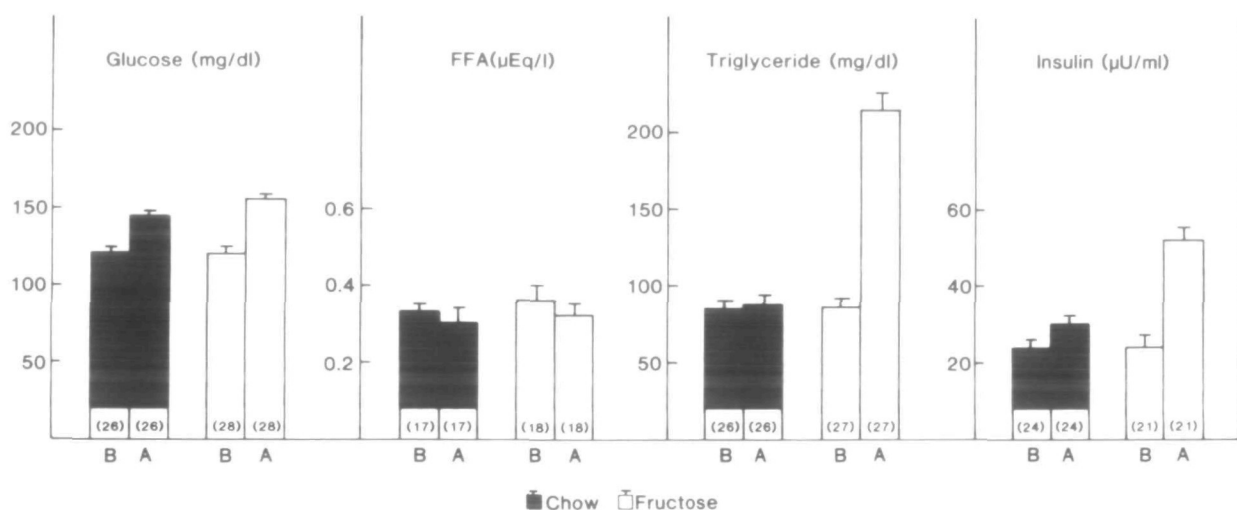


FIGURE 2. Mean (\pm SEM) plasma glucose, free fatty acid (FFA), triglyceride, and insulin concentrations in rats fed either chow or the high fructose diet. Measurements were made before (B) and after (A) the introduction of the fructose diet. The number of animals in each group is shown in parentheses. Both triglyceride and insulin concentrations were increased in rats fed the high fructose diet compared with values in controls ($p < 0.001$).

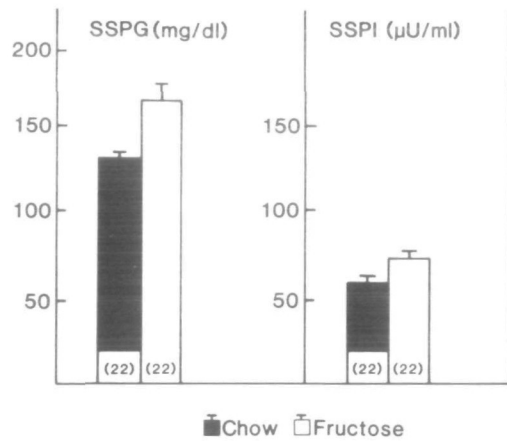


FIGURE 3. Mean (\pm SEM) steady state plasma glucose (SSPG) and insulin (SSPI) concentrations during the insulin suppression test in rats fed chow or fructose. The number of animals in each group is shown in parentheses. The SSPG concentration was higher in the fructose-fed group than in controls ($p < 0.001$).

was confined to blood pressure; the hyperinsulinemia and hypertriglyceridemia produced by fructose in normal rats were not diminished by clonidine administration at a dose that normalized blood pressure.

Discussion

The results of this study indicate that insulin resistance, hyperinsulinemia, and hypertriglyceridemia develop in a relatively short time when normal rats are fed a high fructose diet, as has been seen previously.^{13, 14} In addition, we have shown that these metabolic changes are associated with an increase in systolic blood pressure. These data differ substantially in sev-

eral respects from the majority of previous published studies of the relationship between carbohydrate feeding and hypertension. Most importantly, the ability of fructose to increase blood pressure could be demonstrated in rats that were not genetically destined to become hypertensive. Furthermore, hypertension developed in rats fed a fructose-enriched diet that contained an amount of sodium comparable to that in the control diet. Finally, in contrast to previous reports in which sucrose had been shown to increase blood pressure in rats,^{1-5, 7} fructose was used in the current studies. Thus, it seems apparent that the phenomenon of carbohydrate-induced hypertension is not limited to the administration of one specific carbohydrate to one highly inbred strain of rats, but is rather the reflection of a more general effect of carbohydrate ingestion on blood pressure regulation.

In general, previous studies have focused on an examination of one or another facet of the changes associated with feeding a diet high in refined carbohydrate (usually sucrose) to rats. For example, sucrose diets have been used to induce insulin resistance in rats, which leads to hyperinsulinemia and hypertriglyceridemia.^{8, 9} The mechanism by which sucrose leads to insulin resistance is unclear. Also, sucrose diets have been found to activate sympathetic nervous system activity and elevate blood pressure in rats.^{5, 6} The possible interrelationship between these changes has not been explored. For example, one might speculate that the primary alteration induced by sucrose feeding is activation of sympathetic nervous system activity. This change might then lead to hypertension, and since catecholamines may oppose insulin action, a state of insulin resistance could develop. Alternatively, it is possible that alterations in insulin action are the primary consequences of sucrose feeding and that the other effects of sucrose are secondary. Indeed, at this point it is unclear if all the known effects of sucrose are actual-

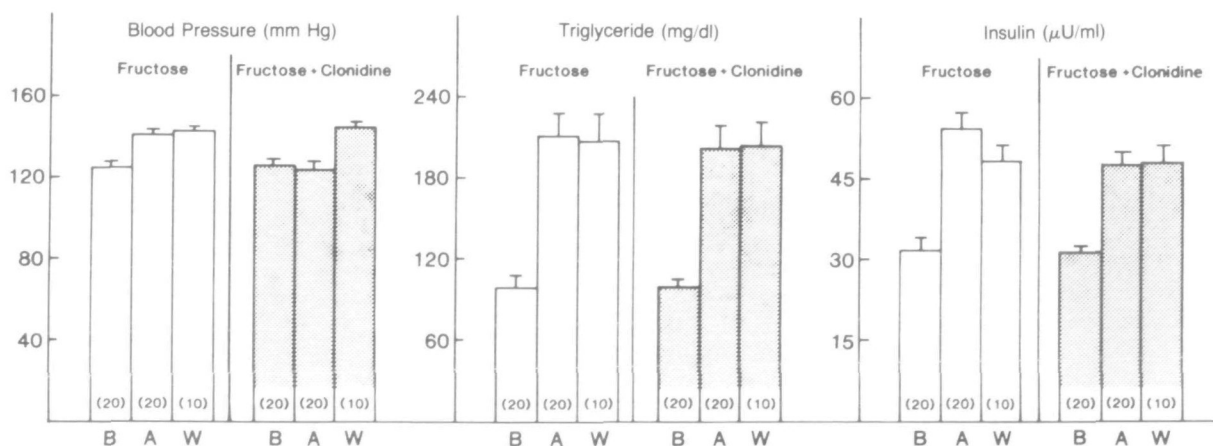


FIGURE 4. Mean (\pm SEM) blood pressure, triglyceride, and insulin in fructose-fed rats treated with either control vehicle (\square) or clonidine (\blacksquare). Measurements were made before clonidine treatment was started (B), at the end of the period of clonidine administration (A), and 3 days after the withdrawal of clonidine (W). The number of animals in each group is shown in parentheses. Clonidine reversibly prevented the development of hypertension in the fructose-fed animals, but it had no effect on the development of hypertriglyceridemia or hyperinsulinemia.

ly causally connected. Our experiments with clonidine were designed to determine if suppression of sympathetic activity with this drug modified the hypertension, hyperinsulinemia, and hypertriglyceridemia induced by the fructose diet. We found that the metabolic abnormalities developed in the presence of a dose of clonidine sufficient to normalize blood pressure in these rats. This dose of clonidine probably has a major suppressive effect on sympathetic nervous system activity. However, we did not wish to use a larger dose since clonidine doses of 1.25 $\mu\text{g/ml}$ or greater in drinking water have been shown to suppress growth of rats.²³ Our results with clonidine suggest that if fructose induces an elevation in sympathetic nervous system activity (as does sucrose), then this change is *not* the cause of the insulin resistance, hyperinsulinemia, and hypertriglyceridemia, which appear to develop in an independent fashion. The apparent absence of effect of clonidine on glucose and insulin concentrations suggests that theoretically possible actions of clonidine, such as stimulation of α_1 -adrenergic receptors in liver (increasing glycogenolysis) and α_2 -adrenergic receptors in islets (suppressing insulin secretion), did not occur to any degree in these rats.

The ability of clonidine to prevent hypertension in these rats does not mean that elevated sympathetic nervous system activity is actually the cause of hypertension in fructose-fed rats. On the other hand, an acute increase in insulin level has also been shown to stimulate catecholamine secretion during euglycemic clamp studies,²⁴ consistent with the view that enhanced sympathetic nervous system activity may be involved in the development of fructose-induced hypertension. However, it also seems necessary to consider the possibility that metabolic changes associated with carbohydrate diets, other than an increase in catecholamine secretion, may be involved in the genesis of the hypertension. Specifically, hyperinsulinemia is associated with the development of fructose-induced hypertension in rats, and similar changes have been described in hypertensive humans.¹⁰⁻¹² Although associations cannot prove causality, they can provide the basis for a new hypothesis. For example, an acute increase in plasma insulin concentration has been shown to reduce sodium excretion in dogs²⁵ and humans.²⁶ In light of these observations the possibility that the insulin resistance and hyperinsulinemia produced by high carbohydrate diets may lead to hypertension by modifying sodium balance deserves consideration. In either formulation, hyperinsulinemia appears to play a central role in the development of carbohydrate-induced hypertension in rats and an analogous situation may also exist in people. Although highly speculative at this time, this hypothesis is both consistent with available data and amenable to experimental testing. As such, it seems to us to be an idea worth pursuing.

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