

Cardiovascular Responses to Hypertonic NaCl Injection Into the Anteroventral Third Ventricle Region in Rats With Fructose-Induced Hypertension and Insulin Resistance

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Introduction and objectives. To investigate the hemodynamic sympathetic response evoked by NaCl microinjection into the third ventricle anteroventral brain area (AV3V) in rats long-term fed with high fructose diet.

Methods. Twelve male rats received 60% fructose enriched diet for 6 months. Control rats (n=12) received regular diet.

Results. Fructose diet increased ($P<.01$) body weight, plasma glucose, triglycerides, cholesterol, insulin, systolic (SBP), and diastolic blood pressure (DBP). Basal heart rate (HR) did not change. AV3V microinjection of 2 μ L of hypertonic 1.5 M NaCl in fructose fed rats increased SBP 44.64 (3.6) mm Hg, DBP 19.9 (2.4) mm Hg and HR 66.2 (8.4) beats/min over basal values ($P<.01$). In control rats, smaller responses were observed, SBP increased 28.33 (3.10) mm Hg, DBP 13.0 (1.9) mm Hg and HR 23.0 (5.0) beats/min over basal values ($P<.01$).

Conclusions. Long-term fructose diet in rats induces cardiovascular hyperactivity of AV3V neurons to sodium chloride, and is associated to hypertension and insulin-resistance.

Key words: Hypertension. Autonomic nervous system. Glucose. Insulin.

Respuestas cardiovasculares al NaCl hipertónico inyectado en la región anteroventral del tercer ventrículo de ratas con hipertensión e insulinoresistencia inducidas por fructosa

Introducción y objetivos. Evaluar la respuesta simpática desencadenada por microinyección de NaCl 1,5 mol (2 μ l) en la región anteroventral del tercer ventrículo cerebral de ratas alimentadas con fructosa a largo plazo.

Métodos. Se usaron ratas macho de la cepa Sprague Dawley. El grupo control (n = 12) recibió dieta convencional y el grupo experimental, fructosa al 60% en comida convencional (n = 12) durante 6 meses.

Resultados. La fructosa produjo aumento de peso corporal, hipertrigliceridemia, hipercolesterolemia, hiperuricemia, hiperinsulinemia e hipertensión arterial, sin cambios en la frecuencia cardíaca basal. La respuesta hipertensiva por microinyección de 2 μ l de NaCl 1,5 mol/l en la región anteroventral del tercer ventrículo cerebral (AV3V) fue mayor en las ratas alimentadas con fructosa; la presión arterial sistólica (PAS) aumentó 44,64, $\pm$ 3,6 mmHg y la diastólica (PAD) 19,9 $\pm$ 2,4 mmHg ($p < 0,01$); en el grupo control la PAS aumentó 28,33 $\pm$ 3,10 mmHg y la PAD 13,0 $\pm$ 1,9 mmHg sobre los valores basales ($p < 0,01$). La frecuencia cardíaca aumentó 23,0 $\pm$ 5,0 lat/min en el grupo control y 66,2 $\pm$ 8,4 lat/min ($p < 0,01$) en el grupo con fructosa.

Conclusiones. La administración de fructosa a largo plazo en la dieta produce hiperreactividad simpática del área AV3V al cloruro de sodio hipertónico, asociada con el desarrollo de hipertensión y resistencia insulínica.

Palabras clave: Hipertensión. Sistema nervioso autónomo. Glucosa. Insulina.

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INTRODUCTION

In attempts to induce changes in experimental animals similar to those seen in metabolic syndrome in humans, rats have been fed long-term diets rich in fructose,¹ saccharose,² or lipids.³ Those fed a fructose-rich diet for 1⁴ to 4 weeks⁵ have been reported to manifest symptoms observed in humans suffering from this syndrome, such

ABBREVIATIONS

AV3V: region: anteroventral third ventricular region
DBP: diastolic blood pressure
HOMA: homeostatic model assessment
SBP: systolic blood pressure

as obesity, insulin resistance, hyperinsulinemia, hypertriglyceridemia, and high blood pressure.⁶ Such patients are at increased risk of suffering a cardiovascular event^{7,8}; they also suffer dyslipidemia, abdominal obesity, high blood pressure, hyperuricemia, and are more sensitive to dietary salt because of reduced sodium excretion at the kidney.^{9,10} In addition, they show increased vascular reactivity to noradrenalin¹¹ and insulin resistance associated with increased sympathetic activity. All these factors contribute to the development of high blood pressure.¹² Insulin resistance is nearly always accompanied by high plasma insulin concentrations; the ensuing mitogenic activity in the smooth muscle fibers of the arteries contributes to the increase in peripheral resistance and the development of atherosclerosis.¹³⁻¹⁵ Metabolic syndrome has also been associated with a reduction in baroreceptor gain, which helps maintain blood pressure high.¹⁶ Further, patients with this syndrome suffer hypertriglyceridemia, a reduction in their high density lipoprotein cholesterol (HDL-C) levels, and an increase in the level of low density lipoprotein cholesterol (LDL-C).¹⁷⁻¹⁹ All these factors are associated with endothelial dysfunction, which impairs arterial vasodilatory function, further increasing the risk of atherosclerosis.^{20,21}

Stimulation of the rat anteroventral third ventricular (AV3V) region of the brain with hypertonic NaCl increases the blood pressure and heart rate via efferent sympathetic nerve activity.²² However, these responses have not been explored in rodents fed a fructose-rich diet. The aim of the present work was to determine their magnitude in rats with experimental metabolic syndrome induced by feeding them a fructose-rich diet over 6 months, a longer period than that used by other authors.

METHODS

The experimental animals used were male Sprague-Dawley rats with a baseline weight of 200-250 g. These were housed and maintained according to international animal research guidelines. A 12L/12D photoperiod was established. All rats were weighed weekly using a balance designed for small animals.

DIET

Twelve rats were fed 60% fructose with their normal compressed food (Ratarina®) for 6 months. All animals had free access to filtered tap water. Twelve control rats were fed Ratarina® without fructose under the same conditions.

Biochemical Assays

Blood samples were taken at the end of the sixth month dietary period. Plasma was separated and the concentrations of glucose, triglycerides, total cholesterol, and uric acid determined using a standard, automated laboratory technique (Spin React Lab®, Model 180). Plasma insulin was determined by ultrasensitive enzyme linked immunosorbent assay (ELISA) using the 1,3 Ultrasensitive kit (ALPCO®, USA). Insulin resistance was calculated using the homeostatic model assessment equation (HOMA)²³⁻²⁵ for humans after a 12 h fast.

Determination of Blood Pressure and Heart Rate

Blood pressure and heart rate were measured non-invasively every month using a digital tail plethysmograph (Letica, Modelo 5001®, Spain). After 6 months of high fructose diet, the rats showed high blood pressure, hyperinsulinemia, and dyslipidemia, and were used to assess the hemodynamic effects of microinjecting hypertonic NaCl into the AV3V region of the brain.

Microinjection of Hypertonic NaCl Into the AV3V

Rats fed on both diets were anesthetized with 35 mg/kg pentobarbital, and the right femoral artery and vein cannulated with PE-50 polyethylene catheters. The arterial catheter allowed the continuous recording of the systolic and diastolic blood pressures (SBP and DBP respectively) using a strain gauge transducer. The venous catheter was used for the administration of solutions or drugs. Heart rate was recorded using a tachograph (Letica, Modelo CAR 300, Spain) activated by pulse waves.

The animals' heads were held in a stereotaxic apparatus (David Kopf Instruments®, USA). The coordinates used for locating the AV3V (taking the bregma as a reference) were: anteroposterior 1.0 mm, lateral 0.5 mm, depth 7.5 mm.²⁵

Two μ L microinjections of 1.5 M NaCl were given over 2 min using a 0.2 mm diameter stainless steel Hamilton® syringe. The dead space was taken into account to correct the injected volume.

Blood pressure and heart rate were recorded continuously before microinjection of the hypertonic NaCl and afterwards until baseline values were restored.

Control rats were microinjected with 2 μ L of isotonic artificial cerebrospinal fluid²²; this causes no hemodynamic changes (results not shown).

Statistical Analysis

Data are expressed as means (standard error [SE]). Differences between means were analyzed using the Student *t* test for non-paired data. The Student *t* test for paired data was used to compare the differences between groups before and after microinjection of the NaCl. ANOVA was used to detect possible significant differences between the data for different groups. All calculations were performed using SPSS v. 11 software.

RESULTS

After 6 months on the fructose-rich diet, the body weight of the rats thus treated increased significantly to 505 (37) g compared to 375 (26) g ($P<.05$) for the rats fed the control diet. After a 12 h fast, the former rats showed the following plasma variable values: blood sugar 110.2 (5.1) mg/dL compared to 63.0 (2.2) mg/dL in the control animals, plasma insulin 429 (8.3) μ U/mL compared to 18.3 (7.4) μ U/mL, triglycerides 175.5 (4.68) mg/dL compared to 65.5 (7.8) mg/dL, total cholesterol 106.2 (16.4) mg/dL compared to 40.3 (3.8) mg/dL, and uric acid 2.8 (0.3) mg/dL compared to 1.4 (0.4) mg/dL; all these differences were significantly different ($P<.01$). The HOMA index, calculated as

$$\text{HOMA} = \frac{(\text{fasting plasma insulin } [\mu\text{U/mL}] \times \text{fasting blood sugar } [\text{mM/L}])}{22.5}$$

was significantly greater ($P<.01$) in the animals fed the fructose-rich diet (11.63 compared to 2.78 for the control diet rats).

Blood Pressure and Paseline Heart Rate

After 6 months on the fructose-rich diet, the SBP of the rats was 152.0 (2.0) mm Hg compared to 120.4 (3.5) mm Hg for the control rats ($P<.01$), and their DBP was 116.4 (2.13) mm Hg compared to 74.58 (2.49) mm Hg ($P<.01$). The baseline heart rate of the rats of the 2 dietary groups did not differ significantly (345.5 [7.67] beats/min for the fructose-rich diet rats, and 344 [6.70] beats/min for the control animals; $P>.05$).

Hemodynamic Changes Caused by Microinjection of the Hypertonic 1.5 M NaCl Into the AV3V Region

After 6 months administration of the control or fructose-rich diet, 2 μ L of 1.5 M NaCl were microinjected into the AV3V. The fructose diet rats showed an increase in

SBP over baseline values greater than that seen in the control diet rats (44.64 [3.6] mm Hg compared to 28.33 [3.1] mm Hg; $P<.01$) (Figure 1). In the control diet rats, the DBP increased by 13.0 (1.9) mm Hg over baseline compared to 19.9 (2.4) mm Hg ($P<.01$) in the rats fed the fructose-rich diet (Figure 2).

The heart rate increased by 23.0 (5.0) beats/min over baseline in the control diet rats, and by 66.2 (8.4) beats/min in the fructose-rich diet rats ($P<.01$) (Figure 3).

These responses lasted longer in the fructose-rich diet rats than in the control diet rats (70 [7] min compared to 45.5 [5.5] min; $P<.01$).

DISCUSSION

The 6-month fructose-rich diet led to plasma metabolic changes greater than those reported by other authors in studies in which this diet was followed for between 2 weeks and 1 month. The reason for extending the dietary period in rats lies in the fact that human diets can remain unchanged over long periods; for rats, 6 months represents about 1/5 of their lives. The fructose-rich diet increased plasma glucose concentrations of the corresponding rats by 74.6% over that of the control diet rats. This increase is much greater than that achieved when diets are fed for shorter periods (9.5% after 2 weeks⁴ and 11.5%-12% after 1 month^{5,26}). Clearly, this longer administration period provides a greater, more prolonged stimulus to the beta cells of the pancreas.^{1,26,27} This likely explains the greater plasma insulin levels seen in the present work (2.3 times greater than in the control group compared to just 0.41% - 2.16% seen when fructose-rich diets were provided for 7¹-30⁴ days).

The increase in plasma insulin concentrations was insufficient to normalize blood sugar levels due to the simultaneous development of insulin resistance, similar to that seen in diabetes type 2.^{28,29}

Insulin resistance was shown in the present work via the increase in the HOMA index, despite its limitations as a measure for estimating insulin sensitivity.^{24,30} Other authors have reported insulin resistance in rats fed diets with high fructose contents for shorter periods.^{1,5}

The increase in plasma triglycerides in the fructose-diet rats is the result of their greater carbohydrate intake and insulin resistance; this is also observed in other models and in human metabolic syndrome. The underlying mechanism is a reduction in the synthesis and storage of triglycerides in the adipose tissue provoked by a reduction in the activity of lipoprotein lipase in this tissue, and a reduction in glycogen production at the liver.³¹

The increase in plasma cholesterol seen in the present fructose diet rats is similar to that seen in others fed a diet enriched in carbohydrates.³²

The fructose-rich diet led to an increase in body weight due to the accumulation of fat in the adipose tissue; at the end of the experiments, autopsy revealed (macroscopically) the extensive accumulation of

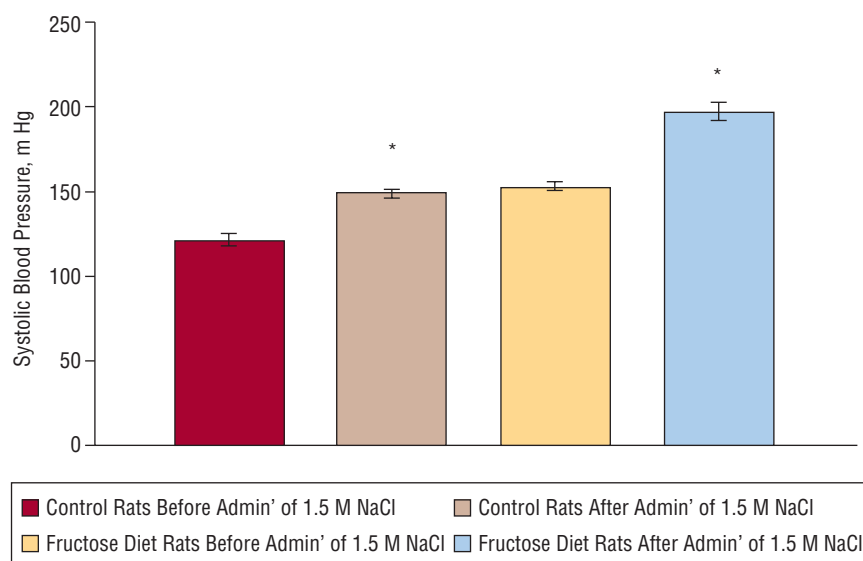


Figure 1. Effect of microinjecting 2 μ L of 1.5 M NaCl into the anteroventral third ventricular region on systolic blood pressure in the control rats (n=12) and in those fed the fructose-rich diet (n=12). The bars show the mean; the vertical lines represent the standard error of the mean. * P <.01 compared to baseline prior to the microinjection of hypertonic NaCl.

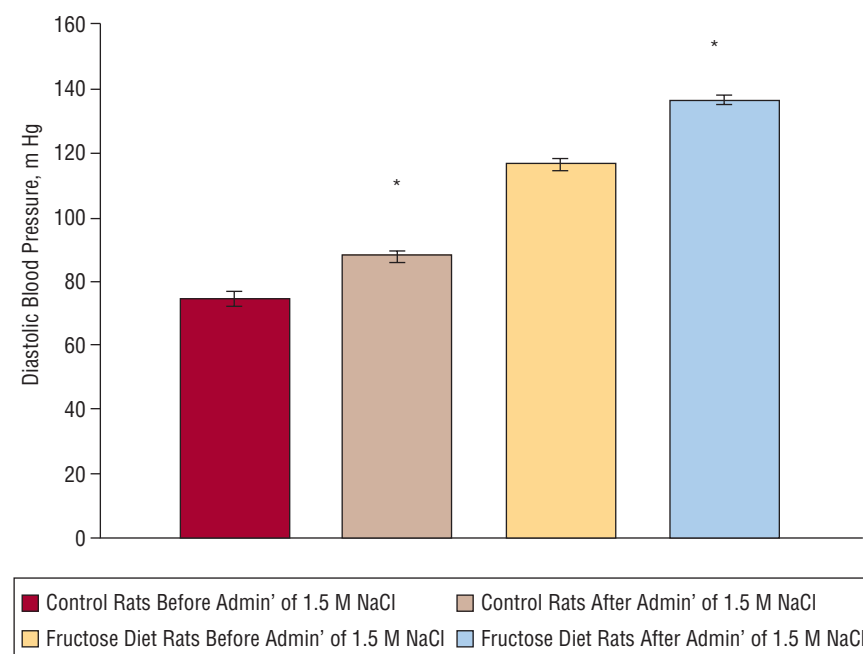


Figure 2. Effect of microinjecting 2 μ L of 1.5 M NaCl into the anteroventral third ventricular region on diastolic blood pressure in the control rats (n=12) and in those fed the rich-rich diet (n=12). The bars show the mean; the vertical lines represent the standard error of the mean. * P <.01 compared to baseline prior to the microinjection of hypertonic NaCl.

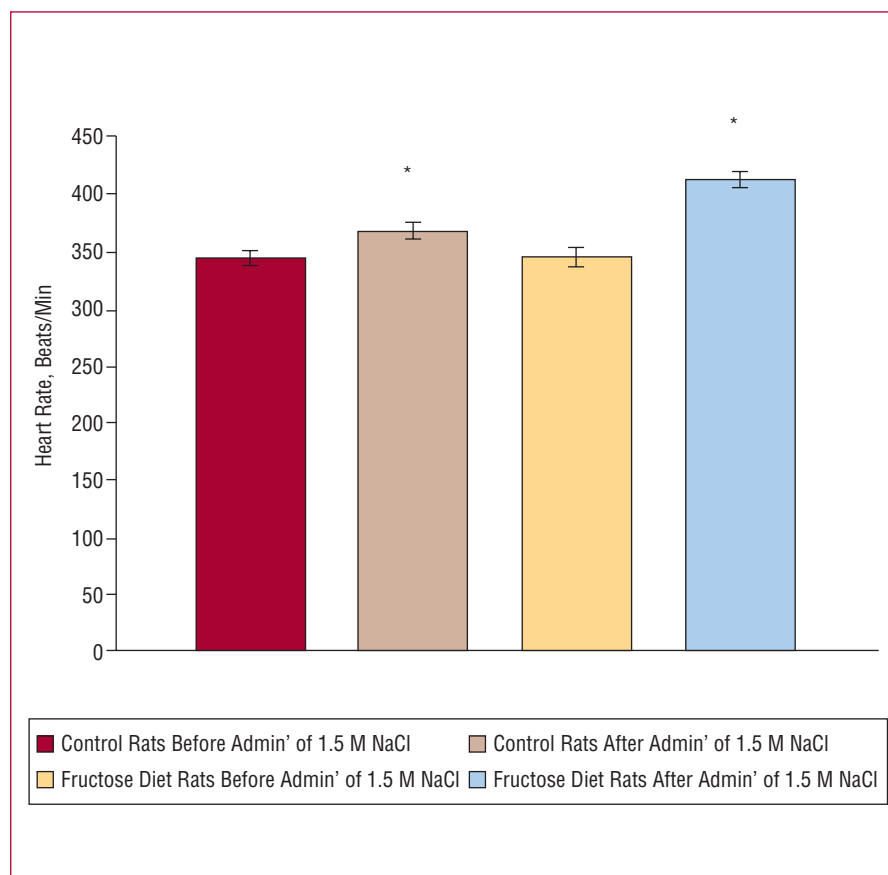


Figure 3. Effect of microinjecting 2 μ L of 1.5 M NaCl into the anteroventral third ventricular region on heart rate in the control rats (n=12) and in those fed the rich-rich diet (n=12). The bars show the mean; the vertical lines represent the standard error of the mean.

* $P < .01$ compared to baseline prior to the microinjection of hypertonic NaCl.

visceral adipose tissue around the abdominal organs compared to the control rats. In agreement with this finding, obese patients with predominantly visceral fat deposits are reported to show greater sympathetic activity.³³ This mechanism contributes to the development of high blood pressure in humans, and probably explains the hypertension recorded in the present fructose diet rats.

The fructose-rich diet induced insulin resistance through the repetitive stimulation of the pancreatic beta cells, a consequence of which is an increase in the plasma insulin concentration. The increased interaction between insulin and its receptors at the target tissues (such as the muscles and adipose tissue) provokes the autophosphorylation of tyrosine residues in the receptor's internal domains³⁴ as well as reductions in the expression and distribution of cellular glucose transporters.^{35,36}

Other mechanisms that might explain the hypertension seen in the rats fed the fructose-rich diet include: an increased reabsorption of sodium at the kidney induced

by hyperinsulinemia,^{37,38} increased arterial reactivity to endothelin,^{20,27,39} a reduction in endothelial vasoconstrictory action,¹⁸⁻²¹ and sympathetic hyperactivity induced by sodium reaching the neurons of the AV3V region, as shown in this work. The increase in sympathetic discharge induced by NaCl is probably 1 of the factors that maintains blood pressure high in the fructose-rich diets rats. However, the mechanism by which the AV3V neurons become hyperactive towards NaCl is unclear. One possibility is a sensitizing effect provoked by high plasma insulin levels passing through the AV3V region, where there is no blood-brain barrier. The AV3V region includes the subfornical organ and the organum vasculosum of the lamina terminalis, which show increased permeability to high molecular weight substances due to the absence of the above barrier.^{40,41} In agreement with this argument, microinjections of insulin into the subfornical organ and the surroundings of the AV3V region have been reported to increase the blood pressure.⁴²

CONCLUSIONS

1. Long-term fructose rich-diet led to a metabolic state involving insulin resistance and high blood pressure.

2. This diet induces an exaggerated hypertensive response when the AV3V region is stimulated with hypertonic NaCl (1.5 M).

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