Effects of L-Arginine on Blood Pressure and Metabolic Changes in Fructose-Hypertensive Rats

Aydın Tay, A. Tanju Özçelikay, and V. Melih Altan

In the present study, we examined the effects of chronic L-arginine treatment on plasma insulin levels and systolic blood pressure (SBP) in fructose-fed (F) rats. Fructose feeding resulted in hyperinsulinemia and elevated blood pressure when compared with that in controls (plasma insulin, 311.3 ± 11.4 μmol/L, control 164.4 ± 11.8 μmol/L, P < .05; SBP, 135.4 ± 4.2 mm Hg control 105.5 ± 1.3 mm Hg, P < .05). L-arginine treatment of fructose-hypertensive rats prevented the development of hyperinsulinemia and hypertension (plasma insulin, 200.1 ± 7.5 μmol/L; P < .05 compared with that in F rats; SBP, 108.0 ± 0.9 mm Hg; P < .05 compared with F rats). However, treatment with L-arginine did not influence any of these parameters in control rats. Statistical analysis of the data of plasma insulin level and SBP, revealed a significant correlation between these two variables. On the other hand, L-arginine treatment of F rats prevented the increased glucose and insulin concentrations in response to oral glucose challenge. L-arginine treatment also prevented the decrease in insulin sensitivity of F rats. These results indicate that L-arginine treatment is able to prevent fructose-induced hypertension and hyperinsulinemia. Our data also suggest a strong relationship between hyperinsulinemia and hypertension in this hypertensive rat model. Therefore, the antihypertensive effect of L-arginine could be, at least in part, the result of the restoration of plasma insulin levels by its vasodilator ability to increase blood flow to insulin sensitive tissues. Am J Hypertens 2002;15:72–77 © 2002 American Journal of Hypertension, Ltd.

Key Words: Insulin resistance, hyperinsulinemia, hypertension, L-arginine, fructose.

Insulin resistance and hyperinsulinemia have been demonstrated frequently to coexist in hypertensive patients. Subsequent findings suggest that these defects in glucose metabolism may play a role in the development of hypertension, dyslipidemia, and coronary artery disease. The association between insulin resistance and hypertension has also been documented in several models of rodent hypertension, including the fructose-fed (F) rats. Rats consuming a high-fructose diet become hypertensive, insulin resistant, hyperinsulinemic, and hypertriglyceridemic. Although the precise mechanism has not been elucidated, it has been proposed that hypertension in fructose-fed rats is secondary to the development of insulin resistance and hyperinsulinemia. L-Arginine infusion, on the other hand, has been shown to produce peripheral vasodilation in healthy subjects and to have antihypertensive effects in hypertensive patients. A recent report suggests that L-arginine infusion restores the defective insulin-mediated vasodilation observed in obese and non-insulin-dependent diabetes mellitus (NIDDM) patients. Further, insulin sensitivity was found to be improved in those patients. The aim of the present study was therefore to examine the effects of chronic L-arginine treatment on plasma insulin levels and systolic blood pressure (SBP) in F rats.

Methods

General Protocol

Male Sprague-Dawley rats weighing 200 to 250 g were divided into four groups: control untreated (C, n = 7), control L-arginine–treated (CT, n = 7), fructose-fed untreated (F, n = 11), and fructose L-arginine–treated (FT, n = 10). The rats were housed in individual cages in a room at the constant temperature of 22° ± 1°C with a fixed 12 h light–dark cycle and were allowed free access to regular laboratory rat chow and water. At week 0, baseline values of plasma glucose, plasma insulin, plasma triglycerides, and SBP were measured. Starting at week 0, the rats in CT and FT groups were kept on drinking water containing L-arginine (1 mg/mL). The rats in the F and FT groups, on the other hand, were given 10% fructose solution. Food
intake, fluid intake and body weights of the rats were recorded every week. Systolic blood pressure, plasma glucose, and plasma triglycerides were measured weekly during the study period. Plasma samples were analyzed bi-weekly for insulin. At the end of the study, an oral glucose tolerance test (OGTT) was performed. Insulin sensitivity was calculated by comparing the insulin/glucose ratio among the experimental groups at weeks 2, 4, 6 and 8.

**Systolic Blood Pressure Measurement**

Systolic blood pressure measurements were performed in conscious rats by indirect tail-cuff method using a fully automatic blood pressure analyzer (MAY 9610, Commat Ltd., Ankara, Turkey). The rats were placed in a constant-temperature (32°C) chamber for 30 min and then put into a rat holder. Eight SBP measurements were carried out in each animal. With the maximal and minimal values being rejected, an average of the six such readings was taken as the individual SBP. To validate readings obtained by the indirect tail-cuff method, at the end of the study the SBP was also measured directly by intra-arterial cannulation. Comparison of the mean values of direct (110.6 ± 4.5 mm Hg) and indirect (113.3 ± 5.8 mm Hg) SBP measurements of rats in all groups showed a correlation of 97%.

**Biochemical Measurements**

Blood samples (0.5 mL) from 5-h–fasted rats were collected into heparinized tubes by snipping the tails and were centrifuged for 15 min at 3330 g to separate plasma. Plasma glucose and triglyceride levels were determined by using appropriate enzymatic colorimetric assay kits (Clonital, Carvico, Italy). Plasma insulin levels were measured by standard radioimmunoassay techniques using a commercial kit available from DPC (Diagnostic Products Corp., Los Angeles, CA).

**Oral Glucose Tolerance Test**

At the end of week 8, plasma insulin and glucose concentrations were measured in response to an oral glucose load. On the day before the test (at 6 pm), food was removed in all groups and fructose solution in F and FT groups was replaced with drinking water. On the morning of the test, a blood sample (min 0) was drawn from the tail. Each animal then received an oral glucose load at a concentration of 2 g/kg of a 40% (weight/volume) solution of glucose by oral gavage. Blood samples were collected at 15, 30, 60, and 90 min after the oral glucose load. Blood samples were immediately centrifuged and stored at −20°C until assay for plasma glucose and insulin.

**Statistical Analysis**

Statistical analysis was performed according to one-way analysis of variance (ANOVA) followed by Neuman-Keuls test. Values are expressed as means ± SEM. The relationship between plasma insulin concentration and SBP in the F and FT groups at weeks 2, 4, 6, and 8 was evaluated by determining the correlation coefficient (r). A probability of $P < .05$ was taken to indicate a significant difference between means.

**Results**

The rats in the F group exhibited hyperinsulinemia, hypertension and increased plasma triglyceride levels compared with those in the C group (average of weeks 1 to 8) (plasma insulin: F, 311.3 ± 11.5 v C, 164.3 ± 11.9 pmol/L; SBP: F, 135.4 ± 4.2 v C, 105.5 ± 1.3 mm Hg; plasma triglycerides: F, 1.94 ± 0.11 v C, 0.95 ± 0.04 mmol/L; $P < .05$) (Fig. 1A–C). Treatment of the F rats with L-arginine (FT group) prevented the development of hyperinsulinemia (200.1 ± 7.5 pmol/L; $P < .05$ compared with F; Fig. 1A) and hypertension observed in this group (108.0 ± 0.9 mm Hg; $P < .05$ compared with F, Fig. 1B), but had no effect on triglyceride levels (1.64 ± 0.10 mmol/L compared with F, Fig. 1C). The treatment did not alter any of these parameters in the CT group (Fig. 1A–C).

Caloric intakes of the rats were calculated based on the daily food and fluid intakes and the caloric values of rat chow (2.6 Kcal/g) and fructose (4 Kcal/g). In the F group, there was a significant increase in fluid intake, but a significant decrease in food intake compared with C group. However, the caloric intakes of the F and C groups were not statistically different. Therefore, body weight gain in the F and C groups was similar. L-arginine treatment did not cause any change in above parameters in the CT and FT groups. In addition, plasma glucose levels were found to be similar in all groups (Table 1). Insulin sensitivity in the F group was lower than that in the C group. In the FT group, L-arginine treatment increased the sensitivity to the level in C group. However, L-arginine did not cause any change in insulin sensitivity of CT group (Fig. 2).

The profiles of plasma glucose and plasma insulin during the OGTT are shown in Fig. 3A and B, respectively. Basal plasma glucose concentrations (min 0) were comparable in all groups. However, plasma glucose levels were found to be higher in the rats from the F group than in those from the other groups at 15, 30, 60, and 90 min after the oral glucose load (Fig. 3A). L-Arginine treatment prevented the increased response to the OGTT in FT rats. Basal plasma insulin concentrations were greater in the F rats as compared with those in the other groups. Plasma insulin levels were also found to be higher in the F rats than in the other groups at each time point tested (Fig. 3B). Plasma concentrations of insulin in FT group were intermediate between the F and the two control groups at 15 and 30 min after the oral glucose load. The exaggerated insulin responses were decreased to control levels by L-arginine treatment in the FT rats at 60 and 90 min after the oral glucose load.

Fig. 4 shows a relationship between plasma insulin concentration and SBP according to data recorded in the F
and FT rats. Because the correlation (r) was computed to be 0.6305, there was a positive correlation between plasma insulin concentration and SBP in the F and FT groups.

**Discussion**

This study demonstrates that chronic L-arginine treatment prevents fructose-induced hypertension and hyperinsulinemia in rats. It also shows that L-arginine treatment is effective in improving glucose tolerance in F rats.

Various mechanisms underlying the development of hypertension in hyperinsulinemic rats have been proposed, including insulin-mediated changes in vascular smooth muscle tone. Insulin, in addition to its effects on carbohydrate and lipid metabolism, may also be involved in the regulation of arterial blood pressure (BP) and vascular smooth muscle tone. Insulin infusion has been demonstrated to increase limb muscle blood flow in humans. Acute insulin infusion in lean subjects has also been reported to cause a rise in peripheral blood flow with an EC50 of about 40 μL/mL. These findings indicate that insulin may exert potent vasodilator effects at physiologic concentrations. The vasodilator effect of insulin, however, is markedly impaired when there is a certain degree of insulin resistance, as in obesity or hypertension, and even more in NIDDM. Insulin has also been shown to attenuate the contractile responses to several vasoactive amines. However, this effect of insulin is blunted in F rats as well.

From these findings, one can assume that the suppression of insulin-mediated vasodilation in states of insulin resistance may contribute to the development of elevated BP. Indeed, chemically diverse drugs that lower plasma insulin levels have been reported to decrease BP in hypertensive rats. Among these drugs are vanadium, metformin, and pioglitazone. Furthermore, the antihypertensive effects of these drugs were reversed by restoring plasma insulin levels to those that existed before treatment. These results indicate that there appears to be a strong association between hyperinsulinemia and hypertension in experimental models of hypertension. In fact, statistical analysis of the data of plasma insulin level and SBP obtained in the present study revealed a significant correlation between these two variables. Therefore, the antihypertensive effect of L-arginine could, at least in part, be the result of the restoration of plasma insulin levels. Our finding that L-arginine treatment prevents the decrease in insulin sensitivity of F rats by assessment of insulin/glucose ratios supports this conclusion. Furthermore, treatment with L-arginine of F rats, in addition to its effect of keeping these animals in a normoinsulinemic state throughout the study, also prevented exaggerated insulin concentrations in response to oral glucose challenge. These findings are somewhat surprising, as L-arginine is known to stimulate endogenous insulin secretion from β-cells. Therefore, the mechanism by which L-arginine can suppress the plasma insulin levels of F rats to normal in the present study is uncertain.

As discussed above, compounds with insulin-sensitizing properties lead to concurrent decreases in plasma insulin levels and BPs of F rats. Thus, the antihypertensive
Effects of these agents have been attributed to their ability to counter hyperinsulinemia. In addition, several antihypertensive-vasodilator agents including angiotensin converting enzyme inhibitors and α-adrenoceptor antagonists also improve insulin sensitivity and reduce plasma insulin levels. The ability of these agents to improve insulin sensitivity, in contrast to those of insulin sensitizers, has been suggested to be an indirect outcome of drug-induced vasodilation and, hence, an increased blood flow to insulin-sensitive tissues. Therefore it has been proposed that on one hand, insulin resistance results in vasoconstriction, whereas on the other, vasoconstriction results in decreases in blood flow to insulin target tissues, which further worsens insulin resistance. It has been reported, however, that systemic infusion of L-arginine reduces BP in healthy rats.

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Values expressed as mean ± SEM.

* P < .05 v C and CT groups.

**FIG. 2.** Insulin sensitivity in control (C, open bars), control l-arginine-treated (CT, striped bars), fructose (F, filled bars), and fructose l-arginine-treated (FT, cross-hatched histograms) groups. Insulin sensitivity was estimated by comparing the insulin to glucose ratios (5-h fasted) at weeks 2, 4, 6, and 8. Values are expressed as mean ± SEM. *P < .05 v all other groups.

**FIG. 3.** Bar graphs show plasma glucose (mmol/L) (A) and plasma insulin (pmol/L) (B) levels in response to an oral glucose load in control (C, open bars), control l-arginine-treated (CT, striped bars), fructose (F, filled bars) and fructose l-arginine-treated (FT, cross-hatched bars) groups. Values are expressed as mean ± SEM. *P < .05 v all other groups.
FIG. 4. Correlation between systolic blood pressure and plasma insulin concentrations in all rats in fructose– (○) and fructose L-arginine-treated (▲) groups at weeks 2, 4, 6, and 8.

These findings that both insulin resistance and endothelial dysfunction occur before hypertension in fructose-treated rats suggest that endothelial dysfunction might be one of the mechanisms linking insulin resistance to elevated BP. Dietary supplementation with L-arginine has been reported to reverse dysfunctional arginine/nitric oxide pathway in the endothelium of spontaneously diabetic bio-breeding rats, whereas it had no effect on endothelium-dependent relaxation in control rats. In addition, we have demonstrated previously that chronic L-arginine treatment of streptozotocin-diabetic rats had both protective and reversible effects on diabetes-induced alterations in vascular reactivity and BP. Therefore, if a defect in the use of L-arginine by endothelium to produce NO does exist in certain pathophysiologic processes including fructose-induced hypertension, it could reasonably be predicted that endothelial function might be improved by increasing dietary L-arginine concentrations. However, the Km of endothelial nitric oxide synthase (eNOS) is far below the intracellular L-arginine concentrations. As a result, eNOS should be saturated in endothelial cells and, thus, increasing the extracellular L-arginine concentrations should not increase NO production any further. Interestingly, NO production by vascular endothelial cells under physiologic conditions has been demonstrated to be increased by extracellular L-arginine, despite the saturating intracellular concentrations of amino acid. Although the exact mechanisms underlying this phenomenon have yet to be explained, it has been proposed that L-arginine in endothelial cells might be sequestered in one or more pools that are poorly accessible to eNOS, whereas extracellular L-arginine transported into the cells is preferentially delivered to eNOS. However, it seems unlikely that this mechanism operated in our experiments, as chronic L-arginine treatment was observed to have no influence on BP in control rats. In addition, tetrahydrobiopterin (BH₄), an absolute cofactor requirement for eNOS activation, can modulate the Km for arginine in intact cells. BH₄ deficiency increases the Km for L-arginine. Recently, it has been reported in rat femoral artery that inhibition of BH₄ synthesis, results in an attenuation in the vasodepressor effect of insulin. If BH₄ deficiency does also occur in F rats, it is plausible that in states of insulin resistance this cofactor defect could be one of the factors that contributes impaired endothelium-dependent relaxation. Although the findings of the present study do not allow us to answer this question, the observations mentioned above may raise the possibility that L-arginine levels in endothelial cells from rats with certain diseases could take place at a limited Km concentration for eNOS which could be restored by L-arginine treatment.

In conclusion, our results suggest that hyperinsulinemia may be linked to the pathogenesis of hypertension in a fructose-hypertensive rat model. Although the exact mechanisms by which L-arginine prevents fructose-induced hypertension and hyperinsulinemia in rats remain to be defined, these effects might be the indirect consequences of...
its vasodilator effect and, hence, an increased blood flow to insulin sensitive tissues. The potential effect of excess L-arginine on NO biosynthesis, on the other hand, may be a contributing factor.

References


