Chronic Thromboxane Synthase Inhibition Prevents Fructose-Induced Hypertension

Denise Galipeau, Emi Arikawa, Inna Sekirov, John H. McNeill

Abstract—To investigate the role of thromboxane A₂ in the development of hypertension in the fructose-fed rat, we treated male fructose-fed rats with dazmegrel (a thromboxane synthase inhibitor) and monitored blood pressure, fasting plasma parameters, and insulin sensitivity for 7 weeks. Systolic blood pressure was measured each week using tail plethysmography, and an oral glucose tolerance test was performed at the end of the study to assess insulin sensitivity. Treatment with a 60% fructose diet and dazmegrel (100 mg · kg⁻¹ · d⁻¹ via oral gavage) was initiated on the same day. Plasma triglyceride levels increased 2-fold in both fructose- and fructose/dazmegrel-treated groups, and plasma insulin levels tended to be higher in these groups, although not significantly. Systolic blood pressure increased significantly throughout the study in the fructose-fed group only (132±3 versus 112±4 mm Hg in control rats, 118±2 mm Hg in control-treated rats, 116±2 mm Hg in fructose-treated rats). Both fructose groups demonstrated a higher peak insulin response to oral glucose challenge and had 40% to 60% lower insulin sensitivity index values. The results of this study show that treatment with a thromboxane synthase inhibitor, dazmegrel, can prevent the development of hypertension but does not improve insulin sensitivity or other fructose-induced metabolic impairments. Based on these data, we conclude that the potent vasoconstrictor thromboxane is involved in the link between hyperinsulinemia/insulin resistance and hypertension. (Hypertension. 2001;38:872-876.)

Key Words: insulin ■ thromboxane ■ blood pressure ■ rats, inbred strains ■ fructose ■ endothelin-1

Hypertension is often found in both humans and animal models to be associated with hyperinsulinemia and insulin resistance.¹ ² On the basis of this observation, the “insulin hypothesis” was developed, which proposes that these 2 metabolic impairments are directly related to the cause of hypertension in such individuals. The fructose-fed hypertensive rat (FHR) is a model of acquired hypertension that also exhibits insulin resistance, hyperinsulinemia, and hypertriglyceridemia.³ Several mechanisms have been proposed to mediate the link between hyperinsulinemia/insulin resistance and hypertension in the FHR.⁴ The sympathetic nervous system is believed to be involved because both chemical sympathectomy⁵ and treatment with rilmenidine, an agent that decreases sympathetic outflow,⁶ have been shown to prevent fructose-induced hypertension.

Another hypothesis that has attracted much interest is that defects in the cardiovascular actions of insulin that affect endothelial function link hypertension to hyperinsulinemia/insulin resistance. The vascular endothelium plays a key role in the regulation of vascular tone via the synthesis and release of various contracting and relaxing factors. Workers at several laboratories have demonstrated that endothelium-dependent relaxation of various vascular tissues is impaired in FHR.⁷ ⁸ ⁹ This observation has been attributed to defects in vasodilatory mechanisms associated with NO⁸ and the endothelium-derived hyperpolarizing factor.⁹ Furthermore, we have shown that the endothelium-dependent vasodilation response to insulin is abolished in aortas of FHR.¹⁰ Alternatively, defects in endothelium-derived contracting factors have been suggested to play a role, particularly those related to endothelin-1 (ET-1). The treatment of FHR with the ET-1 receptor antagonist bosentan has been shown to prevent hypertension in this model, and vascular ET-1 levels are elevated in fructose-fed rats.¹¹ Furthermore, the reactivity of mesenteric arteries to ET-1 from these rats is altered.¹² An increase in the expression of both ET-1 peptide and its ET₁ receptor subtype (which mediates vascular contraction) was recently demonstrated in this model of hypertension.¹³ Because insulin stimulates the secretion and expression of both ET-1 and its receptor,¹⁴ ¹⁵ it is possible that hyperinsulinemia provides a constant stimulus for elevated ET-1 production and therefore increases blood pressure via its vasoconstrictor actions.

Thromboxane A₂ (TXA₂) is another potent vasoconstrictor derived from the endothelium. Studies have shown that renal and/or vascular production of TXA₂ may be increased in several experimental models of hypertension. In both hyperinsulinemic spontaneously hypertensive rats (SHR)¹⁶ and rats chronically infused with insulin,¹⁷ the development of hypertension can be prevented by treatment with a thromboxane...
synthase inhibitor. In addition, insulin has been shown to potentiate the response of coronary blood vessels in vitro to TXA2, suggesting that in vivo, hyperinsulinemia may be linked to hypertension via enhancement of the actions of TXA2.

We designed the present study to investigate the role of thromboxane in the development of hypertension associated with hyperinsulinemia and insulin resistance in the FHR. We evaluated the effects of a thromboxane synthase inhibitor, dazmegrel (UK 38,485), on plasma insulin, glucose, triglyceride, thromboxane, and prostacyclin concentrations; systolic blood pressure; and insulin sensitivity. Furthermore, we investigated the effects of ET-1 on the vascular production of thromboxane.

Methods

Animals
Four experimental groups of 5-week-old Wistar rats (Charles River, Montreal, Canada) were used in this study: control (C, n=6), control dazmegrel-treated (CT, n=6), fructose-treated (F, n=8), and fructose/dazmegrel-treated (FT, n=8). At age 6 weeks, both fructose-fed groups (F and FT) were started on a diet of 60% fructose, and dazmegrel treatment (CT and FT) began concurrently at 100 mg \cdot kg^{-1} \cdot d^{-1} suspended in 1% carboxymethyl cellulose administered via oral gavage for 7 weeks.

Blood Pressure Study Procedures
Systolic blood pressure was measured via the tail-cuff method before treatment and weekly throughout the study period as previously described. Fasting (5 hours) plasma insulin, glucose, and triglyceride levels were measured at study weeks 0, 2, 4, and 6. An oral glucose tolerance test (OGTT) was performed after the animals were fasted overnight at study week 7. Glucose (1 g/kg) was administered via oral gavage, and blood samples were collected at 0, 10, 20, 30, and 60 minutes.

Vascular Thromboxane Production
At termination, aortas were excised, with care taken to not damage the endothelium. Each ring was placed into a glass test tube that contained modified Krebs-Ringer buffer. After a 60-minute incubation period, ET-1 (10^{-7} \text{mol/L}) was added to each test tube. Aliquots of buffer were removed before and 15 minutes after ET-1 challenge for assay of thromboxane B2 (TXB2) and 6-keto-prostaglandin F1α (6-keto-PGF1α) as described later.

Biochemical Analyses
All blood samples were collected from the tail vein, except samples for the determination of plasma TXB2 and 6-keto-PGF1α, the stable metabolites of TXA2 and prostacyclin, respectively, which were obtained via cardiac puncture. Cardiac puncture samples were collected into polypropylene tubes containing 0.95 mL EDTA (0.05 mol/L) and 0.05 mL indomethacin (0.04 mol/L) to inhibit platelet generation of prostaglandins ex vivo. Plasma insulin was determined with a radioimmunoassay kit (Linco Research), triglycerides with an enzymatic colorimetry kit (Sigma Chemical Co), and glucose with a Beckman Glucose Analyzer II. Samples containing TXB2 and 6-keto-PGF1α were first extracted using Ampep C2 minicolumns (Amersham) before assay with enzyme immunoassay kits purchased from Amersham.

Reagents
Fructose diet was obtained from Teklad Laboratory Diets. Indomethacin, EDTA, and methyl formate were purchased from Sigma Chemical Co. Hexane was obtained from Fisher Scientific. Dazmegrel was a generous gift from Pfizer.

Statistical Analysis
All data are presented as mean±SEM. For data with multiple time points, variables were analyzed by the general linear model ANOVA. An unpaired t test was also used to separately compare the effect of fructose treatment on insulin sensitivity within dazmegrel-treated and untreated groups. Area under the curve (AUC) values were calculated using the trapezoidal rule, and insulin sensitivity indexes (ISIs) were calculated using the formula ISI=100[(mean plasma glucose×mean plasma insulin)]/fasting plasma glucose×fasting plasma insulin). A 1-way ANOVA was used to examine AUC and ISI values. Mean values were considered significant at P<0.05. When a mean difference was detected, a Newman-Keuls multiple comparison test was applied.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

Results

General Characteristics
The body weight of each group did not differ at any time point, and the pattern of food and fluid intake was generally similar for all groups throughout the study as shown previously (data not shown). Fasting parameters are given in Table 1. Plasma glucose values did not differ between groups except at week 2, when the F group had slightly but significantly elevated plasma glucose levels compared with those of the control group. Animals fed fructose were hypertriglyceridemic compared with control groups within 2 weeks of the start of the diet and continued to be so throughout the study period. Plasma insulin levels also tended to be elevated in both fructose-fed groups throughout the study, but this difference did not reach statistical significance.

| TABLE 1. Plasma Concentrations of Glucose, Insulin, and Triglycerides in C, CT, F, and FT Rats |
|-------------------|-----------------|-----------------|-----------------|-----------------|
|                   | C               | CT              | F               | FT              |
| Glucose, mmol/L   |                 |                 |                 |                 |
| Basal             | 7.7±0.1         | 7.6±0.2         | 8.0±0.1         | 7.6±0.2         |
| Week 2            | 7.7±0.1         | 7.8±0.2         | 8.6±0.2*        | 8.3±0.2         |
| Week 4            | 7.2±0.1         | 7.5±0.2         | 7.9±0.1         | 7.8±0.2         |
| Week 6            | 7.5±0.2         | 7.4±0.1         | 7.7±0.3         | 7.6±0.1         |
| Insulin, pmol/L   |                 |                 |                 |                 |
| Basal             | 210±26          | 256±15          | 217±22          | 207±21          |
| Week 2            | 182±26          | 221±17          | 363±43          | 391±129         |
| Week 4            | 165±33          | 213±16          | 345±43          | 376±57          |
| Week 6            | 194±36          | 248±25          | 386±73          | 309±23          |
| Triglycerides, mmol/L |             |                 |                 |                 |
| Basal             | 1.4±0.2         | 1.2±0.1         | 1.2±0.1         | 1.5±0.2         |
| Week 2            | 1.4±0.1         | 1.1±0.1         | 2.8±0.2*        | 3.2±0.4*        |
| Week 4            | 1.7±0.1         | 1.5±0.1         | 3.2±0.4*        | 3.2±0.2*        |
| Week 6            | 1.7±0.2         | 1.4±0.2         | 3.6±0.4*        | 3.2±0.4*        |

*P<0.05 vs respective control diet group (C or CT).

Blood Pressure
Systolic blood pressure was significantly increased in the F group by the fifth week of feeding with fructose (Figure 1).
Treatment with dazmegrel prevented the increase in blood pressure caused by the fructose diet. Dazmegrel treatment by itself did not affect blood pressure in non–fructose-fed rats.

**OGTT Responses**

In response to an oral glucose challenge, both fructose-fed groups responded by secreting significantly more insulin, as indicated by a greater phase 1 peak insulin response and AUC, compared with control groups (Figure 2). The plasma glucose profile, on the other hand, was similar among all groups (Figure 3). A comparison of the ISIs (calculated from OGTT data) shows that fructose diet significantly impaired insulin sensitivity (Figure 4). The CT group had a significantly lower ISI than the control group, but the FT group had an even lower ISI than the CT group, indicating that fructose diet contributed to the severe impairment in insulin sensitivity observed in the FT group.

**Vascular Production of TXB₂ and 6-Keto-PGF₁α**

Basal secretions of TXB₂ and 6-keto-PGF₁α from the aorta did not differ between groups and did not significantly increase with time in the absence of any stimulus (data not shown). On the addition of 10⁻⁷ mol/L ET-1, an increase in thromboxane metabolites was detected in the buffer solution from all groups (9.3±2.6, 4.9±1.3, 15.0±1.7, and 7.8±1.7 pg/mg tissue for C, CT, F, and FT, respectively). The vascular TXB₂ production observed in the F group was significantly greater (P<0.001) and was normalized by treatment with dazmegrel.

**Discussion**

The results of this experiment demonstrate that treatment with dazmegrel, an inhibitor of thromboxane synthase, prevents the development of fructose-induced hypertension in male rats. The FHR exhibits the characteristics of “metabolic syndrome X,” namely, hyperinsulinemia, hypertriglyceridemia, insulin resistance, and hypertension. We, and workers from other laboratories, have shown that the treatment of FHR with various compounds that improve insulin sensitivity, such as metformin, vanadium compounds, and pioglitazone, also ameliorated the hypertension that typically develops in this model. Furthermore, increasing insulin levels to those seen before treatment with the insulin sensitizer vanadyl sulfate reverses the beneficial effects of treatment on blood pressure. These experiments provide evidence for the causal role of hyperinsulinemia and insulin resistance in hypertension in FHR.

Because the factors that we believe are responsible for hypertension in this model, namely, hyperinsulinemia and insulin resistance, were not altered in this experiment by dazmegrel treatment, thromboxane may be involved in the link between these 2 conditions. A previous study with a hypertensive model induced by chronic insulin infusion also demonstrated that hypertension could be prevented by the administration of U63557A, a different thromboxane synthase inhibitor. Our experiment shows that fructose-induced hypertension is also dependent on TXA₂ synthesis and shows for the first time that plasma levels of thromboxane metabolites are increased in this type of hypertension. Under normal conditions, prostacyclin production in tandem with TXA₂ typically provides a counterregulatory mechanism to the vasoconstriction and procoagulation effects of thromboxane. However, we have shown that levels of the prostacyclin

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Systolic blood pressure. At least 3 measurements were taken for each individual rat each week. Values are mean±SEM. *P<0.05 vs C; #P<0.05 vs FT.

![Figure 2](http://hyper.ahajournals.org/)

**Figure 2.** Plasma insulin response during an OGTT and AUC. Animals were gavaged with 40% glucose (1 g/kg), and blood samples were obtained from the tail vein at the times indicated. Values are mean±SEM. *P<0.05 vs respective control group (C or CT).

![Figure 3](http://hyper.ahajournals.org/)

**Figure 3.** Plasma glucose response during an OGTT and AUC. Animals were gavaged with 40% glucose (1 g/kg), and blood samples were obtained from the tail vein at the times indicated. Values are mean±SEM. No significant differences were noted.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C</th>
<th>CT</th>
<th>F</th>
<th>FT</th>
</tr>
</thead>
<tbody>
<tr>
<td>TXB₂, pmol/L</td>
<td>445±70</td>
<td>192±3</td>
<td>810±151*</td>
<td>178±13</td>
</tr>
<tr>
<td>6-Keto-PGF₁α, pmol/L</td>
<td>454±51</td>
<td>475±38</td>
<td>569±97</td>
<td>553±30</td>
</tr>
<tr>
<td>Ratio</td>
<td>0.98±0.12</td>
<td>0.46±0.04†</td>
<td>1.40±0.20*</td>
<td>0.33±0.04</td>
</tr>
</tbody>
</table>

*P<0.05 vs all other groups.
†P<0.05 vs C.
TXA2. Although it is not yet known whether the same coronary arteries, potentiates the vasoconstrictive actions of insulin resistance.

Hypertensive models associated with hyperinsulinemia and provide strong evidence for the role of thromboxane in stimulating the production of TXA2 and prostacyclin from endothelial cells. ET-1 has been shown to inhibit the synthesis of TXA2 in vascular tissue and that this effect is greater in FHR than in control animals. These data lend support to our hypothesis that there is an interaction between ET-1 and TXA2 in this model of hypertension. Furthermore, preliminary data from our laboratory demonstrate that cyclooxygenase (COX) inhibition reduces norepinephrine-induced contraction in aorta from FHR, but not from control rats, suggesting that there is enhanced production of COX-derived vasoconstrictor products, possibly TXA2, in vascular tissue of FHR (unpublished data). However, platelets also produce TXA2, and it is possible that the increase in plasma levels observed in this experiment are from this source. Further experiments are required to elucidate the nature and cell types involved in the interactions among insulin, ET-1, and TXA2 in fructose-induced hypertension.

Interestingly, dazmegrel treatment caused a reduction in insulin sensitivity in normal rats not fed fructose. This is due to a prolonged phase 1 insulin response after the initial glucose challenge, compared with control. Both fructose groups demonstrate different insulin secretion patterns, with the peak of the first phase insulin response twice that of control, which is reflected in an increase in AUC and a reduction in ISI. We believe that the decrease in insulin sensitivity in the CT group was not severe enough to affect blood pressure, because the ISI value obtained for this group is within the typical range for male control rats in our previous experiments. The ISI values for C and CT groups in this experiment were 22 ± 5 and 14 ± 1, respectively, compared with 15 ± 1 for control normotensive, insulin-sensitive rats from previous experiments (unpublished data). Calculation of ISI values from OGTT data has been shown to correlate highly with the euglycemic hyperinsulinemic clamp method, considered the gold standard for the assessment of insulin sensitivity.

In summary, the results of the present study provide evidence for the role of thromboxane in the development of hypertension associated with hyperinsulinemia. Fructose treatment is associated with hyperinsulinemia, insulin resistance, hypertension, and elevations in plasma thromboxane levels. Long-term treatment of FHR with dazmegrel, a thromboxane synthase inhibitor, lowered plasma thromboxane levels and completely prevented the increase in blood pressure caused by the fructose diet but did not affect plasma insulin levels or insulin sensitivity. The mechanisms by which hyperinsulinemia stimulates TXA2 production may be related to elevations in ET-1, which is also observed in this form of hypertension. Further experiments into the mechanisms of hypertension associated with hyperinsulinemia are beneficial, because they may provide insight into new targets for therapy of essential hypertension in the human population.

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**Figure 4.** ISI values obtained from OGTT data. Values are mean ± SEM. *P < 0.05 vs C (ANOVA); #P < 0.001 vs CT (unpaired t test).
References

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