Genetic and dietary interactions: role of angiotensin AT$_{1a}$ receptors in response to a high-fructose diet

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Farah V, Elased KM, Morris M. Genetic and dietary interactions: role of angiotensin AT$_{1a}$ receptors in response to a high-fructose diet. Am J Physiol Heart Circ Physiol 293: H1083–H1089, 2007. First published April 20, 2007; doi:10.1152/ajpheart.00106.2006.—The renin-angiotensin system (RAS) has been implicated in the cardiovascular complications of diabetes. We showed that a high-fructose diet increases blood pressure and plasma angiotensin and impairs glucose tolerance. We investigated the role of angiotensin AT$_{1a}$ receptors in the development of fructose-induced cardiovascular and metabolic dysfunction. Male angiotensin AT$_{1a}$ knockout (AT1aKO) and wild-type (AT1aWT) mice with arterial telemetric catheters were fed a standard diet or one containing 60% fructose. Fructose increased mean arterial pressure (MAP) in AT1aWT mice only during the dark phase (8% increase). In AT1KO mice, fructose unexpectedly decreased MAP, during both light and dark periods (24 and 13% decrease, respectively). Analytical methods were used to measure systolic arterial pressure (SAP) and pulse interval (PI) variability in time and frequency domains. In fructose-fed AT1aWT mice, there was an increase in SAP variance and its low-frequency (LF) domain (11 ± 3 vs. 23 ± 4 mmHg², variance, and 7 ± 2 vs. 17 ± 3 mmHg², LF, control vs. fructose, P < 0.004). There were no changes in SAP variance in AT1aKO mice. Depressor responses to a$_{1}$-adrenergic blockade were augmented in fructose-fed AT1aWT compared with AT1aKO mice. Fructose inhibited glucose tolerance with a greater effect in AT1aWT mice. Fructose increased plasma cholesterol in both groups (P < 0.01) and reduced ANG II in AT1aKO mice. Results document prominent interactions between genetics and diet with data showing that in the absence of angiotensin AT$_{1a}$ receptors, a fructose diet decreased blood pressure.

blood pressure; heart rate; autonomic nervous system; insulin; spectral analysis; insulin resistance; diabetes

THERE IS EVIDENCE that associates the increasing incidence of insulin resistance with high caloric consumption and physical inactivity. There is also data that links consumption of high levels of fructose, present in carbonated soft drinks and processed foods, to insulin resistance along with obesity, hypertension, and lipid abnormalities (3). Since cardiovascular pathologies are thought to be the most deleterious outcome in the insulin resistant syndrome, more information on the relationship among diet, glucose intolerance, and cardiovascular disease is required.

Experimental models in mice and rats have been used to study the interactions among diabetes, insulin resistance, and hypertension (10, 15, 23, 27, 32, 33, 36, 59). It is clear that long-term consumption of a high-fructose diet in animals leads to metabolic disturbances, most important being a state of insulin resistance and lipid abnormalities (high cholesterol and triglycerides). However, there is also an association between a high-fructose diet and hypertension. In rats that are fed a high-fructose diet, there is insulin resistance, hyperinsulinemia, and increased blood pressure (10, 25, 32, 33). We conducted studies in mice and found that a high-fructose diet produced an increase in blood pressure that was observed only during the dark period (active period for mice) (15). This circadian-related increase in blood pressure may serve as an early symptom of the prediabetic syndrome. The hypertension was associated with sympathetic activation, increased plasma angiotensin II (ANG II) levels, and increased expression of brain stem angiotensin AT$_{1a}$ and tyrosine hydroxylase mRNA (15).

The role of the renin-angiotensin system (RAS) in the pathogenesis of diabetes-induced cardiovascular pathologies is a critical issue because of clinical and experimental data that validate the beneficial effects of treatment with antagonists of the RAS. Angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARB) are the preferential methods of management of hypertensive diabetic patients (22, 55). In a large clinical trial, ACE inhibitors were reported to be useful in the prevention of heart failure in patients with type 2 diabetes (1). A reduction in the risk of development of cardiovascular pathologies was also noted in diabetic patients treated with ACE inhibitors or ARBs (2, 9). There also appeared to be a preventative action of ARBs against the development of diabetes in persons with hypertension or heart failure (35, 63). Animal studies demonstrated that blockade of the RAS resulted in a reduction in the development of atherosclerotic lesions in diabetic mice (5). AT$_{1}$ receptor blockers or a retroviral vector containing AT$_{1}$ receptor antisense DNA prevented or attenuated the hypertension in fructose-fed rats (24, 33, 44). A high-fructose diet in mice also increased circulating levels of ANG II, increased angiotensin AT$_{1}$ expression, and produced a nocturnal hypertension (15, 53). We also found that the RAS was activated in streptozotocin-induced diabetes and that the diabetes-associated hypertension was absent in angiotensin AT$_{1a}$ receptor-deficient mice (13, 62).

The objective of the present study was to explore the role of angiotensin AT$_{1a}$ receptors in the development of fructose-induced glucose intolerance, hypertension, and sympathetic activation in mice. We present the hypothesis that in the absence of angiotensin AT$_{1a}$ receptors, there will be attenuation or a lack in the cardiovascular and metabolic sequelae.

METHODS

Animals. Experiments were performed in male mice lacking AT$_{1a}$ receptors (AT1aKO) and wild-type mice (AT1aWT) weighing...
25–30 g. The original breeding pairs for the AT1aKO colony were derived from the strain developed by Ito et al. (28) at Duke University, (Durham, NC). The same breeding strategy was maintained, breeding C57BL+/− with SV129+/− and using the F2 animals for experiments. This means that all mice have the same genetic and environmental background. A breeding colony for the strain, including PCR genotyping, was established at Wright State University. Mice were housed individually at 22°C with a 12:12-h dark-light cycle (0500–1700, lights on). Mice were given ad libitum access to tap water and randomly assigned to either standard pellet diet (58% carbohydrate, 12% fat, and 30% protein) or a diet containing high fructose (67% carbohydrate, 98% of which is fructose, 13% fat, and 20% protein). There were four groups of animals: AT1aKO and AT1aWT groups fed standard diet, and AT1aKO and AT1aWT groups fed the fructose diet. Carotid telemetric catheters were inserted at week 7 and cardiovascular measurements were made at week 8 of the dietary treatments. Animals were killed by decapitation with collection of blood and tissues. Wright State University’s Laboratory Animal Care and Use Committee approved all experiments.

Measurement of cardiovascular parameters. Cardiovascular parameters were recorded by radiotelemetry using a probe inserted into the carotid artery. For the surgical procedure, mice were anesthetized with ketamine-xylazine (120:20 mg/kg im). Probes (model TA11PA-C20; Data Sciences International, St. Paul, MN) were inserted into the left common carotid artery, with the transmitter body positioned subcutaneously on the right flank. The details of surgical procedure as performed in our laboratory were described previously (16). Recordings were made after the mice had fully recovered from surgery (5–7 days). Blood pressure recordings were made during the light (0900–1300) and dark periods (2000–2200). The pressure signal was sampled for 30 min at 5 kHz. Heart rate (HR) or pulse interval (PI) was calculated from the pressure signal.

Pharmacological blockade. To examine autonomic influences, we tested the effect of α1-adrenoceptor blockade. α1-Adrenoceptor blockade was induced by prazosin (0.5 mg/kg ip, injected during the dark period; Sigma-Aldrich, St. Louis, MO). Steady-state blood pressure responses were determined (10–20 min after intraperitoneal injection), and data are presented as the percent change from baseline level.

Variance and spectral analysis. The overall variability of PI and SAP was assessed in the time domain by means of variance. PI and SAP fluctuations were assessed in the frequency domain using autoregressive spectral analysis, as described elsewhere (8, 12, 16, 30, 37). Briefly, PI and SAP series were divided in segments of 300 beats and overlapped by 50%; a spectrum was obtained for each of the segments via the Levinson-Durbin recursion, with the model order chosen according to Akaike’s criterion, ranging between 10 and 14. The oscillatory components were quantified in the low- (LF; 0.1 to 1 Hz) and high-frequency ranges (HF; 1 to 5 Hz). The LF band for SAP and PI oscillations was similar to that used by Janssen et al. (29). However, in contrast to these authors, we set the upper limit for the HF band at 5 Hz, since no spectral component was found beyond this frequency. The power spectrum density was calculated for each recognizable component in the LF and HF bands by integrating the spectrum of the components. Segments that presented very slow oscillations (<0.1 Hz), which contributed to >70% of the overall variability, were considered nonstationary and discarded from the study.

Glucose tolerance test. Glucose tolerance test (GTT) was performed after 8 wk on the dietary regimes using an intraperitoneal injection of glucose (1.5 g/kg). Animals were fasted for 6 h, and blood samples were taken from a cut made on the tip of the tail at baseline and 15, 30, 60, and 90 min after the glucose load. Glucose concentrations were determined using an Accu-Check Advantage blood glucose monitor (Roche Diagnostic, Indianapolis, IN).

Plasma insulin, ANG II, and lipids. Blood was obtained by decapitation (0900–1100). Plasma insulin levels were measured by radioimmunoassay in 20-μl aliquots of plasma using a commercial kit (Linco Research, St. Louis, MO). ANG II was measured in plasma as described previously (15). Briefly, 50-μl plasma aliquots were extracted using phenylisilsilica columns, and the reversed phase-extracted ANG II was measured in duplicates using an RIA kit supplied by ALPCO Diagnostics (Windham, NH). Total plasma cholesterol (HDL and LDL) and triglycerides were determined by enzymatic assays provided by Sigma (St. Louis, MO). The end products are colored complexes that are read spectrophotometrically. Absorbance is directly proportional to lipid concentrations.

Statistical analysis. Results are means ± SE. The data were compared using analysis of variance followed by the Newman-Keuls test for multiple comparisons or Student’s t-test where appropriate. Differences were considered statistically significant when P < 0.05.

RESULTS

Body weight increased equally in the groups over the study period (Table 1). At the end of 10 wk of a high-fructose diet, non-fasting blood glucose levels and plasma insulin were not different (Table 1). With regard to the lipid profile, there was an increase in plasma cholesterol but not triglycerides in the fructose groups (Table 1). Plasma ANG II levels were decreased in fructose-fed AT1aKO mice (P < 0.05) (Fig. 1).

The GTT showed that the fructose diet impaired glucose tolerance with a greater effect in AT1aWT than in AT1aKO mice (Fig. 2). The area under the curve for the GTT was increased in fructose-fed AT1aWT but not AT1aKO mice (P < 0.05, Fig. 2).

MAP and HR were recorded during the light and dark periods (Fig. 3). As expected, AT1aKO mice had reduced MAP compared with AT1aWT mice (P < 0.03). The fructose diet increased MAP in AT1aWT mice, but only during the dark (active) phase (P = 0.02). In AT1aKO mice there was an unexpected depressor response to fructose consumption that occurred during both light (P < 0.001) and dark periods (P =

Table 1. Body weight and metabolic parameters in AT1aWT and AT1aKO mice fed either standard diet or high dietary fructose

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Initial, g</th>
<th>Final, g</th>
<th>Glucose, mg/dl</th>
<th>Insulin, ng/ml</th>
<th>Cholesterol, mg/dl</th>
<th>TGL, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT1aWT</td>
<td>Control</td>
<td>23±1</td>
<td>28±1</td>
<td>145±6</td>
<td>1.5±0.1</td>
<td>104±7</td>
<td>121±8</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>21±1</td>
<td>30±1</td>
<td>141±10</td>
<td>1.2±0.1</td>
<td>120±11*</td>
<td>83±13</td>
</tr>
<tr>
<td>AT1aKO</td>
<td>Control</td>
<td>20±2</td>
<td>32±1</td>
<td>139±5</td>
<td>1.4±0.1</td>
<td>100±8</td>
<td>102±10</td>
</tr>
<tr>
<td></td>
<td>Fructose</td>
<td>24±1</td>
<td>32±1</td>
<td>154±8</td>
<td>1.3±0.1</td>
<td>136±9*</td>
<td>83±8</td>
</tr>
</tbody>
</table>

Body weight, blood glucose, insulin, cholesterol, and triglyceride (TGL) were measured in nonfasted angiotensin AT1a wild-type (AT1aWT) or knockout (AT1aKO) mice after 10 wk on the control or high-fructose diet. Values are means ± SE; n = 8–10 mice/group. *P < 0.05, control AT1aWT vs. fructose-fed AT1aWT and control AT1aKO vs. fructose-fed AT1aKO.
Spectral analysis was used to evaluate SAP variability in time and frequency domains. AT1aWT mice showed enhanced SAP variance in response to dietary fructose, but only during the dark period (Fig. 4). The results were similar to those for MAP. There was a dramatic increase in SAP variance \( (P \leq 0.004; \text{a } 2\text{-fold increase}) \) and in the LF domain \( (P \leq 0.001; \text{an almost } 3\text{-fold increase}) \) in fructose-fed AT1aWT mice. In AT1aKO mice there was an increase in MAP variance during the dark period \( (P \leq 0.05) \), but no changes were noted for the LF component.

Table 2 shows group data for PI variability in time and in frequency domains. ANOVA showed that fructose treatment significantly reduced PI variability in both time (variance) and frequency domains (HF and LF components). There was no effect of genetic state.

To evaluate the effects of the fructose diet on autonomic control of blood pressure, we tested the blood pressure responses to \( \alpha_1 \)-adrenoceptor blockade induced by prazosin. Prazosin significantly reduced MAP in both groups. Figure 5 shows that the depressor response was much greater in fructose-fed AT1aWT mice compared with control AT1aWT mice \( (P < 0.02) \). However, there was no significant difference in depressor responses after prazosin in control AT1aKO vs. fructose-fed AT1aKO mice.

**DISCUSSION**

A high-fructose diet is associated with metabolic and cardiovascular dysfunction, which may be related to activation of the RAS. We examined the role of angiotensin AT1a signaling in the mediation of the changes in blood pressure and glucose tolerance by using a receptor gene deletion model. A key result is that the fructose-induced blood pressure increase was converted in AT1aKO mice to a depressor response. We present a hypothesis that activation of vasodilator components of the RAS in the absence of angiotensin AT1a input leads to the reduction in blood pressure. Data also show an amelioration of the metabolic effects of fructose consumption in AT1a gene-deficient mice. This supports the idea that angiotensin receptors may mediate the glucose/insulin feedback system and explains the beneficial effects of angiotensin AT1 blockers on glucose tolerance.

The angiotensin AT1a receptor is the predominant angiotensin receptor type mediating pressor and autonomic control of the circulation (8, 28). In the receptor’s absence, there is lowered blood pressure, increased drinking, increased sympathetic activity, and increased osmotic responsiveness (8, 28, 42, 46, 47). There is also feedback between the angiotensin receptors and the processing enzymes of the RAS. This is clearly seen in AT1aKO mice, which demonstrates increases in plasma renin activity and ANG II levels (7, 13, 58).

In relation to the diabetic syndrome, there is evidence for activation of the RAS in human and animal models. This has led to the routine use of ACE antagonists and ARBs as the method of choice in managing diabetic hypertension (22).
Pharmacological studies in rats showed that treatment with an angiotensin AT1 blocker lowered the increased blood pressure produced by a high-fructose diet (24, 32, 33). There is evidence for fructose-induced activation of the RAS, as seen in increases in plasma ANG II, likely mediated by ACE (15), increased expression of angiotensin AT1a receptors (53), and activation of the vascular angiotensin system (53).

The truly provocative finding in our study was that fructose consumption in angiotensin AT1a-deficient mice did not elevate blood pressure but, unexpectedly, lowered MAP by almost 25%, or 20 mmHg. Similar results were seen when we administered losartan to fructose-fed mice, an enhanced depressor response to ANG AT1 blockade (52). We hypothesize that the mechanism of the effect is related to activation of the RAS in the absence of the ability to stimulate angiotensin AT1a (pressor) receptors. The effect may be related to interactions with the peptide-processing scheme so that there are increased levels of vasodilator peptides. We begin with the fact that AT1aKO mice have high levels of circulating ANG II, which may be further elevated by the fructose diet. Our group (15) previously reported that plasma ANG II is elevated in fructose-fed mice. Indeed, there is evidence that diabetes is associated with ACE activation (13). The high levels of ANG II could be metabolized to a vasodilator peptide, such as ANG-(1–7) via ACE2 (14, 61).

In accordance with this idea, we observed a significant reduction in plasma ANG II levels in fructose-fed AT1aKO mice, suggesting that in the absence of angiotensin receptors there was enhancement in the metabolism of ANG II. ACE2 is present in a variety of tissues and is activated by inhibitors of the RAS (14, 21).

**Table 2. Pulse interval variability in time and frequency domains in control and fructose-fed mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>Treatment</th>
<th>Variance</th>
<th>LF</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT1aWT</td>
<td>Light</td>
<td>Control</td>
<td>35±4</td>
<td>7±3</td>
<td>2±3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fructose</td>
<td>11±2</td>
<td>2±1</td>
<td>7±1</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>Control</td>
<td>25±4</td>
<td>3±1</td>
<td>15±3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fructose</td>
<td>24±7</td>
<td>6±2</td>
<td>13±3</td>
</tr>
<tr>
<td>AT1aKO</td>
<td>Light</td>
<td>Control</td>
<td>43±4</td>
<td>11±4</td>
<td>21±5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fructose</td>
<td>22±4</td>
<td>8±1</td>
<td>13±2</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>Control</td>
<td>33±8</td>
<td>5±2</td>
<td>19±4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fructose</td>
<td>30±6</td>
<td>3±1</td>
<td>20±3</td>
</tr>
</tbody>
</table>

Values are means ± SE. Low-frequency (LF) power oscillations, 0.1 to 1.0 Hz; high-frequency (HF) power oscillations, 1.0 to 5.0 Hz. There were significant main effects of treatment (control vs. fructose) for variance [F(1, 51) = 7.8, P < 0.01], low frequency [F(1, 51) = 5.9, P < 0.02], and high frequency [F(1, 51) = 6.8, P < 0.02].
Another consideration is whether the increased circulating levels of ANG II in the AT1aKO model may activate AT2 receptors to produce vasodilatation. Experimental studies have demonstrated a role for angiotensin AT2 receptors in reducing blood pressure in rats (45, 54). However, recent studies have suggested that AT2 receptors are not involved in the depressor response to losartan treatment in fructose-induced diabetes (25).

In addition, treatment with an AT2 antagonist in AT1aKO mice produced no change in blood pressure (46), and brain AT2 receptors were not altered in AT1aKO mice (41).

An association between insulin resistance and hypertension has been demonstrated in animal and humans studies (11, 20, 34, 40, 51). In fact, there is a correlation between plasma insulin concentrations and the severity of hypertension (20). Data also suggest that insulin resistance is implicated in the etiology of essential hypertension. Similar to insulin resistance, many studies have demonstrated that consumption of a high-fructose diet leads to hypertension associated with insulin resistance, obesity, and lipid abnormalities (3). Epidemiological studies suggest that this may be a causative factor in the epidemic of diabetes seen in the United States and other Western countries. Studies in rats have shown that fructose feeding produces a moderate hypertension associated with glucose intolerance and dyslipidemia (10, 24, 26, 33). Our group (15) reported that a high-fructose diet in C57BL mice produced no change in blood pressure (46), and brain AT2 receptors were not altered in AT1aKO mice (41).

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An important issue in the study of long-term blood pressure control in mice is the method of measurement. We and others (4, 8, 16) have reported on the utility of the radiotelemetric method for chronic, day/night blood pressure measurements in mice. The key issues are as follows: 1) it provides high-fidelity recordings that can be used for evaluation of autonomic balance, and 2) it provides the ability to conduct longitudinal experiments under low-stress conditions. This was emphasized in a previous study of fructose feeding in mice in our laboratory. In this situation, the changes in blood pressure were moderate (increase of ~15 mmHg) and were only observed during the dark period (15). In the absence of chronic, high-fidelity measurements, it would not be possible to detect these blood pressure changes.
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