Gonadectomy prevents endothelial dysfunction in fructose-fed male rats, a factor contributing to the development of hypertension

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Vasudevan, Harish, Prabhakara Reddy Nagareddy, and John H. McNeill. Gonadectomy prevents endothelial dysfunction in fructose-fed male rats, a factor contributing to the development of hypertension. Am J Physiol Heart Circ Physiol 291: H3058–H3064, 2006. First published June 30, 2006; doi:10.1152/ajpheart.00598.2005.—Insulin resistance has been shown to be associated with increased blood pressure (BP). The sex hormones estrogen and testosterone have opposing effects in the development of increased BP. Since testosterone has been implicated in increased BP following insulin resistance, we have tried to dissect out the effects of insulin resistance on endothelium-dependent vasorelaxation in the presence and absence of testosterone. Both gonadectomized and sham-operated male Wistar rats fed with a high-fructose diet developed insulin resistance, but BP increased only in the sham-operated rats. Reintroduction of testosterone in vivo restored the increase in BP, thereby abolishing the protective effects of gonadectomy. Fructose feeding did not affect plasma testosterone levels. Insulin resistance induced endothelial dysfunction in the mesenteric arteries of sham-operated rats, which was prevented by gonadectomy, thus suggesting a key role for testosterone in the pathogenesis of secondary vascular complications. Subsequent to blocking the actions of endothelium-dependent hyperpolarizing factor (EDHF), relaxation to acetylcholine (ACH) was lower in sham-operated fructose-fed rats compared with other groups, suggesting the involvement of nitric oxide (NO) in vasorelaxation. Inhibition of NO synthesis nearly abolished the ACh-evoked relaxation in both fructose-fed groups, thus suggesting a testosterone-independent impairment of EDHF-mediated relaxation. The improvement in endothelial function following gonadectomy could be ascribed to a NO component, although plasma nitrite and nitrate levels were unchanged. In summary, testosterone is essential in vivo for the development of endothelial dysfunction and hypertension secondary to insulin resistance, suggesting a facilitatory role for testosterone in increasing BP in fructose-fed male rats.

insulin resistance; fructose-fed rat; blood pressure; testosterone; nitric oxide; endothelium-derived hyperpolarizing factor

APPROXIMATELY 24% of the total American population suffer from some stage of the metabolic syndrome (7), which is a cluster of abnormalities related to disturbances in the normal carbohydrate-lipid metabolic system. These abnormalities include abdominal obesity, hypertriglyceridemia, low levels of high-density lipoprotein, insulin resistance, and hypertension (18). Among these, insulin resistance and hypertension are two key factors that occur in parallel in both humans (6, 24, 25, 30) and in rodents (3, 20). On the basis of observations in both clinical and experimental settings, it was hypothesized that the insulin resistance-associated metabolic impairments are directly related to the development of hypertension (24, 25, 27). In addition to other mechanisms such as increased sympathetic discharge (36) and elevation of vasoconstrictor substances (8), endothelial dysfunction plays a key role in the pathogenesis of vascular complications and hypertension secondary to insulin resistance (2, 16, 21). Our laboratory has previously demonstrated attenuated endothelial relaxation to ACh in fructose-fed rats (37–39). Further, in separate studies, the actions of endothelial vasodilators nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF) have been shown to be impaired in the vasculature following insulin resistance. Thus, insulin resistance is associated with a loss in the endothelium-dependent relaxation.

Sex differences influence the progression of insulin resistance and hypertension. Studies from our laboratory using fructose-fed rats have shown a greater degree of insulin resistance in males compared with females (9, 10). Further, only males and not females developed hypertension following fructose feeding. Removal of estrogen by ovariectomy in female fructose-fed rats induced insulin resistance and hypertension (9), thus suggesting a protective role for estrogen in the prevention of insulin resistance and hypertension.

The role of testosterone in the induction of hypertension is debated. While clinical reports are controversial in this matter, studies in animals indicate a strong association between the presence of testosterone in vivo and the development of hypertension. Blood pressure (BP) was decreased in spontaneously hypertensive rats (SHR) following the loss of testosterone by gonadectomy or by blocking testosterone action by antiandrogen therapy (28). Our laboratory has recently demonstrated that androgens are necessary for the development of hypertension in males following insulin resistance (31). In male rats fed with fructose for 8 wk, gonadectomy prevented the rise in blood pressure although there was no change in the insulin resistance. However, there are few reports as to how gonadectomy influences the vascular reactivity. Studies have shown decreased pressor responses to various vasoconstrictors in the isolated arteries of gonadectomized rats (11, 32). In addition, Ba et al. (1) showed increased relaxation to ACh in the arteries of rats subsequent to blocking testosterone action. However, the contributions of specific endothelial vasoactive agents following blockade of testosterone have not been investigated. Additionally, the effects of gonadectomy on endothelial function, as well as the factors involved in normal and insulin-resistant states, are yet to be reported.

The present study aims to extend our current findings in the pathogenesis of hypertension with a focus on the changes

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occurring in vasoactive function in isolated blood vessels of gonadectomized rats secondary to insulin resistance. We report that replacing testosterone in gonadectomized insulin-resistant rats restores the state of hypertension. Further, the loss of testosterone prevents the development of endothelial dysfunction occurring secondary to insulin resistance.

MATERIALS AND METHODS

Animals. In both studies described below, male Wistar rats, obtained from Charles River, Montreal, Canada, were used. Two-thirds of the rats had their testes surgically removed at the age of 5 wk while the rest were sham operated at the same age before shipment. The rats were maintained under regular light-dark cycle with ad libitum access to food and water. The rats were acclimatized for 1 wk in the animal facility at the Faculty of Pharmaceutical Sciences, University of British Columbia, and cared for as per the guidelines outlined by the Canadian Council on Animal care (CCAC) and the American Physiological Society in the Guiding Principles in the Care and Use of Animals. The protocol for animal use was approved by the Animal Care Committee of the University of British Columbia. In one-half of the rats, the starch present in normal laboratory rat chow was replaced with a diet enriched with fructose (66%), which was obtained commercially as a preformed diet (Teklad Labs, Madison, WI). Feeding this high-fructose-containing diet has been shown in previous studies to induce insulin resistance and hypertension (15, 26).

Research design. In study 1, rats were divided into four groups: sham-operated normal chow-fed control (C; n = 10), sham-operated fructose-fed (F; n = 10), gonadectomized normal chow-fed (G; n = 10), and gonadectomized fructose-fed rats (GF; n = 10). Before initiation of the fructose diet, basal systolic blood pressure was measured in conscious rats using the indirect tail cuff method as explained in later sections. Subsequently, blood was collected from the rats following a 5-h fast for measuring glucose, insulin, and testosterone.

Following the increase in BP after 6 wk of fructose feeding, an oral glucose-tolerance test was performed on the rats as previously described (9). At termination, the rats were euthanized by a single injection of 65 mg/kg ip pentobarbital (Somnotol) followed by opening of the chest cavity. Blood was obtained by cardiac puncture for measuring testosterone. The superior mesenteric artery was isolated, removed, and cleaned of excess adipose and connective tissues.

To confirm that the presence of testosterone was indeed responsible for the development of hypertension, we studied the changes in blood pressure in fructose-fed rats subsequent to exogenous provision of testosterone. In study 2, sham-operated and gonadectomized male Wistar rats were divided into six experimental groups: sham-operated chow-fed control (C; n = 4), sham-operated fructose-fed (F; n = 5), gonadectomized normal chow-fed (G; n = 4), gonadectomized fructose-fed (GF; n = 4), gonadectomized normal chow-fed and testosterone-treated (GT; n = 4), and gonadectomized fructose-fed and testosterone-treated rats (GFT; n = 5). The rats were started on the 66% fructose-enriched diet as described in study 1. To extend our previous findings, which demonstrate the role of testosterone in the development of hypertension, we determined the changes in insulin sensitivity and blood pressure on reintroduction of testosterone. Following 5 wk of normal chow or fructose feeding, nine of the gonadectomized male rats (GT, n = 4; and GFT, n = 5) were injected intraperitoneally with 10 mg/kg per day (Univet suspension). The rats were injected daily for 5–6 wk. Blood was withdrawn after 7 days of treatment for measuring testosterone. After 5 wk of treatment, i.e., study week 10, the blood pressure was measured and a truncated oral glucose tolerance test was performed the following week. Briefly, blood was collected at time points of 0, 10, 30, and 90 min subsequent to glucose ingestion. The animals were euthanized, and blood was collected by cardiac puncture for analysis of plasma testosterone.

Measurement of blood pressure and assessment of insulin resistance/sensitivity. Systolic blood pressure was measured in conscious rats by using the indirect noninvasive tail-cuff method as previously described (4, 9). Insulin sensitivity was estimated after the oral glucose challenge using the formula of Matsuda and DeFronzo (19) using 100 as constant.

\[
ISI = \frac{100}{(\text{fasting glucose} \times \text{fasting insulin}) \div (\text{mean glucose} \times \text{mean insulin})}
\]

where ISI is the insulin sensitivity index.

Studies on vascular reactivity. Tissue rings each of length 3–4 mm with intact endothelium were dissected from the superior mesenteric arteries and appended onto glass hooks, which were then mounted in a 20-ml isolated tissue bath containing carboxygenated (95% O2-5% CO2) Krebs-Ringer buffer at 37°C as described previously (8, 38). Tissues were assessed for changes in contractile response to phenylephrine (PE) (10−8 to 10−4 mol/l), after which they were precontracted with the 70% of the effective dose (ED70) of PE, and relaxation responses to increasing concentrations of ACh (10−5 to 10−4 mol/l) were obtained. Alterations in responses to ACh were compared between fructose-fed animals and rats maintained on standard lab diet, in the presence and absence of testosterone, respectively.

NO/EDHF mediation and ACh-induced relaxation. The drugs charybdotoxin (CTX) and apamin, taken together, block the calcium-sensitive potassium channels (KCa), the opening of which is necessary for EDHF-dependent hyperpolarization to occur. To evaluate the changes in NO-mediated vasodilation during insulin resistance and its individual contribution to the relaxation process, vessels were incubated with a combination of 10−8 mol/l CTX and 0.25 μmol/l apamin for 20 min, after which relaxation to ACh was studied on tissues precontracted with the ED70 dose of PE.

After being washed, the tissues were incubated with the NO synthase (NOS) inhibitor Nω-nitro-L-arginine methyl ester (L-NAME; 10−4 mol/l) for 20 min, and responses to ACh in precontracted tissues were obtained as mentioned above.

Alterations in tissue responses to PE were calculated in terms of milligrams tension per square millimeter cross-sectional area. Changes in responses to ACh were reported as percent relaxation of contraction induced by PE.

Blood collection. Blood samples were collected from the tail vein at week 0 for determination of plasma glucose, insulin, and testosterone. At termination, blood was collected by cardiac puncture into plastic centrifuge tubes containing 2% EDTA and 0.04 mol/l indocyanine green and centrifuged at 4,500 rpm for 25 min at 4°C. Plasma was aliquoted and stored at −80°C for measuring testosterone and nitrite/nitrate (NOx) concentrations.

Biochemical parameters. Plasma glucose was measured using a Beckman Glucose Analyzer II. Insulin was measured in the plasma using commercially available rat-specific radioimmunoassay (RIA) kits from Linco Diagnostics, while testosterone was measured using RIA kits from MP Biomedicals.

Plasma NOx analysis. Plasma was ultrafiltered though a 30-kDa molecular mass cutoff filter (Ultrafree-MC centrifugal filter units, Millipore, Bedford, MA). Nitrite and nitrate (NOx) levels in the plasma were determined using a commercially available colorimetric assay kit (Cayman Chemical, Ann Arbor, MI) on the basis of the Griess reaction.

Chemicals and reagents. All chemicals unless otherwise mentioned were of reagent grade and purchased from Sigma (St. Louis, MO). PE, ACh, and L-NAME were dissolved in Krebs-Ringer, while CTX and apamin were dissolved in freshly distilled water.

Statistical analysis. All data were analyzed using one-way ANOVA. Data involving multiple time points were subject to the Generalized Linear Models ANOVA using the NCSS 2000 statistical software. For all the results, Newman-Keuls test was used as post hoc test.
RESULTS

Physical parameters. Fructose feeding did not affect the body weights of either the gonadectomized or the sham-operated rats. Feeding and drinking patterns were uniform in all the groups. Treatment with testosterone did not affect the body weights in the fructose-fed rats (see Table 1, study 2).

Changes in insulin sensitivity. Analysis of the ISI values showed significant overlap between the two studies. Therefore, we have combined the data from both studies in Table 2. Glucose and insulin values subsequent to glucose challenge (Fig. 1, A and B) were consistent with previous studies (31, 35), and the comparison of ISI values calculated revealed attenuated insulin sensitivity in the fructose-fed rats (Table 2). Treatment with testosterone (GFT) did not affect the insulin sensitivity in gonadectomized fructose-fed rats (Table 2).

Changes in blood pressure. At the start of fructose feeding, all rats had systolic blood pressure values below 120 mmHg. When blood pressure was measured 6 wk following fructose feeding, there was a significant elevation in the blood pressure of sham-operated rats fed with fructose (F, 137 ± 0.7 mmHg vs. C, 110 ± 1.5 mmHg). In contrast, fructose failed to elevate the blood pressure in gonadectomized animals (GF, 114 ± 1 mmHg) (Table 2). However, gonadectomy by itself did not induce any change in the blood pressure of normal-chow fed rats (G, 113 ± 3 mmHg).

Before the start of testosterone injection, after 5 wk of fructose feeding, there was no significant rise in the blood pressure. At study week 10 (5 wk postinjection), the testosterone-treated gonadectomized fructose-fed rats (GFT) showed an increase in BP (133 ± 2 mmHg) while the control (C) and the gonadectomized groups that were not fructose fed and testosterone treated (G, GF, and GT) were normotensive. The values of blood pressure in the GFT rats were comparable with those from F. Thus testosterone treatment negated the positive effects of gonadectomy on the blood pressure.

Plasma testosterone levels. Similar to insulin sensitivity, we have combined the data from both studies (C, F, G, and GF) in

Table 1. Changes in body weights in normal chow-fed and fructose-fed intact rats, in normal chow-fed and fructose-fed gonadectomized rats, and in normal chow-fed and fructose-fed gonadectomized rats with testosterone implantation

<table>
<thead>
<tr>
<th>Time</th>
<th>Study 1</th>
<th>Study 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body weight, g</td>
<td>Body weight, g</td>
</tr>
<tr>
<td>0 week</td>
<td>C 309 ± 3</td>
<td>F 305 ± 4</td>
</tr>
<tr>
<td></td>
<td>G 277 ± 4</td>
<td>GF 273 ± 3</td>
</tr>
<tr>
<td>8 weeks</td>
<td>C 661 ± 20</td>
<td>F 639 ± 20</td>
</tr>
<tr>
<td></td>
<td>G 568 ± 16</td>
<td>GF 551 ± 13</td>
</tr>
</tbody>
</table>

Values are means ± SE. Experimental groups in study 1 were sham-operated + normal chow-fed control (C), sham-operated + fructose-fed (F), gonadectomized + normal chow-fed (G), and gonadectomized + fructose-fed (GF) rats. Experimental groups in study 2 were C, F, G, GF, normal chow-fed + gonadectomized + testosterone implanted (GT), and fructose-fed + gonadectomized + testosterone implanted (GFT). Statistical analysis was done by 1-way ANOVA followed by Newman-Keuls post hoc test, P < 0.05.

Table 2. ISI, SBP, and plasma testosterone in rats following fructose feeding

<table>
<thead>
<tr>
<th>Group</th>
<th>ISI</th>
<th>SBP, mmHg</th>
<th>Testosterone, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>21.1 ± 2.3</td>
<td>110 ± 1.5</td>
<td>2.55 ± 0.3</td>
</tr>
<tr>
<td>F</td>
<td>9.4 ± 1.2*</td>
<td>137 ± 0.7†</td>
<td>4.53 ± 0.9</td>
</tr>
<tr>
<td>G</td>
<td>15.4 ± 1.6</td>
<td>113 ± 3</td>
<td>ND</td>
</tr>
<tr>
<td>GF</td>
<td>7.5 ± 0.9*</td>
<td>114 ± 1</td>
<td>ND</td>
</tr>
<tr>
<td>GT</td>
<td>14.0 ± 3.5</td>
<td>113 ± 3</td>
<td>1.17 ± 0.6</td>
</tr>
<tr>
<td>GFT</td>
<td>7.1 ± 0.8*</td>
<td>133 ± 2†</td>
<td>12.01 ± 6.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. ISI, insulin sensitivity index; SBP, systolic blood pressure. Statistical analysis was done by one-way ANOVA followed by Newman-Keuls post hoc test, P < 0.05. *Significantly different from C and G. †Significantly different from C, G, GF, and GT. ND, not detected.
Table 2. Testosterone was undetectable in gonadectomized animals. In both the studies, testosterone levels were unaffected by the change in diet (Table 2). In the injected rats (GFT), testosterone levels at the end of 1 wk posttreatment were 2.3 ± 1.0 ng/ml. The levels were as high as 12 ± 6 ng/ml at termination, which was not statistically different compared with the group receiving normal chow and injected with testosterone (GT).

Vascular reactivity studies. In study 1, however, subsequent to KCl challenge, the mesenteric arteries of all groups were contracted with increasing concentrations of PE (10⁻⁹ to 10⁻⁴ mol/l). Although the contraction to PE was robust and ranged from 4,500 to 6,000 mg/mm², there was no difference in contraction in any of the groups.

ACh relaxed the precontracted arterial rings up to 85% in both sham-operated as well as gonadectomized rats fed on normal chow (C and G). In concentrations from 10⁻⁴ to 10⁻³ mol/l, the relaxation to ACh was impaired in sham-operated F compared with C and G groups (Fig. 2). Response to ACh in the GF group was similar to that of C and G groups. Thus the loss of testosterone prevented endothelial dysfunction to ACh following fructose feeding.

We were interested in studying the contribution of specific endothelial vasodilators, which were involved in mediating this increased ACh-induced vasorelaxation in GF. Most of the studies on mesenteric arteries have reported a moderate to negligible role for prostacyclin (PGI₂) in the vasodilatory process (5, 17). Thus the two main endothelial agents involved in mediating vascular relaxation are NO and EDHF. With the hypothesis that the relaxation observed on blockade of NO is due to EDHF action and vice versa, we evaluated vascular reactivity of arterial rings (obtained in study 1) to ACh in the presence of specific NO or EDHF inhibitors.

![Image](http://ajpheart.physiology.org/)

Fig. 2. Fructose feeding (F) reduces relaxation to ACh in the mesenteric arteries, which is improved by gonadectomy (G and GF). Relaxation responses were obtained to ACh (10⁻⁹ to 10⁻⁴ mol/l) after precontraction with 70% of the effective dose (ED₉₀) of phenylephrine (PE) in the 4 experimental groups: C (n = 5), F (n = 5), G (n = 5), and GF (n = 5). Inset: AUC values for the curve. All values are presented as means ± SE. *P < 0.05, F vs. C, G, and GF.

Table 3. AUC of percent relaxation to ACh in the four experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>PE + ACh</th>
<th>PE + ACh (CTX + Apa)</th>
<th>PE + ACh (l-NAME)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>271 ± 14.5</td>
<td>123 ± 13*</td>
<td>119 ± 27.6*</td>
</tr>
<tr>
<td>F</td>
<td>133 ± 21†</td>
<td>70 ± 17.1†</td>
<td>20 ± 13.7*</td>
</tr>
<tr>
<td>G</td>
<td>295 ± 22</td>
<td>151 ± 14.2*</td>
<td>193 ± 31.7*</td>
</tr>
<tr>
<td>GF</td>
<td>224 ± 24.4</td>
<td>136 ± 20.5*</td>
<td>43 ± 18.3†‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 for each group (C, F, G, GF). PE, phenylephrine; CTX, charybdotoxin; Apa, apamin; l-NAME, Nω-nitro-l-arginine methyl ester; AUC, area under the curve. Statistical analysis was done by Generalized Linear Models ANOVA, P < 0.05. *Significantly different from PE + ACh in all 4 treatment groups. †Significantly different from C, G, and GF (PE + ACh). ‡Significantly different from GF [PE + ACh (CTX + Apa)].

Effects of EDHF blockade on ACh-induced vascular relaxation. Preincubation of the tissues with the Kcs channel blockers CTX (10⁻⁶ mol/l) and apamin (0.25 μmol/l) significantly depressed the relaxation in all the groups compared with the responses observed before inhibition of Kcs (Table 3). However, despite the decrease, the relaxation in C, G, and GF groups was appreciable and significantly higher than those observed in F (Fig. 3 and Fig. 3, inset). The fructose-fed sham-operated animals (F) exhibited significantly lower relaxation compared with the other groups, suggesting a decrease in the contribution of NO.

Effects of inhibition of NO synthesis on ACh-induced vascular relaxation. Incubation with the NOS inhibitor l-NAME (10⁻⁶ mol/l) for 20 min inhibited the ACh-evoked relaxation in the mesenteric arteries of all groups. Responses to PE were exaggerated in l-NAME-treated tissues compared with the responses before l-NAME treatment. Similar to the results...
observed with CTX + apamin, l-NAME decreased the relaxation in C and G groups compared with ACh-induced responses before treatment (Table 3). Relaxation to ACh was nearly abolished in both the fructose-fed groups (F and GF) (Fig. 4). However, the degree of relaxation in both the normal chow-fed groups (C and G) was greater compared with F and GF as indicated by the area under the curve values (see Fig. 4, inset).

The effects of each drug response were evaluated in individual groups. Selective inhibition of EDHF/KCa channel or NO synthesis in the C and G groups produced a significant decrease in relaxation to ACh. However, the tissues showed up to 50% relaxation in either case. The ACh-evoked relaxation pattern in the GF rats was similar to that of C and G groups. However, inhibiting NO induced a marked fall in the relaxation of both F and GF. In the sham-operated fructose-fed rats (F), inhibition of EDHF action decreased the relaxation to a greater degree compared with the relaxation observed in GF group (Table 3).

Changes in plasma NOx levels. The level of plasma NOx at termination was not statistically significant in any of the groups (C, 28.8 ± 1.9; F, 17.8 ± 3.6; G, 34.9 ± 4.9; GF, 28.8 ± 6.1 μmol/l, respectively). Although the means do not differ from each other when analyzed by one-way ANOVA, a comparison among individual groups using unpaired t-test revealed a significant difference between C and F groups. While the data are supportive of a potential association between fructose feeding and decreased NOx levels in hypertension, further studies are warranted to investigate this interesting lead.

**DISCUSSION**

In the present study, we attempted to extend our understanding of the role of testosterone in mediating hypertension and how this was reflected in changes in the vascular tone. Our results offer evidence that, following insulin resistance, endothelial dysfunction and hypertension develop only in the presence of testosterone. Increasing testosterone levels by injection in gonadectomized and fructose-fed rats reversed the protective effect conferred by gonadectomy. The prevention of endothelial dysfunction by gonadectomy contributes to protection against hypertension. Further, this vasoprotective effect of gonadectomy may be mediated by an increased contribution of endothelial NO.

Our laboratory has recently demonstrated that fructose-induced insulin resistance is independent of testosterone (31). The present results are in agreement and demonstrate that hyperinsulinemia and attenuated insulin sensitivity are observed in fructose-fed rats (F and GF) compared with normal chow-fed rats (C and G). However, all rats were normoglycemic. The changes in ISI values reflect this decrease in insulin action (Table 2). Although the effects of varying levels of testosterone on insulin action are unclear in rats (13, 29), the data suggest that testosterone levels do not affect insulin sensitivity. We have also demonstrated that both sham-operated (physiological levels of testosterone) and gonadectomized rats (absence of testosterone) develop insulin resistance subsequent to fructose feeding (Table 2). Additionally, testosterone levels were unchanged in the sham-operated groups fed on either normal chow (C) or on fructose-enriched chow (F), indicating that fructose feeding per se does not affect testosterone levels. Our results therefore show insulin sensitivity to be independent of testosterone in fructose-fed male rats, in agreement with previous reports (31, 34).

The results have reproducibly demonstrated an increase in blood pressure in fructose-fed rats with intact testes. In fructose-fed rats, the presence of testosterone, whether endogenous or exogenous, permitted an increase in blood pressure (Table 2). These observations confirm previous results indicating that testosterone is associated with the development of hypertension in insulin-resistant rats (31). Previous reports have demonstrated elevated blood pressure in male and female rats that have been injected with androgens for 10–15 days (22, 23). We believe that the development of hypertension is independent of circulating testosterone levels because plasma testosterone levels were unchanged in the fructose-fed rats compared with normal chow-fed groups. Only gonadectomy prevented the increase in BP. Decreasing testosterone levels by estradiol treatment induced a fall in BP, which was greater than controls (35). Furthermore, we also found that treatment with the antiandrogen flutamide prevents the increase in blood pressure in fructose-fed rats, although plasma testosterone levels were elevated (13 ± 2.05 ng/ml; unpublished data). Therefore we suggest that insulin resistance-induced hypertension per se does not affect circulating testosterone levels. Rather, the mere presence of testosterone is sufficient for the induction of hypertension in fructose-fed rats. We also suggest that this testosterone-facilitated increase in BP may be a receptor-mediated effect. The hypothesis is supported by reports from our and other laboratories in which treatment with flutamide resulted in a fall in both SHR and fructose-fed rats (28). The involvement of 5-dihydrotosterone, which is the active metabolite of testosterone, is presently unclear. Although inhibition of 5α-reductase did not reduce hypertension in SHR (28), Nakagawa et al. (22) have reported elevated blood pressure in

![Fig. 4. Selective inhibition of nitric oxide synthesis using Nω-nitro-L-arginine methyl ester (l-NAME) showed that KCa channel function is impaired in both intact and gonadectomized rats fed with fructose. Relaxation responses to ACh were estimated after incubating with 10−6 mol/l l-NAME (20 min) and precontracting with EDβ2 PE in C (n = 5), F (n = 5), G (n = 5), and GF (n = 5). Inset: AUC values for the curves. All values are presented as means ± SE. *P < 0.05, G and C vs. F and GF.](https://ajpheart.physiology.org/doi/10.1152/ajpheart.00335.2006)

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ravs treated with 5-dihydrotestosterone for 2 wk (22). Presently, no studies using androgen antagonists have been reported in animals with subphysiological levels of testosterone (i.e., <1 ng/ml). Such an experiment may reveal the presence of a threshold level for testosterone action.

Our laboratory has previously reported that endothelium-dependent relaxation to insulin as well as ACh is depressed in the mesenteric arteries of fructose-fed rats (37, 38). Both NO (33) and EDHF (17) functions have been shown to be attenuated by insulin resistance in separate studies. However, the relaxation responses to ACh in gonadectomized fructose-fed rats and the contributions of NO and EDHF are unclear. We studied the effects of ACh on the mesenteric arteries from intact and gonadectomized rats fed with fructose. On comparing the responses of the tissues to incremental doses of ACh, endothelium-dependent relaxation was significantly decreased in the sham-operated fructose-fed rats (F) compared with other groups (Fig. 2). This is in agreement with the previous reports indicating depressed vasorelaxation following insulin resistance (33, 37). In the GF rats, relaxation to ACh was greater compared with F and was about the same as C and G groups (Fig. 2). Thus our study is the first to report a beneficial effect of gonadectomy in the vasculature of insulin resistant rats. Our data suggest that subsequent to insulin resistance, the presence of testosterone is essential for the induction of endothelial dysfunction. Although we do not have results regarding the androgen receptor function in insulin resistance, we believe that the attenuated endothelial function in males may be due to downstream effects of testosterone receptor activation. The role of androgen receptor blockade on hypertension and associated endothelial function in subsequent studies remains to be examined.

On the basis of previous reports that indicated an insignificant role for prostacyclin in the relaxation of rat mesenteric arteries (5, 17), we hypothesized that NO and EDHF/KCa are the two major vasorelaxant pathways involved. Inhibiting either of them would depress responses to ACh, with the resultant relaxation reflecting the function of the other vasodilator. Relaxation to ACh was impaired in fructose-fed rats following inhibition of NO synthesis (Fig. 4). Interestingly, l-NAME abolished the NO-dependent relaxation in the GF rats to a similar degree as the relaxation in sham-operated F rats. This suggests that the impairment in EDHF action associated with insulin resistance is unaffected by gonadectomy (Fig. 4). Further, the responses to ACh in the normal chow-fed rats (C and G) were greater in the presence of l-NAME compared with the fructose-fed rats (F and GF). Thus the EDHF component was unaffected in either of the sham-operated and gonadectomized rats in the absence of fructose. The role of insulin resistance-induced changes in attenuating EDHF action could be assessed by evaluating vascular reactivity of male fructose-fed rats treated with insulin sensitizers such as metformin or thiazolidinediones.

Blocking EDHF action using CTX + apamin resulted in depressed ACh-evoked relaxation in all the groups, which was in agreement with previous reports (17). The attenuation in relaxation was observed to a lesser degree in GF compared with F rats (Fig. 3, A and B). Since EDHF action was blocked, the observed relaxation reflects a NO-dependent vasorelaxation process. However, the levels of plasma NO, do not offer a clear indication of a NO-dependent mechanism being involved. Experiments need to be performed using NO donors such as sodium nitroprusside to determine the specific contributions of NO to vascular sensitivity in intact and gonadectomized rats.

In conclusion, endothelial dysfunction occurs secondary to insulin resistance and subsequently leads to hypertension. NO and EDHF-dependent vasorelaxation are impaired following attenuated endothelial function. Our studies indicate that the presence of testosterone is essential for disturbing the equilibrium between endothelial vasoactive agents. While the loss of testosterone elevates NO-dependent relaxation in insulin-resistant animals, it does not influence the EDHF-regulated component. Additional studies are needed to investigate the testosterone-dependent pathways recruited secondary to insulin resistance, which support the increase in blood pressure. Studies in the kidney and the peripheral vasculature (14) have revealed an androgen-dependent increase in blood pressure following upregulation of the renin-angiotensin system, which promotes tubular reabsorption of Na+ (23). Additionally, testosterone has also been shown to influence the synthesis of vasoconstrictors such as 20-hydroxyeicosatetraenoic acid, a hydroxylated arachidonate derivative (12, 22). However, the role of testosterone in regulating these pathways in the presence of insulin resistance and secondary endothelial dysfunction is yet to be ascertained.

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