Differential regulation of insulin resistance and hypertension by sex hormones in fructose-fed male rats

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Vasudevan, Harish, Hong Xiang, and John H. McNeill. Differential regulation of insulin resistance and hypertension by sex hormones in fructose-fed male rats. Am J Physiol Heart Circ Physiol 289: H1335–H1342, 2005. First published June 10, 2005; doi:10.1152/ajpheart.00399.2005.— Differences in gender are in part responsible for the development of insulin resistance (IR) and associated hypertension. Currently, it is unclear whether these differences are dictated by gender itself or by the relative changes in plasma estrogen and/or testosterone. We investigated the interrelationships between testosterone and estrogen in the progression of IR and hypertension in vivo in intact and gonadectomized fructose-fed male rats. Treatment with estrogen significantly reduced the testosterone levels in both normal chow-fed and fructose-fed rats. Interestingly, fructose feeding induced a relative increase in estradiol levels, which did not affect IR in both intact and gonadectomized fructose-fed rats. However, increasing the estradiol levels improved insulin sensitivity in both intact and gonadectomized fructose-fed rats. In intact males, fructose feeding increased the blood pressure (140 ± 2 mmHg), which was prevented by estrogen treatment. However, the blood pressure in the fructose-fed estrogen rats (125 ± 1 mmHg) was significantly higher than that of normal chow-fed (113 ± 1 mmHg) and fructose-fed gonadectomized rats. Estrogen treatment did not affect the blood pressure in gonadectomized fructose-fed rats (105 ± 2 mmHg). These data suggest the existence of a threshold value for estrogen below which insulin sensitivity is unaffected. The development of hypertension in this model is dictated solely by the presence or absence of testosterone. In summary, the development of IR and hypertension is governed not by gender per se but by the interactions of specific sex hormones such as estrogen and testosterone.

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despite being ascribed opposite roles in the regulation of blood pressure, no studies to date have attempted to investigate the interactions and interdependence between testosterone and estrogen in males and females and how the loss of one hormone influences the actions of the other in normal and insulin-resistant states.

The aim of this study was to determine whether a critical balance between estrogen and androgen in males is needed for the regulation of insulin sensitivity and blood pressure. We hypothesized that the balance between testosterone and estrogen in male rats affects the induction of IR and subsequent development of hypertension.

MATERIALS AND METHODS

Animals. The fructose hypertensive rat model was used in the studies outlined below. Male Wistar rats were obtained from Charles River, Montreal, Canada. The rats were cared for as per the guidelines outlined by the Canadian Council on Animal Care and by the American Physiological Society in the “Guiding Principles in the Care and Use of Animals.” Subsequent to arriving, the rats were acclimatized in the animal facility at the Faculty of Pharmaceutical Sciences, University of British Columbia, for 1 wk before treatment. The starch present in the animal facility was of reagent grade and purchased from Sigma (St. Louis, MO).

Measurement of blood pressure and assessment of IR and sensitivity. In the first study, male rats with intact testes were divided into four experimental groups: normal chow-fed control (C), control rats treated with estrogen (CE), fructose-fed (F), and fructose-fed estrogen treated (FE). Before the introduction of fructose, the basal blood pressure was measured by the noninvasive tail-cuff method (see Measurement of blood pressure and assessment of IR and sensitivity). In addition, changes in body weight and food intake were estimated and the animals were euthanized, and blood was collected from the tail vein. The rat chow in groups C and F and FE was replaced with the diet containing fructose. All the rats were allowed ad libitum access to food and water throughout the 6 to 7 wk of treatment. Estrogen treatment was carried out by the implantation of estrogen pellets (0.5 mg for 60 day release; Innovative Research of America, Sarasota, FL). With the rats under light halothane anesthesia, the pellets were subcutaneously implanted by using a trochar under aseptic conditions. The implantation was performed on the same day as the initiation of fructose feeding. The food consumption of the rats was assessed once a week along with body weights. Because of the unexplained deaths of two rats in the FE group, plasma blood glucose was measured in the surviving rats during the subsequent 2 wk using Accucheck glucose strips (Roche).

In the second study, sham-operated and gonadectomized male Wistar rats were initially divided into four experimental groups, namely, sham-operated chow-fed control (C; n = 5), sham-operated fructose-fed (F; n = 5), gonadectomized normal chow-fed (G; n = 4), and gonadectomized-fructose-fed (GF; n = 16). After 6 wk of feeding fructose, one-half of the GF rats were subcutaneously implanted with a 0.5-mg estrogen pellet (60 day release, Innovative Research of America) to form the GFE group (5th experimental group). All implantations were carried out under light halothane anesthesia. One rat died due to halothane, but the procedure was successful in the remaining animals. Thus the final GFE group totaled n = 7 rats. 17β-Estradiol levels in the plasma were estimated at 3 and 10 days after implantation. Blood pressure was measured in all five groups, i.e., C, F, G, GF, and GFE, 2 wk subsequent to implantation at the end of study week 8. Subsequently, changes in insulin sensitivity were assessed (see Measurement of blood pressure and assessment of IR and sensitivity). The animals were euthanized, and blood was collected for measuring plasma testosterone and 17β-estradiol.

Measurement of blood pressure and assessment of IR and sensitivity. Systolic blood pressure was measured in conscious rats using the indirect noninvasive tail-cuff method as previously described (4, 10). An oral glucose tolerance test (OGTT) was performed in the week after the blood pressure measurement. After the oral glucose challenge, insulin sensitivity was estimated with the formula of Matsuda and DeFronzo, using 100 as a constant: insulin sensitivity index (ISI) = 100/square root [(fasting glucose × fasting insulin) × (mean glucose × mean insulin)] (24). Among all methods used, the values obtained by OGTT offered the best correlation with values from the euglycemic hyperinsulinemic clamp (24).

Blood collection. Blood samples were collected from the tail vein at week 0 for estimation of plasma glucose, insulin, testosterone, and 17β-estradiol. On termination, blood was collected by cardiac puncture into plastic centrifuge tubes containing 2% EDTA and 0.04 M indomethacin and centrifuged at 4,500 rpm for 25 min at 4°C. Plasma was aspirated and stored at −80°C for measuring testosterone, 17β-estradiol, and triglycerides (TGs).

Biochemical parameters. Plasma glucose was estimated by using a Beckman Glucose Analyzer II, whereas TGs were measured colorimetrically by using a commercially available kit. Plasma insulin was measured by using commercially available RIA kits from Linco Diagnostics, whereas 17β-estradiol and testosterone were measured by using RIA kits from MP Biomedicals.

Statistical analysis. All data were analyzed by using one-way ANOVA. Data involving multiple time points were subject to the General Linear Model ANOVA using the NCSS 2000 statistical software. For all the results, the Newman-Keuls test was used as a post hoc test. The value of P < 0.05 was taken as the level of significance. All results are reported as means ± SE.

RESULTS

Physical appearance, body weight, and food intake. Two animals from the FE group died during study 1 due to hypoglycemia. The remaining rats in the FE group, although hypoglycemic at 2 wk, improved over subsequent weeks of the study (data not shown). We observed a significant decrease in the testicle size of male rats subsequent to treatment with estrogen. At termination, the estrogen-implanted male rats (CE and FE) had significantly lower body weights compared with the nonimplanted groups (Table 1). This was observed from the second week after estrogen implantation until the end of the study week 8.

Table 1. Body weights and food intake in intact male rats treated with estrogen and fructose-fed for 7 wk

<table>
<thead>
<tr>
<th>Physical Parameters</th>
<th>C</th>
<th>CE</th>
<th>F</th>
<th>FE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>574 ± 18</td>
<td>458 ± 11*</td>
<td>561 ± 18</td>
<td>466 ± 16*</td>
</tr>
<tr>
<td>Food intake/day, g</td>
<td>32 ± 1</td>
<td>26 ± 1*</td>
<td>31 ± 1</td>
<td>26 ± 1*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Body weights were measured at end of 7 wk of fructose feeding (termination). Four experimental groups were control rats (C, n = 9), estrogen-treated rats (CE, n = 10), and fructose-fed estrogentreated rats (FE, n = 8). Statistical analysis was done by one-way ANOVA followed by Newman-Keuls post hoc test. *P < 0.05, CE and FE vs. C and F.
Table 2. Changes in body weights in gonadectomized fructose-fed male rats treated with estrogen

<table>
<thead>
<tr>
<th>Groups</th>
<th>C</th>
<th>F</th>
<th>G</th>
<th>GF</th>
<th>GFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 wk</td>
<td>499±26</td>
<td>478±10</td>
<td>464±29</td>
<td>453±20</td>
<td>422±8</td>
</tr>
<tr>
<td>9 wk</td>
<td>534±30</td>
<td>528±12</td>
<td>493±32</td>
<td>510±22</td>
<td>379±8*†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Body weights were measured at end of 6 wk of fructose feeding (before estrogen treatment) and at end of 9 wk (termination). Five experimental groups were control sham-operated, normal chow-fed rats (C, n = 5), sham-operated, fructose-fed rats (F, n = 5), gonadectomized normal chow-fed rats (G, n = 4), gonadectomized fructose-fed rats (GF, n = 8), and gonadectomized fructose-fed estrogen-treated rats (GFE, n = 7). Statistical analysis was done by one-way ANOVA followed by Newman-Keuls post hoc test. *P < 0.05, GFE vs. C, F, G, and GF; †P < 0.05, GFE (9 wk) vs. GFE (6 wk).

Table 3. Plasma sex hormone levels in intact male rats treated with estrogen and fructose-fed for 7 wk

<table>
<thead>
<tr>
<th></th>
<th>C</th>
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<tbody>
<tr>
<td>17β-Estradiol, pg/ml</td>
<td>29.5±4.32</td>
</tr>
<tr>
<td>Testosterone, ng/ml</td>
<td>2.47±0.29</td>
</tr>
<tr>
<td>Testosterone/estradiol</td>
<td>0.0996±0.013</td>
</tr>
</tbody>
</table>

Sex Hormone Levels in Plasma, 50 days

<table>
<thead>
<tr>
<th></th>
<th>CE</th>
</tr>
</thead>
<tbody>
<tr>
<td>17β-Estradiol, pg/ml</td>
<td>39.8±2.36*</td>
</tr>
<tr>
<td>Testosterone, ng/ml</td>
<td>1.65±0.25*</td>
</tr>
<tr>
<td>Testosterone/estradiol</td>
<td>0.0439±0.01*</td>
</tr>
</tbody>
</table>

Values are means ± SE. 17β-Estradiol and testosterone were measured at end of 7 wk of fructose feeding (termination). Four experimental groups were C (n = 9), CE (n = 10), F (n = 10), and FE (n = 8). Statistical analysis was done by one-way ANOVA followed by Newman-Keuls post hoc test. *P < 0.05, CE vs. C and FE vs. F (diet controls); †P < 0.01, FE vs. C and CE; ‡P < 0.001, F vs. C and CE.
pressure was unchanged in both control groups (C and CE) after 6 wk (Fig. 3).

In the second study, after 2 wk of estrogen treatment, at 8 wk after fructose feeding, the F rats had higher blood pressure compared with that in the C rats. Blood pressure was unaffected in the gonadectomized fructose-fed rats (GFE and GF) regardless of the presence or absence of estrogen (Fig. 4).

DISCUSSION

IR is the initial stage of the metabolic syndrome, which is suggested to result from complex interactions between genetic and environmental factors. Feeding rats a high-fructose diet induces IR, hyperinsulinemia, hypertriglyceridemia, and hypertension (11). In humans (14) as well as rodents (29), females are less susceptible to developing hypertension compared with males. A similar profile was observed in the induction of IR in Wistar rats, wherein females fed with fructose did not develop IR and hyperinsulinemia compared with age-matched males (10, 11). The protective effects of estrogen (10, 11) and permissive effects of testosterone (32) in the development of hypertension secondary to IR have been demonstrated in separates studies. However, little is known regarding the effects of hormonal balance in vivo on the development of IR and hypertension in either of the sexes. In males, the absolute presence or absence of testosterone is responsible for the changes in blood pressure secondary to IR. Second, in males, estrogen may be required to attain a threshold level to observe its preventive effects on IR and hypertension.

Effects of sex hormone balance on induction of IR in male rats. Fructose-fed male rats with intact testes developed IR after 6 wk of fructose feeding, as indicated by hyperinsulinemia and lower ISI compared with that in the C rats. This result agrees with the previous reports (9, 36). Estrogen elevated the insulin sensitivity in male fructose-fed rats (Fig. 1C). This is supported by results from our laboratory (33) that indicate a similar effect of estrogen on insulin sensitivity in ovariectomized rats. Estrogen treatment resulted in a lower food intake and weight gain in male rats (Table 1). Treatment with estrogen significantly reduced the testis size in male rats, suggesting decreased testicular activity. In a study by Valigora et al. (34), chronic estrone treatment in male SHR reduced plasma

<table>
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<tr>
<th>Table 4. Plasma 17β-estradiol levels at end of 3, 10, and 30 days (termination) following estrogen implantation</th>
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<tr>
<td><strong>Plasma 17β-Estradiol Levels, pg/ml</strong></td>
</tr>
<tr>
<td><strong>Time, days</strong></td>
</tr>
<tr>
<td>****</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>30</td>
</tr>
</tbody>
</table>

Values are means ± SE. Five experimental groups were C (n = 5), F (n = 5), G (n = 4), GF (n = 8), and GFE (n = 7). Statistical analysis was done by one-way ANOVA followed by Newman-Keuls post hoc test. *P < 0.05, F vs. C and GF vs. G (diet controls) at termination; †P < 0.05, GFE vs. C, F, G, and GF.

Fig. 1. A: plasma insulin values in oral glucose tolerance test (OGTT). Plasma insulin levels were the highest in untreated fructose-fed (F) rats compared with levels in other groups, whereas normal control Chow-fed male rats (C) treated with estrogen (CE) had the lowest insulin levels. Estrogen decreased insulin levels in both CE (n = 10) and F rats treated with estrogen (FE, n = 8) compared with levels in F rats (n = 10). *P < 0.05, F vs. C, CE, and FE; †P < 0.05, F vs. CE. B: plasma glucose values in OGTT. No significant changes in glucose levels were observed. C: insulin sensitivity index (ISI) for C (n = 9), CE (n = 10), F (n = 10), and FE (n = 8) rats. Estrogen improved ISI in FE rats compared with that in F rats, though less than that in CE rats. F rats had the least ISI. *P < 0.05, FE vs. F; †P < 0.05, FE vs. CE; ‡P < 0.01, CE vs. C; †P < 0.001, CE vs. F; §P < 0.05, F vs. C. All values are means ± SE.
testosterone levels from 1.2 to 0.1–0.2 ng/ml. A similar profile was observed in our studies wherein 17β-estradiol levels were elevated, whereas testosterone levels fell subsequent to estrogen treatment (Table 3). Interestingly, plasma estradiol levels were higher in both F and GF rats, which were untreated, compared with those in the C and G rats, respectively (Tables 3 and 4). Thus fructose feeding seems to retard the fall in estradiol levels, although the resulting higher level of estradiol was still unable to prevent IR secondary to fructose feeding (Table 4). This finding seems to contradict the currently held belief that estrogen enhances insulin sensitivity. To date, we have not found any studies that have investigated the effects of diet-induced IR on changes in plasma estradiol levels. Although it may be speculated that the fructose-enriched diet affects estradiol synthesis in males, we have no data concern-
ing this issue. Additional studies are needed to look at the changes in the levels and actions of estradiol under states of excess carbohydrate feeding. Second, potential IR-induced changes in the “bioavailability” of estrogen in males need to be determined. The levels of circulating estradiol in male rats (Tables 3 and 4, respectively), although in the same range as that reported in female estrous rats (2, 31), did not prevent the induction of IR in the presence of fructose. Estrogen treatment did not change plasma TGs in the fructose-fed rats. Studies have previously shown that estrogen increases both hepatic and circulating plasma TGs (16), which is reversed by ovariectomy or by estrogen antagonism (23). Furthermore, Galipeau et al. (10) have previously demonstrated that fructose-fed female rats develop hypertriglyceridemia but not IR or hypertension. Thus the data agree with the current literature and suggest either no change or an increase in plasma TG levels in the presence of estrogen.

Effects of sex hormone balance on development of hypertension secondary to IR in male rats. Changes in the sex hormone balance in males and females may be a predisposing factor for hypertension. In earlier studies (10), the induction of IR in ovariectomized rats caused hypertension. Plasma testosterone was not measured in these studies. However, other groups have shown elevated testosterone levels in ovariectomized SHR compared with levels in estrous controls (8), which indirectly support our hypothesis. Similar increases in testosterone levels have been reported in women suffering from polycystic ovary syndrome (17) or preeclampsia (22) in addition to postmenopausal women with or without cardiovascular disease (30). Reintroduction of estrogen reduced the blood pressure and androgen levels in each case. However, it is unclear whether the increased estrogen levels or the concomitant decrease in testosterone mediated the fall in blood pressure. In the present study, insulin-resistant intact male rats developed hypertension similar to ovariectomized rats (Fig. 3). Although estrogen treatment prevented the development of hypertension in FE rats, this effect was only partial because the blood pressure in these rats (125 ± 1 mmHg) was greater than that in C and CE rats (113 ± 1 and 108 ± 1.3 mmHg, respectively). In comparison, GF rats, in which testosterone was absent, had a lower blood pressure (112 ± 1.5 mmHg) compared with that in the FE rats. Furthermore, estrogen treatment failed to modify the blood pressure of gonadectomized rats (GFE, 105 ± 2 mmHg vs. GF, 112 ± 1.5 mmHg). These results support previous findings from McNeill’s laboratory (32) that only a complete loss of testosterone in vivo prevents the development of hypertension. Furthermore, fructose feeding tends to increase estradiol, but in the presence of testosterone, blood pressure is elevated. Decreasing testosterone partially or totally prevents hypertension, although IR is unchanged. A similar situation has been reported in SHR where only the complete loss of testosterone by gonadectomy (5) and not reducing its levels (34) prevented hypertension. Thus the blood pressure lowering effects of estrogen in male fructose-fed rats could merely be an outcome of the estrogen-induced improvement in insulin action, because it has been previously shown that improving the insulin sensitivity lowers blood pressure in both experimental (36, 37) and clinical settings (13, 27). Studies need to be conducted to validate the importance of testosterone to the development of hypertension.

One such experiment would be to treat ovariectomized fructose-fed rats with the antiandrogen flutamide. Because fructose induces IR and hypertension in ovariectomized rats (10), concomitant blockade of the testosterone receptor could shed light on the contribution of testosterone to hypertension. Based on these and other results, we may understand more as to how sex hormones counteract each other’s effects on hemodynamics and vascular reactivity.

Effects of estrogen implantation on development of IR and hypertension in gonadectomized rats. Basal 17β-estradiol, although higher in gonadectomized rats compared with that in sham-operated groups, did not prevent the induction of IR subsequent to fructose feeding. Insulin sensitivity was improved (Fig. 2C) only when supraphysiological levels of estrogen were present (Table 4). Regardless of the presence or absence of IR in both intact and gonadectomized untreated rats, estradiol levels decreased at termination compared with basal values. We suggest this decrease in estradiol to be an age-dependent phenomenon, which has been previously shown in mature male rats (6). In addition, the presence or absence of testosterone did not influence plasma estradiol levels in any of the groups. Thus from our results, it could be speculated that there exists a threshold level of plasma estrogen in both males and females below which the rats become susceptible to developing IR.

In the absence of testosterone, estrogen implantation reduced the plasma insulin levels in the GFE compared with levels in the GF rats in response to an oral glucose load (Fig. 2A). Furthermore, blood pressure was unchanged subsequent to estrogen treatment in the GFE rats (Fig. 4). These results are in agreement with previous studies (32) indicating the dependency of blood pressure increase on androgens. Thus estrogen did not affect blood pressure in the absence of testosterone. However, additional studies need to be conducted to verify whether the homeostatic pathways recruited by estrogen in males are similar or different than those in females.

Our studies do not offer a comprehensive solution as to whether the pathogenesis of IR and hypertension is governed by differences in sex or by the changes in hormonal balance when testosterone and estrogen coexist in vivo. The problem is further complicated by the fact that no studies have previously focused on the effects of changes in sex hormone equilibrium on hypertension, thus lacking precedent. The estrogen pellets used in these studies were designed to release ~8.3 μg/day for 60 days. However, the pellets rapidly released estrogen inside the body (in CE, FE, and GFE, respectively), which was not in accordance with the manufacturer’s claim. Thus we were unable to achieve steady-state levels of estrogen over time. Experiments will have to be performed in male and ovariectomized fructose-fed rats, taking into account the changes in testosterone levels after acute estrogen injection. The present results, however, give an idea of the possible shift in the balance between estrogen and testosterone and the resultant effects on insulin sensitivity and blood pressure.

In conclusion, our studies attempted to resolve which hormone, estrogen or testosterone, dictates the predisposition to hypertension in males. In male fructose-fed rats with intact testes, estrogen treatment prevented hypertension although the blood pressure was higher than that of the C and GF rats. Plasma testosterone was reduced, but not entirely, in the intact
males after estrogen treatment. However, estrogen improved insulin sensitivity in both intact and gonadectomized animals. In conclusion, we suggest that testosterone is essential for the development of hypertension secondary to IR in this model. This facilitatory effect of testosterone is unaffected by physiological levels of estrogen. Testosterone by itself may not affect insulin sensitivity, but it is required for the development of hypertension secondary to IR. Thus IR may be responsible for shifting the hemodynamic equilibrium in favor of testosterone-dependent prohypertensive mechanisms by attenuating vasorelaxant pathways. Furthermore, we suggest that there may exist a threshold level for estrogen below which it does not prevent IR and hypertension. Similar studies focusing on the specific changes in sex hormones and their actions in intact and ovariectomized rats are required to compare and confirm our current findings about the differential roles of estrogen and testosterone in vivo.

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