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.—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 estrogen 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.

hyperinsulinemia; fructose hypertensive rat; gender; estrogen; testosterone

THE METABOLIC SYNDROME is composed of a cluster of cardiovascular risk factors including abdominal obesity, hypertriglyceridemia, low levels of high-density lipoprotein, insulin resistance (IR), and hypertension (21). A key factor contributing to the metabolic syndrome is IR and subsequent hyperinsulinemia, which is often associated with hypertension in both humans (7, 28) and several animal models (3, 25). Differences in gender have been shown to influence the progression of IR and consequently hypertension (11, 12). Studies using fructose-fed rats (3, 36) have demonstrated that the degree of IR developed in males is greater than that in females (12). Because hypertension was observed secondary to IR, the changes in blood pressure were dictated by sex-dependent differences in insulin sensitivity (11). Furthermore, this differential development of IR and hypertension was ascribed to the presence of estrogen (10). Clinical evidence supports the above findings because men and postmenopausal women demonstrate a higher risk of developing hypertension compared with premenopausal women (14). Insulin sensitivity and glucose tolerance are improved in postmenopausal women subsequent to treatment with estrogen alone or in combination with progesterone (18). Thus experimental evidence and short-term clinical studies support the role of estrogen in preventing the development of IR and hypertension. However, the effects of long-term hormone replacement therapy on cardiovascular health have been controversial, and the reasons are yet to be fully understood (1, 15, 19, 26, 38).

Taken together, the physiological differences arising due to gender variation assume a significant role in the development of IR and hypertension. However, the effects of estrogen replacement have been studied mainly in ovariectomized rats. Testosterone is present in women, albeit in lower amounts than men, and is responsible for governing certain phenotypes associated with men. In vivo, it has been recently shown that testosterone is essential in male rats for the development of hypertension secondary to IR (32). Although the individual contributions of estrogen and testosterone to the development of IR and hypertension have been investigated, little is known as to how they affect insulin sensitivity and blood pressure in the presence of each other. Do estrogen and testosterone complement or counteract each other in vivo? Furthermore, how does the endogenous residual estrogen or testosterone in gonadectomized male or female rats, respectively, influence insulin sensitivity and blood pressure?

Testosterone has been strongly implicated in the development of IR and hypertension in women with polycystic ovary syndrome (17, 30, 35) or preeclampsia (22), which is similar to IR and hypertension observed in women after menopause. In a recent study by Fortepiani et al. (8), both 18-mo-old ovariectomized spontaneously hypertensive rats (SHR) and intact very old female (postestrus) SHR had significantly higher levels of testosterone compared with young estrous rats, which had higher circulating estrogen and lower levels of testosterone, respectively. The mean arterial pressure was significantly higher in males and 18-mo-old ovariectomized rats compared with that in control young estrous rats. These results provide an indication that the unmasking of testosterone-mediated effects after the loss of estrogen may be responsible for the increased mean arterial pressure in these rats. On the other hand, male rats, despite having estrogen present in the plasma, are susceptible to developing IR independent of testosterone levels. Thus,
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.

**Chemicals and reagents.** All chemicals, unless otherwise mentioned, were of reagent grade and purchased from Sigma (St. Louis, MO).

**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 hypo-glycemic 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

<table>
<thead>
<tr>
<th>Physical Parameters</th>
<th>C</th>
<th>CE</th>
<th>F</th>
<th>FE</th>
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</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), control estrogen-treated rats (CE, n = 10), fructose-fed rats (F, n = 10), and fructose-fed estrogen-treated 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.
study. The animals’ weights stabilized after 3 wk, although they were significantly lower than those in the C and F groups throughout the study. There was no difference between the body weights of the C and F groups. Thus fructose feeding per se did not affect the changes in weights. Estrogen-implanted rats consumed less food compared with food consumed in the untreated groups (Table 1).

In the GFE group, a lower weight gain was observed compared with weight in the other groups on termination at the end of 3 wk (Table 2) after estrogen implantation (study week 9). Further estrogen induced a weight loss in the GF rats when compared with the values before treatment.

**Plasma TG levels.** Seven weeks of fructose feeding elevated the plasma TG levels (F, 2 ± 0.4 vs. C, 0.6 ± 0.1 mmol). FE rats did not show a reduction in TG levels (1.6 ± 0.2 mmol). CE rats (0.5 ± 0.1 mmol) did not show any change in TG levels compared with levels in the C rats.

**Plasma 17β-estradiol and testosterone levels.** Plasma 17β-estradiol levels were elevated in both CE and FE compared with levels in C and F rats, respectively (Table 3). Correspondingly, testosterone levels dropped significantly in the estrogen-treated groups at termination. Curiously, although fructose feeding did not alter the testosterone levels in F compared with C rats, estradiol levels in F were higher than levels in the C rats. However, this difference in estradiol did not prevent the development of IR in these rats. The testosterone-to-estradiol ratio was significantly higher in the untreated animals compared with the ratios in the estrogen-implanted groups. Estrogen reduced the testosterone-to-estradiol ratio in the CE and FE groups, respectively (Table 3).

Before fructose feeding, gonadectomized rats had significantly higher plasma estradiol levels (87.2 ± 3.9 pg/ml) than those of the sham-operated males (66.7 ± 3.3 pg/ml). At termination, the F and GF groups had higher plasma estradiol compared with that in the C and G groups, respectively. As shown in Table 4, there was a marked release of estradiol from the pellet on the third day after estrogen implantation. 17β-Estradiol values were >3,000 pg/ml at the end of 3 days. After 10 days of treatment, although estradiol levels were significantly higher than those in the other groups, there was a significant decrease compared with the values on day 3. Estradiol was the highest in the GFE group compared with that in other groups until the rats were terminated.

We did not detect any changes in testosterone in both the sham-operated groups (C and F) used in the study (data not shown). However, in the untreated and estrogen-treated gonadectomized rats, testosterone levels were undetectable.

**Assessment of IR and sensitivity.** Rats fed with fructose for 6 to 7 wk developed hyperinsulinemia within 10 min of ingestion of glucose, which was sustained for 60 min (Fig. 1A). Glucose levels were elevated in all the groups 10 min after dosing. However, there was no significant difference among the groups with respect to body glucose disposal pattern over the entire 90 min of observation (Fig. 1B). Estrogen prevented hyperinsulinemia in FE rats and maintained insulin levels at par with controls. The ISI was significantly lower in the fructose-fed group compared with that in the other groups, indicating the presence of IR in these animals. Estrogen treatment improved the insulin sensitivity in fructose-fed rats as indicated by elevated ISI (Fig. 1C).

The gonadectomized fructose-fed rats (8 wk), which were treated with estrogen for 2 wk, did not become insulin resistant, because they had a higher ISI compared with that in the F and GF groups, indicating normal insulin sensitivity (Fig. 2C). After 2 wk of treatment, estrogen reduced plasma insulin in the GFE group to levels comparable with the C and G groups (Fig. 2A, inset). Furthermore, glucose levels in the GFE group were significantly lower than levels in the remaining groups (Fig. 2B).

**Blood pressure.** In the first study, after 6 wk of fructose feeding and estrogen treatment, systolic blood pressure was higher in the F rats (140 ± 2 mmHg) compared with that in the C rats (113 ± 1 mmHg). Blood pressure was also higher in FE rats (125 ± 1 mmHg). However, the blood pressure of FE rats was significantly lower compared with that in the F rats.

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>Sex Hormone Levels in Plasma, 50 days</th>
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<tbody>
<tr>
<td>C</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>17β-Estradiol, pg/ml</td>
</tr>
<tr>
<td>Testosterone, ng/ml</td>
</tr>
<tr>
<td>Testosterone/estradiol</td>
</tr>
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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.
Chronic estrone treatment in male SHR reduced plasma insulin levels, decreasing testicular activity. In a study by Valigora et al. and weight gain in male rats (Table 1). Treatment with estrogenized rats. Estrogen treatment resulted in a lower food intake and weight gain in male rats. A similar effect of estrogen on insulin sensitivity in ovariectomized rats is supported by results from our laboratory that indicate a lower insulin sensitivity in male fructose-fed rats (Fig. 1). This agrees with the previous reports that estrogen elevated the plasma insulin sensitivity in male fructose-fed rats, as indicated by hyperinsulinemia and lower ISI compared with that in the C rats. This result after 6 wk of fructose feeding, as indicated by hyperinsulinemia. Estrogen treatment resulted in a lower blood pressure compared with that in the C rats. Blood 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. In humans as well as rodents, 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. The protective effects of estrogen and permissive effects of testosterone in the development of hypertension secondary to IR have been demonstrated in separate studies. However, little is known regarding the effects of hormonal balance in vivo on the development of IR and hypertension in either sex. In the present set of studies, we have shown for the first time that it is not merely the differences in gender per se but the interactions between estrogen and testosterone that influence the development of IR and hypertension in either sex. 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. Estrogen elevated the insulin sensitivity in male fructose-fed rats. This is supported by results from our laboratory 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. Treatment with estrogen significantly reduced the testis size in male rats, suggesting decreased testicular activity. In a study by Valigora et al., chronic estrone treatment in male SHR reduced plasma pressure was unchanged in both control groups (C and CE) after 6 wk (Fig. 3).

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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 ± 1.5 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 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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