Female rats are protected against fructose-induced changes in metabolism and blood pressure

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Galipeau, Denise, Subodh Verma, and John H. McNeill. Female rats are protected against fructose-induced changes in metabolism and blood pressure. Am J Physiol Heart Circ Physiol 283: H2478–H2484, 2002; 10.1152/ajpheart.00243.2002.—The objective of this study was to determine whether the effects of a fructose diet, which causes hyperinsulinemia, insulin resistance, and hypertension in male rats, are dependent on sex. Blood pressure was measured via the tail-cuff method, and oral glucose tolerance tests were performed to assess insulin sensitivity. Blood pressure in female rats did not differ between fructose-fed and control rats at any time point (126 ± 5 and 125 ± 3 mmHg at week 9 for fructose-fed and control rats, respectively) nor was there a difference in any metabolic parameter measured. Furthermore, the vascular insulin resistance that is present in male fructose-fed rats was not observed. After ovariectomy, fructose caused a significant change in systolic blood pressure from baseline compared with fructose-fed ovary-intact rats (change of 21 ± 5 vs. −2 ± 4 mmHg). The results demonstrate that females do not develop hypertension or hyperinsulinemia upon fructose feeding except after ovariectomy, suggesting that female sex hormones may confer protection against the effects of a fructose diet.

Hypertension is often found to be associated with hyperinsulinemia and insulin resistance in both humans and animal models (4, 23). A common animal model used to study the interaction between insulin and hypertension is the fructose hypertensive rat (FHR). This is a form of acquired hypertension that also exhibits insulin resistance, hyperinsulinemia, and hypertriglyceridemia (15, 26). This model is useful for studying the effects of hyperinsulinemia and insulin resistance on blood pressure (BP) because these defects develop in the absence of any body weight gain. Several mechanisms have been proposed to link hyperinsulinemia and insulin resistance to hypertension in FHR, including sympathetic nervous system activation (30), defects in endothelial function (16, 17, 21, 22, 31), vascular insulin resistance (32, 33), and increased production and/or activity of endothelin-1 (ET-1) (29) or thromboxane A2 (TxA2) (6).

Evidence suggests that there are differences between males and females in the response to being fed with a high carbohydrate diet. For example, it has been shown that, in contrast to males, female rats do not develop hypertriglyceridemia or insulin resistance after being fed with sucrose (13). A separate study investigating the effects of high sucrose on growth and development of juvenile rats demonstrated that both male and female rats develop hypertension; however, the degree of BP increase was greater in males than in females (14). It was uncertain whether the elevated BP in females was related to impairments in insulin sensitivity or if there were any differences between sexes in terms of insulin sensitivity. These studies raise an important question regarding the effects of fructose in female rats and whether hyperinsulinemia and insulin resistance lead to hypertension as in male rats.

On the basis of previous reports, it has not been possible to discern what role sex plays in the relationship between hyperinsulinemia/insulin resistance and hypertension, if any. Given the differences in the incidence and pathogenesis of cardiovascular disease in men and women (9), we hypothesized that sex may affect the relationship between hyperinsulinemia/insulin resistance and hypertension. To investigate this hypothesis, we designed experiments to clarify the effect of a high-carbohydrate (fructose) diet in both male and female rats on the development of hyperinsulinemia, insulin resistance, and hypertension. Furthermore, we examined the role of the sex hormones in the response to a fructose diet in females and examined vascular responses to insulin.

METHODS

BP Study 1

Two experimental groups of Wistar rats (University of British Columbia Animal Care) were used in this study: female control (C; n = 8) and female fructose treated (F; n = 8). Pilot groups of male control (M) and fructose-treated (MT)
FEMALE RATS DO NOT DEVELOP FRUCTOSE-INDUCED HYPERTENSION

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rats (n = 4) were followed at the same time to ensure that we would observe hyperinsulinemia and hypertension as in all our previous experiments but were not included in the statistical analyses. At the age of 6 wk, treatment groups were started on a diet of 60% fructose for 9 wk (Teklad Laboratory Diets; Madison, WI), whereas control groups were maintained on normal laboratory rat chow. Systolic BP was measured before treatment and weekly throughout the study period via the tail-cuff method as previously described (1, 6). Blood samples for determination of 5-h fasted plasma insulin, glucose, and triglycerides were obtained at study weeks 0, 2, 5, and 7. An oral glucose tolerance test (OGTT) after an overnight fast was performed at study weeks 4 and 8. Glucose (1 g/kg) was administered by oral gavage, and blood samples were collected at the times of 0, 10, 20, 30, and 60 min. All blood samples were collected from the tail vein.

In Vitro Vascular Reactivity Procedures

These experiments were performed with the same protocols described previously for experiments with male FHR (32) and used rings of aortic tissue excised from female rats fed with a control or fructose diet for a period of 11 wk (n = 8 rats/group). Indomethacin (10⁻⁵ M) was included in the buffer solutions for all experiments. Changes in tension were detected with a force transducer and recorded on a Grass polygraph machine (model 79D). The experimental protocol, in brief, included a 60-min equilibration period, challenge with KCl (40 mM), and confirmation of a functioning endothelium with norepinephrine (NE; 10⁻⁶ M) and ACh (10⁻⁶ M). A concentration-response curve to NE (10⁻⁹–10⁻⁵ M) was then constructed, followed by incubation with 100 μM/kg insulin (diluted in 0.1% albumin) for 2 h before a second NE concentration-response curve. Tension is expressed as a percentage of the maximum response to NE.

BP Study 2

Four groups of 15-wk-old female Wistar rats were used in this experiment (n = 8 rats/group): control (C), fructose fed (F), ovariectomized (Ovx), and Ovx + fructose fed (F+Ovx). Ovariectomy was accomplished with bilateral dorsal incisions to expose and remove the ovaries as previously described (11). The F and F+Ovx groups began a 60% fructose diet that started on the same day as the ovariectomy, whereas the C and Ovx groups received normal diet. Systolic BP was measured weekly beginning at week 2 as above. Blood samples were collected after a 5-h fast at study weeks 0, 2, 4, and 6. At week 7, an OGTT was performed as described above. At termination, ovariectomy was confirmed by visual inspection, and blood was collected via cardiac puncture for measurement of plasma total estrogens (TE).

Biochemical Analyses

Plasma insulin and TE were determined with radioimmunoassay kits (Linco Research and ICN Biochemicals, respectively), triglycerides were determined with an enzymatic colorimetric kit (Sigma; St. Louis, MO), and glucose was determined with a Beckman Glucose Analyzer II.

Reagents

Unless otherwise stated, all chemicals were reagent grade and were purchased from Sigma.

Statistics and Data Analysis

All data are presented as means ± SE. Area under the curve (AUC) values were calculated using the trapezoidal rule, and insulin sensitivity indexes (ISI) were calculated using the following formula: ISI = 100/square root [(fasting glucose × fasting insulin) × (mean glucose × mean insulin)] (20). Values for pD₂ (−log M) and the curve maximum for each agonist were calculated by nonlinear regression analysis of the concentration-response curves. For data with multiple time points, variables were analyzed by general linear model ANOVA. A one-way ANOVA was used to examine AUC, ISI, and pD₂ values. Mean values were considered significant at P < 0.05. When a mean difference was detected, a Newman-Keuls multiple-comparison test was applied.

RESULTS

BP Study 1

Animal characteristics. Fructose did not affect body weight or food and fluid intake in either sex (data not shown). After 7 wk of fructose feeding, fasted plasma insulin values were not significantly different between C and F groups, although values did increase significantly from basal in the F group (Table 1). Plasma triglyceride values at week 7 were approximately three times higher in FT rats compared with C group values (Table 1). In the pilot male groups, the expected increases in plasma insulin and triglyceride levels were observed in the MT group (insulin: MT, 2.7 ± 0.4 ng/ml and M, 1.8 ± 0.4 ng/ml; triglycerides: MT, 6.3 ± 0.4 mM and M, 1.2 ± 0.3 mM). Plasma glucose was not affected by the fructose diet in either sex (data for female rats are presented in Table 1).

Blood pressure. An age-related increase in systolic BP was observed in female rats; however, it was not significantly different between F and C groups at any time point (C: 126 ± 5 mmHg and F: 125 ± 3 mmHg at week 9; Fig. 1). In the male cohort, BP began to increase in the MT group compared with the M group by 3 wk of treatment and continued to increase throughout the study (115 ± 5 vs. 150 ± 5 mmHg at week 9).

Oral glucose tolerance test. Fructose diet did not affect the plasma glucose profile or the plasma insulin profile in female rats at either of the time points studied. The data from the latter time point, week 8, are presented in Fig. 2. A calculation of ISI for the female groups also demonstrated no differences (C: 41.1 ± 8.3

Table 1. Plasma parameters of female rats fed with control or fructose diets

<table>
<thead>
<tr>
<th></th>
<th>Control Diet (n = 7)</th>
<th>Fructose Diet (n = 8)</th>
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<tbody>
<tr>
<td>Glucose, mM</td>
<td></td>
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<tr>
<td>Basal</td>
<td>6.8 ± 0.1</td>
<td>6.3 ± 0.3</td>
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<tr>
<td>Week 7</td>
<td>7.2 ± 0.2</td>
<td>7.5 ± 0.2</td>
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<tr>
<td>Insulin, ng/ml</td>
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<tr>
<td>Basal</td>
<td>1.08 ± 0.31</td>
<td>0.57 ± 0.06</td>
</tr>
<tr>
<td>Week 7</td>
<td>1.11 ± 0.12</td>
<td>1.50 ± 0.18†</td>
</tr>
<tr>
<td>Triglycerides, mM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>0.83 ± 0.1</td>
<td>0.86 ± 0.10</td>
</tr>
<tr>
<td>Week 7</td>
<td>0.68 ± 0.10</td>
<td>2.20 ± 0.52*</td>
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Values are means ± SE; n = no. of rats. Animals were fasted for 5 h before blood samples were obtained from the tail vein. *P < 0.05 vs. control diet; †P < 0.05 vs. basal.
and F: 36.3 ± 6.6 at week 8, \( P = 0.56 \). As expected, insulin levels peaked higher in the MT group compared with the M group.

**In Vitro Vascular Reactivity Study**

In female rats, insulin did not significantly affect vascular smooth muscle contraction induced by NE in endothelium-intact aortas from either diet group (Fig. 3). In contrast, previous experiments in our lab with aortic rings obtained from male rats show that insulin significantly reduced the sensitivity (pD\(_2\) values of 7.49 ± 0.04 vs. 6.65 ± 0.15, \( P < 0.05 \)) and maximum response (−25 ± 3%) to NE using the same experimental conditions. Upon fructose feeding, the vasodepressor effect of insulin was abolished in male rats (S. Verma and J. H. McNeill, unpublished observations). We have previously reported this phenomenon in male rats using angiotensin II as the agonist (32).

**BP Study 2**

**Animal characteristics.** Ovariectomy of female rats was successful as indicated by visual inspection and by a significant decrease in plasma TE activity at termination in Ovx groups [47 ± 4 and 54 ± 3 vs. 31 ± 2 (\( P < 0.05 \)) and 28 ± 2 (\( P < 0.05 \)) pg/ml for C and F vs. Ovx and F+Ovx, respectively]. Body weights of Ovx rats (Ovx and F+Ovx) tended to be higher throughout the study period, but this was not significant until termination (370 ± 9 and 344 ± 21 vs. 424 ± 15 and 439 ± 17 g for C and F vs. Ovx and F+Ovx, respectively). All groups were normoglycemic, but F+Ovx rats were hyperinsulinemic compared with Ovx rats alone (Table 2). Even though there was a trend toward higher insulin levels in the F group, this was not different from baseline values nor any other group at the same time point. Plasma triglycerides were elevated in both the F and F+Ovx groups, and this increase was more severe in the F group.

**Fig. 1.** Systolic blood pressure in female rats fed with a control (C) or fructose (F) diet (data for pilot male control (M) and fructose-treated (MT) cohorts are indicated for comparison). Blood pressure was measured via a tail cuff at baseline and once weekly for 9 wk. Values are means ± SE.

**Fig. 2.** Plasma insulin (A) and glucose (B) in response to oral glucose challenge in C and F groups after 8 wk of fructose diet. Glucose (1 g/kg) was administered via oral gavage, and blood samples were obtained from the tail vein at the times indicated. No significant differences were observed between the female groups at either week 4 or 8. (Data for week 4 not shown).

**Fig. 3.** Effect of insulin (100 mU/ml) on the response to norepinephrine (NE) in endothelium-intact aortas from female rats fed with a control (A) or fructose diet (B). No significant differences were observed.
Blood pressure. The only group to experience a significant increase in BP during the study was the F+Ovx group (Fig. 4). Systolic BP at study week 7 of the C, Ovx, F, and F+Ovx groups was 118 ± 3, 120 ± 5, 116 ± 3, and 135 ± 6 mmHg, respectively.

Insulin sensitivity. While the fructose groups tended to have elevated plasma insulin values, denoted by the AUC, this was not significant (112 ± 16, 167 ± 19, 131 ± 16, and 183 ± 28 ng·ml⁻¹·min⁻¹ for C, F, Ovx, and F+Ovx). Plasma glucose profiles were nearly identical (AUC: 526 ± 11, 536 ± 15, 558 ± 20, and 555 ± 6 mmol·l⁻¹·min⁻¹ for C, F, Ovx, and F+Ovx). A comparison of the ISI values indicates that the F+Ovx group was insulin resistant compared with Ovx rats, but there was no significant difference between C and F rats (Fig. 5).

DISCUSSION

This study demonstrates that female rats are protected against the metabolic defects and hypertension typically produced by fructose feeding in male rats. Hyperinsulinemia and insulin resistance are believed to be the primary defects that cause hypertension in the fructose-fed male rat model (1, 18, 26, 28). On the basis of this hypothesis, it follows that hypertension may not have developed in the fructose-fed female rats because the effects of fructose on metabolism are less severe in females. In a study by Hulman et al. (14), the BP of juvenile female rats fed with sucrose was reported to be elevated, although the BP increase was not as severe as in males. While these authors showed that the male rats used in their study demonstrated insulin resistance, they did not measure any metabolic parameters in the female group. Another group has reported that sucrose-fed female rats do not develop changes in insulin or triglyceride levels and are not insulin resistant, as measured by the euglycemic hyperinsulinemic clamp (13). Given the hypothesis that high carbohydrate-induced hypertension is related to insulin resistance, these two reports in sucrose-fed females would appear to be conflicting. To our knowledge, this is the first report measuring BP and insulin sensitivity simultaneously in both sexes of fructose-fed rats. The data presented here are in closer agreement with the latter study (13), which unfortunately did not measure BP changes in response to sucrose.

We also examined the vascular actions of insulin in female rats. Because female rats do not develop hypertension after being fed with fructose, we did not expect to observe differences in the vascular actions of insulin between female diet groups. However, an interesting and novel observation made in this study was that in
female rats, insulin does not have any significant effect on vascular smooth muscle contraction. With the use of the same experimental conditions in male rats, insulin reduces contraction in control rats, but this response is blunted after fructose feeding (Verma and McNeil, unpublished observations, and Ref. 32). This response was observed only in endothelium-intact tissues and implies that there are defects in insulin action as an endothelium-dependent vasodilator in the aorta of male FHR. Others have also demonstrated defects in endothelium-dependent relaxation in FHR (16, 17, 21, 22, 31). This may be a mechanism that contributes to the development of hypertension in this model. If the hypothesis that hyperinsulinemia/insulin resistance is related to hypertension only in males is true, then it may be due to sex differences in the role and function of insulin as a cardiovascular hormone.

In both groups of intact female rats, the fructose diet failed to cause any increase in BP, and neither glucose tolerance nor insulin sensitivity were affected. However, increases in plasma triglyceride concentrations were observed. It should be noted that the increase in plasma triglycerides in response to fructose usually develops in male rats of this age within 2–3 wk of the fructose diet being started and levels can be as high as five to six times those of control rats (1, 6, 27–29). In this experiment, the increase in plasma triglycerides required 5 wk to develop in the female rats and was relatively mild compared with that seen in males (~3-fold). While this is merely a qualitative comparison, it does suggest that there may be quantitative and duration-dependent differences between sexes in this response. Some investigators have proposed that the hypertension observed in the fructose-fed model actually depends more on the presence of hypertriglyceridemia rather than hyperinsulinemia or insulin resistance (25). Our data would refute this hypothesis because hypertension was absent in female rats, despite the presence of elevated triglycerides.

Two possible explanations may be postulated for the sex differences described here. First, there may be mechanisms present only in male rats necessary to facilitate the effects of fructose on metabolism, or, second, female rats may possess countermechanisms that protect against the adverse effects of fructose. These mechanisms, if present, may be linked to sex hormones. Referring again to the two earlier studies in sucrose-fed rats, an increase in BP was observed in the study with juvenile rats as the experimental age group (14). A possible explanation for the discrepancy between our study and theirs may be the difference in hormonal status between juvenile and mature rats. If estrogen is indeed protective against the adverse effects of high carbohydrate feeding, BP may increase in high carbohydrate-fed juvenile female rats because estrogen levels would likely not play a role until sexual maturity.

In general, physiological concentrations of estrogen have beneficial effects on lipoproteins, insulin, and glucose metabolism (8, 19). Estrogen can improve insulin action in the liver, muscle, and adipose tissue by increasing glycogen deposition, glucose uptake, and lipogenesis (8). In postmenopausal women, estrogen replacement therapy has been shown to reduce fasting glucose and insulin concentrations, an indirect measure of insulin sensitivity (5). Interestingly, the only adverse effect of estrogen on lipid metabolism is to increase hepatic triglyceride synthesis and circulating plasma triglyceride levels (2, 10). This may explain why the primary metabolic change observed in fructose-fed females was an increase in plasma triglyceride levels, because the effects of fructose and estrogen would be synergistic in this respect.

To determine whether female sex hormones were involved in preventing the effects of fructose on metabolism and BP, we examined the response to fructose in Ovx female rats. This experiment demonstrated that female rats, in the absence of normal levels of ovarian sex hormones, develop an increase in BP as seen in male rats after being fed with fructose. Although it is well established that estrogen has many cardiovascular benefits (12), a lack of sex hormones alone in Ovx animals did not cause a significant change in BP. In addition, ovariectomy itself also did not significantly affect any of the metabolic parameters measured in this experiment, but, comparing the two ovariectomized groups, insulin sensitivity was significantly reduced and fasting insulin levels were significantly increased upon feeding with fructose. Our data indicate that the combination of fructose diet and estrogen/sex hormone deficiency are required to elevate BP in female rats.

For this experiment, we performed an ovariectomy to create an estrogen-deficient state because it is the hormone we believe to be more important in protecting against the effects of fructose. As indicated by the total plasma estrogen concentrations, the Ovx rats in this experiment did have significantly lower levels of the hormone; however, it was not completely absent. The data indicate that estrogens were still being secreted from another source in our Ovx model, likely the adrenal cortex or adipose tissue. It is also important to note that the ovaries are a source of other hormones in addition to estrogen, such as progesterone and androgens, that could impact upon multiple other hormone systems, which also affect metabolism and the cardiovascular system. It cannot be determined from this experiment how these other hormone systems were affected by the ovariectomy. While these results help to rule out other genetic or physiological mechanisms not related to sex hormones, further experiments with Ovx rats and estrogen treatment protocols are planned to identify the specific hormones and possible mechanisms involved. This is supported by data from a recent study demonstrating that estradiol treatment prevents the increase in BP observed in female Ovx rats fed with a high fat sucrose diet (24). While this animal model also exhibits obesity, which has an important impact on BP in itself, this result indicates that estrogens may also be beneficial in our fructose model of insulin resistance and hypertension.
Female sex hormones may be protective against hypertension in fructose-fed rats by protecting against the development of hyperinsulinemia and insulin resistance. In a companion study, we have shown that female rats with normal sex hormone levels do not develop insulin resistance upon chronic exogenous insulin treatment as severely as males do (7). On the other hand, female sex hormones may be protective against hypertension despite the presence of hyperinsulinemia and/or insulin resistance. Indeed, hyperinsulinemia does not seem to be a factor because there was little difference in fasting plasma insulin values between the two fructose-fed groups (F and F + Ovx), whereas only the F + Ovx group was hypertensive. This indicates that insulin resistance in the F + Ovx rats may be more important. Previous data from our laboratory do support that insulin resistance, rather than hyperinsulinemia, has more impact on the development of hypertension secondary to fructose feeding. We have shown that the fructose diet produces insulin resistance and hypertension in streptozotocin-induced diabetic rats, despite the hyperinsulinemic nature of this model (3). Tissue-specific insulin resistance may be another facet to this complex interplay, but unfortunately we have not been able to specifically measure insulin sensitivity in various tissues in this study. We used the oral glucose tolerance method, which tends to reflect resistance to glucose uptake on a whole body level. The ISI we calculated from our OGTT data is a composite index of whole body insulin sensitivity that includes mathemetic representations of both hepatic and peripheral tissue insulin sensitivity (20). Because fasting plasma insulin levels, which are a refection of hepatic insulin sensitivity, were not different, it is possible that the insulin resistance observed in F + Ovx rats is due to peripheral resistance to glucose uptake. If female sex hormones are protective against hypertension through prevention of insulin resistance, it may be through the actions of these hormones at this tissue level.

In summary, this study demonstrates that the effects of a fructose diet on metabolism and BP are dependent on sex. Female rats are protected against fructose-induced hypertension, unlike their male counterparts, and the mechanisms responsible for this protection appear to be related to female sex hormones. Furthermore, there appears to be a sex difference in the vascular actions of insulin, which may also be involved in the mechanisms responsible for the sex differences observed in this experiment. The results of these experiments represent a novel finding into the interrelationship among hyperinsulinemia, insulin resistance, and hypertension. The potential existence of sex differences in this intriguing association might help elucidate the mechanisms involved and are worthy of further investigation.

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