Relationship among hyperinsulinemia, insulin resistance, and hypertension is dependent on sex

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Galipeau, Denise M., Linfu Yao, and John H. McNeill. Relationship among hyperinsulinemia, insulin resistance, and hypertension is dependent on sex. Am J Physiol Heart Circ Physiol 283: H562–H567, 2002. First published April 18, 2002; 10.1152/ajpheart.00238.2002.—Hyperinsulinemia and insulin resistance have been linked to hypertension; however, the influence of sex on this relationship has not been well studied. The purpose of this experiment was to compare the effects of chronic insulin treatment on insulin sensitivity and blood pressure in male and female rats. Male and female Wistar rats were treated with insulin (2 U/day) via subcutaneous sustained release implants for 5 wk. Systolic blood pressure was measured via the tail-cuff method before and after treatment, and insulin sensitivity was assessed with an oral glucose tolerance test. The insulin sensitivity of female rats was 4.5-fold greater than male rats. Chronic insulin treatment impaired insulin sensitivity in both sexes; however, this occurred to a greater degree in male rats. Blood pressure increased in male rats treated with insulin only. The results demonstrate that hyperinsulinemia and insulin resistance are associated with hypertension in male rats only. Therefore, the link between these conditions appears to depend on sex.

blood pressure; insulin sensitivity; male; female; vasculature

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HYPERINSULINEMIA AND RESISTANCE to the glucose-lowering effects of insulin (insulin resistance) are often found to be associated with hypertension in both humans and several animal models (7, 24, 29). From this observation, the “insulin hypothesis” was developed, which proposes that these metabolic impairments are directly related to the cause of hypertension in such individuals. Several mechanisms appear to be involved in the link between these conditions involving the sympathetic nervous system (1, 32), renal handling of sodium (6), and vasoconstrictor hormones such as endothelin-1 (31) or thromboxane (TxA2) (8, 18).

While the relationship between hyperinsulinemia/insulin resistance and hypertension is well established in males, very few experiments have investigated this association in females. Studies using the high-carbohydrate sucrose-induced model of insulin resistance and hypertension have demonstrated conflicting results in female rats (12, 14). With the use of a similar fructose-fed model, we were unable to show any appreciable effect of this high-carbohydrate diet on metabolism or blood pressure in female rats, unlike male rats (unpublished observations). Given the differences in incidence and pathogenesis of cardiovascular disease in men and women and the numerous effects of estrogen on metabolism and the cardiovascular system (10, 19, 26, 28), we hypothesized that sex may affect the relationship between hyperinsulinemia/insulin resistance and hypertension. Because our fructose-fed model did not demonstrate hyperinsulinemia or insulin resistance, we designed experiments to examine the effects of hyperinsulinemia on insulin sensitivity and blood pressure in male and female rats using an exogenous chronic insulin treatment protocol to examine this hypothesis. As a secondary objective, we aimed to explore the mechanisms potentially responsible for any sex differences we observed and therefore examined vascular reactivity in the experimental animals at termination of the experiment. Because TxA2 has recently been identified as a mediator of hypertension in hyperinsulinemic, insulin-resistant models (8, 18), we were particularly interested in the vascular reactivity to U-46619, a stable TxA2 analog.

METHODS

Animals and research design. Four groups of 6-wk-old Wistar rats were used (University of British Columbia Animal Care): male (M, n = 6), male treated (MT, n = 10), female (F, n = 6), and female treated (FT, n = 10). Treatment with bovine insulin via subcutaneous sustained release implants, designed to continuously release a dose of 2 U/day for 60 days, began at an age of 7 wk (Linplant, Linshin Canada; Toronto, Ontario, Canada). This dosage regimen produced comparable levels of hyperinsulinemia in males and females despite the difference in body weight (Table 1). Similar insulin treatment regimens have previously been shown to elevate blood pressure and cause insulin resistance in male rats (3, 4, 15, 22). A pilot study indicated that the drinking water of insulin-treated animals must be supplemented with 10% glucose for the first 4 days of treatment to avoid severe hypoglycemia. Control animals also received 10% glucose to control for possible effects on blood pressure during this period. Body weight and food...
and fluid intake were monitored daily during the first 4 days and weekly thereafter.

**Study procedures.** Systolic blood pressure was measured via the tail-cuff method before and at the end of the treatment period in animals trained for the procedure as previously described (2). We have validated this method in our laboratory by comparing it to direct intra-arterial measurements and have found these measurements to agree to within 5 mmHg. Fasting (16 h) plasma glucose, insulin, and triglycerides were measured pre- and postinsulin treatment from blood samples obtained from the tail vein. After treatment (study week 5), an oral glucose tolerance test (OGTT) was performed (1 g/kg glucose via oral gavage) as previously described (8). One female and three male rats implanted with insulin were removed from the study because fasted plasma insulin levels were <1 ng/ml, suggesting failure of the implant. Another male rat was excluded from the study due to poor hindlimb muscle development and weakness, thus impacting on the rat’s ability to walk and feed normally. Final rat numbers (n) included in the statistical analysis were n = 6 (M, MT, and F) and n = 9 (FT).

**Vascular reactivity study.** At termination, rats were anesthetized with pentobarbital and thoracic aortas were excised. Tissues were placed in cold Krebs-Ringer buffer, cut into 5-mm rings, and prepared as previously described (31). Each ring was placed under a resting tension of 2 g on stainless steel hooks and equilibrated for 60 min. One ring of each pair of endothelium-intact or -denuded rings was incubated with 100 mU/ml insulin (pork/beef, diluted in buffer containing 0.1% albumin) for 2 h. Concentration-responses curves to U-46619, a stable TxA2 mimetic (10^{-9}–10^{-5} M), and norepinephrine (10^{-9}–10^{-5} M) were then constructed, washing between each curve. Changes in tension were detected with a force transducer and recorded on a Grass polygraph machine (model 7D). Tension responses are expressed normalized to cross-sectional area (CSA; in g/mm^2), calculated as CSA (in mm^2) = weight (in mg)/length (in mm) × density (in mg/mm^3). The density was assumed to be 1.05 mg/mm^3.

**Biochemical analyses.** Plasma insulin was determined by radioimmunoassay (Linco Diagnostics), triglycerides were determined with an enzymatic colorimetry kit (Sigma; St. Louis, MO), and glucose was determined with a Beckman Glucose Analyzer II.

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

**Statistics.** Data are presented as means ± SE. For data with multiple time points, variables were analyzed by the general linear model ANOVA. An unpaired t-test was also used to compare the effect of insulin treatment on insulin sensitivity within treated and control groups separately. Area under the curve (AUC) values were calculated using the trapezoidal rule and insulin sensitivity indexes (ISI) were calculated with a formula that correlates highly to results obtained from the euglycemic hyperinsulinemic clamp technique (21). A one-way ANOVA was used to examine AUC and ISI 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**

**General characteristics.** Male rats (M and MT) had higher body weights than female rats (F and FT) at all time points in the study (Fig. 1). Insulin treatment did not affect body weight in females but caused small, although significant, weight gain in males by 2 wk of treatment. During the initial 4 days of insulin treatment and during the time in which drinking water was supplemented with 10% glucose, fluid intake increased and food intake in all groups dropped slightly. Females drank significantly more than males during this pe-

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**Table 1. Plasma parameters of male and female rats chronically treated with insulin**

<table>
<thead>
<tr>
<th></th>
<th>Insulin, ng/ml</th>
<th>Glucose, mM</th>
<th>Triglycerides, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretreatment</td>
<td>0.26 ± 0.02</td>
<td>5.9 ± 0.2</td>
<td>1.1 ± 0.03</td>
</tr>
<tr>
<td>Posttreatment</td>
<td>0.21 ± 0.09</td>
<td>6.2 ± 0.3</td>
<td>1.8 ± 0.1*</td>
</tr>
<tr>
<td>FT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretreatment</td>
<td>0.48 ± 0.16‡</td>
<td>6.3 ± 0.1</td>
<td>1.2 ± 0.04</td>
</tr>
<tr>
<td>Posttreatment</td>
<td>1.95 ± 0.11†‡</td>
<td>3.4 ± 0.4‡‡</td>
<td>1.3 ± 0.1‡‡</td>
</tr>
<tr>
<td>M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretreatment</td>
<td>0.25 ± 0.02</td>
<td>5.5 ± 0.2</td>
<td>1.2 ± 0.01</td>
</tr>
<tr>
<td>Posttreatment</td>
<td>0.60 ± 0.05</td>
<td>6.8 ± 0.2</td>
<td>1.7 ± 0.3*</td>
</tr>
<tr>
<td>MT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretreatment</td>
<td>1.08 ± 0.20‡‡</td>
<td>6.4 ± 0.2</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>Posttreatment</td>
<td>2.05 ± 0.28‡‡</td>
<td>4.8 ± 0.5‡‡</td>
<td>1.9 ± 0.1†‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. F, female; FT, female treated; M, male; MT, male treated. *P < 0.05 vs. pretreatment; †P < 0.05 vs. same sex control group; ‡P < 0.05 vs. opposite sex.
riod. After removal of glucose from the drinking water, food and fluid intake stabilized near baseline levels. At some time points during the study, females treated with insulin ate significantly more than F and MT rats; however, the pattern of food intake remained similar throughout the study.

Plasma insulin, glucose, and triglycerides. Fasting values of glucose, insulin, and triglycerides are shown in Table 1. Animals treated with insulin had significantly higher plasma values for insulin compared with their respective control groups. MT rats had unexplained higher insulin values at baseline than control, although it is of note that the same insulin dosage regimen resulted in very similar plasma insulin values for both the MT and FT groups. Plasma glucose values were significantly lower in treated rats compared with controls and lower in FT rats than in MT rats. A small but significant increase in plasma triglycerides from baseline was observed in both the male and female control groups; this may be related to the placebo vehicle, palmitate. Females treated with insulin had lower plasma triglycerides compared with treated males.

Blood pressure. Systolic blood pressure, measured after 4 wk of treatment, was significantly higher in hyperinsulinemic male rats only (Fig. 2). Mean posttreatment values for the F, FT, M, and MT groups were as follows, respectively: 120 ± 3, 120 ± 3, 123 ± 2, and 137 ± 6 mmHg.

Insulin sensitivity. Results from the OGTT performed at week 5 are illustrated in Figs. 3 and 4. Control male and female rats had similar plasma glucose profiles; however, plasma insulin was significantly higher in males than in females throughout the glucose challenge. This indicates that female rats required less insulin to handle the same glucose load and therefore implies that females have enhanced insulin sensitivity compared with males. Chronic insulin treatment in both male and female rats resulted in a reduction in the OGTT glucose profile, although this was reduced significantly more so in females than males despite similar insulin responses. This observation again supports that females were more insulin sensitive relative to their male counterparts in the treatment cohort. Furthermore, a comparison of ISI values derived from the OGTT data shows that female rats were highly sensitive to insulin compared with male rats and that chronic insulin treatment reduced insulin sensitivity in both males and females. A comparison of the MT and FT groups indicates that insulin sensitivity was impaired to a greater degree in hyperinsulinemic males than in females [F: 68 ± 20 (P = 0.0004 vs. all other groups), FT: 13 ± 2 (P = 0.016 vs. MT), M: 15 ± 1, and MT: 8 ± 1].

Vascular reactivity. Neither chronic hyperinsulinemia nor acute insulin treatment affected the response to U-46619 between same-sex groups. A striking sex difference was noted, however, with respect to sensi-
tivity to U-46619 [pD2 values in endothelium-intact tissues, control conditions: M, 10.14 ± 0.44 (P < 0.0001); MT, 9.39 ± 0.68 (P < 0.0001); F, 8.02 ± 0.34; and FT, 7.72 ± 0.16]. The potency of this agonist was approximately twofold greater in males compared with females regardless of the presence or absence of acute insulin incubation (Fig. 5). In contrast, no significant differences between sex or treatment groups were noted in the response to norepinephrine (Fig. 6).

**DISCUSSION**

The results of this experiment demonstrate that chronic insulin treatment can impair insulin sensitivity in both male and female rats; however, only male hyperinsulinemic rats experienced a rise in blood pressure. We believe that the mechanism(s) of hypertension in this male rat model are directly related to hyperinsulinemia and insulin resistance. These results correlate with results from our laboratory demonstrating that female rats fed with fructose, another model of hyperinsulinemia, insulin resistance, and hypertension, do not develop hypertension like their male counterparts (unpublished observations).

Strikingly, the female control rats in this experiment had an ISI 4.5-fold greater than male controls. This result and observations made during the OGTT indicate that female rats are more sensitive to insulin compared with male rats. First, despite achieving similar plasma insulin levels with treatment, fasting glucose levels were suppressed to a greater extent in the FT group compared with the MT group (Figs. 3 and 4). Second, although glucose responses were similar for the F and M groups during the OGTT, the female group required less than one-half the amount of insulin to maintain these glucose levels (Figs. 3 and 4). To our knowledge, this is the first report demonstrating a sex difference in insulin sensitivity in rats, but this does agree with previous observations made in humans. An experiment comparing age-matched male and female subjects demonstrated that females were inherently more sensitive to insulin as assessed by the euglycemic insulin clamp (23). The enhancement in insulin sensitivity in females was accounted for by a 50% greater rate of insulin-stimulated glucose uptake in femoral muscle tissue. Another study investigating the ability of insulin to suppress free fatty acid concentrations in humans has also demonstrated that females respond with greater sensitivity than males (20). Such sex differences in insulin sensitivity may be related to differences in adipose distribution and/or lipid metabolism (5). With the use of chronic exogenous insulin treatment, we were able to impair insulin sensitivity in female rats, although compared with chronically hyperinsulinemic males, hyperinsulinemic females remained significantly more sensitive to insulin. A study that employed a chronic insulin treatment regimen via an osmotic pump in female rats demonstrated that hyperinsulinemia actually increased muscle glycogen synthesis with no change in insulin-stimulated glucose uptake compared with control (11). Blood pressure was not assessed in this study. An important difference between this experiment and ours was that the rats were also adrenalectomized and treated with propranolol and corticosterone replacement to prevent elevations in adrenergic activity. This may explain why we...
were able to show an impairment in insulin sensitivity and they were not.

Several possibilities may explain the lack of any significant rise in blood pressure of hyperinsulinemic female rats. First, there may be a critical degree to which insulin sensitivity must be impaired before adverse effects on blood pressure can be observed and, if so, the conditions used in this experiment may not have sufficiently impaired insulin sensitivity in female rats. Hyperinsulinemic females had greater sensitivity than hyperinsulinemic males and in fact were comparable to the male control group, which also did not show any elevation in blood pressure. On the other hand, the percent reduction in insulin sensitivity was clearly greater in females than in males, and another explanation may be that hyperinsulinemia and insulin resistance are not associated with high blood pressure in females. The mechanisms which link hyperinsulinemia/insulin resistance to hypertension may be absent in females or there may be compensatory mechanisms that counteract the adverse effects of hyperinsulinemia/insulin resistance on blood pressure. The latter hypothesis is supported by data demonstrating that sucrose (13, 25) or fructose (unpublished observations) will induce hypertension in female rats after ovariectomy, suggesting that female sex hormones may be protective against the adverse cardiovascular effects of hyperinsulinemia and insulin resistance.

Obesity is another factor known to negatively impact upon blood pressure. In this experiment, insulin treatment did result in a significant body weight gain in male rats, although this difference was small and the distribution of values overlapped in each group (M: 308–420 g, MT: 380–452 g). Therefore, we believe this is not responsible for the blood pressure response observed in this experiment.

TxA₂ has been identified as a potential mediator involved in the development of hypertension secondary to hyperinsulinemia and insulin resistance. It has been shown that treatment with a TxA₂ synthase inhibitor prevents the development of hypertension in male rats infused with insulin (18) and hyperinsulinemic, insulin-resistant fructose hypertensive rats (8). At the moment, the exact mechanism by which TxA₂ contributes to the rise in blood pressure in these models of hypertension is not fully elucidated; however, our experiments have demonstrated an increase in both the plasma levels and vascular synthesis of TxA₂ (8). To explore the possible mechanisms responsible for sex differences in the relationship among hyperinsulinemia, insulin resistance, and hypertension, we examined vascular reactivity to the TxA₂ analog U-46619. In this experiment, we observed a striking sex difference in the vascular response to U-46619 but not to norepinephrine. Male aortas were twofold more sensitive to the TxA₂ analog U-46619 compared with those of females. This difference in potency was not affected by removal of the endothelium, which indicates that the function of vascular smooth muscle itself differs, rather than modulation by the endothelium. Neither chronic insulin treatment nor acute incubation with insulin affected the response to U-46619 in either sex. This finding is in contrast to the observation that insulin increases contraction to U-46619 in the male porcine coronary artery (33) but may be related to species or tissue differences. The lack of an enhanced responsiveness to U-46619 in tissue from hyperinsulinemic male rats does not preclude a role for TxA₂ in the development of hypertension in this model. As mentioned above, we have detected increases in the synthesis of TxA₂ in the vasculature of the fructose hypertensive rat, indicating that the abnormality may be related to the strength of the TxA₂ signal rather than the response. In interpreting our data, it should also be noted that the aorta is considered a conduit blood vessel and we are presently conducting studies using other resistance arteries from this model to determine regional differences.

Data from the literature indicate that sex differences in the sensitivity to TxA₂ apply to various blood vessel types and species. The same sex difference in the actions of U-46619 have been shown in the rat aorta and canine coronary and renal arteries (16, 17). Furthermore, it has also been shown that infusion of U-46619 in rats can cause a pressor response in male rats but not in female rats (27). Because TxA₂ appears to be involved in the pathogenesis of hypertension associated with hyperinsulinemia (8, 18), such a profound sex difference in the vascular actions of this hormone provides an appealing explanation for why female rats do not develop hypertension after chronic hyperinsulinemia. It is expected that females would be less susceptible to developing hypertension secondary to hyperinsulinemia because they have significantly reduced sensitivity to this factor.

In summary, this study provides evidence that the link between hyperinsulinemia/insulin resistance and hypertension is dependent on sex. Female rats have an inherently greater sensitivity to insulin compared with male rats, and, whereas chronic insulin treatment impairs insulin sensitivity in both males and females, this occurs to a greater degree in male rats. Elevations in blood pressure, however, are only observed in hyperinsulinemic male rats. A potential explanation for the lack of an increase in blood pressure in female rats may be that vascular tissue from female rats is less sensitive to the vasoconstrictor TxA₂. This is not to say, however, that hypertension does not affect females. Rather, it suggests that the pathological mechanisms of hypertension may differ between males and females. Very few experiments have investigated the effect of sex on the relationship among hyperinsulinemia, insulin resistance, and hypertension in humans. The results of this study, at the very least, indicate that this question warrants further study and could indicate the need for new sex-based treatment strategies that might ultimately improve success at blood pressure control.

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REFERENCES