Gender Differences in the Dietary Lard-Induced Increase in Blood Pressure in Rats

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Abstract—We investigated the difference between male and female rats in the increase in blood pressure (BP) when they were fed a lard-enriched diet. We also investigated the effect of a gonadectomy, with or without testosterone treatment, on the dietary lard-induced increase in BP. Wistar-strain male or female rats were bilaterally castrated or ovariectomized. Some of them were implanted subcutaneously with silicon tubes containing crystalline testosterone. Each group was fed either chow alone or chow in which 50% of the energy content was from substituted lard. Systolic blood pressure (SBP) was determined weekly during each 7- or 11-week feeding period. A steady-state plasma glucose method was used to determine the insulin sensitivity. The dietary lard-induced increase in SBP was observed at 5 weeks after the start of the feeding in the sham-operated male rats. In the sham-operated female rats, SBP did not change from the basal values until 11 weeks into the experimental period. Castration eliminated the dietary lard-induced increase in SBP. Ovariectomy had no effect on SBP throughout the experimental period in both diet groups. In castrated males given testosterone, SBP increased in a dose-dependent manner. In ovariectomized females given testosterone, SBP increased significantly in the lard-enriched diet group. The dietary lard developed an insulin resistance in both the sham-operated and gonadectomized rats. However, SBP increased only in the sham-operated male rats. These results suggest that the dietary lard-induced increase in SBP depends on the presence of testosterone. The development of insulin resistance by dietary lard triggers the increase in SBP. (Hypertension. 2002;39:1015-1020.)

Key Words: blood pressure ■ rats ■ insulin resistance ■ diet ■ hormones ■ gender

A sexually dimorphic pattern has been observed in relation to blood pressure. The prevalence of essential hypertension is higher in men than in age-matched premenopausal women. In hypertensive rat models, such as spontaneously hypertensive rats (SHR) and Dahl-salt hypertensive rats, males have higher blood pressures than do females.

Although the mechanisms of the gender difference in regulating blood pressure have not been completely elucidated, some studies have demonstrated that androgen has a potential role. It was reported that an increase in blood pressure was attenuated by castration in the hypertensive rat model. Furthermore, the relationship between androgen and pressure-natriuresis is suggested to play a part in the mechanism of these genetic forms of hypertension. However, whether or not male hormones may increase blood pressure remains uncertain in the other forms of hypertension.

We recently verified that some fat-enriched diets led to the development of an increase in systolic blood pressure (SBP) in male rats compared with pair-fed control animals. In the study, the chronic feeding of 8-week-old male rats with a lard-enriched diet increased SBP 5 weeks after the start of the feeding. And the chronic lard feeding induced hyperinsulinemia and led to the development of insulin resistance. An insulin sensitivity enhancer, troglitazone, an antidiabetic agent, reversed the effect, suggesting that insulin resistance is involved in the mechanism of dietary lard-induced increase in SBP.

Sex hormones influence insulin sensitivity. Testosterone treatment was reported to induce insulin resistance in women and to increase insulin messenger RNA levels in castrated male rats. Therefore, it is conceivable that there may be a gender difference in the dietary lard-induced increase in BP.

In the present study, we measured changes in the SBP of male and female rats that were fed a lard-enriched diet. We also investigated the effect of gonadectomy, with or without testosterone treatment, on the development of dietary lard-induced hypertension. Furthermore, to study whether testosterone modulates the hypertension by increasing the insulin level or decreasing insulin sensitivity, we measured the serum level of insulin and quantified insulin sensitivity using an insulin suppression technique.

Materials and Methods

Animals

Wistar-strain male and female rats (8 weeks old, weighing from 200 to 220 g and from 150 to 170 g, respectively) were obtained from Nippon SLC Co (Shizuoka, Japan) and were maintained in a...
controlled environment (lights on at 08:00 hours; lights off at 20:00 hours; temperature at 22±2°C). They were housed individually in plastic cages and provided with food and water ad libitum until pair feeding was begun.

The rats were bilaterally castrated, ovarietomized (OVX), or sham operated under an ether anesthesia. Some of the castrated male and OVX female rats were implanted subcutaneously with 8-, 16-, or 32-mm silicon tubes (2.5 mm inner diameter, 4.0 mm outer diameter; Kaneka Medix Co) containing 157, 314, or 628 mm³ of crystalline testosterone (T, Sigma Chemical Co). Plasma levels of testosterone have been reported to correlate with the lengths of implanted tubes. Animals were randomly assigned to the experimental groups (8 animals per group): Study 1, (a) sham-operated male, (b) castrated male (implanted with a tube containing crystalline cholesterol placebo), (c) castrated + T-implanted male; Study 2, (d) sham-operated female, (e) OVX female (implanted with a tube containing crystalline cholesterol placebo), (f) OVX + 32-mm T-implanted female.

Body weights (BW) and SBP were determined weekly throughout the experiment, from 2 weeks after castration or OVX. BP measurement was determined according to the methods of Yoshioka et al. In brief, SBP was measured in the tail region between 13:00 and 17:00 hours using an electronic sphygmomanometer (T-30 Rat Mouse Manometer-Tachometer, UNICOM Inc) after the rats were warmed at 37°C for 20 minutes. After the experiments, the rats were anesthetized with pentobarbital (50 mg/kg, ip) and decapitated, and their bilateral seminal vesicles were removed so they could be weighed.

Animals used in this study were maintained in accordance with the Animal Experimentation Guide of Nagoya University School of Medicine.

**Diet Protocol**

Diet protocol was determined according to the methods of Yoshioka et al. Three weeks after castration or OVX, each group was fed either chow (Oriental Yeast Co, Ltd) alone (control) or a mixture of chow and lard (Nippon Formula Feed Manufacturing Co, Ltd). In the latter diets, 50% of the dietary energy was derived from lard and the remaining 50% was derived from chow on the basis of an energy content for chow of 3.7 kcal/g and an energy content of 9.0 kcal/g for lard. We pair-fed the animals by providing a specified amount of food, and the food was replenished on a daily basis throughout the 7- or 11-week feeding period.

**Blood Sampling Procedure and Insulin Suppression Test (IST)**

Blood sampling procedure was determined according to the methods of Tamaya et al. After the 7- or 11-week feeding period, blood samples were obtained under an unrestrained, unanesthetized condition.

Steady-state plasma glucose (SSPG) and insulin (SSPI) levels were determined by the previously reported method. In short, the procedure was begun between 12:00 and 14:00 hours. Rats were anesthetized with pentobarbital (50 mg/kg, ip). Rats received a continuous infusion (1.0 mL/h) of epinephrine (0.08 mg/kg per minute), propranolol (1.7 mg/kg per minute), glucose (8 mg/kg per minute), and insulin (25 μU human insulin/kg per minute). Two hours after the start of the infusion, and then at 15, 30, 45, and 60 minutes after the first sampling, blood samples were collected. SSPG and SSPI levels were calculated from the mean of the 5 blood samples.

**Assays**

Plasma glucose concentration was determined via the immobilized enzyme membrane/H₂O₂ method using the glucose analyzer Antsense II (Bayer Medical Co, Ltd). Plasma immunoreactive insulin (IRI) and testosterone levels were assayed by the radioimmunoassay method with commercial insulin and testosterone assay kits, respectively (SRL Co). The insulin assay used human or rat insulin as the standard for SSPI or IRI, respectively. The intra- and interassay coefficients of variation were 1.5% and 4.61% for 1.4 ng/mL on rat insulin and 157 mm³ on human insulin assay, 4.3% and 8.5% for 1.4 ng/mL on rat insulin assay, and 6.1% for 1.64 ng/mL and 11.6% for 0.93 ng/mL on testosterone assay, respectively.

**Data Analysis**

Data are reported as the mean±standard error (SE). Statistical analysis was carried out by factorial analysis of variance (ANOVA), unless otherwise noted. The effect of testosterone implantation in the castrated male rats was analyzed by 2-factor ANOVA, incorporating a nested design with the dose nested within the treatment, the rats nested within the diet, and an interaction term (diet×treatment). When the differences between the groups were statistically significant, post hoc pairwise comparisons were performed via the Fisher protected least significant difference method. A level of $P<0.05$ was considered statistically significant.

**Results**

There was no significant difference among the groups in terms of SBP before the beginning of the special diet (Figures 1a through 1d). Also, there was no significant change in the SBP of any of the rats fed chow alone in comparison with each baseline throughout the feeding period (by paired $t$ test: Figures 1a and 1b). In the sham-operated male group, lard feeding significantly increased SBP at 5 weeks after the start of the special diet, and SBP continued to increase until 7 weeks, when it was approximately 5 to 10 mm Hg higher than that of the chow control group (Figure 1a). In the castrated male groups, lard-enriched diet did not cause any significant change in SBP compared with the chow-alone control group. In the female rats, lard feeding or OVX caused no significant change in the SBP throughout 11 weeks (Figure 1b). Figure 1c shows the effect of testosterone treatment on SBP in the castrated male rats fed a lard-enriched diet. Implantation with a tube containing crystalline cholesterol placebo did not...
affect SBP in comparison with that of the sham-operated male rats. In the 32- or 16-mm T-implanted male groups, SBP significantly increased at 6 to 7 weeks and 7 weeks, respectively, compared with that of the placebo-implanted control. In the castrated male groups, the 8-mm T-implantation did not cause any significant change in SBP compared with the placebo-implanted group. Figure 1d demonstrates the effect of testosterone treatment on SBP in the OVX female rats fed a lard-enriched diet. There was no significant change in SBP in the OVX females without testosterone implantation throughout the 11 weeks of feeding (as determined by the paired t test). SBP in the 32-mm T-implanted group significantly increased at 3 to 7 weeks. The levels of SBP at 5 to 7 weeks were comparable to those of the sham-operated male group fed a lard-enriched diet according to the same schedule.

In the male rats, neither the lard feeding, castration, nor testosterone treatment affected the change in BW (Figures 2a and 2b). In the female rats, BWs in the OVX groups were greater than those in the sham groups from 4 weeks until the end of the experiments (Figure 2c). Dietary lard increased BW significantly from 8 to 11 weeks (as determined by two-factor ANOVA). In the OVX+T-implanted female group, a lard-enriched diet increased BW compared with the OVX females with tubes containing crystalline cholesterol placebo from week 4 (Figure 2d).

Testosterone implantation increased plasma levels of testosterone (Figure 3a) and the volumes of seminal vesicles (Figure 3b) dose-dependently (P<0.0001). However, the diet did not affect either of these parameters (P=0.2689 and 0.6633, respectively), and diet×treatment interaction did not reach the statistically significant difference (P=0.6769 and 0.9474, respectively). Post hoc pairwise comparisons showed that there were significant differences in plasma testosterone levels and seminal vesicle volumes between the 8-mm T and 16-mm T groups (P<0.0001 in both parameters) and between the 16-mm T and 32-mm T groups (P<0.0001 in both parameters). Plasma testosterone levels in OVX rats with 32-mm T were comparable to those of sham-operated male rats in both diet groups (Figure 3c).

Neither the testosterone implantation nor the lard feeding had a significant effect on the plasma glucose level in the castrated male rats, as determined by 2-factor ANOVA (treatment P=0.2043; diet P=0.1634; treatment×diet interaction P=0.9421; data not shown). In OVX female rats fed
with a lard-enriched diet, testosterone implantation did not affect the plasma glucose level (data not shown).

Figure 4a shows the effects of lard feeding and castration on the plasma levels of IRI in male rats. Castration did not affect the IRI levels ($P=0.9218$). Lard feeding increased the IRI levels significantly ($P=0.0011$), and diet$\times$ treatment interaction was not statistically significant ($P=0.1033$). The effects of testosterone treatment and lard feeding on the plasma levels of IRI in the castrated male rats are depicted in Figure 4b. Testosterone implantation did not affect the IRI levels ($P=0.4485$). However, lard feeding increased the levels significantly ($P=0.0006$), and diet$\times$ treatment interaction was not statistically significant ($P=0.7548$). Figure 4c demonstrates the effects of lard feeding and OVX on the plasma levels of IRI in female rats. OVX increased the plasma levels of IRI, but the difference did not reach statistical significance ($P=0.0946$). Lard feeding increased the IRI levels significantly ($P=0.0031$), and there was no significant interaction in the diet$\times$ treatment ($P=0.7794$). Testosterone im-

Figure 4. Effects of CAST (a), CAST with T (b), OVX (c), and OVX with T (d) on levels of plasma immunoreactive insulin. See Figure 1 for other details.

Figure 5. Effects of CAST (a), CAST with T (b), OVX (c), and OVX with T (d) on levels of steady-state plasma glucose. See Figure 1 for other details.
plantation did not affect the IRI levels ($P=0.4725$, Figure 4d). However, lard feeding increased the levels significantly ($P=0.0065$), and diet$\times$ treatment interaction was not statistically significant ($P=0.4245$).

Figure 5a shows the effects of lard feeding and castration on the plasma levels of SSPG in male rats. Castration increased the plasma levels of SSPG ($P=0.0404$). Lard feeding increased the SSPG levels significantly ($P=0.0016$), and there was no significant interaction in the diet$\times$treatment ($P=0.1381$). The effects of testosterone treatment and lard feeding on the plasma levels of SSPG in the castrated male rats are depicted in Figure 5b. Testosterone implantation did not affect the SSPG levels ($P=0.3936$), however, lard feeding increased the levels significantly ($P<0.0001$), and diet$\times$treatment interaction was not statistically significant ($P=0.4563$). Figure 5c demonstrates the effects of lard feeding and OVX on the plasma levels of SSPG in female rats. OVX did not affect the plasma levels of SSPG ($P=0.4776$). Lard feeding increased the SSPG levels significantly ($P=0.0108$), and there was no significant interaction in the diet$\times$treatment ($P=0.9384$). Testosterone implantation did not affect the IRI levels ($P=0.3621$, Figure 5d). However, lard feeding increased the levels significantly ($P=0.0004$), and diet$\times$treatment interaction was not statistically significant ($P=0.6114$).

**Discussion**

The present study suggested the presence of gender differences in dietary lard-induced increase in SBP. The dietary lard-induced increase in SBP was observed at 5 weeks after the start of the feeding in the sham-operated male rats. However, in the sham-operated female rats, SBP did not change from the basal values until 11 weeks into the experimental period.

Castration eliminated the increase in SBP induced by dietary lard. In contrast, OVX had no effect on the SBP in females. These data support a role for testicular factors, including androgen, in mediating the increase of BP in males. They also suggest that it is not the presence of ovarian factors, including estrogen, in females that protected them from developing the increase in BP.

We further addressed the question of the role of androgens, especially testosterone, in dietary lard–induced increase in SBP in male rats. In castrated males given testosterone, SBP increased in a dose-dependent manner, and 32-mm T-tube implantation increased it to an extent similar to that in the sham-operated male rats (118±0.33 and 118±0.95 mm Hg, respectively). The plasma testosterone levels in the castrated rats implanted with 32-mm T-tubes was also comparable to that in the sham-operated male rats. These findings strongly support a role for testosterone in the increase of SBP induced by dietary lard in male rats. In a hypertensive rat model, castration is reported to attenuate the development of hypertension, suggesting a role for androgens in mediating the higher BP, which is consistent with our result.

The other question we addressed in the study was whether testosterone mediates dietary lard–induced increase in SBP in female rats. In OVX females given testosterone with a 32-mm T-tube, lard feeding increased their SBP to the level of 118±0.58 mm Hg. Their plasma testosterone level was 1.88±0.29 ng/mL, which was comparable to that of the sham-operated male rats (1.59±0.43 ng/mL). These results suggest that, in both male and female rats, testosterone is essential to the dietary lard–induced increase in SBP.

However, in the castrated male rats, the plasma levels of testosterone in the rats fed a lard-enriched diet were not significantly different from those of the chow-fed groups at any of the doses of testosterone treatment, suggesting that dietary lard did not induce increase in SBP as a result of an increase in plasma testosterone levels.

In the sham-operated male group and the castrated male group implanted with 32-mm T-tubes, the lard-enriched diet significantly increased SBP at 5 and 6 weeks, respectively. However, in the OVX female rats implanted with T-tubes, SBP significantly increased at 3 weeks. We do not know why SBP increased earlier in the female rats than in the male animals. It was reported that an early increase in fat mass was associated with an impairment of insulin action. In our previous study, a dietary lard-induced increase in SBP developed when visceral fat accumulation reached a certain threshold (under submission). The differences in visceral fat amounts could be a reason for the discrepancy between the male and female rats.

In the OVX+$T$-implanted rats, BW was significantly greater than that in the OVX+$T$-placebo rats, which could be responsible for the increased SBP in the OVX+$T$ rats. However, the OVX+$T$-implanted rats that were fed chow did not develop the increase in SBP, and their BWs were comparable to those in the OVX+$T$ rats that were fed the lard-enriched diet throughout the experiment (data not shown). Therefore, it is unlikely that the greater BW gain in the OVX+$T$ rats fed lard was the cause of the increase in SBP.

We have verified that insulin resistance is involved in the mechanism of dietary lard–induced increase in SBP in intact male rats. In the present study, however, dietary lard did induce insulin resistance and hyperinsulinemia in the castrated male, the sham-operated female, and the OVX female groups, but did not increase SBP. It was suggested that the dietary lard–induced increase in SBP depends on the presence of testosterone and that the development of insulin resistance by dietary lard triggers the increase in SBP.

In the castrated rats that were fed chow, SSPG levels were significantly higher than in the sham-operated rats that were fed chow, suggesting that castration itself induced insulin resistance. This result is consistent with a cross-sectional report that low levels of testosterone play some role in the development of insulin resistance and subsequent type 2 diabetes. Although the mechanism is not known, the absence of testosterone may induce insulin resistance by an increase in the visceral fat mass, because testosterone works to decrease the abdominal fat mass.

In summary, a gender difference in dietary lard–induced increase in SBP in the rat was observed. The increase in SBP can be attenuated by castration and can be restored by testosterone supplementation, suggesting that the dietary lard–induced increase in SBP depends on the presence of testosterone.
testosterone and that the development of insulin resistance by dietary lard triggers the increase in SBP.

**Perspectives**

We recently verified that aging accelerates development of dietary lard–induced increase in SBP in rats, suggesting the increased susceptibility to insulin resistance in older rats (under submission). The prevalence of essential hypertension is higher in postmenopausal than in premenopausal women, which suggests that female sex hormone protects against the development of hypertension. Although the effects of estrogen on lipid and lipoprotein metabolism, glucose metabolism, and hemostasis, as well as its direct cardiac and vascular effects, are considered as possible explanations, estrogen is also known to have some effects on insulin sensitivity. The present study suggests that testosterone may trigger the mechanism by which the increase in BP is induced by development of insulin resistance. Dietary lard is reported to stimulate the sympathetic nervous system (SNS), and the increased activity of the SNS is one of the mechanisms of hypertension.20 Also, the central nervous system (CNS), where androgen receptors exist, is involved in BP regulation.21,22 Therefore, testosterone may act on the CNS to regulate BP. Age-related changes in the CNS have been studied extensively. Alzheimer’s disease (AD) is one of the most common types of dementia. Degeneration of the cholinergic system in the hippocampus is a feature of AD pathology, and alteration of BP is reported in AD. Hormone replacement therapy is reported to be effective in the treatment of dementia or cardiovascular disease. These results support the hypothesis that sex hormones in the CNS are involved in the regulatory mechanism of BP. If the central mechanism of the development of the dietary lard–induced increase in BP and the role of sex hormones in this mechanism can be elucidated, new approaches to the treatment of hypertension in the elderly may be expected.

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**References**


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