The Thesis

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Effects of Gender, Gonadectomy and Sex Hormones on Growth and Cholesterol Metabolism in Pigs and Rats

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DEPARTMENT OF ANIMAL BIOTECHNOLOGY
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We hereby recommend that the above thesis be accepted in partial fulfillment of the requirements for the degree of doctor of philosophy.

Committee

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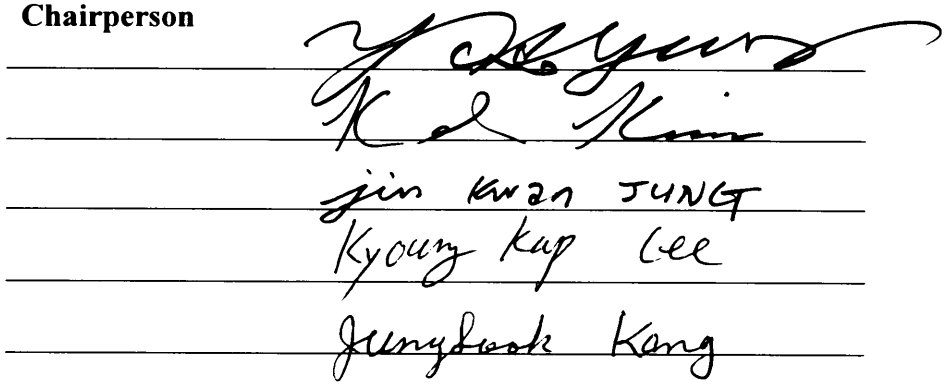

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ABSTRACT

Eight experiments were conducted to determine the effects of gender, gonadectomy and sex hormones on growth and cholesterol metabolism in Landrace pigs and Sprague Dawley rats. In Exp. 1, 20 3-wk-old and 20 10-wk-old rats were divided into four groups of 10 rats each, respectively: young and adult male and female. All groups were fed a hypercholesterolemic diet (HCD) containing 0.5% cholesterol and 0.2% cholate for 1 wk. For Exp. 2, 16 male (163 g) and 16 female rats (135 g), both at the same age (35 d), were divided into four groups of 8 rats each, respectively: sham-operated male and female, and gonadectomized (GNX) male and female. These rats had free access to a HCD for 4 wk. In Exp. 3, 24 male (130 g) and 24 female (124 g) GNX rats were assigned to eight groups of 6 rats each, respectively: sham-operated male and female, GNX male and female, GNX+17β-estradiol (E_2) male and female, and GNX+17α-methyltestosterone (MT) male and female. Rats were injected once every week intramuscularly with MT or E_2 (7 mg/kg body weight). Rats had free access to a commercial chow diet without supplementary cholesterol for 4 wk. In Exp. 4, 24

GNX female rats (115 g) were divided into three groups of eight each: GNX, GNX+E_2 and GNX+MT, hormones injected weekly at 7 mg per kg body weight. Rats were fed a NCD for 3 wk. For Exp. 5, 18 male (165 g) and 18 female (135 g) rats were divided into six groups of six rats each: sham-operated, GNX and GNX+MT (100 mg/kg diet), each consisting of 6 of each sex. Rats were fed a HCD for 4 wk. In Exp. 6, 10 male (177 g) and 10 female (154 g) rats both at the same age (5 wk) were allowed to have free access to a HCD for 4 wk. For Exp. 7, five sham-operated and five GNX female Landrace pigs (26 kg) were allowed to have free access to a diet for 70 d. In Exp. 8, 10 male (104 kg) and 10 female (98 kg) pigs were assigned to four groups of five pigs each: sham-operated male and female, and GNX male and female. Pigs were fed a HCD containing 0.5% supplementary cholesterol and 0.1% cholate for 10 d. Both genders of 3-wk-old rats fed a HCD showed hypercholesterolemia, whereas only females of 10-wk-old rats had hypercholesterolemia, compared to the 10-wk-old male rats ($P < 0.01$) (Exp. 1). Average daily gain (ADG) was higher ($P < 0.01$) in sham-operated male rats than in their female counterparts, but GNX reversed the suppressed growth in females. Plasma total

cholesterol levels in female rats were twice that of males when rats were fed a HCD (Exp. 2). Intramuscular injection of E_2 suppressed growth both in GNX females and males, whereas MT administration did not significantly affect ADG in GNX rats. Plasma cortisol levels in sham-operated females were much higher ($P < 0.01$) than in their male counterparts, but this gender difference disappeared when rats were gonadectomized (Exp. 3). E_2 injection increased ($P < 0.01$) plasma estradiol and cortisol levels, and relative uterine weight in GNX female rats. Regression analysis showed that plasma cortisol concentration ($r = 0.72$, $P < 0.01$) and uterine weight ($r = 0.84$, $P < 0.01$) were highly correlated with plasma estradiol levels (Exp. 4). GNX increased ($P < 0.01$) plasma cholesterol level in male rats, but had no effect ($P > 0.05$) in female rats. MT administration to GNX male and female rats decreased the plasma cholesterol level by 23 and 45%, respectively (Exp. 5). Plasma total bile acids concentrations were higher ($P = 0.08$) in females than in male rats. Fecal excretion of total bile acids was much higher ($P < 0.01$) in males than in females (Exp. 6). GNX also increased average daily gain ($P < 0.04$), but had no effect ($P > 0.44$) on feed efficiency during the growing-finishing period in female pigs (Exp 7). There

were no significant differences in the plasma total or HDL cholesterol levels and HDL/(LDL+VLDL) ratio between genders or treatments before feeding a HCD. Plasma cholesterol levels in pigs fed a HCD for 10 d were much higher in sham-operated females than in their male counterparts (Exp. 8). Results indicate that estrogen suppresses growth, perhaps through inducing the secretion of hormones that increase with stress, and that testosterone appears to be responsible for the lower plasma cholesterol level in male than in female animals with hypercholesterolemia.

Ⅰ. Introduction

Males grow faster and also are larger in mature size than their female counterparts in most mammals. Sex hormones are believed to be behind the gender difference in growth rate. Early studies (Kitay, 1961, 1963; Critchlow et al., 1963; Smith and Norman, 1987; Lesniewska et al., 1990; Burgess and Handa, 1992) showed gender differences in cortisol secretion, indicating that estrogen elevates basal levels of cortisol, corticosterone and adrenocorticotropin, and their responses to various stimuli in rats. Short-term estradiol treatment to young men increased cortisol and norepinephrine concentrations in the saliva following stress over a placebo control (Kirschbaum et al., 1996). Studies (Tarttelin and Gorski, 1973; Wallen et al., 2001) have shown that steroid hormones influence growth in rats, although the mechanism is not clearly known.

In the Framingham study, the risk of cardiovascular disease (CVD) was shown to significantly increase in women who had taken estrogen (Wilson et al., 1985). More recently, Manhem et al. (1996) reported that estrogen administration resulted in an enhanced

cardiovascular response to mental stress in young menstruating women. However, estrogen has long been known to exert cardioprotective effect (Barrett-Connor and Bush, 1991), although the precise mechanism underlying its benefits is unclear (Sudhir et al., 1997). Gender differences have also been reported in plasma cholesterol level in rats (Lee et al., 1999) and guinea pigs (Fernandez et al., 1995), when they were fed hypercholesterolemic diets.

Conflicting results have been reported on the effect of sex hormones on plasma cholesterol level in rats fed normocholesterolemic diets, e.g., increasing (Arjmandi et al., 1997) or decreasing (Lundeen et al., 1997) effect of estradiol replacement on plasma cholesterol level. Handa et al. (1997) demonstrated that plasma free testosterone level was associated with lower levels of HDL-C and that estradiol was related to elevated levels of HDL-C levels in Japanese men in their early fifties, supporting that testosterone may be causally associated with atherosclerosis in men through altered lipoprotein metabolism. However, to my knowledge, no studies have clearly demonstrated that testosterone is related to the gender difference in plasma cholesterol level in animals fed hypercholesterolemic diets. Therefore, a series of

experiments was conducted to determine the effects of gender, gonadectomy and sex hormones on growth and cholesterol metabolism using the rat and pig as model animals.

Ⅱ. Literature review

1. Effects of gender, gonadectomy and sex hormones on growth.

Growth rate and feed efficiency are main economical concerns in the livestock industry. Although males grow faster and also are larger in mature size than their female counterparts in most mammals, scientific bases for the gender difference in growth have not been well established.

Sex hormones are believed to be behind the gender difference in growth rate. Female rats show greater adrenocorticotropin (ACTH) and corticosterone responses to stress and also secrete higher basal levels of corticosterone than do male rats (Kitay, 1961; Critchlow et al., 1963; Le Mevel et al., 1979). This gender difference was abolished by ovariectomy and was reinstated by estradiol administration (Le Mevel et al., 1979). The gender difference in the pattern of cortisol secretion was also reported in the nonhuman primate macaques, and estradiol implant in castrated male macaques elicited a female pattern of plasma cortisol levels (Smith and Norman, 1987; Norman et al., 1992).

In healthy men, short-term treatment with estradiol led to enhanced hypothalamic-pituitary-adrenal (HPA) and sympathetic responsiveness to psychological stress, resulting in increased ACTH, cortisol and norepinephrine concentrations in the saliva compared to the placebo (Kirschbaum et al., 1996). Corticosterone and ACTH levels after a 5-second footshock stress with one mamp were much higher and maintained for a prolonged time in ovariectomized rats when administered with estradiol, compared to those found without estrogen replacement (Burgess and Handa, 1992). They indicated that estrogen treatment results in a loss of the glucocorticoid receptor's ability of autoregulation (Burgess and Handa, 1992).

However, estrogen has been reported to have both stimulatory and inhibitory effects on HPA functions, depending on the time after ovariectomy and different doses of estradiol (Luber et al., 1991; Redei et al., 1994). Cortisol as well as catecholaminergic responses to stress has also been known to vary with estrous cycle in women (Marinari et al., 1976; Hastrup and Light, 1984; Collins et al., 1985) and in rats (Viau and Meaney, 1991; Carey et al., 1995). Studies (Tarttelin and Gorski, 1973; Wallen et al., 2001) have shown that steroid hormones influence growth in rats, although the

mechanism is not clearly known, and testosterone is known to have anabolic effect in rats (Hagar and Kalkhoff, 1989).

2. Effects of gender, gonadectomy and sex hormones on cholesterol metabolism.

Cholesterol homeostasis in animals' body is associated with various factors such as genes, diets and hormones. A feedback control is known to exist in the synthesis of cholesterol in vivo. However, when absorbed cholesterol exceeds body's need, it accumulates in the body and may become a causing factor of CVD including atherosclerosis.

Our recent study (Lee et al., 1999) showed a marked gender difference in plasma cholesterol levels when rats were fed a HCD and we speculated that ovarian activities might be responsible for the increased plasma cholesterol level. Interestingly, Munilla and Herrera (1997) reported that pregnant rats showed a much higher hypercholesterolemic response to dietary cholesterol than do virgin rats. They speculated that the increased plasma cholesterol level may be due to a decreased number of LDL receptors, or an increased

entry rate of cholesterol-bearing lipoproteins in the circulation.

Estrogen administration results in an enhanced cardiovascular response to mental stress in young menstruating women (Manhem et al., 1996). However, on the basis of observational studies on estrogen replacement, estrogen has long been considered to have cardioprotective effect (Barrett-Connor and Bush, 1991), although the precise mechanism underlying its benefits is unclear (Sudhir et al., 1997).

Estrogen administration decreased plasma total and HDL cholesterol levels over the basal level in a dose-dependent manner in adult ovariectomized rats fed a commercial chow but not in 19-d-old immature intact rats (Lundeen et al., 1997). Furthermore, estrogen administration increased the LDL-receptor mRNA and the receptor protein in rats (Srivastava et al., 1993; Parini et al., 1997). In theory, this increased receptor protein should depress cholesterol synthesis and in turn plasma cholesterol levels. The hypocholesterolemic effect of estrogen seems to be highly variable among studies. Lundeen et al. (1997) found no estrogen effect at levels below 0.01 mg (injected daily for 4 d per kg body weight), but at a higher level (1 mg), plasma cholesterol was almost disappeared

(<10 mg/100 mL).

By contrast, estradiol implant (50 mg for 7 d) in chicks increased plasma triacylglycerol levels 45 fold and cholesterol levels 6 fold compared to the control (Park and Cho, 1988). Estrogen's involvement in lipoprotein metabolism (e.g., an increased ratio of HDL to LDL cholesterol) has also been implicated in its role in cardioprotection (Wagner et al., 1991; Campos et al., 1997).

Carter et al. (1997) reported that cholesterol absorption efficiency is a highly regulated process and the regulation may also be controlled by distinct genetic factors. The more elevated plasma LDL-C levels observed in female guinea pigs fed a HCD may be due to a gender-associated effect related to reduced ability to maintain hepatic cholesterol homeostasis with high dietary cholesterol (Fernandez et al., 1995). Marsh et al. (1999) reported that estradiol protected LDL receptor-deficient (LDRL-/-) female mice from atherosclerosis and this protection was independent of changes in plasma cholesterol levels. However, under conditions in which there is an increase in peroxidase activity, estradiol may act as a proxidant and promote the oxidation of LDL-C (Santanam et al., 1998).

There have been conflicting reports on the role of testosterone in cholesterol metabolism; the effect differs, depending on different model animals, age, gender and the route of testosterone administration. Several studies have shown that testosterone has adverse effect on cholesterol metabolism by altering lipoprotein profiles. Total testosterone concentrations and sex hormone-binding globulin (SHBG) are significantly associated with LDL size in men (Haffner et al., 1996).

Testosterone, per se, if administered at sufficiently high doses and for long durations, can significantly raise total cholesterol, triacylglycerol, LDL-C and Apo-B and, at the same time, lower HDL-C in androgenized women, indicating that male predilection for CVD may be due to adverse effects of higher androgen levels on lipid and lipoprotein profiles (Goh et al., 1995). Anderson et al. (1995) also showed that HDL-C was significantly depressed by intramuscular injection of testosterone enanthate (200 mg per week for 12 months) in men while there were no changes in plasma total and LDL cholesterol or triacylglycerol levels during treatment.

More recently, Handa et al. (1997) demonstrated that plasma free testosterone level was associated with lower levels of HDL-C

and that total estradiol was related to elevated levels of HDL-C levels in Japanese men in their early fifties, supporting that testosterone may be causally associated with atherosclerosis in men through altered lipoprotein metabolism. Male hamsters fed a HCD containing 0.05% cholesterol develop greater elevations in plasma total cholesterol and greater concentrations of plasma HDL-C, and greater development of early aortic atherosclerosis versus female hamsters (Wilson et al., 1999).

On the contrary, testosterone replacement therapy in hypogonadal and elderly men showed a beneficial effect on cardiovascular system, decreasing total cholesterol and atherogenic fraction of LDL-C without significant alterations in HDL-C levels or its subfractions HDL_2-C and HDL_3-C (Zgliczynski et al., 1996). Tchernof et al. (1997) also showed that increased testosterone levels were associated with reduced triacylglycerol and Apo B; total and LDL cholesterol concentrations; and increased HDL/total cholesterol and HDL_2-C/HDL_3-C ratios.

Hypotestosteronemia was observed in Chinese male patients with CVD, and positive correlation of plasma testosterone level with plasma HDL-C, but negative correlation with plasma Lp(a) level

was found, suggesting that testosterone has a protective effect against atherosclerosis (Zhao and Li, 1998). These conflicting results led us to study the gender differences in cholesterol metabolism using pigs and rats.

Ⅲ. Materials and methods

1. Animals and diets.

Animal care. Sprague Dawley rats (Korea Institute of Chemistry, Tae-Jun, Korea) in rat experiments were housed individually in suspended wire cages in a room maintained at 20-23 C with a 12-hr light (0700 to 1900) and 12-hr dark (1900 to 0700) cycle. Rats had a free access to water and diets (Table 1). For pig experiments, Landrace pigs were kept individually in pens with 60%-slatted concrete floor (9 m^2) and allowed to have free access to water and diets (Table 2). All animal management and sampling procedures were in accord with the NIH Guide for the Care and Use of Laboratory Animals (Publ. no 85-23 rev.).

Exp. 1. The effect of gender and age on cholesterol metabolism in young and adult rats was determined using 40 rats (average initial body weight of young (3 wk) and old (10 wk) male and female was 52.9 and 52.9, and 249.7 and 190.9 g, respectively). A HCD was received by all groups. During the 7 d of feeding trial, body weight and feed intake were recorded.

Table 1. Composition of the experimental diet used for Exp. 1, 2, 4[a], 5[b] and 6 (as-fed basis)

Ingredient	%	Ingredient	%
Casein	20.0	L-Methionine	0.3
Corn starch	44.3	Vitamin mix[c]	1.0
Lard	5.0	Mineral mix[c]	3.5
Corn oil	5.0	Cholesterol	0.5
Sucrose	20.0	Cholic acid	0.2
Choline chloride	0.2	Total	100

[a]For normocholesterolemic diet, corn starch was substituted for cholesterol and cholic acid in Exp. 4.

[b]For testosterone administration, 17α-methyltestosterone (100 mg/kg diet) was added at the cost of corn starch in Exp. 5.

[c]AIN (1976) purchased from Halan Teklad, Madison, WI.

Exp. 2. This study was conducted to assess the effect of gender and gonadectomy (GNX) on growth and plasma cholesterol levels and body composition. Sixteen male (163 g) and 16 female rats (135 g), both at the same age (35 d), were divided into four groups of 8 rats each, respectively: sham-operated and GNX male and female. These rats had free access to water and a HCD for 4 wk. Rats were weighed every other d and feed intake was monitored.

Exp. 3. This experiment was conducted to confirm the result of Exp. 2, and also to further study the sex hormone effect in

GNX rats, 24 male (130 g) and 24 female (124 g) rats divided into eight groups of 6 rats each, respectively: sham-operated male and female, GNX male and female, GNX+17β-estradiol (E_2) male and female, and GNX+17α-methyltestosterone (MT) male and female. Rats were injected once every week intramuscularly (thigh area) with 0.1 mL of 20% (v/v in saline) ethanol solution alone or 0.1 mL of 20% ethanol solution containing either MT or E_2 (7 mg/kg body weight), which was dissolved in absolute ethanol first and then diluted with saline. Rats had free access to water and a commercial chow (normocholesterolemic diet, NCD) for 4 wk. Body weight and feed intake were monitored. After the feeding trial, livers were taken and weighed to calculate relative liver weight (100 x liver weight/body weight).

Exp. 4. This experiment was carried out to verify the effects of gender on growth, and also to determine the effect of MT or E_2 administration on growth, plasma cortisol level and uterine weight using 24 GNX female rats (115 g at 4 wk of age) divided into three groups of eight rats each: GNX, GNX + E_2 and GNX + MT. Rats were injected once every week intramuscularly with either MT or E_2 (7 mg/kg body weight), which was prepared using the same

method described in Exp. 3. Rats were fed a NCD for 3 wk after 7-d recovery from surgery. At the end of feeding trial, rats were killed and uteri isolated and weighed to confirm the effectiveness of injected E_2.

Exp. 5. This experiment was done to reassess the effects of gender on cholesterol metabolism, and also to determine the effect of MT in a diet fed GNX rats on plasma cholesterol level, assuming that testosterone is responsible for the lower plasma cholesterol level in male than in female animals. Eighteen male (165 g) and 18 female (135 g) rats, both at the same age (5 wk), were divided into six groups (3 x 2) of six rats each: sham-operated, GNX and GNX + MT (100 mg/kg diet), each consisting of six males and six females. Rats had free access to water and a HCD for 4 wk.

Exp. 6. The effects of gender on bile acids excretion in rats fed a HCD were determined using 10 male (177 g) and 10 female (154 g) rats both at the same age (5 wk), which were allowed to have free access to a HCD for 4 wk. Body weight and feed intake were monitored, and feces was collected every other day.

Table 2. Composition of the experimental diet used for Exp. 7 and 8[a] (as-fed basis)

Ingredient	%	Ingredient	%
Corn	28.9	Tallow	3.2
Wheat	38.0	Molasses	3.0
Wheat bran	0.5	Vitamin mix[b]	0.1
Soybean meal	23.5	Mineral mix[b]	0.1
Limestone	1.4	Salt	0.3
Monocalcium P	1.0	Total	100
Chemical composition (calculated)			
CP, %	17.7		
ME, kcal/kg	3,272		
Ca, %	0.61		
P, %	0.57		

[a]0.5% cholesterol and 0.1% cholic acid were added at the cost of corn in Exp. 8.

[b]Provided the following per kg of diet: Fe, 60 mg; Cu, 15 mg; Mn, 25 mg; Zn, 60 mg; I, 0.20 mg; Se, 0.25 mg; vitamin A, 8,000 IU; vitamin D3, 1,500 IU; vitamin E, 30 IU; vitamin K, 1.5 mg; vitamin B1, 1.0 mg; vitamin B2, 4.0 mg; vitamin B6, 2.0 mg; vitamin B12, 0.02 mg; pantothenic acid, 7.5 mg; Niacin, 20 mg; Biotin, 0.1 mg; Folic acid, 0.6 mg.

Exp. 7. This trial was done to confirm the effect of GNX on growth and feed efficiency in female pigs. Five sham-operated control and five GNX Landrace female pigs (26 kg) were allowed to have free access to water and a diet (Table 2) for 70 d. Body weight and feed consumption were recorded every other wk, and ADG, ADFI and gain/feed over the 70-d period calculated.

Exp. 8. The effect of gender and GNX on plasma

cholesterol level in pigs was examined using 10 male (104 kg) and 10 female (98 kg) Landrace pigs divided into four groups: sham-operated male and female, and GNX male and female. Pigs were kept individually in pens with 60%-concrete floor (9 m^2) and allowed to have free access to water and a HCD (Table 2) containing 0.5% supplementary cholesterol and 0.1% cholic acid for 10 d.

2. Gonadectomy, hormone administration, plasma sample preparation and carcass measurement.

Testicles were removed through a small incision in the tip of the scrotum after ligation of the spermatic cords. Ovaries were removed through a dorsal paramedial incision at the level of the lower poles of the kidneys, and the incision was closed with stitches for pigs or wound clips using an autoclip applier (Stoelting Co., Wood Dale, IL) for rats. The intact control animals were sham-operated leaving the organs intact. These procedures were performed in pigs at 10 d of age or in rats at 3 wk of age under light ether anesthesia. The stitches and wound clips were removed 7 d after surgery. At the end of feeding trials and at both the beginning

and end of 10-d feeding period in Exp. 8, blood samples were collected from jugular vein into vacutainer tubes containing EDTA after 16-h fasting. Plasma was prepared from the blood samples by centrifugation, and stored at -20℃ for later analysis. In Exp. 3 and 4, rats were killed 1 d after the third weekly injection of hormones.

After killed in Exp. 2, rat carcass were divided into small pieces, lyophilized, ground in liquid nitrogen, and stored at -70℃ until analysis. In Exp. 7, back fat thickness was measured at two spots of 11^{th} thoracic and 1^{st} lumbar vertebra, and the average value was used. Dressing percentage was calculated dividing slaughter weight by live weight.

3. Analysis of plasma cholesterol and triacylglycerol, and plasma and fecal bile acids.

Total cholesterol, high-density lipoprotein (HDL) cholesterol and triacylglycerol concentrations in the plasma were determined using commercial assay kits (International Reagent Corp., Tokyo, Japan for the former, and WAKO Pure Chemical Ind., Osaka, Japan for the latter two) according to the manufacturer's

instruction. Low-density lipoprotein (LDL) cholesterol was calculated by subtracting HDL cholesterol from total cholesterol.

Total bile acids in the plasma and feces were measured using commercial assay kits (Daiichi, Japan) and biochemical analyzer (HITACHI Photometer 4020, Japan) in Exp. 6. For the determination of bile acids in the feces, 2 mL of diethylether was added to 0.5 g fecal sample, and the mixed samples were shaken and centrifuged at 1,400 x g for 5 min. The supernatants were collected and the remaining residues were extracted with diethylether the second time and the supernatants were added to the first extracts. The extracted samples were dried using N_2 and stored at -70℃ until analysis. At the beginning of measurement, the dried samples were resolved in 1.2 mL diethylether.

4. Plasma hormone assay.

Plasma estradiol, testosterone and cortisol concentrations were determined using the radioimmunoassay kits (Diagnostic Products Corp., Los Angeles, CA), and a γ -counter (COBRA, Packard, Meriden, MD), according to the manufacturer's instructions.

5. Statistical analysis.

The student *t*-test was used to assess the effect of gender or GNX on growth and cholesterol metabolism in Exp. 6 and 7. One-way (Exp. 4) or two-way (Exp. 1, 2, 3, 5 and 8) ANOVA was also applied to the analysis of data using SAS package (SAS, 1988, SAS Inst. Inc., Gary, NC). In ANOVA, the sources of variation for all variables were young and adult for each sex (2 x 2) in Exp. 1; sham-operated and GNX for each sex (2 x 2) in Exp. 2 and 8; sham-operated, GNX, GNX + E_2 and GNX + TM for each sex (4 x 2) in Exp. 3; GNX, GNX + E_2 and GNX + TM in Exp. 4; sham-operated, GNX and GNX + TM for each sex (3 x 2) in Exp. 5. When the *F*-value in ANOVA was significant, the Duncan's multiple range test was used to compare individual means. A Pearson correlation analysis was done to calculate correlation coefficient (r) between plasma estradiol and cortisol levels or uterine weights in Exp. 4.

Ⅳ. Results

Exp. 1. ADG and gain to feed ratio were higher ($P < 0.01$) in male than in female rats over 1-w feeding period. Both genders of 4-wk-old rats fed a HCD for 1 wk showed hypercholesterolemia (501 and 484 mg/100 mL plasma for male and female, respectively), showing no difference between genders. In adult rats, however, plasma total cholesterol levels were much higher ($P < 0.01$) in female than in male rats (277 vs 157 mg/100 mL plasma) (Table 3).

Exp. 2. ADG was higher ($P < 0.01$) in sham-operated male rats than in their female counterparts, but GNX reversed the suppressed growth in females. Plasma total cholesterol levels in female rats were twice that of males when rats were fed a HCD and the ratio of HDL to LDL cholesterol level tended to be opposite to the total cholesterol level, but these gender differences seemed to disappear when rats were gonadectomized. Triacylglycerol levels were not different between genders or treatments (Table 4). GNX increased ($P < 0.05$) the crude fat contents of carcass in both genders, but decreased ($P < 0.05$) crude ash contents, whereas crude protein

contents were not different between genders or treatments (Table 5).

Table 3. Effect of gender on weight gain and plasma cholesterol levels in young and adult rats fed a hypercholesterolemic diet for 1 wk [a] - Exp. 1

Item	Young (4 wk)		Adult (11 wk)	
	Male	Female	Male	Female
Weight gain[bc], g/d	5.4[f] ± 0.24	4.7[g] ± 0.17	3.7[h] ± 0.25	2.9[i] ± 0.16
Gain/feed[bc], g/g	0.50[f] ± 0.01	0.46[g] ± 0.01	0.20[h] ± 0.01	0.18[h] ± 0.01
Rel. liver weight[b], %	4.6[f] ± 0.10	4.6[f] ± 0.10	3.5[g] ± 0.06	3.3[g] ± 0.03
TC[bde], mg/100 mL	501[f] ± 39.9	484[f] ± 49.0	157[h] ± 18.0[c]	277[g] ± 32.9

[a]Values are means with SE of 10 rats (initial body weight for males and females of young and adult rats was 52.9 and 52.9, and 249.7 and 190.9 g, respectively).

[b]Treatment effect ($P < 0.01$).

[c]Gender effect ($P < 0.01$).

[d]Gender x treatment interaction ($P < 0.05$).

[e]Total cholesterol.

[fghi]Within a row, means without a common superscript letter differ ($P < 0.01$).

Table 4. Effect of gender and gonadectomy on weight gain, feed efficiency, and plasma cholesterol levels in rats fed a hypercholesterolemic diet for 4 wk[a]- Exp. 2

Item	Sham-operated[b]		GNX[b]	
	Male	Female	Male	Female
Weight gain[cd], g/d	6.1[i] ± 0.48	3.8[j] ± 0.27	5.2[i] ± 0.35	5.6[i] ± 0.14
Gain/feed[cd], g/g	0.40[i] ± 0.02	0.30[j] ± 0.01	0.37[i] ± 0.01	0.37[i] ± 0.01
TC[e], mg/100 mL	113[j] ± 12.1	390[i] ± 56.3	177[j] ± 20.2	399[i] ± 61.2
HDL-C[f]	8.8[i] ± 3.7	32.9[j] ± 2.7	37.5[ij] ± 3.31	9.5[i] ± 8.1
LDL+VLDL-C[g]	65[j] ± 12.2	357[i] ± 57.3	140[j] ± 20.7	360[i] ± 58.7
HDL-C/VLDL-C[c]	1.2[i] ± 0.48	0.11[j] ± 0.01	0.31[j] ± 0.05	0.12[j] ± 0.02
TG[h], mg/100 mL	59.1 ± 6.41	62.3 ± 4.11	55.9 ± 4.3	52.4 ± 2.8

[a]Values are means with SE of 8 rats (the average initial body weight of males and females was 163 g and 135 g, respectively).

[b]Sham-operated or gonadectomized.

[c]Gender effect ($P < 0.01$).

[d]Gender x treatment interaction ($P < 0.01$).

[e]Total cholesterol.

[f]High density lipoprotein-cholesterol.

[g]Low density + very low density lipoprotein-cholesterol.

[h]Triacylglycerol.

[ij]Within a row, means without a common superscript letter differ ($P < 0.05$).

Exp. 3. GNX increased ($P < 0.01$) average daily gain compared to that of sham-operated female rats. Intramuscular injection of E_2 once every week markedly suppressed growth both in

GNX females and males, whereas MT administration did not significantly increase ADG in GNX rats. Gain/feed in GNX rats was decreased ($P < 0.01$) by E_2 injection compared to the other treatments. E_2 injection once every week increased ($P < 0.001$) relative uterine weight in GNX rats. Plasma cortisol levels in sham-operated females were much higher ($P < 0.01$) than in their male counterparts, but this gender difference disappeared when rats were gonadectomized. No significant differences were found in the plasma total or HDL cholesterol levels and HDL/(LDL+VLDL) cholesterol ratio between the genders or treatments when rats were fed a NCD (Table 6).

Table 5. Effect of gender and gonadectomy on body composition in rats fed a hypercholesterolemic diet for 4 wk[a]- Exp. 2

Item	Sham-operated[b]		GNX[b]	
	Male	Female	Male	Female
Crude protein, %	68.6 ± 1.6	66.8 ± 0.8	64.3 ± 0.7	66.1 ± 1.3
Crude fat, %	18.6 ± 1.5^{d}	20.2 ± 0.9^{d}	24.4 ± 0.7^{c}	21.8 ± 1.1^{cd}
Crude ash, %	13.0 ± 0.3^{cd}	14.0 ± 0.2^{c}	12.3 ± 0.5^{d}	13.5 ± 0.4^{cd}

[a]Values are means with SE of 8 rats (the average initial body weight of males and females was 163 g and 135 g, respectively).

[b]Sham-operated or gonadectomized.

[cd]Within a row, means without a common superscript letter differ ($P < 0.05$).

Table 6. Effect of gonadectomy and estradiol or testosterone injection on weight gain, feed efficiency, liver size, and plasma cholesterol and cortisol levels in rats fed a normocholsterolemic diet for 4 wk[a] - Exp. 3

	Sham-operated		GNX[b]		GNX+E_2[b]		GNX+MT[b]	
Item	Male	Female	Male	Female	Male	Female	Male	Female
Weight gain, g/d[cde]	5.6^{j} ± 0.29	3.1^{l} ± 0.43	5.8^{j} ± 0.20	4.7^{k} ± 0.19	2.3^{m} ± 0.16	1.2^{n} ± 0.11	5.6^{j} ± 0.16	5.0^{jk} ± 0.13
Gain/feed, g/g[cde]	0.30^{m} ± 0.01	0.19^{l} ± 0.01	0.29^{m} ± 0.01	0.26^{lm} ± 0.01	0.14^{k} ± 0.01	0.09^{j} ± 0.01	0.29^{m} ± 0.01	0.27^{lm} ± 0.01
Rel. liver wt, %[f]	2.6^{j} ± 0.05	2.5^{j} ± 0.06	2.6^{j} ± 0.04	2.5^{j} ± 0.03	3.4^{k} ± 0.05	3.5^{k} ± 0.09	2.4^{j} ± 0.03	2.5^{j} ± 0.04
TC, mg/100 ml[g]	124 ± 22.2	131 ± 17.1	186 ± 33.2	198 ± 24.7	166 ± 16.8	135 ± 9.6	158 ± 13.3	167 ± 22.2
HDL-C[h]	50.1 ± 9.4	53.6 ± 9.4	77.0 ± 4.2	81.0 ± 4.4	75.4 ± 11.4	84.1 ± 5.5	64.8 ± 7.0	63.9 ± 17.9
LDL+VLDL-C[i]	74 ± 15.1	77 ± 18.4	109 ± 32.9	117 ± 26.2	91 ± 27.4	51 ± 9.2	93 ± 9.5	103 ± 11.1
HDL/LDL+VLDL	0.67 ± 0.20	0.69 ± 0.44	0.70 ± 0.22	0.68 ± 0.15	0.82 ± 1.4	1.63 ± 0.43	0.69 ± 0.07	0.61 ± 0.33
Cortisol, μg/100ml	0.51^{k} ± 0.09	1.22^{j} ± 0.27	0.32^{k} ± 0.09	0.69^{k} ± 0.08				

[a]Values are means with SE of 6 rats (initial body weight for males and females was 130 and 124 g, respectively.

[b]Rats were gonadectomized and injected weekly with 17β -estradiol or 17α -methyltestosterone (7mg/kg body weight), respectively.

[c]Gender effect ($P < 0.01$). [d]Treatment effect ($P < 0.01$). [e]Gender x treatment interaction ($P < 0.01$).

[f]Relative liver weight = 100 (g liver weight/g body weight).

[g]Total cholesterol. [h]High density lipoprotein cholesterol.

[i]Low density + very low density lipoprotein cholesterol.

[jklmn]Within a row, means without a common superscript letters differ ($P < 0.05$).

Exp. 4. Intramuscular injection of E_2 once every week markedly suppressed growth in GNX female rats (4.67 vs 1.18 g/d), whereas MT injection slightly increased growth. Gain/feed was increased ($P < 0.01$) by GNX compared to the sham-operated (0.21 vs 0.15), and decreased ($P < 0.01$) by E_2 replacement (0.10 vs 0.20) in female rats. Compared with the control, E_2 injection increased plasma estradiol (18,600 vs 172 pg/100 mL) and cortisol (1.18 vs 0.59 μg/100 mL) levels measured 1 d after the injection in GNX female rats. E_2 injection once every week increased ($P < 0.001$) relative uterine weight in GNX female rats. Plasma cortisol or testosterone level measured 1 d after injection of MT was not different from the control (Table 7). A Pearson correlation analysis showed that plasma cortisol concentration ($r = 0.72$, $P < 0.01$) and uterine weight ($r = 0.84$, $P < 0.01$) were highly correlated with plasma estradiol levels (Table 8).

Exp. 5. Average daily gain was different ($P < 0.01$) between genders in sham-operated rats, but GNX increased growth in female rats compared to the sham-operated control. MT administration in a diet slightly increased weight gain in GNX females, and feed efficiency showed the same trend as the weight

gain. Plasma total cholesterol levels of female rats were twice that of males and the ratio of HDL to LDL+VLDL cholesterol level tended to be opposite to the total cholesterol level. GNX increased ($P < 0.01$) plasma cholesterol level in male rats, but had no effect ($P > 0.05$) in female rats. MT administration to GNX male and female rats decreased the plasma cholesterol level by 23 and 45%, respectively. Plasma triacylglycerol levels were not different between genders or treatments (Table 9).

Exp. 6. ADG, ADFI, gain/feed and consequently, total cholesterol intake were higher ($P < 0.01$) in males than in females. Female rats also showed hypercholesterolemia (530 vs 332 mg/100 mL plasma). Plasma total bile acid concentrations in female rats were higher than those found in males although not significant ($P = 0.08$). Fecal total bile acid excretion was much higher ($P < 0.01$) in males than females (Table 10).

Table 7. Effect of estradiol or testosterone injection on plasma sex hormone and cortisol concentrations in female rats fed a normocholesterolemic diet for 3 wk[a] - Exp.4

Item	GNX[b]	GNX + E_2[c]	GNX + MT[d]
Estradiol[e,f], pg/mL	172 ± 67[i]	18,600 ± 3,100[h]	227 ± 122[I]
Testosterone[e], ng/mL	<0.01	<0.01	<0.01
Cortisol[e,f], μg/100 mL	0.59 ± 0.17[i]	1.18 ± 0.25[h]	0.65 ± 0.14[I]
ADG, g/d	4.67 ± 0.83[h]	1.88 ± 0.43[i]	5.04 ± 0.60[h]
Gain/feed, g/g	0.20 ± 0.04[h]	0.10 ± 0.02[i]	0.22 ± 0.03[h]
Uterus weight[f,g], %	0.05 ± 0.07[i]	0.79 ± 0.28[h]	0.06 ± 0.03[i]

[a]Values are means with SE of 8 rats (initial body weight, 115 g).

[b]Gonadectomized and intamuscularly injected once every week with 0.1 mL of 20% ethanol solution.

[c]Gonadectomized and intramuscularly injected once every week with 0.1 mL of 20% ethanol solution containing 17β-estradiol (7 mg/kg body weight).

[d]Gonadectomized and intramuscularly injected once every week with 0.1 mL of 20% ethanol solution containig 17α-methyltestosterone (7 mg/kg body weight).

[e]Hormone concentrations were measured 1 d after the third weekly injection of hormones.

[f]Correlation coefficient (r) between estrogen and cortisol or uterus was 0.72 ($P < 0.01$) or 0.84 ($P < 0.01$), respectively.

[g]Relative uterine weight = 100 (g uterus/g body weight).

[hi]Within a row, means without a common superscript letter differ ($P < 0.01$).

Table 8. Correlation analysis - Exp. 4

Item	Cortisol	ADG	Uterus Weight
Estradiol	0.720**	-0.877***	0.837***
Cortisol	-	-0.758**	-

** $P < 0.01$, *** $P < 0.001$

Table 9. Effect of gender, gonadectomy or 17α-methyltestosterone administration on the growth and plasma cholesterol levels in rats fed a hypercholesterolemic diet for 4 wk [a] - Exp. 5

Item	Sham-operated[b]		GNX[c]		GNX+MT[d]	
	Male	Female	Male	Female	Male	Female
ADG[e,f,g], g	7.5^{l} ± 0.36	3.7^{n} ± 0.32	5.8^{m} ± 0.33	5.4^{m} ± 0.16	5.6^{m} ± 0.20	6.8^{l} ± 0.33
Gain/Feed[e,f,g], g/g	0.23^{l} ± 0.004	0.15^{n} ± 0.008	0.27^{lm} ± 0.01	0.21^{lm} ± 0.006	0.20^{m} ± 0.007	0.22^{lm} ± 0.006
TC[f,g,h]	279^{n} ± 41.3	561^{lm} ± 58.2	511^{lm} ± 75.2	601^{l} ± 81.2	395^{lmn} ± 50.2	330^{mn} ± 51.2
HDL-C[f,i]	96^{o} ± 11.2	135^{lmn} ± 7.4	147^{lm} ± 17.3	161^{l} ± 11.3	114^{mno} ± 14.3	103^{no} ± 10.3
LDL-C[f,g,j]	183^{n} ± 31.4	425^{l} ± 53.3	364^{lm} ± 62.4	440^{l} ± 71.1	281^{lm} ± 46.4	220^{n} ± 44.1
HDL-/LDL-C	0.55 ± 0.05	0.33 ± 0.03	0.41 ± 0.06	0.37 ± 0.06	0.41 ± 0.08	0.47 ± 0.04
TG[k]	44.2 ± 3.2	58.1 ± 12.1	51.5 ± 6.3	41.2 ± 9.2	59.3 ± 8.2	61.4 ± 2.3
Testosterone, ng/mL	1.7 ± 0.44	<0.01	<0.01	<0.01	<0.01	<0.01

[a]Values are means with SE of 6 rats fed a hypercholesterolemic diet for 4 wk (average initial body weight of males and females was 165 g and 135 g, respectively).

[b]Sham-operated control.

[c]Gonadectomized.

[d]Gonadectomized and fed a diet containing 17α-methyltestosterone (100 mg/kg).

[e]Gender effect ($P < 0.01$).

[f]Treatment effect ($P < 0.01$).

[g]Gender x treatment interaction ($P < 0.01$).

[h]Total cholesterol, mg/100 mL.

[i]High density lipoprotein-cholesterol, mg/100 mL.

[j]Low density + very low density lipoprotein-cholesterol, mg/100 mL.

[k]Triacylglycerol, mg/100 mL.

[lmno]Within a row, means without a common superscript letters differ ($P < 0.05$).

Exp. 7. GNX increased ADG ($P < 0.04$) compared with that of sham-operated female pigs during the growing-finishing period, but had no effect ($P > 0.44$) on feed efficiency. Back fat thickness and dressing percentage tended to be higher in GNX females than in sham-operated females (Table 11).

Table 10. Effect of gender on plasma cholesterol levels and bile acids excretion in rats fed a hypercholesterolemic diet for 4 wk[a] - Exp. 6

Item	Male	Female
ADFI, g	21.4 ± 0.57[c]	15.3 ± 0.92
ADG, G	7.2 ± 0.33[c]	3.5 ± 0.20
Gain/feed, g/g	0.33 ± 0.009[c]	0.23 ± 0.006
Total cholesterol input, g/28d	3.0 ± 0.07[c]	2.1 ± 0.41
Rel. cholesterol input[b]	0.78 ± 0.007	0.82 ± 0.029
Total cholesterol, mg/dL	332 ± 23.0[c]	530 ± 9.1
Plasma bile acids, μmol/L	19.9 ± 6.1	39.9 ± 8.1
Fecal bile acids, μg		
Per g feces	1488 ± 73.1	1287 ± 104
Per total feces	15211 ± 1255[c]	8911 ± 924

[a]Values are means with SE of 10 rats (initial body weight for males and females was 177.7 and 154.4 g both at 5 wk of age, respectively).

[b]Relative cholesterol intake = 100 (g total cholesterol intake/g final body weight).

[c]Mean values differ between genders according to the student *t*-test ($P < 0.01$).

Exp. 8. There were no significant differences in the plasma total or HDL cholesterol levels and HDL/(LDL+VLDL) ratio

before feeding a HCD between genders or treatments. However, when they were fed a HCD for 10 d, plasma cholesterol levels were much higher in sham-operated females than in their male counterparts (161 vs 104 mg/100 mL plasma). GNX significantly increased the plasma cholesterol level in male but not in female pigs. HDL/(LDL+VLDL) ratio appeared to be higher in males than in females, and was not influenced by GNX in both male and female pigs (Table 12).

Table 11. Effect of gonadectomy on daily weight gain and feed efficiency in female pigs[a] - Exp. 7

Item	Sham-operated[b]	GNX[b]
Initial BW, kg	26.1 ± 1.6	26.4 ± 1.8
ADFI, kg	2.4 ± 0.05	2.6 ± 0.09
ADG, kg	0.88 ± 0.03[c]	1.01 ± 0.04
Gain/feed	0.37 ± 0.02	0.39 ± 0.03
Carcass traits		
Back fat thickness[3], mm	20.4 ± 3.7	23.6 ± 4.4
Dressing percentage	71.2 ± 1.6	73.2 ± 1.4

[a]Values are means with SE of 5 pigs.

[b]Sham-oprated or gonadectomized at 10 d of age and group-fed until the experiment began at 26 kg of body weight.

[c]Mean values differ between sham-operated and GNX groups according to the student *t*-test ($P < 0.05$).

Table 12. Effect of gender and gonadectomy on plasma cholesterol levels in adult pigs fed a hypercholesterolemic diet for 10 d[a] - Exp. 8

Item	Sham-operated[b]		GNX[b]	
	Male	Female	Male	Female
Feed intake, kg/d	3.60 ± 0.25	3.06 ± 0.16	3.12 ± 0.11	2.89 ± 0.07
TC[c]				
Before feeding C	97 ± 3.5	111 ± 5.0	105 ± 5.1	107 ± 3.6
After feeding C[f]	104[h] ± 9.6	161[g] ± 12.6	136[gh] ± 10.1	160[g] ± 11.6
HDL-C[d]				
Before feeding C	44.4 ± 1.4	44.0 ± 1.5	43.0 ± 2.1	38.8 ± 2.8
After feeding C	44.5 ± 6.8	38.2 ± 5.1	49.8 ± 3.6	41.8 ± 2.0
LDL+VLDL-C[e]				
Before feeding C	52.4 ± 4.9	67.0 ± 6.2	62.0 ± 4.5	68.2 ± 5.2
After feeding C[f]	59[h] ± 11.7	122[g] ± 14.6	86[gh] ± 8.9	118[g] ± 11.5
HDL/(LDL+VLDL)				
Before feeding C	0.89 ± 0.11	0.68 ± 0.08	0.70 ± 0.05	0.59 ± 0.09
After feeding C[f]	0.74[h] ± 0.07	0.34[g] ± 0.04	0.57[gh] ± 0.03	0.37[g] ± 0.02

[a]Values are means with SE of 5 pigs (the average initial body weight, 100.7 kg).

[b]Pigs were sham-operated or gonadectomized at 10 d of age and fed a diet without supplementary cholesterol and cholate before the hypercholesterolemic diet was fed for 10 d.

[c]Total cholesterol, mg/100 mL.

[d]High density lipoprotein-cholesterol, mg/100 mL.

[e]Low density + very low density lipoprotein-cholesterol, mg/100 mL.

[f]Gender effect ($P < 0.01$).

[gh]Within a row, means without a common superscript letter differ ($P < 0.01$).

V . Discussion

1. The effects of gender, gonadectomy and sex hormones on the growth.

The present studies clearly demonstrated that suppressed growth in female animals is mostly due to their ovarian activity or estrogen secretion. Ovariectomy increased weight gain in female pigs and rats, but estradiol replacement in GNX female rats markedly suppressed growth, while elevating plasma cortisol levels (Tables 6 and 7). Similar results have been reported by others (Tarttelin and Gorski, 1973; Wallen et al., 2001) showing increased growth by ovariectomy, but suppressed growth by estradiol replacement in female rats.

Plasma cortisol concentration was highly correlated with plasma estradiol level ($r = 0.72$, $P < 0.01$). Different from estradiol, 17α-methyltestosterone injection had no effect on cortisol or testosterone concentrations in the plasma sampled 1 d after the injection (Table 7). Considering that the half-life of testosterone is much shorter than that of estradiol and that testosterone was

measured 1 d after 17α-methyltestosterone was injected, it is not surprising that no significant amounts of testosterone were detected in GNX female rats.

Female rats are known to show greater adrenocorticotropin (ACTH) and corticosterone responses to stress and also have higher basal levels of corticosterone than do male rats (Kitay, 1961; Critchlow et al., 1963; Le Mevel et al., 1979). This gender difference was abolished by ovariectomy and was reinstated by estradiol administration (Le Mevel et al., 1979). Estradiol implant in GNX males elicited a female pattern of plasma cortisol level in the nonhuman primate macaques (Norman et. al., 1992).

In healthy men, short-term treatment with estradiol led to enhanced hypothalamic-pituitary-adrenal (HPA) and sympathetic responsiveness to psychological stress, resulting in increased ACTH, cortisol and norepinephrine concentrations in the saliva compared to the placebo (Kirschbaum et al., 1996). Burgess and Handa (1992) showed that corticosterone and ACTH levels after a 5-second footshock stress with 1-mamp current were much higher and maintained for a prolonged time when ovariectomized rats were administered with estradiol. However, estrogen has been reported

to have both stimulatory and inhibitory effects on HPA functions, depending on the time after ovariectomy and different doses of estradiol (Luber et al., 1991; Redei et al., 1994). Cortisol as well as catecholaminergic responses to stress have also been known to vary with estrous cycle in women (Marinari, 1976) and in rats (Viau and Meaney, 1991).

Together, these data and others' indicate that estrogen induces the secretion of hormones that increase with stress, in turn resulting in growth suppression. These hormones likely change animal's energy use toward catabolic direction. This hypothesis appeared to be evident because gain/feed was increased by GNX and decreased by estradiol replacement in female rats (Tables 6 and 7). Although gain/feed was not significantly increased in female pigs, the average gain/feed and ADG were improved 5% (0.39 vs 0.37) and 15% (1.01 vs 0.88) by ovariectomy, respectively (Table 11). Therefore, ovariectomy appears to be an economically viable practice to improve growth and feed utilization of female farm animals. In fact, ovariectomy has long been practiced by pig farmers in some areas of China as a means of improving growth and pork quality (Shu-tang Feng, email: xmskyczy@public3.bta.net.cn,

personal communication). In contrast to estrogen, testosterone is known to have anabolic effect in rats (Hagar and Kalkhoff, 1989). Our current study showed that MT administration increased ($P < 0.01$) ADG in GNX female rats, but not in GNX male rats (Table 9). Our data indicate that estrogen is a hormone that increases metabolic rates and consequently depletes body energy reserves that may otherwise be used for weight gain.

2. The effects of gender, gonadectomy and sex hormones on the cholesterol metabolism.

Studies have shown marked gender differences in plasma cholesterol levels in Sprague Dawley rats (Lee et al., 1999) and in guinea pigs (Fernandez et al., 1995) when animals were fed hypercholesterolemic diets. We speculated that ovarian activities might be responsible for the hypercholesterolemic response to dietary cholesterol in female animals. Unexpectedly, our experiments showed that: 1) ovariectomy or estradiol replacement had no effect on plasma cholesterol levels in adult female rats fed either normo- or hypercholesterolemic diets; and 2) young rats (28 d

of age) fed a HCD for 1 wk had hypercholesterolemia in both male (501 mg/100 mL plasma) and female (484). These findings clearly suggest that the gender difference in cholesterol metabolism does not appear before sexual maturity and is not related to ovarian activities.

Our next question was on the effect of testis or testosterone on cholesterol metabolism and we used the rat and pig as model animals to find that testosterone secreted by males is responsible for the gender difference in plasma cholesterol level. GNX of male rats increased the plasma cholesterol level to that of females and MT treatment markedly decreased the cholesterol level of GNX male (23%) and female (45%) rats (Table 9), clearly demonstrating that testosterone suppresses plasma cholesterol level in animals fed hypercholesterolemic diets. However, our pig study showed that differences in plasma cholesterol level between male and female (104 vs 161 mg/100 mL plasma) or between sham-operated and GNX male pigs (104 vs 136) were less drastic compared to those found in rat studies (279 vs 561 and 279 vs 511, respectively). The difference in the magnitude of plasma cholesterol level between the two species may be due to the different length of time when hypercholesterolemic diets were fed (28 d for rat study vs 10 d for

pig study), the different type of diet or interspecies variation in cholesterol metabolism. Strain-specific responses to hypercholesterolemic diets have been shown in plasma and liver cholesterol levels in rats (Bottger et al., 1996).

There have been conflicting reports on the role of testosterone in cholesterol metabolism; the effect differs, depending on different model animals, age, gender and the route of testosterone administration. Several studies have shown that testosterone has adverse effect on cholesterol metabolism by altering lipoprotein profiles. Total testosterone concentrations and sex hormone-binding globulin (SHBG) are significantly associated with LDL size in men (Haffner et al., 1996). Testosterone, per se, if administered at sufficiently high doses and for long duration, can significantly raise total cholesterol, triacylglycerol, LDL-C and Apo-B and, at the same time, lower HDL-C in androgenized women, indicating that male predilection for CVD may be due to adverse effects of higher androgen levels on lipid and lipoprotein profiles (Goh et al. 1995). Anderson et al. (1995) also showed that HDL-C was significantly depressed by intramuscular injection of testosterone enanthate (200 mg per week for 12 months) in men while there were no changes in

plasma total and LDL cholesterol or triacylglycerol levels during treatment.

More recently, Handa et al. (1997) demonstrated that plasma free testosterone level was associated with lower levels of HDL-C and that total estradiol was related to elevated levels of HDL-C levels in Japanese men in their early fifties, supporting that testosterone may be causally associated with atherosclerosis in men through altered lipoprotein metabolism. Male hamsters fed a HCD containing 0.05% cholesterol developed greater elevations in plasma total cholesterol and greater concentrations of plasma HDL-C, and greater development of early aortic atherosclerosis versus female hamsters (Wilson et al., 1999).

On the contrary, testosterone replacement therapy in hypogonadal and elderly men showed a beneficial effect on cardiovascular system, decreasing total cholesterol and atherogenic fraction of LDL-C without significant alterations in HDL-C levels or its subfractions HDL_2-C and HDL_3-C (Zgliczynski et al., 1996). Tchernof et al. (1997) also showed that increased testosterone levels were associated with reduced triacylglycerol and Apo B; total and LDL cholesterol concentrations; and increased HDL/total cholesterol

and HDL_2-C/HDL_3-C ratios. Hypotestosteronemia was observed in Chinese male patients with CHD, and positive correlation of plasma testosterone level with plasma HDL-C, but negative correlation with plasma Lp(a) level was found, suggesting that testosterone has a protective effect against atherosclerosis (Zhao and Li, 1998).

Further studies are needed to clarify the mechanism by which testosterone suppresses plasma cholesterol level in animals fed hypercholesterolemic diets, e.g., through regulation of all or some of the following: LDL receptor, HMG-CoA reductase and 7α-hydroxylase in the liver, possibly at the gene transcription level. Dietary cholesterol has been known to decrease the activity and mRNA of LDL receptor and HMG-CoA reductase, and increase those of 7α-hydroxylase (which is suppressed by dietary cholate) in rats (Roach et al., 1993) and mice (Dueland et al., 1993).

Estrogen administration was reported to increase the LDL-receptor mRNA and the receptor protein in Sprague Dawley rats (Srivastava et al. 1993, Parini et al. 1997). In theory, this increased receptor protein should depress cholesterol synthesis and in turn plasma cholesterol levels. However, Fernandez et al. (1995) showed that hepatic HMG-CoA reductase activity was much higher

in female than in male guinea pigs when they were fed a hypercholesterolemic diet, suggesting that females are less sensitive than males in feedback control mechanism of cholesterol synthesis. Our data also suggest likewise. Plasma cholesterol level in female rats was twice as much as that in males when rats were fed a HCD, but no gender difference was found with a normocholesterolemic diet (124 mg/100 mL plasma for male vs 131 for female).

Estrogen's involvement in lipoprotein metabolism (e.g., an increased ratio of HDL to LDL cholesterol) has also been implicated in its role in cardioprotection (Wagner et al., 1991; Campos et al., 1997). Beneficial effect of estrogen on CVD seems to be contradictory to our findings that female pigs and rats had much higher plasma cholesterol levels than males when they were fed a HCD, and ovariectomy had no significant effect on the plasma cholesterol level. If estrogen plays a role at all in reducing CVD in females, it may be through actions on other than controlling blood cholesterol levels. Naito et al. (1995) indicated that the effect of sex hormones on lipid metabolism is not likely to account for the sex difference in CVD, on the basis of the reports: 1) men with premature myocardial infarction was shown to have increased

estrogen levels (Phillips, 1976), and 2) men who had received high doses of estrogen showed an increased frequency of cardiovascular events (Veterans Administration Cooperative Urological Research Group, 1967; Coronary Drug Project Research Group, 1976).

Estrogen may partly contribute to cardioprotection through enhancing the secretion of hormones that increase metabolic activities, as exercise plays a role in cardioprotection (Lindheim et al., 1994). The proposed hypothesis in the present study may be further supported by an increased relative liver weight by estrogen injection in GNX rats, both male and female rats in this study. A study done by Srivastava et al. (1993) with male Sprague Dawley rats fed a normocholesterolemic diet showed that subcutaneous injection of estradiol (5 mg/kg body weight daily for 5 d) significantly increased relative liver weight (1.5 times the placebo control), decreased growth (to 85% of the control), and plasma total cholesterol (to 6%) and triacylglycerol (to 59%). Antioxidant activity of estrogen has been suggested us its role in cardioprotection (Walsh et al., 1999), although no inhibitory action on LDL oxidation was found at physiological concentrations (Santanam et al., 1998).

Overall results of these studies indicate that ovarian activity

suppresses growth in female animals and thus ovariectomy (or inhibition of estrogen secretion) can be an economically viable method to improve growth and possibly feed efficiency in female pigs, but we need to develop a simple method of neutering female pigs either through ovariectomy or nullifying the ovarian activity. Testis (testosterone) is responsible for the lower plasma cholesterol level in male than in female animals with hypercholesterolema. Further studies are required to clarify what stage of cholesterol metabolism testosterone is involved in; i.e., depression of cholesterol synthesis (e.g., HMG-CoA reductase gene expression), or enhancement of cholesterol oxidation and excretion (e.g., cholesterol 7α -hydroxylase gene expression).

Ⅵ. Literature cited

AIN (American Institute of Nutrition). 1976. Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies. J. Nutr. 107:1340-1348.

Anderson, R. A., E. M. Wallace, and F. C. W. Wu. 1995. Effect of testosterone enanthate on serum lipoproteins in man. Contraception 52:115-119.

Arjmandi, B. H., D. A. Khan, S. S. Juma, and A. Svanborg. 1997. The ovarian hormone deficiency-induced hypercholesterolemia is reversed by soy protein and the synthetic isoflavone, ipriflavone. Nutr. Res. 17:885-894.

Barrett-Connor, E., and T. L. Bush. 1991. Estrogen and coronary heart disease in women. J. Am. Med. Assoc. 265:1861-1867.

Bottger, A., M. den Bieman, Æ. Lankhorst, H. A. van Lith, and L. F. M. van Zutphen. 1996. Strain-specific response to hypercholesterollaemic diets in the rat. Lab. Anim. 30:149-157.

Burgess, L. H., and R. J. Handa. 1992. Chronic estrogen-induced alterations in adrenocorticotropin and corticosterone

secretion, and glucocorticoid receptor-mediated functions in female rats. Endocrinol. 131:1261-1269.

Campos, H., B. W. Walsh, H. Judge, and F. M. Sacks. 1997. Effect of estrogen on very low density lipoprotein and low density lipoprotein subclass metabolism in postmenopausal women. J. Clin. Endocrinol. Metab. 82:3955-3963.

Carey, M. P., C. H. Deterd, J. De Koning, F. Helmerhorst, and E. R. De Kloet. 1995. The influence of ovarian steroids on hypothalamic-pituitary-adrenal regulation in the female rat. J. Endocrinol. 144:311-321.

Chen W-J. L., F. W. Hays, and J. R. Overfield. 1970. Effects of dietary ascorbic acid on performance and plasma cholesterol levels of growing swine. J. Anim. Sci. 31:63-66.

Collins, A., P. Eneroth, and B. M. Landgren. 1985. Psyconeuroendocrine stress responses and mood as related to the menstrual cycle. Psychosom. Med. 47:512-527.

Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, Savoy, IL.

Coronary Drug Project Research Group. 1976. The coronary

drug project: initial findings leading to modifications of research protocol. J. Am. Med. Assoc. 214:1303-1313.

Critchlow, V., R. A. Liebelt, M. Bar-Sela, W. Mountcastle, and H. S. Lipscomb. 1963. Sex difference in resting pituitary-adrenal function in the rat. Am. J. Physiol. 205:807-815.

De Deckere, E. A. M., W. J. Kloots, and J. M. M. Van Amelsvoort. 1993. Resistant starch decreases serum total cholesterol and triacylglycerol concentrations in rats. J. Nutr. 123:2142-2151.

Fernandez, M. L., D-M. Sun, M. A. Tosca, and D. J. Mcnamara. 1994. Citrus pectin and cholesterol interact to regulate hepatic cholesterol homeostasis and lipoprotein metabolism: a dose response study in guinea pigs. Am. J. Clin. Nutr. 59: 869-878.

Fernandez, M. L., M. Vergara-Jimenez, A. L. Romero, S. K. Erickson, and D. J. McNamara. 1995. Gender differences in response to dietary soluble fiber in guinea pigs: effects of pectin, guar gum, and psyllium. J. Lipid Res. 36:2191-2202.

Goh, H. H., D. F. M. Loke, and S. S. Ratnam. 1995. The impact of long-term testosterone replacement therapy on lipid and lipoprotein profiles in women. Maturitas 21:65-70.

Haffner, S. M., M. Laakso, H. Miettinen, L. Mykkanen, P.

Karhapaa, and D. L. Rainwater. 1996. Low levels of sex hormone-binding globulin and testosterone are associated with smaller, dencer low density lipoprotein in normoglycemic men. J. Clin. Endocrinol. Metab. 81:3697-3701.

Hager, S. R., and P. K. Kalkhoff. 1989. Effects of parenteral testosterone nutrition on insulin action in perfused hindlimb muscle of female rats. Metabolism 38:431-434.

Handa, K., H. Ishii, S. Kono, K. Shinchi, K. Imanishi, H. Mihara, and K. Tanaka. 1997. Behaviocular stress responses: modulation as a function of menstrual cycle phases. J. Psychosom. Res. 28:475-483.

Hastrup, J. L., and K. C. Light. 1984. Sex Differences in Cardiovascular Stress Responses: Modulation as a Function of Menstrual Cycle Phases, J. Psychosom. Res. 28:475-483.

Kirschbaum, C., N. Schommer, I. Federenko, J. Gaab, O. Neumann, M. Oellers, N. Rohleder, O. Untiedt, J. Hanker, K. M. Pirke, and D. H. Hellhammer. 1996. Short-term estradiol treatment enhances pituitary-adrenal axis and sympathetic responses to psychosocial stress in healthy young men. J. Clin. Endocrinol. Metab. 81:3639-3443.

Kitay, J. I. 1961. Sex differences in adrenal cortical secretion in the rat. Endocrinol. 68:818-824.

Kitay, J. I. 1963. Pituitary-adrenal function in the rat after gonadectomy and gonadal hormone replacement. Endocrinol. 73:253-260.

Lee, C. E., D. H. Koh, Y. M. Chang, and K. I. Kim. 1999. Effects of sex and diets containing alfalfa, laver or pine-needle meals on the plasma cholesterol level in rats. Nutritional Sciences (Korean Society of Nutritional Sciences) 2:46-50.

Le Mevel, J. C., S. Abitbol, G. Beraud, and J. Maniey. 1979. Temporal changes in plasma adrenocorticotropin concentration after repeated neurotropic stress in male and female rats. Endocrinol. 105:812-827.

Lesniewska, B., B. Miskowiak, M. Nowak, and L. K. Malendowicz. 1990. Sex differences in adrenocortical structure and function. XXVII. The effect of ether stress on ACTH and corticosterone in intact, gonadectomized, and testosterone- or estradiol-replaced rats. Res. Exp. Med. 190:95-103.

Lindheim, S. R., M. Notelovitz, E. B. Feldman, S. Larson, F. Y. Khan, and R. A. Lovo. 1994. The independent effects of exercise

and estrogen on lipids and lipoproteins in postmenopausal women. Obstet. Gynecol. 83:167-172.

Luber, A. H., C. V. Mobbs, M. Muramatsu, and D. W. Pfaff. 1991. Estrogen receptor messenger RNA expression in rat hypothalamus as a function of genetic sex and estrogen dose. Endocrinol. 129:3180-3186.

Lundeen, S. G., J. M. Carver, M. L. McKean, and R. C. Winneker. 1997. Characterization of the ovariectomized rat model for the evaluation of estrogen effects on plasma cholesterol levels. Endocrinol. 138:1552-1558.

Manhem, K., L. Hansson, I. Milsom, M. Pilhall, and S. Jern. 1996. Estrogen and progestagen modify the hemodynamic response to mental stress in young women. Acta Obstet. Gynecol. Scand. 75:57-62.

Marinari, K. T., A. I. Leshner, and M. P. Doyle. 1976. Menstrual cycle status and adrenocortical reactivity to stress. Psychoneuroendocrinol. 1:213-218.

Marsh, M. M., V. R. Walker, L. K. Curtiss, and C. L. Banka. 1999. Prptection against atherosclerosis by estrogen is independent of plasma cholesterol levles in LDL receptor-deficient mice. J. Lipid

Res. 40: 893-900.

Munilla, M. A., and E. Herrera. 1997. A cholesterol-rich diet causes a greater hypercholesterolemic response in pregnant than in nonpregnant rats and does not modify fetal lipoprotein profile. J. Nutr. 127: 2239-2245.

Naito, M., T. Hayashi, and A. Iguchi. 1995. New approaches to the prevention of atherosclerosis. Drugs 50:440-453.

National Research Council. 1985. Guide for the Care and Use of Laboratory Animals. Publication no. 85-23 (rev.), National Institute of Health, Bethesda, MD.

Norman, R. L., C. J. Smith, J. D. Pappas, and J. Hall. 1992. Exposure to ovarian steroids elicits a female pattern of plasma cortisol levels in castrated male macaques. Steroids 57:37-43.

Parini, P., B. Angelin, and M. Rudling. 1997. Importance of estrogen receptors in hepatic LDL receptor regulation. Arteriosclerosis Thrombosis Vascular Biol. 17:1800-1805.

Park, J. R., and B. H. S. Cho. 1988. Changes in plasma lipids, lipoproteins, triglyceride secretion and removal in chicks with estrogen implants. Lipids 23:329-333.

Phillips, G. B. 1976. Evidence for hyperestrogenemia as a

risk factor for myocardial infarction in men. Lancet 2:14-18.

Phillips, J. G., and W. Poolsanguan. 1978. A method to study temporal changes in adrenal activity in relation to sexual status in the female laboratory rat. Endocrinol. 77:283-291.

Redei, E., L. Li, I. Halasz, R. F. McGivern, and F. Aird. 1994. Fast glucocorticoid feedback inhibition of ACTH secretion in the ovariectomized rat: Effect of chronic estrogen and progesterone. Neuroendocrinol. 60:113-123.

Roach, P. D., S. Balasubramaniam, F. Hirata, M. Abbey, A. Szanto, L. A. Simons, and P. J. Nestel. 1993. The low-density lipoprotein receptor and cholesterol synthesis are affected differently by dietary cholesterol in the rat. Biochim. Biophys. Acta 1170:165-172.

Santanam, N., R. Shern-Brewer, R. McClatchey, P. Z. Castellano, A. A. Murphy, S. Voelkel, and S. Parthasarathy. 1998. Estradiol as an antioxidant: incompatible with its physiological concentrations and function. J. Lipid Res. 39:2111-2118.

Smith, C. J., and R. L. Norman. 1987. Influence of the gonads on cortisol secretion in female rhesus macaques. Endocrinol. 121:2192-2198.

Srivastava, R. A. K., D. Bauman, and G. Schonfeld. 1993. In vivo regulation of low-density lipoprotein receptors by estrogen differs at the post-transcriptional level in rat and mouse. Eur. J. Biochem. 216:527-538.

Sudhir, K., M. D. Esler, G. L. Jennings, and P. A. Komesaroff. 1997. Estrogen supplementation decreases norepinephrine-induced vasoconstriction and total body norepinephrine spillover in perimenopausal women. Hypertension 30:1538-1543.

Tarttelin, M. F., and R. A. Gorski. 1973. The effects of ovarian steroids on food and water intake and body weight in the female rat. Acta Endocrinol. 72:551-568.

Tchernof, A., F. Labrie, A. Belanger, D. Prud'homme, C. Bouchard, A. Tremblay, A. Nadeau, and J-P. Despres. 1997. Relationship between endogenous steroid hormone, sex hormone-binding globulin and lipoprotein levels in men: contribution of visceral obesity, insulin levels and other metabolic variables. Atherosclerosis 133:235-244.

Veterans Administration Cooperative Urological Research Group. 1967. Treatment and survival of patients with cancer of the prostate. Surg. Gynecol. Obstetr. 124:1101-1017.

Viau, V., and M. J. Meaney. 1991. Variations in the hypothalamic-pituitary-adrenal response to stress during the estrous cycle. Endocrinol. 129:2503-2511.

Wagner, J. D., T. B. Clarkson, R. W. St Clair, D. C. Schwenke, C. A. Shively, and M. R. Adams. 1991. Estrogen and progesterone replacement therapy reduces low-density lipoprotein accumulation in the coronary arteries of surgically postmenopausal cynomonkeys. J. Clin. Invest. 88:1995-2002.

Wagner, W. D., and L. L. Jackson. 1983. Phytoestrogen from pinus ponderosa assayed by competitive binding with 17β -estradiol to mouse uterine cytosol. Theriogenology. 19:507.

Wallen, W. J., M. P. Belanger, and C. Wittnich. 2001. Sex hormones and the selective estrogen receptor modulator tamoxifen modulate weekly body weights and food intakes in adolescent and adult rats. J. Nutr. 131:2351-2357.

Walsh, B. A., A. E. Mullick, R. L. Walgem, and J. C. Rutrige. 1999. 17beta-estradiol reduces tumor necrosis factor-alfa-mediated LDL accumulation in the artery wall. J. Lipid Res. 40:387-396

Wilson, P. W. P., R. J. Garrison, and W. P. Castelli. 1985. Postmenopausal estrogen use, cigarette smoking, and cardiovascular

morbidity in women over 50: Framingham Study. New Engl. J. Med. 313:1038-1043.

Wilson, T. A., R. J. Nicolosi, C. W. Lawton, and J. Babiak. 1999. Gender differences in response to a hypercholesterolemic diet in hamsters: effects on plasma lipoprotein cholesterol concentrations and aortic atherosclerosis. Atherosclerosis. 146:83-91.

Zgkiczynsky, S., M. Ossowski, J. Slowinska-Srzednicka, A. Brzezinska, W. Zgliczynski, P. Soszynski, E. Chotkowska, M. Srzednicki, and Z. Sadowski. 1996. Effect of testosterone replacement therapy on lipids and lipoproteins in hypogonadal and elderly men. Atherosclerosis 121: 35-43.

Zhao, S-P., and X-P. Li. 1998. The association of low plasma testosterone level with coronary artery disease in Chinese men. International Journal of Cardiology 63:161-164.

要　　約

性, 生殖腺 摘出 및 性 호르몬이 成長 및 콜레스테롤 代謝에 미치는 影響을 究明하기 위하여 쥐와 돼지를 대상으로 總 8 번의 試驗을 實施하였다. 試驗 1 에서는 性 成熟 前과 後에 암 · 수 間 콜레스테롤 代謝 및 成長 差異를 究明하기 위하여 어린 쥐(3 주령) 20 마리와 성쥐(10 주령) 20 마리를 性別 또는 나이별로 要因配置(2 x 2) 하여 高콜레스테롤飼料(0.5% cholesterol + 0.2% cholate)를 1 주 동안 給與하였다. 試驗 2 에서는 性 또는 生殖腺 摘出(Gender or GNX)이 成長 및 콜레스테롤 代謝와의 關係를 究明하기 위하여 35 일령 수쥐(163 g) 16 마리와 암쥐(135 g) 16 마리를 GNX 與否 및 性別로 要因配置(2 x 2) 하여 高콜레스테롤飼料를 4 주 동안 給與하였다. 試驗 3 에서는 性 호르몬이 成長 및 콜레스테롤 代謝에 미치는 影響을 確認코자 수쥐 (130 g) 24 마리와 암쥐(124 g) 24 마리를 이용하여 암수별 對照區, GNX, GNX + 17β-estradiol (E_2) 및 GNX + 17α-methyltestosterone(MT) 區로 要因配置(4 x 2) 하여 一般飼料를 4 주 동안 給與하면서 각각의 호르몬을 1 주 1 회 체중 kg 당 7 mg 씩 筋肉注射 하였다. 試驗 4 에서는 試驗 2 와 3 의 結果를 再 確認하고 호르몬과 cortisol 및 子宮 무게 등과의 關係를 究明코자 4 주령 24 마리의 卵巢 摘出한 암쥐(115 g)를 GNX, GNX + E_2 및 GNX + MT 區로 完全任意配置하여 一般飼料를

3 주 동안 給與하면서 각각의 호른몬을 試驗 3 에서와 같이 投與하였다. 試驗 5 에서는 testosterone 과 콜레스테롤 代謝와의 關係를 再確認하기 위하여 4 주령 수쥐(163 g) 18 마리와 암쥐(135 g) 18 마리를 암수별로 對照區, GNX, GNX + MT 區로 要因配置(3 x 2) 하여 高콜레스테롤飼料를 給與 함과 동시에 MT 處理區는 飼料 kg 당 17α-methyltestosterone 100 mg 씩 4 주 동안 투여하였다. 試驗 6 에서는 암 · 수 間 bile acids 分泌에 차이가 있는지를 究明하기 위하여 5 주령 10 마리의 수쥐(177 g)와 10 마리의 암쥐(154 g)를 4 주동안 高콜레스테롤飼料를 給與하였다. 試驗 7 에서는 卵巢 摘出이 돼지의 成長과 屠體特性에 미치는 影響을 究明코자 암퇘지(26kg, Landrace) 10 마리를 각각 對照區와 GNX 區로 處理하여 70 일 동안 成長 試驗을 實施하였다. 試驗 8 에서는 돼지를 이용하여 性 또는 生殖腺 摘出이 콜레스테롤 代謝에 미치는 影響을 再 確認코자 암(98 kg) · 수(104 kg) 각각 10 마리를 이용하여 암수별 對照區와 GNX 區로 處理하여 高콜레스테롤飼料를 10 일 동안 給與 하였다.

高콜레스테롤飼料를 給與할 때 어린 쥐에서는 암 · 수 모두 高콜레스테롤血症을 보였으나 성쥐에서는 암쥐가 수쥐보다 콜레스테롤 濃度가 높게($P < 0.01$) 나타났다(試驗 1). 試驗 2 의 結果 암쥐는 수쥐에 비해 日當增體量과 飼料效率이 각각 60% 와 65%에 지나지 않았으나 生殖腺 摘出 암쥐는 生殖腺 摘出 수쥐와 成長에 있어 差異를 보이지

않았다. 또한 高콜레스테롤飼料를 給與했을 때 암쥐가 수쥐에 비해 2 倍 이상의 血 中 콜레스테롤 濃度를 보였다($P < 0.01$). 生殖腺 摘出 쥐에 E_2 의 投與는 成長率을 減少시켰으나 MT 의 投與는 약간 增加시키는 傾向을 보였고 血 中 cortisol 濃度는 암쥐에서 높게 ($P < 0.01$) 나타났으며 이런 性別 差異는 生殖腺 摘出한 쥐에서는 나타나지 않았다. 一般飼料를 給與한 쥐에서는 암 · 수 間 血 中 콜레스테롤 濃度는 差異가 없었다(試驗 3). 또한 E_2 의 投與는 生殖腺 摘出한 암쥐에서 血 中 cortisol 濃度를 100%까지 增加 시켰으며 子宮 무게 또한 크게 增加 시켰다. 또한 血 中 estradiol 濃度와 cortisol 및 子宮 무게와 有意的으로($P < 0.01$) 높은 相關關係(r = 0.72)를 보였다(試驗 4). 高콜레스테롤飼料를 給與할 때 수쥐의 生殖腺 摘出은 血 中 콜레스테롤 濃度를 크게 增加 시켰으나($P < 0.01$) MT 의 투여는 生殖腺 摘出한 수쥐에서 23%, 암쥐에서는 45%까지 血 中 콜레스테롤 濃度를 크게 減少시켰다(試驗 5). 高콜레스테롤飼料를 給與하는 상태에서 血 中 總 bile acids 濃度는 有意的 差異는 없었지만(P = 0.08) 암쥐가 높게 나타났고, 분 중 총 bile acids 濃度는 수쥐가 높게($P < 0.01$) 나타났다(試驗 6). 돼지 試驗에서도 生殖腺 摘出은 암퇘지의 成長率을 向上시켰으나($P < 0.01$) 屠體特性 등은 處理 間 差異를 보이지 않았다(試驗 7). 또한 돼지에 高콜레스테롤飼料를 給與했을 때도 쥐 試驗에서와 같이 암퇘지가 血 中 콜레스테롤 濃度가 높게

나타났으며($P < 0.05$), 수퇘지에서의 生殖腺 摘出은 血 中 콜레스테롤 濃度를 增加 시켰다(試驗 8).

本 硏究 結果 estrogen 은 成長率과 飼料效率을 크게 減少시키는데 그 原因은 cortisol 과 같은 스트레스 호르몬의 分泌를 增加시키기 때문으로 思料된다. 따라서 卵巢 摘出은 estrogen 의 分泌를 根本的으로 遮斷하기 때문에 家畜에서 成長率 과 飼料效率을 向上시키는 效率的인 方法으로 利用될 수 있을 것으로 보인다. 또한 testosterone 은 高콜레스테롤血症을 가지고 있는 動物에 있어 血 中 콜레스테롤 濃度를 減少시킨다.

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