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Effect of Ovary(Estrogen) on Growth and Cholesterol Metabolism in Female Animals

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ABSTRACT

Studies were conducted to determine the effect of gender, gonadectomy (GDX), or sex hormones on growth and cholesterol metabolism in rats. Young adult(130~160g) intact males and females, GDX males and females, with or without 17β-estradiol or 17α-methyltestosterone administration, were fed a diet supplemented with 0.5% cholesterol plus 0.2% cholate(cholesterol diet, CD) or a commercial chow(normal diet, ND) for four week. Weight gain, feed efficiency, and plasma cholesterol, cortisol, estrogen and testosterone levels were determined. Average daily gain of females was about 60% of that of intact males over a 4-wk feeding period, but the average daily gain of GDX females was almost equal to that of males. Weekly estradiol injection(7mg/kg body weight) depressed average daily gain by 60 and 75% in GDX male and female rats, respectively. Plasma cholesterol levels were 2~3 times higher in females than in males when rats were fed CD, but no gender difference was found when they were fed ND. The gender difference in plasma cholesterol levels was persistent even in GDX rats. The average plasma cortisol level of females was twice that of males(1.22 vs 0.51μg/100mL) but the gender difference disappeared when females were ovariectomized(0.69μg/100mL), and estrogen replacement increased the cortisol level back to that of intact females(1.18μg/100mL). The plasma estrogen level was found to be highly correlated with the plasma cortisol level($r=0.72$, $P<0.01$). Results indicate that estrogen depresses growth, perhaps through inducing the secretion of hormones that increase metabolic rates. The hypercholesterolemic response of female rats to dietary cholesterol, which was not influenced by ovariectomy, suggests that physiological events not directly related to ovary affect the sex-related cholesterol metabolism.

KEY WORDS : rats, ovariectomy, estrogen, growth, cholesterol

INTRODUCTION

For most mammals, males appear to grow faster and also are larger in mature size than their female counterparts. Sex hormones are believed to be behind the gender difference in growth rate. Early studies(Kitay 1961, 1963, Clitchlow 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 cortisol, corticosterone and adrenocorticotropin levels, 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).

In the Framingham study, the risk of cardiovascular disease 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). Our recent studies(Lee 1996) showed much higher plasma cholesterol level in female than in male rats when rats were fed a hypercholesterolemic diet(CD). This result raised an important question on the role of estrogen in reducing cardiovascular diseases. Naitio et al.(1995) proposed that the effect of sex hormones on lipid metabolism is not likely to account for the sex difference in cardiovascular disease. Furthermore, in the 1995 Ancel Keys lecture, Barrett-Conner(1997) indicated that plasma estrogen levles do not explain coronary heart disease risk in either sex.

To our knowledge, no studies have shown direct evidence that estrogen is responsible for the slow growth of females and that the control mechanism of cholesterol metabolism differs between genders. Therefore, we conducted a series of experiments using rats as a animal model to determine the effect of gender, gonadectomy or sex hormones on growth, feed efficiency, and plasma cholesterol and cortisol levels when they were fed either normo-or hypercholesterolemic diet.

RESULTS

1. Effect of ovary(estrogen) on growth in rats

Average daily gain was different($P<0.01$) between genders in intact rats, but

ovariectomy completely reversed the depressed growth in female rats(Tables 1 and 2). Weekly intramuscular injection of estradiol markedly depressed growth in GDX rats(5.8 vs 2.3 and 4.7 vs 1.2g/d for male and female rats, respectively), whereas testosterone injection had no effect(Table 3). Feed efficiency showed the same trend as that of weight gain. Weekly estradiol injection increased($P<0.001$) relative liver and uterus weights compared to the other groups(Tables 2 and 3). The average plasma cortisol level in females was higher than twice that found in males, but it decreased to that of males by ovaryectomy(Table 2). Injection of estradiol increased the plasma cortisol level to twice the control value in OVX females(0.59 vs 1.18μg/100mL) when it was measured 1 d after the injection(Table 3). Regression analysis showed that plasma estrogen levels were highly correlated with plasma cortisol concentration ($r=0.74$, $P<0.0001$). Plasma cortisol or testosterone levels were not increased by injected testosterone when measured 1 d after the injection.

Table 1. Effect of gender and gonadectomy on weight gain, feed efficiency, and plasma cholesterol, triacylglycerol and urea levels in rats fed a hypercholesterolemic diet for 4 weeks[1]

Item	Intact		Gonadectomized		SE	P
	Male	Female	Male	Female		
Weight gain (g/d)[2,3]	6.1[a]	3.8[b]	5.2[a]	5.6[a]	0.16	0.0001
Gain/feed (g/g)[2,3]	0.40[a]	0.30[b]	0.37[a]	0.37[a]	0.009	0.0001
TC (mg/100mL)[4]	113[b]	390[a]	177[b]	399[a]	3.4	0.0001
HDL-C (mg/100mL)[5]	48.8[a]	32.9[b]	37.5[ab]	9.5[ab]	1.55	0.0005
LDL+VLDL-C(mg/100mL)[6]	65[b]	357[a]	140[b]	360[a]	23.9	0.0001
HDL-C/LDL+VLDL-C[2]	1.26[a]	0.11[b]	0.31[b]	0.12[b]	0.07	0.0001
TG (mg/100mL)[7]	59.1	62.8	55.9	52.4	1.91	0.2400
Urea (mg/100mL)[2]	6.3[b]	8.1[a]	7.1[ab]	7.5[ab]	0.19	0.0005

[1] Values are means with pooled standard errors(SE) of 8 rats of the same age(initial body weight for males and females was 163 and 134g, respectively).
[2] Gender effect($P<0.01$).
[3] Gender x treatment interaction($P<0.01$).
[4] Total cholesterol.
[5] High density lipoprotein-cholesterol.
[6] Low density + very low density lipoprotein cholesterol.
[7] Triacylglycerol.
[ab] Values in the same row with no common superscripts differ($P<0.05$) and those without superscripts do not differ($P>0.05$).

Table 2. Effect of gender, gonadoectomy and gonadectomy plus weekly injection of estradiol or testosterone on weight gain, feed efficiency, liver size, and plasma cholesterol and cortisol levels in rats fed a commercial diet for 4 weeks[1].

Item	Intact		Gonadectomized		GDX+Estradiol[2]		GDX+Testosterone[2]		SE	P
	male	female	male	female	male	female	male	female		
Weight gain(g/d)[3,4,5]	5.6^{a}	3.1^{c}	5.8^{a}	4.7^{b}	2.3^{d}	1.2^{e}	5.6^{a}	5.0^{ab}	0.25	0.0001
Gain/feed(g/g)[3,4,5]	0.30^{d}	0.19^{c}	0.29^{d}	0.26^{cd}	0.14^{b}	0.09^{a}	0.29^{d}	0.27^{cd}	0.02	0.0001
Rel. liver wt(%)[6]	2.62^{a}	2.59^{a}	2.63^{a}	2.50^{a}	3.40^{b}	3.50^{b}	2.47^{a}	2.50^{a}	0.05	0.0001
TC(mg/100ml)[7]	124	131	186	198	166	135	158	167	7.7	0.14
HDL-C(mg/100ml)[8]	50.1	53.6	77.0	81.0	75.4	84.1	64.8	63.9	3.58	0.13
LDL+VLDL(mg/100ml)[9]	74.3	77.2	109.1	117.4	91.0	51.3	93.2	103.2	7.0	0.44
HDL-C/LDL+VLDL-C	0.67	0.69	0.70	0.68	0.82	1.63	0.69	0.61	0.21	0.26
Cortisol(μg/100ml)	0.51^{b}	1.22^{a}	0.32^{b}	0.69^{b}					0.10	0.0005

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

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

[3] Gender effecf($P<0.01$).

[4] Teatment effecf($P<0.01$).

[5] Gender x teatment interaction($P<0.01$).

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

[7] Total cholesterol.

[8] High density lipoprotein cholesterol.

[9] Low density + very low density lipoprotein choleserol.

[abc] Values in the same row with no common superscripts differ($P<0.05$) and those without superscripts do not differ($P<0.05$).

2. Effect of ovary (estrogen) on cholesterol metabolism in rats

Plasma total cholesterol levels in females were higher than thrice that of males when they were fed CD and the ratio of HDL to LDL cholesterol level tended to be opposite to the total cholesterol level(Table 1), but this gender difference disappeared when they were fed ND(Table 2). Gonadectomy or exogenous sex hormones did not influence the plasma cholesterol level(Tables 1 and 2). Plasma triacylglycerol levels were not different between genders or between treatments, but plasma urea levels increased generally with decreasing feed efficiency(Table 1), indicating that intact females consuming excessive protein compared to males or OVX females.

Table 3. Effect of estradiol or testosterone injection on plasma sex hormone and cortisol concentrations in OVX rats[1,2,3]

Item	OVX[4]	OVX+est[5]	OVX+TEST[5]	SE	P
Estradiol(pg/mL)	172[a]	18,633[b]	227[a]	1846	0.0001
Testosterone[6](ng/mL)	-	-	-		
Cortisol(μg/100mL)	0.59[a]	1.18[b]	0.65[a]	0.06	0.0001
Average daily gain(g)	4.67[a]	1.88[b]	5.04[a]	0.31	0.0001
Gain/feed(g/g)	0.20[a]	0.10[b]	0.22[a]	0.01	0.0001
Rel. uterus weight[7](%)	0.05[a]	0.79[b]	0.06[a]	0.07	0.0001

[1] Values are means with pooled standard errors(SE) of 8 female rats(initial body weight, 115g).
[2] Hormone concentrations were measured 1 d after the third weekly hormone injection.
[3] Correlation coefficient(r) between estradiol and cortisol or uterus was 0.72($P<0.01$), or 0.84($P<0.001$), respectively.
[4] Ovariectomized.
[5] 17β-estradiol of 17α-methyltestosterone(7mg/kg body weight) was intramuscularly injected weekly in OVX rats.
[6] Less than 0.01ng/mL Plasma
[7] Relative uterus weight = 100(g uterus weight/g body weight)

DISCUSSION

Our studies clearly demonstrate that depressed growth in females compared to males is mostly due to their ovarian activity(Tables 1 and 2). Estrogen secreted in the ovary appeared to be responsible for the growth depression because the injection of estradiol markedly depressed growth in GDX male and female rats(Table 2), while elevating plasma cortisol levels(Table 3).

Female rats are known to show greater adrenocorticotropin(ACTH) and cor-

ticosterone 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). Estradiol implant in castrated males elicited a female pattern of plasma cortisol level in the nonhuman primate macaques(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). Similarly, Burgess and Handa(1992) showed that corticosterone and ACTH levels after a 5-second footshock stress with one mamp were much higher and maintained for a prolonged time when OVX rats were administered with estradiol. In addition, they found that estrogen treatment resulted in a loss of the glucocorticoid receptor's ability of autoregulation.

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 & Meaney 1991, Carey et al. 1995).

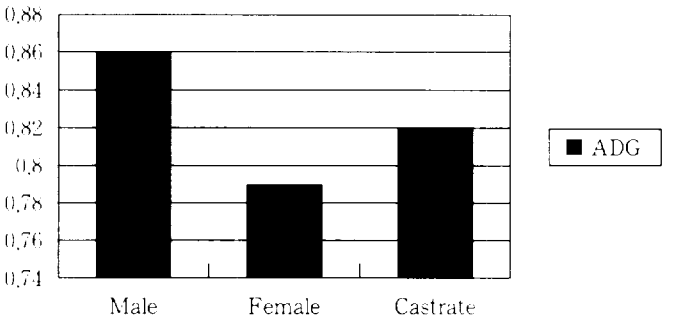

Figure 1. Influence upon growth performance of three sexes in pigs.

Taken together, our data and others' (Kitay 1963, Ramaley 1976, Phillips and Poolsaguan 1978, Le Mevel et al. 1979) clearly indicate that estrogen induces the secretion of hormones that increases metabolic rates, in turn resulting in growth depression. In general, female farm animals show low growth rate com-

pared with their male counterparts(Figure 1). Therefore, ovariectomy may be an economically viable practice to improve growth of female farm animals. In fact, ovariectomy has been widely practiced by pig farmers in some areas of China as a means of improving growth and pork quality(Shu-tang Feng, personal communication).

Recent studies have shown marked gender difference in plasma cholesterol levels in Sprague Dawley rats(Lee 1996) and in guinea pigs(Fernandez et al. 1995, Figure 2). When animals were fed CD, we speculated that ovarian activities might be responsible for the hypercholesterolemic response to dietary cholesterol in female animals. Unexpectedly, ovariectomy had no significant effect on plasma cholesterol levels in rats fed either CD(Tables 1) or ND(Table 2).

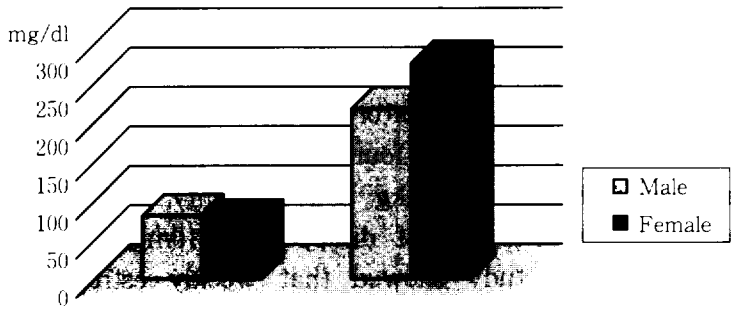

Figure 2. Effect of gender on plasma cholesterol level in rats fed low(0.04%) or high (0.25%) cholesterolemic diet(Fernandez et al. 1995).

Reports of an increased risk of cardiovascular disease in women who had taken estrogen(Wilson et al. 1985) and also enhanced cardiovascular response to mental stress in young menstruating women after administration(Manhem et al. 1996), led us to speculate that estrogen may not be beneficial in cardioprotection. However, observational studies on estrogen replacement have indicated taht estrogen has a 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 OVX rats(Sprague Dawley) fed a commercial chow but not in 19-d-old immature intact rats(Lundeen et al. 1997). We also found no gender difference($P>0.05$) in plasma cholesterol levels in 28-d-old rats fed CD for one w(Table 6). Results of our studies suggest that the gender difference in plasma cholesterol level in response to

dietary cholesterol appears after sexual maturity but is not related to gonadal activities.

Estrogen administration was reported to increase the LDL-receptor mRNA and the receptor protein in Sprague Dawely 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 fed CD, suggesting that females are less sensitive than males in feedback control mechanism of cholesterol synthesis. We consider that the gender difference in the feedback control is not related to sex hormones because our data showed that ovaryectomy had no significant effect on plasma cholesterol level in rats fed either CD or ND.

The hypocholesterolemic effect of estrogen seems to be highly variable among studies maybe due to different species(or strains), age of animals and dose of estrogen used. Lundeen et al.(1997) found no estrogen effect at levels below 0.01mg(injected daily for 4days per kg body weight), but at a higher level (1mg), plasma cholesterol was almost disappeared(<10mg/100mL) in Sprague Dawley rats. The present study showed that weekly estrogen injection into OVX rats with a comparable dose(7mg injected weekly per kg body weight) did not have a significant effect. Both these studies were done with Sprague Dawley rats fed NCD. By contrast, estradiol implant(50mg for seven days) in chicks increased plasma triacylglycerol levels 45 folds and cholesterol levels 6 folds 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). In contrast to the result of Lundeen et al.(1997) that estrogen replacement decreased plasma total and HDL cholesterol levels in OVX rats, we found no significant effect of estrogen replacemen on plasma total or HDL cholesterol levels(Table 2). Our study also showed that the ratio of HDL/LDL+VLDL cholesterol was much higher in males than in females when rats were fed CD(Table 1), but the gender difference was not shown when fed ND(Table 2). The ratio appears to be more relavant to plasma total cholesterol concentrations than to gender or sex hormones.

Beneficial effect of estrogen on cardiovascular diseases seems to be contradictory to our findings that female rats had a much higher plasma cholesterol levels than did males when they were fed CD, and ovariectomy had no effect on

the plasma cholesterol level. If estrogen plays a role at all in reducing cardiovascular disease in females, it may be through actions on other than controlling blood cholesterol levels. Naito et al.(1995) indicated that the effect of sex sex hormones on lipid metabolism are not likely to account for the sex difference in cardiovascular disease, 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 Progect Research Group 1970).

Estrogen may partly contribute to cardioprotection through enhancing the secretion of hormones that increase metabolic activities perhaps the same way as exercise plays a role in cardioprotection(Lindheim et al. 1994). Our proposed hypothesis may be further supported by an increased relative liver weight by estrogen injection in GDX male and female rats(Table 3). A study done by Srivastava et al.(1993) with male Sprague Dawley rats fed ND showed that subcutaneous injection of estradiol(5mg/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%). Although obtained with supraphysiological doses of estrogen, these data support our contention that estrogen is a hormone that increases metabolic rates and consequently depletes body energy reserves that may otherwise be used for weight gain.

Different from estradiol, testosterone injection had no effect on cortisol or testosterone concentrations in the plasma sampled 1 d after the injection(Table 3). Considering that the half-life of testosterone is much shorter than that of estrogen and that testosterone was injected into(OVX) female rats, it is not surprising that no significant amounts of testosterone were detected in the blood taken 1 d after injection.

CONCLUSION

Female rats grow more slowly than their male counterparts because estrogen depresses growth by inducing the secretion of hormones that increase metabolic rates. The hypercholesterolemic response to dietary cholesterol in female rats,

which was not influenced by ovariectomy, suggests that physiological events not related to ovary control the sex-related cholesterol metabolism, e.g., absorption, synthesis and catabolism. Futher studies on this matter are warranted.

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