

Gender-Related Differences in the Level of Serum Lipids in Meishan Pigs

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Levels of serum lipids such as total cholesterol (T-CHO), free cholesterol (F-CHO), low density lipoprotein cholesterol (LDL-C), triglycerides (TG), and phospholipids (PL) were examined in both sexes of Meishan pigs. In young adult pigs (4- to 5-month-old), levels of all above lipids mentioned were significantly higher in females than in males, while no such sex difference was observed until the age of 3 months. In young adult male pigs castrated at the age of 1 month, levels of these lipids were almost the same as levels in corresponding female pigs. In addition, no sex differences in the levels of high density lipoprotein cholesterol and non-esterified free fatty acids were observed. The present findings indicated that androgen might act as a down-regulator for the levels of serum lipids, such as T-CHO, F-CHO, LDL-C, TG and PL, in pigs.

Key words— serum lipid, pig, sex difference, cholesterol, triglyceride, phospholipid

INTRODUCTION

Pigs are often used in pharmacological and toxicological studies, because anatomical and physiological characteristics are close to those of humans. In particular concerning cardiovascular anatomy, there is a high similarity between pigs and humans, and the pig is considered as a universal standard an-

imal for general cardiovascular studies.¹⁾

There are many breeding strains of pigs, including Chinese breed Meishan pigs and European White breeds, which have different physiological characteristics. As judged on the basis of body weight gain, growth is faster in European White breeds than in Meishan pigs.²⁾ On the other hand, puberty is earlier in Meishan pigs than in European White breeds,^{3–5)} and Meishan pigs are more prolific than European White breeds.^{6,7)} Another distinctive feature is that Meishan pigs have a lot of adipose tissue, as compared with European White breeds.⁸⁾ However, there is little information about the age-dependent change in serum lipid level in pigs and any sex-differences.

In the present study, we examined the age-dependent change in the levels of serum lipids, including total cholesterol (T-CHO), triglycerides (TG), non-esterified free fatty acids (NEFA), free cholesterol (F-CHO), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and phospholipids (PL) in both sexes of Meishan pigs, and demonstrated gender-related differences in the levels of T-CHO, TG, F-CHO, LDL-C and PL.

MATERIALS AND METHODS

Animals— All animals were handled with humane care under the guidelines of National Institute of Agrobiological Sciences and National Institute of Livestock and Grassland Science (Tsukuba, Japan). Meishan pigs were bred and kept at the National Institute of Livestock and Grassland Science. Pigs were fed a commercial grain diet and provided with water *ad libitum*. Two male pigs were castrated at the age of 1 month and used as castrated male pigs after 4 months of the castration. Average body weight of pigs in each experimental group is shown in Table 1.

Male and female BALB/c mice were purchased from Japan SLC, Inc. (Hamamatsu, Japan) and used at 7 weeks of age. Body weight (mean \pm S.D., $n = 5$) at the age of 7 weeks was 23.9 ± 1.4 g in males and 20.0 ± 0.9 g in females. Mice were kept in plastic cages in an air-conditioned room with a 12 hr light/dark cycle, and given a basal diet, MF (Oriental Yeast, Co., Tokyo, Japan), and water *ad libitum*.

Blood samples were collected from individual

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Table 1. Body Weight of Meishan Pigs Examined in the Present Study

Age	Body weight (kg)		
	Male	Female	Castrated male
2 days	0.9 ± 0.1 (3) ^a	1.0 ± 0.2 (3)	
1 week	1.8 ± 0.1 (3)	1.6 ± 0.3 (3)	
2 weeks	2.0 ± 0.2 (3)	2.3 ± 0.2 (3)	
1 month	7.1 ± 0.9 (3)	7.8 ± 0.7 (3)	
2 months	16.7 ± 3.0 (3)	19.0 ± 0.7 (3)	
3 months	27.2 ± 3.8 (3)	29.8 ± 4.2 (3)	
4 months	44.1 ± 5.1 (3)	42.0 ± 3.5 (3)	
5 months	48.2 ± 5.6 (4)	52.7 ± 4.1 (4)	61.8 ± 7.5 (2)

The values shown represent the mean ± S.D. in both sexes of intact pigs and the mean ± S.E. in castrated male pigs. *a*) The values shown in parentheses represent the number of pigs examined.

Table 2. Lipid Content in Serum of 5-Month-Old Meishan Pigs

Sex	Animal No.	Content in the serum						
		T-CHO (mg/dl)	TG (mg/dl)	NEFA (mEq/l)	F-CHO (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	PL (mg/dl)
M	4	72.5 ± 8.7	20.8 ± 5.1	0.16 ± 0.07	16.3 ± 1.5	29.3 ± 3.8	36.4 ± 5.3	84.8 ± 12.7
F	4	98.3 ± 9.4**	41.3 ± 9.2**	0.14 ± 0.06	19.9 ± 1.8*	51.0 ± 1.4**	43.0 ± 1.2	119.5 ± 7.0**
CM	2	103.5 ± 3.5	42.0 ± 5.0	0.12 ± 0.01	22.8 ± 4.0	52.0 ± 1.0	44.5 ± 1.9	117.5 ± 3.5

The values shown represent the mean ± S.D. in both sexes of intact pigs and the mean ± S.E. in castrated male pigs. M; male, F; female, CM; castrated male. **Significantly different from intact male pigs (M): * $p < 0.05$, ** $p < 0.01$.

pigs between 10:30 am and 13:30 pm. Serum was separated from the collected blood by centrifugation at $1500 \times g$ for 15 min at 4°C, and stored at -80°C until use.

Measurement of Lipid Contents in the Serum—Levels of serum T-CHO, TG, NEFA, F-CHO, LDL-C, HDL-C, and PL were measured with a 7020 Automatic Analyzer (Hitachi, Tokyo, Japan). All reagents suitable for the assays were purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan. Blood of mice was diluted 2-fold with phosphate-buffered saline for measurement of NEFA because of over scaling.

Statistical Analysis—Significant differences were evaluated using the Student's *t*-test.

RESULTS AND DISCUSSION

In both sexes of Meishan pigs, body weights after birth were gradually increased until at least 5 months of age, and their body weight at the age of 5 months was about 50 kg in both sexes of pigs (Table 1). In addition, the body weight of 5-month-old male pigs castrated at the age of 1 month was slightly higher compared with the corresponding in-

tact male pigs.

In young adult (5-month-old) Meishan pigs, the levels of serum T-CHO, TG, F-CHO, LDL-C and PL were significantly higher in females than in males, while no such sex differences were observed in the levels of serum NEFA and HDL-C (Table 2). In addition, levels of serum lipids in castrated male pigs were almost the same as levels in corresponding female pigs. These findings suggest the possibility that androgen might down-regulate levels of serum lipids such as T-CHO, TG, F-CHO, LDL-C and PL in pigs.

Therefore, an age (sex maturation)-dependent change in the level of serum lipids was next examined. As shown in Fig. 1, significant sex differences in the level of serum lipids such as T-CHO, TG, F-CHO, LDL-C and PL were clearly observed in young adult pigs (4- to 5-month-old), and level of serum lipids was higher in females than in males. On the other hand, no such sex difference was observed until 3 months of age, although sex differences in the level of serum LDL-C and PL was temporally observed at the ages of 2 months and 2 weeks, respectively. These findings also indicate that androgen could down-regulate the levels of the serum lipids in the pigs.

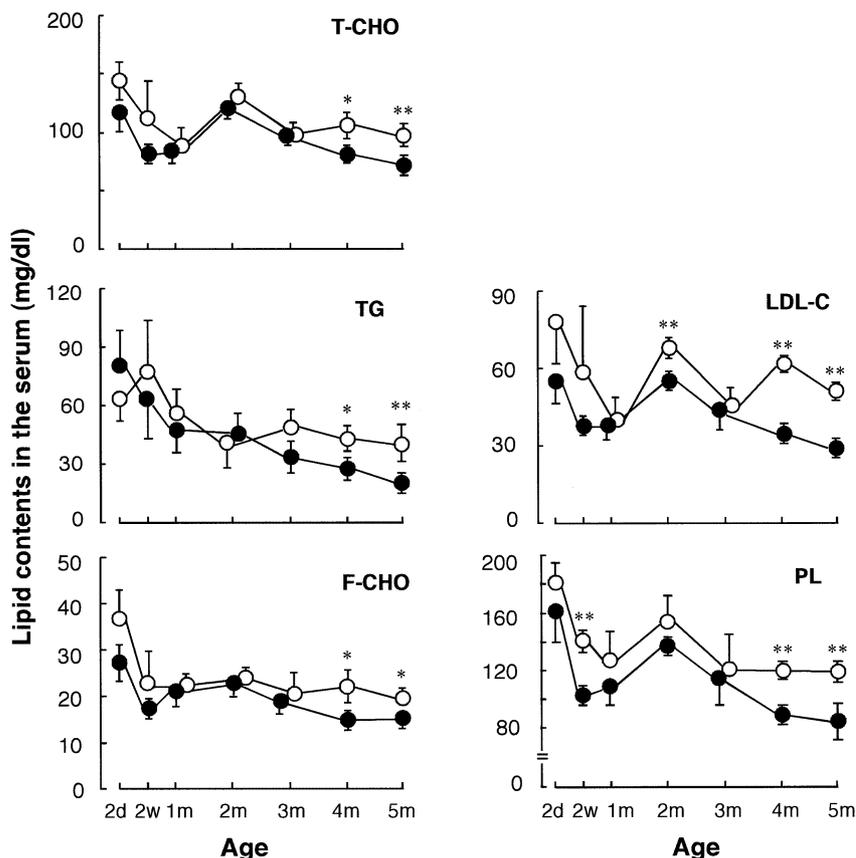


Fig. 1. Age-Related Changes in Levels of Serum Lipids in Meishan Pigs

Levels of serum T-CHO, TG, F-CHO, LDL-C, and PL in both sexes of Meishan pigs were measured at 2 days (d), 2 weeks (w) and 1–5 months (m) after birth as described in MATERIALS AND METHODS. Closed and open circles show the mean of male and female pigs, respectively, and bars represent S.D. ***, Significant differences from age-matched male pigs: * $p < 0.05$, ** $p < 0.01$, respectively.

Table 3. Lipid Content in Serum of 7-Week-Old BALB/c Mice

Sex	Animal No.	Content in the serum		
		T-CHO (mg/dl)	TG (mg/dl)	NEFA (mEq/l)
M	5	118.6 ± 9.2	118.0 ± 23.3	3.27 ± 0.46
F	5	86.2 ± 8.6**	87.0 ± 12.3*	3.06 ± 0.39

The values shown represent the mean ± S.D. M; male, F; female. ***, Significantly different from the corresponding male mice: * $p < 0.05$, ** $p < 0.01$.

On the contrary, in young adult (7-week-old) BALB/c mice, the level of serum T-CHO and TG was significantly higher in males than in females (Table 3), suggesting that in mice, androgen might up-regulate the serum lipid levels. In addition, mouse phenome database (<http://phenome.jax.org/pub/cgi/phenome/mpdcgi?rtn=docs/home>) reveals that levels of serum T-CHO and TG in mice, including C3H/HeJ, DBA/2J, and C57BL/6J, are higher in males than in females. Consequently, species difference between pigs and mice might result from differences in the expression level and/or activ-

ity of androgen-dependent enzymes responsible for biosynthesis and/or metabolism of these lipids, although the exact mechanism remains unclear.

The level of serum NEFA (about 3 mEq/l) in BALB/c mice was much higher than the level (about 0.15 mEq/l) in Meishan pigs (Tables 2 and 3), whereas the level (about 4.7 mg/dl) of plasma LDL-C previously reported in mice⁹⁾ is much lower than the level (25–50 mg/dl) in pigs (Table 2). In addition, levels of NEFA and LDL-C in human serum are reported to be around 0.5 mEq/l¹⁰⁾ and 120–130 mg/dl,¹¹⁾ respectively, and resemble those in pigs rather than those in mice.

In conclusion, we demonstrated a gender-related difference in the level of serum lipids such as T-CHO, TG, F-CHO, LDL-C and PL in Meishan pigs, and further suggested that the gender-related difference could occur in an androgen-dependent manner. Because it has been reported that endogenous sex hormones, such as estrogen, progesterone, and androgen, contribute to development of cardiovascular disease,^{12, 13)} Meishan pigs might be useful

animals for understanding the gender-related differences in human cardiovascular diseases.

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