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Sex Differences of Serum Lipid Profile in Novel Microminipigs

HIROAKI KAWAGUCHI[1*], TOMONOBU YAMADA[2,3*], NAOKI MIURA[4*], MICHIKO NOGUCHI[5], HIROYUKI IZUMI[3], NORIAKI MIYOSHI[1] and AKIHIDE TANIMOTO[2]

[1]Laboratory of Veterinary Histopathology, [4]Veterinary Teaching Hospital, [5]Laboratory of Domestic Animal Internal Medicine, Joint Faculty of Veterinary Medicine, Kagoshima University, Kagoshima, Japan; [2]Department of Molecular and Cellular Pathology, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan; [3]Drug Safety Research Laboratories, Shin Nippon Biomedical Laboratories, Ltd., Kagoshima, Japan

Abstract. *Swine have been used extensively in biomedical research, with a significant increase in recent decades. Minipigs are increasingly becoming an especially attractive animal model in life science research because of their physiological and anatomical similarities to humans. The Microminipig (MMPig) has emerged as a novel and small minipig for non-clinical pharmacological/toxicological use. The MMPig is docile, weighs less than 10 kg in early maturity, and has an easily manageable size. In this study, we report on sex and age patterns in serum biochemistry parameters, including lipid analysis items and lipid profiles in healthy MMPigs. In total, 58 males and 67 females aged 0-34 months underwent serum biochemistry parameter measurements. Most parameters showed no effect of age or sex (although some did). Lipid analyses showed that the serum levels of total cholesterol, but not those of triglycerides (TG), were consistently higher in females at 0-34 months of age. Lipid profiles in 5-month-old MMPigs were investigated in greater detail. Serum low-density lipoprotein-cholesterol (LDL-C) values were higher in females. The percentage of LDL-C against total cholesterol was also higher, although high-density lipoprotein-cholesterol was lower, in females. There were no sex differences in the TG fraction. Although the sex difference in the serum lipid profile remains unexplained, the reference values obtained in this study could help facilitate the use of MMPigs in life science research.*

Swine have been used extensively in biomedical research, with a significant increase in recent decades. More than 60,000 pigs are used for research in a year in the EU (1, 2); however, they are not yet widely used in Japan. Minipigs are increasingly becoming an especially attractive animal model in life science research because of their physiological and anatomical similarities to humans (3, 4). In particular, the number of minipigs used in cardiovascular and skin research is increasing (5, 6). Minipigs can be classified by adult body weight (BW) into a light category weighing 35-70 kg, which includes the Göttingen, Yucatan, and Sinclair strains, and a heavier category weighing 70-90 kg, which includes the Hanford strain (1). The Microminipig (MMPig; Fuji Micra Inc., Shizuoka, Japan) has emerged as a novel and small minipig for non-clinical pharmacological/toxicological use (3, 7). The MMPig is docile, with a BW in early maturity of less than 10 kg, and of a good manageable size for an experimental animal (3, 8, 9). The founder of the MMPig strain was a female (named "Catherin") bred from mating a pot-bellied pig with a minipig of another type (3). The use of MMPigs in pharmacological/toxicological experiments includes: an established atherosclerosis model induced by diet control (high fat and high cholesterol diet) (4, 10), and evaluation in a dermal phototoxicity study (6). Recently, we reported that general hematological and biochemical parameters in MMPigs were similar to those in Göttingen and Yucatan minipigs (8, 11-14). To expand on our previous study, we investigated differences by age and sex in biochemistry parameters and lipid profiles of healthy MMPigs to obtain reference data, which will be essential for future life science research.

*These Authors contributed equally to this study and should be regarded as co-first authors.

Correspondence to: Hiroaki Kawaguchi, DVM, Ph.D. Laboratory of Veterinary Histopathology, Joint Faculty of Veterinary Medicine, Kagoshima University, 1-21-24, Korimoto, Kagoshima 890-0065, Japan. Tel: +81 992858720, Fax: +81 992858722, e-mail: k3038952@kadai.jp and Akihide Tanimoto, MD, Ph.D. Department of Molecular and Cellular Pathology, Kagoshima University Graduate School of Medical and Dental Sciences, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan. Tel: +81 992755263, Fax: + 81 992646348, e-mail: akit09@m3.kufm.kagoshima-u.ac.jp

Key Words: Lipid profile, porcine serum biochemistry, sex difference, swine.

Table I. *Age-specific values in serum biochemistry in Microminipig*.

Parameter	Unit	Gender	Age (months)						
			0 (M=4, F=3)	1-3 (M=5, F=12)	4-6 (M=7, F=7)	7-9 (M=15, F=22)	10-12 (M=7, F=5)	13-24 (M=14, F=10)	25-34 (M=6, F=8)
Aspartate aminotransferase	IU/l	M+F	43.3±18.9	43.4±11.4	37.9±25.2	42.1±33.3	37.0±7.7	38.5±13.7	40.5±21.9
		M	40.5±14.8	43.6±10.1	43.0±36.1	33.7±7.3	39.7±6.5	42.8±13.3	40.2±13.6
		F	47.0±26.5	43.3±12.3	32.7±4.4	47.9±42.1	33.2±8.3	32.6±12.4	40.8±27.5
Alanine aminotransferase	IU/l	M+F	29.4±5.0	40.9±14.4	54.6±17.8	47.8±18.5	42.8±11.7	45.2±12.0	46.1±12.5
		M	28.8±5.4	38.0±12.7	45.0±16.1	39.1±10.9	44.6±13.3	47.9±9.8	46.2±14.0
		F	30.3±5.5	42.2±15.4	64.1±14.6*	53.7±20.4**	40.2±9.8	41.5±14.3	46.1±12.3
Alkaline phosphatase	IU/l	M+F	2399.3±1138.7	863.9±228.3	584.6±239.8	468.0±191.1	600.7±223.6	455.3±232.1	322.4±137.6
		M	2797.8±1440.6	897.4±249.7	662.4±186.4	546.9±230.7	710.6±228.8	488.9±276.9	285.2±114.2
		F	1868.0±189.7	849.9±228.9	506.7±275.2	414.2±140.1	446.8±90.4*	408.3±150.5	350.3±154.2
Creatinine kinase	IU/l	M+F	1185.7±1027.8	404.6±237.3	677.5±1280.6	863.4±1431.3	827.6±1081.0	557.2±474.0	937.3±1250.2
		M	953.0±412.3	329.2±82.7	990.1±1814.5	656.8±806.6	1033.7±1409.2	589.6±478.2	716.2±697.8
		F	1496.0±1631.5	436.0±275.3	364.9±180.9	1004.2±1739.8	539.0±236.8	511.9±489.9	1103.1±1575.2
Total bilirubin	mg/dl	M+F	0.097±0.136	0.008±0.011	0.021±0.023	0.027±0.023	0.039±0.028	0.034±0.022	0.039±0.027
		M	0.095±0.150	0.012±0.008	0.017±0.017	0.025±0.015	0.047±0.029	0.026±0.019	0.023±0.012
		F	0.100±0.148	0.006±0.012	0.024±0.028	0.029±0.027	0.028±0.026	0.045±0.022*	0.051±0.029*
Urea nitrogen	mg/dl	M+F	10.3±2.5	12.2±2.2	12.6±2.4	13.6±2.9	14.8±4.1	15.3±3.6	15.4±3.6
		M	8.8±2.0	13.0±2.1	13.3±2.5	14.5±3.1	16.6±4.3	16.4±3.8	14.6±2.0
		F	12.4±1.3*	11.9±2.3	11.8±2.3	13.0±2.6	12.1±1.9	13.9±2.8	16.0±4.6
Creatinine	mg/dl	M+F	0.62±0.04	0.88±0.21	0.82±0.25	0.80±0.19	0.89±0.18	1.17±0.40	1.08±0.28
		M	0.61±0.04	0.84±0.10	0.74±0.25	0.87±0.19	0.99±0.15	1.19±0.45	1.27±0.29
		F	0.64±0.04	0.90±0.24	0.89±0.25	0.76±0.19	0.75±0.11*	1.14±0.33	0.93±0.17*
Total cholesterol‡	mg/dl	M+F	202.4±155.8	82.2±8.9	85.9±18.9	74.1±14.6	83.3±12.3	80.3±14.6	77.6±22.9
		M	112.5±8.4	73.8±6.6	74.3±6.4	66.0±12.7	82.0±10.7	74.9±14.0	60.2±14.2
		F	322.3±187.0	85.7±7.3**	97.4±20.5*	79.8±13.3**	85.2±15.5	87.7±12.5*	90.8±19.3**
Triglycerides	mg/dl	M+F	102.6±63.9	42.6±12.9	41.9±13.4	40.9±19.5	41.3±28.0	44.1±14.8	35.4±13.8
		M	116.0±85.2	41.4±8.7	33.7±6.2	33.5±9.4	35.1±14.9	41.6±13.5	30.2±9.8
		F	84.7±22.9	43.1±14.7	50.0±14.1*	46.0±23.0*	49.8±40.8	47.6±16.4	39.4±15.6
Total protein	g/dl	M+F	6.0±0.7	6.3±0.6	7.8±0.6	8.0±0.6	8.0±0.5	8.1±0.8	8.2±0.8
		M	6.0±0.5	6.0±0.3	7.7±0.5	7.9±0.6	8.1±0.5	8.2±0.9	8.0±0.7
		F	6.0±1.0	6.5±0.7	7.9±0.7	8.0±0.7	8.0±0.5	8.0±0.4	8.4±1.0
Albumin	g/dl	M+F	3.9±0.4	4.0±0.6	4.4±0.3	4.3±0.4	4.9±0.3	4.7±0.4	4.5±0.4
		M	3.9±0.4	4.4±0.3	4.5±0.3	4.3±0.5	5.0±0.3	4.6±0.5	4.6±0.4
		F	3.9±0.5	3.9±0.6	4.4±0.4	4.3±0.5	4.7±0.3	4.7±0.3	4.3±0.4
Globulin	g/dl	M+F	2.1±0.3	2.3±0.9	3.4±0.8	3.7±0.8	3.2±0.5	3.4±0.7	3.8±1.0
		M	2.1±0.1	1.7±0.2	3.3±0.6	3.7±0.9	3.1±0.7	3.6±0.8	3.4±0.5
		F	2.1±0.5	2.6±0.9**	3.6±0.9	3.7±0.8	3.3±0.3	3.2±0.5	4.1±1.3
Albumin-globulin ratio	ratio	M+F	1.9±0.2	2.0±0.8	1.4±0.3	1.2±0.4	1.6±0.4	1.4±0.3	1.2±0.3
		M	1.9±0.2	2.7±0.5	1.4±0.3	1.3±0.4	1.7±0.5	1.3±0.3	1.4±0.2
		F	1.9±0.2	1.7±0.7*	1.3±0.3	1.2±0.4	1.5±0.2	1.5±0.3	1.2±0.4
Glucose	mg/dl	M+F	136.9±38.9	98.1±16.4	88.1±12.5	82.5±10.2	84.2±7.2	88.9±20.2	89.6±20.8
		M	148.8±50.7	110.4±18.4	86.7±8.4	84.5±11.2	82.0±5.9	86.5±25.0	78.8±6.1
		F	121.0±5.3	93.0±13.2*	89.6±16.2	81.2±9.5	87.2±8.3	92.2±10.5	97.6±24.6
Phosphorus	mg/dl	M+F	11.3±1.0	8.1±1.0	6.8±0.7	6.5±0.7	6.1±0.6	6.3±0.6	5.5±0.6
		M	11.2±1.4	8.4±0.6	6.7±0.7	6.4±0.5	6.0±0.6	6.1±0.7	5.2±0.3
		F	11.5±0.5	8.0±1.1	6.9±0.7	6.5±0.8	6.3±0.5	6.4±0.5	5.8±0.7
Calcium	mg/dl	M+F	11.8±0.4	10.8±0.5	10.7±0.4	10.5±0.4	11.0±0.4	10.6±0.5	10.3±0.3
		M	11.6±0.3	11.1±0.4	10.8±0.3	10.4±0.4	11.1±0.4	10.6±0.7	10.3±0.4
		F	12.0±0.4	10.6±0.5	10.6±0.4	10.5±0.5	10.9±0.4	10.7±0.3	10.2±0.2
Sodium	mEq/l	M+F	144.6±3.4	143.5±2.3	144.6±2.3	144.3±5.2	146.2±2.4	145.6±2.7	145.1±2.7
		M	144.3±3.0	144.4±1.7	145.7±2.4	144.7±3.3	146.9±2.9	145.9±3.2	146.7±3.4
		F	145.0±4.6	143.2±2.6	143.6±1.9	144.0±6.2	145.2±1.1	145.1±1.8	143.9±1.2
Potassium	mEq/l	M+F	6.1±1.1	5.8±1.1	5.6±0.7	5.7±0.6	5.9±0.5	5.8±0.7	5.4±0.6
		M	6.0±1.3	6.0±1.1	5.9±0.4	5.6±0.4	5.9±0.5	5.8±0.7	5.5±0.6
		F	6.3±1.1	5.8±1.1	5.4±0.8	5.8±0.7	5.9±0.7	5.7±0.7	5.3±0.6
Chloride	mEq/l	M+F	106.6±2.1	106.2±1.8	103.4±3.1	102.3±5.0	102.9±2.9	102.8±2.9	102.3±2.3
		M	107.5±1.7	106.0±2.2	103.9±3.3	102.2±3.2	103.3±3.3	102.4±3.1	103.7±2.2
		F	105.3±2.3	106.3±1.7	103.0±3.0	102.4±6.1	102.4±2.5	103.3±2.7	101.3±2.0

M, Males; F, females. ‡7-9 months of age (M=15, F=21). *$p<0.05$, **$p<0.01$: significantly different from males.

Table II. *Analysis of serum lipid metabolism markers, cholesterol and triglyceride fractions in Microminipigs aged five months.*

Parameter	Unit	Age 5 months of age	
		Males (n=5)	Females (n=5)
T-Cho	mg/dl	77.8±11.3	94.8±3.7*
Free-Cho	mg/dl	16.4±2.5	20.8±1.1**
CE	mg/dl	61.4±8.9	74.0±3.1*
Triglycerides	mg/dl	35.4±6.6	54.4±8.2**
HDL-C	mg/dl	40.8±10.1	39.4±7.5
LDL-C	mg/dl	31.2±5.7	49.2±6.4**
VLDL-C	mg/dl	4.0±1.2	4.0±1.0
CM-C	mg/dl	1.6±0.5	2.2±0.4
Cholesterol fraction			
HDL-C	%	52.2±6.8	41.6±7.3*
LDL-C	%	40.6±6.8	52.2±7.3*
VLDL-C	%	5.2±1.6	4.0±1.0
CM-C	%	2.0±0.0	2.2±0.4
Triglycerides fraction			
HDL Triglyceride	%	13.8±4.1	12.6±2.5
LDL Triglyceride	%	38.8±5.1	40.4±5.3
VLDL Triglyceride	%	30.8±5.0	29.2±5.0
CM Triglyceride	%	16.6±4.7	17.8±8.0

T-Cho: Total cholesterol, Free-Cho: free cholesterol, CE: cholesterol ester, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, VLDL-C: very low-density lipoprotein cholesterol, CM-C: chylomicron cholesterol. *$p<0.05$, **$p<0.01$: significantly different from males.

Materials and Methods

All animals were maintained in the same animal housing unit at 24±3˚C and relative humidity at 50±20%, with a 12 h light/dark cycle in the breeder's facility. The dedicated space for each animal was 0.5-1.2 m^2. Restricted feeding of a porcine diet (Marubeni Nisshin Feed Co., Tokyo, Japan) was set as previously reported (8). Tap water was available *ad libitum*. The animals used in this study were found to be in good health and free of clinical signs of illness. They were not given any treatment or medication other than vaccination through the study. All animals were vaccinated against mycoplasmal pneumonia of swine (MPS), porcine pleuropneumonia (APP), and swine erysipelas (SE) at 0, 1-2, and 3 months old, respectively. All protocols were approved by the Ethics Committee of Animal Care and Experimentation, Kagoshima University (A09001) and the research was performed according to the Institutional Guidelines for Animal Experiments and in compliance with the Japanese Law Concerning the Protection and Control of Animal, (Law No. 105 and Notification No. 6).

Blood samples were collected from the cranial vena cava of 125 conscious animals (58 male and 67 females) aged 0-34 months under fasted conditions. Zero month of age was not newborn and over three weeks of age. It was possible for handlers to hold all MMPigs without causing them stress and/or pain while other technicians collected blood from them. For measurement of 19 serum biochemical parameters (Table I), serum was obtained by centrifugation (room temperature, 1710 × *g*, 15 min) and examined with an automatic analyzer (JCA-BM8; JEOL Co., Ltd., Tokyo, Japan). Lipid profiles [high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), very low-density lipoprotein-cholesterol (VLDL-C) and chylomicron] were investigated in 5-month-old MMPigs (five males and five females) by analyzing serum samples with an electrophoresis processing analyzer (Epalyzer 2; Helena Laboratories Japan Co., Ltd., Saitama, Japan). The cholesterol ester (CE) value was calculated as: CE=total cholesterol (T-Cho) – free cholesterol (Free-Cho).

All data are presented as the mean±SD and the statistical significance of any difference was assessed by F-test and Student's *t*-test or Welch's *t*-test, and $p<0.05$ was considered significant.

Results

As shown in Table I, most biochemistry parameters were not affected by sex. A small number of parameters revealed sex differences, although these differences were not consistent at 0-34 months of age. However, lipid analyses showed that the serum levels of T-Cho, but not those of triglycerides (TG), were consistently higher in females. Moreover, the alkaline phosphatase, total bilirubin, T-Cho, TG, and glucose levels in both male and female MMPigs at 0 months of age were higher than those at 1–34 months of age while the alanine aminotransferase level was lower.

As shown in Table II, lipid profile analyses showed that the serum levels of T-Cho, Free-Cho, CE, TG, and LDL-C were higher in females. The percentage of LDL-C against T-Cho was also higher, although that of HDL-C was lower, in females. There were no sex differences in the TG fraction.

Discussion

Most biochemistry parameters were not affected by age or sex. The levels of some parameters, such as aspartate aminotransferase, alkaline phosphatase etc. fluctuated by age as those obtained in Göttingen minipigs (11, 12, 15). In lipid analyses, the levels of serum T-Cho in female MMPigs were consistently high at 0-34 months of age, while serum TG levels were not. This sex difference was similar to that obtained in Göttingen minipigs (11, 12). In addition, the serum levels of T-Cho, total protein, albumin and glucose in MMPigs were also higher than those in Göttingen minipigs. The serum levels of T-Cho, total protein, albumin and glucose in male and female Göttingen minipigs aged six months were 51.4±7.7 mg/dl and 75.7±16.6 mg/dl, 6.1±0.3 g/dl and 6.2±0.4 g/dl, 3.4±0.2 g/dl and 3.3±0.2 g/dl, 57.7±7.2 mg/dl and 57.7±7.2 mg/dl, respectively (11). These phenomena may have been related to nutrition; the MMPigs were provided with feed corresponding to 1-8% of BW compared with usual figure of 2-3% for minipigs (15). The higher T-Cho levels in both male and female MMPigs at 0 months of age than at 1-34 months of age were probably due to the diet in the lactation and weaning periods, when porcine milk, which has a high fat content (about 5-6%), was included in the diet provided (16).

We investigated lipid profiles in greater detail in MMPigs at five months, which is considered the most likely age of use in life science research. The serum levels of T-Cho, Free-Cho, CE, TG, and LDL-C were higher in females. These high levels of lipid metabolism markers in female MMPigs may be related to greater lipolytic sensitivity in females as in humans (17). Women have higher HDL-C levels than men due to female hormones, while a tendency for lower HDL-C levels in female MMPigs was revealed. The tendency for lower HDL-C level in female MMPigs may be due to their young age because in children a similar tendency was revealed (18). This phenomenon may be related to the fact that the percentage of LDL-C against total cholesterol was also higher in female MMPigs, while that of HDL-C was lower. We believe these sex differences in the lipid profile of MMPigs are new findings, since we have not encountered any previous reports of them.

Although breeders have been making efforts to expand their supply, minipigs including MMPigs are not yet widely used in life science research mostly because of a lack of accumulated reference data, which are essential for any field of life science research (19). The reference values for serum lipid analysis items and lipid profiles, including those showing sex differences, obtained in this study could facilitate the use of MMPigs in life science research.

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