Table IV. *Atherosclerosis score according to Stary classification (experiment B).*

Group:	I (Normal diet)			II (Special diet)		
Animal no.:	1	2	3	4	5	6
LAD artery	–	–	–	–	II	–
LCX artery	–	–	–	II	II	II
RCA	–	–	–	II	II	II
Pulmonary artery	–	–	–	–	–	–
Aortic arch	–	–	–	II	II	II
Common carotid artery	–	–	–	–	–	–
Thoracic aorta	–	–	–	–	–	II
Abdominal aorta	–	–	–	II	II	II
External iliac arteries	–	–	–	II	II	II
Internal iliac arteries	–	–	–	–	II	–
Renal artery	–	–	–	II	–	–
Rostral cerebral artery	–	–	–	–	–	–
Internal carotid artery	–	–	–	–	–	–
Caudal communicating artery	–	–	–	–	–	–
Basilar artery	–	–	–	–	–	–
Ventral spinal artery	–	–	–	I	II	II

LAD: Left anterior descending, LCX: left circumflex, RCA: right coronary artery.

As shown in Table IV, the degree of atherosclerosis was evaluated by the Stary classification and no atherosclerotic lesions were observed in the systemic arteries in group I. The lesions in animals of experiment B (group II) were less severe than those in experiment A and no stenosis, calcification, or hemorrhage was observed in this experiment.

In the liver, fewer foamy cells infiltrating the sinusoid were observed as a finding with low severity in one animal in group II without any accompanying fatty change in the hepatocytes.

Discussion

Experiment A revealed that all diets induced similar degrees of hypercholesterolemia and atherosclerosis within a short period of just three months in MMPigs. Serum levels of T-Cho and LDL-Cho reached peaks of approximately 1,000 and 400 mg/dl, respectively, at week 2.

Since fecal cholesterol and TG excretions were slightly lower in animals fed the HF/HC/SC diet than those fed the HF/HC diet, it is suggested that SC slightly stimulated cholesterol and TG absorption in the MMPigs. However, endogenous bile acid including SC was sufficient for cholesterol and TG absorption because the HF/HC and HF/HC/SC diets induced similar degrees of hypercholesterolemia. This suggests that the supplemental dietary SC may not be necessary for the induction of hypercholesterolemia. Actually, an adverse effect of SC, an increase in the severity of the fatty change in the hepatocytes, was highest in the animals fed the HF/HC/SC diet.

Low cholesterol (0.5%) supplementation was considered sufficient for the induction of atherosclerosis in MMPigs because all diets induced similar hypercholesterolemia in the MMPigs, and all these animals showed a similar degree of atherosclerotic lesions. It is considered that the high cholesterol content (5%) may have been excessive because fecal cholesterol excretion in the animals fed HF/HC and HF/HC/SC diets was higher than that in the animals fed HF/LC/SC. This suggests the possibility that a high-fat and low-cholesterol diet without SC may be suitable for an MMPig model of atherosclerosis.

The diet-induced atherosclerotic lesions seen in MMPigs in this study (such as fibrous cap and calcification) were considered to be very similar to those seen in humans because of their location and histopathological characteristics, as previously described (20). Many animals, such as rabbits and swine, have been reported to develop similar atherosclerotic lesions in the coronary arteries, thoracic and abdominal aorta, and other arteries after being provided with a similar diet (3, 4, 7, 14, 20, 32, 33). However, atherosclerosis in the cerebral arterial circle and basilar artery, a finding known to be related to cerebral stroke, was also seen in MMPigs with each of the three diets in this study. This interesting result suggests that the MMPig is potentially suitable as an animal for a cerebral stroke model based on atherosclerosis.

In experiment B, hypercholestemia was induced by supplementation with cholesterol alone (0.3% to 5%) and severe hypercholestemia was induced by cholesterol (0.5%) and fat (12%) supplementation (Figure 8). Serum levels of T-

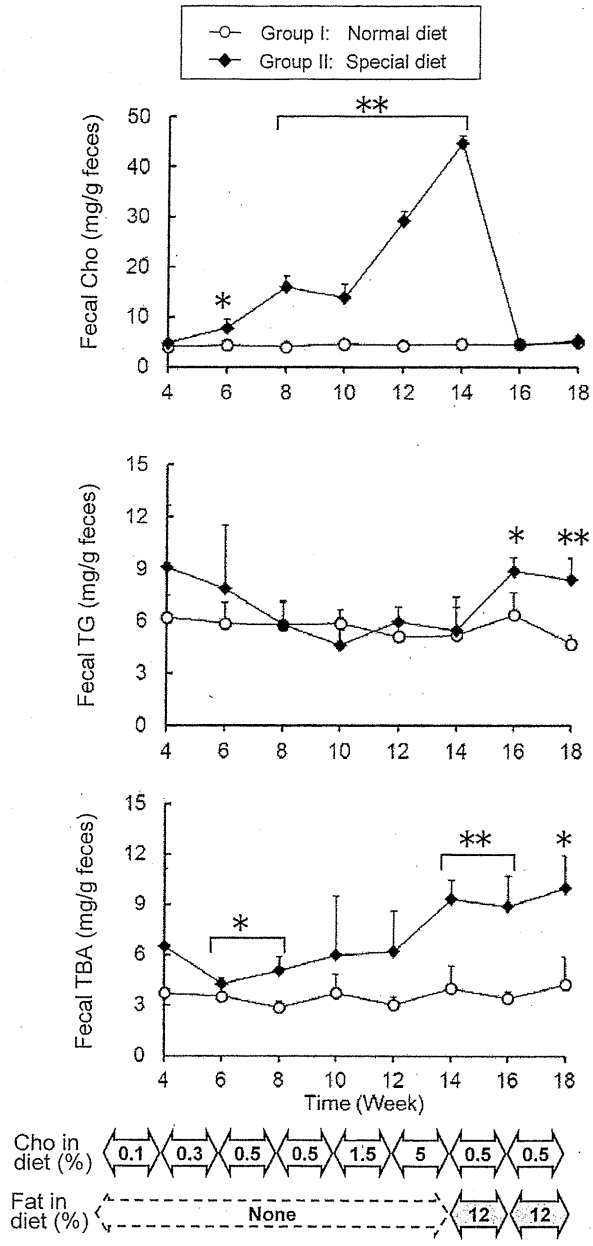

Figure 7. *Experiment B: Fecal analysis. Cho: Cholesterol, TG: triglycerides, TBA: total bile acid.*

Cho and LDL-Cho reached a plateau with 0.5% cholesterol supplementation. The fecal excretion of cholesterol was high in the animals fed 1.5% to 5% cholesterol diets, suggesting there may have been excessive amounts of cholesterol in the diet. Based on these results, it was considered that that the minimal dietary cholesterol content required to induce hypercholestemia in MMPigs is 0.5%. However, the severe hypercholestemia seen in experiment A was not induced when cholesterol alone was dosed to 5%, so the investigation of the dietary regimen was continued with both cholesterol and fat supplementation. Supplementation with cholesterol at 0.5% and fat at 12% proved capable of inducing severe hypercholestemia similar to that seen in experiment A. It is considered that the absorption of cholesterol in MMPigs may be enhanced when both cholesterol and fat are additives (at 0.5% and 12%, respectively), compared with that seen when cholesterol alone was supplemented (at 5%), since severe hypercholestemia was not induced under the latter dietary condition.

The atherosclerotic lesions in animals of experiment B were less severe than those in experiment A, and this was considered to be due to the shorter period of severe hypercholestemia in experiment B; it remains to be determined whether providing a diet with 0.5% cholesterol and 12% fat for 12 weeks can induce atherosclerosis similar to that seen in experiment A.

No fatty changes in the hepatocytes were observed as adverse findings in the liver with the diet of HF/HC alone in experiments A and B, suggesting that such a diet (without SC) may not induce hepatotoxicity.

In conclusion, dietary supplementation of SC was clearly shown nor to be required for the induction of atherosclerosis in the MMPig model, and a diet with cholesterol as the sole additive was judged unable to induce severe hypercholestemia. Moreover, it is suggested that a diet with 0.5% cholesterol and 12% fat may be suitable for the induction of atherosclerosis in the MMPigs. The results of this study show that an appropriate atherosclerosis model can be achieved without hepatotoxicity and demonstrate a cost benefit for research into human atherosclerosis research, for which the MMPig is suggested to be a useful experimental animal.

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References

1 Schoen FJ: Blood vessels. *In*: Robbins and Cotran Pathologic Basis of Disease, seventh Edition. Kumar V, Abbas AK and Faust N (eds.). Philadelphia, USA, Saunders, pp. 511-554, 2005.

2 Washio M, Sasazuki S, Kodama H, Yoshimasu PK, Liu Y, Tanaka K, Tokunaga S, Kono PS, Arai H, Koyanagi S, Hiyamuta K, Doi Y, Kawano MT, Nakagaki MO, Takada K, Nii MT, Shirai K, Ideishi MM, Arakawa MK, Mohri MM and Takeshita A: Role of hypertension, dyslipidemia and diabetes mellitus in the development of coronary atherosclerosis in Japan. Jpn Circ J *65*: 731-737, 2001.

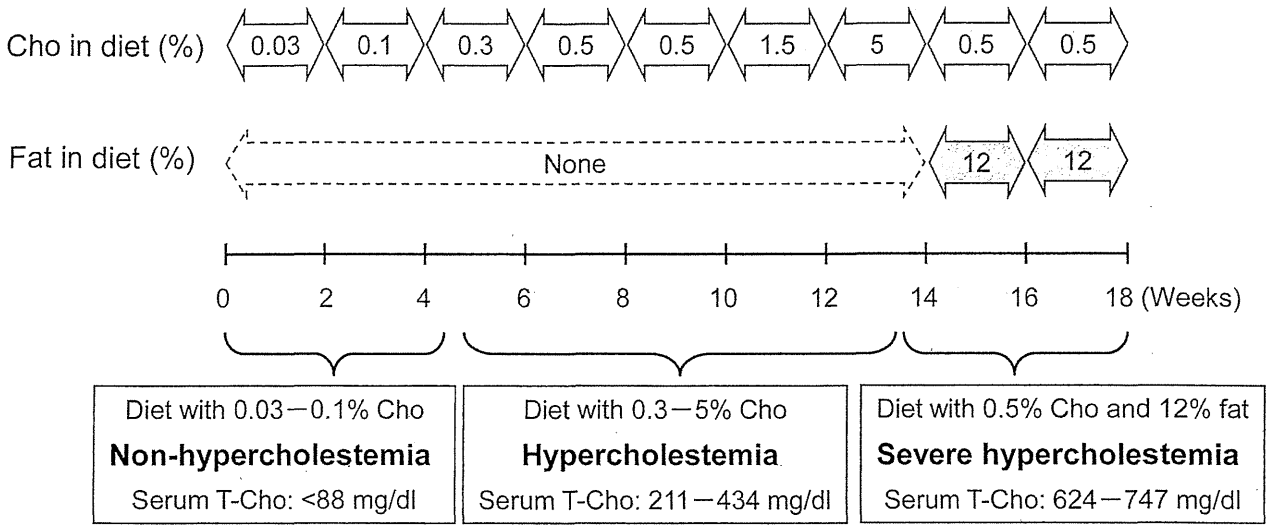

Figure 8. *Experiment B: Study design and result of hypercholestemia. T-Cho: Total cholesterol.*

3 Xi S, Yin W, Wang Z, Kusunoki M, Lian X, Koike T, Fan J and Zhang Q: A minipig model of high-fat/high-sucrose diet-induced diabetes and atherosclerosis. Int J Exp Path *85*: 223-231, 2004.

4 Turk JR, Henderson KK, Vanvickle GD, Watkins J and Laughlin MH: Arterial endothelial function in a porcine model of early-stage artherosclerotic disease. Int J Exp Path *86*: 335-345, 2005.

5 Reddick RL, Zhang SH and Maeda N: Atherosclerosis in mice lacking apo E. Evaluation of lesional development and progression. Arterioscler Thromb *14*: 141-147, 1994.

6 van Vlijmen BJ, van den Maagdenberg AM, Gijbels MJ, van der Boom H, HogenEsch H, Frants RR, Hofker MH and Havekes LM: Diet-induced hyperlipoproteinemia and atherosclerosis in apolipoprotein E3-Leiden transgenic mice. J Clin Invest *93*: 1403-1410, 1994.

7 Hamada N, Miyata M, Eto H, Ikeda Y, Shirasawa T, Akasaki Y, Miyauchi T, Furusho Y, Nagaki A, Aronow BJ and Tei C: Loss of clusterin limits atherosclerosis in apolipoprotein E-deficient mice *via* reduced expression of Egr-1 and TNF-α. J Atheroscler Thromb *18*: 209-216, 2011.

8 Buja LM, Kita T, Goldstein JL, Watanabe Y and Brown MS: Cellular pathology of progressive atherosclerosis in the WHHL rabbit. An animal model of familial hypercholesterolemia. Arteriosclerosis *3*: 87-101, 1983.

9 Shiomi M, Ito T, Tsukada T, Yata T and Ueda M: Cell compositions of coronary and aortic atherosclerotic lesions in WHHL rabbits differ. An immunohistochemical study. Arterioscler Thromb *14*: 931-937, 1994.

10 Suzuki H, Kobayashi H, Sato F, Yonemitsu Y, Nakashima Y and Sueishi K: Plaque-stabilizing effect of pitavastatin in Watanabe heritable hyperlipidemic (WHHL) rabbits. J Atheroscler Thromb *10*: 109-116, 2003.

11 Yamada S, Wang KY, Tanimoto A, Fan J, Shimajiri S, Kitajima S, Morimoto M, Tsutsui M, Watanabe T, Yasumoto K and Sasaguri Y: Matrix metalloproteinase 12 accelerates the initiation of atherosclerosis and stimulates the progression of fatty streaks to fibrous plaques in transgenic rabbits. Am J Pathol *172*: 1419-1429, 2008.

12 Svendsen O: The minipig in toxicology. Exp Toxicol Pathol 57: 335-339, 2006.

13 Bollen PJA, Hansen AK and Rasmussen HJ: Important biological features. *In*: The Laboratory Swine, second edition. Bollen PJA, Hansen AK, Rasmussen HJ and Suckow MA (eds.). New York, USA, CRC Press, pp. 1-14, 2010.

14 Kawaguchi H, Miyoshi N, Miura N, Fujiki M, Horiuchi M, Izumi Y, Miyajima H, Nagata R, Misumi K, Takeuchi T, Tanimoto A and Yoshida H: Microminipig, a non-rodent experimental animal optimized for life science research-Novel atherosclerosis model induced by high fat and cholesterol diet. J Pharmacol Sci *115*: 115-121, 2011.

15 Clauss SB, Walker DL, Kirby ML, Schimel D and Lo CW: Patterning of coronary arteries in wild-type and connexin 43 knockout mice. Dev Dyn *235*: 2786-2794, 2006.

16 Feng Y, Xie Y, Wang H, Chen F, Ye Y, Jin L, Marchal G and Ni Y: A modified rabbit model of reperfused myocardial infarction for cardiac MR imaging research. Int J Cardiovasc Imaging *25*: 289-298, 2009.

17 Osugi T, Saitoh S, Matumoto K, Muto M, Aikawa K, Ohkawara H, Sugimoto K, Kamioka M, Ishibashi T and Maruyama Y: Preventive effect of chronic endothelin type A receptor antagonist on coronary microvascular spasm induced by repeated epicardial coronary artery endothelial denudation in pigs. J Atheroscler Thromb *17*: 54-63, 2010.

18 Kaneko N, Itoh K, Sugiyama A and Izumi Y: Microminipig, a non-rodent experimental animal optimized for life science research: preface. J Pharmacol Sci *115*: 112-114, 2011.

19 Miura N, Kawaguchi H, Nagasato T, Yamada T, Ito T, Izumi H, Shameshima H, Miyoshi N, Tanimoto A and Maruyama I: Coagulation activity and white thrombus formation in the Microminipig. In Vivo *27*: 357-361, 2013.

20 Miyoshi N, Horiuchi M, Inokuchi Y, Miyamoto Y, Miura N, Tokunaga S, Fujiki M, Izumi Y, Miyajima H, Nagata R, Misumi K, Takeuchi T, Tanimoto A, Yasuda N, Yoshida H and Kawaguchi H: Novel Microminipig model of atherosclerosis by high fat and high cholesterol diet, established in Japan. In Vivo *24*: 671-680, 2010.

21 Moghadasian MH: Experimental atherosclerosis: A historical overview. Life Sci *70*: 855-865, 2002.

22 Goodrich JA, Clarkson TB, Cline JM, Jenkins AJ and Del Signore MJ: Value of the micropig model of menopause in the assessment of benefits and risks of postmenopausal therapies for cardiovascular and reproductive tissues. Fertil Steril *79(Suppl 1)*: 779-788, 2003.

23 Zhang C, Yin W, Liao D, Huang L, Tang C, Tsutsumi K, Wang Z, Liu Y, Li Q, Hou H, Cai M and Xiao J: NO-1886 up-regulates ATP binding cassette transporter A1 and inhibits diet-induced atherosclerosis in Chinese Bama minipigs. J Lipid Res *47*: 2055-2063, 2006.

24 Herrmann J, Saguner AM, Versari D, Peterson TE, Chade A, Olson M, Lerman LO and Lerman A: Chronic proteasome inhibition contributes to coronary atherosclerosis. Circ Res *101*: 865-874, 2007.

25 Teng S and Piquette-Miller M: Hepatoprotective role of PXR activation and MRP3 in cholic acid-induced cholestasis. Br J Pharmacol *151*: 367-376, 2007.

26 Iwami K, Fujii N, Suzuka T and Kanamoto R: A crucial role of soybean resistant protein in increased fecal steroid excretion and structural peculiarity of caught bile acids. Soy Protein Res Jpn *5*: 58-62, 2002 (in Japanese).

27 Stary HC, Chandler AB, Glagov S, Guyton JR, Insull W Jr., Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD and Wissler RW: A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation *89*: 2462-2478, 1994.

28 Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W Jr., Rosenfeld ME, Schwartz CJ, Wagner WD and Wissler RW: A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation *92*: 1355-1374, 1995.

29 Stary HC: Natural history and histological classification of atherosclerotic lesions: An update. Arterioscler Thromb Vasc Biol *20*: 1177-1178, 2000.

30 Kawaguchi H, Yamada T, Miura N, Takahashi Y, Yoshikawa T, Izumi H, Kawarasaki T, Miyoshi N and Tanimoto A: Reference values of hematological and biochemical parameters for the world smallest Microminpigs. J Vet Med Sci *74*: 933-936, 2012.

31 Kawaguchi H, Yamada T, Miura N, Noguchi M, Izumi H, Miyoshi N and Tanimoto A: Sex differences of serum lipid profile in novel Microminipigs. In Vivo *27*: 617-21, 2013.

32 Ostlund-Lindqvist AM, Lindqvist P, Bräutigam J, Olsson G, Bondjers G and Nordborg C: Effect of metoprolol on diet-induced atherosclerosis in rabbits. Arteriosclerosis *8*: 40-45, 1988.

33 Uchida M, Ishii I, Inoue C, Akisato Y, Watanabe K, Hosoyama S, Toida T, Ariyoshi N and Kitada M: Kefiran reduces atherosclerosis in rabbits fed a high cholesterol diet. J Atheroscler Thromb *17*: 980-988, 2010.

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Sex Differences of Serum Lipid Profile in Novel Microminipigs

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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.*

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

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.

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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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References

1 Bollen PJA, Hansen AK and Rasmussen HJ: Important biological features. *In*: The Laboratory Swine Second edition. Bollen PJA, Hansen AK, Rasmussen HJ and Suckow MA (eds.). New York, U.S.A., CRC Press, pp. 1-14, 2010.

2 Svendsen O: The minipig in toxicology. Exp Toxicol Pathol *57*: 335-339, 2006.

3 Kaneko N, Itoh K, Sugiyama A and Izumi Y: Microminipig, a non-rodent experimental animal optimized for life science research: Preface. J Pharmacol Sci *115*: 112-114, 2011.

4 Kawaguchi H, Miyoshi N, Miura N, Fujiki M, Horiuchi M, Izumi Y, Miyajima H, Nagata R, Misumi K, Takeuchi T, Tanimoto A and Yoshida H: Microminipig, a non-rodent experimental animal optimized for life science research: Novel atherosclerosis model induced by high fat and cholesterol diet. J Pharmacol Sci *115*: 115-121, 2011.

5 Kamimura R, Miura N and Suzuki S: The hemodynamic effects of acute myocardial ischemia and reperfusion in Clawn miniature pigs. Exp Anim *52*: 335-338, 2003.

6 Yoshikawa T, Takahashi Y, Kawaguchi H, Utsunomiya S, Miura N, Izumi H, Miyoshi N and Tanimoto A: A dermal phototoxicity study following intravenous infusion administration of ciprofloxacin hydrochloride in the novel Microminipigs. Toxicol Pathol *41*: 109-113, 2013.

7 Sugiyama A, Nakamura Y, Akie Y, Saito H, Izumi Y, Yamazaki H, Kaneko N and Itoh K: Microminipig, a non-rodent experimental animal optimized for life science research: in vivo proarrhythmia models of drug-induced long QT syndrome: Development of chronic atrioventricular block model of microminipig. J Pharmacol Sci *115*: 122-126, 2011.

8 Kawaguchi H, Yamada T, Miura N, Takahashi Y, Yoshikawa T, Izumi H, Kawarasaki T, Miyoshi N and Tanimoto A: Reference values of hematological and biochemical parameters for the world smallest Microminpigs. J Vet Med Sci *74*: 933-936, 2012.

9 Takeishi K, Horiuchi M, Kawaguchi H, Deguchi Y, Izumi H, Arimura E, Kuchiiwa S, Tanimoto A and Takeuchi T: Acupuncture improves sleep conditions of minipigs representing diurnal animals through an anatomically similar point to the acupoint (GV20) effective for humans. Evid Based Complement Alternat Med *2012*: 472982, 2012.

10 Miyoshi N, Horiuchi M, Inokuchi Y, Miyamoto Y, Miura N, Tokunaga S, Fujiki M, Izumi Y, Miyajima H, Nagata R, Misumi K, Takeuchi T, Tanimoto A, Yasuda N, Yoshida H and Kawaguchi H: Novel microminipig model of atherosclerosis by high fat and high cholesterol diet, established in Japan. In Vivo *24*: 671-680, 2010.

11 Ellegaard L, Jorgensen KD, Klastrup S, Hansen AK and Svendsen O: Haematologic and clinical chemical values in 3- and 6-month-old Göttingen minipigs. Scand J Lab Anim Sci *22*: 239-248, 1995.

12 Jørgensen KD, Ellegaard L, Klastrup S and Svendsen O: Haematological and clinical chemical values in pregnant and juvenile Göttingen minipigs. Scand J Lab Anim Sci *25*: 181-190, 1998.
13 Rispat G, Slaoui M, Weber D, Salemink P, Berthoux C and Shrivastava R: Haematological and plasma biochemical values for healthy Yucatan micropigs. Lab Anim *27*: 368-373, 1993.
14 Miura N, Kawaguchi H, Nagasato T, Yamada T, Ito T, Izumi H, Shameshima H, Miyoshi N, Tanimoto A and Maruyama I: Coagulation activity and white thrombus formation in the Microminipig. In Vivo *27*: 357-361, 2013.
15 Swindle MM: Biology, handling, husbandry, and anatomy. *In*: Swine in the Laboratory, Second edition. Swindle MM (ed.). Boca Raton, FL, U.S.A., CRC Press Taylor & Francis Group, pp. 1-33, 2007.
16 Klobasa F, Werhahn E and Butler JE: Composition of sow milk during lactation. J Anim Sci *64*: 1458-1466, 1987.
17 Williams CM: Lipid metabolism in women. Proc Nutr Soc *63*: 153-160, 2004.
18 Krishnaveni GV, Veena SR, Hill JC, Kehoe S, Karat SC and Fall CH: Intrauterine exposure to maternal diabetes is associated with higher adiposity and insulin resistance and clustering of cardiovascular risk markers in Indian children. Diabetes Care *33*: 402-404, 2010.
19 Tumbleson ME, Badger TM, Baker PC and Hutcheson DP: Systematic oscillations of serum biochemic and hematologic parameters in Sinclair (S-1) miniature swine. J Anim Sci *35*: 48-50, 1972.

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Original Article

Rapid Development of Atherosclerosis in the World's Smallest Microminipig Fed a High-Fat/High-Cholesterol Diet

A Useful Animal Model Due to its Size and Similarity to Human Pathophysiology

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Aim: **Experimental studies of human atherogenesis require an appropriate animal model that mimics human physiology and pathology. Because swine physiology is similar to human physiology, we developed a hyperlipidemia-induced atherosclerosis model using the recently developed world's smallest Microminipig™.**

Methods: **These animals weigh only 5 kg at 3 months of age, much smaller than any other miniature pig. We found that the administration of a high-fat/high-cholesterol diet containing at least 0.2% cholesterol without cholic acid for as little as eight weeks induces hypercholesterolemia and subsequent atherosclerosis in these animals.**

Results: **The serum levels of low-density lipoprotein cholesterol (LDL-C) and the percent distribution of cholesterol in the LDL fractions were markedly increased. The hepatic expression of LDL receptor and hydroxymethylglutaryl-CoA reductase was coordinately decreased. The cholesteryl ester transfer protein activity, which plays a role in reverse cholesterol transport, was detected in the serum of the Microminipigs. Niemann-Pick C1-like 1 protein was expressed in both the liver and small intestine; however, hepatic apoB mRNA editing enzyme was not expressed. As in humans, and in contrast to that observed in mice, most of the hepatic lipase activity was localized in the liver. These results suggest that the hyperlipidemia-induced gene expression profile linked to cholesterol homeostasis and atherogenesis is similar in Microminipigs and humans.**

Conclusion: **We conclude that the characteristics of the Microminipig, including its easy handling size, make it an appropriate model for studies of atherosclerosis and related conditions.**

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Key words: Microminipig, Atherosclerosis, Cholesterol metabolism, CETP

Introduction

Atherosclerosis is a predominant risk factor for cardiovascular and cerebrovascular events and is closely related to serious morbidity and mortality in developed nations. The recent westernization of lifestyle in Japan, especially the increased caloric intake from a fatty diet, may account for the increasing inci-

dence of cerebral and coronary artery disease. An appropriate animal model that reproduces human physiology and pathology would be ideal for investigating atherosclerosis because the pathogenesis of this disease includes both genetic and environmental factors.

Attempts to develop experimental animal models of atherosclerosis have primarily involved mice and rabbits. However, lipid metabolism in mice is quite different from that observed in humans, as mice are originally resistant to high-fat/high-cholesterol diet (HcD)-induced atherosclerosis. As a result, mice that exhibit hyperlipidemia and atherosclerosis due to a lack of apolipoprotein-E (apoE) or low-density lipoprotein cholesterol receptor (LDLr) genes are often used in atherosclerosis studies[1, 2]. The introduction of an additional transgene and/or the use of gene knockout in apoE- or LDLr-deficient mice are good tools for investigating the effects of specific genes in atherosclerosis[3]. Rabbits, which have a similar lipid metabolism to humans and are very sensitive to HcD with respect to the induction of atherosclerosis, are the next most often used animals. LDLr gene-mutated Watanabe heritable hyperlipidemic rabbits are good models of human familial hypercholesterolemia[4]. The recent development of transgenic rabbits has clarified the effects of various specific genes on the development of atherosclerosis[5, 6].

Swine represent another potentially useful animal model because, unlike mice and rabbits, their anatomy, physiology and habits of feeding and sleep are very similar to those of humans[7]. In general, domestic pigs are often used in medical training and education regarding vascular surgery techniques, arterial intervention, etc. However, their large size hampers handling and maintenance; therefore, they are unsuitable for experimental use in ordinary laboratories. Commercially available experimental miniature pigs (minipigs), such as Clawn, Göttingen, Chinese Bama and Yucatan minipigs are smaller than domestic pigs. Many studies have reported that HcD can induce atherosclerosis in Göttingen, Chinese Bama and Yucatan minipigs as well as domestic pigs[8-12]. These animals, which weigh less than 100 kg by definition, are still too large to be widely used in life science research. The Microminipig™ (MMPig, Fuji Micra Inc., Shizuoka, Japan) has recently been established as an experimental animal[13]. The MMPig is the world's smallest pig, with a body weight of only 5 kg at 3 months of age that remains at less than 10 kg at 7 months of age. Our preliminary study demonstrated that hyperlipidemia-induced atherosclerosis develops in MMPigs fed HcD containing sodium cholate (SC)[14]. Dietary SC is required to accelerate the progression of hyperlipidemia and atherosclerosis, presumably by inhibiting cholesterol excretion into bile; however, it is also known to cause hepatotoxicity[15].

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Aim

The specific aims of the present study were to further expand upon our previous research and detect evidence of close similarities between MMPigs and humans with respect to lipid metabolism and atherogenesis. We specifically investigated whether HcD alone (with no SC) induces atherosclerosis in the MMPigs and determined the minimum cholesterol content required to cause disease. We performed a histological evaluation of hyperlipidemia-induced atherosclerosis and assessed the expression of genes regulating cholesterol metabolism, including LDLr, class B scavenger receptor type I (SR-BI), hydroxymethylglutaryl-CoA reductase (HMGCR), apoB mRNA editing enzyme catalytic polypeptide 1 (APOBEC-1) and Niemann-Pick C1-like 1 protein (NPC1L1), which is expressed in the mammalian small intestine and liver and is critical for intestinal cholesterol absorption[16, 17]. The activity and localization of cholesteryl ester transfer protein (CETP) and hepatic lipase (HL) in the MMPigs were also evaluated and compared with those observed in humans.

Methods

Animals and Diet

Male MMPigs 3 months of age were maintained in a special room under environmental conditions with a room temperature of 24±3℃, a relative humidity of 50%±20% and a 12-hour light/dark cycle. Tap water was available *ad libitum*, and the animals were provided a special diet on a daily basis. The body weight was measured once a week. All protocols were approved by the Ethics Committee of Animal Care and Experimentation at Kagoshima University and performed according to the laws (no. 105) and notifications (no. 6) of the Japanese Government. This study was also performed in accordance with the animal welfare bylaws of Shin Nippon Biomedical Laboratories Ltd., a facility fully accredited by the Associa-

tion for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International and approved by the International Animal Care and Use Committee.

Twenty-two MMPigs were divided into four groups: seven control animals fed a normal chow diet (NcD) and three groups (five animals in each group) fed HcD for eight weeks. The HcD was composed of 12% lard (Miyoshi Oil & Fat, Tokyo, Japan) and 0.2%, 0.5% or 1.5% cholesterol (Wako Pure Chemical Industries, Osaka, Japan) mixed with NcD (Kodakara 73; Marubeni Nisshin Feed, Tokyo, Japan). After eight weeks, all MMPigs were anesthetized and sacrificed via bilateral axillary artery exsanguination.

Blood Pressure

The arterial systolic and diastolic blood pressures were measured at the foreleg with an apparatus meant for human pediatric use according to the Manchette method.

Hematology and Biochemical Analysis

Blood samples were collected once every two weeks for general hematology, biochemistry and lipoprotein profiling. The biochemical parameters measured in the blood samples included the levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, γ-glutamyl transpeptidase, glucose and total bilirubin. The levels of total cholesterol (TC), chylomicrons (CM), very low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were analyzed using an automated agarose gel electrophoresis apparatus (Epalyzer 2, Helena Laboratories, Saitama, Japan).

For measurement of the HL activity, blood was collected from the NcD-fed MMPigs before and 10 minutes after the intravenous injection of sodium heparin (50 unit/kg BW) at 5 and 25 months of age. Serum samples of the pre- and post-heparin fractions were then analyzed using an HL activity assay kit (Progen Biotechnik GmBH, Heidelberg, Germany). The serum CETP expression and activity in the HDL fractions were assayed in the NcD-fed control MMPigs (See **Supplemental text**).

Serum ApoB Profiles in the CM and VLDL Fractions

The similarity between the human and MMPig plasma proteomes was investigated using a method for cross-species detection of lipoproteins (**Supplemental text**). In brief, the CM and VLDL fractions were separated via ultracentrifugation. The samples were then alkylated, digested with trypsin and analyzed on a nano HPLC system coupled with a triple quadrupole mass spectrometer.

Evaluation of Visceral and Subcutaneous Fat

The weight of visceral tissue fat was measured at necropsy. The accumulation of subcutaneous fatty tissue (back fat thickness) was evaluated on computed tomography (CT) performed at the beginning and end of the study. The back fat thickness was measured at the midportion of the level between the lower angles of both scapulae, and the percentage increase in thickness after the eight-week dietary treatment was calculated.

Pathological Examination

At necropsy, the aorta, arteries, heart, liver, kidneys, spleen and small intestine were removed from each animal. The heart, liver, kidneys, spleen and visceral (omental and mesenteric) adipose tissue were weighed. All organs were fixed in 10% phosphate-buffered formalin and routinely processed as paraffin-embedded, 5-μm-thick tissue sections stained with hematoxylin and eosin (H&E) and Elastica-Masson stains. The aortas were longitudinally incised and fixed with 10% buffered formalin for 24 hours, followed by staining with Oil-red O stain for an *en face* analysis. The Oil-red O-stained area relative to the entire surface was calculated using the Image J software program. Immunostaining for atherosclerotic lesions was performed (Envision kit, Dako Cytomation, Kyoto, Japan) on the paraffin-embedded sections using antibodies against smooth muscle actin (anti-α-SMA clone 1A4, ×100; Dako Cytomation) and macrophages (anti-lysozyme rabbit polyclonal antibody, × 2,000; Dako Cytomation).

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

The liver and small intestine were stored in RNAlater immediately following sample collection, and total RNA was extracted using the mirVana miRNA isolation kit (Invitrogen, Carlsbad, CA). The mRNA expression was quantified using qRT-PCR with a TaqMan quantitative PCR analysis (Applied Biosystems, Oyster Bay, NY). **Supplemental Table 1** lists the genes investigated and the primers/probes used for PCR. The primers and probes were either obtained from predesigned gene expression assays or designed based on the sequence information of domestic swine (Applied Biosystems). The expression level of GAPDH mRNA was used as an internal control.

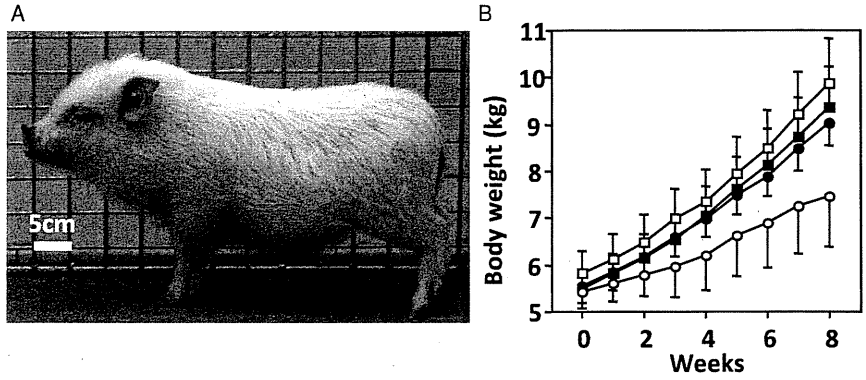

Fig. 1. Growth curves of the MMPigs

(A) Profile of a 5-month-old NcD-fed MMPig (body weight, 7 kg; body length, 60 cm). (B) Growth curves of the NcD-fed control and HcD-fed animals over eight weeks. All animals experienced an increase in body weight; however, no significant differences were observed among the experimental groups. Note that the body weights of HcD-fed animals remained < 10 kg following the administration of the HcD for eight weeks (7 months of age). Open circle, NcD control; closed circle, HcD with 0.2% cholesterol; open rectangle, HcD with 0.5% cholesterol; closed rectangle, HcD with 1.5% cholesterol.

Table 1. Visceral and subcutaneous adiposity in the MMPigs

% cho in diet	0	0.2	0.5	1.5
Relative weight (g/kg)				
Omentum	0.80 ± 0.25	1.78 ± 0.30*	1.96 ± 0.19**	1.78 ± 0.36*
Mesenterium	3.05 ± 0.47	4.30 ± 0.64	4.40 ± 0.35	4.22 ± 0.88
% increase of BFT	147.7 ± 38.8	214.0 ± 18.8	211.2 ± 26.4	234.8 ± 21.8*

The data are presented as the mean ± SE. cho, cholesterol; BFT, back fat thickness *$p<0.05$ and **$p<0.01$ vs. the control (0 % cho diet)

Statistical Analysis

All results are expressed as the mean ± SE. The statistical analysis of the differences between groups was performed using Student's *t*-test, and the results were considered to be significant at $p<0.05$.

Results

Body Weight, Adiposity and Blood Pressure

A 5-month-old male NcD-fed MMPig, 7 kg in body weight, is shown in **Fig. 1A**. The body weight values increased in all groups during the eight-week experimental period. Compared with the NcD-fed MMPigs, the HcD-fed MMPigs showed a more rapid increase in body weight. In contrast, no significant differences in body weight were observed between the NcD- and HcD-fed groups (**Fig. 1B**). The omental fat weight was higher in the HcD-fed animals than in the NcD-fed animals; however, no significant differences in mesenteric adipose tissues were observed. The back fat thickness was significantly increased after eight weeks in the 1.5% cholesterol-fed MMPigs, as compared with that observed in the control MMPigs (**Table 1**). The blood pressure levels, both systolic and diastolic, were similar in the HcD-fed and NcD-fed control MMPigs.

Hematology and Blood Biochemistry

Considering the normal reference data for MMPigs[18, 19], no animals exhibited leukopenia, leukocytosis or anemia after being fed NcD or HcD for eight weeks. No increases in the levels of AST, ALT or LDH occurred in either the NcD- or HcD-fed animals. The levels of ALP, γ-GTP and total bilirubin were moderately increased at scattered time points in the animals fed 0.5% or 1.5% cholesterol. No differences in blood glucose were noted between the HcD-fed and NcD-fed MMPigs.

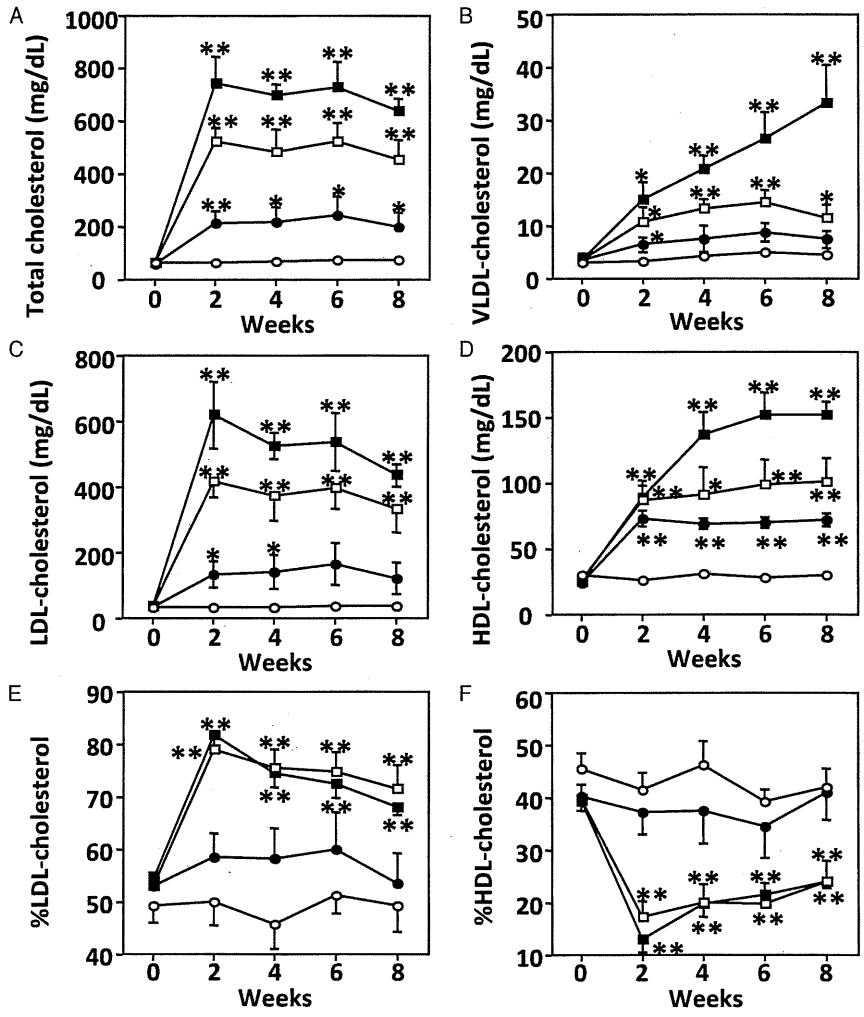

Fig. 2. Serum lipoprotein profiles

(A) Total cholesterol (TC). (B) VLDL-C. (C) LDL-C. (D) HDL-C. TC and each cholesterol fraction in the HcD-fed MMPigs exhibited dose-dependent increases during the eight-week experimental period. The percentages of the cholesterol fractions increased in LDL-C (E) and decreased in HDL-C (F) in the MMPigs fed HcD with 0.5% and 1.5% cholesterol during the eight-week experimental period. Open circle, NcD control; closed circle, HcD with 0.2% cholesterol; open rectangle, HcD with 0.5% cholesterol; closed rectangle, HcD with 1.5% cholesterol. The data are presented as the mean ± SE. $^{*}p<0.05$, $^{**}p<0.01$ vs. the control

Serum Lipoprotein Profile

The MMPigs became hypercholesterolemic after two weeks of the HcD at all cholesterol concentrations (0.2%, 0.5% and 1.5% cholesterol) compared with that observed in the NcD-fed controls (**Fig. 2A**). The TC levels plateaued after two weeks. The VLDL-C and LDL-C levels were increased in the groups fed higher concentrations of cholesterol (0.5% and 1.5% cholesterol;**Fig. 2B** and **2C**), whereas the HDL-C levels increased in all HcD-fed groups during the eight-week experimental period (**Fig. 2D**). The percent distribution of cholesterol with 0.5% and 1.5% cholesterol loading was increased in the LDL fractions and decreased in the HDL fractions at every time point during the experiment (**Fig. 2E** and **2F**). The serum TG levels were similar in all groups.

Expression of LDLr, SR-BI, HMGCR, SREBP-2 and NPC1L1

The hepatic expression of LDLr and HMGCR

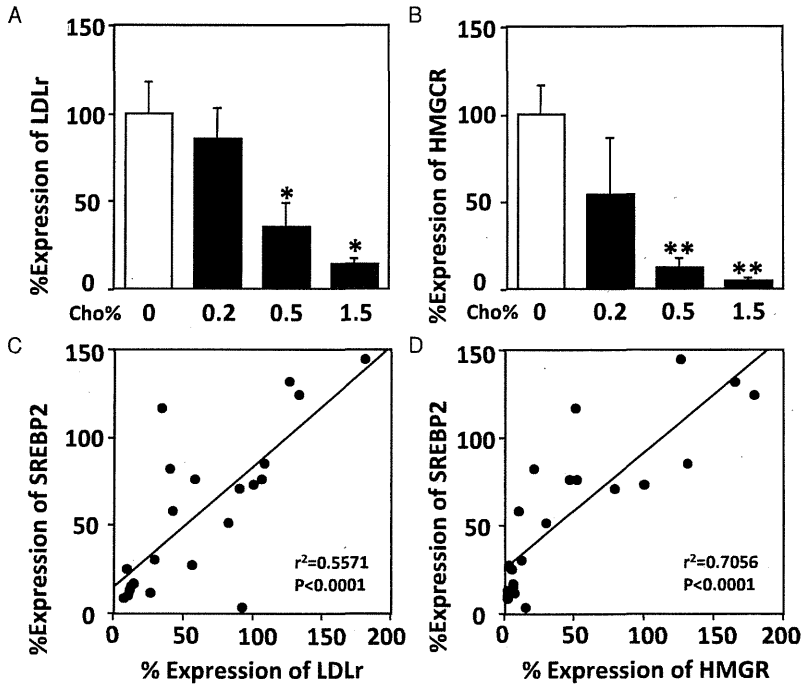

Fig. 3. Hepatic expression of LDLr, HMGCR and SREBP-2

The hepatic expression of LDLr, HMG-CoA reductase and SREBP-2 was analyzed using real-time RT-PCR at the end of the eight-week experiment. The expression of (B) was significantly decreased by HcD containing 0.5% and 1.5% cholesterol. (C) (D) The expression level of the SREBP-2 gene was highly correlated with the LDLr and HMGCR expression. The gene expression levels were calculated as the percentage expression over the level observed in the control NcD-fed group. The data are presented as the mean ± SE. $*p < 0.05$, $**p < 0.01$ vs. the control

was downregulated in the MMPigs fed HcD for eight weeks at higher dietary cholesterol concentrations (0.5% and 1.5% cholesterol; **Fig. 3A** and **3B**). The expression levels of these two genes were highly correlated with that of SREBP-2, irrespective of diet (**Fig. 3C** and **3D**). The expression levels of SR-BI in the liver were unchanged during HcD consumption (**Fig. 4A**). The expression of NPC1L1 in the small intestine was not decreased in the jejunum or ileum in the animals fed the HcD compared with that observed in the controls (data not shown); however, the hepatic expression was markedly reduced in the HcD-fed MMPigs (**Fig. 4B**).

HL and CETP Activity

The HL activity was much higher in the post-heparin fractions vs. the pre-heparin fractions in the NcD-fed MMPigs, thus demonstrating an activity of more than 90% in the circulation following the administration of sodium heparin (**Table 2**). For CETP, the protein expression, detected using Western blotting with antibodies against human CETP, and activity were detected in the HDL fraction in the serum of the NcD-fed MMPigs (**Supplemental Fig. 1**).

Expression of apoB mRNA Editing Enzyme and Serum apoB Profile

Hepatic APOBEC-1 is expressed in mice but not in humans or rabbits, and the editing enzymatic action generates apoB48 to form VLDL, including both apoB48 and apoB100[21]. Under our experimental conditions, the APOBEC-1 expression was detected using qRT-PCR in the small intestine alone and not in the liver in the MMPigs (**Fig. 4C**). We detected apoA1 and apoB48/100 peptides in both the human and swine VLDL and CM fractions (**Supplemental Fig. 2**).

Hyperlipidemia-Induced Atherosclerosis

The *en face* analysis demonstrated that aortic atherosclerotic lesions were significantly increased in the HcD-fed MMPigs at all dietary cholesterol concentra-

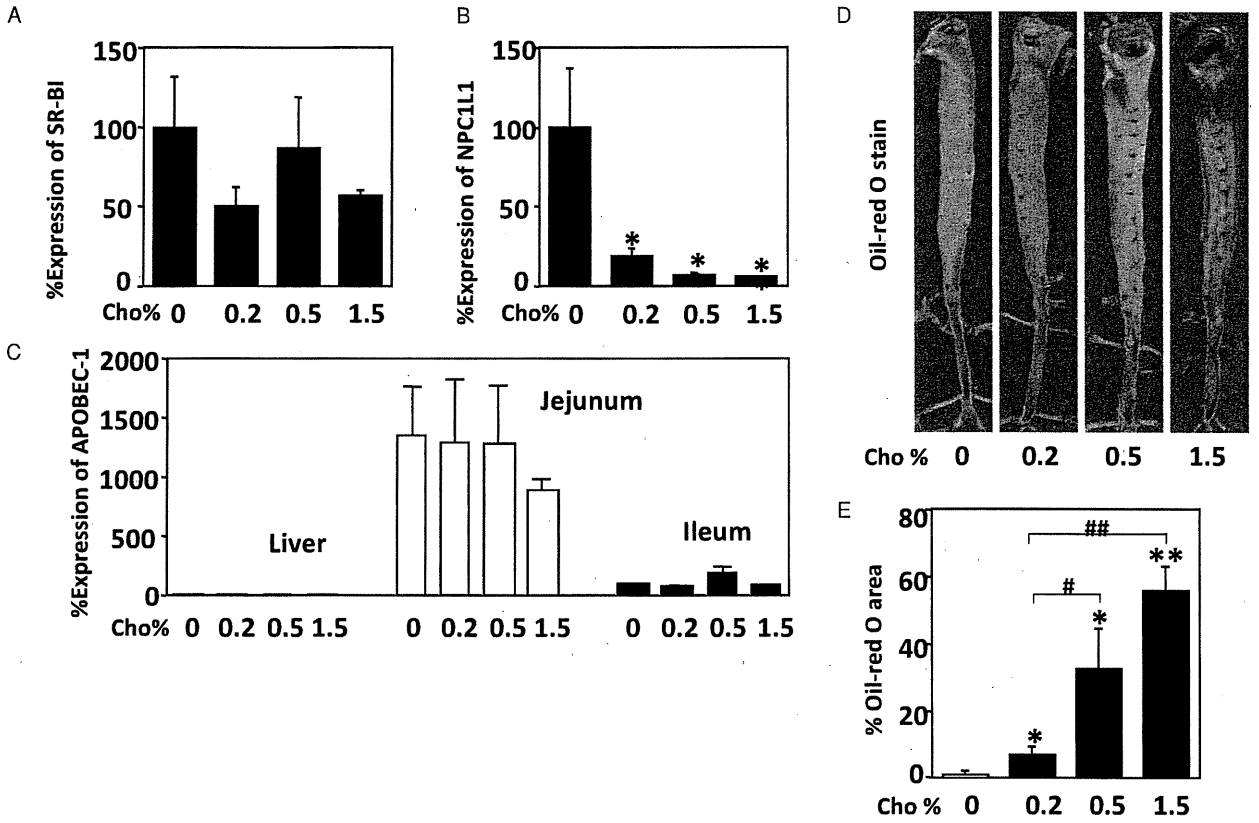

Fig. 4. Hepatic expression of SR-BI, NPC1L1 and APOBEC-1 and quantitative analysis of hyperlipidemia-induced atherosclerosis

(A) The hepatic expression of SR-BI in the HcD-fed groups was similar to that observed in the NcD-fed controls. (B) The hepatic NPC1L1 expression was markedly reduced by HcD feeding. (C) The APOBEC-1 expression was detected in the jejunum and ileum but not in the liver. The gene expression levels were calculated as the percentage expression over the level observed in the control NcD-fed group. (D) Oil-red O-stained atherosclerotic lesions in the NcD-fed and HcD-fed MMPigs after eight weeks. (E) The number of atherosclerotic areas stained with Oil-red-O stain was increased in the HcD-fed MMPigs. The values are presented as the mean ± SE. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. the control. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ between each group.

Table 2. Plasma hepatic lipase activity in the NcD-fed MMPigs

age (month)	25					5			
animal No.	1	2	3	4	mean ± SE	5	6	7	mean ± SE
B.W. (kg)	24.0	24.0	28.0	25.0	25.3 ± 1.6	5.7	5.8	5.7	5.73 ± 0.05
HL activity (pmol/ml/min)									
pre-heparin	0.07	0.24	0.08	0.09	0.12 ± 0.07	0.11	0.15	0.14	0.13 ± 0.02
post-heparin	2.47	2.37	2.66	3.42	2.73 ± 0.41	2.89	1.98	2.93	2.60 ± 0.44
liver-bound	2.40	2.13	2.58	3.33	2.61 ± 0.46	2.78	1.83	2.79	2.47 ± 0.45
% in liver	97.2	89.9	97.0	97.4	95.4 ± 2.8	96.2	92.4	95.2	94.6 ± 1.6

NcD, normal chow diet; B.W., body weight; HL, hepatic lipase

tions but unchanged in the NcD-fed controls (**Fig. 4D** and **4E**). The atherosclerotic lesions developed first at the aortic arch and the entry of the spinal arteries and abdominal aorta and progressed to involve the entire aorta as the cholesterol content in the HcD increased. The aortic atherosclerotic lesions were located in the intima (**Fig. 5A**) and primarily composed of infiltration of foam cells (**Fig. 5B**). Elastica-Masson Tri-

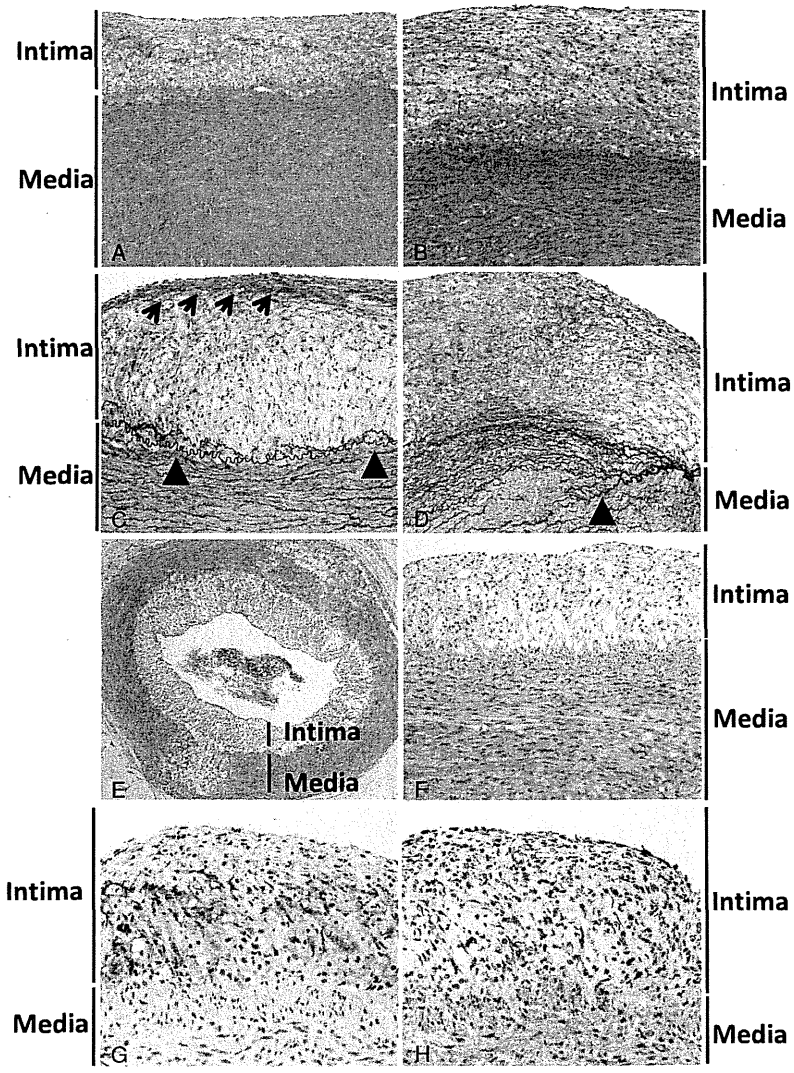

Fig. 5. Atherosclerotic lesions in the aorta and coronary arteries

Aortic atherosclerotic lesions of an MMPig fed HcD for eight weeks. (A) (B) Atheromatous plaque in the abdominal aorta with intimal infiltration of foam cells (H&E stain). (C) The internal elastic laminae are duplicated (arrowheads) and the plaque is covered with a fibrous cap (arrows; Elastica-Masson Trichrome stain). (D) An advanced lesion exhibits disruption of the internal elastic lamina and stratification of newly formed laminae (arrowhead; Elastica-Masson Trichrome stain). (E) Atherosclerotic lesions in the coronary artery of an MMPig fed HcD for eight weeks. (F) Light microscopy of an atherosclerotic lesion in the left main trunk at low and high power (H&E stain). (G) The plaque lesion consists of the accumulation of foam cells positive for the macrophage marker lysozyme. (H) A small number of intimal cells are positive for α -SMA. Medial smooth muscle cells positive for α-SMA were used as an internal control.

chrome staining showed duplication and disruption of the internal elastic lamina (**Fig. 5C** and **5D**). The advanced lesions exhibited fibrous cap formation covering the atheromatous plaques (**Fig. 5C**). The stages of atherosclerosis were varied in the sections examined; however, in general, the abdominal aortas showed a large amount of advanced lesions. The atherosclerotic lesions in the carotid, coronary and femoral arteries were identical to those observed in the aorta (**Fig. 5E** and **5F**). In the coronary arteries, the

proximal portions, located immediately after branching from the aorta, were severely involved in the atherosclerosis; however, a few lesions in the distal and intramuscular arteries were also observed, especially in the higher cholesterol diet-fed groups. The infiltrating foam cells were positive for the macrophage marker (lysozyme) (**Fig. 5G**). The atheromatous lesions also demonstrated small numbers of α-SMA-positive smooth muscle cells (**Fig. 5H**).

Histopathology of other Organs

No significant histological changes were observed in the heart, lungs, spleen or kidneys. Mild fatty degeneration was detected in the liver in the HcD-fed MMPigs at 0.5% and 1.5% cholesterol concentrations (data not shown). Despite the presence of atherosclerotic lesions, no HcD-fed MMPigs experienced thrombosis or spontaneous myocardial or cerebral infarction during the eight-week experiment.

Discussion

Diet-Induced Hypercholesterolemia and Atherosclerosis

This study demonstrated the presence of eight-week HcD-induced hypercholesterolemia and atherosclerosis accompanied by moderate visceral and subcutaneous adiposity in MMPigs. After eight weeks of the administration of HcD containing 1.5% cholesterol, the serum levels of TC reached approximately 600 mg/dL and atherosclerotic areas were seen in approximately 50% of the aortas. The relatively low 0.2% dietary cholesterol concentration was sufficient to induce the formation of aortic atherosclerotic areas in 7.5% of the animals, with TC levels of approximately 200 mg/dL. The consumption of dietary SC, which is often administered to mice in order to induce atherosclerosis[20], was not necessary to enhance the development of hyperlipidemia-induced atherosclerosis in the MMPigs. It is noteworthy that a period of only eight weeks was sufficient to induce atherosclerosis in MMPigs, whereas other miniature pigs require more than three months to develop this condition[8-12].

Serum Lipid Profiles

Both the serum LDL-C and HDL-C levels markedly increased after the administration of the HcD, with a greater increase observed in the LDL-C levels. The percentage distribution of cholesterol into HDL-C was therefore significantly reduced, indicating a proatherogenic lipid profile. This paradoxical increase in HDL-C induced by dietary fat has been reported in both humans and rabbits[21, 22]. Human metabolic studies have shown that the administration of HcD decreases the catabolic rate while increasing the transport rate of apoA-I, resulting in increased HDL-C levels[23]. Similar observations have been made in human apoA-I transgenic mice, in which the administration of HcD increases the HDL-C levels, whereas metabolic turnover studies indicate a decreased catabolic rate and increased transport rate of HDL-cholesteryl ester (CE) and apoA-I[24]. The diet-induced increase in the HDL-C levels may represent an adaptation for enhancing reverse cholesterol transport mediated via the HDL pathway, although the exact mechanism is unclear. The detection of the enzymatic activity of CETP, which regulates a portion of RCT, in the HDL fraction in the MMPigs supports the presence of this possible adaptive mechanism(s).

Serum ApoB Profiles

In this study, the MMPigs, similar to humans and rabbits, did not express hepatic APOBEC-1. The VLDL-C fraction therefore included only apoB100 and not apoB48. In contrast, mice express hepatic APOBEC-1 to generate apoB48-containing VLDL-C and subsequently lower the LDL-C levels[25]. The transfer of the APOBEC-1 gene in the liver in New Zealand White rabbits and Watanabe heritable hyperlipidemic rabbits results in the production of apoB48-containing VLDL-C and a reduction in LDL-C formation[26]. Serum VLDL-C cannot be converted to LDL-C in humans with hypobetalipoproteinemia because it contains truncated apoB50 due to a premature stop codon in the apoB gene[27]. Therefore, the regulation of the size of apoB by hepatic apoB mRNA editing represents a fundamental mechanism for limiting the generation of atherogenic apoB100-containing lipoproteins. The hepatic expression of APOBEC-1 corresponds closely with a low ratio of [VLDL-C + LDL-C] to HDL-C (<0.5) in dogs (0.26), rats (0.41), mice (0.25) and horses (0.44). Mammals that do not express hepatic APOBEC-1 exhibit higher ratios, including humans (1.92), monkeys (0.91) and pigs (1.4). Rabbits (0.32) are an exception to this rule[25]. In this study, the ratio in the NcD-fed MMPigs was 1.31, indicating a proatherogenic lipid profile in this group.

Expression of LDLr, HMGCR and NPC1L1

The blood cholesterol levels are largely determined by LDL-C removal mediated by LDLr and cholesterol synthesis via the HMGCR activity in the liver[28, 29]. The hepatic expression of LDLr and HMGCR is coordinately regulated by the sterol regulatory element-binding protein (SREBP)-2 signaling

pathway; the transcriptional activity of SREBP-2 is usually inhibited when cellular cholesterol is abundant[30-32]. In the present study, the hepatic expression of LDLr, HMGCR and SREBP-2 was correlated and markedly downregulated following the consumption of HcD. The regulation of these genes and the LDL-C-rich lipid profile in MMPigs is therefore very similar to that observed in humans and is in contrast with that observed in mice, in which downregulation gene responses are minimal[30].

Intestinal absorption and biliary excretion are additional closely regulated mechanisms of cholesterol homeostasis[33, 34]. A recently identified NPC1L1 protein that regulates intestinal cholesterol absorption is highly expressed in the small intestine in various species, including humans, rabbits and mice[16, 35]. The NPC1L1 protein is also expressed in the human liver, where it partially regulates biliary cholesterol excretion; however, it is not expressed in the murine liver[33]. In the present study, the NPC1L1 gene was expressed in both the small intestine and liver in the MMPigs. The regulatory gene mechanisms of the NPC1L1 expression in both organs include the SREBP-2 pathway, suggesting that a high level of cholesterol suppresses SREBP-2-regulatory genes, such as LDLr, HMGCR and NPC1L1[36, 37]. Although the NPC1L1 expression was not clearly reduced following the administration of the HcD, the detection of the expression of NPC1L1 in both the liver and intestine supports the conclusion that the gene expression profile linked to cholesterol homeostasis is very similar between MMPigs and humans.

Expression of the CETP and HL Activity

CETP catalyzes the transfer of CE from HDL to apoB-containing lipoproteins and is considered to be a key protein for reverse cholesterol transport[38, 39]. Humans and animals, including rabbits and chickens, with documented atherosclerosis susceptibility have a higher level of CETP activity than atherosclerosis-resistant animals, such as cats, dogs, rats and mice[40]. The development of atherosclerosis is accelerated by an atherogenic diet when CETP is genetically introduced in mice, which are naturally deficient in CETP[41, 42]. CETP and apoB100 double transgenic mice also show increased levels of atherosclerosis[43]. However, the overexpression of CETP in apoC-III or lecithin cholesterol acyltransferase transgenic mice inhibits atherosclerosis[44, 45]. Assessments of the effects of CETP on atherogenesis in mice are limited because the lipoprotein metabolism in mice differs markedly from that observed in humans. The relationship between the CETP activity and the development of human atherosclerosis is also controversial at present. Patients with coronary heart disease have lower levels of large HDL particles and higher levels of small, dense (sd) LDL particles[46-48]. CETP inhibitors, which significantly increase the level of large HDL particles and decrease the level of sdLDL particles, are thought to be effective for reducing the development of atherosclerotic cardiovascular disease[49]. In humans, a deficiency of the CETP activity results in increased plasma HDL-C levels with the generation of large CE-rich HDL particles, supporting this hypothesis[50]. Cases of CETP polymorphism or genetic variation, characterized by a diminished CETP activity, are associated with increased levels of HDL-C, a reduced incidence of coronary heart disease and greater longevity[51, 52]. However, other studies have reported an increased incidence of atherosclerotic cardiovascular and cerebrovascular diseases in CETP-deficient human subjects[53-55]. Furthermore, a recent clinical study showed that the administration of a CETP inhibitor (dalcetrapib) did not reduce the risk of recurrent cardiovascular events even though it increased the HDL cholesterol levels in patients with a recent history of acute coronary syndrome[56]. Whether CETP inhibitors are effective in preventing cardiovascular disease remains a matter of controversy.

Swine express no or very low levels of CETP activity, although they are susceptible to developing atherosclerosis[40]. In the present study, the CETP gene expression was not detected in the MMPigs using RT-PCR with primers designed from human and rabbit CETP cDNA sequences (data not shown). However, the MMPigs expressed CETP-like proteins and the CE transfer activity. Although the presence of a similar CETP gene remains a matter of debate, MMPigs appear to be a useful model for evaluating the relationship between the CETP activity and atherogenesis.

HL hydrolyzes TG in HDL to convert HDL_2 to HDL_3 and is involved in the conversion of IDL to LDL. In this study, the HL activity in the MMPigs was mostly detected in the post-heparin plasma, similar to findings observed in humans and rabbits. This suggests that most HL activity is localized on the surface of hepatic sinusoidal endothelial cells, in contrast to that observed in mice, in which most of the activity is detected in the circulation[57]. HL- and apoE-deficient mice exhibit a lesser degree of atherosclerosis than apoE-deficient mice, indicating the proatherogenic role of murine HL[58]. In LDLr and murine endogenous HL-deficient mice with the transgenic overexpression of human HL, which can bind to the surface of hepatic sinusoidal endothelial cells, the levels of VLDL, IDL and LDL are decreased, subse-

Table 3. Comparison of lipoprotein metabolism in mice, humans, rabbits and MMPigs

	mice	rabbits	humans	MMPigs
Lipoprotein	HDL-rich	LDL-rich	LDL-rich	LDL-rich
CETP	no	yes	yes	yes
HL	70% in circulation	liver-bound	liver-bound	liver-bound
Hepatic apoB editing	yes	no	no	no
Hepatic LDLr	high	down-regulated	down-regulated	down-regulated
Hepatic NPC1L1	no	yes	yes	yes
Diet-induced atherosclerosis	resistant	sensitive	sensitive	sensitive

CETP, cholesteryl ester transfer protein; HL, hepatic lipase; LDLr, LDL receptor; NPC1L1, Niemann-Pick C1-like 1 protein
This table was modified from ref. no. 5 by Fan J. and Watanabe T.

quently reducing atherosclerosis[59]. The overexpression of human HL results in the reduction of HDL and IDL in rabbits[60]. The function of HL appears to be affected by the location in which it is primarily localized (the liver or the circulation).

Conclusion

We herein established a novel swine model of hyperlipidemia-induced atherosclerosis in the world's smallest MMPig, an animal with a cholesterol metabolism very similar to that observed in humans (**Table 3**). We believe that MMPigs represent a potential alternative animal model suitable for studies of metabolic syndrome because the alteration of lipid metabolism is one of the key events in this condition.

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Conflicts of Interest

None.

References

1) Jawien J: The role of an experimental model of atherosclerosis: apoE-knockout mice in developing new drugs against atherogenesis. Curr Pharm Biotechnol, 2012; 13: 2435-2439
2) Wouters K, Shiri-Sverdlov R, van Gorp PJ, van Bilsen M, Hofker MH: Understanding hyperlipidemia and atherosclerosis: lessons from genetically modified apoE and ldlr mice. Clin Chem Lab Med, 2005; 43: 470-479
3) Getz GS, Reardon CA: Animal models of atherosclerosis. Arterioscler Thromb Vasc Biol, 2012; 32: 1104-1115
4) Bilheimer DW, Watanabe Y, Kita T: Impaired receptor-mediated catabolism of low density lipoprotein in the WHHL rabbit, an animal model of familial hypercholesterolemia. Proc Natl Acad Sci U S A, 1982; 79: 3305-3309
5) Brousseau ME, Hoeg JM: Transgenic rabbits as models for atherosclerosis research. J Lipid Res, 1999; 40: 365-375
6) Fan J, Watanabe T: Transgenic rabbits as therapeutic protein bioreactors and human disease models. Pharmacol Ther, 2003; 99: 261-282
7) Forster R, Ancian P, Fredholm M, Simianer H, Whitelaw B: The minipig as a platform for new technologies in toxicology. J Pharmacol Toxicol Methods, 2010; 62: 227-235
8) Goodrich JA, Clarkson TB, Cline JM, Jenkins AJ, Del Signore MJ: Value of the minipig model of menopause in the assessment of benefits and risks of postmenopausal therapies for cardiovascular and reproductive tissues. Fertil Steril, 2003; 79: 779-788
9) Turk JR, Henderson KK, Vanvickle GD, Watkins J, Laughlin MH: Arterial endothelial function in a porcine model of early-stage atherosclerotic disease. Int J Exp Pathol, 2005; 86: 335-345
10) Zhang C, Yin W, Liao D, Huang L, Tang C, Tsutsumi K, Wang Z, Liu Y, Li Q, Hou H, Cai M, Xiao J: NO-1886 upregulates ATP binding cassette transporter A1 and inhibits diet-induced atherosclerosis in Chinese Bama minipigs. J Lipid Res, 2006; 47: 2055-2063
11) Herrmann J, Saguner AM, Versari D, Peterson TE, Chade A, Olson M, Lerman LO, Lerman A: Chronic proteasome inhibition contributes to coronary atherosclerosis. Circ Res, 2007; 101: 865-874
12) Takai H, Miyoshi A, Yamazaki M, Adachi K, Katagiri K,