Myocardial Infarction-Prone Watanabe Heritable Hyperlipidemic Rabbits with Mesenteric Fat Accumulation Are a Novel Animal Model for Metabolic Syndrome

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Key Words

Atherosclerosis • Insulin resistance • Mesenteric fat accumulation • Metabolic syndrome • Myocardial infarction-prone Watanabe heritable hyperlipidemic rabbits

Abstract

Objectives: To examine whether the myocardial infarction-prone Watanabe heritable hyperlipidemic (WHHLMI) rabbit with visceral fat accumulation is a new animal model for human metabolic syndrome, we examined the relationship between mesenteric fat accumulation and insulin resistance, hyperlipidemia and atherosclerosis. **Methods:** Glucose tolerance tests were performed using adult (11- to 15-month-old) and middle-aged (17- to 21-month-old) WHHLMI rabbits fed standard chow restrictedly. In addition, lipoprotein lipid levels, serum C-reactive protein (CRP) levels, mesenteric fat weight and physical and physiological parameters were measured. Mesenteric fat was stained immunohistochemically. **Results:** The mesenteric adipose tissue was positive for monoclonal antibodies against macrophages, C-reactive protein and monocyte chemoattractant protein. In adult rabbits, mesenteric fat correlated to aortic lesion area, insulin resistance, fasting immunoreactive insulin, serum CRP,

abdominal circumference and body weight. In middle-aged rabbits, mesenteric fat correlated to lipoprotein lipid levels in addition to the parameters showing a significant correlation in adult rabbits, excluding aortic lesion area. **Conclusions:** The WHHLMI rabbit with visceral fat accumulation is a new animal model for metabolic syndrome.

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Introduction

Raised serum cholesterol levels are considered a major risk factor for atherosclerosis. However, hypocholesterolemic treatment is effective at preventing coronary events in less than 50% of patients [1]. Therefore, lipid abnormalities can only partly explain the prevalence of the development of coronary heart disease, and the control of factors other than serum cholesterol levels is important to lower the prevalence of heart disease. Metabolic syndrome is associated with an increased risk of both diabetes and cardiovascular disease [2] and has a very high prevalence (24% in the USA and between 24.6 and 30.9% in Europe) [3]. According to the International Diabetes Federation, metabolic syndrome is a disease cluster involving the accumulation of mesenteric fat (visceral fat)

accompanying any two of the following: insulin resistance (or mild hyperglycemia), hypertriglyceridemia, low high-density lipoprotein (HDL) cholesterol levels and raised blood pressure [4].

Studies using genetically modified mice and spontaneous mutant mice have contributed to the finding of adipocytokines, clarifying their role [5], and the molecular mechanisms underlying the development of metabolic syndrome [6]. However, the lipoprotein metabolism in rodents differs markedly from that in humans [7], and there are no rodent models for metabolic syndrome with atherosclerosis, except a few mouse strains with slight atherosclerotic lesions [8, 9]. Recently, a swine model for metabolic syndrome was established using an atherogenic diet [10]. This model is useful for stent experiments, but the correlation between mesenteric fat accumulation and atherosclerosis is unclear. However, in rabbits, lipoprotein metabolism resembles that in humans [11, 12]. The postprandial hyperlipidemic rabbit shows intraperitoneal fat accumulation, insulin resistance and postprandial hypertriglyceridemia [13], mimicking human metabolic syndrome, although no atherosclerotic lesions develop. Recently, Waqar et al. [14] reported that a high-fat diet induced metabolic syndrome and atherosclerosis in rabbits. They clearly demonstrated that their model is similar to human metabolic syndrome. However, they did not analyze the influence of mesenteric fat accumulation or insulin resistance on plasma lipid levels or the degree of atherosclerosis.

At Kobe University, we developed the Watanabe heritable hyperlipidemic (WHHL) rabbit, an animal model of familial hypercholesterolemia [15]. WHHL rabbits show hypercholesterolemia due to a genetic defect of low-density lipoprotein receptors and spontaneous atherosclerosis [12]. We have also developed a myocardial infarction-prone strain (WHHLMi rabbits) by selective breeding of WHHL rabbits [16]. Some WHHL rabbits showed intraperitoneal fat accumulation and insulin resistance, and these findings were improved by a thiazolidinedione derivative, a peroxisome proliferator-activated receptor- γ agonist [17, 18], which is an insulin action enhancer. These studies suggest WHHL or WHHLMi rabbits with intraperitoneal fat accumulation to be a good animal model for examining the relationship between metabolic syndrome and the development of atherosclerosis. In the present study, we examined the relationship between mesenteric fat accumulation and insulin resistance, hyperlipidemia and atherosclerosis, to examine whether the WHHLMi rabbit with mesenteric fat accumulation is a new animal model for human metabolic syndrome.

Materials and Methods

Animal Care and Use

We used 46 WHHLMi rabbits aged 11–15 months (adult) and 52 WHHLMi rabbits aged 17–21 months (middle-aged), as well as 10 adult Japanese white rabbits as a normal control. The WHHLMi rabbits were bred at the Kobe University Graduate School of Medicine. The Japanese white rabbits were obtained from Kitayama Labs, Co. Ltd. (Ina, Japan). The rabbits resided individually in metal cages (550 mm wide, 600 mm deep and 450 mm high) with a flat metal floor and consumed standard rabbit chow (LRC4, Oriental Yeast Co., Ltd., Tokyo, Japan) at 120 g/day and water ad libitum. The animal rooms were maintained under constant temperature ($22 \pm 2^\circ\text{C}$), relative humidity (50–60%), ventilation rate (15 cycles/h) and lighting cycle (12 h light/12 h dark). This study was approved by the Institutional Animal Care and Use Committee (approval numbers: P080111R, P091011R), and animal experiments were conducted in accordance with the Regulations for Animal Experimentation of Kobe University, the Act on Welfare and Management of Animals (Law No. 105; 1973, revised in 2006), Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain (Notification No. 88, 2006) and Fundamental Guidelines for the Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions under the Justification of the Ministry of Education, Culture, Sports, Science and Technology (Notice No. 71, 2006).

Intravenous and Oral Glucose Tolerance Tests

The intravenous glucose tolerance test (IVGTT) was carried out according to methods described previously [17, 18]. After 16 hours' fasting, rabbits were injected with a glucose solution (0.6 g/kg body weight) into a marginal ear vein. Blood samples were collected through ear veins 0, 5, 10, 20, 30, 60 and 120 min after the glucose was administered. The oral glucose tolerance test (OGTT) was performed as described previously [13]. After 16 hours' fasting, rabbits were administered a glucose solution (1.5 g/kg body weight) orally. Blood samples were collected through marginal ear veins 0, 15, 30, 45, 60, 90, 120, 180 and 240 min after the glucose administration. Blood sugar and immunoreactive insulin (IRI) concentrations were assayed. Using fasting blood sugar and IRI levels, homeostasis model assessment-insulin resistance (HOMA-IR) [19] and the Matsuda insulin sensitivity index (ISI) [20], Matsuda and DeFronzo [20] demonstrated that the Matsuda-ISI in the OGTT closely reflects whole-body insulin sensitivity.

Measurements of Body Size and Blood Pressure

Blood pressure in conscious rabbits was monitored directly at the ear central artery. An intravenous infusion catheter (18 G $\times$ 2 inches, SR-FF1851, Terumo, Japan) was inserted into the ear artery. A transducer (DTX Plus DT-4812, Becton Dickinson Inc., N.J., USA) was connected to the catheter via pressure tubing (TPT-12, PT-06 or PT-60, Becton Dickinson). Recordings were made with PowerLab 8/SP (ADInstruments). After euthanasia of the rabbits by intravenous injection of pentobarbital (30 mg/kg), the abdominal circumference at the lowest part of the costal bone and length from shoulder to buttocks were measured. Mesenteric fat, axillary fat, inguinal fat, the aorta and the heart were excised.

Table 1. Baseline data of adult (10- to 15-month-old) and middle-aged (17- to 21-month-old) WHHLMi rabbits

	Adult Japanese white rabbits	WHHLMi rabbits	
		adult	middle-aged
Body weight, kg	3.58 ± 0.10 (10)	3.33 ± 0.04 (46)	3.53 ± 0.05 (52)
Abdominal circumference, cm	38.3 ± 0.9 (10)	38.3 ± 0.40 (46)	39.4 ± 0.42 (52)
BMI, kg/cm/cm × 10,000	25.5 ± 1.4 (10)	25.4 ± 0.54 (40)	26.2 ± 0.48 (49)
Fat accumulation, g			
Mesenteric fat	48.1 ± 9.8 (9)	69.8 ± 4.6 (46)	92.2 ± 6.5 (52) ^{a, b}
Axillary fat	19.0 ± 3.3 (9)	17.6 ± 1.6 (37)	17.3 ± 1.3 (50)
Inguinal fat	22.0 ± 2.6 (9)	27.3 ± 1.8 (37)	33.0 ± 2.4 (50) ^a
Fasting blood sugar, mg/dl	129 ± 2.3 (10)	130 ± 2.3 (35)	117 ± 1.8 (37) ^{a, b}
Fasting IRI, ng/ml	0.82 ± 0.09 (10)	1.29 ± 0.28 (25)	1.60 ± 0.34 (34)
HOMA-IR	6.12 ± 0.72 (10)	10.2 ± 2.4 (25)	11.2 ± 2.6 (34)
Matsuda-ISI on the IVGTT	2.89 ± 0.41 (10)	3.08 ± 0.37 (21)	3.66 ± 0.75 (17)
Matsuda-ISI on the OGTT	2.73 ± 0.32 (10)	3.56 ± 0.46 (21)	3.28 ± 0.57 (18)
Serum total cholesterol, mg/dl	15.0 ± 1.8 (8)	820 ± 39 (41) ^a	761 ± 27 (42) ^a
VLDL cholesterol, mg/dl	3.5 ± 1.8 (8)	88.7 ± 10.5 (16) ^a	107 ± 17 (17) ^a
LDL cholesterol, mg/dl	2.5 ± 0.4 (8)	773 ± 34 (16) ^a	730 ± 35 (17) ^a
HDL cholesterol, mg/dl	9.0 ± 1.5 (8)	13.7 ± 1.1 (16)	13.7 ± 1.1 (17)
Serum triglyceride level, mg/dl	44.9 ± 6.8 (8)	331 ± 34 (40) ^a	323 ± 32 (41) ^a
VLDL triglyceride, mg/dl	21.5 ± 5.1 (8)	47.1 ± 5.2 (16) ^a	67.3 ± 13.7 (17) ^a
LDL triglyceride, mg/dl	4.5 ± 1.9 (8)	196 ± 13 (16) ^a	230 ± 19 (17) ^a
HDL triglyceride, mg/dl	24.1 ± 2.3 (8)	18.6 ± 2.1 (16)	17.9 ± 1.2 (17)
Whole triglyceride/HDL cholesterol ratio	11.3 ± 5.3 (8)	18.1 ± 1.8 (16)	27.1 ± 4.6 (17) ^a
Systolic blood pressure, cm Hg	116 ± 6.5 (5)	122 ± 3.1 (16)	121 ± 4.3 (15)
Diastolic blood pressure, cm Hg	87.5 ± 2.9 (5)	85.0 ± 1.8 (16)	79.0 ± 2.3 (15)
Surface lesion area of aorta, %	0	75.1 ± 3.4 (46) ^a	88.2 ± 0.8 (52) ^{a, b}
Serum CRP, µg/ml	7.6 ± 0.7 (7)	34.7 ± 5.9 (20)	86.2 ± 40 (17)

Values in parentheses represent the number of rabbits analyzed. Data are presented as means ± SEM. Statistical analyses were carried out with Scheffe's multiple comparison test.

^a p < 0.05 versus Japanese white rabbits; ^b p < 0.005 versus adult WHHLMi rabbits. BMI = Body mass index [body weight/(length between shoulder and buttocks)² × 10,000]; IVGTT = intravenous glucose tolerance test; OGTT = oral glucose tolerance test; VLDL = very low-density lipoprotein; LDL = low-density lipoprotein; HDL = high-density lipoprotein; CRP = C-reactive protein.

Preparation of Histological Sections

After the adipose tissue and heart were weighed, they were fixed with a 10% buffered formalin solution and embedded in paraffin. Each section was sliced serially to a thickness of 4 µm. Sections were stained immunohistochemically with monoclonal antibodies specific for rabbit monocytes/macrophages (RAM-11, Dako A/S, Glostrup, Denmark), monocyte chemoattractant protein-1 (MCP-1; Dako A/S) and C-reactive protein (CRP; Dako A/S). Immunohistochemical staining was carried out using a Dako Envision+ kit according to the manufacturer's instructions, accompanied by hematoxylin counterstaining.

Evaluation of Atherosclerotic Lesions of the Aorta

All parameters for atherosclerotic lesions were measured by computer-assisted color image analysis (Image-Pro Plus, version 4.5, Media Cybernetics Inc., Silver Spring, Md., USA). Aortic atherosclerosis was evaluated using the percentage surface area of lesions on the whole aorta (surface area of lesion/surface area of the whole intima × 100) [21].

Preparation of Plasma Lipoproteins and Biochemical Analyses

Lipoproteins were fractionated by ultracentrifugation (very low-density lipoprotein (VLDL), density <1.006 g/ml; low-density lipoprotein (LDL), 1.006 g/ml < density < 1.063 mg/dl; HDL, density >1.063 g/ml) [22]. Total cholesterol and triglyceride levels were assayed by enzymatic methods. Blood sugar levels were assayed with Antsense III (Horiba Ltd., Kyoto, Japan). Serum insulin levels and CRP levels were assayed with ELISA kits (Rat Insulin ELISA kit and Rabbit CRP ELISA kit, Shibayagi Co., Ltd., Shibukawa, Japan).

Statistical Analyses

Data are presented as means ± SEM. Statistical analyses were carried out for mean values with Student's t test or Welch's t test, or for the mean values among multiple groups with Scheffe's multiple comparison test, and for frequency with a χ² test. Correlation analyses were carried out with Pearson's correlation test. A value of p < 0.05 was considered statistically significant.

Table 2. Correlation between accumulation of fat and physical or physiological parameters of adult (11- to 15-month-old) and middle-aged (17- to 21-month-old) WHHLMI rabbits

	Adult rabbits						Middle-aged rabbits					
	mesenteric fat			subcutaneous fat			mesenteric fat			subcutaneous fat		
	n	r	p value	n	r	p value	n	r	p value	n	r	p value
Age	46	0.013	n.s.	37	0.373	0.014	52	-0.078	n.s.	50	-0.005	n.s.
Body weight	46	0.349	0.002	37	0.405	0.007	52	0.682	<0.001	50	0.481	<0.001
Abdominal circumference	46	0.327	0.027	37	0.319	0.037	52	0.646	<0.001	50	0.440	0.001
BMI	46	0.112	n.s.	34	0.306	0.055	50	0.541	<0.001	49	0.240	n.s.
Subcutaneous fat	37	0.363	0.017				50	0.649	<0.001			
Blood pressure												
Systolic	16	-0.200	n.s.	15	0.129	n.s.	15	0.343	n.s.	13	0.083	n.s.
Diastolic	16	-0.310	n.s.	15	-0.021	n.s.	15	0.257	n.s.	13	0.245	n.s.
Average	16	0.319	n.s.	15	-0.042	n.s.	15	0.268	n.s.	13	0.176	n.s.
Percentage surface lesion area of aorta	46	0.331	0.025	37	0.043	n.s.	52	-0.085	n.s.	50	-0.176	n.s.

Subcutaneous fat was measured as the sum of inguinal fat and axillary fat. Correlation analyses were carried out with Pearson's correlation test. A value of $p < 0.05$ was considered statistically significant. BMI = Body mass index; n.s. = not significant.

Results

Baseline Data of WHHLMI Rabbits

There were no significant differences in baseline data, except for serum or lipoprotein lipid levels and atherosclerotic lesions, between adult WHHLMI rabbits and Japanese white rabbits (table 1). In middle-aged WHHLMI rabbits, the accumulation of fat in the mesentery and inguinal region was increased and blood sugar levels were lowered compared with values in adult WHHLMI rabbits and Japanese white rabbits.

Accumulation of Mesenteric Fat in WHHLMI Rabbits

Figure 1a and b shows the accumulation of intraperitoneal fat in middle-aged WHHLMI rabbits compared to Japanese white rabbits (fig. 1c, d). In the mesenteric fat of WHHLMI rabbits, RAM-11-positive cells (monocytes/macrophages) were observed in swollen adipocytes with lipid (fig. 1e) and were positive for MCP-1 (fig. 1f) and CRP (fig. 1g, h).

Glucose Tolerance Tests

Figure 2 shows results of glucose tolerance tests in adult rabbits. Changes in blood sugar levels were similar among the three groups. However, after glucose administration, serum IRI levels were markedly higher in WHHLMI rabbits with high fasting IRI levels than in WHHLMI and Japanese white rabbits with normal fast-

ing IRI levels. As a result, the HOMA-IR value was significantly higher and the Matsuda-ISI in the OGTT and IVGTT was significantly lower than the values in rabbits with normal fasting IRI levels (fig. 2g, h, j-l). Similar results were observed in middle-aged rabbits (fig. 3). These results suggest that WHHLMI rabbits with high fasting IRI levels show insulin resistance.

Correlation between Mesenteric Fat Accumulation and Other Parameters

As shown in table 2, mesenteric fat was correlated to body size and accumulation of subcutaneous fat (axillary fat + inguinal fat) in adult and middle-aged WHHLMI rabbits. However, a significant correlation with aortic lesions was observed only in the adult rabbits. Subcutaneous fat accumulation did not correlate to aortic lesion area despite showing significant correlation to parameters of body size. As shown in table 3, mesenteric fat correlated to most blood chemical parameters but did not correlate to blood sugar and HDL triglyceride in middle-aged rabbits. These results may suggest that mesenteric fat accumulation affects insulin sensitivity or response, lipoprotein metabolism and atherosclerosis. However, a significant correlation with mesenteric fat accumulation in adult rabbits was observed only for fasting IRI, HOMA-IR and serum CRP levels. Although we analyzed rabbits aged 11–15 months and 17–21 months, age did not correlate to mesenteric fat accumulation or any other parameter (data

Fig. 1. Visceral fat accumulation in WHHLMI rabbits and immunohistochemical staining of mesenteric adipose tissue. **a, b** Photographs of the accumulation of intraperitoneal fat and mesenteric fat in a WHHLMI rabbit (female, 18 months old). **c, d** Photographs of the accumulation of intraperitoneal fat and mesenteric fat in a Japanese white rabbit (male, 15 months old). **e-h** Photomicrographs of immunohistochemical staining of mesenteric adipose tissue of the WHHLMI rabbit.

Fig. 2. Glucose tolerance tests in rabbits aged 10–15 months. **a, b** Blood sugar levels and serum IRI levels during the OGTT. **c, d** Blood sugar levels and serum IRI levels during the intravenous glucose tolerance test (IVGTT). **e–h** Results of the OGTT. **i–l** Results of the IVGTT. Data are presented as means $\pm$ SEM. Statistical analyses were carried out with Scheffe's multiple comparison test. ^a $p < 0.05$ compared with normal rabbits; ^b $p < 0.05$ compared with WHHLM rabbits with low fasting IRI levels. BS = Blood sugar.

not shown) in these age ranges. Therefore, the influence of age in these analyses was minor. Similar findings were observed in correlation analyses between subcutaneous fat and metabolic parameters, except serum CRP levels.

Correlation between the Index of Insulin Resistance in the OGTT or Fasting IRI Levels and Other Parameters

Fasting IRI levels correlated to body size, lipoprotein lipid levels and serum CRP levels in the middle-aged group but did not correlate to aortic atherosclerosis in the

adult group (table 4). On the other hand, the Matsuda-ISI, an index of insulin sensitivity in the OGTT, showed a significant correlation to aortic atherosclerosis in adult WHHLM rabbits ($r = -0.477$, $p = 0.029$) and the ratio of whole triglyceride level to HDL cholesterol ($r = -0.549$, $p = 0.028$) and serum CRP levels ($r = -0.646$, $p < 0.001$) in middle-aged WHHLM rabbits. These results suggest that hyperinsulinemia and insulin resistance are independent in WHHLM rabbits.

Fig. 3. Glucose tolerance tests in WHHLM rabbits aged 17–21 months. **a, b** Blood sugar levels and serum IRI levels during the OGTT. **c, d** Blood sugar levels and serum IRI levels during the intravenous glucose tolerance test (IVGTT). **e–h** Results of the OGTT. **i–l** Results of the IVGTT. Data are presented as means $\pm$ SEM. Statistical analyses were carried out with Student's t test or Welch's t test. Statistical significance was set at $p < 0.05$. BS = Blood sugar.

Discussion

WHHLM rabbits with high fasting IRI levels showed an accumulation of mesenteric fat and insulin resistance even with restricted feeding on normal chow. The mesenteric fat accumulation correlated to aortic atherosclerosis, body size, insulin sensitivity, lipoprotein metabolism and plasma CRP levels.

In WHHLM rabbits, the accumulation of fat at the mesentery correlated with aortic lesion area in the adult

group but not in the middle-aged group (table 2). This difference may be due to the extremely advanced atherosclerotic lesions in the middle-aged rabbits. Actually, the percentage area of lesions on the aortic surface was $75.1 \pm 3.4\%$ in the adult group and $88.2 \pm 0.8\%$ in the middle-aged group (table 1). In the adult group, the percentage area of aortic lesions was $67.3 \pm 5.7\%$ ($n = 21$) in rabbits with less than 60 g of mesenteric fat and $84.1 \pm 3.6\%$ ($n = 15$) in rabbits with more than 80 g of mesenteric fat ($p = 0.018$). The mean age was 13.2 ± 0.3 months in each

Table 3. Correlation between accumulation of fat and biochemical parameters of adult (11- to 15-month-old) and middle-aged (17- to 21-month-old) WHHLMi rabbits

	Adult rabbits						Middle-aged rabbits					
	mesenteric fat			subcutaneous fat			mesenteric fat			subcutaneous fat		
	n	r	p value	n	r	p value	n	r	p value	n	r	p value
Fasting blood sugar	21	-0.162	n.s.	15	0.331	n.s.	37	0.352	0.033	32	0.206	n.s.
Fasting IRI	21	0.520	0.016	15	-0.197	n.s.	34	0.493	0.003	32	0.535	0.002
HOMA-IR	21	0.479	0.028	15	-0.160	n.s.	34	0.502	0.002	32	0.442	0.011
Matsuda-ISI on the IVGTT	21	-0.335	n.s.	15	0.1180	n.s.	17	-0.570	0.018	15	-0.353	n.s.
Matsuda-ISI on the OGTT	21	-0.307	n.s.	15	0.145	n.s.	17	-0.570	0.017	15	-0.646	0.009
Lipoprotein cholesterol												
VLDL	19	-0.331	n.s.	16	-0.184	n.s.	17	0.834	<0.001	15	0.475	0.074
LDL	19	-0.032	n.s.	16	0.119	n.s.	17	0.570	0.021	15	0.689	0.005
HDL	19	-0.280	n.s.	16	-0.219	n.s.	17	-0.555	0.026	15	-0.271	n.s.
Whole cholesterol	19	-0.110	n.s.	16	-0.071	n.s.	17	0.701	0.002	15	0.667	0.007
Lipoprotein triglyceride												
VLDL	19	0.236	n.s.	16	-0.134	n.s.	17	0.855	<0.001	15	0.399	n.s.
LDL	19	0.138	n.s.	16	0.025	n.s.	17	0.825	<0.001	15	0.309	n.s.
HDL	19	0.410	n.s.	16	0.625	0.010	17	0.066	n.s.	15	0.146	n.s.
Whole triglyceride	19	0.233	n.s.	16	0.020	n.s.	17	0.836	<0.001	15	0.367	n.s.
Whole triglyceride/HDL cholesterol ratio	19	0.268	n.s.	16	0.187	n.s.	17	0.781	<0.001	15	0.238	n.s.
Serum CRP	20	0.455	0.044	17	0.211	n.s.	17	0.601	0.023	12	0.139	n.s.

Subcutaneous fat was measured as the sum of inguinal fat and axillary fat. Correlation analyses were carried out with Pearson's correlation test. A value of $p < 0.05$ was considered statistically significant. IVGTT = Intravenous glucose tolerance test; OGTT = oral glucose tolerance test; VLDL = very low-density lipoprotein; LDL = low-density lipoprotein; HDL = high-density lipoprotein; CRP = C-reactive protein; n.s. = not significant.

Table 4. Correlation between fasting IRI and physical or biochemical parameters in WHHLMi rabbits

	Adult rabbits			Middle-aged rabbits		
	n	r	p value	n	r	p value
Body weight	21	0.133	n.s.	18	0.488	0.047
Abdominal circumference	21	0.190	n.s.	18	0.389	n.s.
Body mass index	21	-0.192	n.s.	16	0.403	0.012
Lipoprotein cholesterol						
VLDL	18	-0.287	n.s.	16	0.552	0.027
LDL	18	-0.234	n.s.	16	0.658	0.006
HDL	18	-0.158	n.s.	16	-0.416	n.s.
Lipoprotein triglyceride						
VLDL	18	0.454	n.s.	16	0.818	0.005
LDL	18	0.324	n.s.	16	0.690	<0.001
HDL	18	-0.125	n.s.	16	0.054	n.s.
Whole triglyceride/HDL cholesterol ratio	18	0.303	0.22	16	0.719	0.002
Blood pressure						
Systolic	15	0.218	n.s.	14	0.109	n.s.
Diastolic	15	-0.315	n.s.	14	0.002	n.s.
Average	15	-0.320	n.s.	14	0.030	n.s.
Aortic lesion area	21	0.326	n.s.	18	-0.184	n.s.
Serum CRP	19	0.0912	n.s.	16	0.825	<0.001

Correlation analyses were carried out with Pearson's correlation test. A value of $p < 0.05$ was considered statistically significant. VLDL = Very low-density lipoprotein; LDL = low-density lipoprotein; HDL = high-density lipoprotein; CRP = C-reactive protein; n.s. = not significant.

group. These results strongly suggest that mesenteric fat accelerates the progression of aortic atherosclerosis in adult rabbits. Previous studies suggested that adipocytokines derived from mesenteric fat can affect dyslipidemia, glucose metabolism, hypertension, inflammation and thrombogenesis [23]. These secondary disorders are related to atherogenesis [5, 10, 23]. Although several studies demonstrated that a high-fat diet induced obesity, insulin resistance, intraperitoneal fat accumulation and slight atherosclerosis [8–10, 14], there are no studies on the correlation between mesenteric fat accumulation and atherosclerosis. In addition, Matsuzawa [24] pointed out that mesenteric fat has an important influence on dyslipidemia and atherogenesis because it supplies free fatty acids to the liver through the portal veins. Since atherosclerotic lesions enlarged to cover most of the aortic surface due to hypercholesterolemia in middle-aged rabbits, the influence of mesenteric fat accumulation might be small. In adult rabbits, the Matsuda-ISI of the OGTT correlated negatively to aortic lesions but fasting IRI levels did not. These results indicate that insulin resistance affects the progression of atherosclerosis. Although we cannot explain the present results, insulin resistance may accelerate atherosclerosis through adipocytokines secreted from adipocytes of mesenteric fat [5].

In WHHLMi rabbits, mesenteric fat accumulation correlated to the Matsuda-ISI, HOMA-IR and fasting IRI levels as in other animal models reported previously [25]. Although Matsuda-ISI values of adult rabbits did not correlate with mesenteric fat accumulation, those of middle-aged rabbits showed a significant correlation. The weight of mesenteric fat was 69.8 ± 4.6 g ($n = 46$) in adult rabbits and 92.2 ± 6.5 g ($n = 52$) in middle-aged rabbits ($p = 0.006$). These results suggest that insulin resistance of WHHLMi rabbits developed with an increase in mesenteric fat accumulation. In addition, our previous study demonstrated that metabolic syndrome-like symptoms in WHHL rabbits with high fasting IRI levels were ameliorated by the administration of troglitazone, a thiazolidinedione derivative [18]. These results suggest that WHHLMi rabbits with mesenteric fat accumulation correspond to human metabolic syndrome.

In middle-aged WHHLMi rabbits, mesenteric fat correlated positively to apolipoprotein B-containing lipoprotein lipid levels and negatively to HDL cholesterol levels. The mechanisms of the development of dyslipidemia in patients with metabolic syndrome or obesity were summarized previously [26]. The ratio of plasma triglyceride to HDL cholesterol showed a high correlation similar to that in obese individuals showing insulin resistance

[27, 28]. It is well known that HDL cholesterol negatively correlates to cardiovascular diseases. In mice, HDL cholesterol levels were increased by a high-fat diet [29, 30] or increased or unchanged by obesity [31, 32]. An increase in HDL cholesterol levels was also observed in rat [33, 34] and swine [10] models. However, HDL cholesterol levels were decreased in normal rabbits fed a high-fat diet, and overexpression of lipoprotein lipase improved insulin resistance in rabbits fed a high-fat diet [35–37]. These differences in changes in HDL cholesterol levels may be due to species differences [12]. The results of previous studies suggest that rabbit models for metabolic syndrome or insulin resistance have the advantage of both an increase in triglyceride levels and a decrease in HDL cholesterol levels due to the progression of mesenteric fat accumulation.

In the present study, plasma CRP levels correlated to mesenteric fat accumulation in both adult and middle-aged WHHLMi rabbits and to the Matsuda-ISI and fasting serum IRI levels in middle-aged rabbits. In mesenteric fat, CRP and MCP-1 were positive and macrophages had infiltrated, suggesting inflammation. On the other hand, serum CRP levels did not correlate to aortic atherosclerosis. These results suggest that serum CRP levels in WHHLMi rabbits affected the progression of metabolic syndrome or insulin resistance. Similar findings were observed in patients with metabolic syndrome [38]. In rabbits fed a high-fat diet, serum CRP levels increased, accompanying insulin resistance and with an increase in intraperitoneal fat accumulation [14]. In rodents, CRP was not detected in the circulation, and the inflammatory marker in that species is serum amyloid P component rather than CRP as in humans and rabbits [39]. These findings suggest the advantage of a rabbit model for metabolic syndrome.

In conclusion, since the characteristics of WHHLMi rabbits with large amounts of mesenteric fat resemble those of human metabolic syndrome even with restricted feeding of normal chow, the accumulation of visceral fat and insulin resistance in WHHLMi rabbits may be controlled by genetic factors. WHHLMi rabbits with mesenteric fat accumulation may be a new animal model for human metabolic syndrome.

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